Systematized Survey on the History of Drug Discovery with Technical Development

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Kohei Umezu

■Abstract

This Survey on the Systemization of Industrial Technologies for Medicines systematically investigates pharmaceutical technologies by tracing the history of the advancement. It starts with crude drugs (natural drugs derived from minerals, plants, and animal substances), which have been used since the dawn of civilization. Then modern science discovered the causes of many diseases, which led to research and development of new medicines with specific mechanisms of action to eliminate those causes. Although few crude drugs are used at present, some of their ingredients are highly effective and others have unique mechanisms of action. These ingredients have made a significant contribution to modern pharmaceutical drug discovery as a basis for the advances of various technologies.

This paper consists of three chapters. The second chapter, History of Disease and Medicines, outlines the history of pharmaceutical drugs used from the earliest civilizations to the present day.

This chapter consists of four sections. The first section is History from Ancient to Early Modern Times, which narrates the history of crude drugs used together with remedies from witchcraft as well as the fight against humankind's greatest enemy, pandemic diseases, in Ancient Mesopotamia, China, and India through Greece and Rome to the dawn of modern science.

The second section, The Dawn of Modern Science and Medicine describes how modern science identified and isolated the active ingredients from crude drugs and the history of new pathogen discovery.

The third section, The Origin of Modern Pharmaceutical Drug discovery and Development explains how basic sciences including immunology, chemotherapeutics, and pharmacology, have evolved into the modern medical system for the scientific drug development. This section also examines the circumstances of the pharmaceutical industry in Japan which started behind Western countries.

The fourth section, From New Medicines to Improved Medicines and Innovative Medicines, describes the process of the rapid evolution in Japan of pharmaceutical development after the Second World War using the cases of β-receptor antagonists and digestive, anti-inflammatory, and central nervous system medications as example.

The third chapter, Technological Advancement of Pharmaceutical Drug Discovery and Development through Systems Science, illustrates the process of drug development and technological innovation driven by advances in science. In the late 20th century, drug development became too complex to be accomplished just by pharmacologists and specialists in organic synthesis, evolving into a multidisciplinary science that necessitated a wide range of expertise. This process of evolution is also presented in the third chapter.

The fourth chapter discusses the advances of pharmaceutical drug discovery and development from modern times to the present using the example of lifestyle-related diseases, which have attracted attention as one of the most common health issues in current society. The drugs that have undergone research and development for lifestyle-related diseases are systematized, especially medications for diabetes, hypertension, hyperlipidemia, thrombosis and clotting disorders, and hyperuricemia.

In addition to drugs for lifestyle-related diseases discussed in this paper, there are numerous medicines available for a wide range of diseases such as central and peripheral nervous system disorders, cardiovascular diseases, heart failure, respiratory diseases, urinary tract disorders, hormonal and endocrine disorders, digestive tract disorders, rheumatism and inflammation, allergic and immune disorders, sensory disorders, bacterial and viral diseases, cancers, orphan and rare diseases, and medications such as anesthetics in surgery. The details of these drugs will be described in other papers.

This paper outlines the history of drug development such as β -receptor antagonists, and digestive, central nervous system, and anti-inflammatory related drugs, as mentioned above, because these were the first drugs created by the modern pharmaceutical drug discovery and development system and thus are closely related to advances of pharmaceutical development technology. This technology has evolved rapidly since the Second World War, and is becoming an established system at present.

Advancement in genetic engineering has enabled us to utilize the genes of different species since around1972. Application of this biotechnology has opened up the way to develop the biological medicines, which are now growing in number. This progress is briefly summed up in this paper, and the details such as the antibody medicine will be covered in another opportunity.

This figure (Fig. 0.1) outlines the discussion of the present paper. The history of medicines for infectious diseases and that of other medicines are shown separately in two chronological flow charts.

Analytical studies on the ingredients of crude drugs started in the 19th century. Afterword, advancements infields of sciences such as pharmacology and organic chemistry led to establishment of the pharmaceutical drug discovery and development process, and a variety of new drugs have been developed, with some being based on theory and some being discovered by chance. Researchers first tackled the development of simple medicines that were easy to develop by using pharmaceutical technology, and then gradually expanded their efforts to include medicines for complex diseases. After the human genome was first mapped in 2000, the attention of researchers has recently focused on personalized and regenerative medicine.

A scientific approach to the infectious diseases, involving immunotherapy and chemotherapy, also emerged in the 19th century, when many pathogens were discovered after years of fighting against this enemy. Since the invention of antibiotics in the-mid 20th century, treatment of infectious diseases has been significantly improved, saving countless

lives.

In conclusion, this survey systematizes the technologies for developing medications by tracing the advances from crude drugs to scientific development of modern medicines along with the historical background.

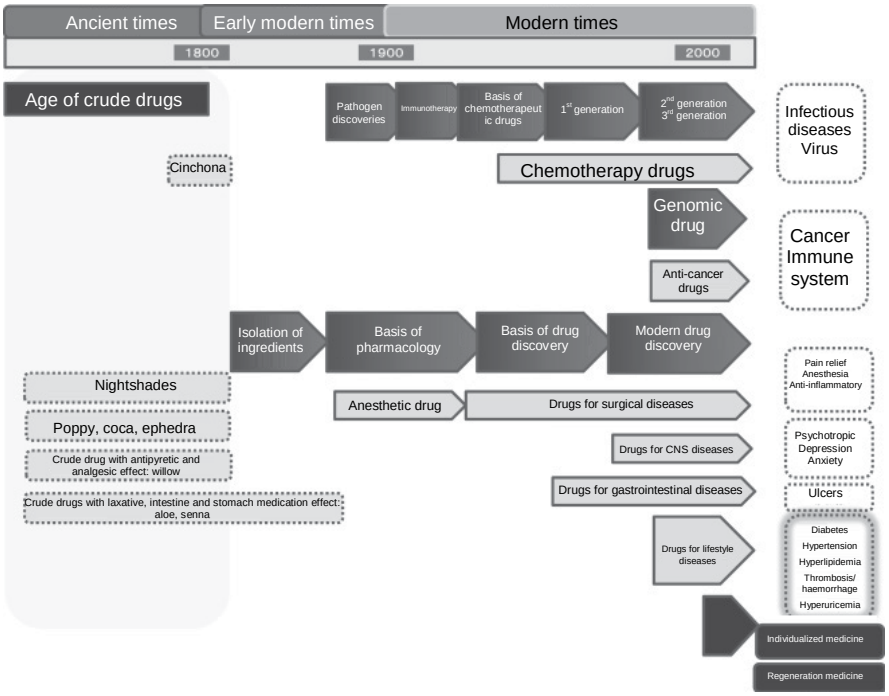

Fig. 0.1 Composition of Systematized Survey on the History of Drug Discovery with Technical Development.

■ Profile

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1969	Graduated from Kyoto University, Faculty of Pharmaceutical Sciences
1971	Graduated from Kyoto University, Graduate School of Pharmaceutical Sciences Started working for Mitsubishi Chemical Industries, Central Research Laboratory
1977	Studied at Michigan State University, Department of Pharmacology
1978	Studied at Yale University, Yale School of Medicine, Department of Pharmacology
1980	PhD in Pharmacology at Kyoto University
1996	Head of the Product Planning Department, Mitsubishi Chemical Corporation Pharmaceuticals and Diagnostics Company
2000	Director of Mitsubishi-Tokyo Pharmaceuticals and Director of the Medical Research Institute
2001	Executive Officer and Director of Research, Mitsubishi Pharma Corporation
2002	Executive Officer of Mitsubishi Chemical Corporation and Director of the Healthcare Planning Center, concurrent Director of Mitsubishi Chemical Safety Institute
2004	Managing Director and Head of the Clinical Trials Department, Head of the Food Products Division in charge of Research and Development, Mitsubishi Kagaku Bio-Clinical Laboratories
2007	Director and Managing Executive Officer, Head of the Business Development Office in charge of the Anti-Doping Center
2008	Visiting Professor at Wayo Women's University
2009	Appointed Consultant to Mitsubishi Chemical Medience
2010	Retired from Mitsubishi Chemical Medience

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1 Introduction

In 2012, the average life expectancy in Japan was 86.4 years for females and 79.9 years for males. According to the "2010 Annual Changes in Life Expectancy taken from Complete Life Tables", a resource produced every five years by the Ministry of Health, Labour and Welfare based on the national census, the life expectancy was 42.8 years for males and 44.3 years for females in the period from 1891 to 1898, the earliest life table recorded. In the period from 1926 to 1930, the life expectancy was 44.8 years for males and 46.5 years for females. Even by 1935, the life expectancy was still surprisingly short, at 46.9 years for males and 49.6 years for females. By 1955, the figures had risen to 63.6 years for males and 67.8 years for females; by 2010, they had risen to 79.6 years for males and 86.3 years for females.

While it is not as straightforward as simply comparing Meiji-period data with the data of today, this resource does indicate that there was a rapid increase in life expectancy after the Second World War, whereas even in the late 1930s and early 1940s people were not expected to reach 50 years of age. To what could this change be attributed? It is a fact that infant mortality is closely tied to life expectancy; however, the life expectancy only increased by three to four years in the 44-year period from 1899 to 1935, despite improvements in hygiene and nutrition accompanying advances in education from the late 19th century through to the late 1920s. Alongside these improvements in environmental and hygiene conditions, medicine is thought to have played a significant role in the rapid increase in post-war life expectancy.

The 2011 "Vital Statistics" by the Ministry of Health, Labour and Welfare record the mortality rates by leading causes of death from 1899 to 2009 (Figure 1.1). The mortality rates for the 110-year period shown in this diagram indicate a significant change on either side of the blank period representing the Second World War (Pacific War). The top three leading causes of death before the war were pneumonia, gastroenteritis and tuberculosis, followed by cerebrovascular diseases (cerebral hemorrhage, stroke). Given that the top three leading causes of death were infectious diseases, with gastroenteritis encompassing diseases such as cholera, typhoid fever, dysentery and diarrhea, it is evident that the mortality rate from infectious diseases was extremely high.

After the war, there was a rapid decrease in the mortality rate from these types of infectious diseases. This underlines the importance of improved hygiene and nutrition conditions, as well as the emergence of antibiotics and antibacterial medicines. With the arrival of penicillin to combat infection, as well as streptomycin, p-aminosalicylate (PAS) and isoniazid, among others, to combat tuberculosis, the change in mortality rates demonstrated the ground-breaking efficacy of these medicines. Thus, the life expectancy in Japan began to increase after the war. Second- and third-generation antibiotics and antibacterial medicines became the mainstay of the Japanese pharmaceutical industry and stayed that way for a long time. With the infectious diseases that had been the top leading causes of death now able to be remedied, cerebrovascular diseases became the top leading causes of death. Since at that time there were no refrigerators as yet, the Japanese were using large quantities of salt in homemade miso and pickles that could last up to six months. These high amounts of salt are believed to have been a leading cause of hypertension in those who were eating these products on a daily basis. Epidemiological and medical studies eventually identified a link between hypertension and cerebrovascular diseases and people began to realize how important it is for ordinary individuals to take steps to lower their blood pressure. A series of blood pressure medications began to be developed to reduce blood pressure using pharmacological action mechanisms; as blood pressure was brought under proper control, the number of people suffering from cerebrovascular diseases declined. Blood pressure medications played a significant role in reducing the mortality rate from these diseases.

Stomach ulcers and duodenal ulcers had been incurable in Japan since the late 19th century; even after the war, they required surgery. The discovery of H_2-receptor antagonist oral drugs improved treatment efficacy to the point where surgery was no longer required. Meanwhile, as the economy developed and lifestyles became more stable with adequate food and clothing, there was a gradual increase in lifestyle-related diseases. In the coming era, diabetes, hyperlipidemia, obesity and other lifestyle-related diseases began to draw attention. The so-called "metabolic syndrome" exacerbated the hardening of the arteries and insulin resistance, ultimately leading to ischemic heart disease or cerebrovascular disease. A succession of hyperlipidemia medications and cholesterol-lowering medications began to be developed, as a major cause of hardened arteries is excessive cholesterol or triglycerides in the blood. Statins, a class of cholesterol-lowering medications invented in Japan, demonstrated a powerful efficacy, receiving worldwide acclaim as a breakthrough drug, and made a significant contribution to reducing ischemic heart disease. Several other new drugs developed in Japan have also produced good results as diabetes medications, anticoagulants and antiplatelet drugs.

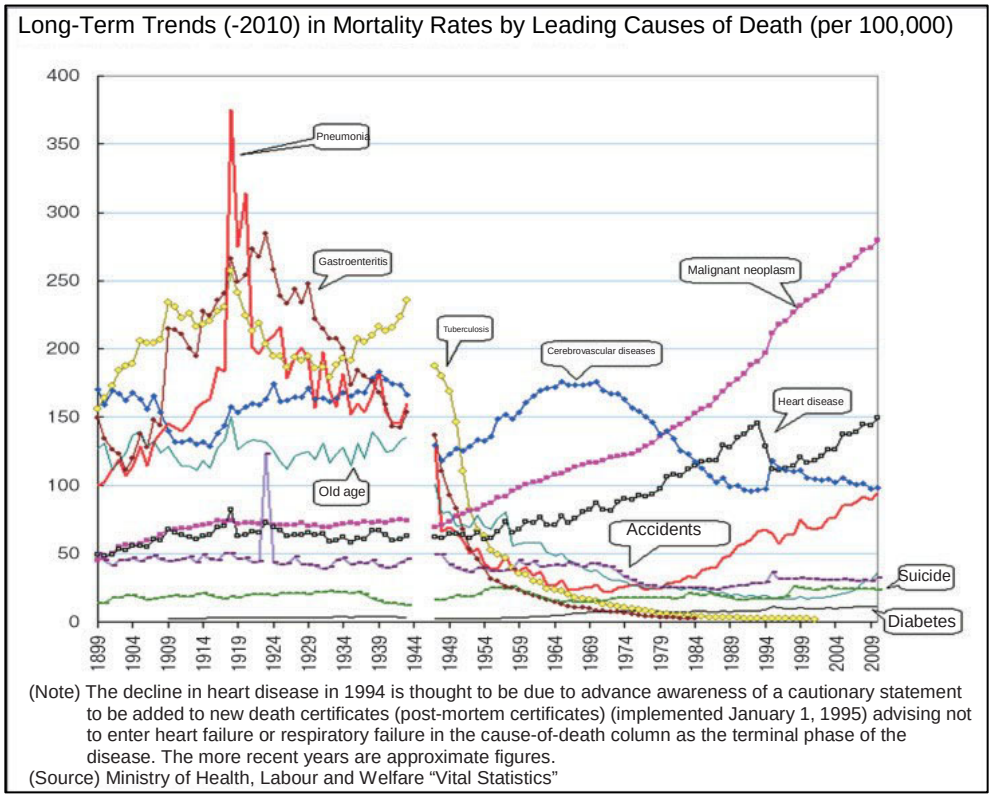
Despite growing public awareness, we have entered an age of fine dining and insufficient exercise. Since 2007, the Ministry of Health, Labour and Welfare has implemented specific physical examinations as a countermeasure against metabolic syndrome, attempting to raise greater public awareness and also reduce ever-increasing medical expenses, but the result thus far has been less than satisfactory. Nevertheless, inadequate as it may be, education and new drugs also have some effect; the increase in mortality rate due to lifestyle-related diseases is no longer as rapid as it once was.

Meanwhile, the leading cause of death shifted to cancer (malignant neoplasm) from around 1980. Unlike infectious

diseases, cancers are not caused by microbes from outside the body, but by changes in the body's own cells. Advances in molecular biology and genetic engineering have made it evident that not all cancers are the same and that they have different properties when manifesting in different organs, as well as that anticancer medications react differently in different individuals, so that one medication is not effective against all cancers. We have now entered an era of ongoing research and development on medications for different cancers, or anticancer medications that target individual genes and genetic makeups.

Although medications have played an extremely significant role in maintaining healthy lives for people amidst these changing times, they in fact have quite a short history; it has only been since the end of the Second World War that theoretically and scientifically effective medications have come to be produced.

This survey report retraces the history of medications for diseases suffered by humans and provides a systematic examination of developments in pharmaceutical technologies, from the crude drugs and remedies from witchcraft in ancient times to the drug discoveries of today supported by modern science. The hope is that this will serve as a reference resource for the next generation of young technologists and researchers in looking back at the steps taken by the pioneers in the industry and thereby play a role in future pharmaceutical research and development.



(Note) The decline in heart disease in 1994 is thought to be due to advance awareness of a cautionary statement to be added to new death certificates (post-mortem certificates) (implemented January 1, 1995) advising not to enter heart failure or respiratory failure in the cause-of-death column as the terminal phase of the disease. The more recent years are approximate figures.
(Source) Ministry of Health, Labour and Welfare "Vital Statistics"

Fig. 1.1 Mortality Rates in Japan by Leading Causes of Death

2 History of Disease and Medicines

Chapter 2 outlines the history of diseases suffered by humans and the medications used to combat them.

The first section of this chapter discusses human history from ancient to early modern times as the age of using natural products (crude drugs) and provides a summary of those crude drugs as well as the diseases most feared at each point in time. The next section discusses the dawn of modern science and medicine as the age of advances in organic synthetic chemistry and isolating and identifying ingredients, in which modern science began and the main substances (drugs) in natural products started being extracted, isolated and identified, and infection-causing pathogens started being identified. The following section discusses the origin of modern pharmaceutical drug discovery and development, in which developments in pharmacology and biochemistry made it possible to start analyzing the actions mechanisms of drugs, through to modern drug discovery supplemented by synthesis technology based on organic chemistry. The final section in the chapter examines the evolution of drugs and the advances in drug discovery from new medicines to improved medicines and innovative medicines, discussing successive discoveries of new drugs since the Second World War, aided by modern drug discovery technology, through to the age of the so-called genomic drug discovery from the late 1990s onwards.

2.1 History from Ancient to Early Modern Times (Age of Using Crude Drugs)

2.1.1. History of Drugs (Ancient Times to 19th Century)

Living in ancient times before agriculture, our ancestors would have been desperate to secure food for survival. They would have eaten whatever plants or animals they could get their hands on, and through their experiences would have classified these as edible or inedible, having inevitably come into contact with poison at some point, experienced food poisoning from eating decomposing matter and suffered from infectious diseases. During that process, they would have come to know that certain plants, animals or minerals were effective at relieving pain and fever. They would have also come to know how to make use of plants that cause vomiting or diarrhea, and would have passed this information on to their descendants. Eventually, people started planned hunting in groups as well as learning how to farm; over a long period of time, ancient civilizations were born.

As these ancient civilizations developed in various places around the world, people started accumulating and systematizing their knowledge of drugs (crude drugs) derived from natural plant, animal and mineral substances. Usage of these crude drugs including grinding and extracting by mixing with hot or cold water, drying and eating, and applying to the affected area as a paste.

Around 5000 BP in Mesopotamia, the Sumerians are reported to have engraved the names of various medicinal animals, plants and minerals on clay tablets in cuneiform. Medical treatment in this region at that time was intertwined with astrology; people believed that everything, including the fate of humans, was determined by the movement of the stars, which represented the will of the gods. These clay tablets, together with some slightly more recent clay tablets, record more than 250 species of plants, more than 180 species of animals and more than 120 kinds of minerals used for crude drugs. Poppy and henbane were used to relieve pain, cinnamon for gastronomical problems, pomegranate for expelling parasitic worms and almonds and licorice for suppressing coughs. These drugs were not used on their own, but were combined in close connection with incantations and witchcraft [(1)].

This information was passed on to the Egyptians and eventually compiled together. The Ebers Papyrus, a medical compendium written in the 13th Egyptian dynasty around 3500 BP, records 700 types of crude drugs, including medications for wounds, skin diseases, women's diseases, laxatives and emetics. The curing of diseases usually involved the driving out of demons of sickness dwelling inside the body, often through the prescribing of emetics, laxatives or enemas. In some cases, animal dung, rotting flesh or fat was used to achieve this. A number of other papyri have been discovered with crude drugs recorded in them, although they are from other eras [(1)].

Hippocrates, who lived in the fifth and fourth centuries BCE, was a representative physician of the Classical Greek era; his ideas are still highly valued to this day. One of his greatest achievements was separating medicine from primitive superstitions and witchcraft and developing it into an empirical science with high regard for clinical practice and observation. Assuming that diseases were some kind of natural disorder, he emphasized the use of medicine to assist the natural healing power of the body. He is said to have treated patients with only limited drugs, such as laxatives, emetics and diuretics.

Some of these ideas have been carried through into modern medical and pharmaceutical science, which is why Hippocrates is referred to as the "father of modern medicine". A text entitled *The Oath*, regarding the morality and objectivity of physicians, is included among his collected works; this has been carried down to the present day as the Hippocratic Oath.

Medications used at the time included milk or oriental melons as laxatives, sodium carbonate or honey for enemas, a mixture of honey and vinegar as an emetic and spring onions or sea onions as diuretics. Imperial Rome imported valuable drugs and Oriental medicines from across the vast expanse of its occupied territories and many complicated formulations of crude drugs were invented [(1, 2, 3)].

The first century CE brought Pedanius Dioscorides, known as the world's first pharmacologist [(4)]. His five-volume *Materia Medica*, the world's first scientific work on pharmacognosy and pharmacology, lists 600 types of drugs and records the habitats, proper names and alternative names of medicinal plants, as well as accurate

sketches of them.

Medication therapy was popular in Imperial Rome, leading to a chaotic state of disarray as various different factions of medicine emerged alongside growing numbers of traders in counterfeit medicines. The second century brought Claudius Galenus, known as Galen, whose adherence to the Hippocratic notion of the natural healing power of the body led to a revival of Hippocratic medicine. Galen is believed to have authored over 500 treatises on an expansive range of topics, including anatomy, physiology, etiology, pathology, diagnoses and treatments. In the field of pharmacology, he recorded different types of drugs, their effects and their formulation. While Galen made some significant achievements in the development of contemporary medicine, his works are permeated with his own views and biases. However, Galen's works on medicine remained the authoritative works on the subject throughout the Middle Ages to the Renaissance; under the domination of these authoritative works, an age of no significant medical discoveries or inventions continued. Later generations of pharmacologists continued to term their own herbal preparations as Galenic formulations, even into early modern times, in an attempt to benefit from Galen's authority, even when those formulations contradicted Galen's works.

Three million people are said to have died in the Crusades, these heavy casualties occurring in the Dark Ages before the development of science. While universities started being established in various places throughout Europe from the 12th century onwards, the university in Bologna became famous for surgical medicine.

However, surgical procedures date back to ancient times. The Sumerians are believed to have had the technology for trephining and removing tumors. Human dissections are believed to have been carried out in Imperial Alexandria, accompanied by advancements in surgical techniques, such as performing surgical operations using an infusion of mandragora as an anesthetic. The revival of surgical techniques in the 12th century included the use of the sap from medicinal plants as an anesthetic. Human dissection, which had not been practiced since the Alexandrian era, revived in 1302 for medical research purposes. The foundation for the systematic study of anatomy was laid by Mondino de Luzzi in his 14th century work *Anathomia corporis humani* [2, 3, 5].

Pharmacists and pharmacies were coming into being throughout Europe from the 11th to the 14th centuries. The study of anatomy had developed to the point of noting errors in Galen's works, but his authority had yet to be contested. Andreas Vesalius, regarded as the founder of anatomy, is said to have caused an uproar in scientific circles in 1543 with his publication of *De humani corporis fabrica*, the first work on human anatomy [1:94-96].

Retracing the history of crude drugs reveals that they have been used since ancient times the entire world over in conjunction with witchcraft. While in many cases these crude drugs would have been of dubious effectiveness in and of themselves, in some cases as modern science has isolated the active ingredients it has shown that there was a rational reason for their usage, with an empirically proven medicinal effect, such as opium from poppies or henbane and belladonna in the nightshade family. Crude drugs from all kinds of natural products would have been tested to try to alleviate pain, cramps and fevers from acute illnesses, empirically showing which ones had a strong medicinal efficacy. However, while crude drugs with strong medicinal efficacy could alleviate pain or cramps, there would have been very little progress made in terms of causal therapy to take effect on the cause of the ailment.

Surgical techniques have been practiced since ancient times. Although crude drugs with strong medicinal efficacy were also used since the very early days, the anesthesia effect was not the same as that used in modern medicine, often involving widespread paralysis of the central nervous system. The study of anatomy was essential, not only for surgical techniques, but also for the development of medical science; however, it was strongly discouraged in an era in which religion and medicine were intertwined.

The oldest work on Chinese medicine is the *Rites of Zhou*, complied by Duke Wen of Zhou around the 3rd or 4th century BCE. Although primarily written about the government system of the Zhou Dynasty, it mentions a technique used by doctors involving "five poisons and five medicines". The "five medicines" are grass, wood, corn, stone and insects, while the "five poisons" are mineral poisons. This idea was also carried over into *The Devine Farmer's Classic of Materia Medica*, the oldest work on herbal medicines, written in the Three Kingdoms period of China (3rd century CE). Although the author of this work is unknown and the original text has been lost, Tao Hongjing of the Liang Dynasty compiled his three-volume *Reading of the Divine Farmer's Classic of Materia Medica* around 500 CE based on this work and later a further seven-volume edition. These works record more 730 types of medicines.

Of the medicines recorded in *The Divine Farmer's Classic of Materia Medica*, 365 are categorized three ways into the noble or upper herbs, the commoner or middle herbs and the lower herbs. The 120 upper herbs are designated as non-toxic, life-enhancing drugs that can aid longevity, able to be taken long term. The 120 middle herbs are designated as health-enhancing drugs, some non-toxic and some toxic. The 125 lower herbs are designated as treatment drugs, but highly toxic and not able to take long term. In Chinese medicine, medicinal herbs are not determined on the efficacy of the herb in and of itself, but on the idea of interaction, so that certain effects are achieved through certain combinations [6, 7].

Archaeological findings at ancient Indus Valley Civilization sites discovered in the lower reaches of the Indus River indicate that there was quite a highly advanced ancient civilization in this region around 5000 years ago.

The Brahma sutras were compiled around 3500 years ago. One of these, the *Ayer Veda*, suggests that a traditional medical system was established around 3000 years BP. This work categorizes medicine into eight different disciplines, including surgery, internal medicine, pediatrics and psychiatry. Treatment methods include "taking and vomiting medicine", "cleansing the intestines using diarrhea or an enema" and "blood-letting at bad points". Crude drugs were used in complex combinations and in large quantities. The idea was that "there is nothing that exists on the earth that cannot be used as medicine". A medical treatise written by Sushruta, a 4th-century Indian physician records in detail

various animal toxins found in tropical environments, such as in snakes and frogs, as well as methods for counteracting those poisons [8, 9].

There are thought to be more than 2000 different kinds of crude drugs found around the world; other than some regional variations, there are no significant fundamental differences between the crude drugs found in different regions.

In Japan, according to *Kojiki* and *Nihon Shoki*, Takamimusubi, one of the three deities of creation, is revered as the god of medicine. His son, Sukuna-Biko-Na, worked together with Ōnamuchi-no-mikoto, known for healing the Hare of Inaba, to try to treat the diseases of humankind in a demonstration of divine virtue [10]. As in other parts of the ancient world, illness in ancient Japan was the work of gods and devils and witchcraft was a principal method of medical treatment. As in other ancient civilizations, crude drugs were used experimentally as an added measure.

From the 4th century onwards, as the Yamato Imperial Court began to have increasingly more exchange with the Korean Peninsula, medical and pharmaceutical science from the Asian continent began to spread to Japan. In 553, Emperor Kimmei sent an envoy to Baekje requesting Beon Yangpung and Jeong Yuta be sent to Japan as experts in medicinal herbs. In 563, Chiso of Wu was naturalized and presented the emperor with 164 books, including works on Chinese medicine.

Shitennōji Temple was constructed during the reign of Empress Suiko and incorporated a dispensing apothecary, where medicinal herbs were cultivated. Zenna, the son of Chiso of Wu, who had gifted Emperor Koutoku with milk, is regarded as the father of medication production in Japan.

Systematically produced medicines were imported to Japan by envoys to the Sui and Tang Dynasties of China. Chinese monk Jianzhen finally reached Japan on his sixth attempted voyage at the invitation of Emperor Shoumu, bringing Buddhism with him, as well as many medicines. Around 60 of those medicines from that time still exist and are held among the artifacts at the Shōsō-in treasure house in Nara, cataloged as “various pharmaceuticals”. In the late 1940s, Yasuhiko Asahina led an investigation on the medicines held at the Shōsō-in. The study confirmed the existence of 29 types of medicine and determined that most of these were imported products [11:45-47, 12:337-338].

As Tang Dynasty medical science began to be introduced to Japan with the sending and receiving of envoys, Korean medicine gradually began to go to into decline in Japan and was completely overtaken by Chinese medicine by the Edo Period.

Japanese Names of Medical Herbs, written in the Heian Period, lists 1025 types of medicines and is thought to be Japan’s oldest book on medicinal herbs. While many of the medicines are also recorded in the contemporary work *Ishinpō*, the content draws references from Korean medical works and *The Newly Revised Pharmacopeia* from Tang China.

The battles throughout the Kamakura and Azuchi-Momoyama Periods prompted the development of medicines to treat wounds. The arrival of Western culture in the 16th century brought surgical techniques and Galenic medicine, discussed above. However, the main external medicines were ointments for cleaning wounds, salves and compress pastes [13].

The more stabilized society of the Edo Period ushered in a golden age of medical and pharmaceutical products. A uniquely Japanese branch of “experimental Chinese medicine” began to flourish, known as Kampo medicine. This was founded by Chinese medical practitioner Todo Yoshimasu based on the *Treatise on Cold Injury*, compiled by Zhang Zhongjing in the Later Han Dynasty. Everyday people were buying medicines, ranging from medicinal plants bought from the medicine vendors of Toyama to medications bought from drug wholesalers, while doctors were referring to *The Divine Farmer’s Classic of Materia Medica*, the *Compendium of Materia Medica* written by Li Shizhen and other classic Chinese medical texts to formulate their own medications and dispensing these to their patients.

Edo-Period medicines were also known as Tang medicine, since many of them were imported from China. Syphilis, tuberculosis and leprosy were rampant during this time. Sarsaparilla root was often used to treat syphilis, while ginseng or cinnamon was used to treat tuberculosis and chaulmoogra was used to treat leprosy. The earliest record of syphilis in Japan is found in the medical treatise *Gekkai Roku*, written by Shūkei Takeda in 1512. In the Edo Period, Dokushōan Nagatomi, a well-traveled doctor, wrote that eight out of ten invalids in Edo, Nagasaki and Osaka were suffering from syphilis [15:25]. The biggest Chinese medicine import during the Edo Period was sarsaparilla root; the fact that it was used to treat syphilis indicates how rampant syphilis was at the time. It was later replaced by a similar species of sarsaparilla grown in Japan [14].

The *Latest Reports on Pharmacology* by Tatuo Kariyone records that “sarsaparilla root was formerly used as an alternative against syphilis and rheumatism, but now has very little medicinal use” [16]. Syphilis is caused by infection from *Treponema pallidum* bacteria; no substance found in sarsaparilla root has been identified as having any properties that have any effect on the *Treponema pallidum* bacteria.

The three main infectious diseases were dysentery, typhoid fever and cholera. Although rhubarb, Chinese ephedra, Baikal skullcap, licorice and dried aconite root were used to treat these diseases, it is presumed that these crude drugs probably had no direct bacteria-killing effect.

An examination of crude drugs around the world from ancient civilizations through to early modern times reveals that during the several millennia before modern science identified the causes of diseases and the action mechanisms of the drugs needed to treat them, natural plant, animal and mineral products were used medicinally alongside witchcraft and religion all the world over, although there was much in common among the natural products used.

Although it is difficult to categorize those diseases from the perspective of modern medicine, they can be categorized by crude drug usage. The following discusses: (i) medications for digestive system diseases; (ii) medications for diseases causing pain, fever or cramps; (iii) medications relating to surgical procedures, such as treating wounds or removing tumors; (iv) medications for infectious diseases; and (v) medications for lifestyle-related diseases, such as diabetes and arteriosclerosis.

(i) Throughout the ancient world, the cause of diseases was beyond human understanding. A common idea all around the world was the use of cathartics, laxatives or vomiting/diarrhea-inducing drugs to expel the disease from the body. Although the prescriptions used to rid patients of diseases were not always theoretically consistent with the cause of disease, this method presumably would have worked to expel toxins from the body in the case of food poisoning or accidental ingestion of poison. It may also have been effective to some extent in the case of infectious diseases affecting the digestive system.

While there are many crude drugs that can benefit a stomach affected by minor ailments of the digestive system, the placebo effect also cannot be overlooked, as shall be mentioned later.

(ii) Fevers, pains and cramps caused by infectious diseases and various other diseases suffered by humans could presumably have been eased by crude drugs such as willow bark, which has an antipyretic and analgesic effect, or by nerve paralysis using plants in the nightshade family that strongly affect the central nervous system, or in modern pharmacological terms, have an acetylcholine-like effect or anti-acetylcholine-like effect. Poppies and ergot fungi, which induce mental exhilaration, were also presumably used for pain control in the treatment of various diseases. However, the use of crude drugs with strong neurological effects would surely have also caused some major side effects in patients.

(iii) Since ancient times, wounds have been commonplace in battle and in everyday life. While medicated pastes and sometimes surgery were used to treat wounds, there was no concept of sterilization until more recent times. As discussed in (ii), crude drugs with strong neurological effects were used to alleviate pain and inflammation.

There are ancient records from all over the world of tumors also being removed by surgery. The idea has existed since ancient times that the body will return to normal after removing a tumor or damaged tissue. However, although crude drugs were used, none of them would have had a very strong anti-inflammatory effect. Records indicate that nightshades and poppies containing morphine and codeine were used for analgesia and as a substitute anesthetic.

In Japan, when surgical techniques developed in the Azuchi-Momoyama Period, serious wounds were bound up to prevent bleeding and smelling salts (stimulants) administered. Styptics were used to stop bleeding and wounds were cleansed with ointments. Cuts in muscle tissue and broken bones were dressed and splinted; arrows and bullets were removed with the aid of medications [(5)].

However, it stands to question what the life extension rate was among patients treated by surgery in an age in which there was no concept of disinfection or sterilization. Only those with strong natural healing power would have survived; even then the prognosis would have been extremely poor.

The various effects of early crude drugs presumably often included a placebo effect [(Note 1)]. Given that the placebo effect is surprisingly strong even in modern medicine, it must have been far greater when combined with witchcraft to treat patients in ancient and medieval times.

Hippocrates shrewdly observes that “clinical symptoms are natural reactions to a disease, and a temperature is nothing more than this reaction; the natural power given to individuals is to progress towards treatment and fight against disease” and that “it is nature that heals diseases; the human body has been given natural healing power. The art of medicine is to aid this; that is the fundamental method of treatment” [(17)]. This natural healing probably also includes a mental placebo effect.

Although there are still many cases today in which a strong placebo effect appears during drug therapy at acute stages, there is also a theory supported by meta-analysis [(Note 2)] that the placebo effect disappears with long-term administration and that it cannot be used as a primary form of treatment [(18)]. There are a number of reports on the “magnitude of the placebo effect”. According to a report [(19)] put together by the Japanese Society of Gastroenterology, “Beecher’s oft-cited paper [(20)] reports that the placebo effect improved post-operative pain and coughing by an average of 35.2±2% in 15 tests”. In a 1994 article, Kienle notes that the reviews on the placebo effect include a report indicating a more than 90% improvement for stomach/duodenal ulcers. However, Kienle also states that there is no basis for the purported magnitude of natural healing caused by the placebo effect alone [(21)]. Clinical trials to evaluate the efficacy of a drug are carried out using the same drug in different places around the world; however, reports indicate that the placebo effect differs significantly depending on the region and the time [(22)].

The findings from these current reports reveal that the placebo effect is very wide ranging and can surprisingly be as high as 80-90% effective on certain ailments.

Meanwhile, papers that question the placebo effect reveal a higher placebo effect in tests conducted with fewer people in the placebo group and a lower placebo effect as the group size increases. Further, although some subjective effects are recognized, it has a lower effect on objective indicators [(17)].

There has been much recent discussion on the benefits of placebos in terms of the ethical aspects of clinical trials [(Note 3)]. There is also thought to be a need to continue to analyze the scientific efficacy of the effect in diseases susceptible to it and to consider utilizing the effect. It would not be denying the historical contribution made by the crude drugs of the past to include the placebo effect among the effects of those crude drugs. Meanwhile, it also cannot be denied that this placebo effect that is found only in humans may have been one of the reasons that kept us from discovering truly effective crude drugs until relatively recently in our history.

(iv) There are countless different kinds of infectious diseases. They are able to take large numbers of human lives at a time and have caused pandemics many times all around the world. Although various crude drugs would have been used alongside witchcraft and surgical procedures to treat these diseases and drive out the demons, none of these crude drugs would have provided any miracle cure. It is now known that certain fungi have powerful antibacterial properties, but there is no record of this effect being utilized until modern times.

Before the causes of dreaded infectious diseases had been discovered and with no knowledge of how to prevent them, treatment would have simply involved waiting for the patient to recover naturally. It was not until the 20th century that salvarsan, sulfonamides, penicillin and other treatment drugs appeared. However, it does seem strange that our human ancestors did not encounter "blue mold", as they must have trialed thousands or even tens of thousands of different animal, plant and mineral products as medications.

(v) Based on ancient records and artifacts found at archeological sites, lifestyle-related diseases such as arteriosclerosis and diabetes are presumed to have existed since ancient times, although the causes of these diseases were not identified until the 20th century. Before that, people with hyperlipidemia, hypertension, arteriosclerosis or other "silent diseases" would have died from heart attack or stroke without even knowing they were sick. They probably had no fear of lifestyle-related diseases.

The age of experimental treatment methods passed down since ancient times was a long era in human history. The dawn of modern science ushered in a significant qualitative change in medicine.

Note 1: An effect produced by a placebo drug made from ingredients that should not have any effect.

Note 2: Meta-analysis: a method of gathering and performing comprehensive statistical analysis on the results of multiple separately-conducted comparative randomized clinical trials. There are pros and cons to this analysis.

Note 3: There is ongoing debate on the ethical issue of dividing patients in clinical trials into two groups and not actually administering medicine to the placebo group.

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2.1.2. History of Infectious Diseases (ancient times – 19th century)

Infectious diseases claimed countless human lives from ancient to medieval times. In ancient times, there would have been fewer opportunities for contact between clans and therefore less widespread contagion of infectious diseases (pandemics). As civilizations advanced, there was greater exchange of people and goods, which also meant increased opportunities for people to come into contact with infectious diseases. Infectious diseases began to spread with the advancement of civilization, rapidly broadening the extent of the harm caused by them.

The following examines the main infectious diseases and their histories.

Bubonic Plague

Bubonic plague (Black Death) is an invasive systemic infectious disease caused by Gram-negative bacillus *Yersinia pestis*. It is transmitted by fleas and also through airborne transmission. In the majority of cases of bubonic plague in humans, the bacteria spread to the regional lymph nodes, causing necrosis and boils. It then spreads to the liver, lungs and bone marrow via the lymph and the blood, causing septicemia.

Bubonic plague has a very long history. Detailed descriptions of it have been found in Egyptian papyri dating to 4300 BP, as well as in the classic *Romance of Sinuhe*. The plague bacillus *Yersinia pestis* has also been found in the lungs and liver of ancient Egyptian mummies. A papyrus records that when the plague broke out in an ancient Egyptian stone quarry, the place was quarantined off until everyone in it had died. Of the pandemics found in recorded history, the earliest widespread contagion of an infectious disease presumed to be bubonic plague broke out in the Eastern Roman Empire (Byzantine Empire) in 542-543 CE.

There was an outbreak of bubonic plague in China from around 1320 to 1330; it also spread throughout the Islamic world during the Mamluk Sultanate, around the same time as it came to Europe. Records indicate that more than one third of the population of Europe was lost to bubonic plague in the 14th century. This is a fearsome story. The flourishing trade between east and west on the Eurasian continent under the control of the Mongol Empire is believed to be one of the underlying factors for this pandemic; the danger of infectious diseases in an age without antibiotics is well known [(1:97-132, 2:32-36)]. There are no accounts of bubonic plague spreading to Japan until 1899, when bubonic plague was spreading throughout Asia. In 1896, Shibasaburō Kitasato, president of the Institute for Infectious Diseases, and Tanemichi Aoyama, professor of internal medicine at Tokyo Imperial University Medical College, were sent to Hong Kong to research prevention methods. Kitasato discovered the plague bacillus *Yersinia pestis* in 1896 [(3:220-221)]. There is no record of any bubonic plague patients in Japan after 1926.

Malaria

Malaria is a chronic disease caused by the protozoan *Plasmodium*, carried by mosquitoes of the genus *Anopheles*. Symptoms manifest in the form of sudden violent shaking accompanied by fever, headache and nausea. This continues for four to five hours, after which the temperature returns to normal for two or three days, then the symptoms return. In severe cases, it can cause impaired consciousness, kidney failure and death.

Malaria appears in ancient records from China and other places in Asia. The *Atharvaveda*, compiled in India around 3500 BP, records a ritual for a malaria curse, while a bronze inscription from Yin Dynasty China dating to 1400 BCE contains a character signifying “malarial fever”. The armies of ancient Greece also had trouble with malaria, although the tropical coastal areas of the Mediterranean were not the only endemic areas for malaria, as the low-humidity areas of southeastern England were similarly afflicted [(1:133-172)]. In 1902, English doctor Ronald Ross was awarded the Nobel Prize in Physiology or Medicine for being the first to discover the protozoan *Plasmodium* in the alimentary canal of *Anopheles* mosquitoes.

Malaria long went without a remedy. Around 1640, plants of the genus *Cinchona*, native to the South American Andes, were imported to Europe as a specific medicine for malaria. However, it was not until 1820 that a substance known as quinine was isolated from *Cinchona* bark for use as a specific medicine for malaria.

Ancient Japanese records make frequent mention of a disease described as malarial fever; this is thought to be the same disease as present-day malaria. The character used in Japanese for malarial fever appears in the Taiho Code. It would seem that malaria has been common in Japan since ancient times. Records reveal that Kujou Kanezane, author of *Gyokuyō*, Fujiwara no Teika, author of *Meigetsuki*, and even Taira no Kiyomori suffered from malaria [(3:74-76)]. The disease was prevalent in the low wetlands of Western Japan until early modern times, but has been less prevalent since the Meiji Period.

Smallpox

Smallpox (variola) was a fearsome disease from ancient to early modern times, with no means of treatment. Due to its high mortality rate and the fact that survivors were left with scars, it has been one of the most feared infectious diseases all the world over. Caused by the variola virus and with a very high mortality rate, symptoms include fever, vomiting, lower back pain and rash all over the body. It appears to have been affecting humans for the past 10,000 years, with smallpox pockmarks even noted on the faces of Egyptian mummies [(4)].

The unfortunate story is well known of Francisco Pizarro bringing the virus to the Americas during Spain’s invasion of the South American continent in the 16th century and decimating the indigenous Incan population, who had no

immunity to the disease. Other records include that of an outbreak in India in 1770 that claimed three million lives.

Meanwhile, it was discovered at various places around the world that people could become immune to smallpox. Without knowing the scientific basis for it, people all over the world, including Persian nomads and inhabitants of India, China and the Georgia region of Russia, were inoculating healthy people with pus taken from an infected person to cause a mild onset of the disease and thereby making them immune to it. In the late 18th century, it was found that people who had contracted the bovine disease cow pox (humans could contract it, but it was mild and left no scarring) would not contract smallpox. This led to the development of a smallpox vaccine by Edward Jenner in 1796 [5].

Japan had its own outbreak of smallpox during the Nara Period. This smallpox outbreak, which started in 737, was the most dreadful disease that ancient Japan had experienced. It spread across the entire country from Chikushi in Fukuoka to the ancient capital of Nara, taking countless lives, as the people had no immunity to it. That same year, the disease took the lives of all four sons of Fujiwara no Fuhito, who had been a powerful key figure in the political administration of his day. The imperial edict to erect the great statue of Buddha at Todaiji Temple was issued in 743, immediately following the smallpox epidemic. This was an attempt by Emperor Shoumu to entreat the power of Buddhism and the great Buddha to break free from these epidemics, famine, social unrest and political turmoil [3:34-36, 6:131-134].

Meanwhile, Emperor Shoumu issued orders through the Department of State in 737 instructing every province to take preventative measures against the disease. The treatment measures were as follows: “Once smallpox symptoms appear, do not drink cold water even though you may be so hot you feel like you are burning. Once the scabs stop appearing, the fever will subside, but it may be complicated by diarrhea. Wrap the belly and back in cloth to warm it; do not cool it. Although the common people may have no beds, they should not sleep directly on the ground. Take rice gruel, not fish or fruit. Un-boiled water is strictly off limits. It is essential to eat, even if it is difficult. Take no pills or powdered medicine.” These directions are extremely detailed and comprehensive. It is interesting to note that medicine is regarded as ineffective [7:39-40]. Patients had no choice but to wait for a natural recovery.

Just like Europe and China had, Japan also suffered many subsequent outbreaks. By the Edo Period, it was a disease that everyone had encountered. It had a high mortality rate among children; according to a detailed study by Dr. Keizo Suda of the death registers held by temples in the Hida district of Gifu, 68 of the 93 victims of smallpox in a village of 2,733 people in 1804 were children aged 1-5 years [8].

Jenner’s cow pox vaccine made it as far as Kwangtung in Qing dynasty of China by 1805, but took a little longer to reach Japan. Leading doctors in Japan learnt what vaccinations were by reading the Chinese books of the day. A smallpox outbreak in 1847 prompted two programs to introduce cow pox vaccinations to the domains of Echizen and Saga. Although the Echizen program did not succeed in being implemented, in 1849 Saga acquired some active cow pox from Batavia (Jakarta) with the help of O. G. J. Mohnike, a Dutch doctor in Nagasaki. One of the Saga Domain doctors, Sōken Narabayashi, promoted the vaccine after successfully testing it on his own children, thus marking the beginning of vaccination in Japan [9].

Time went on, and in 1958 the World Health Organization (WHO) embarked on a campaign to eradicate the disease through a world-wide smallpox eradication program, deploying a surveillance [Note 1] and containment strategy of administering the vaccine in the vicinity of infected people. As a result, smallpox dramatically decreased and was declared eradicated in May 1980 by the WHO. Since then, the smallpox virus is believed to no longer exist naturally. This is the first infectious disease to be successfully eradicated by humans.

Tuberculosis

Tuberculosis is caused by the human-type tubercle bacillus *Mycobacterium tuberculosis*, an acid-fast bacterium. It causes inflammation of the lungs and other internal organs, which eventually fester, destroying the tissue. (Humans can also be infected by a bovine tubercle bacillus, which is also highly pathogenic; in Japan this is kept under control by the *Act on Bovine Tuberculosis Control* and there have been no reports of bovine tuberculosis in humans.)

The existence of tuberculosis as a disease has been known since ancient times. The oldest and most well-known proof of this is an Egyptian painting depicting characteristic the deformation of spinal tuberculosis (Pott’s disease). A female skeleton with spinal tuberculosis has been discovered at the predynastic site of Adaima (6500-5100 BCE). Studies on mummies have also confirmed that tuberculosis was a common disease in Egypt around 3700-1000 BCE. X-rays have also revealed tuberculosis lesions on a female mummy from the Mawangdui site in China, dated around the second century BCE [2:69-73, 11]. According to a study by Aoki, human tuberculosis probably adapted from the bovine tuberculosis bacillus found in cows, pigs, sheep and goats on entering the human body through the consumption of meat or the consumption of milk, which can very commonly contain high amounts of the tuberculosis bacillus [10]. Based on the above, the tuberculosis bacillus is thought to have first affected humans around 7000 years BP.

In Japan, people have suffered from pulmonary tuberculosis since ancient times. The earliest evidence of tuberculosis in Japan is two human bones with signs of advanced spinal tuberculosis, found among 5000 human bones uncovered at the Yayoi-period Aoya-Kamijichi site in Tottori. While numerous human skeletons have been unearthed from Jōmon period sites, no evidence of

tuberculosis has been found among them, indicating that the disease was brought to Japan by migrants from the Asian continent. It was prevalent among the nobility in the Heian Period, even mentioned in *The Pillow Book*, but the conditions of that time were not conducive to chronic infection and dramatic increases in patient numbers. Repeated minor outbreaks occurred and died away up to and into the Edo Period [10, 11]. Dramatic increases in patient numbers started occurring in the Meiji Period, when the population became more concentrated in urban areas and the promotion of industry led to harsher working conditions.

Influenza

Three types of influenza affect humans: A, B and C, all belonging to the *Orthomyxoviridae* family of negative-sense single-stranded RNA viruses. The virus infects airway epithelial cells and develops into a respiratory illness. It has a high mortality rate, with complications including pneumonia, brain inflammation and heart failure.

Outbreaks of diseases presumed to be influenza have been observed since the Greek and Roman Empires. Hippocrates famously described an incident strongly suggestive of influenza in 412 BCE: "one day, many villagers suddenly contracted a fever with trembling and much coughing. This mysterious illness spread quickly throughout the village, causing fear among the villagers, but it went as quickly as it had come" [12].

The influenza virus was brought to Japan from the continent. Dr. Shizu Sakai writes in *Yamai ga Kataru Nihonshi*, "we see that influenza had already spread to Japan in 862 from an account in *Sandai Jitsuroku* that 'many people suffered from violent coughing and a great number died'" [3:111-120]. Outbreaks of violent coughing are recorded in history books from the Heian Period (1150 CE) and the Kamakura Period (1233 CE). Envoys from overseas are known to have come to Japan in both of those years; it is very likely that they brought viruses with them from overseas.

In the Edo Period, there were no more outbreaks after the 1614 outbreak until 1730. This is presumed to be because Japan had entered a period of national isolation and had no contact with other countries. Even today, treatment for influenza includes health management, vaccination and antiviral drugs at the early stages of infection; there are no drugs that will completely get rid of the virus after onset.

Spanish influenza caused a worldwide pandemic in 1918-1919, claiming over 20 million lives. Five hundred thousand people died in the United States; according to *Influenza*, a report published in 1922 by the Central Sanitary Bureau of the Japanese Ministry of Home Affairs, 385,000 Japanese lost their lives (the population at the time was 56.7 million) [14]. Other worldwide influenza pandemics have occurred since then as well. The influenza virus mutates very easily and can mutate in other mammals as well, making it one of the most vigilance-requiring pathogens known today.

Measles

Like smallpox and influenza, measles is caused by a virus. The measles virus is a single-stranded RNA virus in the *Morbillivirus* genus of the *Paramyxovirinae* subfamily. As it is genetically similar to the bovine virus rinderpest, there is a theory that it evolved from this into a human virus around 10,000 years BP with the domestication of cattle. It is also assumed that its earliest endemic area was somewhere in the Middle East around 3000 years BP. It is highly contagious; once it spreads to a region, most of the people in that area will be infected. Once infected, patients experience a tingling or stinging sensation in the throat or on the skin; this sensation is termed *hashikai* in the Kansai dialect. Patients then experience fever accompanied by conjunctivitis and coughing, later followed by high fever and rash all over the body. Like smallpox, after a person has contracted it once they are then immune to it for life; however, there has never been a way to cure it and many people have lost their lives or gone blind because of it [15].

In Japan, the *Fusō Ryakuki* records an outbreak in 998 CE, at the height of power of Fujiwara no Michinaga. Another major outbreak is recorded in 1256 during the Kamakura Period, but at that time there was nothing that could be done except pray to the gods and change the name of the era. There are 13 known instances of measles outbreaks during the Edo Period, coming in 10-20 year cycles; it is said that no man or woman of any age was unaffected. It is presumed that many deaths occurred as a result of complications from other infections as well as the measles itself [3:192-202].

As was the case with smallpox, humanity was saved from measles by a vaccine. The development of inactivated vaccines and avirulent vaccines has now reduced the number of measles patients. The WHO is currently implementing a measles eradication program targeting measles as the next disease to eliminate after smallpox.

Syphilis

Syphilis flared into virulence in Europe at the end of the 15th century, taking countless lives since with a vehemence that has not been seen since among venereal diseases. Thought to have been brought back with Columbus from Haiti, two thirds of the officers and men are said to have been infected by syphilis as it spread throughout Europe by means of the women who accompanied the soldiers.

Syphilis is a chronic infectious disease caused by infection from the spirochaete bacterium *Treponema pallidum*. It has an incubation period of around three months, followed by a secondary stage of headache, fever and tiredness, accompanied by the outbreak of syphilitic lesions all over the body and arthritis. Eventually, the disease enters a latent stage, leaving only a swelling of the lymph gland. Patients may enter a tertiary stage three years after infection, affecting all of the organs, causing tissue damage and the collapse of the nose or other parts of the body. A fourth stage can occur after ten years, causing damage to the brain and spinal cord, leading to paralytic dementia and loss of coordination of movement.

In 1493, while Columbus was reporting the success of his expedition to Queen Isabella in Barcelona, syphilis was spreading throughout the city and then across all of Europe at an incredible speed. Syphilis made its appearance in the Kansai region of Japan in 1512, transmitted either from Ming China or by Japanese pirates, and soon spread throughout the whole country. This took place before the Portuguese arrived at Tanegashima in 1543.

Mercury patches and fumigation, long used in Europe to treat scabies, were used as treatment, but many people died of mercury poisoning from the treatment process [16:100-123]. In the Edo Period, sarsaparilla was used as a specific crude drug, but it would have had little effect, as discussed previously.

Dysentery

An orally transmitted infection caused by the *Shigella* bacillus, named after Kiyoshi Shiga, who discovered it in 1898. Infection by the *Shigella* bacillus damages the tissues of the intestinal epithelium; infected macrophages and inflammatory cytokines released by intestinal epithelium cells cause hemorrhagic diarrhea and fever.

Shizu Sakai writes that "from an account in *Sandai Jitsuroku*, it is thought that dysentery spread to Japan from the Chinese continent at an early stage. There are surprisingly few records of dysentery" [3]. Dysentery seems to have spread when the body was weakened by the spread of other diseases rather on its own. Fujiwara no Sanesuke, author of *Shōyūki*, used expensive myrobalan plums to treat dysentery. This crude drug from India was hung from poles with string, "more to appease the evil spirits than as a medicine" [2:187-191]. Most people would have had no choice but to wait for the worst to be over.

Cholera

Cholera is an orally transmitted infection caused by the bacteria *Vibrio cholerae*. It takes hold and multiplies in the lower small intestine; cholera toxins produced at the site of infection invade other cells, causing severe diarrhea and vomiting, which leads to dehydration.

Although cholera had existed in Asia since ancient times, it was not until the 19th century that there were any signs of a worldwide pandemic. Cholera is thought to be endemic to the lower reaches of the Ganges River, a region ranging from Bengal in India to Bangladesh. The oldest record of cholera dates from around 300 BCE. While there are records of epidemics thought to be cholera in 7th century China and 17th century Java, there were no worldwide pandemics until 1817. The first outbreak of cholera in Japan was in 1822. *Koan no Kusuribako* (Medicine Box of Koan) notes that a pill made from tiger skulls, believed to kill evil spirits, was developed and distributed in Doshomachi, the wholesale drug district of Osaka [13]. The instructions for this pill were to "learn disease prevention, use fumigation when a disease comes and finally crush and take". An analysis of pills still in existence revealed that they contained arsenic; they would have probably had no effect against cholera.

In 1858, the US frigate Mississippi arrived in Nagasaki from China, bringing cholera with it. Booklets recommending the treatment of cholera with quinine and opium, then known to be specific medicines for malaria, were translated into Japanese, printed in Nagasaki and distributed throughout the country. However, although quinine performed spectacularly in preventing infections, from a scientific standpoint, it was not a cure-all for all infectious diseases. Cholera broke out again in 1877, starting in Yokohama and Nagasaki [17].

On contracting infectious diseases, people in ancient and medieval times would have had no recourse other than symptomatic treatment, as the existing crude drugs were unable to eliminate the pathogenic microbes causing the disease. Humanity started winning this battle in modern times, as economic development led to environmental hygiene and developments in nutrition led to sufficient nourishment to allow the body to build resistance, as well as the emergence of antibacterial drugs and antibiotics to kill the pathogens.

From ancient times to modern, infectious diseases have been our greatest enemy, in Japan and Europe alike. Being an isolated island nation has meant that epidemics prevalent in Europe and across the Eurasian continent have not spread to Japan immediately; however, these eventually did spread to Japan, borne by bacteria and viruses aboard trading vessels travelling to and from the continent. Smallpox, measles, influenza and other viral diseases caused repeated pandemics from ancient times to the Edo Period with no crude drugs offering any cure. For gastrointestinal diseases (typhoid fever, dysentery), the main medications were stomachic or intestinal Chinese remedies or Chinese remedies with antipyretic or analgesic effects.

While nutrition, hygiene and specific medicines played a major role in safeguarding against infectious diseases, there were no adequate countermeasures in Japan from ancient to early modern times. Edward Sylvester Morse, who discovered the Ōmori shell midden, arrived in Japan in 1887 and recorded his impressions in *Japan Day by Day*. "Somewhat astonished at learning that the death-rate of Tokyo was lower than that of Boston, I made some inquiries about health matters. I learned that dysentery and cholera infantum are never known here; some fevers due to malaria occur, but are not common; rheumatic troubles show themselves among foreigners after several years' residence. But those diseases which at home are attributed to bad drainage, imperfect closets, and the like seem to be unknown or rare, and this freedom from such complaints is probably

due to the fact that all excrementitious matter is carried out of the city by men who utilize it for their farms and rice-fields. With us this sewage is allowed to flow into our coves and harbors, polluting the water and killing all aquatic life; and the stenches arising from the decomposition and filth are swept over the community to the misery of all". Hygiene conditions may have been surprisingly good in the Edo Period [(18)].

Note 1: Surveillance – Survey monitoring. Here it refers to investigating trends in infectious diseases.

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2.2 Dawn of Modern Science and Medicine (Advances in Organic Synthetic Chemistry and Isolating and Identifying Ingredients)

2.2.1. Age of Isolating Active Ingredients in Crude Drugs (1800 CE)

(Advances in Organic Synthetic Chemistry and Isolating and Identifying Ingredients)

Breaking away from the medieval and into the modern started with the anthropocentrism movement, which had begun during the Renaissance in 15th century Europe. By the mid-16th century, modern natural science had begun to develop through scientific methodologies that emphasized mathematics and empirical results, distinct from conventional philosophy and theology. The field of medicine also began to undergo significant changes, influenced by modern science.

Although some of the crude drugs derived from natural products used for medication since ancient times demonstrated some strong effects, many of them were used in the form of mixture extracts. Medieval doctors had a number of different drugs and would prescribe them in complicated formulations. There are records from the Renaissance period recording as many as 60 types of crude drugs being prepared in a solemn ceremony. As medical science progressed, doctors gradually began to break away from these "shotgun" drug preparations. Modern pharmacopeia started being compiled from the 16th century onwards in many countries and a number of superstition-based substances were done away with [(1)].

In the 19th century, the advancement of modern science made it possible to isolate and identify the ingredients of conventional crude drugs, determine their structure and produce synthetic substances using organic chemistry. Pharmacists in Europe in the early decades of the 19th century isolated morphine, strychnine, quinine and other organic compounds from medicinal plants, thus paving news roads of development in organic chemistry. Justus Freiherr von Liebig followed on from the efforts of these pharmacists and further developed organic chemistry, as well as training up a number of other notable organic chemists.

Many of the alkaloids isolated from crude drugs had strong pharmacological activity and created issues when directly administered to patients; however, quite a few of them are still being used in clinical trials with different dosage regimens (discussed below: see Table 2.6). These alkaloids were also used as a pharmacological research tool to investigate the action mechanisms of drugs and have made a significant contribution to the development of modern

pharmacology.

The following sections discuss “substances discovered and isolated from crude drugs”.

Morphine

Throughout history the world over, opium has taken center stage as an unrivalled drug for alleviating pain and inducing sleep. It appears on Sumerian clay tablets, ancient Egyptian manuscripts, Greek epic poetry and Dioscorides’ pharmacopoeia.

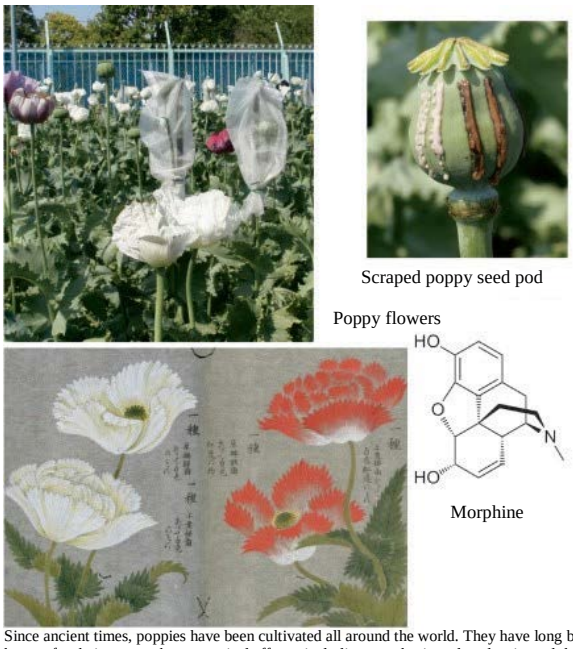
Once a poppy has bloomed, its petals fall off within 10-20 days. Scratching the skin of the immature seed pod causes a milky substance to ooze out (see Figure 2.1). This oxidizes into a dark clay-like substance known as raw opium. Cultivation of opium poppies started in the region around Turkey and was brought from there to Mesopotamia. It later spread to the surrounding regions as a result of Alexander’s eastern expedition and the spread of Islam. In Japan, cultivation of opium poppies started in Aomori during the Edo Period.

Opium’s ability to take away the effects of pain has been well known since ancient times, although the reason for its efficacy long remained a mystery, despite the efforts of countless physicians. As modern science began attempting to clarify the medicinal ingredients in crude drugs from the Renaissance onwards, it would seem that opium was the natural choice for a starting point.

However, although opium was a crude drug that had demonstrated outstanding efficacy since humankind discovered, it was difficult to deal with due to being highly addictive, as historically evidenced by the Opium Wars. Opium is a compound of several alkaloids, with the main ingredient being morphine, with a molecular weight of 285, successfully isolated by German pharmacist Friedrich Wilhelm Adam Sertürner in 1817 [(Note 1)]. However, morphine has a very complex, three-dimensional structure; defining its chemical composition and structural arrangement meant waiting for advances in organic chemical technology. Prof. Robert Robinson of the University of Manchester presented a structure the same as the one we know today in 1925, actually 120 years after Sertürner first isolated morphine. In 1947, Robinson received the Nobel Prize in Chemistry for his alkaloid research. Although today there is a branch of medicine dedicated to pain and active research is being carried on analgesics, very few drugs have been developed that are as effective as morphine at alleviating pain.

The side effects of morphine include nausea, vomiting and severe constipation due to decreased gastrointestinal peristaltic movement (when using morphine for medical treatment, other drugs must be taken concomitantly to control these side effects). Arabian doctors in the Saracen Empire used opium to treat dysentery; the strong antidiarrheic effect of opium was indispensable for stopping the strong diarrhea caused by dysentery, which was very infectious. Even today, morphine is used for an antitussive and to treat diarrhea, although it remains essential in medical treatment for its role as an analgesic [(2, 3, 4)].

Opium contains more than 20 types of alkaloids. Codeine (content in opium 0.4-1%) was isolated in 1832, while papaverine (content in opium 0.4-0.8%) was isolated in 1848. Codeine has one fourth of the analgesic effect of morphine and is still used clinically today as an antitussive, a sedative, an analgesic and a symptomatic treatment for severe diarrhea. Modern pharmacology has confirmed that papaverine has a phosphodiesterase [(Note 2)] inhibiting effect; it is still used today as an anticonvulsant. Narcotine (4-7%) was isolated by Jean-François Derosne; used today as a central antitussive, it was renamed noscapine as it has no narcotic action [(2, 4)].



Since ancient times, poppies have been cultivated all around the world. They have long been known for their strong pharmaceutical effects, including anesthesia and analgesia, and their addictive properties. Morphine, a key ingredient in the opium harvested from the poppy seed pod, was isolated by Friedrich Sertürner in 1803. Pictures of poppies have been found in Japanese books on medicinal plants.

Top photos: Poppy flowers and seed pod: The Tokyo Metropolitan Medicinal Plants Garden (Kodaira) has a poppy garden enclosed within a double fence. Photograph courtesy of Prof. Yoshihito Okada.

Bottom photo: From Honzō Zufu (Vol. 23). Image supplied by the Tokyo National Museum.

Fig. 2.1 Morphine Isolated from Opium Harvested from Poppies.

Acetylcholine-Related Alkaloids

Acetylcholine plays a very important role in the body as a neurotransmitter in a number of different nervous systems, including the central nerves, peripheral nerves, autonomic nerves and somatic nerves. The discovery of certain alkaloids, discussed below, made a significant contribution to rapid advancements in neuropharmacology in relation to acetylcholine.

The rhizomes and roots of Japanese belladonna *Scopolia japonica*, the leaves of henbane *Hyoscyamus niger*, the leaves of devil’s snare *Datura tatula* and the roots of deadly

nightshade *Atropa belladonna*, all members of the nightshade family *Solanaceae*, have long been used all around the world as crude analgesics, antispasmodics, antitussives and anesthetics [(Note 3)]. The ancient Sumerians are known to have used poppies and henbane for pain relief; first-century Greek physician Pedanius Dioscorides also recorded their anesthetic effect. In Japan, surgeon Seishū Hanaoka is known for being among the first in the world to perform surgical procedures using general anesthetics. In 1804, Hanaoka performed a partial mastectomy for a breast cancer patient under general anesthetic using a herbal mixture containing six types of herbs, mainly devil's trumpets (*Datura*) and wolf's bane (*Aconitum*), having confirmed the general anesthetic effect of this mixture through trial and error.

In the medieval Western world, belladonna was also widely used by women as a beauty treatment, as it would dilate the pupils. It is one of the so-called mydriatic drugs.

Into the modern era, pharmacists extracted and identified various ingredients from this family of plants with its extremely diverse range of effects. These alkaloids include atropine, ℓ-hyoscyamine (atropine is a racemic mixture of (d- and ℓ-) hyoscyamines) and scopolamine. Each of these has their own strong antiacetylcholine effect and some of their component ingredients are still used in medical treatment today.

Later, physostigmine and iso-physostigmine were isolated from the calabar bean *Physostigma venenosum* in the *Leguminosae* family, while muscarine was isolated from the fly agaric *Amanita muscaria* and pilocarpine from the leaves of jarabondi *Pilocarpus jaborandi*. Each of these alkaloids has an effect on acetylcholine neurotransmission and they have played a pivotal role in the advancement of modern pharmacology (discussed later).

Currently, the crude drug "Scopolia Rhizome" listed in the Japanese Pharmacopeia is described as containing not less than 0.29% total alkaloids (hyoscyamine, scopolamine), as well as other related alkaloids, with Japanese belladonna *Scopolia japonica* as the plant of origin. Similarly, "Belladonna Root" is described as containing not less than 0.4% hyoscyamine, as well as several other alkaloids besides atropine and scopolamine. The crude drug "Datura" is derived from devil's snare *Datura tatula* and devil's trumpet *Datura stramonium*, used by Seishū Hanaoka; however, it is dangerous and has been prohibited from private use. Accordingly, it does not appear in the Japanese Pharmacopeia (see Figures 2.2 and 2.3) [(2, 6, 7)].

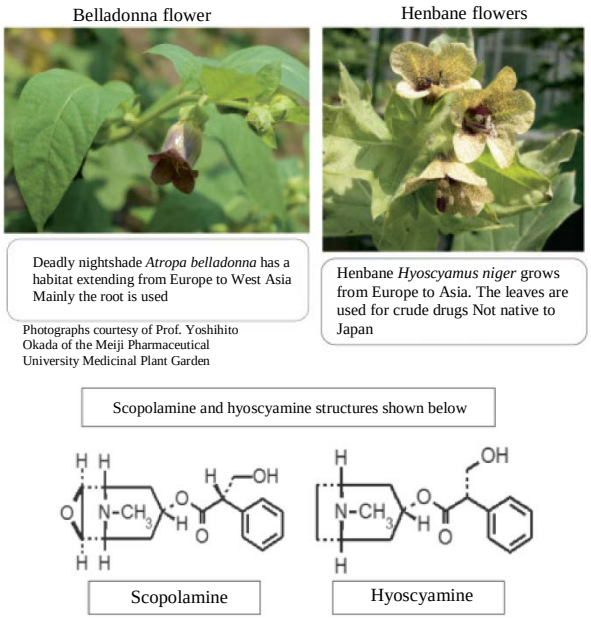


Fig. 2.2. Active Substances Found in Plants in the Family *Solanaceae* with Acetylcholine-Like Effects on Nerves (1).

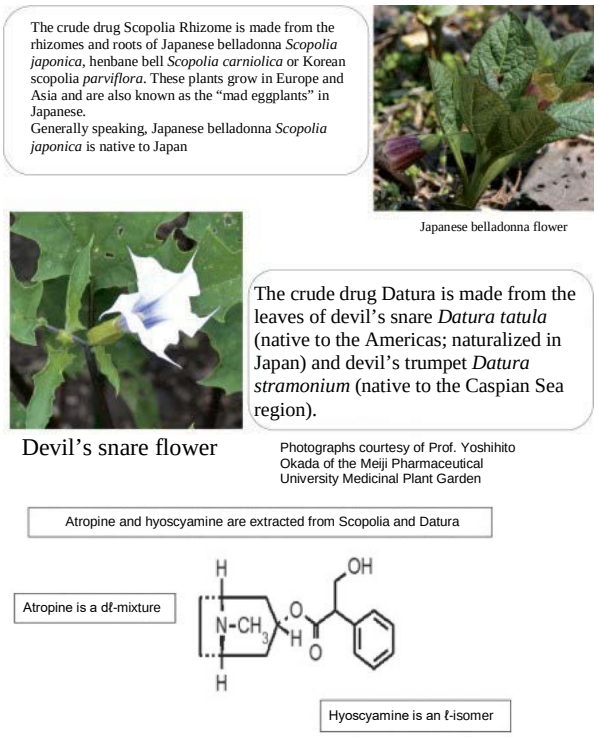


Fig. 2.3. Active Substances Found in Plants in the Family *Solanaceae* with Acetylcholine-Like Effects on Nerves (2).

Atropine, Hyoscyamine and Scopolamine

Atropine was isolated from belladonna root in 1833 by German pharmacists Philipp Geiger and Ludwig Hesse. Modern science has shown it to have an antagonistic effect on the neurotransmitter acetylcholine in acetylcholine receptors. This knowledge has sped up research on receptors

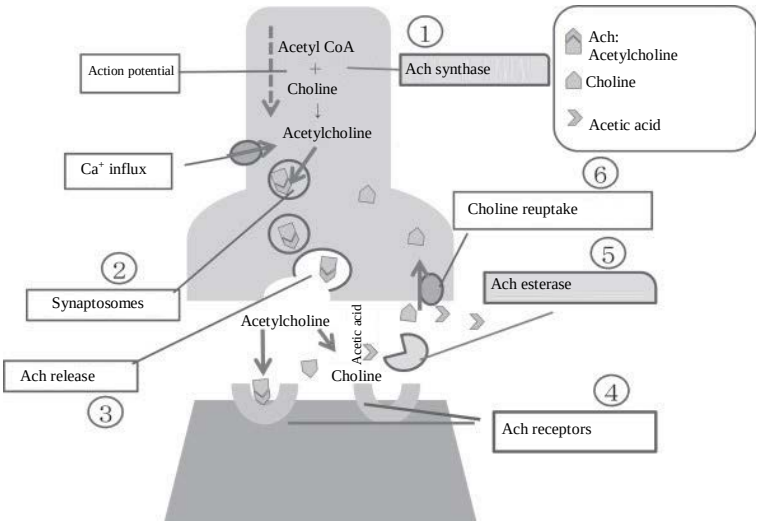
and sympathetic nervous systems, thereby contributing greatly to the development of modern pharmacology. Atropine is a racemic mixture; hyoscyamine is its ℓ-isomer, also isolated from belladonna by Geiger and Hesse in 1833. Its structure was defined by Albert Ladenburg in 1879. Ladenburg also isolated scopolamine from henbane leaf in 1881. It was found that these alkaloids suppress the action of the parasympathetic nerve by acting against the "muscarinic receptors" affected by the neurotransmitter acetylcholine (an anticholinergic action), thereby suppressing gastrointestinal motility and also increasing the heart rate.

Atropine is used today as an antispasmodic drug with an anticholinergic action, while scopolamine is currently the strongest anticholinergic drug used for motion sickness [4, 7].

Physostigmine, Neostigmine, Pilocarpine and Muscarine

Physostigmine was isolated from the calabar bean in 1864 by Julius Jobst and Oswald Hesse. It was later clarified that since physostigmine and neostigmine (discoverer unknown) have an inhibiting action on cholinesterase, a catabolic enzyme of acetylcholine, they prolong the activity of acetylcholine and thereby systemically enhance the action of acetylcholine (Barger and Stedman: 1923). Consequently, these could be used as antidotes to drugs that suppress acetylcholine in the nerves. Neostigmine is also used as a symptomatic treatment for myasthenia gravis.

Muscarine was first isolated from the fly agaric *Amanita muscaria* in1869 by Oswald Schmiedeberg and Richard Koppe. Its pharmacological action was found to be that of a parasympathomimetic agonist, binding to the acetylcholine receptor in the membrane of the postganglionic nerve and mimicking the action of the neurotransmitter acetylcholine. This receptor is a muscarinic acetylcholine receptor; this alkaloid led to the discovery that there are two types of acetylcholine receptors. The other acetylcholine receptor is the "nicotinic acetylcholine receptor", which is affected by nicotine (see Figure 2.4).



- ○ Nerve endings join to "other neurons" and "muscle cells"; these junctions are called synapses. There is a gap at the join.
- ○ Within the nerve cell, information is transmitted by electric stimulation; at the synapse, information is transmitted by the release of chemical substances. Acetylcholine (Ach) and noradrenaline are stimulation transmitters secreted into the synapses.
- ○ Cells transmitting the information are called "presynaptic cells", while those receiving the information are called "postsynaptic cells". Muscle cells and the like are postsynaptic cells.

1) Ach is synthesized within neurons from acetyl CoA and choline.
2) The synthesized Ach is stored within synaptosomes.
3) Ca ions influx by electric stimulation and Ach is released outside the neuron.
4) The released Ach moves to the Ach receptors and stimulation is transmitted to the postsynaptic cell.
5) Ach released into the intercellular gap is broken down by the catabolic enzyme cholinesterase, forming choline and acetic acid.
6) Choline reuptake mechanism operates to recycle it back to the presynaptic cell.

Alkaloids extracted from crude drugs served as tools to clarify the action of the Ach system.
- ○ In 4), atropine acts against Ach with an Ach inhibiting action. Scopolamine acts the same way.
- ○ In 4), pilocarpine demonstrates an Ach-like action (nonselective agonist).
- ○ In 4), muscarine demonstrates a selective Ach-like action on the muscarinic acetylcholine receptors (selective agonist).
- ○ Physostigmine and neostigmine inhibit Ach esterase in 5). As a result, the Ach activity is prolonged.

Fig. 2.4. Acetylcholine (Ach) Metabolism at Acetylcholinergic Nerve Endings.

Pilocarpine was discovered in the leaves of jarabondi *Pilocarpus jaborandi* in the *Rutaceae* family by Ernest Hardy in 1874. It was found to act as a nonselective muscarinic agonist.

Curare

When Europeans began travelling to South America in the 16th century, reports came back of the native inhabitants of South America using poisoned arrows. This poison, with lethal effects ranging from motor paralysis to dyspnea, was known as curare and drew much attention in Europe. In 1844, Claude Bernard reported that curare blocked stimuli from the brain, causing the loss of normal muscle control.

Meanwhile, it was found that the native inhabitants used different formulations in different locations, with combinations of several dozen different ingredients from various different plants. Analysis of these ingredients took some time; they were not fully identified until 1935 in a paper by Harold King (8:282-289).

Several substances have been isolated from curare. The quaternary base ($N\text{-}R^+$) structure of one of these substances, tubocurarine, has been the subject of much attention; it has been developed into surgical muscle relaxants decamethonium and suxamethonium, as well as the hypotensive drug hexamethonium (no longer used). Tubocurarine has been found to have an antagonistic effect on acetylcholine in the peripheral nerves and a skeletal muscle relaxant effect in the limbs that paralyzes the motor nerves (10).

Foxglove

Foxglove *Digitalis purpurea* is a perennial plant native to Europe, known to be poisonous since ancient times. In 1775, William Withering found from folklore prescriptions that foxglove leaves have a diuretic effect. French chemist Claude-Adolphe Nativelle isolated and identified digitoxin in 1869.

Foxglove formulations were complexes of several cardiac glycosides and were used as specific medicines for heart failure due to their powerful cardiotonic effects. Although several chemical compounds were isolated, many of them had a narrow safety margin between intoxicating dose and effective dose. Simple substances digitoxin and deslanoside have relatively wider safety margins and are still used today as cardiotonics (4, 5).

Strophanthin

A compound used for poisoned arrows since ancient times among native inhabitants of Africa. Strophanthin was extracted from the seeds of plants in the *Apocynaceae* and *Buddlejaceae* families by Pierre-Joseph Pelletier et al. in 1818. Strophanthin contains many impurities; in 1888, François Arnaud found it to contain ouabain (g-strophanthin), cymarin and other glycosides (Note 4) (16). While it has a cardiotonic effect similar to foxglove (Note 5), it is highly toxic, with side effects including nausea, vomiting and cardiac arrhythmia (8:71-75).

Cocaine

The people of the ancient Inca Empire are believed to have been chewing coca leaves around 3000 BCE to increase their heart rate and quicken their breathing as a means of coping with the thin air in the Andean highlands. Coca is a palatable drug that quickly creates physical dependency. The ingredients in coca leaves proved difficult to analyze; however, reports indicate that “German chemist Friedrich Gaedcke isolated cocaine from the coca leaf in 1855, further validated in 1859 by German organic chemist Albert Niemann” (8:21-22). Although cocaine abuse is now an issue faced by more than three million Americans, it is still used clinically for its local anesthetic effect, discovered by Carl Schroff and Louis-Gustave Demarle in 1862.

Ergot

In times of food shortage in Europe, it was found that people who ate moldy rye bread with ergot fungus *Claviceps purpurea* growing on it would experience restricted blood circulation, leading to gangrene. An Assyrian clay tablet from 600 BCE records that “there are poisonous nodules on the ears of grains”. This mold acts on the central nervous system, causing confusion, drowsiness and convulsions. However, around 2400 BP it was also found that when taken in appropriate measures, this moldy bread could work to lower the uterus of a pregnant woman and it was used by midwives to assist delivery.

It was not easy to isolate the substances causing these diverse effects. Ergotoxine, ergotamine and ergotaminine were refined and isolated in 1906, although continued efforts were made to isolate the many other substances contained. Ergotoxine is made up of multiple substances and has a powerful vasoconstrictive effect. Ergonovine was isolated in 1935 and was found to have a uterine smooth muscle contraction effect.

Lysergic acid diethylamide (LSD), a derivative from this fungus, is similar to mescaline derived from American cacti. Rather than being a drug for the uterus or for migraines, it acts on the central nervous system and has also become a social issue, as it causes hallucinations [8:92-108]. Its pharmacological effects include acting as an agonist of the serotonin, noradrenaline and dopaminergic neurons [10].

Salicylic Acid

The analgesic and antipyretic effects of willow are widely known around the world. Records exist among Hippocrates' works, as well as in Sumerian, Assyrian and Egyptian writings. Native Americans also used various willow trees for their analgesic and antipyretic effects. Even the Chinese and Japanese knew of the use of "willow sticks for toothache".

In Europe, the medicinal use of willow as a crude antipyretic was forgotten through disuse, despite the demand for willow increasing in the medieval period. It was not until early modern times that the antipyretic effect of willow was rediscovered by Edmund Stone, in 1793.

Later, in 1830, French pharmacist Henri Leroux isolated the antipyretic ingredient in willow and named it salicin. Salicylic acid is a substance isolated from salicin. Salicylic acid was used on patients suffering from rheumatoid arthritis. In 1897, Felix Hoffmann of German company Bayer acetylated salicylic acid to organically synthesize acetylsalicylic acid. Also known as aspirin, this drug has made the most significant contribution to humankind in history [9].

Salicylic acid itself has a skin collagen softening effect, as well as suppressing on *Trichophyton* fungi, which cause athlete's foot. It is currently used in ointment, liquid or medicated bandage form to treat psoriasis and keratosis, as well as warts, calluses, corns and athlete's foot [2, 4].

Ephedrine

Bluestem joint fir *Ephedra equisetina* is a shrub in the genus *Ephedra* that is used in Chinese medicine as a diaphoretic, antitussive and antipyretic. It has also had various applications in Western medicine for its sympathomimetic effects. Its main ingredient, ephedrine, was extracted and identified in 1885 by Nagayoshi Nagai, who later became the first president of the Pharmaceutical Society of Japan. Dr. Nagai also successfully achieved a large-scale synthesis method for it, which became widely used. Ephedrine has a bronchodilator effect and was used until recently to prevent asthma attacks, saving a lot of patients from a lot of pain. While a gentler derivative, dl-methylephedrine hydrochloride, is currently used as a bronchodilator in prescription medicine, ephedrine itself is still used as an over-the-counter cold remedy [2, 4].

Quinine

Quinine kills malaria-causing protozoa by obstructing polymerization and has saved countless lives. It is still used as a backup medication for Plasmodium infections that have become immune to other drugs.

Cinchona plants are native to the South American Andes, where the native inhabitants used the bark as an antipyretic rather than to treat malaria. Although malaria has existed in Europe since ancient times, it did not originally exist in the Americas; it is presumed to have spread to the Americas with the Europeans. The effects of cinchona bark in treating malaria were later discovered by chance; it started being imported to Europe in 1640 [8].

Portuguese doctor Bernardo Gomes isolated crystals from quinine in 1811, naming the substance cinchonine. In 1820, pharmacist Pierre-Joseph Pelletier et al. found that Gomes' crystals were made up of quinine and cinchonine; by 1908 they had isolated the quinine and determined its chemical structure. Total synthesis of it was achieved in 1944 by Robert Burns Woodward, who was awarded the Nobel Prize in Chemistry in 1965 for his achievements in chemosynthesis, including success with strychnine, reserpine and colchicine.

Reserpine

Reserpine is a crude-drug-derived compound that has made a significant impact on modern pharmacology. It is extracted from Indian snakeroot *Rauwolfia serpentina* in the *Apocynaceae* family, used as a crude drug in the Indian region, although it was not until 1952 that its ingredients were isolated by Johannes Muller and Emil Schlittler. Having been used in India since ancient times as a treatment for psychosis, insomnia and confusion, attention was drawn to its powerful pharmacological effects and it became the subject of Western research, initially as an antipsychotic drug. Later, it was found that it not only had an inhibitory effect on mental stimulation, but also hypotensive and sedative effects as well. Reserpine became a major pharmacological research tool in the 1950s; at one stage, it was widely used clinically as a hypotensive [4, 10].

Contributions by Japanese Scientists

The late 19th century saw the beginning of Japan's Meiji Period and notable efforts made by Nagayoshi Nagai and other Japanese scientists. The new Meiji government implemented policies to promote industry, while the country as a whole was actively absorbing new culture from the West. The government issued a directive steering the field of medicine away from Chinese medicine and towards incorporating a German-medicine-centered approach. Young Japanese scientists and doctors also made active efforts to study overseas to gain a foundation in Western medicine, leading to many discoveries and inventions.

Meanwhile, back on Japanese soil, much energy was being invested into isolating and analyzing the ingredients in Japan's own crude drugs. Yasuhiko Asahina isolated and identified the ingredients in the lacquer tree *Toxicodendron vernicifluum* (1906), while Kōjirō Makoshi did the same for aconite *Aconitum japonicum* (1909). In 1909, Yoshizumi Tahara extracted tetrodotoxin from the ovaries of pufferfish in the *Tetraodontidae* family. Kyōsuke Tsuda et al. of Sankyo determined the chemical structure of tetrodotoxin in 1964. Heizaburō Kondō identified the ingredients in red spider lily *Lycoris radiata* in 1927 [(11)]. The isolation of these active ingredients by Japanese researchers has contributed to the advancement of pharmaceutical science and pharmacology.

Thiamine (Vitamin B_1)

During Japan's Edo Period, there was an ailment known as "Edo disease", which started as a peripheral nervous system disorder and led to circulatory failure, eventually leading to weakness in the lower limbs and ultimately death from heart failure. This sickness was rare in agricultural communities, but often broke out in urban districts such as Edo and Kyoto; it is now known to be beriberi. At the end of the 17th century, rice prices had stabilized and people were regularly getting three meals a day. The citizens of Edo, who had been eating unrefined brown rice and other grains, started eating refined white rice with the bran removed. While this pure white rice was a luxury, the people of the day had no way of knowing that it would lead to vitamin B_1 deficiency. The disease did not exist in Europe, but continued to affect many Japanese soldiers even into the Meiji Period, causing great concern to the Meiji government, whose policies were to increase national prosperity and military power.

According to military hygiene records kept by the Army Ministry Medical Bureau in the First Sino-Japanese War, 1 in 3.85 hospitalized soldiers were suffering from beriberi. This means that patients with beriberi accounted for between one quarter and one third of all hospitalized soldiers, including those suffering from war wounds. The Russo-Japanese War followed, and beriberi recurred in a similar manner. Casualty numbers rose to 210,000, with beriberi accounting for around half of this figure [(15)]. Other records indicate that beriberi accounted for as much as 11.24 times as many casualties as war wounds [(13)].

Dutch pharmacologist Christiaan Eijkman, who conducted research in Java in 1888, reported that beriberi does not strike people with unrefined brown rice in their diet. However, Japanese research in this area fell far behind, with the Japanese medical community and army persisting with theories that it was caused by infection or poison. Later, studies by naval physician Kanehiro Takaki and Kiyoshi Shiga et al. found that beriberi was linked to diet rather than infection, while Jinnosuke Tsuzuki, returning from studying in Europe, confirmed that rice bran contained a substance that could prevent and treat beriberi [(13)].

In 1910, Umetarō Suzuki successfully isolated the active ingredient from the beriberi preventing component in rice bran. He named this ingredient oryzanin in 1912.

Casimir Funk had isolated an active ingredient from rice bran in 1911 and named it a vitamin. This ingredient was vitamin B_1, which had earlier been isolated by Umetarō Suzuki [(14)]. However, it was Eijkman who received the Nobel Prize in Physiology or Medicine in 1929 for his research achievements on beriberi and vitamin B_1.

In 1923, as many as 27,000 Japanese died from beriberi. This figure dropped in later years as people came to realize the importance of vitamin B_1, although it was not until the emergence of a number of vitamin B_1 formulations in the late 1950s that the figure dropped below 1000 [(15)]. By 1959, beriberi had an incidence rate of around 0.1%.

Adrenaline

Jōkichi Takamine and his assistant Keizō Uenaka began extracting and studying adrenaline using discarded livestock organs, successfully extracting a crystal in 1900. Like acetylcholine, the discovery of adrenaline led to significant developments in research on neurotransmitters and receptors and laid the foundation for modern pharmacology. Adrenaline itself was also used as a styptic in all kinds of surgical procedures and made a significant contribution to developments in medical and pharmaceutical science (see Section 2.3.4 below for details).

From the 19th century into the 20th century and to the present day, "alkaloids isolated from crude drugs" have been utilized, with their efficacy noted in the Japanese Pharmacopeia. However, these have not been great in variety or number and it is mainly new drugs formulated based on the action mechanisms of these alkaloids that are used in clinical practice.

There are currently 219 crude drugs listed in the Pharmacopeia. Table 2.1 lists the alkaloids isolated from crude drugs, while Table 2.2 shows the alkaloids derived from crude drugs listed in the Pharmacopeia.

Table 2.1 Alkaloids Isolated from Crude Drugs (Note 6)

Year discovered	Ingredient	Discovered by	Crude drug
1803	Morphine	Sertürner	Opium
1818	Strychnine	Pelletier and Caventou	Poison nut tree
1820	Quinine	Pelletier and Caventou	Cinchona bark
1820	Colchicine	Pelletier and Caventou	Meadow saffron
1821	Caffeine	Runge	Coffee beans
1828	Nicotine	Posselt	Tobacco leaves
1832	Codeine	Robiquet	Opium
1833	Atropine	Geiger and Hesse	Deadly nightshade
1833	Hyoscyamine	Geiger	Henbane
1842	Papaverine	Geiger	Opium
1855	Cocaine	Gaedcke	Coca leaves
1864	Physostigmine	Jobst and Hesse	Calabar bean
1869	Muscarine	Schmiedeberg and Koppe	Fly agaric
1869	Digitoxin	Nativelle	Foxglove
1874	Pilocarpine	Hardy and Gerrard	Jaborandi
1881	Scopolamine	Ladenburg	Hyoscyamus niger, devil's trumpet
1885	Ephedrine	Nagai	Bluestem joint fir
1909	Tetrodotoxin	Tahara	Pufferfish ovaries

Table 2.2 Alkaloids Derived from Crude Drugs Listed in the Japanese Pharmacopeia, 16th Edition

Crude drug of origin	Pharmaceutical compound extracted from crude drug	Efficacy / Effect (Pharmacopeia)
Opium	Morphine hydrochloride	Narcotic analgesic
	Codeine phosphate	Narcotic analgesic / antitussive / antidiarrheal
	Noscapine hydrochloride	Non-narcotic antitussive
	Papaverine hydrochloride	Visceral smooth muscle antispasmodic for gastritis / biliary tract diseases
Cinchona	Quinine hydrochloride	Antimalarial drug
	Quinidine hydrochloride	Antiarrhythmic
Coca	Cocaine hydrochloride	Local anesthetic
Saffron	Colchicine	Gout suppressant
Foxglove	Digitoxin	Cardiotonic
Japanese lime flower	Santonin	Anthelmintic
Tea	Caffeine	Non-narcotic antitussive
Ergot	Ergotamine tartrate	Non-narcotic antitussive (hypertensive migraine)
	Ergometrine maleate	”
Ephedra herb	Ephedrine hydrochloride	Antitussive agent (bronchial asthma)
Digenea	Kainic acid	Anthelmintic
Jaborandi	Pilocarpine hydrochloride	Miotic glaucoma medication
Indian snakeroot	Reserpine	Hypertensive emergency (cerebral hemorrhagic attack, etc.)
Scopolia rhizome	Atropine sulfate	Antispasmodic / mydriatic / poison antidote
Belladonna root	Scopolamine hydrobromide	Antiparkinsonian

Around 30 crude drug ingredients are listed in the Pharmacopeia as standalone pharmaceutical compounds.
Most of the pharmaceutical compounds listed here can be chemically synthesized; however, the majority of them are extracted and isolated to be used directly as pharmaceutical raw ingredients.
The crude drugs listed here are those mentioned in this paper.
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Note 1: Date calculation error: Sertürner wrote a paper on the discovery of morphine in 1817, but this paper was ignored in Germany, as two French pharmacists had already isolated the active ingredient in opium. One of these pharmacists, Charles Derosne, presented a paper the active ingredient in 1803; this was later found to be a narcotine mixture. The other pharmacist, Armand Séguin, published a report on trace crystals from opium in 1814, but these were low in purity, while Sertürner's crystals were superior in quality and quantity. According to Ishizaka, Sertürner reported not only his isolating the crystals, but also his testing of morphine on live subjects, including himself. Other reports state that Sertürner first isolated a basic substance from opium in 1803, while yet other reports state that he did so in 1805 (3:75, 8:314-322).

Note 2: Broadly speaking, an enzyme that hydrolyzes phosphodiester bonds. More specifically, an enzyme that hydrolyzes cyclic phosphodiester bonds.

Note 3: Plants in the nightshade family *Solanaceae*: Tomatoes, eggplants and potatoes are members of the *Solanum* genus in the *Solanaceae* family; devil's snare is a member of the *Datura* genus in the *Solanaceae* family; henbane is a member of the *Hyoscyamus* genus in the *Solanaceae* family; deadly nightshade is a member of the *Atropa* genus in the *Solanaceae* family; Japanese belladonna is a member of the *Scopolia* genus in the *Solanaceae* family. Japanese belladonna is also known as the "mad eggplant" in Japanese, as it can cause abnormal behavior if ingested.

Note 4: A molecule in which a sugar is bound to a non-sugar compound within a plant substance.

Note 5: Recent studies indicate that it inhibits the Na^+ pump in cardiac muscle cells, thereby increasing the Na^+ concentration in the cell. This results in an increase in the Ca^{2+} concentration in the cell, ultimately strengthening the myocardial contractility.

Note 6: Different records indicate different discoverers and different years of discovery, because it is sometimes difficult to determine who discovered what and when.

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2.2.2. Age of Discovering Pathogens

Around 50 years after active ingredients started being isolated from crude drugs, a succession of infection-causing pathogenic microbes started being discovered in what is now bacteriology. Specific pathogens for at least 21 diseases were discovered between 1879 and 1900. Table 2.3 shows the main pathogen discoveries.

Charles Louis Alphonse Laveran was awarded the Nobel Prize in Physiology or Medicine for his research on diseases caused by malaria protozoa in 1907. This was the second time a Nobel Prize was awarded in relation to malaria-causing protozoa, the first being Ronald Ross (discussed previously). This indicates the level of worldwide attention paid to malaria at the time. That same year, Karl Joseph Eberth discovered the typhoid fever bacterium *Salmonella typhi*. Robert Heinrich Hermann Koch discovered the tubercle bacterium *Mycobacterium tuberculosis* in 1882 and the cholera bacterium *Vibrio cholerae* the following year. In 1884, Arthur Nicolaier discovered the tetanus bacterium *Clostridium tetani*.

There were also some notable efforts by Japanese doctors and scientists. Shibasaburō Kitasato, who had studied in Germany under Robert Koch, successfully cultivated a pure culture of tetanus bacterium *Clostridium tetani* in 1889, together with Emil Adolf von Behring. They were also the first in the world to discover serotherapy, developing antiserums for diphtheria and tetanus toxins. This achievement resulted in the awarding of the first Nobel Prize in Physiology or Medicine in 1901, although it was only

awarded to Behring (discussed later). Another of Kitasato's achievements was his discovery of the plague bacillus *Yersinia pestis* in 1894, having been sent by the Meiji government to Hong Kong, which was under attack by the bubonic plague.

Doctor and microbiologist Kiyoshi Shiga, who studied under Shibasaburō Kitasato, discovered the dysentery bacillus *Shigella* [Note 1], which was named after him (discussed previously). In 1913, Hideyo Noguchi discovered the syphilis spirochete *Treponema pallidum* by studying brain specimens from patients who had suffered from terminal neurosyphilis that had led to motor ataxia and arthropathy. Noguchi also achieved research results in snake venom serology, proving that Peruvian wart and Oroya fever are both symptoms of Carrion's disease. However, several of his published pathogen-specific reports were later repudiated.

Table 2.3 Pathogen Discoveries

Year discovered	Pathogen	Discovered by
1849	Bacillus anthracis	Aloys Pollender
1873	Mycobacterium leprae	Gerhard Hansen
1879	Neisseria gonorrhoeae	Albert Neisser
1880	Malaria plasmodium	Charles Laveran
1880	Salmonella typhi	Karl Eberth
1882	Mycobacterium tuberculosis	Robert Koch
1883	Vibrio cholerae	Robert Koch
1883	Corynebacterium diphtheriae	Edwin Klebs
1884	Clostridium tetani	Arthur Nicolaier
1884	Staphylococcus	Alexander Ogston
1885	Escherichia coli	Theodor Escherich
1886	Streptococcus pneumoniae	Albert Fraenkel
1894	Yersinia pestis	Shibasaburō Kitasato & Alexandre Yersin
1897	Shigella	Kiyoshi Shiga
1900	Salmonella paratyphi	Hugo Schottmüller
1905	Treponema pallidum	Fritz Schaudinn & Erich Hoffman
1906	Bordetella pertussis	Jules Bordet
1909	Salmonella typhi	Charles Nicolle

Excerpts taken from the following materials and other sources

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Note 1: Kiyoshi Shiga reported his discovery in Japanese in the Japanese Journal of Bacteriology in 1897 and published a paper in German in 1898.

2.3 The Origin of Modern Pharmaceutical Drug Discovery and Development

While alkaloids were isolated from crude drugs and pathogens identified, medical treatment had to start with means of dealing with the pathogenic bacteria. Following on from Edward Jenner's late-18th-century method of vaccination, doctors gradually started discovering vaccines and dealing with pathogens, thus laying the foundations of immunology.

The idea of chemical treatment took hold in this era and formed the basis for modern drug discovery. Drugs to fight pathogens living in the human body would have been a very easy field to get involved in.

Numerous alkaloids were discovered from the time of Sertürner onwards and were used against all kinds of diseases. Many of these substances were too toxic to be used on their own and were put to more practical use in new fields of study. The discipline of pharmacology developed as researchers began to examine the detailed mechanisms of action as to why these drugs were effective.

Pharmacology initially developed from research on drugs that act on the nervous system. This began with analyses on the parasympathetic nerves using atropine, scopolamine and physostigmine, which act on acetylcholine, and was followed by studies on sympathetic nerves using adrenaline. However, it was not until after the Second World War that the molecular-level mechanisms of neurotransmission were identified.

In contrast to the isolation of ingredients from plant-based crude drugs, endocrine hormones were being extracted from animal organs. Alongside neuropharmacology, studies on endocrine hormones facilitated pathological analysis and contributed to advances in pathophysiology and pharmacology.

2.3.1. The Foundations of Immunotherapy and Chemotherapy

Smallpox has been one of humanity's greatest enemies, assaulting the world since ancient times. All the while, people all over the world have been trying to find ways to prevent it. The Chinese in the Ming Dynasty found that if a healthy individual was inoculated with pus from the scabs of an infected individual, the healthy individual would not contract smallpox. It was also found that dressing a healthy infant in clothing worn by an infected infant would prevent contagion. It was further found among nomads in the Persian region that individuals who had contracted cow pox would not contract smallpox.

Based on these anecdotes, in 1796 Jenner inoculated an eight-year-old boy with pus collected from the hands of a milkmaid. Two months later, he inoculated the boy with smallpox, but the boy was not infected. Jenner published these results in a pamphlet in 1798, an epoch-making publication in the history of human medicine. However, these results appear to have been largely ignored by the medical community at the time. This is not an isolated incident; the dawn of modern science saw many outstanding inventions and discoveries ignored or opposed, unable to oust existing misconceptions.

Nevertheless, this discovery by Jenner laid the foundation for immunology. Research on immunization as a method of treatment was carried from the late 19th century onwards, before the appearance of synthetic drugs [1, 2, 3].

Louis Pasteur, known as the father of modern microbiology, came up with the idea of fighting invading microbes with serum containing substances that would kill the bacteria. In 1888, George Henry Falkiner Nuttall observed a substance that had a destructive power against microbes but that lost its activity at 50-55˚C. This became the basis for theories on liquids in immunology. In 1901, Jules Jean Baptiste Vincent Bordet (awarded the Nobel Prize in Physiology or Medicine in 1919) was the first to observe the phenomenon of complement fixation. Around the turn of the 20th century, Russian zoologist Elie Metchnikoff confirmed that macrophages kill microbes. Thus, the foundation was laid for immunology and advances were made in research on attenuated vaccines, antitoxin serums and other areas of immunotherapy.

Pasteur devised this immunization technique from infectious animal diseases. He successfully tested a fowl cholera vaccine in 1880 and a rabies vaccine in 1885. Although the rabies vaccine worked for canine rabies as well as human rabies, there were not many instances of the vaccine or serotherapy working successfully against the human rabies pathogen.

By contrast, Robert Koch of Germany took the plunge into human infectious diseases, but was ultimately unsuccessful in his efforts with diphtheria, typhoid fever and tuberculosis. Shibasaburō Kitasato studied under Koch and discovered an antitoxin for the tetanus bacillus *Clostridium tetani* in 1890 with Emil Behring. He later had success with serotherapy, producing antiserum by administering animals with successive small doses of bacteria. He and Behring used this method on diphtheria and published their results. Behring was later awarded the first Nobel Prize in Physiology or Medicine in 1901 for his research on diphtheria antitoxin; Kitasato was short-listed, but was not awarded the prize (mentioned previously). One of the reasons for this was that there was no concept at the time of awarding the Nobel Prize for collaborative research.

From the late 19th century to the early 20th century, the greatest leading cause of infant mortality in Europe was diphtheria, due to myocardial damage caused by the diphtheria toxin once infected. The discovery of diphtheria antitoxin was a very significant achievement.

Paul Ehrlich entered the field of chemotherapeutics, having achieved some good results in developing methods for testing immune serum efficacy and methods for staining tissue and cells using pigment (1889: director of the Institute for Serum Research and Serum Testing in Frankfurt). Ehrlich believed that infectious diseases that could not be treated with antiserum or vaccine therapy should be able to be targeted with chemical substances, stating that “the aim of experimental therapeutics is to discover a magic bullet that will react only to foreign parasites and not affect the host” (1).

Working under Ehrlich, Kiyoshi Shiga discovered a pigment (trypan red) in 1904 that would inhibit the multiplication of trypanosomes that cause sleeping sickness in humans.

In 1909, bacteriologist Sahachirō Hata, also working under Ehrlich, experimented on animals to confirm the effects of a chemical compound synthesized by Alfred Bertheim. It was at this time that the relationship was established between synthesis and evaluation in modern drug discovery terms. Arsenic compound 606 was confirmed to have a powerful effect; in 1910, German pharmaceutical company Hoechst marketed this compound under the name of Salvarsan as a specific medicine for syphilis. It is thought that Hata was chosen to work with Ehrlich as a joint researcher on the challenging task of anti-syphilis treatments for his long-standing research in Japan on bubonic plague and communicable disease control. In 1908, Ehrlich was awarded the Nobel Prize in Physiology or Medicine for his theory on antigen-antibody reactions (Note 1).

Oswald Schmiedeberg, a forerunner in pharmacology, discovered in 1878 that muscarine, a crude drug alkloid, muscarine had a similar effect on the heart as electrical stimulation of the vagus nerve (mentioned previously). He also reported on the hypnotic properties of urethane in 1885. He conducted comprehensive research on the drugs and poisons of the day (experimental research on the medical efficacy of active ingredients) and in 1883 published the future pharmacology classic *Fundamentals of Pharmacology*. Well known for taking on and training students from the West, Schmiedeberg also had a pioneering influence on pharmacology in Japan, with many Japanese also learning under him, including Haruo Hayashi (founding chairman of the Japanese Pharmacological Society) and Kurata Morishima (Dean of Kyoto University College of Medicine, pharmacologist and discoverer of many active ingredients from crude drugs).

As pharmacology advanced, pre-existing chemical compounds began to be trialed as drugs, resulting in discoveries of anesthetics (such as chloral hydrate and chloroform) and antianginals (such as amyl nitrite). Later, organic chemists began actively synthesizing a number of compounds in search of compounds with powerful medical efficacy. This led to the discovery of antipyrine, phenacetin, acetanilide and other analgesic-antipyretics.

At the time, Ehrlich gave an address to the German Chemical Society acknowledging the benefits of medicines, but criticizing the use of pharmacological ideas for drug discovery, noting that “drugs discovered by pharmacological methods act on the symptoms of a disease and do not treat the disease in the true sense” and that “there is no sense in experimenting without using pathological animals. Quinine and mercury for syphilis are supreme drugs. Pharmacology has nothing to contribute to this in terms of research methods” (1:234-235).

Although diseases can be caused by pathogens from outside the body and disorders within the body, Ehrlich only focused on diseases caused by pathogens from outside the body. However, research advances were yet to be made on diseases caused by disorders within the body and it was not

until the 1960s onwards that the use of the pathological animals proposed by Ehrlich saw some success on diseases other than infections.

The basis of modern drug discovery is to find drugs that kill pathogens in vitro, check their efficacy on infected animals and then administer them to patients. However, there was very little science available in those days on applying such a system to drugs other than antibacterial (chemotherapeutic) drugs. By contrast, the pathogen infection model was already soundly tested and so it was the natural course to take for drug discovery to start with anti-pathogens [1, 2, 3].

Quite some time later, in 1935, Gerhard Domagk of Germany discovered that the red dye Prontosil had an antimalarial effect. He also discovered that Prontosil was also effective on streptococcus; there is a well-known story that he saved his daughter's life by administering her with Prontosil when she contracted streptococcus. Prontosil became the topic of global attention as a chemotherapeutic equal to Salvarsan for the syphilis spirochete and quinine for malaria.

Later, a group from the Pasteur Institute in France conducted a thorough study on Prontosil in opposition to Germany. The Domagk faction in Germany conducted research targeting azo dyes with the idea that "the efficacy of Prontosil lies in the azo dye, the source of the red coloring, which binds to the sulfonamide group". The French faction removed the azo group from Prontosil to synthesize a white sulfonamide, with the idea that "the sulfonamide group is essential, while the azo group is unnecessary". This compound had a higher medicinal efficacy and lower toxicity than the red sulfonamide. Sulfonamide derivatives are easy to synthesize; on the basis of this information, a succession of sulfa drugs started being developed all around the world. Prontosil was a so-called "prodrug" [Note 2], demonstrating its efficacy after metabolizing to its active ingredient, sulfamine [1].

Sulfa drugs is a general term for antibacterial or chemotherapeutic drugs with a "sulfonamide functional group ($-S(=O)_2-NR_2$)". (They are not called antibiotics since they are not of biological origin. For the purpose of this study, synthetic compounds with an antibacterial effect are treated as chemotherapeutic drugs.) Domagk was later awarded the Nobel Prize in Physiology or Medicine for his development of Prontosil, although he declined it in accordance with German state policy at the time [1:265].

According to a general review in 1940, there had been 3000 types of sulfamine compounds synthesized to date, signaling the arrival of the sulfa drug era, with drugs such as sulfapyridine and sulfathiazole used on a wide range of bacterial infections, including *Pneumococcus* and *Shigella* [1:267].

Company records indicate that many Japanese pharmaceutical companies also caught hold of the information on Prontosil and leapt straight into research, development and trial manufacture, resulting in successful compound synthesis [4, 5, 6, 7]. At the time, Japan had no restrictions on substance patents. Yamanouchi Pharmaceutical released Gerison® (sulfamine powder) in Japan in 1937, followed by the two-sulfonamide-group Albasil® (sulfathiazole powder) in 1938. Daiichi Pharmaceutical released Therapol® in 1937 and the two-sulfamine-group Two-Group Therapol® in 1939 (discussed later).

In 1928, Alexander Fleming observed bacteriolysis in a petri dish cultivation of *Staphylococcus* contaminated with *Penicillium notatum* and conjectured that the bacteriolysis was caused by a substance released by the mold. He named this substance "penicillin". While the story is very well-known, this penicillin was a mixture that could not be mass produced. A project team was organized in 1938 to isolate and identify the substance and carry out clinical trials. Mass production was successfully achieved by Howard Florey and Ernst Chain of the "Oxford group" [8].

The case with penicillin was the first time that a substance of microbial origin was observed to have a powerful antibacterial effect, rather than a synthetic antibacterial, which were the focus of the time. This marked the dawning of antibiotics. Penicillin also had the added benefit of being easier to use with fewer side effects than the synthetic antibacterial drugs of the day. With the capability to kill a number of pathogens that humanity had never been able to conquer, penicillin became a world-saving hero, rescuing countless patients from all kinds of infections. Penicillin also saved the lives of many soldiers wounded in the Second World War. Since then, full-scale efforts have been made to screen for antibiotics, with research and development continuing to advance in order to find other mold or fungi products with powerful medicinal efficacy. In 1945, Fleming, Florey and Chain were awarded the Nobel Prize in Physiology or Medicine for their invention of penicillin. The "Oxford group" project formed the basis for the antibiotics research and development process [9].

In 1882, Robert Koch discovered the pathogen behind tuberculosis, one of humanity's most troublesome infectious diseases since ancient times. At the time, the Industrial Revolution meant workers were converging in urban areas, with harsh working conditions, poor nutrition and adverse environments; around 1870, 1% of the population had died of tuberculosis. After discovering the pathogen, Koch published his ideas on "tuberculin treatment" in 1890, anticipating that it would work for immunotherapy. However, the effect deteriorated and did not result in a tuberculosis drug. A number of other compounds emerged at this time, purported to be effective against various kinds of tuberculosis in the medieval sense, but none of them lived up to expectations; it was quite a long time after that before a tuberculosis drug appeared.

Swedish scientist Jörgen Lehmann noticed that salicylic acid actually promoted the multiplication of the tubercle bacillus and anticipated that an analogous compound of salicylic acid would have an inhibitory effect on the bacillus. This idea is the exact concept behind modern drug discovery. In 1944, it was finally found that para-aminosalicylic acid (PAS) is effective against the tubercle bacillus. In Japan, Tanabe Seiyaku started producing this in 1950 [(7)].

In 1944, Selman Waksman discovered that ray fungus contains streptomycin, an antibiotic produced by microbes and a specific medicine for tuberculosis [(Note 3)]. Streptomycin showed good efficacy against gram-negative bacteria, against which penicillin and sulfa drugs had no effect, and had a dramatic effect on improving the symptoms of pulmonary tuberculosis.

However, in many cases the remaining tubercle bacilli in the lung lesions developed resistance around 40 days after treatment with streptomycin and the symptoms remerged and started to deteriorate even with continued treatment.

PAS, mentioned above, had little effect in treating tuberculosis on its own, but combined with streptomycin it improved the treatment rate by inhibiting the emergence of antibiotic-resistant bacteria, thus making it possible to provide an effective anti-tuberculosis treatment to patients who previously could only hope and pray for a cure. This treatment started being used in Japan around 1952 [(10)].

In 1946, Gerhard Domagk of German pharmaceutical company Bayer, who had discovered the sulfa drug Prontosil, discovered that isonicotinic acid hydrazide (INH, isoniazid) was effective against the tubercle bacillus. A triple-drug regimen of isoniazid, pyrazinamide and streptomycin was perfected, resulting in a dramatic decrease in deaths from tuberculosis.

However, even triple-drug therapy was mainly concerned with inhibiting bacteria. The complete eradication of bacteria began with the appearance of rifampicin in the 1970s. A “cocktail treatment” of streptomycin added to a triple-drug regimen of rifampicin, isoniazid and pyrazinamide showed outstanding results in eradicating tubercle bacilli from lesions.

Screening for antibiotics was the earliest system used for drug discovery. Researchers would cultivate a particular microbe in a petri dish, add a compound and determine the medicinal efficacy by observing whether the compound killed or inhibited the growth of the microbe. This was a simple system of evaluation; it was found in later years that a vast amount of information on endogenous diseases in human organs is required. The course taken by drug discovery has been a natural consequence of starting with the development of chemotherapeutics [(9, 10)].

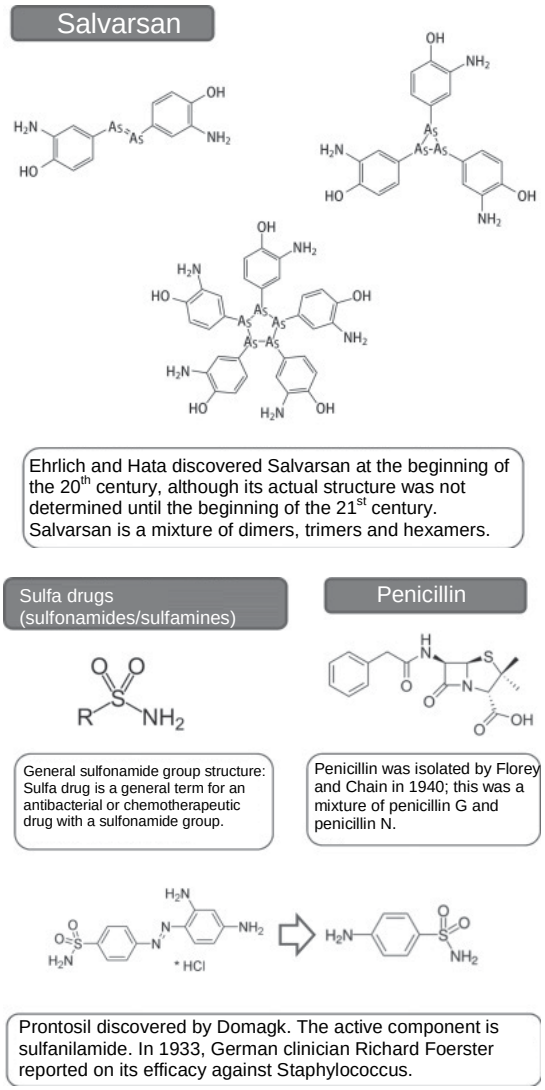

Fig. 2.5. Discovery of Chemotherapeutics and Antibiotics.

Note 1: The proper chemical structure of Salvarsan was not identified until quite recently in 2005. Salvarsan is actually not a single dimer compound, as per the structure proposed by Ehrlich, but a mixture of a cyclic trimer and a pentamer; it has been found to act in the body by oxidizing into monomers (see Figure 2.5. Discovery of Chemotherapeutics and Antibiotics. The following literature discusses trimers and pentamers. {Lloyd N.C., Morgan H.W., Nicholson B.K. and Ronimus R.S.: The composition of Ehrlich’s Salvarsan: Resolution of a century-old debate. Angew Chem. Int. Ed. Engl. 44 941-944 (2005)}).

Note 2: Some drugs have no or little medicinal effect on their own, but have a powerful medicinal effect once metabolized and activated in the body. These precursors are called prodrugs.

Note 3: Although Waksman was awarded the Nobel Prize in Physiology or Medicine in 1952, it was actually medical student Albert Schatz who did the planning and experimenting to discover streptomycin. Waksman was his nominal supervisor. Schatz insisted on being billed as the discoverer; this developed into a lawsuit. The court ruled that Waksman and Schatz were the co-discoverers of streptomycin and a settlement was reached.

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2.3.2. Advances in Endocrine Research

By the end of the 19th century, two important keys to unravelling the mystery of vital phenomena had been found: endocrines and nutrients.

The idea of incretions from organs having an effect on tissues in the human body was not new; it had been proposed by anatomist Frederik Ruysch in the late 17th century. By the mid-19th century, a wealth of research was being carried out on the adrenal glands, gonads and other endocrine organs in the form of experimental resections and anatomical observations using animal organs. This helped to clarify the relationship between various endocrine disorders and in vivo substances.

In 1836, English medical scientist Richard Bright conducted a study on kidney disease, hypertension and heart disease. His colleague Thomas Addison discovered "Addison's disease", in which lesions form in the adrenal glands and not enough hormones are produced, eventually resulting in death. This observation came 60 years before the discovery of the hypertensive substance renin. In 1849, German anatomist Arnold Berthold found that the crests of castrated roosters would grow to maturity if the bird were transplanted with testes from another bird. Based on the fact that microscopy revealed no nerves clinging to the gonads and the fact that sperm cells were present, he conjectured that the gonads had an effect on the emergence of secondary sex characteristics unrelated to the nerves and that the changes were triggered by a chemical substance. This developed into the concept of endocrine hormones.

While alkaloids with various different powerful effects had been extracted from crude drugs, hormones were the first in vivo substances to be extracted from animal organs. The adrenal glands and the pancreas were the first target organs. In 1907, Jōkichi Takamine and his assistant Keizō Uenaka isolated the first hormone, adrenaline, from the adrenal medulla. Prior to that, Edward Albert Sharpey-Schafer and George Oliver had found in 1895 that an extract from the adrenal medulla had vasoconstrictive, hypertensive and brochodilating effects. The target substance was variously named adrenaline and epinephrine and there was some controversy between Japan and the United States as to who had discovered it first [1, 2:217-220] (mentioned later).

The Industrial Revolution that had begun in the late 18th century led to dramatic developments in the mining and manufacturing industry, as well as population influx into larger urban areas. Supplying food to support these populations became a major social issue and prompted developments in nutritional science. Besides humans, the raising of livestock was also an important issue and studies were carried out on the relationship between food and nutrition. In 1837, William Prout proposed the idea of three main nutrients. The concept of metabolism of in vivo substances came into being in the mid-19th century, as did the concepts of inorganic salts and vitamins [2].

Insulin

Diabetes is recorded in the ancient Egyptian document *Papyrus Ebers* and is presumed to have been known as a disease that causes blindness and eventually death at a time when humans' food situation was by no means adequate. Ancient Indian doctors are also thought to have been aware that the urine of sufferers from this disease had a sweet smell. Medical works from ancient China also have clear descriptions of symptoms including dry mouth, increased urination and sugar in the urine.

This disease was even known in ancient Japan. An ¥80 postage stamp was issued in 1994, bearing the image of an insulin crystal and Fujiwara no Michinaga to commemorate the 15th World Diabetes Congress. It has been conjectured from *Shōyūki*, the journal of Fujiwara no Sanesuke, that the influential Heian-Period figure Fujiwara no Michinaga

suffered from diabetes. *Mananpō*, written in the Kamakura Period, also records a disease with the symptoms of diabetes. At that time, there were no crude drugs that were effective against diabetes, nor any method of treating it. It was not until the 19th century that the relationship between the pancreas and diabetes was recognized and the cause of diabetes identified.

In 1864, Paul Langerhans discovered the islets of Langerhans, so named by Gustave-Édouard Laguesse of France in 1893. In 1889, Oscar Minkowski and Joseph von Mering reported that symptoms similar to those of human diabetes occurred in dogs with the removal of the pancreas. In 1903, Eugene Lindsay Opie reported that a hormone was produced by the islets of Langerhans. However, isolating and identifying the causative agent insulin took some time, due to the fact that insulin is a protein; it was not until 1920 that insulin was discovered by Frederick Banting and his assistant Charles Best. There was fierce competition at the time to discover insulin. In *Shin Insulin Monogatari*, Kōsaku Maruyama notes that prior to the discovery by Banting and Best, 400 medical scientists and researchers had attempted to discover insulin, with five of these pulling out after failing on the brink of obtaining insulin [(3)].

Diabetes sufferers show remarkable improvement when treated with the administration of insulin; people marveled at this effect the world over (1922). Pharmaceutical companies immediately started mass producing substances extracted and isolated from bovine and porcine pancreases. Frederick Banting, who discovered insulin, and John Macleod, who supported the research, were awarded the Nobel Prize in Physiology or Medicine in 1923. Although there was some objection to Macleod's being awarded the Nobel Prize, having only been a financial support and not involved in planning or conducting experiments, Banting would not have had any success without his support [(4)].

In 1956, British analytical chemist Frederick Sanger determined the amino acid sequence of insulin and identified the molecular structure of insulin as a peptide with a molecular weight of 5087 made up of 51 amino acids in two strands. Sanger was one of the greatest contributing scholars to life science and was awarded the Nobel Prize in Chemistry twice, for his work on the identification of the structure of insulin and for his work on DNA sequencing.

The side effects experienced by patients as a result of immunoreaction to the porcine and bovine insulin were resolved with the emergence of human insulin. By 1979, gene recombination technology made it possible to produce human insulin easily and safely. Keiichi Itakura of Japan was the first in the world to produce insulin using genetic engineering (mentioned later).

Steroid Hormones

In 1924, Edgar Allen and Edward Doisy experimented on rats that had had their ovaries removed, injecting them with an extract containing estrogen (also called female hormone; a general term for several hormones). Advances in endocrinology were based on the phenomenon of symptoms caused by the removal of the testes or ovaries improving with the administration of substances extracted from gonads.

Adolf Friedrich Johann Butenandt isolated estrogen from the urine of pregnant women in 1929 and androsterone (a male hormone) from the urine of men in 1931 (He was nominated for the Nobel Prize in Chemistry in 1939, but turned it down in line with Nazi German policy. He was awarded the prize after the Second World War). William Allen and George Corner isolated progesterone (a luteal hormone) in 1934. Edward Doisy, who discovered estrogen, was awarded the Nobel Prize in Physiology or Medicine in 1943. While these steroid hormones first identified were sex hormones, at the time the gonads were thought to be central to the phenomenon of reproduction. Later research revealed the brain-pituitary gland-genitalia system.

Mayo Clinic chemist Edward Kendall isolated the steroid cortisone from the adrenal cortex (itself inactive, cortisone activates as it converts into cortisol). Its structure was determined by Tadeus Reichstein. There is a well-known account of physician Philip Hench of the same clinic administering cortisone to a 13-year-old girl suffering from rheumatism, resulting in a miraculous recovery, earning cortisone a reputation as a magic bullet.

The effects of the adrenal cortex hormone are wide-ranging and their mechanisms of action are not fully known, but it is currently regarded as an indispensable drug capable of achieving complete response against immune and inflammatory diseases that would otherwise be incurable. Kendall was awarded the Nobel Prize in Physiology or Medicine in 1950 for the discovery of the adrenal cortex hormone and his achievements in clarifying its structure and function, along with Hench and Reichstein [(1, 2, 5)]. It was more technically difficult to isolate unknown substances from animal organs than it was to isolate ingredients from crude drugs. Factors included the stability of ingredients, the quantity of ingredients (biologically active substances in particular existed in microscopic quantities), the extraction technology and the handling of the proteins; in some cases, isolation and purification meant waiting for the technology to advance.

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2.3.3. The Basis of Drug Discovery through Pharmacology and Medicinal Chemistry

While various chemical compounds have been isolated from crude drugs through advances in modern science, advances have also been made in fields for evaluating the medicinal efficacy of those compounds. The field involving clarifying the mechanism of action of drugs is called pharmacology; collaborative research relationships have been established in this field with synthetic organic chemists who synthesize drugs. The field of synthetic organic chemistry for synthesizing drugs is known as medicinal chemistry.

In 1868, T. Lauder Brunton published a paper entitled “On the Use of Nitrite of Amyl in Angina Pectoris”. This is thought to be the first case of a chemical compound synthesized by an organic chemist being used as a drug.

In the 1860s, Thomas Richard Fraser and organic chemist Alexander Brown examined the relationship between chemical structure and medicinal action for a number of alkaloids (including strychnine, codeine and morphine) using medicinal chemistry techniques, the basis of modern drug discovery. They found that despite being structurally similar, the newly-synthesized analogous compounds had a reverse effect or antagonistic action (a competing action that negates the original action). The information gained from these derivatives and their medicinal action provided a basis for the structure-activity relationship and led to the idea of agonists and antagonists.

The anitmetabolic phenomenon was already known in vitaminology and chemotherapy; under the autonomic nervous system, the theory extended into the field of pharmacology as well. The in vivo substances of note included adrenaline, acetylcholine and histamine.

In 1872, Oswald Schmiedeberg (previously mentioned) became the first professor of pharmacology at the University of Strasbourg. Lauded as the father of modern pharmacology, one of the student from abroad that studied under him was John Abel.

In the United States, the first university course in pharmacology was established in 1890 at the University of Michigan; the founding professor was John Abel. Abel built up a track record of brilliant achievements, including isolating epinephrine from the adrenal medulla (1898), isolating histamine from the pituitary gland (1919) and crystallizing insulin (1926). One of his students, Reid Hunt, discovered acetylcholine in an adrenal gland extract in 1906 [1, 2, 5, 6].

Adrenaline

In 1856, at a time when physiological societies were raising the idea of endocrines, E. F. Alfred Vulpian used a color staining method he had devised himself to confirm the presence of a unique substance secreted by the adrenal medulla that had very powerful effects in ultra-trace amounts. While researchers from a number of different countries competed in the attempt to isolate this substance, it was another 44 years until success was achieved by Japanese researchers Jōkichi Takamine and Keizō Uenaka [3, 4].

John Abel, mentioned previously, reported the isolation of epinephrine in 1897 in a German publication; Jōkichi Takamine also reported the isolation of adrenaline in 1900. European researchers as well as the pharmaceutical company Parke-Davis [Note 1], boasting state-of-the-art facilities for the time, confirmed the results on adrenaline reported by Keizō Uenaka, a young 24-year-old scientist and pharmacist who had just been employed by Takamine. Epinephrine was not extracted using Abel’s method, and Takamine and Uenaka were named as the discoverers.

However, after Takamine’s death, Abel stirred up controversy in the *Johns Hopkins Hospital Bulletin*, the *American Journal of Physiology*, *Science* and other scientific publications, saying that his methodology had been stolen by Takamine and Uenaka. However, Uenaka’s experiment notebook [Note 2] confirms that Takamine and the team obtained a crystal in 1900, thus defending their honor. American academic circles use the name epinephrine to this day, although in Europe the name adrenaline is used. In Japan, however, the name epinephrine was long used as the official name until it was corrected to adrenaline in the *Pharmacopeia* in 2006. What a story [Note 3]!

In 1894, George Oliver and Edward Albert Schafer found that the adrenal medulla contains a substance that constricts the blood vessels in the skin and organs, causing an increase in blood pressure and relaxation of the bronchial tube. This substance was the same adrenaline that was discovered by

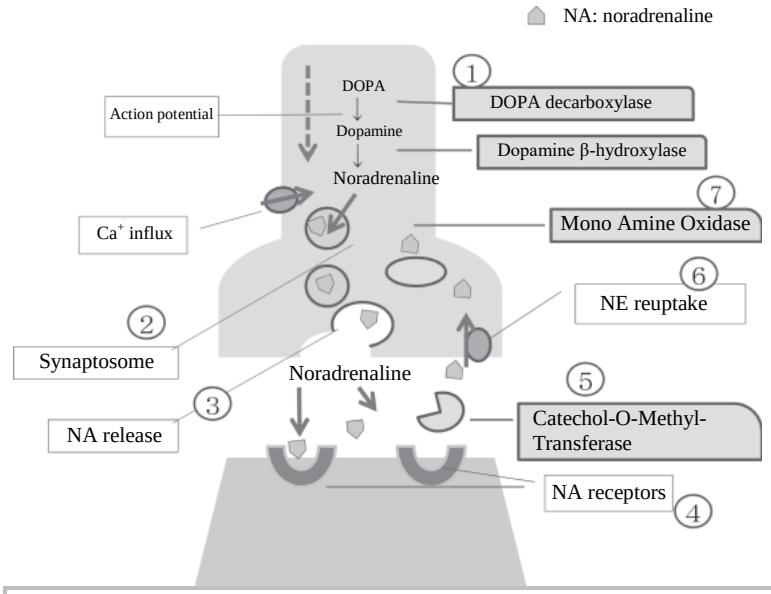
Takamine and Uenaka; its later synthesized analogous compound phenylethylamine had an antagonistic action (agonist action). In the 1930s, these agonists and antagonists were used in a number of studies aimed at clarifying the medicinal action of the sympathetic nervous system [1, 2, 5, 6].

In contrast to the voluntary somatic nervous system (motor nerves / sensory nerves), the involuntary autonomic nervous system controls involuntary functions, such as blood circulation, respiration, digestion, perspiration/thermoregulation, endocrine functions and reproductive functions. The autonomic nervous system works in accord with the endocrine system, a regulatory mechanism using hormones, to maintain homeostasis in the body.

The autonomic nervous system comprises sympathetic nerves and parasympathetic nerves; adrenaline and noradrenaline are the neurotransmitters for the sympathetic nerves, while acetylcholine is the neurotransmitter for the parasympathetic nerves. Acetylcholine was discovered in 1914 by Henry Dale; its function as the neurotransmitter for the parasympathetic nerves was identified by Austrian physiologist Otto Loewi, whose research laid the foundation for pharmacology in the United States. These two men later received the Nobel Prize in Physiology or Medicine in 1936 for their achievements in the theory of chemical transmission of nerve impulses (mentioned previously).

It was not known at the time whether the transmission of information at the nerve endings (synapses) was chemical or electrical. On finding that it is relatively dependent on adrenaline, Loewi hypothesized that neurotransmission occurs by means of a chemical substance. He eventually conducted an in vitro experiment in 1921 on two frog hearts, one with the vagus nerve attached and one without, and conjectured that stimuli are transmitted by a chemical substance. Although this experiment is well-known, the substance was later identified as acetylcholine rather than adrenaline. Foundational neuropharmacological research was carried out from 1930 to 1940, with experiments conducted on muscarine, an acetylcholine nerve agonist, antagonists tubocurarine and atropine and cholinesterase inhibitor physostigmine [5, 6].

Fig. 2.6 shows the metabolism of noradrenaline at the sympathetic nerve endings.



(1) DOPA turns into dopamine through the action of DOPA decarboxylase. Dopamine turns into noradrenaline (NA) through the action of dopamine *β*-hydroxylase.
(2) NA is stored in the synaptosome.
(3) NA is released from the synaptosome by electrical stimulus transduction.
(4) The released NA causes various physiological phenomena by acting on the postsynaptic cell receptors.
(5) NA is metabolized by the catabolic enzyme COMT.
(6) Unmetabolized NA is collected by the presynaptic cell through the reuptake system.
(7) NA is metabolized by the enzyme (MAO) in the synapse.

DOPA: L-3,4-DihyrOxy PhenylAlanine
COMT: Catechol-O-Methyl-Transferase
MAO: Mono Amine Oxidase

Fig. 2.6. Metabolism of Noradrenaline (NA) at the Sympathetic Nerve Endings.

Histamine

It took a long time to discover histamine, an allergy-induced in vivo substance [8]. Many researchers, including George Oliver at the end of the 19th century, reported on inducing hypotension in animals by injecting them with extracts from tissue (thyroid gland, blood, nerve tissue). While organic chemist Adolf Windaus synthesized histamine in 1907 and examined its medicinal action, it was not until 1911 that it was isolated in an in vivo experiment by George Barger and Henry Hallett Dale. A succession of reports on the physiological action of histamine followed. In 1919, John Abel and Bennosuke Kubota reported the presence of histamine in the pituitary gland and gastrointestinal mucosa [9:562].

Research was also being carried out on the relationship between anaphylaxis and histamine. The final proof came in 1932, with two laboratories, Wilhelm Siegmund Feldberg et al. and Carl Albert Dragstedt et al., both reporting that damaged tissue releases histamine. Based on the idea of adrenaline and acetylcholine antagonists, research began on histamine antagonists in the hopes of finding a substance that would protect people from the shock caused by the histamine. In 1939, Daniel Bovet et al. synthesized an antihistamine substance (929F). After that, many compounds were found with antihistamine effects, establishing the basis for the structure-activity correlation. Bovet was awarded the Nobel Prize in Physiology or Medicine for his "discoveries relating to synthetic compounds that inhibit the action of certain body substances, and especially their action on the vascular system and the skeletal muscles" [6].

Advances in Pharmacological Experimentation

The use of animals to test medicinal efficacy dates back the ancient Greeks. In the 19th century, dogs, cats and birds were used as laboratory animals. Later, advances in pharmacology began to be achieved through the use of rats in experiments on the endocrine system as well as to test the efficacy of drugs acting on the central nervous system and to observe behavioral changes. A number of different species, including rabbits, dogs, monkeys and chimpanzees, began to be used in addition to rats, mice and guinea pigs as pathological models for various different human diseases.

The Royal Society for the Prevention of Cruelty to Animals (RSPCA) was founded in England in 1824; in the United States, the American Society for the Prevention of Cruelty to Animals (ASPCA) was founded in 1866. The use of animals in research was long regarded as an exemption to the related laws; many pathological animals were developed through hybridization and gene transfer technology. However, in the mid-20th century, limitations started being placed on the use of animals for efficacy and safety testing; in vitro experiments (experiments in test tubes using cells, tissue, enzymes and receptors) began to be increasingly carried out instead. The use of genetically engineered pathological rats and other animals is currently very restricted.

In the modern era of the 19th century, humans were sometimes used to test medicinal efficacy. In the early days, there were no adequate means of evaluating efficacy, nor any adequate safety measures. It was not until 1942 that an idea similar to the clinical trials of today emerged in a biological experiment on digitalis in humans using a non-invasive electro-cardiogram to examine the effects of digitalis. Subsequently, the criteria for experiments on human subjects were gradually clarified and improved into what they are today.

The usual method used for testing the effects of drugs today is in vitro experimentation using organs or tissue; this dates back to 1900. Henrick Magnus established this method of pharmacological experimentation by suspending strips of small intestine in a glass tube (Magnus tube) containing physiological saline with oxygen bubbling through it and observing the movements of the tissue using a polygraph (which at the time comprised a rotating drum coated in soot). In 1904, Jean-François Heymans similarly experimented on mammalian hearts, while Claude Bernard experimented on nerve-muscle preparations. While the Magnus tube was simple, the discovery that tissue and organs could be brought into a living state in vitro was ground-breaking. This experimentation technique is still used today [6].

Sterilization and anesthetics revolutionized surgical procedures. Anesthetics derived from crude drugs had been used in Europe, but were not completely effective. In 1846, William Morton successfully performed a tooth extraction using ether as an anesthetic; in 1847, James Simpson successfully performed major obstetric surgery using chloroform. In 1869, Otto Liebreich hypothesized that chloral hydrate would have an anesthetic effect because it releases chloroform in the presence of an alkali. After a number of clinical trials, he reported it as a new hypnotic/anesthetic (mentioned previously).

Organic chemists in Germany later synthesized a number of compounds, including salicylic acid, aspirin, acetanilide, phenacetin, antipyrine and other antipyretic analgesics [7, 8].

From the late 19th century to the early 20th century, advances were made in analytics and organic chemistry and biochemistry was developed. Progress was made in research on in vivo amino acids and organic acids, proteins and nucleic acid. In 1910, Albrecht Kossel was awarded the Nobel Prize in Physiology or Medicine "in recognition of the contributions to our knowledge of cell chemistry made through his work on proteins, including the nucleic substances". The Nobel Prize in Chemistry was awarded to Otto Wallach "in recognition of his services to organic chemistry and the chemical industry by his pioneer work in the field of alicyclic compounds".

Research on diseases continued with advances in immunology. Charles Richet was awarded the Nobel Prize in Physiology or Medicine in 1913 “in recognition of his work on anaphylaxis”, while Jules Bordet received the award in 1919 “for his discoveries relating to immunity”.

Although these were remarkable scientific developments, in terms of drug discovery, no outstanding new drugs emerged until after the Second World War as the modern drug discovery process was perfected, offering thorough evaluation of compounds isolated from crude drugs and their related compounds. This is thought to be due to a number of different factors, such as the fact that the science for analyzing pathological mechanisms was not yet far enough advanced, the fact that there were no clear objectives for the drugs and the fact that medicinal chemistry lacked an adequate level of technology to synthesize the various different samples to be evaluated, not to mention the fact that there was no set methodology for theoretical advances in the structure-activity correlation and it often depended on chance.

Note 1: In 1900, Thomas Aldrich of Parke-Davis isolated a compound from the adrenal medulla with the empirical formula of $C_9H_{13}NO_3$. Although this was only CH_2 less than the empirical formula $C_{10}H_{15}NO_3$ of the adrenaline reported by Takamine et al., Aldrich regarded the two as the same substance, assuming the Takamine et al. substance to contain impurities. Despite having been Abel’s assistant, he also considered the empirical formula of epinephrine $C_{17}H_{15}NO_4$ to contain impurities.

Note 2: Uenaka’s notebook has been registered by the National Museum of Nature and Science and certified as a future heritage asset.

Note 3: *Horumon Hantā* by Mitsuo Ishida goes into more detail on this course of events [(4)].

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2.3.4. Medicines in Japan (Part 1: Meiji Period to Second World War)

Western medical science and medicines were introduced to Japan in earnest from the Meiji Period of the late 19th century onwards. However, even during the Edo Period in the early 18th century under national seclusion, the study of Western knowledge still penetrated Japan and had an effect. Konyō Aoki was ordered by shogun Tokugawa Yoshimune to start studying Dutch in 1740. The first autopsy in Japan was carried out in 1771; observers Genpaku Sugita, Ryōtaku Maeno and Junan Nakagawa were surprised at how different the actual organs were from those they had studied in books. They were very impressed by the detail of the Western book *Ontleedkundige Tafelen* and worked on completing its Japanese translation, *Kaitai Shinsho*.

In 1823, Dr. Philipp Franz von Siebold came to Japan from Holland to teach Western medical treatment. He used around 230 kinds of crude drugs, including *Zingiber* (ginger), *Mentha* (mint), *Camphora* (camphor), cinnamon bark, gum arabic and cloves, and is said to have amazed Japanese physicians with their effectiveness. While there are doubts as to whether these drugs really had such amazing medicinal efficacy, there is no doubt that some of the Western scholarship and science brought to Japan by the Dutch in the late 18th and early 19th century started laying the foundation for Japan’s modernization. Another view asserts that the Dutch would not have been able to bring the most advanced medical technology and medicine to Japan, as the Dutch were not as advanced in chemistry and pharmaceutical science as the Germans, the French or the English [(1)].

An examination of journals and records that describe the drug situation at that time reveals that according to *Koan no Kusuribako* (Medicine Box of Koan) [(2:46-47)], the drugs used most commonly by Dr. Ogata Kōan were “mugwort (anthelmintic from which santonin was later isolated)”, “rhubarb (cathartic, from which sennoside was later isolated; rhubarb is also one of the medicines held at the Shōsō-in and analysis was performed on the ingredient sennocide)”, “cinnamon bark”, “licorice (from which glycyrrhizin was isolated)” and “scopolia rhizome”. Dr. Kōan seems to have mainly treated his patients with anthelmintics, laxatives and scopolia rhizome, which acts on the central nervous system.

At the end of the Edo Period in the mid to late 19th century, people in many different fields came to Japan from overseas, each writing an account of the situation in Japan from their own perspective. Russian naval captain Vasily Golovnin, taken prisoner by the Japanese in 1811, wrote that “Japanese

physicians pay very little attention to the regimen of the sick" and that "medicine could not operate to advantage with such food". He also noted that "all Japanese carried antidiarrheals" (*Memoirs of a Captivity in Japan*). Commodore Matthew Perry of the United States East India Squadron wrote in his *Narrative of the Expedition of an American Squadron to the China Seas and Japan* that "the native physicians… have not availed themselves at all of *post mortem* examinations… superstition is in the way. …Without such examinations, it is obvious that the knowledge of the physician and surgeon must be but imperfect at best. …Their drugs are mostly animal and vegetable; they are too little acquainted with chemistry to venture upon mineral remedies. They study medical botany, however, with great attention, and their remedies are said to be generally efficacious" [2:118-124].

Dutch physician Johannes Pompe van Meerdervoort arrived in Nagasaki in 1857 and spent five years in Japan. He had a significant impact on Japan at the dawning of a new era in medical treatment. Pompe van Meerdervoort appealed to the shogun with the need to establish hospitals. In his book *Vijf Jaren in Japan*, he noted that "surgery is done using salves; there is no understanding at all of surgical procedures. Knowledge of science is nonexistent, but there was high enthusiasm for learning among the young medical students." Pompe van Meerdervoort also wrote of ancient Japanese medicine that "unfortunately I could not even glean any medical principles from them, as I believe there is a lack of training schools", noting that "the common people treat their own ailments, using moxa and acupuncture to release the toxic gases from within the body" [3:301-302].

British consul-general Rutherford Alcock came to Japan in 1859. In his work *The Capital of the Tycoon*, he noted that "There are many skin diseases in Japan. People go to hot springs for treatment. The home remedy of 'moxa' is performed once a year to eradicate all kinds of diseases" [2:118-124]. These records provide a good estimation of the medicine and medical treatment situation in Japan at the end of the Edo Period.

Around the time that advances in modern science in the West were contributing to the isolation of medicinally-efficacious active substances from various crude drugs and the discovery of pathogenic microbes, the Japanese government was transitioning to the new Meiji administration. An examination of Meiji-era trends in Japanese pharmaceutical companies reveals a transition by drug wholesalers from Chinese medicine to Western medicine once the isolation period ended and an increase in transactions of drugs imported from the West. The company histories of various drug developers indicate that after the Meiji Reformation they faced a monumental decision of whether to keep working with Chinese and Japanese medicine or to switch to Western medicine. However, many records state that companies "were zealous to absorb knowledge of Western medicines and actively absorbed the new knowledge" [5, 6].

With the influx of Western medicine, counterfeit drugs and low-purity medications also began to appear on the market. The Meiji government established pharmaceutical laboratories for testing imported drugs in an effort to ensure the supply of genuine drugs (1874-1876). In 1889, the government promulgated the *Regulations on Pharmaceutical Operations and Products*.

A medical system, including a licensing system for physicians, had been announced in 1874, while the Pharmacopeia, which set the criteria for medical and pharmaceutical products, had been established in 1886.

Aware that they had to do more than just import drugs to ensure the quality of Western medicines, drug wholesalers started transitioning into specialized manufacturers of Western medicines. The major drug wholesalers Tanabe Gorobē, Takeda Chōbei and Shiono Gisaburō each started their own drug manufacturing enterprises, although many of their early products were poorly manufactured (iodine was particularly problematic). Realizing that to compete against the West they had no choice but to establish a government-subsidized pharmaceutical company, in 1883 they jointly established Dainippon Pharmaceutical with support from the government.

This was Japan's first pharmaceutical company. Domestic drug production started with Nagayoshi Nagai, the discoverer of ephedrine, as chief of drug manufacture, overseeing everything from installing machinery to producing medicines. Meanwhile, influential drug brokers and wholesalers in Osaka established Osaka Pharmaceutical in 1897. However, the operation of Dainippon Pharmaceutical was impeded by state-influenced company bureaucracy and in 1899 it was taken over by Osaka Pharmaceutical [8]. Dainippon Pharmaceutical continued working on the formulation of ephedrine, later launching this Japanese discovery in 1927 as the bronchodilator and antitussive "Nagai" [1, 4, 8].

Adrenaline, discovered by Jōkichi Takamine, was re-imported back to Japan in 1902, having already been launched on the American market. Sankyo Shoten had launched Takadiastase, another of Takamine's discoveries, in 1898. Takadiastase is a digestive drug that is frequently mentioned in Sōseki Natsume's novel *I Am a Cat*. In 1913, Jōkichi Takamine was appointed the first president of Sankyo Shoten.

Diastase (amylase) is a highly active enzyme that hydrolyzes starch into disaccharides and monosaccharides. It is used in fermentation-related industries. Jōkichi Takamine and Tetsukichi Shimizu successfully extracted and powdered diastase from koji mold *Aspergillus oryzae* and patented it in the United States in 1894. This is the product Takadiastase. The product was marketed in the United States the following year by George Davis of Parke-Davis as a powdered digestive medicine [7]. Until then, all extracts had been concentrated syrups, which were troublesome and inconsistent to deal with. The powdered form also made it easier to combine with other drugs, making it a popular product since it was first launched and it is still available on the market today [18].

Vitamin B, first discovered from rice bran by Umetarō Suzuki in 1910, was marketed by Sankyo Shoten as Oryzanin®. Later, this drug was found to be effective against beriberi, a very serious disease in Japan at the time, and the number of patients suffering from beriberi steadily began to

decline. While all kinds of vitamin pills were available on the market in the early 1940s before the Pacific War broke out, there was even greater credence in vitamins after the war and companies started researching and discovering thiamine (B_1) derivatives (mentioned later).

The revision of the *Patent Act* in 1909 and the adoption of the process patenting system made it possible for the domestic pharmaceutical industry to adapt the processes used for new drugs developed overseas by more advanced nations and market the products in Japan provided they had been produced in Japan. As a result, research and development of new drugs in Japan fell behind. The outbreak of the First World War in 1914 made it difficult to import drugs from Germany and other countries. The resulting sudden steep rise in the cost of Western drugs created a greater need for domestic production. The government regulated the medical and pharmaceutical products leaving the country and provided financial incentives for pharmaceutical products, including allowing the use of German patents as a wartime exception (*International Property Act in Wartime*). Manufacture of vital drugs began in earnest, including Salvarsan (antisyphilitic), salicylic acid, sulfa drugs, local anesthetics, anesthetics and anticonvulsants. The domestic production of Salvarsan in particular sparked intense competition, drawing in the best Japanese technology and expertise of the day.

Dr. Katsuzaemon Keimatsu, former director of the medical division of the South Manchuria Railway Central Experimental Laboratory, started working on a Salvarsan prototype in January 1915. By April, he had successfully synthesized it and started commercial production in Japan. He established "Arsemin Shokai" (which became Daiichi Pharmaceutical in 1918) and launched Salvarsan under the brand name of Arsemin®. However, Salvarsan was administered by injection and, being insoluble, would cause pain to patients if too much was administered. The formulation was improved and marketed as Neo-Arsemin®. Daiichi Pharmaceutical devised a dosage form, which significantly increased sales.

Umetarō Suzuki of Tokyo Imperial University College of Agriculture established a method for manufacturing Salvarsan in March 1915, assisted by a grant from the Ministry of Finance. The product was launched by Sankyo in September that year under the brand name of Arsaminol®.

Tōru Iwadare, under the supervision of Prof. Kōchi Matsubara of the Tokyo Imperial University College of Science, completed a prototype in April 1915 and invested his own funds to have it launched by Banyu Ltd. (later Banyu Pharmaceutical) under the brand name of Eramisol®.

Dr. Mitsuru Kuhara of the Kyoto Imperial University College of Science and Engineering (and the first president of the Chemical Society of Japan) completed a prototype in May 1915 and had it marketed by Kyoto Shinyakudo (now Nippon Shinyaku) as Saviol®.

This flurry of developmental activity gives us a sense of the energy and vigor befitting the dawning era of domestic production of new drugs [12:5-7, 17:252-253].

Domestic production of sulfa drugs in the 1920s and 1930s in the early Showa Period saw eight major industry players playing off against each other [8, 9]. Daiichi Pharmaceutical, established in 1918, marketed the first domestically-produced sulfa drug in 1937 under the brand name of Therapol®. Therapol was synthesized by Kazuo Miyatake not long after he started working there; he later went on to become the company president. According to the company's history, the chief engineer saw the compound in the German organic chemistry journal *Justus Liebigs Annalen der Chemie* and said to Miyatake, "I have seen this stuff – could you make it at once?" Miyake then "made it as quickly as possible. There were no animal experiments in those days. This was Therapol®, the first such product to be made domestically" [12].

At almost the same time, Yamanouchi Pharmaceutical marketed Gerison® (sulfamine powder) in 1937 and produced the two-sulfonamide-group Albasil® the following year. By 1949, Yamanouchi Pharmaceutical was the top producer of sulfa drugs in Japan [14].

Daiichi Pharmaceutical also released the two-sulfamine-group Two-Group Therapol in 1939, followed by the sulfapyridine Therapyridine® in 1942.

Takeda Pharmaceutical, Banyu Pharmaceutical, Shionogi Seiyaku, Tanabe Seiyaku, Sankyo and Dainippon Pharmaceutical all started researching and developing sulfa drugs at around the same time, each marketing their own respective products.

Tanabe Seiyaku launched the topical non-steroidal anti-inflammatory Salomethyl® with methyl salicylate as the main ingredient in 1921 after having found out that methyl salicylate was the main ingredient in the well-known French pain-relief ointment Baume Bengué® [5].

After the First World War, the pharmaceutical industry no longer had to depend on imported products as it had done and was able to use domestically-produced drugs as its production staple. However, the government's policy of prioritizing domestic production through initiatives such as the process patenting system for overseas drugs came at the expense of investment into Japanese research and development of new drugs, thereby hindering any new drug inventions by the Japanese.

In the 1930s and 1940s, there was a rapid increase in antiseptics formulated from acridine derivatives, azo dyes and organomercury compounds; these were marketed by Banyu Pharmaceutical, Takeda Pharmaceutical, Daiichi Pharmaceutical, Tanabe Seiyaku, Yamanouchi Pharmaceutical, Shionogi Seiyaku and other companies [5, 6, 7, 11, 12, 13].

The 1931 edition of *Jōyō Shinyaku Shū* (Nippon Shinyaku ed.) reveals the common diseases of the time, as well as the medical and pharmaceutical products used to treat them. According to this data, drugs for infections accounted for 30% of all drugs (see Figure 2.10). There were many dermatological, otolaryngological and gynecological drugs, while analgesics and antipyretics accounted for 10% and anticonvulsants 9%. There was not yet the same mention of drugs for treating diabetes, hypertension, hyperlipidemia or other 21st-century lifestyle-related diseases [9].

(Table 2.4 shows the types and ratios [Note] of drugs administered in 1931 and has been re-tabulated by the author based on information taken from *Jōyō Shinyaku Shū* (Nippon Shinyaku ed.) and *Nihon Iyaku Sangyō-shi* (pp.74-75).)

Table 2.4 Types and Ratios of Drugs Administered in 1931

	Types	Ratio
(1) Antimicrobial / chemotherapeutic / antibiotic	570	30%
(2) Surgery related	94	5%
(3) Analgesic / antipyretic / anticonvulsant	189	10%
(4) Gastrointestinal	170	9%
(5) Lifestyle-related disease	20	1%
(6) Circulatory / antianginal	139	7%
(7) Antitussive	73	4%
(8) Vitamins / nutritional supplements	173	9%
(9) Other (dermatological / otolaryngological / gynecological)		34%

The Japanese also had issues with the treatment of sewerage; many people carried roundworm and threadworm from ancient to early modern times. Since the discovery of santonin from mugwort in 1830, it has been used by many researchers in pharmacology and clinical studies. In Japan, Hisomu Ichinose and Katsura Takada investigated ways to extract santonin from sea wormwood; domestic production started in 1929 and it has been used to eradicate roundworm since then [15]. In 1953, Tsunematsu Takemoto extracted kainic acid from the crude drug red algae *Digenea simplex* [16]. While kainic acid is an amino acid and used for eradicating roundworm and threadworm, it is known to bind to the glutamate receptors, thereby increasing nerve stimulation, causing paralysis.

Once news of the curative effects of penicillin reached Japan from Western Europe despite it being wartime [Note 1], collaborative research began in the public and private sectors, including research by Hamao Umezawa, promoted by Major Katsuhiko Inagaki of the Army Medical College. The Penicillin Committee was inaugurated in 1944, comprising members from the government and the academic world, and industrial production of penicillin began in December 1944 with the country still at war. Industrial production started at the Morinaga Food Industry factory at Mishima; Banyu Pharmaceutical also started manufacturing it not long after that. By 1945, Japan had gone from a penicillin production capacity of several dozen bottles a day to around 200 bottles a day. According to *Hekiso / Nihon Penishirin Monogatari*, "wartime production peaked in August 1945 at the end of the war". There is no record of the production volume; most of it is presumed to have been used on the battlefield [10].

While the 1930s saw the start of domestic production, there were few original Japanese drugs manufactured at that time; it was not until after the war that new drugs invented by the Japanese began to appear. Notwithstanding, Japan did make some achievements during this time, including the discovery of the cardiotonic vitacampher by Kenzō Tamura and Morizō Ishidate and the discovery of analeptic amines as a result of ephedrine research by Yukito Ōta and Fujio Egami. Incidentally, records indicate that the analeptic amine Philopon® was announced in the *Journal of the Pharmaceutical Society of Japan*, Vol. 139 in 1894 by Nagayoshi Nagai as "ephedra research substance 33" and was marketed by Dainippon Pharmaceutical in 1941 [4, 8].

The main thrust of drug discovery in postwar Japan was research and development of chemotherapeutic drugs [Note 2]. This became the mainstay of the Japanese pharmaceutical industry, with universities, government research institutions and private companies discovering a succession of new antibiotics and synthetic antimicrobials. This trend continued until the 1990s, as Japan topped the world in the administration of antibiotics, antimicrobials and other chemotherapeutic drugs.

Note 1: In 1943, the German clinical bulletin *Wiener Klinische Wochenschrift* containing news of the "antimicrobial substance penicillin obtained from microbes" was brought by the U-boat *511* from Axis power Germany; in 1944, the news that "Churchill made a complete recovery from pneumonia with penicillin" was brought to Japan by the Asahi Shimbun Buenos Aires correspondent. This marked the beginning of penicillin development in Japan. Although these are well-known stories, the medical journal was not in fact brought to Japan by a German U-boat. During the Second World War, Japan sent five submarines to Germany, three of which made it. *Hekiso / Nihon Penishirin Monogatari* confirms that the information was brought back to Japan by the *I-8*, the only one of these submarines to return to Japan. At Churchill's request, he was actually treated with sulfa drugs (or sulfon drugs, as the German reading was more prevalent at the time), which had proven effectiveness, rather than penicillin [10].

Note 2: Chemotherapeutic treatment refers to the treatment of infectious diseases using chemical drugs. Antibiotics refers to "antibacterial, antifungal, antiviral and anticancer drugs derived from substances produced by microbes". In this paper, chemotherapeutic drugs include antibacterial, antifungal, antiviral and anticancer drugs derived from chemical drugs.

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2.4 From New Medicines to Improved Medicines and Innovative Medicines

2.4.1. Medicines in Japan (Part 2: Post-Second World War)

As previously mentioned, development progress was made in postwar Japan on antibiotics, chemotherapeutic drugs and vitamins, the foundation having already been laid. Given the fact that the two leading causes of death in Japan since the late 19th century were infectious diseases and beriberi, it would have been only natural to start postwar drug developments with vitamins and drugs to fight infection.

Although pharmaceutical production started up again soon after the war as drug production facilities had not been as hard-hit by war damage as other industries, production volumes were small due to shortages of ingredients and materials. Meanwhile, the demand for medical and pharmaceutical products increased dramatically after the war due to the poor public health situation. With inadequate public health, drugs such as penicillin and sulfamine were designated as high-priority bacterial pharmaceuticals, with priority given to their production. There was also a demand for increased production of dermatological drugs as well as local anesthetics, analgesics and anticonvulsants, which were in high demand in surgery, ophthalmology and dentistry. Regulated distribution of medical and pharmaceutical products continued until 1952.

Around 35 new Japanese drugs (new ingredients) were recognized in the 1950s, including 10 antibiotics and chemotherapeutic drugs, 3 bacteria-derived anticancer drugs and 3 vitamin formulations. The same trend continued in the 1960s, with another 35 new Japanese drugs recognized, including 9 antibiotics and chemotherapeutic drugs, 7 vitamin formulations and 2 anticancer drugs (1).

Social demand aside, companies were also competing technologically to produce improved drugs from the available lead compounds for chemotherapeutic drugs and antibiotics, having honed their methods of evaluation to perfection. The situation was the same for vitamins as well. (* The difference between drug production and drug discovery: Up until the 1980s, companies in the pharmaceutical industry were often referred to as drug manufacturing companies; in the 1990s, as drug research and development began to require a greater accumulation of science and technology (system science), the research and development of new drugs began to be referred to as "drug discovery" rather than "drug production". Companies that focused on discovering new drugs began to be referred to as drug discovery companies. Currently, the research and development of new drugs, including formulation improvement and drug delivery systems (DDS), is often referred to as drug production.)

Antibiotics

Research and development on penicillin continued to progress after the war. The Japan Penicillin Research Association was founded in 1946, while the Japan Penicillin Association was established around the same time by private companies planning to manufacture the product. There were 39 founding member companies, many of them principally engaged industries other than pharmaceuticals, such as food, brewing, chemistry and spinning.

The production of penicillin required chemical engineering technology. Pharmaceutical companies lacked the experience and facilities, prompting companies from the chemical, food and brewing industries to expand into the production of antibiotics. At the three-year commemoration of the Japan Penicillin Association in 1949, Captain Samson of the GHQ said in his congratulatory address, "There are presently only three countries in the world that are able to supply their own penicillin. Japan is one of them. Three years ago I asked you to 'meet your domestic demand' and to 'make first-rate penicillin' and you have succeeded admirably. I would like to make a third request of you: export it to the world" (2:225-226). This came true the following year. The outbreak of the Korean War raised the demand for penicillin; with the American military purchasing it, penicillin production increased dramatically and overseas exports began. While this was partly due to luck, it also indicates the superiority of Japanese fermentation and chemical engineering technology.

Streptomycin production required even larger fermentation equipment and facilities; the pharmaceutical companies again lacked the necessary experience and facilities. Domestic production began in 1950 with licenses granted to five organizations in other industries (Meiji Seika, Kyowa Hakko, Kaken Chemicals, Shimane Chemical and the Nippon Institute for Biological Science).

As antibiotics began to replace chemotherapeutic drugs, signs began to appear of antibiotic overuse. Incidents of death by penicillin shock in 1956 called product quality and administration methods into investigation. In 1958, Hamao Umezawa (National Institute of Health) and Meiji Seika developed the epoch-making antibiotic kanamycin using

domestic technology, although other newly-developed antibiotics were later imported from overseas. The successive appearance of penicillin-resistant bacteria and other antibiotic-resistant bacteria meant the Japanese drug discovery industry had to progress from developing first-generation antibiotics to developing second- and third-generation antibiotics.

Many of Japan's developments in antibiotics and chemotherapeutic drugs were world-class new drugs. This is a very significant area in the history of drug discovery, although for the purposes of this paper these are summarized together under "lifestyle-related diseases" rather than discussed individually in detail.

Vitamins

While it is understandable amidst postwar food shortages that beriberi ranked as one of the top two leading causes of death alongside tuberculosis, it may be surprising that all ten of the new vitamin formulations mentioned previously were thiamine (vitamin B_1) derivatives.

Postwar Japan was very receptive to vitamin formulations to combat nutritional deficiencies stemming from food shortages. During this time, many different companies were marketing multivitamin formulations made up of all kinds of vitamins, sparking the over-the-counter vitamin boom. Vitamin formulations remained the highest-selling pharmaceutical product in postwar Japan for 12 years [3] (see Table 2.5).

A succession of products hit the market. Takeda Pharmaceutical launched Panvitan® in 1950, while Tokyo Tanabe Seiyaku launched Vitaplex® and Taisho Pharmaceutical launched Vitacolin® in 1951, Shionogi Seiyaku launched Popon-S® in 1952 and Sankyo launched Minevital® in 1953.

In 1952, Motonori Fujiwara et al. of the Kyoto University Faculty of Medicine discovered allithiamine, comprising vitamin B1 bonded to alliin, a substance found in garlic. They gained some attention for their theory that this would be effective against arthritis. Backed by this theory, Taizō Matsukawa et al. of Takeda Pharmaceutical developed the active vitamin B_1 prosulthiamine (Alinamin®) in 1954. This sparked competition to develop new vitamins, such as octotiamine (Noivita®) by Fujisawa Pharmaceutical and benfotiamine by Sankyo in 1961, bisbentiamine (Beston®) by Tanabe Seiyaku in 1962, cetotiamine hydrochloride (Dicetamin®) by Shionogi Seiyaku in 1965, cycotiamine (Cometamin®) by Yamanouchi Pharmaceutical in 1966 and bisibuthiamine by Taisho Pharmaceutical in 1967, each new drug being approved by the Ministry of Health and Welfare. As a result, pharmaceutical companies made significant profits from vitamin formulations and were able to invest those profits into establishing research laboratories for the pharmaceutical industry [1, 3].

Table 2.5 Pharmaceutical Products Sales in Postwar Japan

Year	¥100 million	Product with highest production volume
1957	1,251	antibiotics
1958	1,345	vitamins
1959	1,493	vitamins
1960	1,760	vitamins
1961	2,181	vitamins
1962	2,656	vitamins
1963	3,411	vitamins
1964	4,232	vitamins
1965	4,576	vitamins
1966	5,071	vitamins
1967	5,633	vitamins
1968	6,890	vitamins
1969	8,425	vitamins
1970	10,253	antibiotics

Anticancer Drugs

Following the Second World War, government research institutions joined forces with private companies in research on cancer, which had rapidly rose in incidence to become the top leading cause of death by 1979. Several effective anticancer drugs were discovered by furthering the research done on antibiotics and antibacterial drugs in the 1950s and 1960s.

Sarkomycin was discovered by Hamao Umezawa (National Institute of Health & Meiji Seika) in 1954, while mitomycin was discovered by Tōju Hata (Kitasato Institute & Kyowa Hakko) in 1955. These drugs are well known as typical early antibiotic-type anticancer drugs. The anticancer drugs of the day were nonselective, inhibiting the functional activity of normal cells as well as cancer cells; many inhibited cell division and many had very strong side effects. Later, early detection and surgical removal became the main means of treating cancer, while rapid advances in molecular biology and molecular cytology in the latter half of the 20th century enabled science to identify cancer-causing mechanisms. What had previously been lumped together under the name of "cancer" was actually found to be the result of several stages of complex mechanisms at work. Scientists began to understand that cancer could not be explained as the result of a single mechanism like other diseases (although, of course, many other diseases are likewise not the result of one simple mechanism).

Advances in genetic engineering gradually revealed that there are different causes for types of cancers and that there are differences between individual patients due to genetics. The field of oncology, one of the major focal areas for the future, is seeing significant results by drug companies using advanced biotechnology to develop new drugs. Antibody formulation is one such development that is bringing a ray of hope into the field of oncology.

Social Background to the Pharmaceutical Industry

The stability of the Japanese postwar economy was in crisis due to deflation. The outbreak of the Korean War in 1950 brought with it a sudden demand for vast amounts of munitions and strategic resources; these special procurements caused a boom in the Japanese economy. Following this, the pharmaceutical industry entered an age of free competition.

Although the Japanese economy plateaued for a while after the Korean War, a rapid increase in exports in 1955 caused another economic boom. During this time, pharmaceutical company production divisions started out in new directions of technological innovation, capital investment and advances in automation. In 1956, drug production yield rose to ¥103.7 billion, the first time it had exceeded ¥100 billion, a 16% increase from the previous year. In 1957, the Japanese economy was showing signs of a recession, but this had little impact on the pharmaceutical industry, which continued at a growth rate of 21% resulting in a drug production yield of ¥125.1 billion [3, 4].

Japan implemented a national health insurance program in 1927 and by 1945 it had 4 million members. In 1958, the *National Health Insurance Act* was amended to make membership obligatory; by 1961, it had been implemented across the entire country and medical insurance for all citizens had been achieved. This boosted the medical examination rate and also increased the drug production yield. These figures grew by more than 20% each year from ¥176 billion in 1960 to ¥457.6 billion by 1965 (see Figure 2.7) [3].

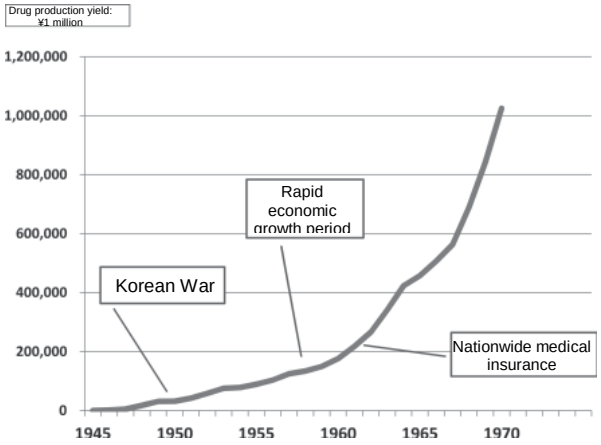

Fig. 2.7. Trends in Pharmaceutical Industry Production Yield.

The pharmaceutical industry in Japan was historically not "new drug oriented". At that point in time, the mechanisms causing many of the world's diseases had not yet been fully identified and the process of research and development for drug discovery was by no means perfect. All the Japanese pharmaceutical industry had perfected was the ability to re-create pharmaceutical products imported from the West. Unlike in the West, patented in Japan were granted based on production methods. A product could be patented as long as a different method of production was used to produce it. In the 1950s, pharmaceutical products in all different fields were being introduced, from antibiotics, tuberculosis remedies and sulfa drugs through to antihistamines, hormones and psychoneurotic drugs. By contrast, only a few drugs were being produced through derived technology, including kanamycin (Meiji Seika), Alinamin (Takeda Pharmaceutical), Sinomin (Shionogi Seiyaku) and Adona (Tanabe Seiyaku).

After this had continued for some time, a substance patenting system was adopted in 1976. Drug production companies had to make the shift from the existing research and development approach, which was based around developing imitations of known pharmaceutical products using improved production methods, to one of developing new products, thus metamorphosing into so-called drug discovery companies.

In the 1960s, people were becoming highly safety conscious and Japan saw the emergence of a major social issue: pollution and contamination caused by the rapid industrial development during the postwar economic boom. Safety evaluation criteria were far from perfect in Japan in the 1950s and 1960s; in some cases, data taken from overseas was all that could be used for approval. The pharmaceutical industry experienced a succession of major "incidents of harmful side effects" and people started paying close attention to the safety of medical and pharmaceutical products. Eventually, even vitamins and health supplements came under scrutiny, despite having long been consumed in large quantities.

There was a succession of incidents involving serious side effects or even death in large numbers of patients, including the penicillin shock in 1950, the global thalidomide scandal in 1959, subacute myelo-optico-neuropathy (SMON) in 1955-1970, chloroquine retinopathy in 1962-1971 and the ampoule cold medicine incident in 1964 (see Table 2.6). This sequence of harmful side effects incidents made people think about the state of medicines in Japan. The criteria for drug discovery were carefully reexamined as a countermeasure against harmful side effects from drugs, with increasing demand for safety testing.

The *Pharmaceutical Affairs Act* was also amended as a result of these incidents. This Act sets the criteria for the administration of pharmaceutical products, including the licensing of medical and pharmaceutical products. It was amended after the war in 1948 and in 1961; however, the amended version lacked provision for the "efficacy of drugs" and the "safety of drugs". The amendment to the Act in 1979 clearly specified its "aim to ensure quality, efficacy and safety", reflecting the public opinion of the time [3].

The Good Manufacturing Practice (GMP) *Regulations for Manufacturing Control and Quality Control of Drugs* were enacted in 1980, followed by the Good Laboratory Practice (GLP) *Regulations for Conducting Non-Clinical Drug Safety Trials* in 1983. The safety trials required for applying for production approval from the Ministry of Health and Welfare (now Ministry of Health, Labour and Welfare) had to be carried out in accordance with GLP.

However, the side effect safety of internal medicines cannot be verified by restricted animal tests or by administering them to limited numbers of patients. The criteria for safety testing continued to increase for decades afterwards as companies continued to add their own criteria as well as those directed by the government.

Table 2.6 Drug Side Effect Incidents: Major Side-Effect Incidents in Japan

Incident Period	Incident	Cause	Symptoms	Number of victims (confirmed)
1950 - 1955	Penicillin shock	Penicillin	Shock	100 affected
1959 - 1962	Thalidomide scandal	Thalidomide	Birth defects	>309 affected
1964 - 1965	Ampoule cold medicine	Pyrine drugs	Shock	>700 affected; 11 deaths
1955 - 1970	SMON	Quinoform formulation	Neuropathy	11,033 affected
1965 - 1970	Coralgil incident	Coralgil	Systemic lipidosis	>20 deaths; >1,000 affected
1962 - 1971	Chloroquine retinopathy	Chloroquine	Visual impairment and vision loss	>3,000 affected
1970 - 1975	Myositisis	Intramuscular injection	Injection site muscle contraction	10,000 affected
1972 - 1986	Vitamin K injection shock	Additive (HCO-60)	Shock	>14 deaths
1983 - 1987	Hopate encephalopathy	Hopantenic acid	Metabolic encephalopathy	>14 deaths

(* These are the Japanese figures; thalidomide is estimated to have caused birth defects in 6,000 children in West Germany and 500 children in the United Kingdom.)
(Cited from *Nihon Iyakuhin Sangyō Kindai-shi* (Modern History of the Japanese Pharmaceutical Industry) p.130. Originally from Beppu H., Hama R. & Murai N.: Nikkei Science. pp.20-21 (Dec. 1991).)

The strict safety standards pushed up the research and development costs for drug discovery and there were serious risks in the industry. Later, with individual drug discovery companies being unable to obtain sufficient data for assuring human safety, they began to investigate the creation of a collaborative drug evaluation system, compiling safety data from candidate compounds no longer being developed and drugs no longer being sold by other companies.

The drug discovery system involved more than evaluating medicinal efficacy and synthesizing compounds; advances were also made in safety testing to ensure safety and methods for evaluating pharmacokinetics. The framework of modern drug discovery was nearing completion in the Japanese drug discovery industry.

Completion of the Framework of Modern Drug Discovery

Around this time, progress was being made in identifying drug mechanisms and successive new drugs were being produced using new mechanisms. Prompted by this, Japanese companies also started competing to discover new drugs.

As mentioned previously, the top-producing pharmaceutical products in the 1960s were vitamins and antibiotics. The 1970s finally saw the emergence of drugs for the central nervous system, circulatory system and second-generation antibiotics developed by drug companies using their own drug discovery technologies [1, 3]. According to *Takeda Nihyakunen-shi*, the company history of Takeda Pharmaceutical, the company's pharmacology department established specialized divisions for anticancer drugs in 1953 and for central nervous system drugs in 1954, followed by the successive establishment of specialized divisions for analgesic and anti-inflammatory drugs, endocrine system drugs and digestive system drugs from 1961 to 1967. These divisions worked on projects together with the chemical synthesis group and the fermentation group, thus forming the basis of the company's research system [6]. Safety research came a little later, as "the drug safety center was established in 1973, while the pharmacology department was incorporated into the pharmacokinetics department in 1978, forming the research system that is in place today". Other drug discovery companies started similarly organizing their departments around the same time or slightly later.

Sections 3 and 4 of Chapter 2 above have outlined the history of drugs from the latter half of the 19th century through to about the 1960s in terms of "The Origin of Modern Pharmaceutical Drug Discovery and Development" and "From New Medicines to Improved Medicines and Innovative Medicines". A review of the history of drug development reveals that as the technologies of drug discovery systems have grown more complete, drugs discovery has advanced beyond the realm of easy, large-market initiatives with clear disease identification, as shown below. Drugs in the following four areas have emerged through drug discovery in the modern drug discovery system.

(1) Antibiotics, antibacterials and other drugs for diseases caused by external microbes
(2) Drugs that act on the central or peripheral nervous systems (including surgery-related drugs)
(3) Digestive system drugs
(4) Drugs for inflammation or allergies

Following on from these, "lifestyle-related diseases" began gaining attention, with research and development focusing on drugs such as:

(5) Anti-hypertensives, diabetes medications, lipid-lowering drugs, antithrombotic drugs and hyperuricemia drugs

Into the 21st century, genomic drug discovery and advances in biotechnology drove progress in research and development into areas yet to be explored by the academic world and (niche) areas with less demand. These drugs included:

(6) Niche-area drugs, cancer/immunity-related drugs and drugs in areas currently lacking specific medicines

The "Detailed Discussion" in Chapter 4 outlines the research, development and marketing of drugs in Japan from

after the war to the present day. The chapter opens with a discussion on “drugs for lifestyle-related diseases”, namely, anti-hypertensives, diabetes medications, lipid-lowering drugs, antithrombotic drugs and hyperuricemia drugs, and examines how outcomes have been achieved by postwar Japanese drug discovery companies.

Detailed discussions on drugs for other diseases shall be presented at another opportunity.

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2.4.2. Completion of Modern Pharmacology

From 1940 to 1960, rapid developments were made in Western pharmacology in relation to drugs for the central nervous system, anti-inflammatory drugs, cardiovascular drugs and digestive system drugs. The basic technologies and processes for new drug discovery and development were complete.

In 1946, Ulf Svante von Euler and Peter Holtz demonstrated that noradrenaline acts as a neurotransmitter in the mammalian sympathetic nervous system [(1)].

Using the explanation that adrenergic agonists isoproterenol, adrenaline and noradrenaline act differently on different tissues, Raymond Ahlquist hypothesized in 1948 that “the action of noradrenaline takes effect through two different adrenergic receptors, α and β”. Interestingly, his theory was so unique that his first paper on it was not accepted for publication in the authoritative American publication *Journal of Pharmacology and Experimental Therapeutics* (JPET).

Around the same time, Julius Axelrod contributed to the development of neurochemistry with his discovery of the process whereby noradrenaline stored at the ends of the synapses is released from the storage granules by electrical stimulation and then either metabolized by enzymes or reabsorbed by the storage granules and reused. This study laid the groundwork for later research on selective serotonin reuptake inhibitors (SSRIs); Axelrod was awarded the Nobel Prize in Physiology or Medicine in 1970, along with Ulf von Euler (mentioned above) and Bernard Katz, for “their discoveries concerning the humoral transmitters in the nerve terminals and the mechanism for their storage, release and inactivation”.

While “adrenergic receptors” in the smooth muscle and myocardium were known receptors of catecholamines such as adrenaline and noradrenaline, it was not until 1954 that Raymond Ahlquist’s theory of α- and β-type adrenergic receptors was accepted [(3)]. This marked the beginning of a trend in research to split receptors into increasingly more detailed categories, which continues to this day. A-receptors are characterized by causing vasoconstriction, thereby increasing peripheral resistance and raising the blood pressure. B-receptors are further categorized into β_1- and β_2-receptors; β_1-receptor stimulation has been found to cause heart excitation, while β_2-receptor stimulation has been found to cause vasodilation and bronchodilation. Sir James Whyte Black was the first to utilize this theory to synthesize a receptor-specific compound and investigate its medicinal efficacy (mentioned later).

Subsequent research has demonstrated that there are several subtypes of receptors. Initially, receptors were categorized by agonist. Genetic cloning and the ability to synthesize and utilize increasingly superior antagonists have made it possible to identify further receptor subtypes and their molecular characteristics. For example, α-receptors are categorized into α_1- and α_2-receptors; α_1-receptors are further categorized into α_{1A}, α_{1B} and α_{1D}, while α_2-receptors are categorized into α_{2A}, α_{2B} and α_{2C} [(3, 4)].

Currently, the action mechanisms of over 50% of drugs can be explained as (i) mechanisms that either antagonize or stimulate receptors or (ii) mechanisms that inhibit enzymes (see Chapter 3 for details).

The drug discovery process for creating receptor antagonists was completed soon after the war. Examples of useful medications include β-blockers, H_2 antagonists and central nervous system drugs, while anti-inflammatory drugs are a good example of enzyme inhibitors.

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2.4.3. Discovery of β-Blockers and H_2 Antagonists

James Black of Scotland was a professor at the University of Glasgow's School of Veterinary Medicine. Hypothesizing that antianginal drugs could eliminate oxygen deficiency in the coronary vessels by controlling the action of the heart, he started working for British company ICI, where he engaged in collaborative research with organic synthetic chemists to develop the β-receptor blocker propranolol in 1965. At the time, even the existence of β-receptors was only a hypothesis; however, this drug became widely used for angina and other conditions. The drug was developed using the structure-activity relationship; Black obtained propranolol by screening using techniques from the modern drug discovery process.

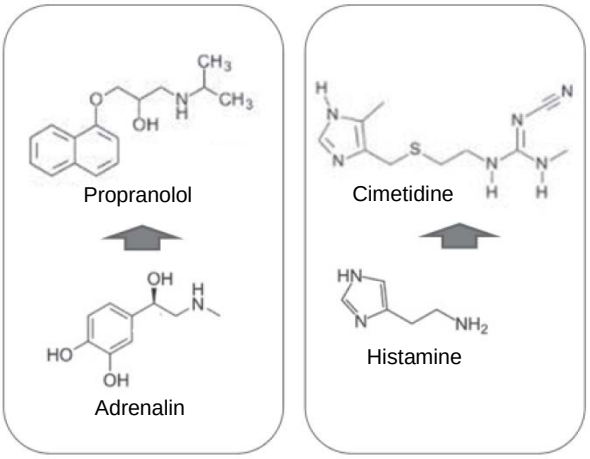
Propranolol was the world's first β-blocker [(1)]. The idea was that since noradrenaline released by the sympathetic nervous system causes constriction by acting on the β-receptors in the smooth muscle and myocardium, blocking these receptors would regulate the heart's pulse and inhibit the constriction of the coronary arteries, causing them to relax. Although the use of propranolol initially proved very effective against angina and other conditions, it was not yet known at the time that different types of tissue have different types of β-receptors. Since propranolol was a non-selective β-blocker, it also blocked the β_2-receptors in the bronchial smooth muscle, causing side effects such as asthma.

Black then turned the focus of his research to studying H_2 receptors. Although it was known at the time that histamines promoted the secretion of gastric acid, histamine antagonists had never been able to inhibit this secretion. (Early histamine antagonists suppressed allergies by acting antagonistically on H_1-type histamine receptors, but did not act antagonistically on the H_2-type receptors involved in gastric acid secretion.)

Black hypothesized that there were two types of histamine receptors, H_1 and H_2, and set about developing the world's first H_2 receptor antagonist. By screening H_2 receptors, he obtained N^{α}-guanylhistamine, a partial histamine receptor antagonist. Using this, he estimated the structure of H_2 receptors and successfully developed burimamide, the first gastric acid inhibitor. From burimamide, he went on to develop the more effective metiamide, followed by the stronger, more effective and less toxic oral anti-ulcer drug cimetidine (launched in the United Kingdom in 1976 by SKF). Black achieved these research results at Smith, Kline & French Laboratories (SKF; now GlaxoSmithKline) [(2)]. For these achievements, Black was awarded the Nobel Prize in Physiology or Medicine in 1988 (see Figure 2.8 for details on Blacks achievements).

In Japan, many people had suffered from gastric ulcers and duodenal ulcers from the Meiji Period onwards, Sōseki Natsume among them; in many cases they proved fatal. The use of H_2 receptor antagonist cimetidine (developed in Japan by SKF and Fujisawa Pharmaceutical) from 1982 allowed people to recover from these ulcers without surgery, which was the usual method of treatment at the time.

Later, further advances were made in identifying the gastric acid secretion mechanism and the target shifted from the H_2 receptors to the proton pump [(Note 2)], with ongoing competition over development. In Japan, the introduction of H_2 blocker famotidine by Yamanouchi Pharmaceutical and second-generation proton pump blocker lansoprazole by Takeda Pharmaceutical brought the medicinal treatment of gastric ulcers and duodenal ulcers almost to completion, to the alleviation of many patients. These drugs made a significant contribution to identifying the causes of gastric ulcers and duodenal ulcers.



Black (UK) derived the β-blocker propranolol from the chemical structure of adrenaline and then produced the antagonist cimetidine from histamine. Propranolol provides relief to sufferers of angina, arrhythmia and hypertension; cimetidine was a ground-breaking new drug that meant surgery was no longer required to treat ulcers.
Black was awarded the Nobel Prize in Physiology or Medicine.

Fig. 2.8 β-Blockers and H_2 Antagonists.

Note 1: Histamines, acetylcholine and gastrin act on their respective receptors on the gastric wall; stimuli are transmitted by the proton pump and gastric acid is secreted. Blocking the proton pump inhibits the secretion of gastric acid regardless of the agonist.

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2.4.4. Anti-Inflammatories (from Aspirin to Selective Cyclooxygenase II Inhibitors)

Anti-inflammatories started with quinine and salicylic acid from crude drugs. Chinchona, a crude drug from South America, is believed to have been brought to Europe in 1630. Chinchona became known as a specific medicine against malaria, for which there was no medicine at all at the time. It was also found to have a strong analgesic-antipyretic effect.

Quinine, the main component in chinchona, was extracted in 1820 by French chemists Pierre-Joseph Pelletier and Joseph Bienaimé Caventou; the French went wild with enthusiasm at their discovery and the country gave these young scientists the unprecedented honor of erecting a commemorative monument to them. The French Académie des Sciences is said to have received donations from all over the world when it erected the statue of the two men on the boulevard St.-Michel in Paris. The statue was destroyed by the Germans in the Second World War, but a replacement was erected in the same location after the war [(9)].

Quinine was in such demand for its powerful antimalarial effect that research began on large-scale synthesis of it as soon as it was extracted. During the course of its synthesis, pyrazolone derivatives began to be synthesized. These pyrazolone derivatives were found to have a powerful analgesic-antipyretic effect; antipyrine (Ludwich Knorr, 1883) and sulpyrine were synthesized from these.

The analgesic-antipyretic effect of these pyrazolone derivatives is now believed to work through the central nervous system. These drugs were found to cause an eruption known as antipyrine exanthema on rare occasions; in severe cases, they were also found to cause leucopenia. Nevertheless, the pyrazolone structure was later used as the basic structure for anti-inflammatory drugs. In 1949, phenylbutazone was released on the market as an anti-inflammatory with fewer side effects. Phenylbutazone was characterized for having an analgesic-antipyretic effect as well as an anti-inflammatory effect.

In 1897, acetylsalicylic acid (aspirin) was synthesized from salicylic acid. It had powerful, clinically-proven analgesic, antipyretic and anti-inflammatory effects. Its mechanism of action was further clarified in the 1960s; it was found to demonstrate its anti-inflammatory effect by inhibiting cyclooxygenase (COX), the first enzyme in the arachidonate cascade, thereby suppressing the production of various inflammation-related prostaglandins (see Figure 2.9) [(1)].

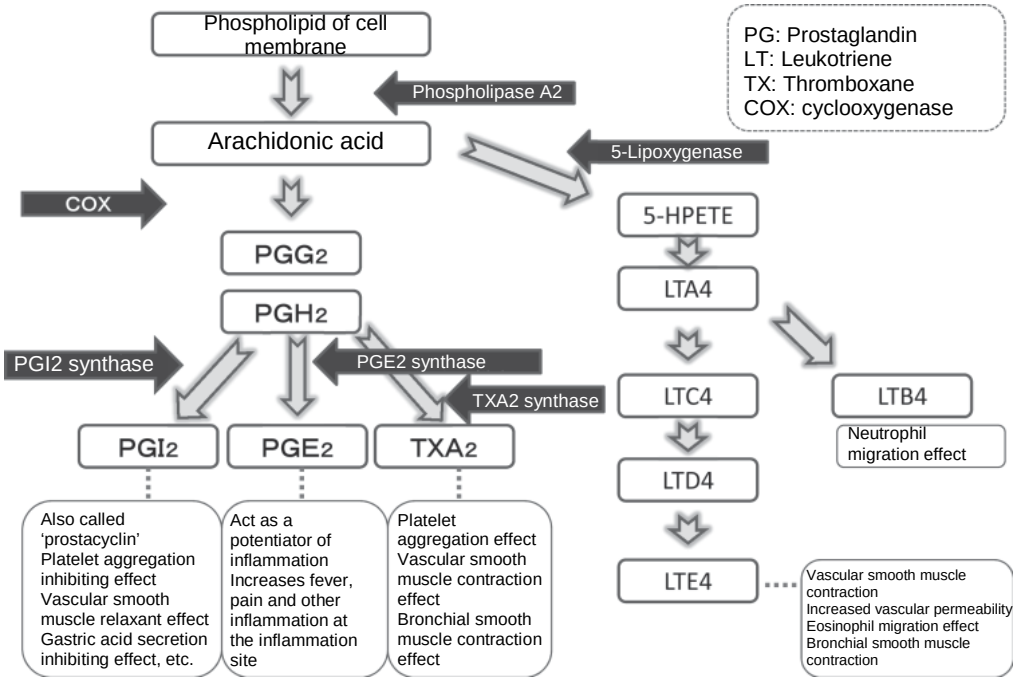

Fig. 2.9 The Arachidonate Cascade.

John Vane was awarded the Nobel Prize in Physiology or Medicine for this research. Aspirin was later found to have a platelet aggregation inhibiting effect and a uricosuric effect. As its field of application has expanded, it has become regarded as one of the drugs that have made the greatest contribution to medical treatment in the history of humanity.

Although antipyrine and sulpyrine similarly end in "pirin/pyrine", their analgesic-antipyretic effects act on the central nervous system and they are not classified as nonsteroidal anti-inflammatory drugs (NSAIDs), which have a COX-inhibiting anti-inflammatory effect.

Indomethacin was researched and developed by Merck and gained some attention as a new pharmaceutical substance that demonstrated an extraordinarily powerful anti-inflammatory effect in animal models. Its powerful anti-inflammatory and analgesic effects were confirmed clinically and it was launched in the United States in 1963. Fierce development competition ensued, with various other phenylpropionic acid and phenylacetic acid derivatives synthesized using this structure as the lead compound.

Since these differed in structure from corticosteroids, which have a powerful anti-inflammatory effect, they were labelled as acidic anti-inflammatories in the nonsteroidal anti-inflammatory drug (NSAID) category [(2)]. While prolonged use of steroids causes a number of side effects, including gastrointestinal ulcers, suppression of immunity, causing susceptibility to infection, and greater susceptibility to osteoporosis, diabetes and hypertension, these side effects are unlikely from nonsteroidal drugs.

Hisao Yamamoto of Sumitomo Chemical investigation methods for synthesizing indomethacin and came up with a new method of synthesizing it by directly closing acylphenylhydrazine into an indole ring, which was previously said to be impossible. This method made it possible to supply a higher-yield, higher-purity product; Sumitomo Chemical licensed the technology back to Merck. In 1967, Sumitomo Chemical started marketing it as an inflammation suppressant for rheumatism [(3)].

The age of importing anti-inflammatories continued in Japan. After indomethacin in 1996 came mefenamic acid, followed by flufenac and ibufenac in 1967, ibuprofen in 1971, diclofenac in 1974, ketoprofen, flurbiprofen and naproxen in 1978 and piroxicam in 1982. On the domestically-produced NSAID market, Sankyo launched loxoprofen in 1986 [(4)].

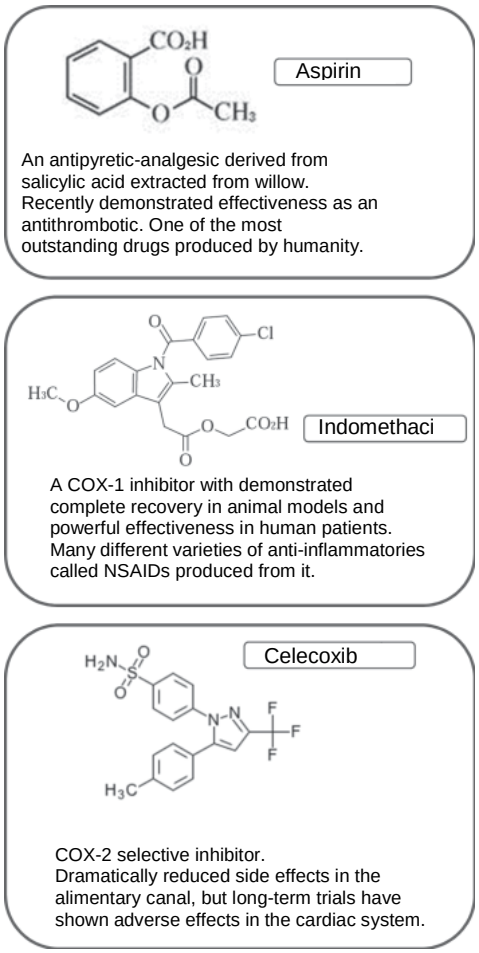

Fig. 2.10 Typical NSAIDs.

However, it was eventually found that acidic anti-inflammatories damage the gastric mucosa and cause other side effects in proportion to their medicinal efficacy. With the Japanese tending to be more susceptible to gastrointestinal disorders, Japanese drug discovery companies embarked on developing anti-inflammatories with different chemical structures from the phenylacetic/phenylpropionic acid-based acidic anti-inflammatories. This resulted in the creation of successive domestically-produced non-acidic NSAIDs.

Daiichi Pharmaceutical launched epirizole in 1970, while Takeda Pharmaceutical launched difenamizole in 1973, Fujisawa launched tiaramide in 1974, Shionogi launched perisoxal in 1978 and Yoshitomi Pharmaceutical launched pranoprofen in 1981. These were known as basic anti-inflammatories; while they had milder gastrointestinal side effects, they were not as powerful as their acidic counterparts in terms of the main anti-inflammatory effect [(3, 4, 6)].

Acidic anti-inflammatories were essentially used to suppress pain at sites of inflammation stemming from rheumatism or osteoarthritis and did not treat the root cause of the disease. Proper anti-rheumatic drugs did not appear until the emergence of low-molecular-weight drugs and

antibody drugs that acted on the immune system. However, dosages were kept low in Japan to reduce the side effects of acidic anti-inflammatories; sales of these drugs increased as they started being used to relieve colds and muscular pain.

When tissue is severely damage, blood vessels at the area of inflammation expand and the blood flow increases, leaking blood cells from the blood vessels into the tissue. Leukocytes, macrophages and mastocytes infiltrate the inflamed tissue and the inflammation progresses. Damaged and inflammatory cells in the inflamed tissue release algesic substances with vascular hyperpermeability effects, such as bradykinin, thromboxane, leukotriene, serotonin and histamine, as well as algesic potentiators, such as prostaglandin. Bradykinin and other algesic substances characteristically produce pain by directly stimulating the receptors, while algesic potentiators, such as prostaglandin E (PGE_2) and prostacyclin (PGI_2), characteristically increase receptor reactivity. Prostaglandins and leukotrienes (generically called prostanoids) are produced from the arachidonate cascade and play an important role in inflammation.

Phospholipase A2 is activated by the cells in the inflamed tissue. The phospholipids in the cell membrane release arachidonic acid, activating cyclooxygenase (Note 2) II (inducible enzyme COX-2), which converts the arachidonic acid into prostaglandin H2 via prostaglandin G2. The production of prostaglandins and thromboxanes (both are termed as prostanoids), which are inflammation-related substances, aggravates the inflammation; however, suppressing the COX-2 also suppresses the production of inflammatory factors downstream (see Figure 2.9).

Meanwhile, constitutive enzyme COX-1 is expressed by the stomach, platelets, kidneys and almost all other tissues; in the gastric wall, it produces prostacyclin, which protects the gastric wall. It also works to maintain blood pressure and circulation in the kidneys.

For example, COX-1 is constantly expressed by the platelets; it has been reported that the platelet-inhibiting effect of aspirin is achieved by inhibiting COX-1. Since aspirin has a platelet-aggregation-inhibiting effect at small doses, it has come to be widely used around the world to prevent the recurrence of cerebral infarction (see Section 4.4, Medications for Thrombosis and Clotting Disorders).

The use of aspirin and indomethacin, which have little enzyme selectivity, has occasionally resulted in serious gastric ulcers as a side effect. In particular, it has caused gastric perforation when used by sufferers of severe rheumatism. Side effects of anti-inflammatory drugs were the third leading cause of death among rheumatism patients in the United States in 1997 [5:120-121].

Studies began on COX-2 specific inhibitors in order to improve these side effects. In 1992, Pfizer marketed the first coxib-type (Note 2) selective COX-2 inhibitor celecoxib (produced by Searle). This reduced the side effects in the gastrointestinal system, as expected, and triggered a global development boom in COX-2 inhibitors. Celecoxib was launched on the market in Japan in 2007 by Yamanouchi Pharmaceutical and Pfizer as an anti-inflammatory and analgesic for rheumatoid arthritis and osteoarthritis.

However, none of the inhibitors were perfectly selective between COX-1 and COX-2; the actual efficacy and side effects were determined by the balance between COX-1 and COX-2 and differed not only due to enzyme selectivity, but also due to tissue affinity. Thus, development competition continued over various anti-inflammatory drugs.

It was later reported that COX-2 is activated in cancer tissue [6], with other theories emerging to say that it promotes the activation of cancer. With Merck guaranteeing its anti-inflammatory effect and safety, rofecoxib (Merck: US brand name Vioxx®; unapproved in Japan) was widely adopted; a clinical study began on its use for the secondary prevention of bowel cancer.

However, the study revealed an unforeseen issue: increased risk of thrombosis or cardiovascular complications such as heart attacks. Rofecoxib was voluntarily recalled in 2004. Many side effects victims and their families took Merck to court; the company eventually ended up paying out $4.85 billion (Note 3).

While it is not yet known whether the increased risk of cardiovascular events is common to all coxib drugs, reported research results suggest a low correlation between celecoxib (Pfizer) and cardiovascular complications [7].

COX-2 inhibitors suppress the production of prostanoids and suppress the production of PGI_2 in vascular endothelial cells. However, they do not suppress the production of thromboxane A2, which promotes platelet aggregation; some theories consider this to be a cause of cardiovascular disorders. Figure 2.10 shows the chemical structures of typical anti-inflammatories aspirin, indomethacin and celecoxib.

Note 1: Cyclooxygenase (COX) is an enzyme involved in the start of the arachidonate cascade. Arachidonic acid is oxidized by cyclooxygenase into PGG and PGH, from which various prostanoids are biosynthesized. COX exists in two forms, I and II.

Note 2: A classification name for new drugs with three-dimensional structures designed to achieve COX-2 selectivity.

Note 3: The findings of the clinical study were published in 2005 after the study was terminated. The trial targeted people within 12 weeks of having had surgery for colorectal cancer. Subjects were divided into a rofecoxib group and a placebo group, 1300 in each, and administered their respective treatments for three weeks. After the trial, 1074 subjects and 1092 subjects from the two groups respectively participated in the study. The combined number of subjects who either died or suffered non-fatal cardiovascular events was 59 in the rofecoxib group and 34 in the placebo group in 32 and 20 events respectively occurring after the study [8].

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2.4.5. Central Nervous System Agonists

Illnesses treated with central/peripheral nerve agonists include schizophrenia, anxiety, nervous disorders, depression and other mental disorders, as well as dementia, epilepsy, Parkinson's disease and other illnesses caused by the degeneration of nerve cells. The drugs used to treat schizophrenia, depression, nervous disorders and anxiety are called psychotropic drugs. There is a wide range of nerve agonists in addition to these, including general and local anesthetics used for surgery and analgesics. All around the world since ancient times, many of the crude drugs used alongside witchcraft had strong effects on the mind and nerves; modern research on the alkaloids extracted from these crude drugs has contributed to advances in neuropharmacology.

Drugs for Schizophrenia

The belief that people suffering from mental illnesses are possessed by evil spirits has persisted throughout the centuries since ancient times. To cure the disease, it was believed necessary to drive out the evil spirits; extreme treatment methods existed through to early modern times and included drilling into the skull or locking patients in a dungeon.

Trialing various different drugs began in the 20th century and included alcohol, chloral hydrate, opium and alkaloids. It was found that administering these to patients in high concentrations could eliminate patient anxiety and induce calm and drowsiness, but could not alleviate hallucinations or other mental disorders. Around this time, radical treatment therapies such as lobotomy (partial surgical removal of the frontal lobe) and electroconvulsive therapy were also being used.

Chlorpromazine was the first antipsychotic drug to be used for diseases affecting the central nervous system. While researching anesthetics for surgery patients, French surgeon Henri Laborit found that antihistamines were effective for alleviating patient fear and anxiety. Based on these findings, the phenothiazine derivative chlorpromazine was discovered at the Rhône-Poulenc laboratory in 1952 by screening peripheral compounds [1]. In 1952, French psychiatrists Jean Delay and Pierre Deniker administered chlorpromazine to schizophrenia patients and found that it had a therapeutic effect of suppressing patient stimulation and reducing hallucinations. The drug was introduced to Japan in 1955.

This new drug demonstrated dramatic effects on schizophrenia patients, heralding the arrival of a new era in mental illness treatment. Stories began to emerge of the drug being administered to patients who had previously stood motionless week after week in the same place in the hospital, or patients who had previously been so violent they had to be restrained, but now no longer needed supervision [2]. It was truly epoch-making.

It consequently became possible to control mental illnesses with medication, thus putting a stop to the previous treatments of electroconvulsive therapy, insulin stimulation and lobotomy. Most patients no longer needed to be kept in confinement, thus reducing the hospitalization rate of schizophrenia patients worldwide.

Unlike previous hypnotic sedatives, this drug was a major tranquilizer; many other phenothiazine-based antipsychotic drugs were later developed using chlorpromazine as the lead compound. In 1958, a compound synthesized in the process of developing an antidiarrheal drug was found to have a strong antipsychotic effect; the compound was haloperidol. Haloperidol has stronger hallucination and delusion suppressing effect than chlorpromazine and is also fast-working; it has consequently been used as a lead compound in the development of butyrophenone-based antipsychotic drugs.

In 1963, pharmacological research revealed that these drugs act on the dopaminergic neurons to block the dopamine receptors (Note 1). Blocking the dopamine D_2 receptors in the nigrostriatal pathway in the midbrain is believed to be the most significant effect in schizophrenia treatment. Drugs were screened in vitro and in vivo to determine the strength of the D_2 receptor blocking; a good correlation was found between the dopamine receptor binding capacity and the clinical dosage.

However, while these antipsychotic drugs worked well for suppressing positive symptoms, strong side effects (extrapyramidal symptoms) similar to the symptoms of Parkinson's disease occurred in proportion to the receptor binding capacity, including shaking (tremors) or rigidity of the hands and feet, immobilization or difficulty in movement and feelings of restlessness.

Also, although the drugs were effective against hallucinations, delusions and other positive symptoms (the manifestation of phenomena that are not normally present), they had hardly any effect against social withdrawal, loss of motivation and other negative symptoms (the loss of phenomena that are normally present when healthy).

These first-generation drugs are known as typical antipsychotics and were successively produced with dopamine antagonism as the indicator. However, it has not been possible to separate the antipsychotic effect from the extrapyramidal side effects.

Research findings showed that not only is chlorpromazine a selective dopamine antagonist, it also has a wide range of side effects, including blocking histamine receptors, serotonin receptors, muscarinic receptors and adrenergic α_1 receptors. It was also found that noradrenaline is a central nervous system transmitter involved in anxiety and restlessness and that it is helpful to block the adrenergic α receptors. The idea came to the fore that treatment drugs for schizophrenia should regulate the balance of various neurotransmitters rather than selectively antagonizing the D_2 receptors.

Clozapine, marketed overseas in 1969, demonstrated a D_2 antagonist effect as well as 5-HT_2 and α_1 blocking effects. It also acted on the histamine H_1 and muscarinic receptors. Researchers found that the main action could be separated from the side effects; this drug served as the basic model for developing next-generation atypical antipsychotic drugs.

It was eventually found that dopamine has presynaptic (Note 2) serotonin receptors that inhibit the release of dopamine. This made it possible to develop drugs that act on both the dopamine receptors and the serotonin receptors.

Risperidone was introduced to Japan in 1996 as a serotonin dopamine antagonist (SDA), effective against both negative and positive symptoms. Perospirone was also introduced to Japan a little later than risperidone. Unlike the conventional antipsychotic drugs, these affected the neurotransmitters, which made them effective against negative symptoms. The extrapyramidal symptoms are surmised to be suppressed by the serotonin inhibiting action.

This was followed by the development of quetiapine and olanzapine. Known as multi-acting receptor targeted antipsychotics (MARTA), these acted on various different neural receptors. These became known as second-generation (atypical) antipsychotics, as opposed to the existing (typical) antipsychotics [3, 4].

Aripiprazole, developed by Ostuka Pharmaceutical in 2006, is a classic example of a domestically-produced, second-generation drug. Since it has a partial dopamine D_2 agonist effect, it has the pharmacological properties of acting as a dopamine D_2 antagonist when the dopaminergic nerves are overactive and as a dopamine D_2 agonist when dopaminergic neurotransmission is low; it is known as a dopamine system stabilizer (DSS). Aripiprazole also has a partial agonist effect on serotonin receptor 5-HT_{1A} and an antagonistic effect on serotonin receptor 5-HT_{2A}. Due to these pharmacological properties, aripiprazole has been reported to be effective for schizophrenia with few extrapyramidal symptoms and no increase in prolactin levels [5].

In 2008, Dainippon Sumitomo Pharma developed blonanserin, a second-generation antipsychotic with a new structure that has a dopamine D_2 receptor blocking effect and a serotonin 5-HT_2 receptor blocking effect with few extrapyramidal symptoms [6].

Although ongoing research and development for new drugs with new mechanisms of action is still ongoing, the root cause of schizophrenia still remains unsolved. Figure 2.11 shows the chemical structures of some representative examples of typical and atypical antipsychotics.

In "Psychosis: Discovery of the Antipsychotic Receptor", Neil Seeman states the following [7:172-178].

"In May 1997 James Watson came to Toronto to give the Andrzej Jus lecture on Neuroscience and Ethics…

At lunch that day Watson said that there were no new ideas in schizophrenia research and that we would all have to wait until the human genome was sequenced…. The human genome has now been sequenced and known for about ten years, but there are no new dramatic breakthroughs in this aspect of DNA research on schizophrenia. In fact, since 1999, there are approximately 50 to 100 genes announced each year as being linked or associated with schizophrenia, but none have stood the test of time in being readily replicated beyond any doubt, and none have stood the test of time as a biomarker for schizophrenia… At lunch that day, it became clear that Watson's intense interest in schizophrenia was presumably related to the fact that his son had schizophrenia. And this brings to mind that the son of Dr. Michael Smith also had schizophrenia. Smith received the Nobel Prize for his invention of "site-directed mutagenesis of DNA". And with the son of Albert Einstein also having schizophrenia, could it be that there is an inherited pattern in the nervous system that may lead to a major creative discovery or to a major thinking disorder? Consider the mathematician John Nash who developed schizophrenia after he did the work that led to his Nobel award."

Antidepressants

Monoaminergic nerves [Note 3] are known to be involved in stimulating overall brain function (see Figure 2.12).

While reserpine was historically used as an anti-hypertensive, American scientist Nathan Kline used it to treat schizophrenia in 1954. However, it was found that the administration of reserpine caused depression-like symptoms. It was later discovered that reserpine has a synaptic vesicle draining effect on dopamine, noradrenaline and other neurotransmitters; it was hypothesized that the lowering of the catecholamine levels in the brain was the mechanism that causes depression.

It was noted that patients administered with iproniazid, a tuberculosis drug developed in 1951, would become lively. Investigation into the cause of this revealed that iproniazid has a powerful inhibitory effect on monoamine oxidase (MAO: an enzyme that intraneurally oxidizes and degrades monoamines); it was presumed that inhibiting the degradation of catecholamines induces a state of excitement.

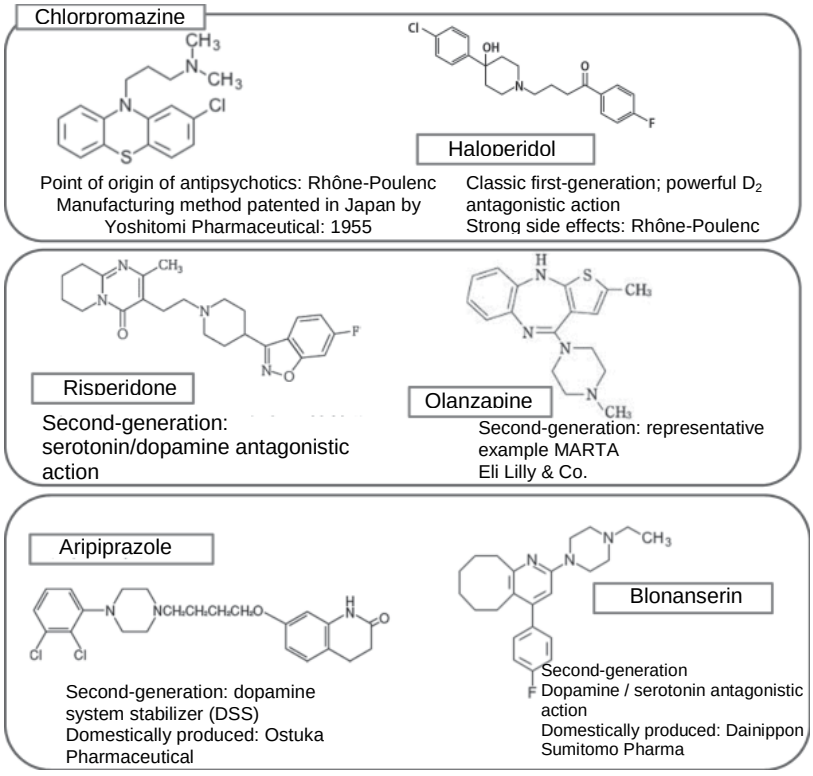

Fig. 2.11 Chemical Structural Formulas of Representative Examples of Antipsychotic Drugs.

In 1958, a clinical trial of the antipsychotic imipramine revealed that it had an antidepressant effect. Imipramine was conjectured to demonstrate this effect by suppressing the reuptake of monoamine neurotransmitters into the nerves, thereby increasing the amount of monoamines in the synaptic cleft; it was theorized that lowering the monoamine levels would produce depression. Imipramine was introduced to Japan in 1959.

These were first-generation (conventional) antidepressants. Since imipramine and amitriptyline have tricyclic structures, they became known as tricyclic antidepressants. In 1956, Joseph Schildkraut of the US National Institute of Mental Health proposed the hypothesis that depression reduces the noradrenaline levels in the brain and that antidepressants restore those noradrenaline levels. The improved tricyclic antidepressants and tetracyclic antidepressants that later emerged are sometimes referred to as second-generation antidepressants [(4)].

However, it was later suggested that serotonin reflects a more pathological condition in the brain of people suffering from depression; research began on selective serotonin reuptake inhibitors (SSRIs). These are also called next-generation antidepressants.

Since the first-generation antidepressants were not specific monoamine reuptake inhibitors, they had various side effects, including anticholinergic effects and adrenaline α_1 blocking effects. The new-generation drugs, however, were specific to the serotonergic nerves; the mechanism of action and the side effects were vastly different from those of the conventional drugs [(2)].

In our complex and advanced modern society, there has been an increase in the number of people showing signs of depression. Successive (i) selective serotonin reuptake inhibitors (SSRIs) and (ii) serotonin noradrenaline reuptake inhibitors (SNRIs) were developed in the West; many of them were introduced to Japan. The former (i) includes fluvoxamine (launched in 1999, developed by Meiji Seika Pharma), paroxetine (launched in 2000, developed by GlaxoSmithKline; Japan's first depression and panic disorder drug) and sertraline (launched in 2006, developed by Pfizer), while the latter (ii) includes milnacipran (launched in 2008, developed by Asahi Kasei Pharma and produced by Pierre Fabre Medicament; Japan's first SNRI) and duloxetine (launched in 2010, Eli Lilly Japan). All of these were introduced products marketed in Japan. Fluoxetine by Eli Lilly & Co. is widely used around the world, but has not yet been approved in Japan.

The reason that Japan has not produced any antidepressants can be surmised to be due to the low demand for these drugs, as the culture in Japan has always been to hide depression and other mental illnesses.

Nevertheless, many aspects of the mechanisms that cause depression are yet unknown; future progress can be expected in new drug development and the identification of mechanisms.

Although monoamine oxidase inhibitors (MAOIs) have also been effective, they have fallen out of use as improved monoamine reuptake inhibitors have emerged, as inhibiting monoamine oxidase also affects other tissues and organs.

More recently, antidepressants have started being administered not only for depression, but also for neurosis and schizophrenia. Figure 2.12 shows the chemical structures of some representative examples of first- and second-generation antidepressants.

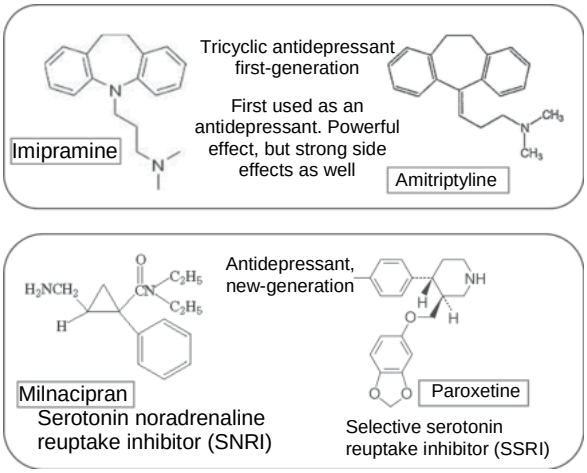

Fig. 2.12 Representative Examples of Antidepressants.

Antiparkinsonians

Parkinson's disease is a neurodegenerative disease with cardinal signs of muscular rigidity, tremors and immobilization. In 1919, it was confirmed that neurodegeneration and neuronal loss occur in the substantia nigra within the brain of people suffering from Parkinson's disease. The substantia nigra produces dopamine, the neurotransmitter for the dopaminergic neurons and it is thought that degeneration in this region causes degradation in dopaminergic nerve function. With the pathogenic mechanism identified, theoretical discussion began on a drug remedy.

Activation of the dopaminergic neurons was essential. Means proposed to achieve this included: (i) increasing the dopamine levels; (ii) inhibiting the metabolic enzymes (catechol-O-methyl transferase [COMT] or monoamine oxidase [MAO]) involved in dopamine degradation; and (iii) reducing the acetylcholine levels in an effort to raise the activity of the dopaminergic neurons through negative feedback.

DOPA (L-3,4-dihydroxyphenylalanine) acts as a neurotransmitter in the brain as it is decarbonized by DOPA-decarboxylase to become dopamine. However, if this is administered externally, the dopamine cannot pass through the blood-brain barrier (Note 4), although the DOPA can. Accordingly, a method was devised whereby DOPA administered orally migrates to the brain and is converted to dopamine by the action of the DOPA- decarboxylase in the brain, thereby supplementing the dopamine deficiency (Note 5). A dopamine formulation (DOPA®) applied clinically in 1967 by George Cotzias was long used as the main drug treatment for Parkinson's disease. By 1980, it was being used in a compound formulation combined with a dopamine decarboxylase inhibitor.

Later, dopaminergic agonist ergot alkaloids were used; the ergot alkaloid bromocriptine was marketed by Sandoz Pharmaceuticals in 1979. Ergot alkaloid derivative pergolide mesylate was released by Pfizer in 1994. Non-ergot derivatives were also developed, having different structures from ergot alkaloids. Doktor Karl Thomae launched talipexole hydrochloride in 1996, while Nippon Boehringer launched pramipexole in 2004. In 2006, GlaxoSmithKline released ropinirole in 2006.

CMOT and MAO inhibitors have also been used. In recent years, these have shown to be effective in extending the life expectancy of patients with Parkinson's disease to be on par with the average lifespan.

However, Japanese companies have made few developments in this field; most of these drugs are imported from overseas.

Central stimulants

In 1885, Nagayoshi Nagai successfully extracted ephedrine from ephedra herb; amphetamine was synthesized from ephedrine in Germany in 1887; Nagayoshi Nagai and Kinnosuke Miura synthesized methamphetamine in 1893.

Amphetamine hit the American market in 1933 as the inhaled asthma drug Benzedrine®. Many people used it as a cough medicine, although it started being abused when people began to discover the amphetamine stimulant effect. Some companies marketed it as a diet drug, as it also had an appetite-suppressing effect. With news of such abuse, the US Food and Drug Administration (FDA) started restricting the prescription of it in 1959.

Methamphetamine, which has far stronger effects than amphetamine, was launched on the German market in 1938, but its harmful effects were soon realized and it was designated as a dangerous drug by the German authorities in 1941.

In Japan, Takeda Pharmaceutical marketed an amphetamine formulation under the brand name Zedrin® in 1941, while Dainippon Pharmaceutical marketed a methamphetamine formulation under the brand name Philopon®. During the Second World War, the Japanese military distributed these pills to munitions factory workers to increase productivity; they were also distributed to soldiers and crew members on night fighters. After the war, these drugs were readily obtainable at drugstores and on the black market; the social issue of increasing drug abuse led to the enactment of the *Stimulants Control Act* in 1951 (8).

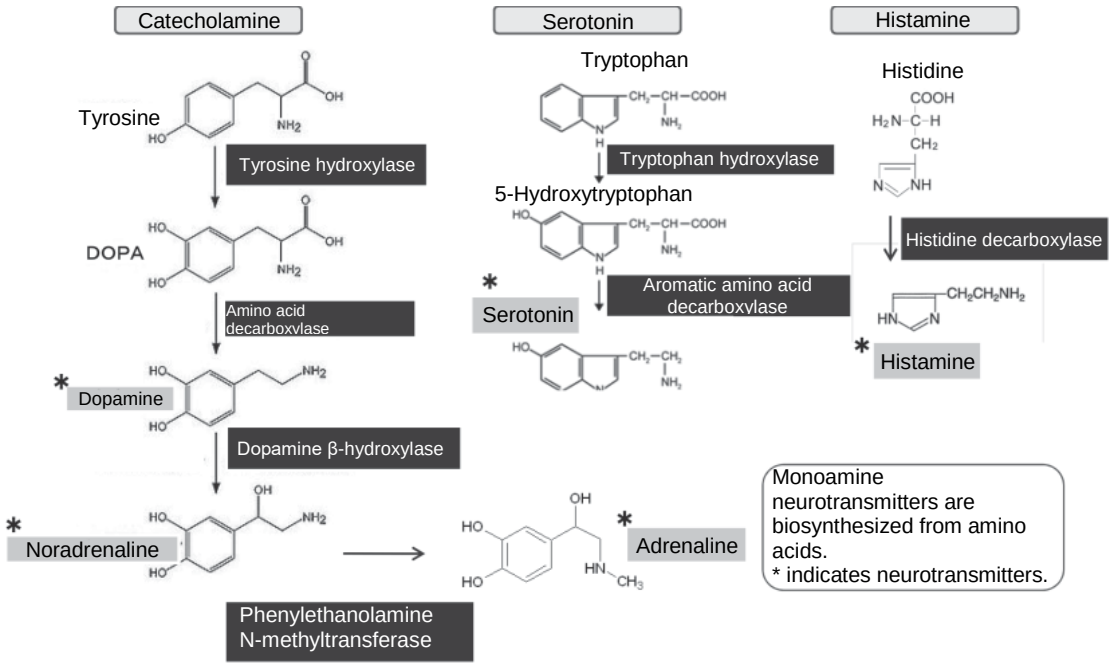

Fig. 2.13 Monoamine Neurotransmitter Biosynthesis Pathways.

Stimulants have been used as reagents in pharmacological experiments to identify pathogenic mechanisms of various mental disorders. Research on mechanisms of action has confirmed that amphetamine forces the release of neurotransmitters noradrenaline and adrenaline from the synaptic vesicles.

Refer to Figure 2.13 for a summary of the biosynthesis of central nervous system agonists (in vivo agonists).

The above has provided a simplified overview of the postwar research and development of some central nervous system drugs; however, there are many more besides these. Further details on central nervous systems drug await another opportunity.

Note 1: Dopamine is a neurotransmitter with a vital role to play in the central nervous system; it is not simply a precursor to noradrenaline. Arvid Carlsson, Eric Kandel and Paul Greengard were jointly awarded the Nobel Prize in Physiology or Medicine in 2000 for "their discoveries concerning signal transduction in the nervous system".

Note 2: A synapse is a point of connection between neurons and other cell types (nerve cells and muscle cells) and contains a gap of around 20nm. Cells on the transmitting side of the electrical stimulus are presynaptic; cells on the receiving side are postsynaptic. More details are given in Figures 2.4 and 2.6.

Note 3: Monoamines include serotonin, noradrenaline, adrenaline, histamine and dopamine. Of these, noradrenaline, adrenaline and dopamine contain catechol groups and are referred to as catecholamines.

Note 4: The blood-brain barrier is a barrier between the brain and the blood. The gap between the endothelial cells in the blood is extremely narrow in the brain and only a few substances, such as glucose, are able to pass through. The blood vessels are also surrounded by glial cells; this also restricts substances from passing through.

Note 5: When DOPA is administered for Parkinson's disease, patients are advised to avoid excessive consumption of foods rich in vitamin B6. This is because vitamin B6 is a coenzyme of DOPA decarboxylase; high amounts of vitamin B6 will activate the enzyme. When the DOPA metabolizes into dopamine in the blood, it will not be able to pass through the blood-brain barrier, thereby losing the effectiveness.

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2.4.6. Advances in Bioscience and Drug Discovery

While the term biotechnology is a combination of the words "biology" and "technology", this is not exactly how it is defined. Accordingly, in this paper, the term "biotechnology" is used to refer to technology that chiefly comprises gene recombination technology and encompasses cell fusion technology and cell/tissue culturing technology, while the term "biopharmaceuticals" is used to refer to polymeric drugs produced using this technology.

The origins of biotechnology in this sense can be traced back to 1953, with the discovery of the complementary double-stranded, double helix structure DNA model by American biologist James Dewey Watson and British physicist Francis Harry Compton Crick. This double helix is said to have been the greatest discovery of the century for unravelling the secrets of life. Watson and Crick were awarded the Nobel Prize in Physiology or Medicine in 1962.

In 1972, Paul Berg (awarded the Nobel Prize in Chemistry in 1980) of Stanford University succeeded in splicing monkey virus DNA (SV40) with *Escherichia coli* DNA and multiplying the result in the *E. coli* culture [(1)]. In 1973, Stanley Norman Cohen of Stanford University and Herbert Wayne Boyer of the University of California, San Francisco created a hybrid plasmid with eukaryotic cell and prokaryotic cell DNA, using restriction enzymes, which cleave DNA, and DNA ligase, which joins DNA strands together, and confirmed that this produced RNA in *E. coli* [(2, 3)]. These technologies were recombinant DNA technologies that enabled humanity to cross the "barrier between species". However, for some reason, Boyer and Cohen were not awarded the Nobel Prize. Another Cohen did receive the Nobel Prize in Physiology or Medicine: Seymour Stanley Cohen in 1986, for discoveries relating to nerve growth factor.

Eventually, the basic technology was completed to be able to produce large volumes of special proteins from the gene produced by inserting other genes into *E. coli* plasmids and multiplying the recombined DNA with the *E. coli*. The first successful case of genetic engineering in Japan was in 1977 [(4)]. The technology was actively incorporated into agriculture and animal husbandry; by 1986, tobacco was being produced that was resistant to disease and pests. By 1994, genetically modified tomatoes were being marketed in the United States.

In the pharmaceuticals industry, the main target areas were polymeric hormones, protein enzymes and cytokines; the first successful biopharmaceutical product was insulin. Despite the ongoing postwar demand for insulin, there were side effects to using porcine or bovine insulin and there was

also the issue of the cost of producing semi-synthetic human insulin from porcine insulin; the hope was to mass produce human insulin.

In the early 1970s in the United States, research began on producing human insulin by mass cultivation after splicing the human insulin gene into microbial plasmids. In 1978, Arthur Riggs and Keiichi Itakura of Genentech and the City of Hope National Medical Center in the United States developed the "proinsulin method" of chemically synthesizing the DNA equivalent of the A and B chains of insulin, splicing these into plasmids to produce proinsulin in *E. coli*, and then using a chemical reaction to produce a double strand. Eli Lilly & Co. adopted this method and in 1983 launched Humalin®, an insulin formulation produced by genetic modification [(5)].

In 1987, Novo successfully developed its own "mini-proinsulin method" of producing insulin precursor mini-proinsulin with yeast fungus and converting it into human insulin. In Japan, approval for human insulin had been granted in 1985. Developments and improvements have continued since then to the time of writing in 2014, with faster-acting, longer-lasting and sustained-acting formulations being achieved by partial modification of amino acids and binding with fatty acids to coordinate the onset and metabolism of the medicinal efficacy, as well as the development of insulin analogs by specialized overseas manufacturers [(6)].

Insulin was followed by genetically-engineering growth hormones and interferons; these began to be used in medical treatment.

Biopharmaceuticals are polymeric drugs comprising mainly proteins; these are produced using animal cells and gene recombination. Given the ease of producing proteins with biotechnology, as mentioned above in the example of insulin, the focus turned to antibody preparations as well as hormones, enzymes, cytokines and other in vivo substances. There were high hopes for biopharmaceuticals and the late 1980s saw growth in the market expanded as epoch-making pharmaceuticals began to appear.

Between 1980 and 1989, new drug approval was given for six types of α, β and γ interferons; six types of growth hormones (somatotropin) were also approved between 1980 and 1990. However, despite being a secret weapon against the previously-incurable Hepatitis C, the early interferons had strong side effects and sales slumped as these were not adopted for clinical use and failed to demonstrate adequate medicinal efficacy.

Other in vivo hormone and enzyme biopharmaceuticals were developed; however, many of these were accompanied by side effects, despite being in vivo substances. Many also failed to demonstrate the anticipated medicinal efficacy. As a result, biopharmaceuticals were limited to niche areas, such as intractable diseases and hereditary enzyme deficiencies. Substances such as α-galactosidase, α-L-iduronidase and blood coagulation factor were used for patients with incurable enzyme deficiencies, but were not widely adopted for general illnesses (see Table 2.7: Biopharmaceuticals Market Table).

Table 2.7 Biopharmaceuticals Market Table [(7)]

		1980-1990	1990-2000	2000-2010	2010-2014
Enzymes					
		0	1	7	1
Blood coagulation fibrinolytic system	tPA	0	2	0	0
	Thrombomodulin	0	0	1	0
	Blood coagulation factor	0	2	2	1
		0	4	3	1
Hormones	Insulin	1	1	5	2
	Growth hormones	3	3		0
	Follicle stimulating hormones	0	0	2	0
	Other	0	3	0	3
		4	7	8	5
Vaccines	Hepatitis	2	1	0	0
	HPV	0	0	1	1
		2	1	1	1
Interferons	α	3	0	3	0
	β	2	0	2	0
	γ	1	0	0	0
		6	0	5	0
Erythropoietin					
		0	2	1	1
Cytokines	G-CSF	0	3	0	0
	Interleukin 2	0	2	0	0
	FGF	0	0	1	0
		0	5	1	0
Antibodies					
		0	1	14	13
Fusion proteins					
		0	0	1	3
Total		12	19	42	30

Erythropoietin (EPO) and granulocyte-colony stimulating factor (G-CSF) were large-scale biopharmaceuticals. Erythropoietin is a glycoprotein hormone with a molecular weight of 30,000; it promotes the differentiation of erythroid stem cells and the production red blood cells. G-CSF is a cytokine with a molecular weight of 19,000; it is a protein that promotes the production of granulocytes and boosts the function of neutrophils.

In 1977, Takaji Miyake of Kumamoto University School of Medicine announced that he had refined EPO from human urine. In 1985, US companies Amgen and Genetics Institute announced the successful production of EPO by gene recombination. Both groups had started researching using Miyake's EPO extracted from urine (10mg of refined erythropoietin from 2.5 tons of urine from aplastic anemia patients).

A patent dispute ensued from 1987 to 1992 between Amgen and Genetics Institute, which had joined forces with Miyake; Amgen was ultimately the winner.

In Japan, Kirin signed a contract with Amgen and started working on domestic clinical development, while Chugai Pharmaceutical did the same with Genetics Institute. In 1990, Kirin was granted manufacturing approval for a human erythropoietin formulation (epoetin-α) made using gene recombination technology as a treatment for renal anemia during dialysis. Chugai gained approval for eopetin-β in 1990 as well, also as an effective treatment for renal anemia during dialysis.

According to the 1990 predictions for the future of the bioindustry in *Survey of Trends in Technology Patent Applications to the Japan Patent Office*, the genomic drug discovery market would be worth ¥5.4 trillion by 2020, gene therapy would be worth ¥2.7 trillion, genetic diagnosis ¥720 billion and regenerative medicine ¥2.3 trillion. In reality, the estimates took a major downturn, with biopharmaceuticals slumping from the 1990s to the 2000s.

However, with the advent of the 21st century, it was found that antibodies that target specific cells in specific proteins have a significant medicinal efficacy in the fields of oncology and immunology (Note 1). A number of companies started investing into research and development in these areas, thus breathing new life and significant development into biopharmaceuticals. At present, the fields of oncology and immunology are the most active fields for biopharmaceuticals.

According to materials produced by the National Institute of Health Sciences Bio Division, 26 types of antibody drugs were marketed between 2000 and 2014 (7). The main key technologies in these antibody drugs were humanization technologies, in which mouse genes are replaced by human genes, and technologies to produce human immunoglobulin using cultivated Chinese hamster ovary (CHO) cells. There have been a number of achievements in these fields; advances in technology have rapidly made it possible to safely mass produce these drug products, while the use of human antibodies has meant drastic reductions in side effects and greater ease in clinical trials.

Biopharmaceuticals have also made it possible to target cancers and autoimmune disorders, which had not been possible to properly deal with using low-molecular-weight drugs. Successive new drug products began to emerge on the market. The 26 antibodies mentioned above included 14 types of anticancer drugs and 6 types of immunity-related biopharmaceuticals used for rheumatoid arthritis, renal transplants and the like (see Table 2.7).

Japanese scientists have made significant contributions in the field of antibodies. The mechanism of how the body produces such a diverse range of antibodies that bind to innumerable proteins using a limited number of genes had long been a mystery in immunology. The question was finally and brilliantly answered with a theory proposed by Susumu Tonegawa, who was awarded the Nobel Prize in Physiology or Medicine in 1987. The theory suggests that variable region genes are formed by an H-chain gene, for instance, randomly selecting and binding to antibody gene fragments present in different areas V, D and J; the combination of these allows a great diversity of antibodies to be produced.

Other known phenomena include immunoglobulin class switching, in which the constant-region portion of a sequence changes when the antibody gene is mature, and somatic hypermutation, which allows further adaptation of the variable region; research by Tasuku Honjo made a significant contribution to understanding these phenomena.

While such technology has made it possible to produce large quantities of human-type monoclonal antibodies, production for antibody drugs requires massive cultivation facilities and significant research and development expenses.

Meanwhile, where biopharmaceuticals have struggled to gain traction, biotechnology has played a major role in the form of tools (supporting technologies) for low-molecular-weight drug discovery. These technologies have included: microarrays and other forms of chip technology; automatic cell separators for separating cells; polymerase chain reaction (PCR) as a method of replicating DNA (Note 2); DNA sequencers for automatically determining base sequences; and peptide sequencers for automatically analyzing the amino acid sequences of proteins.

These technologies have been put to use in genomic drug discovery (Note 3) for discovering and validating new targets for low-molecular-weight drugs. Pathological animal models are essential for evaluating new drugs and many contributions to research and development have enabled by the creation of knockout mice, which have certain genes inactivated, and transgenic mice, which have genes transferred into them. However, with the focus on animal welfare in recent years, research has also gone into methods that substitute the pathological animal models with in vitro testing; biotechnology can be expected to be utilized in this area as well.

Advances have also been made in cell biology, with Yoshio Okada making a ground-breaking and highly-impacting discovery in 1957 with his paper on cell fusion using Sendai virus. It has since been found that cell fusion is possible with other animals; cell fusion has become one of the mainstays of biotechnology, with dramatic progress being made in cellular-level genetics and in research on viral carcinogenesis.

Various cell differentiation and regeneration technologies have emerged, such as gene therapy, cloned animals, embryonic stem cells (ES cells) and induced pluripotent stem cells (iPS cells), pioneered by Shinya Yamanaka, who was awarded the Nobel Prize in Physiology or Medicine in 2012. While the ultimate use for these technologies will be regenerative medicine, there are a number of factors involved and there will be a number of challenges to face, not only in terms of medicinal efficacy and treatment, but also in terms of the mass production of cells, such as ensuring a stable supply of culture fluid free from viruses and other contaminants, steady production using cultivation and refining processes, ensuring product quality, having the relevant inspection and validation systems, reducing timeframes and keeping costs down.

These regenerative-medicine-related technologies are currently being used as tools to support drug discovery. Biotechnology is being accumulated in the target discovery and target validation (see Chapter 3) part of the drug discovery process and can be expected to make a significant contribution to new drug discovery in the 21st century. Initial screening using human cells and tissues has been problematic, but the use of iPS technology is expected to make it possible to evaluate drugs in human systems at the earliest stages of drug discovery. The production of knockout animals and transgenic animals is also being used in drug discovery research as technology has advanced.

Note 1: Antibody drugs: When an antibody specifically binds to a specific protein, that protein ceases to perform its original function. The use of antibodies on key cancer-cell-activating proteins can be expected to have an anti-cancer effect by inhibiting the activation of the cancer cells.

Note 2: Polymerase chain reaction (PCR) is a DNA replicating technology using DNA polymerase, invented by Kary Banks Mullis, who was awarded the Nobel Prize in Chemistry in 1993. It has had a significant impact on molecular biology. It is also known for its use in criminal investigations; the PCR method is used to amplify DNA from billions to hundreds of billions for DNA testing.

Note 3: Genomic drug discovery: The incorporation of genome analysis and genome-related technologies into drug discovery. Genomic drug discovery includes using these technologies in finding and validating drug discovery targets, as well as in methodologies and screening leading to drug discovery. See Chapter 3.

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2.4.7. Medicines in Japan (Part 3: Current Pharmaceuticals Industry)

Changes in the Pharmaceuticals Market

According to the Ministry of Health, Labour and Welfare's *Vision for the Pharmaceutical Industry 2013*, the Japanese pharmaceuticals market was worth ¥9.3 trillion in 2011, with ethical pharmaceuticals accounting for ¥8.7 trillion and the over-the-counter drug market making up the remaining 7%. In 2000, the market was worth ¥6.7 trillion, with ethical pharmaceuticals accounting for 88% at ¥5.9 trillion and over-the-counter drugs accounting for 12%. During the decade in between, there were calls to reduce medical expenses each year; measures included bring down drug prices and promoting the use of generic drugs. Nevertheless, the ethical pharmaceuticals market has continued to grow in terms of turnover; total medical expenditure has continued to increase during this ten-year

period, with drug costs remaining at 20-22% (see Figure 2.14) [(1)].

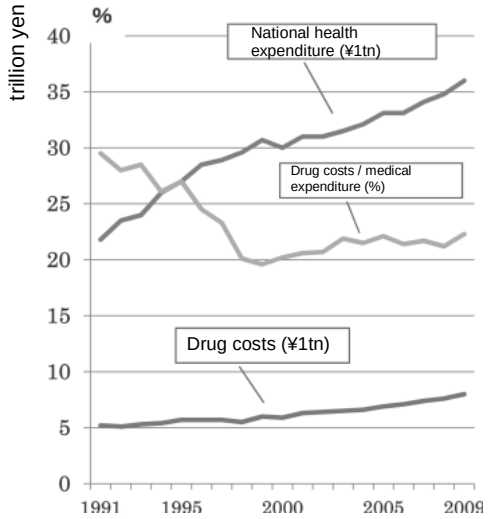

Fig. 2.14 Recent Trends in National Health Expenditure / Drug Costs (1991-2009).
Chart drawn from MHLW document *Vision for the Pharmaceutical Industry 2013* (p. 4, 2013)

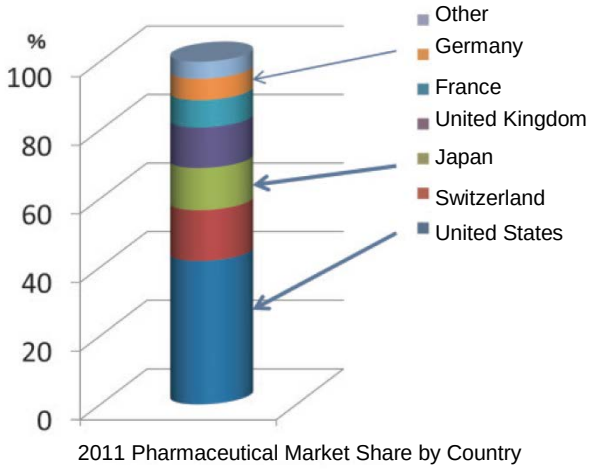

Fig. 2.15 Market Share by Country of the 30 Highest-Selling Pharmaceutical Companies (2011).

(from MHLW document *Vision for the Pharmaceutical Industry 2013*, p. 16, 2013)

Looking back, ethical pharmaceutical production rose from ¥770 billion in 1970 to around ¥3 billion by 1980 (a growth factor of 3.9 in ten years); by 1990, it had risen to ¥4.7 trillion (growth factor of 1.6 in ten years) and by 1995, it had risen to ¥5.2 trillion (growth factor of 1.1 in five years).

The global market hit $726 billion in 2007; by 2011, it had grown to $953 billion, with annual growth in the markets outside of Japan in Asia, Africa and Central and South America. On the world market, Japan has been second only to the United States since the 1980s; its 2011 market share of $111.6 billion accounted for 11.7% of the global market, rivalling the $114 billion of Germany, France and Italy combined.

Between 2007 and 2011, North America (United States and Canada) occupied 36-40% of the global market; during that time, Japan took second place at around 10%. Looking further back, the Japanese market occupied 15% of the world market in 1985, rising to 19% by 1990 and 21% by 1995. Since then, the Japanese market share has started to decline, returning to 15% by 2000 [(2)]. These figures have earned the Japanese a reputation as the world's most medicine-loving nation. After that, the global market started growing in developing nations, giving the Japanese market the appearance of a relative decline.

Figure 2.15 shows the market share of the 30 highest-selling pharmaceutical companies by country. While Takeda Pharmaceutical is the only Japanese company in the top ten, Japan still ranks third highest among the total 30.

Opinions are divided as to how to interpret these figures. Japanese drug discovery companies have continued their research and development focused on pharmaceuticals for the domestic market; this policy has not kept them abreast of global changes. It is important to adopt a global strategy for the future while also maintaining the strategy of drug discovery for the domestic market.

Recent research has found that due to polymorphisms and single nucleotide polymorphisms (SNPs), different individuals produce proteins with different amino acid sequences, meaning that drugs work in different ways in different individuals. These genetic differences not only occur between individuals, but also between races; the difficulty of discovering drugs that work for all people irrespective of race is beginning to be understood. Consideration is being given to the idea of drug discovery branching in two different directions: drugs that can be used by all people and drugs that are administered to specific individuals or races.

Original Drugs and Generic Drugs

There are two types of pharmaceutical products: ethical pharmaceuticals, which require a medical prescription, and over-the-counter (non-prescription) drugs, which can be purchased at any drugstore. Ethical pharmaceuticals comprise original drugs and generic drugs, which are marketed after the patent period of the original drug has expired. In Japan, the original drug market grew from 17% to 23% in volume terms and from 5.9% to 8.8% in value terms between 2007 and 2011 [(1)].

There are substantial costs involved in the research and development of generic drugs; it can cost over ¥10 billion in research and development to produce a single drug, especially if the clinical trial is large in scale. Taking into account the research and development of new drugs that have

been discontinued, this area of research and development is costing tens of billions of yen. The average research and development expense in the pharmaceutical industry in 2011 was 12% of sales, higher by far than the 6.44% in the telecommunications device manufacturing industry, the 6.39% in the electronic circuits and components manufacturing industry, the 5.9% in the electrical machinery and device manufacturing industry and the 4.78% in the automobile and accessories manufacturing industry. This ratio has hardly changed since the 1980s [(4)].

Meanwhile, patients are also becoming more aware, as the administration of generic drugs is being encouraged as a means of reducing costs. An increasing number of leading companies are also starting to sell generic drugs. There is a renewed acknowledgement of the importance of low-priced pharmaceutical products with guaranteed safety and efficacy.

Nevertheless, even under such circumstances, companies need to maintain and increase their turnover for the sake of business. There is a growing emphasis on new drug research and development to play a role in driving growth. Releasing a blockbuster drug on the market requires faultless target setting that hits close to the true cause of a disease; it also necessitates increasing the speed of research and development, which requires a rapid feedback system to determine overall efficacy and side effects, as well as efficient research and development facilities. Much can be expected from companies utilizing their strengths and creativity.

The Domestic Market by Field of Disease

The proportional shipment volumes for the top four drug classifications on the Japanese market for 2011 have hardly changed since the 1990s, with (i) circulatory system drugs (anti-hypertensives, hyperlipidemia medications, heart medications, etc.) accounting for 16.6%, (ii) central nervous system drugs (medications for depression, anxiety, schizophrenia, dementia, antipyretic analgesics, etc.) accounting for 10.8%, (iii) other metabolic drugs (diabetes medications, liver medications, etc.) accounting for 10.3% and (iv) gastrointestinal drugs (antiulcer medications, stomach medications, intestine medications, etc.) accounting for 7.4%.

One major change is that of antibiotics dropping from second place in 1990 to fifth place by 2000 and out of the top ten by 2011. As the hygiene and nutritional environments improved and peoples' general medical knowledge increased, infectious diseases reduced and the administration of unnecessary antibiotics and chemotherapeutics also decreased. The Japanese were also losing faith in antibiotics. Metabolic drugs held fifth position in 1990 and later rose into the top four. Anticancer drugs, which were not numbered among the top ten in 1990, had risen to eighth place by 2005 and to fifth place by 2011 (see Table 2.8).

Table 2.8 Domestic Market Share of Shipments by Drug Classification

1990		2000		2005		2011	
Drug classification (share)							
Circulatory system drugs	13.8 %	Circulatory system drugs	16.9 %	Circulatory system drugs	18.1 %	Circulatory system drugs	16.6 %
Antibiotics	11.2	Gastrointestinal drugs	8.2	Metabolic drugs	9.4	Central nervous system drugs	10.8
Central nervous system drugs	9.2	Central nervous system drugs	8.1	Central nervous system drugs	9.3	Metabolic drugs	10.3
Gastrointestinal drugs	8.9	Metabolic drugs	8.1	Gastrointestinal drugs	8.0	Gastrointestinal drugs	7.4
Metabolic drugs	7.9	Antibiotics	6.2	Skin medications	4.8	Tumor drugs	7.3
Skin medications	5.7	Skin medications	5.9	Antibiotics	4.6	Biological preparations	5.3
Diagnostic agents	5.6	Blood/body fluid medications	5.0	Blood/body fluid medications	4.5	Blood/body fluid medications	5.1
Vitamins	4.7	Biological preparations	4.2	Tumor drugs	4.4	Allergy drugs	4.1
Respiratory drugs	4.5	Sensory organ drugs	3.8	Biological preparations	4.0	Skin medications	4.0
Blood/body fluid medications	3.1	Hormone drugs	3.7	Sensory organ drugs	3.6	Sensory organ drugs	3.5
Other	25.3	Other	29.9	Other	29.4	Other	25.8

(from MHLW documents *Vision for the Pharmaceutical Industry 2013*, p. 14, and *Statistics of Production by Pharmaceutical Industry*)

This table indicates that lifestyle-related diseases and cancers have become the focal diseases of the 21st century. Drugs for lifestyle-related diseases, now more than ever at the focus among pharmaceutical products, have reached a state of near completion from a technology perspective. However, like the course taken by vitamins and antibiotics from the 1950s to the 1980s, these drugs, too, can be expected to follow a similar pattern, passing from an "in-demand new drug stage" to an "ongoing sales stage", followed by "eventual reduce in demand". Consideration will be needed as to how to maintain sales in future.

Gastrointestinal drug sales also generate high figured and have long held a top ranking. While oral antiulcer medications (H_2 inhibitors and proton pump inhibitors) are administered in high numbers, the administration of stomach and intestine medications alongside other drugs also contributes to these figures.

Anticancer drugs are a major target for such concomitant drug administration. This field requires a concept unlike that of conventional pharmaceuticals; consideration needs to be given to "individualized medicine" that incorporates the use of biotechnology and diagnostic examination.

Globalization of the Pharmaceuticals Industry

According to the MHLW's *Statistics of Production by Pharmaceutical Industry*, shipments by foreign-capital enterprises accounted for 36.2% of the total pharmaceutical product shipments on the domestic market in 2011. At 18.6% in 1991, this figure has more than doubled in ten years [(1)].

The proportion of overseas sales by Japanese companies has remained level at around 35-37% of total sales since 2007.

In terms of research and development, according to the latest documentation from the Ministry of Health, Labour

and W2elfare, Japan approved 89 new active ingredients between April 2008 and May 2012, 49.4% of which were researched, developed and produced by Japanese companies, while the remainder were imported from overseas (for itemized details on the new drugs by the Japanese companies, see the Central Social Insurance Medical Council (82nd) Special Committee on Drug Prices, August 22, 2012; expert committee members Kanji Negi and Yoshiaki Kamoya).

From the 1970s onwards there was a shift away from the long-lasting postwar era that gave precedence to imported drugs and an increase in domestically-produced products, although these were mainly improved formulations. While some innovative new drugs did emerge as well, overseas imports have again been on the rise. In an age of increasingly individualized medicines, there is a need to develop drugs for domestic production. Attention needs to be given to finding the balance between globalization (world-perspective strategies) and "Galapagosization" (domestic-focused strategies).

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3 Technological Advancement of Pharmaceutical Drug Discovery (Drug Discovery as a Systems Science)

This paper thus far has discussed the development of pharmaceutical products in chronological sequence; this chapter now focuses on developments in drug discovery processes from a technology point of view.

3.1 Drug Point of Action

The body works to maintain homeostasis; however, when internal or external abnormal information (stimuli) is introduced, it confuses the transmission of information (stimulus transduction), thereby disturbing homeostasis. If this continues, it can cause abnormal conditions in tissues, organs or throughout the body. The role of drugs is to either eliminate the source of the abnormal information causing the condition (e.g. eliminating pathogens) or to bring the abnormal information transmission (signal transduction) back to normal (e.g. eliminating inflammatory substances from a site of inflammation).

Drugs demonstrate their efficacy by inhibiting or promoting the transduction of stimuli within or between cells. For example, enzyme inhibitors demonstrate their medicinal efficacy by inhibiting certain enzymes that are working excessively and thereby prevent the any subsequent mechanisms. Drugs that are receptor antagonists inhibit the transduction of large amounts of stimuli by antagonizing the receptors, thereby returning conditions to normal. Drugs that are receptor agonists invoke the transduction of strong stimuli by stimulating the impaired receptor function, thereby returning conditions to normal. See Figure 3.1.

At molecular level, the point of action of drugs is often protein. According to the literature examining the point of action of drugs, as at 2000, (i) receptors accounted for 45%, (ii) enzymes accounted for 28%, (iii) membrane transport protein accounted for 5% and (iv) nuclear receptors 2%. Thus, almost 80% of drug discovery targets are enzymes or receptors [(1)].

The modern drug discovery process is based on processes used for screening chemotherapeutic drugs. Since chemical treatment targeted pathogenic microorganisms coming from outside of the human body, advances in drug discovery were relatively straightforward.

In the 1950s, James Black discovered histamine H_2 receptor antagonists. These were administered to patients suffering from stomach ulcers and duodenal ulcers, with breakthrough effects. Thus, the modern drug discovery research and development process reached a basic level of completion. This process is summarized in Figure 3.2.

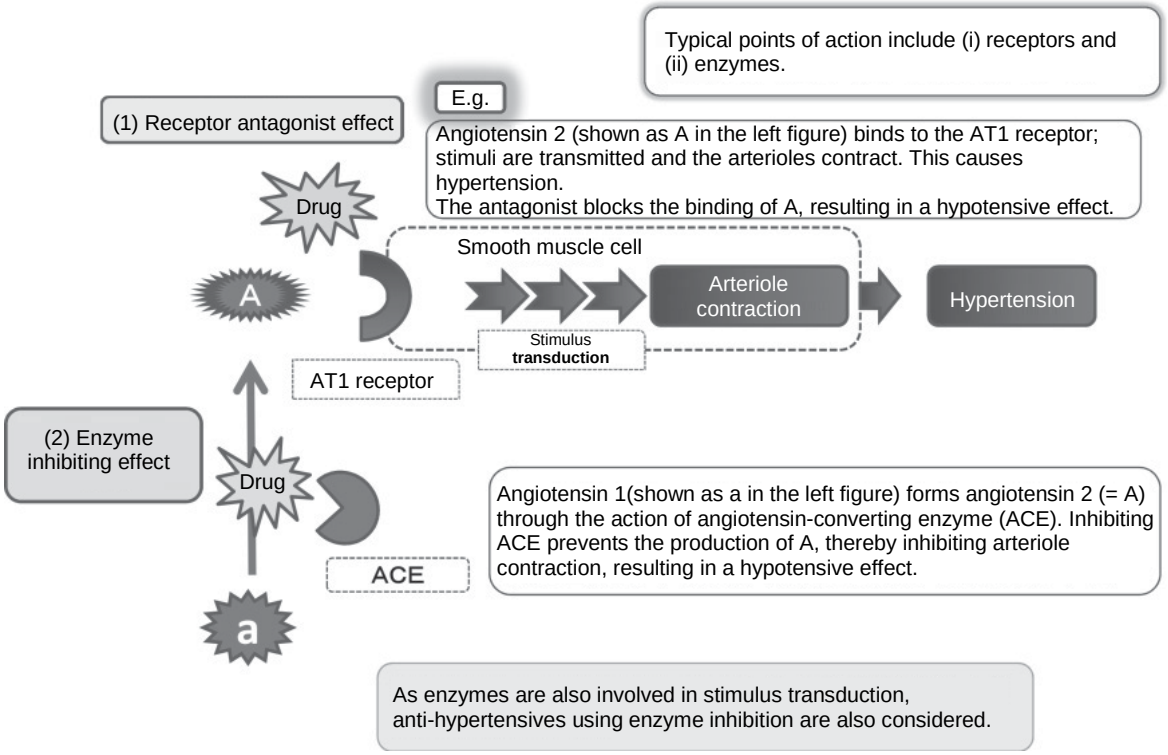
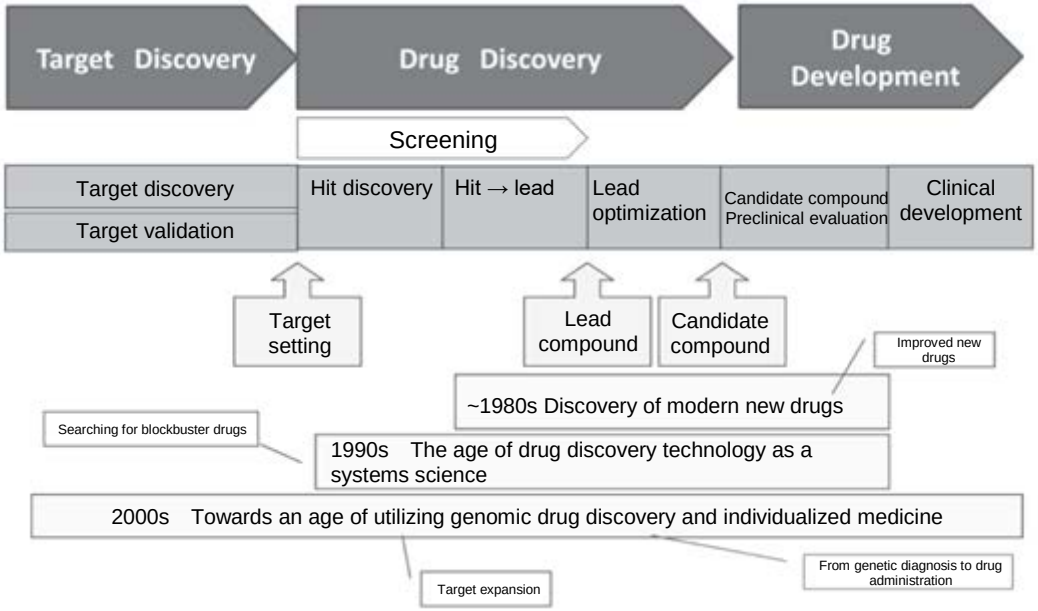


Fig. 3.1 Point of Action of Drugs. (see Section 4.2.4. Renin-Angiotensin System for details on anti-hypertensives)



Until the 1980s, there were more "improved drugs" using known lead compounds than innovative drugs using lead compounds with new structural formulas. By the 1990s, science and technology had built up to the point that drug discovery could be referred to as a systems science. It became possible to narrow down theoretical candidate compounds from greater numbers of compounds. The 2000s saw the dawning of the age of genomic drug discovery, with targets emerging that differed from existing ideas. The ties between clinic and base field grew stronger; patients were selected with coordinated consideration between diagnosis and drugs; drugs began to be administered with more reliable medicinal efficacy. Since it takes 15-20 years for pharmaceutical products to reach the market, the products being marketed in the 2010s are the results of drug discovery research and development in the 1990s. The results of genomic drug discovery are yet to emerge.
In pharmaceutical product research and development, clinical trials are often referred to as "development", while the preceding processes are often referred to as "research".

Fig. 3.2 Drug Discovery Process Development.

3.2 Changes in Drug Discovery Processes

This explanation of the drug discovery process follows the course shown in Figure 3.2.

(1) Target Discovery and Setting

Determining drug targets requires determining the target disease and determining the proteins on which the drug will act (this is best discussed in terms of protein, since most targets are currently proteins). Targets are determined in consideration for disease/patient trends, their necessity in clinical practice and the clarification and differentiation of points of improvement to existing drugs, as well as the points of difference with existing drugs for factors such as sales and profitability from a business perspective.

Receptors or enzymes were chosen as targets in the postwar era; as mentioned previously, these were the main drug targets for a long time. Although there is not necessarily one single target for a certain disease, selecting pre-existing drugs meant the target was the same. There are not any substantially significant differences between these drugs in terms of medicinal efficacy; they are categorized by their side effects and methods of administration. Drugs discovered using this type of target selection are referred to as "improved drugs".

The late 1990s saw the advent of genomic drug discovery and the emergence of the term "target discovery" (Note 1), in which gene analysis is used to find new targets for diseases.

(2) Establishing Evaluation/Selection Systems (Screening Systems)

The so-called screening stage is termed "drug discovery" and encompasses everything from discovering hit compounds to optimizing lead compounds. This process first requires the creation of a screening system. The first stage examines whether or not a compound is possible to be used as a drug. This system must be very simple and repeatable in order to process large numbers of compounds. This is where different companies put their own ingenuity into action.

- *in vitro* testing systems (testing systems carried out in test tubes or Petri dishes; enzymes or receptors are used in crude or refined form; tissues or cells also used)
- *in vivo* testing systems (medicinal efficacy evaluation system using entire animals; pathogenic animal models, etc.)
- *in silico* systems (in genomic drug discovery, a method of screening for hit compounds and lead compounds by computer based on past information)

(3) Lead Compound Setting

Lead compound setting refers to screening large numbers of compounds and then determining the basic skeleton of the compounds to be evaluated. Any compounds exceeding the initial criteria for efficacy, drug metabolism, safety, etc. are

selected as lead compounds.

- Where the lead compound is a known drug

While compound setting is easier with a basic skeleton already in place, it is more difficult to demonstrate any new drug characteristics if the structure of the compound does not greatly differ from existing drugs. Many of the drugs known as improved drugs started with a known lead compound and resulted from the synthesizing and evaluating of peripheral compounds. Although this method is known as Japan's forte, it is very common in the West as well, with numerous improved drugs emerging on the heels of every innovative new drug [(Note 2)].

- Where the lead compound is set by random screening

In the absence of a basic skeleton for reference, lead compounds can be selected by screening compounds at random. A group of compounds used randomly is called a "compound library"; each company has access to their own collection of compounds. More important than the number of compounds in the collection is the variety of basic skeletons; the greater the variety, the higher the chance of the evaluation system making a hit. A company's compound library represents the compound characteristics available to that company and reveals the originality of the company.

Synthetic compounds are not the only targets for screening. Fungus or mold culture filtrates and plant extracts are also used in order to extend the breadth of the basic skeletons available.

While many antibiotics are derived from fungus or mold, the world-class breakthrough drug Pravastatin from Japan and the globally innovative immunosuppressant Tacrolimus by Fujisawa Pharmaceutical were discovered from fungus culture filtrates.

It is rare to discover a perfect lead compound with only a short amount of screening, but it should reveal several compounds with certain levels of pharmacological activity. These are called "hit compounds". Where multiple hit compounds share a common chemical structure (basic skeleton), it means they have a "pharmacophore" [(2)], or "the ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target structure and to trigger (or to block) its biological response" [(Note 3)].

Based on this information, medicinal chemists presume a structure-activity correlation and start to narrow down lead compounds by altering side chain molecules, thereby enhancing the medicinal efficacy and reducing the side effects.

(4) Optimization

Having obtained a lead compound, further chemical modification is carried out in detail on the compound to produce a more active development candidate compound with fewer side effects; this is called "optimization". This is where medicinal chemists need to have ideas and technological prowess.

In the optimization process, medicinal efficacy is evaluated using multiple evaluation systems rather than on a single screening system alone; this includes testing on several pathological animal models. These evaluations not only serve to validate medicinal efficacy, but also to check for toxicity using simple toxicity tests and affirm that the compound has good pharmacokinetic properties. Thus, a development candidate compound is selected. This is a major process requiring coordination between drug evaluation divisions, compound synthesis divisions, safety evaluation divisions and pharmacokinetics [(Note 4)] evaluation divisions. The respective criteria for each division are important; if the criteria are too strict, it will be very difficult to produce a candidate compound, but if the criteria are too lax, the candidate compound will lack originality. Japanese drug discovery companies have had systems in place to connect these divisions since the 1980s (see Section 2.4.1).

(5) Preclinical Testing (Nonclinical Testing)

Once a development candidate compound is selected, the necessary testing for clinical adoption is then carried out in accordance with good laboratory practice (GLP). This testing is known as preclinical testing (or sometimes, nonclinical testing). This includes validating the medicinal efficacy, safety testing, pharmacokinetic testing and setting candidate compound criteria, as well as chronic safety testing, medicinal efficacy testing on animal models and testing large-scale synthesis methods for setting criteria.

GLP is a set of standards applicable to testing laboratories in order to ensure the reliability of nonclinical testing in relation to safety. These were first established in the United States in 1979. In 1981, the OECD formulated GLP standards and indicated that member countries should introduce GLP based on these standards, prompting the establishment of various kinds of GLP in many countries. Japan enacted GLP standards for medical and pharmaceutical products in 1983. These underwent subsequent changes, including being codified into a Ministry of Health, Labour and Welfare ordinance and being adopted for pharmacological safety testing. GLP was written into a ministerial ordinance in 1997 (see Section 2.4.1 above).

There are six GLP programs in Japan: medical and pharmaceutical products / medical equipment; industrial safety and health (*Industrial Safety and Health Law*); new chemical substances (*Chemical Substances Control Law*); agricultural chemicals; feed additives; and veterinary medical and pharmaceutical products / veterinary medical equipment. These are governed by four regulatory authorities [(3, 4, 5, 6)].

Good manufacturing practice (GMP) is a set of standards that determine matters relating to the appropriate manufacturing control and quality control methods to be observed when manufacturing investigational new drugs, as well as the necessary structures and facilities. GMP was established in the United States in 1963 following instances of harmful side effects from drugs. In 1969, the World Health Organization (WHO) endorsed requirements for good practices in the manufacture and quality control of drugs. In Japan, the Ministry of Health and Welfare notified the administrative divisions of Japan of these requirements as criteria for the manufacture and quality control of drugs in 1974. Meanwhile, the Japan Pharmaceutical Manufacturers Association (JPMA) had already established its own independent criteria (JGMP) in 1973, having investigated the establishment of GMP of its own volition. GMP was

promulgated as an ordinance of the Ministry of Health and Welfare in 1980 [7, 8]. A summary of these drug discovery processes is shown in Tables 3.1 and 3.2.

Table 3.1 Drug Discovery Process (Exploratory Research)

Exploratory Research Stage		
Seeking/Creating Candidate Substances		
Target Discovery basic research	Drug Discovery initial exploratory research	Drug Discovery subsequent exploratory research
• selection of drug targets • target validation	• discovering hit compounds • discovering lead compounds	Candidate compound optimization
• concept validation (patenting / competition / market / medical environment / medical needs, etc.) • establishing activity evaluation systems • target validation (verification)	Securing evaluation samples • synthesized / natural / biosynthesized products • combi-chem sample	Drug design • medicinal efficacy • pharmacokinetics (preliminary testing) • safety (preliminary testing)
	Screening test • general screening • in silico screening • HTS	Testing with composite evaluation systems (in vitro/in vivo testing)

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Table 3.2 Drug Discovery Process (Preclinical Testing Stage)

Preclinical Testing Stage	
Validation/Suitability Evaluation of Candidate Compounds for Clinical Trials	
GLP/GMP Conformance	
Synthesis/Bio	validate structure / research substance production methods / secure large volume of samples
Formulation	formulation design / research formulation production methods / produce formulation samples
Quality Assurance	research physical properties and analysis methods / set criteria
	safety testing (substance/formulation)
Medicinal Efficacy	high-order efficacy evaluation / analyze mechanism of action / safety evaluation testing
Pharmacokinetics	investigate measurement methods / investigate pharmacokinetics and metabolites
Safety	general toxicity (single/repeated dose) / TK testing / genotoxicity (mutagenicity) reproductive and developmental toxicity (Seg I, II: after normal clinical stage) / special toxicity

Testing is carried out at GLP-compliant facilities, in accordance with Ministry of Health and Welfare Ordinance No. 21

MBC internal document, revised 2006

(6) Clinical Trials

Clinical trials (clinical testing) are clinical tests carried out from Phase I to Phase III in accordance with GCP.

- Phase I involves safety testing using on a small number of healthy individuals (volunteers) who have provided consent.
- Phase II involves confirming efficacy, safe dosage levels, methods of administration and other factors on a small number of patients who have provided consent.
- Phase III involves confirming new drug efficacy and safety by carrying out double-blind trials and other comparative studies against existing drugs on a large number of patients who have provided consent. Quite a number of clinical trials have groups with several thousand participants in them.

Good clinical practice (GCP) refers to standards relating to the implementation of clinical trials for medical and pharmaceutical products. It is an international set of standards enacted to ensure that clinical trials are carried out appropriately; in Japan, these have been summarized in Ministry of Health, Labour and Welfare ordinances. The international standards aim to ensure that the human rights and safety of volunteers participating in clinical trials are maintained, that medical and pharmaceutical products are developed scientifically and that drug information is recorded accurately during development. Following the promulgation of the *Ordinance on Standards for Clinical Studies of Pharmaceuticals* in 1985, this was established as an administrative directive in 1988 by the director of the Pharmaceutical Affairs Bureau and was put into practice in 1990. The so-called *New GCP Ordinance on Standards for Clinical Studies of Pharmaceuticals*, was enacted in April 1997 and implemented the following year in 1998 [9].

Once medical efficacy, utility and safety have been confirmed, drug discovery companies then apply to the Ministry of Health, Labour and Welfare for production approval. When an application is received, the Ministry reviews the overall structure and refers the application to the Pharmaceutical Affairs and Food Sanitation Council (an advisory body for the Ministry of Health, Labour and Welfare) for deliberation based on the review outcome. Approved pharmaceuticals are granted production and sales approval by the Minister of Health, Labour and Welfare. A drug price is then set and the product is put on the market.

Other basic and applied technologies relating to drug discovery include systems for regulating drug absorption, distribution, metabolism and excretion, for ensuring prompt delivery to and prolonged retention at the target location and for prompt excretion once no longer needed. These make up the drug delivery system (DDS), also called a drug transport system. While some drugs feature cleverly-designed DDSs from the outset, in many cases approval is given to add these systems later to improve any drawbacks identified during clinical trials, such as appending dosage types. A summary of these technologies is provided elsewhere.

The basic drug discovery process discussed thus far has improved and progressed since the 1960s as advances in medical science, molecular biology, biochemistry and pharmacology have made it possible to analyze the causes of diseases at molecular level. Rapid improvements in genetic engineering and molecular biology since the 1970s have also made it possible to analyze diseases at genetic level, with increasing genetic information becoming available. The drug discovery process has become more complex as scientific advances have been incorporated and now involves quite a diverse range of technologies. Table 3.3 summarizes the drug discovery process as it relates to clinical trials.

Table 3.3 Drug Discovery Process (Clinical Trials)

Clinical Trial Stage			
Exploratory Clinical Efficacy Examination to Confirmatory Clinical Efficacy Examination			
Phase I	Phase IIa	Phase IIb	Phase III
tolerability / pharmacokinetics trial on healthy individuals	efficacy / safety trial on patients (pilot trial)	examination of adaptability / symptom dose dependence / optimum application method and dosage, etc.	utility trial / double-blind comparative trial in an expanded clinical trial
(clinical trial: GCP conformance) clinical trial implementation protocol / clinical trial design / biostatistics / institutional review board / clinical trial association structure / initial clinical trial notification and clinical trial consultations / agreement with clinical trial medical institution / informed consent / clinical trialing monitoring / data management / data analysis / auditing and ensuring reliability / writing overall clinical trial reports / application pre-consultation			
(nonclinical trial) chronic toxicity testing (rats, dogs, etc.) / reproductive toxicity testing / carcinogenicity testing			

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In the event that a certain drug is effective, analogs are synthesized based on its chemical structure and evaluated. The dosage may be lessened by increasing the medicinal efficacy, thereby comparatively reducing the side effects. This has been the trend for creating improved drugs in the past. However, with dramatic increases in information about genes and proteins thought to be potential drug discovery targets for certain diseases, it is becoming increasingly important to validate whether or not a given target actually does relate to a given disease.

With it now possible to analyze the causes of diseases at gene level, it has also been found that homeostasis within an organism, or aggregate of multi-phase systems, is regulated through multiple channels and that the target for a single disease is not necessarily a single protein or nucleic acid.

Drug discovery companies have each had to devise ways to address issues, such as which targets to select, how their drugs differ characteristically from other drugs, how to select lead compounds more quickly and at which point to make improvements to safety evaluations and pharmacokinetics and with which kinds of trials.

In the 1990s, biotechnology began to progress rapidly. Following the decoding of almost the entire human base DNA sequence in the international Human Genome Project in 2001 [10, 11], genomic drug discovery research accelerated, with hopes of making effective use of this genomic information to develop new drugs [Note 5]. Disease analyses began to be undertaken at gene level based on the new research sequence of "DNA→ RNA → enzymes / receptors → drugs (proteins)". This was the beginning of pharmacogenomics (drug discovery using gene technology).

New methods were developed in basic research year by year and were added to the basic process, making the drug discovery process more complex and more heavily equipped, which also meant greater research expenses. The cost of pharmaceutical research and development continues to rise as clinical trials grow larger in scale and more complex.

Some examples are given for each process below.

(1) Target Setting

More than half of current pharmaceutical product targets are receptor or enzyme inhibitors (antagonists) or activators. Developments in biotechnology, molecular biology and genetic analysis technology have resulted in the deduction and discovery of successive new potential target genes and proteins, which has meant an excessive increase in new target candidates. Verification testing is being carried out to determine whether or not these new targets actually relate to certain diseases; this process is called target validation.

It is now possible to compare diseased tissue with normal tissue and extract any significantly differing proteins or nucleic acids to use as target candidates [12]. For example, by comparing the expression of a healthy individual with that of a patient and analyzing the difference in terms of mRNA and cDNA, it is now possible to identify the protein that has changed specifically due to the disease and use that protein as a target candidate.

Since proteins are considered to function according to a three-dimensional structure, even if an unknown protein is identified, it is possible to examine its three-dimensional structure and calculate the properties of the new protein based on information from other proteins. It is also possible to predict which molecules and surrounding structures (active center) are likely to be involved in its activity. Although vast amounts of information are now thus required, advances in information technology have made computers commonplace. The technologies involved in this information processing are known collectively as bioinformatics.

While it is necessary to validate whether a deduced protein actually relates to a particular disease, pathological animal models can be used to determine the importance of that protein based on pathological changes that occur when the gene responsible for the target protein is prevented from expressing (using knockout mice, etc.). Although it is difficult to substantiate every candidate protein, if a certain conjecture could be made during in vitro testing, then those proteins have been put forward for use as candidate proteins.

(2) Evaluation Systems and (3) Lead Compound Setting

In this process, the target protein is used to create evaluation systems. The efficacy and accuracy of these systems are crucial. A method has emerged known as high throughput screening (HTS), making it possible to process larger volumes of samples in shorter lengths of time. This method was first put to use in the 1990s by American drug manufacturers. The Society for Biomolecular Screening was established in 1994 as a dedicated HTS organization, followed by the Association for Laboratory Automation in 1996. In Japan, the Drug Discovery Research Automation Association was formed in 1995 and drug discovery companies began to gather together to work on initiatives. To begin with, these focused on technology imports, such as an introduction to the American approach by Nippon Roche. Different companies also worked on their own HTS-integrated evaluation systems using genome technology to determine the efficacy of samples comprising not only enzymes or proteins, but also cells or specific proteins within cells.

HTS involves *in vitro* screening processed by assay robot. While this has significantly improved screening efficiency, it

has also meant the compounds to be evaluated are rate-limited, thus requiring a higher volume of compound samples. Companies have worked hard to build up their own compound libraries, using not only synthetic compounds, but also bacterial culture filtrates.

Combinatorial chemistry, which emerged in the 1990s in relation to synthetic compounds, has made it possible to meet the demand for increased numbers of compounds. Synthesizing individual compounds one at a time was a time-consuming process. For solid-phase synthesis, the substrate for the reaction is bonded to a particular solid-phase simple substance, such as polystyrene, and the reaction is effected by the addition of the reaction reagent dissolved in a solvent. The target final product is obtained from on top of the solid phase during cleanup once the reaction is complete. This allows compounds to be obtained in a shorter length of time and with less complicated post-processing than the conventional liquid-phase synthesis. Based on this principle, the parallel synthesis method and the subsequently-developed split-mix synthesis method have made it possible to synthesize larger volumes of compounds in shorter lengths of time [(13)].

While there were initially high hopes that combinatorial chemistry would revolutionize the field of drug discovery, it had certain drawbacks, such as poor efficiency in the time taken to establish a synthetic pathway and the inability to ensure diversity due to limited numbers of reactions able to be used. Adulteration from byproducts also interfered with the evaluation systems, so this has gradually been put to use at the optimization stage later in the drug discovery screening process.

Another method is also being used, known as virtual screening, in which past data is input into a computer and software is used to predict the structure of the compound.

(4) Candidate Compound Structural Optimization

New ideas and new technologies are also being incorporated into this process to further optimize the compounds thus narrowed down. It is now possible to examine the three-dimensional structure of the active center of target proteins using X-ray analysis during refining and crystallization. Where this once required a certain amount of protein crystals, genetic engineering techniques have made it possible for *E. coli* or similar to express large quantities of proteins that normally only exist in miniscule amounts *in vivo*. The greatest obstacle in X-ray structural analysis has been the process of discovering the conditions for crystallization. With crystals able to be produced, conditions are now all set for the ensuing analysis to be carried out accurately.

Developments in computer technology and the use of synchrotron radiation have resulted in astounding advances in the speed and scope of X-ray structural analysis. SPring-8 is a large-scale synchrotron radiation facility in the Nishi-Harima region bordering Hyogo and Okayama Prefectures. It is one of only three third-generation synchrotron radiation facilities in the world and is able to produce the highest-level synchrotron radiation in the world.

With these new technologies revealing the active center of target molecules, medicinal chemists have begun incorporating them into optimization, combining computer-predicted structures with prior experience and insight (the intuition that comes with experience). The technology for designing drugs from target protein structures is known as structure-based drug design (SBDD) [(Note 6)].

In 1992, the FDA issued guidelines encouraging racemic switching [(14)]. These guidelines indicated that pharmaceuticals with enantiomeric structures should not contain a mixture of enantiomers, but only one enantiomer with medicinal efficacy, in order to prove that the unnecessary enantiomers are harmless when marketed in mixtures.

This determination was made on the basis of a disparity in pharmacokinetic properties of drugs in the past, with some containing isomers with strong medicinal effects and some with strong side effects. The idea was to select drugs with precedence given to both efficacy and safety. (The Japanese blockbuster drugs Cravit and Argatroban have been confirmed by chiral resolution to have only one direction of activity [(15)]. Mentioned later: Section 4.4.2.) Following the issuance of these guidelines, research has been conducted on methods to selectively synthesize only one enantiomer. The BINAP transition-metal-catalyst reaction, invented by 2001 Nobel Prize in Chemistry recipient Ryoji Noyori, makes it possible to selectively react an enantiomer (optical isomer) using the BINAP chiral structure. This has become a useful method for the purpose of racemic switching [(16)]. Yoshiji Takemoto et al. of Kyoto University have also created artificial enzymes using the enzyme reaction principle, making it possible to efficiently synthesize large volumes of enantiomers [(13:68-75)].

(5) Preclinical Testing Genomic Drug Discovery

In the 1970s and 1980s, the selection of candidate compounds at the exploratory stage generally involved simple safety testing and pharmacokinetic testing as the evaluation criteria. However, once GLP-compliant safety testing started being incorporated into preclinical testing on candidate compounds for clinical use, many candidate compounds were dropped due to safety issues or inadequate pharmacokinetics. A number of other candidate compounds have been withdrawn from development as a result of side effects or pharmacokinetic issues during clinical trials (clinical testing).

If a candidate compound is dropped in the latter stage of clinical trials, as much as two years of chronic safety testing (required for new drug applications (NAD)) becomes unnecessary, meaning that the significant costs outlaid in producing large volumes of samples and prolonged high-volume animal testing have been wasted. One reason for reason this is that the evaluation of candidate compounds at the exploratory stage has historically revolved around medicinal efficacy, with not enough evaluation going into safety and pharmacokinetics; this has become a matter for reconsideration.

Consequently, with the advent of the 21st century, more straightforward *in vitro* safety testing without using animals or using small numbers of animals has been carried out so as to avoid endlessly repeating high-volume animal testing. Research to introduce safety evaluations at the early phase of drug discovery by examining biomarkers has begun and is now being implemented. Some safety issues also result from the basic skeleton of the compound; it is now more commonplace to use research methods that make it possible to select and develop a number of different lead compounds with different basic skeletons at the same time.

With the incorporation of high throughput toxicology into safety testing in order to evaluate multiple samples within a short space of time, good use is also being made by utilizing cells of human origin, safety testing on pathological animal models and the use of databases for checking the correlation between compounds and toxicity. There are expectations that iPS cells can be used as a drug discovery tool in the area of *in vitro* drug discovery screening using human cells and in clarifying mechanisms of action.

Toxicogenomics is a new field of science that involves identifying gene expression patterns and using these to clarify mechanisms of toxicity expression. The aim is to validate gene expression patterns and predict the likelihood of toxicity expression with increasing accuracy by gathering vast amounts of known toxicity data and combining this with genomics and bioinformatics technology.

The aim of toxicoproteomics is to predict toxicity expression in cells, tissues and organs by detecting functional proteins secondary to gene expression; this field is being explored for the purpose of predicting toxicity [17, 18].

Drugs administered orally to humans and animals are often absorbed from the digestive tract and transported to the liver via the portal vein and then oxidized (hydration, but an oxygenation reaction) and metabolized by drug metabolizing enzymes. The drug metabolizing enzymes are a group of oxygenases present in microsomes in liver cells and small intestine cells; they form a “super family” known as cytochrome P450. These inherently increase the hydrophilic properties of drugs, poisons and other xenobiotics, making them easier to excrete from the body [19].

Drug metabolizing enzymes in humans encompass dozens of different types of enzymes; the enzymes all have different properties and differ significantly in terms of their substrate (drug) reactivity. Drug metabolizing enzymes can be polymorphisms or single nucleotide polymorphisms (SNPs), depending on the individual; phenotypes also occur, where an atypical enzyme or abnormal enzyme is expressed with reduced or deficient enzyme activity. Accordingly, drug metabolism differs between individuals; this is also one of the main causes of difference in drug efficacy between individuals. It is also necessary to get a proper understanding of the correlation between drugs and drug metabolizing enzymes at the animal testing stage; the hope is to develop drugs for which individual differences in drug metabolizing enzymes can be disregarded. It is also becoming preferable to determine dosage based on the state of an individual’s drug metabolizing enzymes. The idea of personalized medicine has come to the fore (also known as tailor-made medicine, order-made medicine or optimized medicine); although this is still a long way off, the application of pharmacogenomics has begun to place medicine on the road to the future [20].

With a candidate compound determined, the drug metabolism is examined using animal models in pharmacokinetic testing. If an issue is identified during this process, it presents a serious setback to development, just as for safety testing. More than a few pharmaceutical candidate compounds have been withdrawn from clinical developed due to pharmacokinetic defects. According to a 1997 report by the Drug Research Center, of 198 pharmaceutical candidate compounds withdrawn from development during clinical trials in the United Kingdom, 39% were due to pharmacokinetic unsuitability, 30% were due to lack of medicinal efficacy and 21% were due to issues with safety [18:87].

The importance is now becoming recognized of not only carrying out this testing in the latter part of the drug discovery stage, but also carrying out pharmacokinetic testing together with drug efficacy testing in order to ascertain the pharmacokinetic properties. Policies are beginning to be devised to incorporate *in vitro* evaluation systems from the early stages of drug discovery.

(6) The Adoption of Genomic Drug Discovery into Clinical Trials

Despite the increasing research and development costs of drug discovery companies, the number of new drugs being released on the market has actually reduced since the mid-1990s in the United States. One such example is the recalled product Vioxx. Researched and developed by Merck, Vioxx emerged as a selective cyclo-oxygenase II (COX-2) inhibitor with no digestive tract side effects. It was marketed in the United States in 1999, followed by 80 countries worldwide, as an anti-inflammatory and anti-rheumatic; it grew into something of a cash cow for Merck, with sales

reaching around $2.5 billion in 2003. Merck conducted a clinical trial aimed at expanding the application of Vioxx for use as a secondary prevention for duodenal cancer; however, patients who took the drug for 18 months or more were found to be with increased risk of myocardial infarction and other heart disease events. Accordingly, the company announced a voluntary recall on the drug at the end of September that year. This was a major blow for Merck, as it not only suffered the loss of all future profits from the drug, but was also faced with the issue of patient compensation (see Section 2.4.4 Anti-Inflammatories).

A number of drugs other than COX-2 inhibitors were also subjected to voluntary recalls. The growing public awareness of side effects meant a greater demand on companies to take extra care in development. This meant tests to ensure a greater level of safety in clinical trials and at the drug discovery stage. Clinical trials became longer, leading to increased research and development costs.

Table 3.4 shows the major pharmaceutical products worth more than ¥10 billion that had been withdrawn from the American market in the 2000s.

Table 3.4 Pharmaceutical Products Withdrawn from the American Market due to Serious Side Effects

Drug name	Manufacturer	Generic name	Indications	Side effects	Year approved	Year withdrawn
Rezulin	Sankyo	Troglitazone	type II diabetes	liver damage	1997	2000
Propulsid	Janssen	Cisapride	gastrointestinal disease	ventricular tachycardia	1993	2000
Raplon	Organon	Rapacuronium	anesthetic	bronchial spasm	1999	2001
Baycol	Bayel	Cerivastatin	hyperlipidemia	serious myopathy	1997	2001
Vioxx	Merck	Rofecoxib	anti-inflammatory	cardiovascular damage	1999	2004
Bextra	Phizer	Valdecoxib	analgesic	Stevens-Johnson syndrome	2001	2005

Source: Excerpt from Shiew-Mei Huang et al.: Toxicology Mechanisms and methods 16, 89-99 (2006).

Japan started implementing policies to keep drug prices down as a means of curbing the ever-increasing cost of medical treatment. However, stricter auditing to ensure new drugs were safer and more effective meant greater research and development costs. Drug companies now had to think of ways to carry out their research and development more efficiently.

There were hopes that the epoch-making arrival in the 2000s of genomic drug discovery and other new drug discovery technologies would usher in a host of superior pharmaceutical products with globally-innovative mechanisms of action, but in fact, contrary to expectation, no new drugs were developed, other than biopharmaceutical antibodies. The FDA has indicated that despite a number of new discoveries in new basic research in recent years, there are fears that rather than leading to any more useful pharmaceutical products, these will only lead to longer development times and more failed developments due to inadequate efficacy and safety evaluation methods.

The Critical Path Initiative promoted by the FDA in 2004 encouraged the creation of better treatments using more efficient research and development methods with efficacy and side effects worked out in advance, rather than the conventional development method of trial and error. While in the past biomarkers had long been used empirically, many of them had no theoretical backing for predictability. Selecting better biomarkers in future will make it possible to determine medicinal efficacy and predict side effects more accurately during the drug discovery process; there are also hopes that this could be put to use for diagnostic drugs in clinical trials. Selecting more appropriate biomarkers should reduce the pharmaceutical product dropout rate [21, 22].

The biomarkers indicated by the FDA are biochemical or biological reactions produced by the body; these include DNA, m-RNA, proteins, receptors and carbohydrate chains, as well as blood pressure and positron emission tomography (PET) in a broader sense [23].

Selecting appropriate biomarkers will ultimately lead to personalized medicine, in which medicinal efficacy and side effects for an individual can be predicted by genetic diagnosis. However, making any progress in this direction will be no simple matter.

Since personalized medicine means preparing drugs for patients individually, it means an inevitable increase in the number of different types of drugs, as well as having to find out information on patients' gene and protein expression in advance. From a business perspective, this level of diversity may run contrary to efficient research and development. There is also concern that it will increase expenses for the patient. Doctors in particular may face an increased weight of responsibility.

This idea will require diagnostic drugs and treatment drugs to be developed together from early in the exploratory stage. In Japan, these are separate industries and are still at the trial-and-error stage. Although this has been trialed since 2000 by Mitsubishi Chemical Holdings, a group encompassing drug discovery, diagnostic drugs and clinical testing operations, the road ahead is anything but smooth.

Another fundamental issue is whether specifically-limited biomarkers alone can identify people as responders (people for whom the drug has medicinal efficacy) or non-responders (people with no reaction to the drug), due to the fact that multiple enzymes and receptors are involved in causing diseases and in drug metabolism. The same is true for side effects.

It is difficult to develop a drug that will be effective for everyone, regardless of individual differences; however, personalized medicine also presents huge barriers. In Japan, the Japan Pharmaceutical Manufacturers Association established the Japan Pharmacogenomics Consortium (JPG Consortium) in 2003, with 11 member companies taking part. Some results were seen from a clinical pilot trial exploring the SNPs involved in aspirin resistance, but there has been little development since [24].

While Japanese drug discovery companies are taking note of FDA policies, they are approaching those policies with caution. However, other matters outlined in the Critical Path Initiative are already known to Japanese drug discovery companies through their own experiences. Companies will continue to use their own experiences in future developments in drug discovery [25].

Note 1: Target discovery generally refers to determining the point of action (target) of a drug; the discovery of targets in genomic drug discovery is often termed as target discovery.

Note 2: Innovative new drugs, or blockbuster drugs, have recently been referred to as “first in class”. Improved drugs with superior properties are referred to as “best in class”. While it is important to try to produce blockbuster drugs, both to advance science and to prove the instincts of researchers correct, improved drugs are very significant in that they resolve issues found in prior products in terms of side effects and medicinal efficacy. In many cases, it is the improved drugs that experience growth on the market and are administered widely to patients.

Note 3: The International Union of Pure and Applied Chemistry (IUPAC) defines a pharmacophore as “the ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target structure and to trigger (or to block) its biological response”. This concept is not new; it was proposed by Ehrlich in 1909.

Note 4: Pharmacokinetics: Drugs enter the body through various methods of administration (administration), are distributed within the body (distribution), are metabolized by the body (metabolism) and are eventually excreted from the body (excretion). This sequence is referred to as ADME. Ideally, drugs maintain their medicinal efficacy by remaining in the body for a certain length of time, have no metabolite toxicity and are promptly excreted from the body after serving their purpose.

Note 5: The International Genome Sequencing Consortium started in 1990 with the participation of the United States, Japan, the United Kingdom, Germany and other countries. In June 2006, US President Bill Clinton and British Prime Minister Tony Blair announced the results of the decoding at a press conference. Private venture Celera Genomics, led by John Craig Venter, was opposed to the international project and fierce competition ensued to gain precedence in the field. In February 2001, the international project team published the genome sequence in *Nature*, while Celera Genomics published its own results in *Science*. The complete version was made public in 2003, comprising 99% of the human genome sequence with 99.99% accuracy.

Note 6: The optimization of candidate compounds has conventionally been achieved by medicinal chemists using a mixture of experience, hard work, intuition and luck. While there are hopes that computer theory and the latest advances in technology will be put to good use in optimization, new technology alone does not necessarily mean perfect drug discovery and the role played by medicinal chemists is as important now as it ever was. The success probability of producing a single drug is the topic of much discussion. Although the use of robotized synthesis and compound libraries has rapidly increased the number of compounds that can be tested (the number of samples), it does not mean that the success probability has increased; the capabilities of medicinal chemists are as highly regarded as ever.

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4 Detailed Discussion (Lifestyle-Related Diseases)

This chapter addresses lifestyle-related diseases and discusses the history of new drug discovery for diabetes, hypertension, hyperlipidemia, hematologic malignancy and hyperuricemia from the postwar period to the present. Although a great many other pharmaceutical products have been developed for other diseases as well, including antibiotics/antibacterials, central/peripheral nervous system drugs, circulatory system drugs, endocrine system drugs, anticancer drugs, osteoporosis drugs, renal disease drugs and sensory organ drugs, these will need to be discussed on another occasion.

4.1 Medications for Diabetes

According to the 2011 *Patient Survey*, conducted every three years by the Ministry of Health, Labour and Welfare, the total number of diabetes sufferers (patients assumed to be receiving ongoing treatment) was 2.7 million (1.48 million males and 1.22 million females). The number of adults suspected to have diabetes could be as high as 9.5 million. The production value of drugs classified as diabetes drugs has grown in a decade from ¥112.5 billion in 2003 to ¥225 billion in 2012. According to the International Diabetes Federation (IDF), in 2012 there were 371 billion diabetes sufferers worldwide between the ages of 20 and 79; Japan ranked ninth with 7.1 million diabetes sufferers, behind China, India, the United States and other countries.

There are two types of diabetes, according to the pathology. The first is called type 1 diabetes (previously known as insulin-dependent diabetes), in which the beta cells in the islets of Langerhans in the pancreas are destroyed by viral infection, autoimmunity or chemical substances. While it often occurs in young people between the ages of 10 and 20, it has a low occurrence rate in Japan, accounting for only 5% of all diabetes sufferers. Since the body does not produce enough insulin, insulin has to be supplied externally.

The other type is called type 2 diabetes; in addition to genetic predisposition, onset can also be triggered by overeating, lack of exercise and stress. It is thought to be caused by either (1) insufficient insulin from the pancreas or (2) insufficient insulin action due to deterioration in insulin receptor function in the muscles and liver. Onset frequently occurs in people who gain weight in middle age; almost all instances (95%) of diabetes at the present time are this type. Insulin has been administered as a specific medicine to sufferers of type 1 and type 2 diabetes since 1922. Improvements continue to be made to insulin injections.

Other types of diabetes include gestational diabetes and secondary diabetes caused by other diseases; however, these account for very small numbers of patients.

The cells in our bodies act in various ways to keep us alive, using glucose absorbed from the digestive system as an energy source. The blood glucose level in the body increases after eating; the beta cells in the islets of Langerhans in the pancreas are activated by the high blood glucose levels to release insulin. This action causes the glucose to be rapidly taken and used by the tissue cells (liver, muscles, fat cells, brain, etc.), thereby lowering the blood glucose level. During fasting, the body produces a certain amount of glucose, either by breaking down the glycogen stored in the liver and converting it into glucose or by synthesizing glucose (gluconeogenesis) from amino acids, lactic acid and glycerol, and releases it into the bloodstream. Under normal insulin conditions, the production of ketone bodies (beta-hydroxybutyrate, acetoacetate and acetone; abnormal glucose metabolism causes increased compensatory lipid utilization and increased ketone body production) is suppressed. During fasting, ketogenesis occurs, resulting in metabolic acidosis. Hyperglycemia also triggers diuresis with concurrent Na^+ and K^+ loss, eventually resulting in a coma.

As mentioned above, there are thought to be two potential causes for ongoing high blood glucose levels: (1) a shortage of insulin from the pancreas, either by delayed timing in releasing the insulin or by an insufficient amount of insulin being released; (2) the insulin receptors and the subsequent signal transduction not functioning properly despite the presence of insulin (insulin resistance). The latter can also be caused by the tissue reacting to the insulin. Postprandial hyperglycemia, in which the blood glucose levels in the body elevate only after eating, is an indication that the body is not secreting enough insulin; it is thought to be the first stage of deterioration in pancreatic function. If tissue becomes resistant to insulin whether it is in sufficient supply or not, blood glucose levels will remain elevated for longer. One theory proposes that since glucose reacts easily with certain amino acids in some peptides and proteins, prolonged periods of elevated blood glucose levels will cause a glycation reaction (Maillard reaction), during which the proteins in various cells in the tissue will degenerate.

The glycation reaction forms chains, ultimately producing advanced glycation end products (AGEs). While this is not yet fully understood, there are reports indicating that AGEs bond to receptors for AGEs (RAGEs) and cause various impairments. The degenerated proteins are the cause of three

major complications, namely diabetic neuropathy, diabetic retinopathy and diabetic nephropathy, as well as causing arteriosclerosis in major blood vessels and causing heart attacks and strokes. The glycation reaction due to hyperglycemia also increases susceptibility to infection by causing abnormalities in immune cells. The diabetes biomarker is glycated hemoglobin HbA1c (see Figure 4.1).

Hypoglycemia, the reverse of hyperglycemia, often occurs in conjunction with pharmacotherapy for diabetes. People who take insulin injections or hypoglycemic drugs during treatment can experience hypoglycemia if they engage in strenuous activity, skip a meal, or if they accidentally overdose on their medication. Hypoglycemia causes a variety of symptoms, which can also include anything from a loss of consciousness to a coma; accordingly, proper care must be taken [1, 2, 3]. Figure 4.2 shows the operation of insulin and blood glucose in the body.

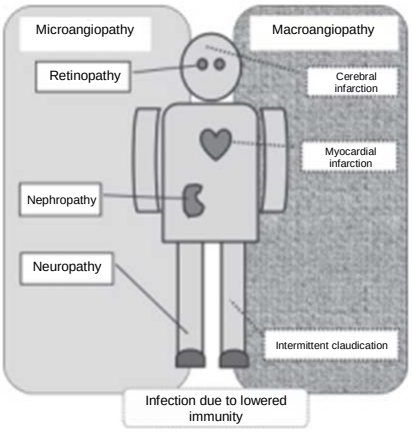
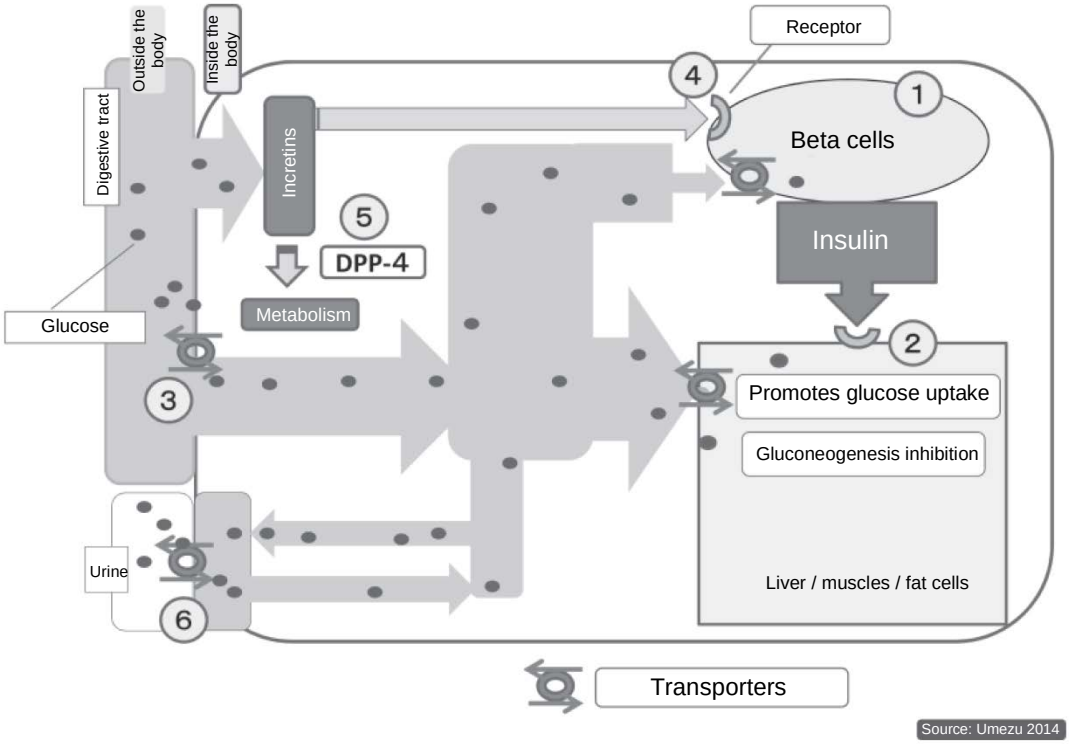


Fig. 4.1 Complications of Diabetes.



- ○ Blood glucose is controlled by insulin.
- ○ Glucose absorbed into the blood from food is taken in by the tissue (liver, muscles, fat cells, brain, etc.) as an energy source.
- ○ During this time, insulin promotes “migration to tissue” and “utilization of glucose within the tissue”.
- ○ Hyperglycemia is sensed and insulin is secreted by the beta cells in the pancreas. Incretins are secreted from the digestive tract as hyperglycemia is sensed and promote insulin secretion in a different process.
- ○ Glucose excreted from the blood into the urine is reabsorbed in the uriniferous tubules and returns into the blood.

①-⑥ show the points of action of diabetes drugs.

- ① SU drugs and glinide drugs act on the beta cells to promote the secretion of insulin.
- ② Insulin sensitizers (biguanides, thiazolidinediones) act on the liver and other tissues.
- ③ Glucose absorption inhibitors (voglibose) inhibit glucose absorption from the digestive tract.
- ④ Incretins promote insulin secretion.
- ⑤ Inhibiting incretin catabolic enzymes increases the efficacy of the incretins.
- ⑥ SGLT2 inhibitors inhibit the reabsorption of urinary sugar into the uriniferous tubules, thereby lowering the blood sugar level.

Fig. 4.2 Operation of Insulin and Blood Glucose.

While lifestyle-related diseases have gained more attention in recent times, there are few records of them from ancient times. However, diabetes is one such disease for which ancient records exist from various different regions.

The Ebers Papyrus, which dates back 3,500 years, records a disease that causes "excessive urination". In Japan, *Shōyūki*, the journal of Fujiwara no Sanesuke, appears to record that Fujiwara no Michinaga died from diabetes. Michinaga's condition is described as "having constant dryness in the mouth, feeble and drinking much water," "emaciated in the body and lacking strength," "tumors forming on the back" and "unable to see" [(4)]. This is thought to be a good representation of the characteristics of diabetes.

On a side note, *Shōyūki* also records in the fifth month of 1016 that "Yorihide said that the death of Michinaga may be imminent" [(4)].

It seems to have been known since ancient times that a disease producing glucose in the urine was caused by insufficient exercise and excessive dietary intake. However, it was not until 1922 that insulin was discovered by Banting et al., while low-molecular-weight drugs to combat diabetes did not emerge until the mid-20th century.

The Story of Insulin states, "Can diabetes be prevented or its commencement postponed in prediabetics? Not all prediabetics go on to develop diabetes. We cannot answer this question at the present time." By the standards of medical and pharmaceutical sciences at the time in the 1940s, there would have been much that was yet unknown regarding the prognosis for diabetes [(5)].

In 1947, glucose tolerance tests were carried out on 3,516 residents of Oxford, Massachusetts in the United States due to the similarity of the age demographics of the town to those of New York. The study found that 70 people, or 2% of the subjects, were diabetic; as many as half of them were unaware of their diabetes. A further 118 subjects (3.5%) made up the pre-diabetic group. Following this, similar tests were carried out in various different countries around the world; it was estimated that around 1-1.5% of Americans and Canadians had diabetes at that time. With diabetes patients in Japan accounting for 2.1% of the population in 2011 and assuming the number of presumed diabetes sufferers to be 7.3% (mentioned above), it is surprising how many diabetes sufferers there were in the developed countries, even just after the war, when the food situation would have been unfavorable [(5:184-189)].

Although *The Story of Insulin*, published in 1962, discusses the history of insulin in detail, it does not mention any research on the action mechanism of insulin at molecular level. While it was known at the time that type 2 diabetes is related to weight and genetics and that there were patients with prediabetes, it was not until the advances in biochemistry and molecular biology in the 1980s that research on insulin receptors, their signal transduction and their connection to blood sugar levels began to accelerate.

In 1988, Gerald Reaven proposed the theory that the lifestyle diseases of obesity, hypertension and diabetes were caused by insulin resistance and poor glucose tolerance, leading to cardiovascular disease (Syndrome-X) [(6)].

The following year, Norman Kaplan named the synergistic deterioration effect of obesity (upper-body obesity), glucose intolerance (diabetes), hypertriglyceridemia (hyperlipidemia) and hypertension as "the deadly quartet" [(7)], triggering a wealth of research on insulin-resistance syndrome. In 1998, the WHO published the diagnostic criteria for what it termed "metabolic syndrome"; this syndrome has become widely known, even in Japan.

Since 2008, the Ministry of Health, Labour and Welfare in Japan has required medical insurers to carry out specific health examinations and provide specific health advice for around 52 million medical insurance subscribers and their dependents, between the ages of 40 and 74, with specific focus on metabolic syndrome. Simple tests, such as blood sugar, fat, blood pressure and girth, are used identify pre-metabolic syndrome individuals, who are then given advance health advice to maintain good health, thereby reducing medical expenses.

However, the implementation rate has been lower than initial predictions, at 38.9% in 2008 and 46.2% by 2012. Of all those tested in 2012, 17.7% were targeted to be given specific health advice, while only 16.4% actually received that advice. While the number of people being medically examined each year is increasing, the general population still remains largely unaware of metabolic syndrome.

4.1.1. The History of Insulin as a Medicine

Insulin for diabetes has been a wonder drug (mentioned previously). In 1923, Eli Lilly & Co. began mass producing insulin from bovine and porcine pancreases; subsequently, production began in countries around the world. Early insulin formulation required refining due the presence of impurities; however, this presented a paradox in that the more it was refined, the more effective the medicine, but the shorter the effective working time. This was because the impurities prevented the insulin from breaking down. Accordingly, research began on how to make insulin effective for longer, eventually resulting in the discovery in 1936 by Hans Christian Hagedorn that protamine, a protein isolated from fish sperm, could be used to prolong the effectiveness of insulin. In 1938, D. A. Scott and A. M. Fisher of Canada reported that the effectiveness of insulin could be prolonged (hypodermic injection twice a day) if the insulin was mixed with protamine as well as the zinc used for insulin crystallization. It was found that by adjusting the ratio of zinc and protamine, the period of effectiveness could be extended from seven hours to three days. This PZI formulation became

extremely widely used, thus improving insulin treatment. This principle is still used today [5:96-103].

However, when it was applied clinically, many patients became hypoglycemic from the PZI regime. Doctors needed a form of insulin somewhere in between the fast-acting crystalline insulin and the prolonged-effectiveness of PZI. Various formulations were devised; Louis Bauman of the Wellcombe Trust developed globin insulin in 1939. Red blood cell globin combines with insulin to produce an intermediate-acting preparation. In 1945, Meller et al. of Novo developed a formulation that did not contain any proteins other than insulin (Lente insulin). In 1946, Hans Christian Hagedorn developed NPH insulin with a reduced amount of protamine. Research on the effectiveness period of insulin has continued since then to the present, with various different formulations being devised and developed. In 1978, Arthur Riggs and Keiichi Itakura used advanced genetic engineering technology to successfully produce human-identical insulin from *E. coli*. In 1985, Eli Lilly started selling insulin produced from *E. coli*, while Novo Nordisk did the same in 1987 with insulin produced from yeast cells. These formulations improved the safety of insulin treatment and also made it possible to start mass producing insulin formulations [5].

Meanwhile, according to *A 50-year History of New Drugs in Japan* by Hikaru Ozawa [9], insulin was in wide use from around the middle of the Second World War and into the postwar era (1940-1956), being produced by Shimizu Seiyaku from *Katsuwonus pelamis*, *Thunnus orientalis* and other bony fish, which were abundant in supply in Japan. The entry for “insulin injections” in the sixth revision of the *Japanese Pharmacopeia* (1951) describes it as an “aqueous solution containing a hypoglycemic ingredient from the Langerhans cells of edible animals or fish”. The introduction of long-acting insulin formations developed in the West during the war was somewhat delayed, with import approval given for protamine zinc insulin in 1951, for globin zinc insulin in 1952, for NPH insulin in 1955 and for Lente insulin in 1956.

The next step in insulin development was the development of insulin that was more physiologically effective. While the insulin secreted from the pancreas is a monomer, the fast-acting insulin was a hexamer that was highly stable in formulations. When the fact-acting insulin was injected, it broke down from a hexamer into dimers and monomers and was transported throughout the entire body in the bloodstream. Since it took around 30 minutes to come into effect, it had to be injected around 30 minutes before eating. Meanwhile, the insulin formulations with additions protamine and zinc to prolong the effectiveness circulated through the body in fixed amounts 24 hours per day; these in no way imitated the natural insulin secretions of the body. Consequently, studies began on “insulin analog formulations” with slightly modified amino acid sequences. Ultra-fast-acting insulin analog formulations, which took effect even more quickly than the fast-acting formulations, were released by Eli Lilly & Co. in 1995, by Novo Nordisk in 1999 and by Aventis (now Sanofi) in 2004 [10].

Meanwhile, devices were being developed to self-administer insulin. The pen-type insulin syringe emerged to combat the inconvenience of carrying around big syringes every day. In 1985, Novo Nordisk launched the world’s first insulin pen, with the insulin formulation held in a cartridge loaded into the pen, and the needle being the only non-reusable part (launched in Japan in 1988). It looked just like a pen. The user would attach the disposable needle to the pen, insert the needle under the skin and then press the injection button once to administer two units of insulin into the body. This dramatically increased its portability and greatly reduced the inconvenience associated with injections. At present, each insulin formulation manufacturer is marketing its own self-administered injection device, with various different syringes and improved modifications [Note 1].

While insulin treatment in Japan has progressed by means of various different types of insulin from the West, it was not until 1981, around 60 years after the discovery of insulin, that patients were granted legal approval to inject their own insulin. Although self-administration of insulin by patients had been a matter of course in the West since insulin was first discovered, it seems that it was harder to accept in Japan, amidst apprehension about patients administering injections with no medical qualifications.

Despite Keiichi Itakura et al. at the American biotechnology venture Genentech successfully producing human insulin by genetic engineering in 1978, it was Eli Lilly, Novo Nordisk and other conventional Western corporations that led the world in the technology for administering insulin to patients; unfortunately, Japanese drug discovery companies trailed behind in this arena [11].

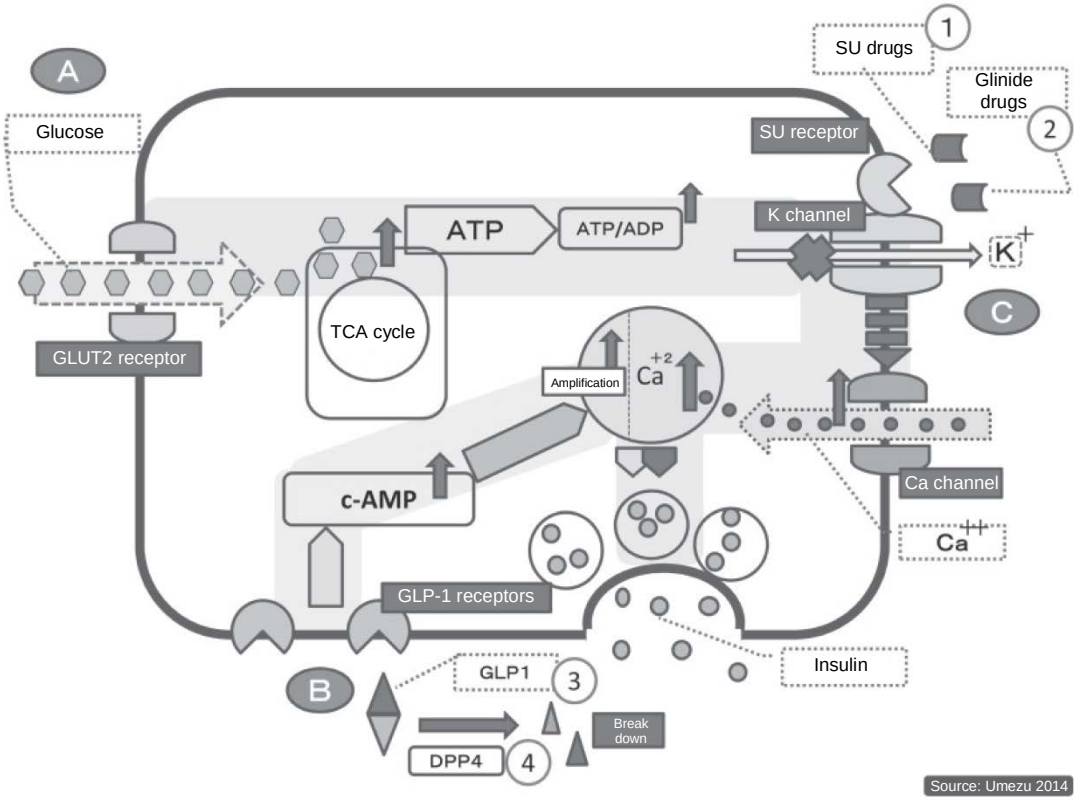
However, with many patients finding self-administered injections inconvenient, research was also continuing on orally administered drugs. Japanese drug discovery companies in particular were achieving results from their dedicated research and development of oral diabetes medications.

4.1.2. Sulfonylurea (SU) Oral Diabetes Medications

In 1942, Marcel Janbon discovered that one of the sulfa drugs used in clinical studies for typhoid fever contained a compound that had a hypoglycemic effect [12]. This compound became the first sulfonylurea oral diabetes drug. On learning this, many chemists started screening in order to synthesize analogous compounds. This resulted in the synthesis of sulfonylurea drugs Carbutamide in 1955 and Tolbutamide in 1956, still used today, by German companies Boehringer Mannheim and Farbwerke Hoechst. Another SU drug, Glibenclamide, emerged in 1969. Glibenclamide is also a sulfonylurea drug and was selected from among around 8,000 similar hypoglycemic compounds out of consideration for its efficacy and level of toxicity.

Around this time, it was reported that new oral diabetes drugs were ineffective against type 1 diabetes and ineffective against serious cases of type 2 diabetes. Tolbutamide was later shown to have a mechanism of action of stimulating the pancreas to release insulin, thereby showing Tolbutamide to be ineffective when the pancreas is not working properly, such as with type 1 diabetes.

Recent research has proposed an arrangement in which the sulfonylurea (SU) receptors exist alongside the ATP-sensitive K^+ channel on the membrane of the beta cells of the pancreas; when SU derivatives bind to the SU receptors, which form part of the K^+ channel, the K+ channel closes and depolarization occurs, whereupon the calcium channel on the membrane opens accordingly and Ca^{2+} (calcium ions) flow into the cells from outside, resulting in the secretion of insulin (see Figure 4.3) [13].



The detailed mechanism of action of diabetes drugs in relation to the release of insulin from the beta cells is now becoming clear.
A: Glucose stimulation route
B: Incretin stimulation route
C: SU (sulfonylurea) receptor route

①-④ show the points of action of diabetes drugs on the beta cells in the pancreas.

Fig. 4.3 Beta Cell Insulin-Releasing Drug Mechanisms.

The first generation of SU drugs to be developed (Tolbutamide, Acetohexamide, etc.) had low potency and had to be administered in high volumes to be effective for treatment; however, administration in high volumes caused hypoglycemia. SU drugs were also problematic in terms of interactivity, in that they bonded strongly with albumin in the blood. The second-generation drug Glibenclamide was introduced to Japan by Hoechst in 1971 and marketed by Chugai Pharmaceutical [14]. Gliclazide was introduced by Servier in 1984 and marketed by Dainippon Pharmaceutical (now Dainippon Sumitomo Pharma). These were more potent than the first-generation drugs and also had lower interactivity with other drugs due to their nonionic bonding properties with blood albumin. Glimepiride (Sanofi-Aventis), which emerged in 2000, is called a third-generation drug, as it works despite poor insulin secretion, its hypoglycemic effect is equal to or better than other SU drugs and it is thought to reduce blood sugar levels by acting outside of the pancreas (promoting glucose use in the muscles and inhibiting glucose release from the pancreas) [15].

Having noted the insulin release effect, Japanese drug discovery companies have focused on research and development of "fast-acting insulin secretagogues", which enhance this effect. These "fast-acting insulin secretagogues" could be called the fourth generation SU drugs, and they have their origins in Japan [16:25] (mentioned later).

While SU drugs are often used together with insulin, side effects can include susceptibility to hypoglycemia or an increase in body weight.

In 1970, the results were published for the University Group Diabetes Program (UGDP), a large-scale clinical test on major SU drugs for side effects [17]. The results showed a 75% increase in cardiovascular deaths in the Tolbutamide treatment group over the placebo group; the use of SU drugs decreased amidst the ensuing debate on SU drug usage. A number of other tests were carried out; the United Kingdom Prospective Diabetes Study (UKPDS), a large-scale clinical trial conducted from 1977 to 1991 (Note 2), dismissed the hypoglycemic effect of SU drugs and the risk cardiovascular death [18]. Nevertheless, results of other small-to-medium-scale tests have confirmed these risks, meaning that further research is still needed.

4.1.3. Fast-Acting Insulin Secretagogues

Japanese characteristically have lower insulin secretion capacity than Westerners; there are many cases of people with abnormal glucose tolerance whose release of insulin after eating is at a slower rate than the elevation in their blood sugar level. Glinides were developed specifically for the Japanese in order to combat this issue. These drugs are characteristically fast-acting, thereby having the effect of increasing insulin secretion within a short space of time. The concentration in the blood peaks within 30 minutes to 2 hours before being metabolized by the liver and then promptly excreted from the kidneys. This characteristically short period of effect is close to the normal insulin secretion pattern. In recent years, studies both in Japan and overseas have reported that postprandial hyperglycemia is an independent risk factor for causing diabetes-related vascular diseases [19].

The idea of preventing postprandial hyperglycemia led to the release of a succession of Japanese products, such as Nateglinide by Ajinomoto in 1999, followed by Mitiglinide by Kissei Pharmaceutical in 2004. This drug was more potent than Nateglinide, but without the side effect of postprandial hypoglycemia. Repaglinide was launched on the market in 2011 by Dainippon Sumitomo Pharma through Novo Nordisk.

The pharmacological effect of glinides is reported as inducing depolarization by binding to sulfonylurea receptor subunits in the ATP-sensitive K^+ channel to inhibit the K^+ channel and ultimately induce insulin secretion [20]. Since these have a less potent effect than SU drugs, they are suitable for mild type 2 diabetes patients with postprandial hyperglycemia. It should be noted that because they are a fast-acting insulin secretagogue, they have no effect unless they are used directly before eating and that they cannot be used in combination with other SU drugs with the same effect. Their characteristically short period of effect is close to the normal insulin secretion pattern.

Figure 4.5 shows the chemical structural formulas of different generations of typical sulfonylurea drugs.

4.1.4. Biguanides

Although C. K. Watanabe of Japan had reported in 1918 on observing a blood glucose effect in guanidine, the forerunner of the biguanides, this was overshadowed by the epoch-making discovery of insulin in 1922 and medical research shifted from that path. It was not until around 1961 that the biguanide Metformin gained a foothold as an oral diabetes medication. In the 1970s, there were lactic acidosis issues with Phenformin, another biguanide, resulting in the maximum administration dosage of metformin being halved. Limitations were placed on its indications and it became poorly rated. The results of the University Group Diabetes Program (UGDP), a large-scale clinical trial for SU drugs and biguanides, were also reported at the end of seven years of treatment [17, 21]. These results showed an increase in significant deaths among the SU drug Tolbutamide group and the biguanide Phenformin group over the control group; amidst growing apprehensions, biguanides and SU drugs declined in use.

It was later reported that Phenformin was the only biguanide to cause lactic acidosis. The double-blind Multicenter Metformin Study (1995) conducted in the United States reported that Metformin was very beneficial for type 2 diabetes accompanying obesity. The Diabetes Prevention Program (2002) also concluded clear vascular event inhibitory effects and diabetes onset prevention effects. On the basis of such results, the safety and utility of Metformin

began to be reconsidered. While the UKPDS also demonstrated that patients administered with Metformin showed improved cardiovascular prognosis [18], it took almost another 30 years for the era of lactic acidosis apprehension to finally come to an end.

Currently, Metformin is often used in conjunction with insulin due to its low potency on its own; however, there are sometimes issues with hypoglycemia due to concomitant administration.

The main pharmacological effects of biguanides take place outside of the pancreas. For example: (i) inhibiting the release of glucose by inhibiting gluconeogenesis in the liver; (ii) boosting the uptake and utilization of glucose by the skeletal muscle and the fat cells; (iii) lowering the rate of absorption of glucose from the digestive tract, thereby improving insulin resistance. Another important characteristic is moderate improvement in hyperlipidemia [2:368-369].

Into the 21st century, there have been many reports on the progress of research on detailed mechanisms of action at molecular level. These findings include increasing the cellular AMP/ATP ratio by inhibiting the mitochondrial respiratory chain complex I, thereby increasing the intracellular AMP to activate the AMP-activated protein kinase (AMPK) and thus improve the glucose metabolism, as well as stimulating GLUT4 transporter migration to the cell membrane and inhibiting the action of glucagon [21, 22].

4.1.5. Alpha-Glucosidase Inhibitors (α-GIs)

The sulfonylurea drug Tolbutamide went onto the market in 1957, followed by the biguanide Metformin in 1961; however, no further "new mechanism" oral diabetes drugs emerged for quite a long time after that. Although various companies continued research and development on new diabetes medications out of concern over the side effects of SU drugs and biguanides, it was quite some time before any new drugs hit the market.

Three decades later, in 1993, the α-glucosidase inhibitor Acarbose (Bayer) finally came onto the Japanese market, followed by the domestic product Voglibose the following year in 1994 by Takeda Pharmaceutical. A succession of other insulin sensitizers started being put into clinical use, including thiazolidines Troglitazone and Pioglitazone and fast-acting insulin secretagogues (glinides) Nateglinide and Mitiglinide. The research and development by Japanese drug discovery companies was finally starting to make some noticeable headway.

While glucose metabolism in the body is usually what comes to mind in relation to diabetes and insulin, the idea of "α-glucosidase inhibitors" is completely different. Carbohydrates are present in food in forms such as polysaccharide starch; these are broken down into oligosaccharides including disaccarides, mostly by the amylase in the saliva and secreted from the pancreas. The disaccharides are then broken down further into glucose by the α-glucosidase (an enzyme group rather than a specific enzyme, including maltase, sucrase and other disaccharidases) present in the small intestinal mucosa. The glucose is transported within the body by glucose transporters, which are unable to transport oligosaccharides or starch. If an inhibitor were to completely inhibit α-amylase and α-glucosidase, no glucose would be absorbed into the body. Since these do not actually block these enzymes 100%, glucose is transported within the body in smaller amounts or at a delayed pace. This creates similar conditions to dietary restrictions by using medication. While experiments on animals have confirmed the validity of blockers, there have been concerns about side effects in the gastrointestinal tract caused by undigested carbohydrates. In fact, Acarbose did cause gastrointestinal side effects, including flatulence, bloating and upset stomach.

Bayer's Acarbose tablets came in 50 mg and 100 mg sizes, while the dosage for the domestically-produced Voglibose was far lower at 0.2 mg and 0.3 mg, which was expected to improve the issue of side effects. Following clinical testing, Takeda Pharmaceutical launched Voglibose on the market one year after Bayer's product. It was used by postprandial hyperglycemic diabetics whose "blood glucose levels did not elevate significantly during fasting, but elevated rapidly after eating". The results were favorable; although there were some complaints of the digestive tract as feared, these were not enough to negate the main medicinal efficacy of the drug.

Takeda Pharmaceutical's *History of Innovative Research and Drug Discovery at Takeda* provides some information on the development history of Voglibose [23]. Basic research began at in 1973 at the Takeda Pharmaceutical laboratory with the aim of producing an anti-obesity agent. The idea was to create "a drug to curb obesity" by inhibiting the absorption of sugar. This was more than a decade before metabolic syndrome became the focus of attention.

The news that German company Bayer had already embarked on an α-glucosidase inhibitor project and discovered the blocker Acarbose (brand name Glucobay®) from culture filtrate in 1977 prompted Takeda Pharmaceutical to launch a full-scale project in its own laboratory.

Screening the compound bank and culture filtrates while taking hints from the structure of Acarbose resulted in the discovery of candidate compounds in 1981. Voglibose was eventually selected from the analogs that were synthesized. At the time, biological laboratories were using pathological animal models in their research to test their anti-obesity agents. New animals, such as KKA^y mice and Wistar fatty rats, were developed as new disease models for obesity and diabetes (see Section 4.1.6 below). Non-obese diabetic rats (streptozocin-administered rats) were also developed to manifest pathology similar to the Japanese. These models were used to demonstrate that Voglibose had an anti-obesity effect six to eight times greater than that of Acarbose, as well as a pancreas protective effect. Having a number of these independently-developed animals could be said to have contributed significantly to research and development.

Comparative studies on the medicinal efficacy of Voglibose and Acarbose revealed that while Voglibose at

low doses strongly inhibited blood glucose level elevation in animals given starch and disaccharides, it did not inhibit blood glucose level elevation in animals given monosaccharides or lactose. Meanwhile, Acarbose was confirmed to be 3,400 times more powerful than Voglibose at inhibiting amylase, resulting in undigested matter being excreted in the feces. This was because Voglibose had a very powerful α-glucosidase-specific inhibiting effect.

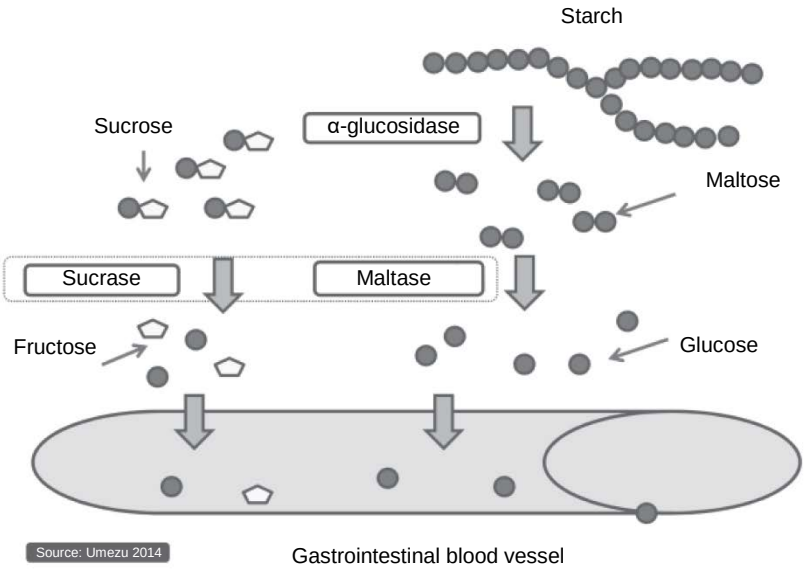
Later, when it came to the domestic phase 1 clinical trial stage of testing on healthy individuals, an issue arose with potential liver cell damage, with indicated elevated levels of liver function indicators aspartate aminotransferase (AST, identical to GOT) and alanine aminotransferase (ALT, identical to GPT). However, Takeda's central research laboratory hypothesized that "enzymes had escaped from the liver cells as a result of protein glucose production mechanisms being activated due to slow glucose absorption". The hypothesis was proven correct when the administration of Voglibose to rats on a high-protein diet inhibited the escaping enzymes.

Although obesity was the top application for Voglibose at the time, development of it as an anti-obesity agent had to be discontinued after severe abnormal abdominal symptoms manifested in a pre-phase 2 clinical trial pilot study in Europe.

Clinical studies later continued with the dosage reduced, the testing protocols modified and the target for the drug amended to "improving diabetic postprandial hyperglycemia". This was more effective than anticipated; thus, the new-concept postprandial hyperglycemia inhibitor Basen® came into being. This success was probably due to (i) the change in clinical indication, but mostly (ii) the skill of the research team at Takeda in developing their own various kinds of pathological animal models for diabetes and obesity at the basic research stage [24, 25]. Figure 4.4 shows the mechanism of action of α-glucosidase inhibitors, while Figure 4.5 shows the chemical structure.

4.1.6. Thiazolidine Derivatives (Insulin Sensitizers)

Diabetes is not only caused by insulin deficiency; it can also be caused by the loss of signal transduction by the insulin receptors in the liver, muscles or fatty tissue, regardless of whether or not enough insulin is being secreted, resulting in no glucose utilization and, consequently, hyperglycemia. This is known as "insulin resistance" and is a cause of metabolic syndrome.



- Starch is metabolized into maltose by α-glucosidase and then into glucose by maltase, and then absorbed into the body.
- Sucrose is metabolized into glucose and fructose by sucrase, and then absorbed into the body.
- Sucrase and maltase are α-glucosidase enzymes that metabolize disaccharides.
- When α-glucosidase is inhibited, the absorption of monosaccharides is inhibited.
- When amylase is inhibited, starch is excreted from the body without being metabolized.
- Compared to Acarbose by Bayer, Voglibose by Takeda (i) specifically inhibited α-glucosidase, and (ii) was not absorbed by the body. As a result, there were fewer side effects such as diarrhea; another significant feature was that since it was not absorbed, there was no buildup of hepatic glycogens and no inhibition of the lysomal enzymes of the liver.

Fig. 4.4 Mechanism of Sugar Absorption Inhibitory Action.

SU drugs and biguanides were the only diabetes drugs for quite some time; there were no diabetes drugs emerging with any new mechanisms of action. When Ciglitazone (Takeda Pharmaceutical) reached the clinical stage in 1981, the concept of “insulin-resistance-improving drugs” was ground-breaking. Many drug discovery companies, both in Japan and overseas, started competing to develop new drugs, screening peripheral compounds and eventually embarking on clinical trials. However, Ciglitazone lacked in medicinal efficacy and its development was discontinued (1983). After that, a succession of drug development companies also discontinued their developments after encountering side effects, presumably due to an issue with the basic thiazolidine structure, which had been the basis for the candidate compounds being developed. Drugs that made it as far as the market included Troglitazone (released 1997) by Sankyo, Pioglitazone (1999), developed by Takeda Pharmaceutical almost in parallel with Thiazolidine, and Rosiglitazone by GSK.

Let us now examine the history of research and development of “insulin-resistance-improving drugs”, a concept that originated in Japan. While having started out as a ground-breaking idea, the history of this new drug development has been one long battle against side effects. As mentioned in Section 4.1.5. Alpha-Glucosidase Inhibitors above, Takeda Pharmaceutical started out in the early 1960s to develop anti-obesity agents and hypolipidemic agents, with the bold new idea that “obesity underlies all lifestyle-related diseases” [(26)].

At the time, pathological animal models for obesity did not exist; however, the researchers at Takeda endeavored to develop them, with the idea that these animals would be needed in order to develop new drugs.

The diabetic KK mouse was produced by Nagoya University in 1963. Pathophysiological studies on this mouse showed a notable elevation in blood insulin concentration when it was overweight, thereby revealing an “insulin resistance” that lowered the effect of the insulin. This was at a time when the concepts of “insulin resistance” and “metabolic syndrome” were still unheard of.

Hyperinsulinemia was successfully induced by means of diet-controlled weight gain; this model corresponded to the prediabetes group in humans. However, due to the effort required to achieve this pathology and the time it took to build up the required number of mice, the researchers at Takeda decided to collaborate with Nagoya University to jointly develop spontaneous-onset mice. Researchers introduced the obesity gene A^y to the KK mice through breeding and by 1967 had successfully developed KKA^y mice (mice with spontaneous onset of obesity and diabetes). By 1974, a stable supply of these mice had been achieved for antidiabetic drug screening. Efforts were also put into developing pathological model rats, which were easier to use in experiments. At the time, it was known that Kyoto University’s spontaneously hypertensive “Wistar Kyoto rat” was mildly insulin resistant. This rat was bred with the genetically obese “Zucker fatty rat” to successfully produce the obese, diabetic “Wistar fatty rat”.

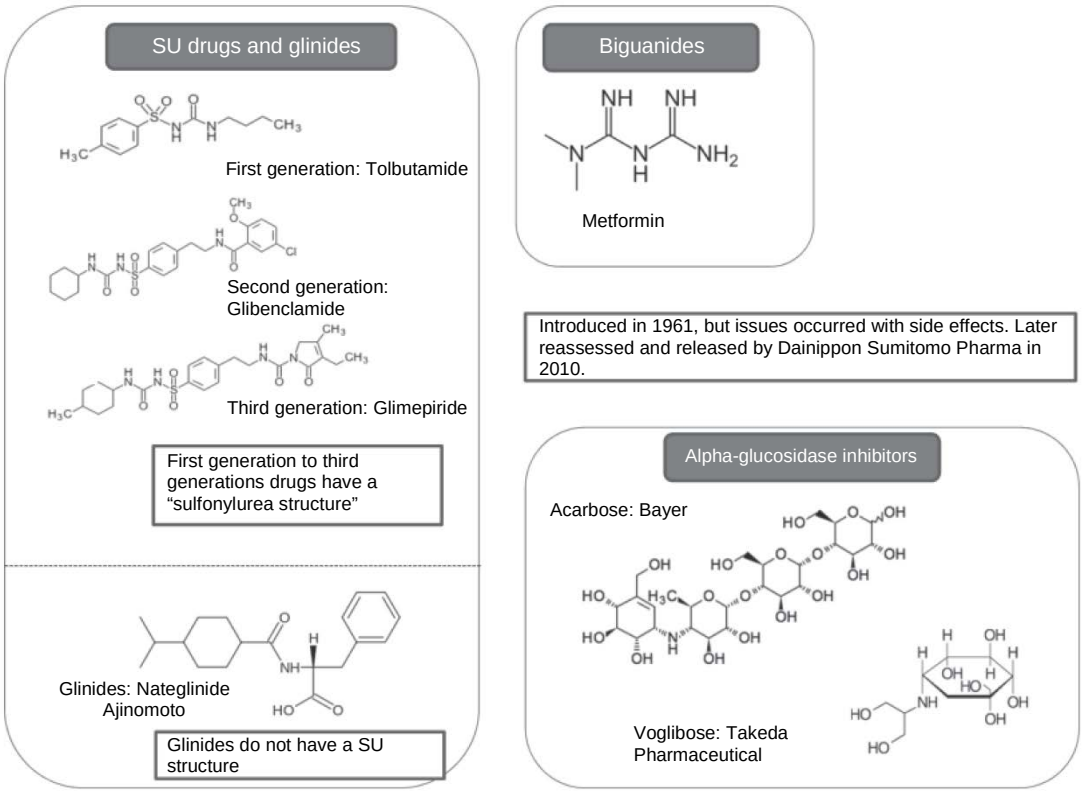


Fig. 4.5 Chemical Structures of Representative Diabetes Drugs [(1)].

These pathological animal models contributed significantly not only to screening, but also to research on the side effect mechanisms of Pioglitazone (released 1999) and to clarifying matters on insulin resistance [26].

Screening was conducted using KKA^{y} mice; of the compounds synthesized using the hypolipidemic clofibrate as the lead compound, AL-294 indicated powerful efficacy. This was used to obtain the compound AL-321, with a thiazolidinedione structure. Further derivatives were synthesized, ultimately producing Ciglitazone, with a cyclohexane ring in a side chain. Although Ciglitazone went to clinical trial in the United States in 1981, it demonstrated no medicinal efficacy; the trial was discontinued in 1983.

However, using the information that Ciglitazone metabolism involves more powerfully active substances, compounds were able to be obtained that were five to eight times more active by replacing the Ciglitazone "cyclohexane ring" with a hydrophilic substituent "pyridine ring". One such substance was Pioglitazone.

Around this time, Troglitazone by Sankyo also went to clinical trial and the competition grew fierce.

The "insulin sensitizer" Troglitazone was developed in Japan based on a completely different concept from Pioglitazone. In 1980, Sankyo started full-scale research on "diabetes medications with insulin sensitivity enhancing action". The hypothesis was that various disorders were caused by lipid peroxides, which increase in production in a diabetic state; *in vitro* screening commenced with the aim of finding a compound that would act to regulate the production of these lipid peroxides.

Having tested several hundred synthetic compounds, the synthesis team discovered the activity of CS-045, which has a Vitamin E partial structure. When this compound was assessed *in vivo*, it had no effect on regular mice. However, when administered into the feed of KK mice, the insulin-resistant pathological animal models with characteristic hyperinsulinemia, the blood glucose levels and insulin concentration in the mice was found to decrease markedly. The compound went into preclinical testing in 1984. Although its absorption rate decreased as the purity increased, greater absorption was achieved through amorphization of the compound's raw material to produce a solid dispersion with an irregular molecule arrangement. The animal experiments confirmed the compound to have the ground-breaking mechanism of action of reducing the blood glucose level by promoting glucose utilization in the liver, muscles and fatty tissue. Joint clinical development began in Japan and the United States. Following the clinical trials, the drug was launched simultaneously in Japan (Noscal®) and the United States (Rezulin®) in 1997; it was also launched in the United Kingdom (Romozin®) six months later. These were the world's first insulin sensitizers to be made available on the market, even earlier than Pioglitazone.

Clinically, the drugs demonstrated superior efficacy in patients who were secreting insulin but whose insulin was functioning poorly. However, Troglitazone was voluntarily recalled from the market in 2000 after some patients presented with liver damage [27].

Meanwhile, Pioglitazone by Takeda Pharmaceutical was jointly developed in the 1980s together with Upjohn (now Pfizer) in the United States. The results of clinical testing in the United States in 1990 indicated that the drug lacked adequate medicinal efficacy; Upjohn withdrew from the venture and development was suspended. However, following negotiation with the FDA and amendment of the clinical trial protocols, the clinical trials resumed in 1995. To ensure safety, 20,000 clinical test cases were carried out. Pioglitazone was later launched back on the market in Japan and the United States in 1999 and in Europe in 2000 [27].

The new idea of insulin resistance improvement using pathological animal model selection became clear-cut in the 1990s. PPARα was discovered as a receptor that activates peroxisome proliferation in the liver; its subtype PPARγ was also discovered. It was found that thiazolidine derivatives are a ligand (agonist) for these. Pioglitazone was found to act on nuclear receptor PPARγ to promote adipocyte differentiation and conversion to small adipocytes, thereby increasing insulin sensitivity and promoting glucose uptake. Reported research results included inhibiting gluconeogenesis in the liver and muscles to lower the blood sugar levels, administering Pioglitazone to reduce Tumor Necrosis Factor-α (TNF-α), Interleukin-6 (IL-6) and other insulin-resistance-promoting adipocytokines, increasing the blood concentration of adiponectin, which has an anti-arteriosclerotic action, and improving vascular endothelium function for macrophages. Although the idea of drugs to improve insulin resistance originated in Japan with an innovative mechanism of action, it is unfortunate that most of the academic research was conducted overseas [27, 28, 29].

With the mechanisms and testing methods made clear, more than ten Japanese drug discovery companies embarked on research and development on insulin sensitizers, although various companies pulled out due to a lack of safety assurance. In the end, the only thiazolidine derivatives left were Rosiglitazone by GlaxoSmithKline (GSK) (marketed in Europe and the United States, but not in Japan) and Pioglitazone by Takeda Pharmaceutical (marketed in Japan, Europe and the United States).

Since these drugs worked to improve insulin resistance, they had a fundamentally different mechanism of action from SU drugs and insulin. Rather than lowering blood sugar levels by taking a single dosage, they tended to lower the insulin concentration. Meanwhile, it was also found that the mechanism of action was related to increases in body weight; since body fluids would accumulate, attention had to be given to the burden placed on the heart.

Although Rosiglitazone (GSK) was marketed in the West as a superior diabetes drug, major issues were raised when the results of a meta-analysis combining a number of tests with different protocols (Note 3) were published by Steve Nissen and Kathy Wolski in the *New England Journal of Medicine* in 2007, entitled "Effect of Rosiglitazone on the Risk of Myocardial Infarction and Death from Cardiovascular Causes" (30). Based on the meta-analysis, the authors concluded that "until more precise estimates of the cardiovascular risk of this treatment can be delineated in patients with diabetes, patients and providers should carefully consider the potential risks of rosiglitazone in the treatment of type 2 diabetes". In response, GSK produced past clinical test cases and strongly argued that a conclusion could not be reached on the meta-analysis results alone. However, the attention drawn to the potential risk of heart disease became an issue for society and GSK took a 38% drop in turnover.

Ultimately, sales were discontinued in Europe, although the FDA initially allowed the drug to continue to be used in the United States with conditions in place; these conditions were lifted in June 2013. However, GSK had taken a severe hit.

Although Pioglitazone sales grew favorably in Japan and the United States as a promising new drug with a novel mechanism of action and efficacy, patients in a clinical trial in Europe (France and Germany) presented with bladder cancer in 2005. It was decided to include bladder cancer as a risk on the package insert (Note 4).

With the results of clinical studies in the United States showed the occurrence of bladder cancer as 6.9 per 10,000 Pioglitazone non-recipients (the control group) and 8.2 per 10,000 Pioglitazone recipients, the FDA has approved the continued sale of Pioglitazone (April 2014).

In August 2014, Takeda Pharmaceutical reported its ten-year epidemiological findings to the Ministry of Health, Labour and Welfare, the FDA and the European Medicines Agency (EMA). According to that report, "the primary analysis found no association between the use of pioglitazone and the risk of bladder cancer".

4.1.7. Incretin-Related Drugs (GLP-1 Analogs)

Following the discovery of insulin, there was a theory that substances other than insulin would lower blood glucose levels; these substances were termed incretins. In 1964, it was reported that after administering glucose to healthy individuals either orally or by intravenous injection to equalize their blood glucose levels and then measuring their blood insulin concentration, those who had been administered glucose orally had overwhelmingly higher insulin concentrations. It was presumed that the digestive tract had some kind of mechanism for secreting insulin due to glucose-related signal transduction from the beta cells in the pancreas.

After that came the discovery of the gastrointestinal hormone gastric inhibitory polypeptide (GIP) in 1969, followed by the discovery of glucagon-like peptide 1 (GLP-1) in 1983.

It was known that GIP is secreted by the K cells in the upper small intestine, whereas insulin is secreted by the pancreas. Once it was found that under diabetic conditions GIP not only lacks adequate insulin secretion promotion action, but also induces obesity by acting to promote fat uptake by fatty tissue, drug discovery companies lost interest in GIP.

Meanwhile, later research found that GLP-1 (a polypeptide made up of 29 amino acids in a particular 7-36 sequence of peptides with an insulin-secretion promoting action) directly acts on the beta cells in a glucose-dependent manner to elevate c-AMP concentration and induce insulin secretion, as well as acting on the alpha cells to inhibit glucagon secretion. While glucose promotes insulin secretion by the beta cells, the action of GLP-1 works as a "potentiator" to enhance the effect of Ca2+ in the insulin secretion mechanism. SU drugs are "initiators" that have the potential to cause hypoglycemia by inducing insulin secretion even in the presence of low glucose levels; by contrast, incretins are "potentiators" that do not promote the secretion of insulin where there are low glucose levels and no elevation in intracellular Ca2+ concentration. Since they only promote insulin secretion when there is an elevation in intracellular Ca2+ concentration, it can be assumed that they would not cause hypoglycemia (31). Research on GLP-1 as a drug has accelerated, with other recent findings showing GLP-1 to inhibit hyperplasia and cell death (apoptosis) in beta cells, as well as inhibiting gastric emptying and appetite loss.

Since the polypeptide GLP-1 can be rapidly metabolized in the blood by the proteolytic enzyme dipeptidyl peptidase-4 (DPP-4) at a rate of 95% within two minutes, only 5% or less of it reaches the original point of action, the pancreas. Accordingly, improved analogs have emerged on the market, having been synthesized to be and less easily broken down and to be administered by injection. Like insulin, the injections are performed by the patient using a pen-type syringe.

Liraglutide and Lixisenatide are GLP-1 analogs with some of the amino acids altered to become less easily broken down by DPP-4. Exenatide (Eli Lilly) is a synthetic compound produced using a 39-amino-acid peptide extracted from the Gila monster *Heloderma suspectum* to change the action site in order to avoid the action of DPP-4. Interestingly, 53% of this peptide found in the salivary gland of the *Varanoidea* superfamily of lizards shares the structure of human GLP-1.

The product was released on the Japanese market in 2010, imported from overseas. No domestic development was undertaken; instead, Japanese drug discovery companies entered the market by researching orally-administered DPP-4 inhibitors. This is characteristic of the Japanese medical industry, which tends to avoid injected medications.

4.1.8. DPP-4 Inhibitors

In 1994, DPP-4 was identified as the enzyme that breaks down incretins. Research began on inhibitors for this enzyme, as inhibiting DPP-4 would enable GLP-1 to survive longer in the blood, thereby enhancing its insulin-secreting action.

Unlike existing medications, DPP-4 inhibitors promoted insulin secretion in relation to glucose levels, thereby preventing hypoglycemia and weight gain. Accordingly, Sitagliptin, launched in the United States by Merck in 2007, gained blockbuster status within three short years of its approval. In terms of Japanese market trends in DPP-4 inhibitors, imported Sitagliptin was the first to hit the market in 2009. Seven new players hit the market between 2010 and 2013, with Vildagliptin (Novartis Pharmaceuticals) and Alogliptin (Takeda Pharmaceutical) released in 2010, Linagliptin (Boehringer Ingelheim Japan / Eli Lilly Japan) released in 2011, Tenegliptin (Mitsubishi Tanabe Pharma) and Anagliptin (Sanwa Kagaku) in 2012 and Saxagliptin (Kyowa Hakko Kirin) in 2013. While the Japanese system for new drug approval took longer than its Western counterparts, resulting in different release times, four of the seven new drugs were developed by Japanese companies, a commendable effort by the Japanese companies in the new drug development competition, considering that their strengths lie in developing improved new drugs. Since the forerunner Sitapgliptin was a renally-eliminated drug excreted in the urine through the kidneys, it could not readily be administered to patients with renal diseases; thus, some demand was noted for biliary-excreted DPP-4 inhibitors. However, lowering the dosage of Sitagliptin made it suitable patients with renal diseases and allowed it to leverage its position of primacy in the market.

Although there are variations between the DPP-4 inhibitors in terms of dosage amount, dosage frequency, excretion pathway, metabolic pathway and half-life in the blood, the utility and side effects can only be revealed through future results. When Japan was in the early post-marketing stage for DPP-4 inhibitors, an issue arose from a case report in which a disturbance of consciousness was caused by serious hypoglycemia following the supplementary administration of a DPP-4 inhibitor to a SU drug.

To clarify and respond to the issue, the Japan Diabetes Society established the “Committee for Proper Use of Incretin and SU Drugs” and issued a recommendation outlining proposed measures to be taken when concomitantly administering SU drugs and DPP-4 inhibitors. The number of serious hypoglycemia incidents has reduced since then. Fig. 4.6 shows the chemical structures of some representative diabetes drugs.

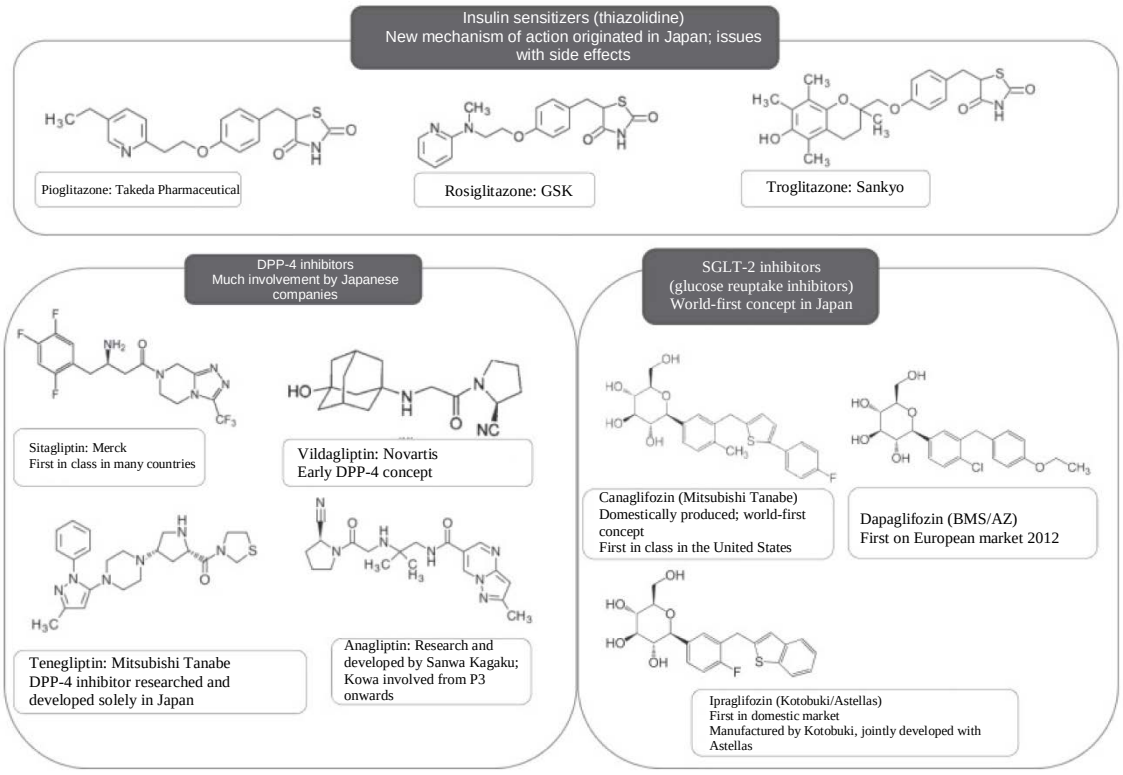


Fig. 4.6 Chemical Structures of Representative Diabetes Drugs [(2)].

4.1.9. Sodium Glucose Transporter-2 (SGLT-2) Inhibitors

Amidst fierce competition over DPP-4 inhibitors, diabetes drugs emerged with other new mechanisms of action. These were the SGLT-2 inhibitors. Canaglifozin, the world's first SGLT-2 inhibitor, gained approval in the United States in 2013; this was a new drug that was Japanese in origin (Mitsubishi Tanabe Pharma). The drug was licensed to Janssen Pharmaceuticals under the Johnson & Johnson umbrella; approval was granted in the United States in March 2013 and sales began immediately.

A polyphenol compound called phlorhizin started being extracted from the bark of pear and apple trees in 1835. In 1966, it was reported that this compound had a renal glycosuria inducing effect by means of a mechanism preventing the reuptake of glucose by the kidneys [(32)]. However, the compound was long disregarded due to its nephrotoxicity.

Some people, however, were interested in its pharmacological effects. Kenji Tsujihara, a researcher at Tanabe Seiyaku, considered the idea of treating a disease in which "glucose is present in the urine" (diabetes) with the reverse: a medication that "excreted glucose through the urine". Of all the many different medications for diabetes, this idea stands out as the most unique.

Although animal experiments using oral administration achieved no results, administration by injection was found to promote urinary sugar excretion and thereby lower the blood glucose level. A research project was commenced in 1990. The reason for the lack of efficacy with oral administration was that phlorhizin is a glycoside that bonds with glucose; when administered orally, it hydrolyzed into phloretin and glucose by the β-glucosidase in the digestive tract and lost its medicinal efficacy. Tsujihara et al. at Tanabe Seiyaku used this compound as a lead to identify the structure-activity correlation; the first results were published in the *Journal of the Pharmaceutical Society of Japan* in 1996 [(33)]. Further results were published the United States *Journal of Medicinal Chemistry* after narrowing down the candidate compound T-1095 [(34)]. Later, cooperative partner Janssen Pharmaceuticals began clinical trials in the United States in 2000.

However, these publications drew the attention of a number of drug discovery companies, both in Japan and overseas, who began their own research and development of these new diabetes drugs. Meanwhile, it was found during clinical testing that the compound T-1095 broke down considerably in the digestive tract, thereby requiring large doses to be administered in order to produce any effect. This threw up a red light for development, with the need for some kind of backup. With other companies catching up and proposing new mechanisms of action, Tanabe Seiyaku opted to withdraw from the frontline of development. At this time, Sumihiro Nomura, a medicinal chemist, drew on his prior knowledge and ingeniously came up with a backup within a short space of time. The result was TA-7284 (Canagliflozin) [(35)]. From late 2006 onwards, clinical testing was resumed overseas by Janssen Pharmaceuticals and in Japan by Tanabe Seiyaku.

Since then, clinical testing has been carried out on more than 10,000 type 2 diabetics. Despite some physicians expressing safety concerns for patients with poor renal function, the drug was deemed to have good overall tolerability and high efficacy; new first-in-class drug approval was granted in the United States. According to analysts, this drug is expected to achieve over $2 billion in annual sales worldwide.

The kidneys contain tissue structures called glomeruli, which filters the blood by means of the arterial blood pressure, with the primary urine excreted through the renal tubule. The renal tubule is divided into the proximal tubule and the distal tubule. As the primary urine passes through, around 90% of the high concentration of glucose contained in it is reabsorbed by a protein transporter known as sodium glucose co-transporter-2 (SGLT-2). The 10% of glucose remaining in the proximal tubule is reabsorbed by SGLT-1, meaning that 100% of the glucose is reabsorbed into the blood.

SGLT-1 is also expressed in the small intestine epithelial cells outside of the glucose-transporting tubular epithelial cells of the kidney. It plays a major role in glucose absorption in the body. Selectively inhibiting SGLT-2 would prevent the reabsorption of most of the glucose from the renal tubule, allowing it to be excreted as urinary sugar, thereby lowering the blood glucose level. Further, SGLT-2 selectivity would mean there would be no hindrance to SGLT-1 reuptake from the renal tubule, nor any hindrance to the absorption of glucose from the alimentary canal, thus no excessive hypoglycemia would be expected.

Despite concerns about infection of the urinary bladder and other areas of the urinary tract, clinical testing has shown fewer side effects than expected. Currently, SGLT-2 inhibitors continue to be developed in Japan; as of 2014, there are five types competing on the market.

These five SGLT-2 inhibitors include some of Japanese origin: Ipragliflozin, jointly researched and developed by Kotobuki/Astellas, Tofogliflozin, manufactured by Chugai Pharmaceutical, and Luceogliflozin, manufactured by Taisho Pharmaceutical. Canagliflozin by Mitsubishi Tanabe Pharma was released on the American market to become the world first.

This gives a sense of strength of the research and development capabilities of Japanese drug discovery companies.

4.1.10. Aldose Reductase Inhibitors

Epalrestat (Kinedak) is an aldose reductase (AR) inhibitor developed by Ono Pharmaceutical. While glucose in the body is mainly metabolized by glycolysis, the polyol metabolic pathway was discovered in 1956, whereby aldose

reductase reduces glucose to sorbitol, which is then metabolized into fructose by sorbitol dehydrogenase (SDH). Although only a little glucose is metabolized by the polyol metabolic pathway, under prolonged diabetic hyperglycemic conditions, AR is activated and the amount of glucose converted into sorbitol increases, thereby increasing the amount of sorbitol produced in the cells. In 1973, Gabbay et al. empirically demonstrated that increased sorbitol plays a serious role in the onset of diabetic neuropathy and also confirmed the presence of AR in the peripheral nerves, the retina, the crystalline lens and the kidneys; this study identified elevated polyol metabolism as an onset mechanism of diabetic complications that had previously had no clearly known cause.

Ono Pharmaceutical worked on researching and developing AR inhibitors, focused on suppressing sorbitol production. In 1992, the company obtained manufacturing approval based on efficacy and results for "improving subjective symptoms (numbness, pain) associated with diabetic peripheral neuropathy, abnormal vibration sensations and abnormal heart rate variability (where glycosylated hemoglobin levels are elevated)".

In 1998, six years after Epalrestat went to market, the Ministry of Health, Labour and Welfare issued a warning about it due to 17 reported cases of impaired liver function in which a causal relationship could not be ruled out. A number of other aldose reductase inhibitors being developed were abandoned. There is as yet no definitive means for preclinical testing to predict liver damage occurring clinically.

Note 1: Source: Novo Nordisk website (2014).

Note 2: The United Kingdom Prospective Diabetes Study (UKPDS) aimed to verify whether normalizing the blood glucose levels of type 2 diabetes patients could suppress the onset/development in blood vessel complications due to diabetes. Testing was carried out in 23 facilities around the United Kingdom, with multiple experiments strictly controlling blood sugar levels and also examining the differences between medications.

Note 3: A protocol is a written procedure outlining the plans and implementation details for a clinical trial.

Note 4: According to the report from the 2nd Pharmaceutical Affairs and Food Sanitation Council Subcommittee on Drug Safety in 2011, the onset of bladder cancer was 14/2,605 (0.5%) in the Pioglitazone group and 6/2,633 (0.2%) in the placebo group. Excluding the onset of bladder cancer within the first year of administration, the onset rate was 6/2,605 (0.23%) in the Pioglitazone group and 3/2,633 (0.11%) in the placebo group. Original reference: Lancet 366:1279, 2005.

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4.2 Medications for Hypertension

According to the 2011 *Patient Survey*, conducted every three years by the Ministry of Health, Labour and Welfare, the total number of hypertension sufferers (patients assumed to be receiving ongoing treatment) was 9.07 million (3.82

million males and 5.26 million females). The number of people with undiagnosed hypertension is assumed to be several times higher. Although there are relatively few subjective symptoms for hypertension, it is well known by most people today to cause stroke and heart disease.

The Japanese Society of Hypertension published the Guidelines for the Management of Hypertension 2014 (JSH 2014) in April 2014. While this revision maintained the diagnostic criteria for hypertension (criteria for commencing hypertension medication treatment) at the existing values of "systolic blood pressure 140mm Hg or higher, diastolic blood pressure 90mm Hg or higher", the antihypertensive target or non-binding target for lowering blood pressure for young and middle-aged hypertension patients was relaxed slightly from 130/85mm Hg to 140/90mm Hg to provide uniform diagnostic criteria (office blood pressure). For elderly patients (75 years and over), the antihypertensive target was modified from 140/90mm Hg to 150/90mm Hg. While it is true that these indicators are all biomarkers, biomarker reference values are an important means of diagnosis. These vary according to age and nationality, which may be confusing to the general public.

Other than beta-blockers, which have been included to date, the four first-line drugs in the guidelines are angiotensin II receptor blockers (ARBs), ACE inhibitors, Ca blockers and diuretics (thiazides and thiazide analogs). Beta-blockers "are recommended as first-line drugs for heart failure, tachycardia, angina pectoris and myocardial infarction".

When hypertension is prolonged : (i) Arteriosclerosis progresses, leading to thickening of the vascular wall and narrowing of the vascular cavity, which results in greater susceptibility to cerebral hemorrhage due to cerebrovascular damage or stroke due to vascular stenosis or occlusion. The statistics for 2009 indicate that there were 130,000 deaths from cerebrovascular diseases and 84,000 deaths from myocardial infarction and ischemic heart disease. Infarction and hemorrhage accounted for 60% and 40% respectively. Subarachnoid hemorrhage is also a serious disease caused by hypertension. (ii) The pumping action of the heart is elevated due to hypertension, resulting in cardiac hypertrophy if prolonged. If this happens, there is a relative decrease in myocardial blood flow, along with thickening of the coronary artery and smaller arteries, increasing susceptibility to myocardial infarction and angina pectoris. (iii) If diabetes, high cholesterol, obesity and other hypertension risk factors become more serious, the incidence rate for ischemic heart disease becomes extremely high. These are well-known facts today.

However, the lifestyle disease hypertension has had a very short history, only becoming known as a risk factor for cerebral hemorrhage and ischemic heart disease since the Second World War. Ca blockers, the ace of anti-hypertensives, only started being administered for hypertension in the 1980s. Since then, however, anti-hypertensives have been well used clinically; according to the 2007 production values by medicinal category listed in the Pharma Japan Handbook 2010 published by Jiho, anti-hypertensives amounted to ¥594 billion, while vasodilators amounted to ¥328.4 billion. These figures were the highest sales figures of the medicinal categories.

According to the website of the Ministry of Health, Labour and Welfare, the average systolic blood pressure value reached its highest point in the mid-1960s, decreasing for both men and women after this peak. One reason for this decrease is the increase in the number of people receiving treatment for hypertensive diseases. In 1955, 61 in 100,000 people were receiving treatment for hypertensive diseases; by 1975, this figure had risen to 475; by 2005, it was 513.

The mortality rates by leading causes of death mentioned at the beginning (from the Vital Statistics survey by the Vital, Health and Social Statistics Office of the Minister's Secretariat of the Ministry of Health, Labour and Welfare) show the number of deaths from cerebrovascular diseases start to rapidly decline from around 1970 onwards.

This suggests that the general population was increasing in awareness of hypertension and paying greater attention to diet and lifestyle, while anti-hypertensives prescribed by doctors also probably played a significant role.

Tracing the history of hypertension research, clinicians appear to have treated hypertension objectively since the invention of the sphygmomanometer by Scipione Riva-Rocci of Italy in 1896. Studies on blood pressure regulation by the central nervous system began in the early 20th century; in 1936, Astley Cooper found that the blood pressure in a dog could be elevated by ligation of the common carotid artery.

At the time, hypertension was not known as a risk factor. In 1954, soon after the appearance of anti-hypertensives, A. M. Fishberg published the opinion that "it is better not to notify hypertensive patients of their blood pressure reading if they have no symptoms" (Fishberg A. M.: Hypertension and Nephritis, 5th ed. Lea & Fibiger, 1954 [(1)]).

According to *Kōketsuatsu no Igaku* [(2)], "in 1944, U.S. President Franklin Roosevelt undertook a strict reduced-salt, weight loss diet to combat his hypertension. Having visited him, British Prime Minister Winston Churchill commented that the President appeared not to eat any meals and looked haggard, thin, aged and lacking vitality. In spite of this strict lifestyle improvement, there were no effective anti-hypertensives in those days and the President passed away from a cerebral hemorrhage in the spring of 1945". This story highlights the importance of anti-hypertensives

(Note 1).

The U.S. government started academic epidemiological surveys on heart diseases in 1948. That same year, a large-scale prospective cohort study was undertaken with the cooperation of the residents of Framingham, Massachusetts, as a countermeasure against rising incidences of cardiovascular complications (Note 2). This was the well-known Framingham Heart Study (see Section 4.3. Medications for Hyperlipidemia). This study recorded blood pressure as a risk factor in the report for the 1960s and 1970s (4:14).

Hypertension falls into two categories: "secondary hypertension", which has a clear cause and accounts for around 10% of patients, and "essential hypertension", which has no identifiable cause and accounts for the majority of patients. However, studies seeking the cause of essential hypertension have found various direct causes that raise or lower the blood pressure.

Blood pressure refers to the pressure applied to the vascular wall and is represented by "cardiac output" × "peripheral vascular resistance". Cardiac output is the heart rate multiplied by the blood volume pumped out by one contraction of the heart; peripheral vascular resistance is proportional to the hardness of the blood vessel and inversely proportional to the cross sectional area of the blood vessel.

Theoretically, reducing these factors would be enough to lower the blood pressure. Early efforts focused on medications to reduce the heart rate and diuretics to lower the blood volume, thereby regulating the cardiac output or reducing the blood volume (see Fig. 4.2.1).

While increased peripheral vascular resistance is mainly due to arteriole vasoconstriction, progress was made on developing specific antagonists targeting the receptors for centrally or peripherally acting sympathomimetic substances as a constricting factor.

In 1949, Euf von Euler identified noradrenaline as a neurotransmitter for sympathetic nerve activity and established the relationship between the sympathetic nerves and hypertension. Reserpine, empirically known to have a hypotensive effect, was found to cause this effect by inhibiting the reuptake of neurotransmitter into the neurotransmitter storage granules in the sympathetic nerve endings (synapses), thereby causing catecholamine depletion. In 1953, Reserpine was approved by the FDA and was widely prescribed in the 1960s and 1970s.

During this time, the idea of blocking the sympathetic nerves was devised as a means of lowering blood pressure, although it is not used today. Sympatholytic agents (such as guanethidine, alpha-methyldopa and clonidine) and ganglionic blockers (such as hexamethonium) were developed. Later, substances causing blood pressure elevation were successively identified, including adrenaline, noradrenaline and angiotensin. Various types of drugs began to be developed to deal with each of these.

In 1967, a large-scale clinical intervention trial (Note 3) was conducted by the Veterans Administration Study (VA) and it was reported for the first time that anti-hypertensive treatment suppressed cardiovascular complications (3). The trial used a comparative study against a placebo group; the drug administration group was given a combination of three of the more potent anti-hypertensives available at the time (hydrochlorothiazide + reserpine + hydralazine).

After that, clinical trials mainly involved diuretics. A 1977 study by the United States Public Health Service (USPHS) indicated that while anti-hypertensive treatment did not prevent serious cardiovascular events in mild hypertension cases, it did prevent the condition from deteriorating. Successive intervention trial results were reported, such as the Australian National Blood Pressure Study (ANBPS) (1980) and the Oslo Study (1980), but these results revealed that although anti-hypertensives significantly suppressed the onset of cerebral hemorrhage, they were ineffective against the onset of ischemic heart disease. Later, beta-blockers were developed and results were reported to indicate a secondary prevention of myocardial infarction, demonstrating their efficacy as an anti-hypertensive. Several comparative studies were conducted comparing beta-blockers and diuretics. Although beta-blockers were found to be effective at the endpoint of heart disease onset, there was no difference in other indicators (4:124-128).

Based on the results of basic research from these clinical trials, efforts continued in drug discovery to reduce the causes of hypertension. Research and development was undertaken on diuretics to reduce body fluids, beta-blockers to lower the heart rate and alpha-blockers and calcium channel blockers to reduce the peripheral artery resistance. The focus fell on renin, secreted from the kidneys, and the related angiotensin; developments included angiotensin II converting enzyme inhibitors (ACE inhibitors) and angiotensin II inhibitors. With the development of drugs with several different mechanisms of action, anti-hypertensives became a highly satisfying field of medicine.

The importance of renal blood pressure has recently been realized; it is now known that there are a number of different factors that directly cause elevations in blood pressure. Anti-hypertensives are administered to patients in accordance with their pathology, with consideration given to the relevance of each factor for that patient. However, even now there is still no established theory as to the cause of essential hypertension, which is dominant further upstream than these direct causes of elevations in blood pressure. Figure 4.7 shows the points of action of anti-hypertensives.

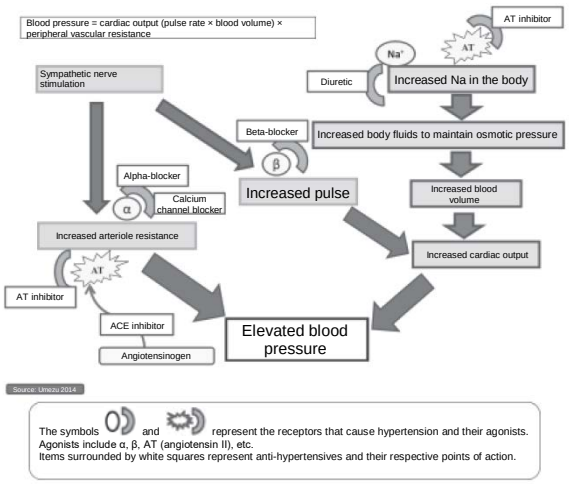

Fig. 4.7 Points of Action of Anti-Hypertensives.

4.2.1. Diuretics

The history of diuretics can be traced back to the early 20th century, when sulfa drugs were found to have a diuretic effect. This was later found to be due to the inhibition of carbonic anhydrase and analogs began to be synthesized. During this time, from around 1957, thiazide diuretics, loop diuretics and potassium-sparing diuretics were developed.

Although these three types of diuretics all act by promoting the excretion of Na^+ and water in the uriniferous tubules, they differ in their point of action. Figure 4.8 shows the points of action of different diuretics.

Thiazide diuretics mainly produce a diuretic effect by inhibiting the reuptake of Na^+ and Cl^- in the distal tubules. Hydrochlorothiazide and trichlormethiazide were used for this; however, these have moderate potency as diuretics due to their poor diuretic action. Nevertheless, since these are cheap and perform adequately as anti-hypertensives, they are still recommended by the American Hypertension Guidelines (JNC7).

Loop diuretics such as furosemide act at the ascending limb of the loop of Henle in the kidney (Note 4). While they do not have a strong hypertensive effect, they do have a strong diuretic effect, which prevents the exacerbation of renal function deterioration; accordingly, they are effective in improving edema and hypertension accompanying renal dysfunction. These drugs increase susceptibility to hypokalemia and hyperuricemia.

Potassium-sparing diuretics promote the excretion of Na^+ and water in the uriniferous tubules by antagonizing aldosterone (a hormone known as a mineral steroid) in the aldosterone-dependent Na^+/K^+ exchange sites in the distal tubules and collecting tubules. Since they inhibit K^+ excretion, they are characterized by no K^+ loss. These drugs include spironolactone and eplerenone. Spironolactone is a compound developed in 1957 by Kagawa et al. of American company G. D. Searle as a result of systematic exploratory research on aldosterone antagonists (23). Eplerenone was developed by Pharmacia (now Pfizer) and selectively inhibits the binding of aldosterone to the mineralocorticoid receptors. Recent research has shown that aldosterone is produced in the heart and vascular cells, as well as the adrenal cortex. Reports also indicate that the mineralocorticoid receptors on which aldosterone acts are found in various sites throughout the body, such as the heart and vascular wall, not just in the kidneys. The importance of aldosterone in cardiovascular diseases has begun to be recognized and there are great expectations for the protective effects of aldosterone antagonists on the internal organs. Clinical trials for eplerenone began in 1997; it was adapted for hypertension in 2007.

Triamterene produces a potassium-sparing diuretic effect by inhibiting amiloride-sensitive Na^+ channels; it can sometimes induce hyperkalemia.

Although diuretics have long been recognized as mild anti-hypertensives, some side effects have also been identified, such as elevated blood glucose and uric acid levels. However, these elevated blood glucose and uric acid levels have been found to be the result of excessive fluid excretion due to overly high dosages of diuretics; low-dosage diuretic usage has now become standard practice.

In the 1980s, alpha-blockers, ACE inhibitors and calcium channel blockers were developed as a means of reliably lowering blood pressure. While these anti-hypertensives were initially administered concomitantly with diuretics, they have none of the metabolic side effects (hypokalemia, hyperuricemia, hyperlipidemia, glucose intolerance) usually associated with diuretics. Patients in Japan have long awaited drugs that were less complicated to use than diuretics and are advocating individualized treatment rather than one-size-fits-all treatment protocols.

Various anti-hypertensives are now being developed with fewer side effects. Clinics are entering an era in which different options are available according to patient pathology. Diuretics are being re-evaluated, although few are being used as standalone treatments due to high incidences of hypokalemia and renal dysfunction.

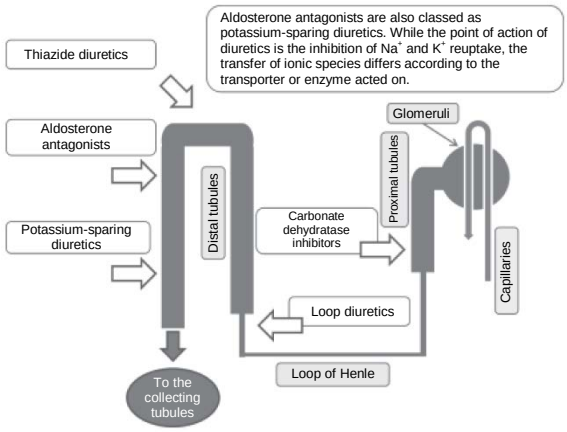

Fig. 4.8 Points of Action of Diuretics

4.2.2. Alpha-Blockers and Beta-Blockers

Adrenergic receptors include α-receptors and β-receptors; the β-receptors include β_1-receptors, which are mainly present in the myocardium, and β_2-receptors, which are present in the bronchial smooth muscle. Blocking the β_1-receptors (i) reduces myocardial contractility, (ii) lowers cardiac output, (iii) inhibits atrioventricular conduction and (iv) reduces renin secretion by the adrenal glands, resulting in a hypotensive effect. However, since β-blockers with no β_1 selectivity block other β-receptors throughout the body, they are more prone to producing various side effects. Specifically blocking the β_2-receptors can cause bronchial

constriction and therefore must be avoided in asthmatic patients.

However, β-receptors are also present in the sympathetic nerves (presynaptic cells); the stimulation of those receptors has been found to be a noradrenaline-releasing mechanism. Non-selective β-blockers are presumed to have a hypotensive effect by inhibiting sympathetic nerve activity. Beta-blockers with partial agonist activity have also emerged, making for even more complex analysis of their pharmacological effects.

The β-blocker propranolol (Zeneca; now AstraZeneca) hit the Japanese market in 1966, indicated for arrhythmia and angina pectoris; in 1978, it was adapted as an anti-hypertensive. The first anti-hypertensive to be approved in Japan as the β-blocker was pindolol (Ciba-Geigy; now Novartis), launched in 1973. Many Japanese pharmaceutical companies were involved in the development of β-blockers; bufetolol (Yoshitomi Pharmaceutical) was the first domestically-produced β-blocker to emerge on the market in 1974. However, it was not adapted as an anti-hypertensive. Other drugs emerged in succession, including indenolol in 1980 by Yamanouchi Pharmaceutical, carteolol in 1981 by Ostuka Pharmaceutical and nipradilol in 1988 by Kowa. These are the first-generation β-blockers.

AstraZeneca then synthesized a drug with greater β_1 selectivity in order to reduce side effects; this was jointly developed with Novartis. The once-a-day medication metoprolol was launched on the Japanese market in 1983. AstraZeneca also released atenolol in 1983, a once-a-day β-blocker with greater selectivity. Metoprolol was superseded by atenolol internally within AstraZeneca.

Nadolol (discovered by Squibb in 1970 and developed by Dainippon Pharmaceuticals) was launched in 1986 with greater compliance as a once-a-day medication, although it was non-selective. In 1990, Tanabe Seiyaku licensed bisoprolol from E. Merck and was granted approval that same year. Although this was the 20th β-blocker on the Japanese market, it achieved a top ranking in sales.

Although β-blockers were also effective for ischemic heart disease and arrhythmia because they lowered the cardiac output to reduce the workload on the heart, they were known as “delicate balance” medications that had to be increased gradually; they were problematic to use, as excessive dosage could increase the burden on the heart and cause various cardiac disorders. Beta-blocker sales began to decline with the eventual emergence of calcium channel blockers and ACE inhibitors.

Analogs would have been simple to synthesize using the β-blocker structure and many companies in Japan announced their candidacy for the first-generation drugs. However, very few second-generation products were produced [(Note 5)].

By contrast, α_1-blockers acted on the arteriole smooth muscle receptors to inhibit smooth muscle contraction, thereby lowering the peripheral vascular resistance and consequently lowering the blood pressure. With α-receptors widely distributed throughout the body, there are many α-receptor subtypes. While α_1-receptors are found in smooth muscle cells, α_2-receptors are also found in presynaptic cells and inhibit the release of catecholamine. Inhibiting these receptors promotes the release of catecholamine, which causes side effects. Accordingly, selective α1-blockers are preferable. First-generation selective α_1-blockers include prazosin (1981, Pfizer) and bunazosin (1985, Eisai), used on patients with hyperlipidemia and abnormal glucose tolerance for their carbohydrate and lipid metabolism improving effects. These gradually fell out of use due to side effects such as orthostatic hypotension.

Drugs developed to block both α- and β-receptors include amosulalol (1988, Ono /Dainippon), arotinolol (1985, Sumitomo) and carvedilol (Boehringer Ingelheim). Arotinolol has a potent β-blocker effect that is eight times more powerful than its α-blocker effect; designed to suppress peripheral vascular resistance, this was a proprietary new anti-hypertensive drug development by Sumitomo Pharmaceuticals directly following the establishment of the company.

Fig. 4.9 shows the chemical structures of some representative diuretics and β-blockers.

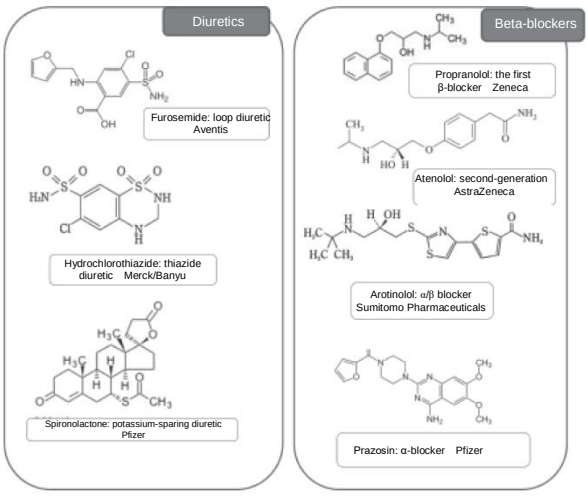

Fig. 4.9 Chemical Structures of Representative Diuretics and Beta-Blockers

4.2.3. Calcium Channel Blockers

The history of calcium channel blockers began with the discovery that the cardiac suppression effect of the newly-developed coronary vasodilator verapamil, unlike

nitroglycerin, suppressed myocardial contraction. While it was reported that atrial cell depolarization was caused by early Na^+ influx and late Ca^{2+} influx, the mechanism of action of verapamil was found to be due to the blocking of Ca^{2+} influx.

The idea of calcium channel blockers was proposed in 1972 by Albrecht Fleckenstein of Germany [(5)], but it gained few supporters, as the main remedies for angina pectoris in Europe at the time were dipyridamole and β-blockers. Further, in 1972, Mototaka Murakami of Kanazawa University reported on the anti-hypertensive effects of nifedipine [(6)].

The Japanese drug diltiazem was the first to substantiate this idea of calcium channel blockers. Diltiazem was released to market in 1974 by Tanabe Seiyaku as an "anti-anginal having a mechanism of action due to blocking Ca^{2+} influx and having a myocardial contraction suppressing effect and a pulse-regulating effect". It later came to be used in 110 countries around the world, with sales peaking at over $100 billion.

According to Tanabe Seiyaku's company history, companies in the West started taking an interest in diltiazem from around 1976 due to its unique mechanism of action. After several international symposia, Japan deemed it to be safe and highly effective. When it was launched in the United States in 1982, it was received as a "life-saving medicine". While diltiazem was originally used for its effectiveness against stable angina by suppressing thick blood vessel spasms, once it was found that exercise-induced angina involves coronary blood vessel spasms, the drug was adapted to be effective against exercise-induced angina as well. In 1982, it was adapted again for hypertension. This was possible due to the fact that its vasodilatory action by inhibiting the influx of calcium to the vascular smooth muscle cells results in a hypotensive effect [(7, 8)].

However, the results of a clinical trial found that "the administration of diltiazem to myocardial infarction patients showed no difference in incidence rate from the non-administered group", thus casting doubt on its effectiveness on angina pectoris and myocardial infarction [(9)]. It was pointed out that although this short-acting drug causes rapid vasodilation, the effect quickly diminishes, which could potentially disrupt the blood pressure due to vasoconstriction. In response, a controlled-release formulation of diltiazem, taken once or twice daily, was developed, thereby improving its effectiveness against angina pectoris and also improving its anti-hypertensive effect. While intense development competition later ensued for calcium channel blockers, the original Japanese diltiazem was a great success as one of the compounds that laid the foundation for clinical utility as an anti-hypertensive with a calcium channel blocking effect.

There are three types of calcium channel blockers with three basic structures: phenylalkylamines (such as verapamil), benzothiazepines (such as diltazem) and dihydropyridines (such as nifedipine). As these grew more highly regarded as effective first-generation calcium channel blockers, issues were raised with side effects and excessive dosage frequency and volume due to shorter half-lives. Demand grew for drugs with milder action and fewer side effects, leading to the emergence of a succession of second-generation drugs that required less frequent dosages. However, recent policies have focused on extending the dosage duration through innovations in dosage form; some attention has even been given to reassessing first-generation calcium channel blockers.

The emerging second-generation drugs included nilvadipine (1989, Fujisawa Pharmaceutical), taken twice daily, and nisoldipine (1990, Bayer), manidipine (1990, Takeda Pharmaceutical), benidipine (1991, Kyowa Hakko with collaborative research by Nagoya City University Faculty of Pharmaceutical Sciences) and barnidipine (1992, Yamanouchi Pharmaceutical), each taken once daily. In addition to these, other domestically-produced drugs were also released on the market, including efonidipine (1994, discovered by Nissan Chemical and jointly researched by Zeria Pharmaceutical), cilnidpine (1995, Fujirebio/Ajinomoto) and aranidipine (1996, Maruko/Taiho). Nitrendipine (Bayer) also emerged in 1998. Thus, the anti-hypertensive market in Japan bloomed to life with a succession of calcium channel blockers following on from the β-blockers.

While these drugs had favorable anti-hypertensive effects, as with nifedipine, there were still a number of drawbacks with side effects as the dosage amount increased, including facial flushing, heart palpitations and headaches, resulting from tension on the reflex sympathetic nerves due to rapid decrease in blood pressure. Later, calcium channel blockers were developed that had greater sustained efficacy and fewer side effects. One such drug was amlodipine (1993, Pfizer), which had a half-life of 36 hours, a far greater duration of action than any of the second-generation drugs. These were the third-generation calcium channel blockers and included azelnidipine (2003, Ube/Sankyo).

There are now dozens of calcium channel blockers in current use and it is possible to select the most appropriate one for the pathology, although these cannot be used on patients with acute myocardial infarction, diabetic nephropathy, congestive heart failure or similar [(10, 11)].

Papers have been published questioning the safety of calcium channel blockers since 1995 as the "calcium channel blocker controversy" has unfolded. A report by Pahor et al. on a series of case-control studies identified the possibility that calcium channel blockers increase the risk of cancer [(12)]. However, these reports have come under repeated criticism for the testing methods and scale used, as well as the fact that the medication history was unclear; the need for longer-term prospective studies has been pointed out.

Meanwhile, the Syst-Eur trial [(13)] and the STONE study [(14)] have confirmed that calcium channel blockers do not increase the risk of cancer. A report on the relative risk of cancer in patients administered with β-blockers [(14)] also stated that calcium channel blockers do not increase the risk of cancer, while a report [(16)] by the Ad Hoc Subcommittee of the Liaison Committee of the WHO/ISH [(Note 6)] and the US hypertension guidelines JNC-VI [(Note 7)] also take a negative opinion regarding the cancer risk of calcium channel blockers.

4.2.4. Renin/Angiotensin Blockers

In 1898, Swedish medical scientists Robert Tigerstedt and Per Bergman noted that administering animals with an extract from the kidneys of other animals caused a blood pressure elevation reaction; they named this substance “renin”, with the idea that it came from the kidneys. In 1934, American medical scientist Harry Goldblatt used animal experiments to test the question proposed by Richard Bright on whether renal glomerular arteriosclerosis precedes hypertension or if arteriosclerosis progresses after hypertension. Goldblatt concluded that hypertension is not caused by arteriolar stenosis, but by a hormone-like substance released by the kidney following stenosis. He also stated that the causative substance was renin, as mentioned by Tigerstedt [2:76-85].

Research on renin continued, with the renin-angiotensin system found to be one of the mechanisms that control blood pressure. Renin (a protease) secreted by the kidneys acts on the angiotensinogen in the blood to produce angiotensin I. This peptide was found to convert into angiotensin II through the action of an enzyme known as angiotensin-converting enzyme (ACE); angiotensin II was found to be one of the most powerful substances in the body for vasoconstriction. Angiotensin III and IV also exist, but their effects are not as potent as angiotensin II. Angiotensin II constricts the blood vessels by binding with the angiotensin II receptors (AT1) in the arteriolar smooth muscle; in the heart, this increases the cardiac output, producing hypertensive conditions. It is also known to have the effect of increasing the glomerular filtration rate by selectively constricting the efferent arterioles of the renal glomeruli and promoting the secretion of aldosterone from the adrenal cortex by binding to the receptors in the adrenal cortex. This aldosterone activity promotes the reuptake of sodium by the collecting tubules in the kidneys, causing a vasopressor effect from an increase in body fluids. Assuming that the renin-angiotensin system plays a significant role in regulating the blood pressure, with hypersecretion of renin causing hypertension, the hypothesis has emerged that interrupting part of this process should lower the blood pressure [17, 18].

To that end, drug discovery research began to focus on (i) preventing the production of angiotensin II by inhibiting ACE, (ii) using angiotensin II receptor AT1 antagonists to inhibit signal transduction and (iii) inhibiting renin activity. As expected, clinical studies found drugs based on (i) and (ii) to have definite hypotensive effects.

ACE Inhibitors

The first ACE inhibitor, captopril (1983, Sankyo/Squibb), was put to the test through widespread clinical use. Various issues began to be reported, including the rapid manifestation of its hypotensive effect, the need to administer it three times a day due to the short duration of its hypotensive effect and the relatively high incidence rate of side effects, including rashes, distorted sense of taste and dry coughing. In 1988, the first domestically-produced ACE inhibitor hit the market in the form of alacepril (Dainippon Pharmaceuticals), with a reduced dosage frequency. This was a captopril prodrug and still contained an SH-group.

The second-generation drug enalapril (1986, Banyu/Merck) had the SH-group present in captopril substituted for a COOH-group to alleviate the side effects. Substituting the SH-group suppressed the rashes and the dry coughing. Various companies in Japan rapidly embarked on new developments to overcome these issues; later, fully-Japanese “non SH-group” ACE inhibitors hit the market. In 1989, delapril (Takeda Pharmaceutical) was launched, with a slow and sustained hypotensive effect. In 1990, Eisai released cilazapril, licensed from Roche Products (UK), which only need to be administered once a day. In 1993, imidapril (Tanabe Seiyaku) was launched as an anti-hypertensive; in 2002 it was approved for treatment of diabetic nephropathy accompanying type 1 diabetes. In 1994, Sankyo launched temocapril as an anti-hypertensive. Temocapril was also biliary and renally excreted and could accordingly be used as a once-a-day prodrug for patients with renal dysfunction.

While angiotensin I converts into the vasopressor substance angiotensin II by means of ACE, angiotensin II is also produced by chymase secreted by mastocytes (Fig. 4.10).

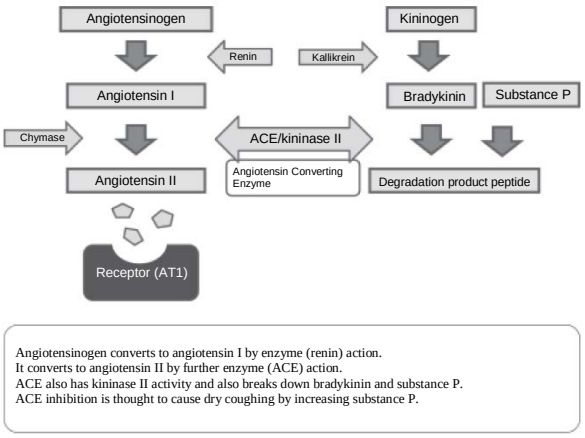

Fig. 4.10 Renin-Angiotensin System and Kinin-Kallikrein System

ACE has two enzyme activities; it acts as kininase II to break down and metabolize bradykinin and substance P. Even in this route ACE (kininase II) acts in a way that elevates blood pressure by breaking down bradykinin, which has a blood pressure lowering effect and an algesic effect. However, the use of ACE inhibitors presumably means not only suppressed production of angiotensin II, but also overproduction of substance P (which acts on the nerves responsible for coughing and swallowing during normal eating and drinking), as it does not break down properly, as well as other side effects, including dry coughing and other various effects of non-angiotensin bradykinin systems [8, 9].

Fig. 4.11 shows the chemical structures of representative calcium channel blocker and ACE inhibitor compounds.

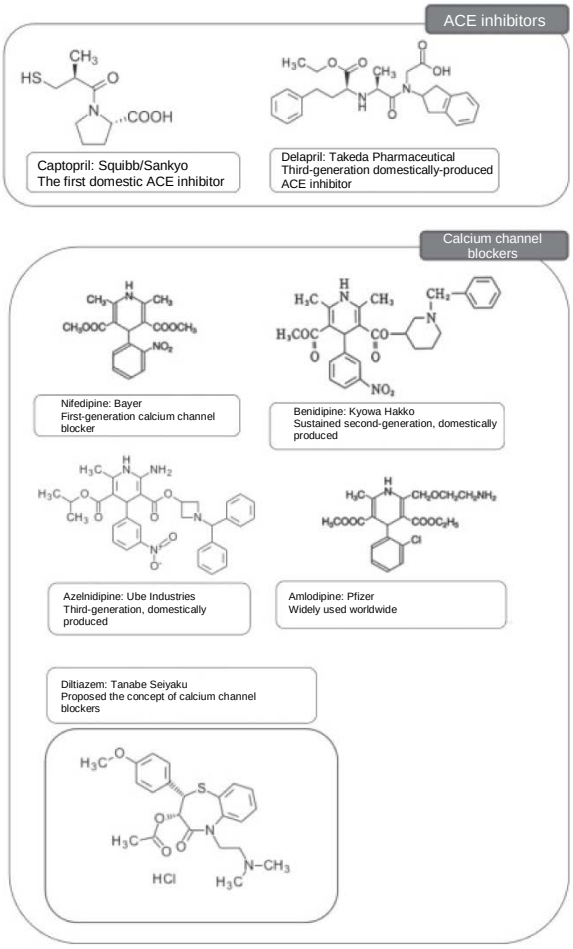

Fig. 4.11 Chemical Structures of Representative Calcium Channel Blockers and ACE Inhibitors

Angiotensin II Receptor Blockers (ARBs)

Research began on angiotensin II receptor blockers with the idea of overcoming the shortcomings of ACE inhibitors. It was already known that angiotensin would be synthesized by other enzymes even if ACE were completely suppressed. It was also predicted that side effects would be caused if ACE inhibitors activated the bradykinin systems, but directly blocking the angiotensin II receptors would eliminate these potential issues, making it possible for such a drug to be used on patients who experienced side effects from ACE inhibitors. Accordingly, drug discovery companies all began to explore this field as well. There were two known types of angiotensin receptors: type I and type II; these anti-hypertensives targeted the type II receptor AT1.

In 1998, losartan (Merck) was released on the Japanese market. Takeda Pharmaceutical launched candesartan the following year. The 4-8mg daily dosage for candesartan was far lower than the 25-50mg daily dosage for losartan and the 40-80mg daily dosage for valsartan, released the following year. Valsartan had been synthesized by Swiss company Novartis Pharma in 1989, but was launched in Japan in 2000 by Ciba-Geigy Japan (now Novartis Pharma). These early first-generation drugs delivered a superior hypotensive effect as anticipated, while also avoiding the side effects of dry coughing and angioedema that had plagued the ACE inhibitors. Later, the second generation of value-added ARBs hit the market. Nippon Boehringer Ingelheim launched telmisartan in 2002, while irbesartan, jointly developed by Shionogi and Dainippon Pharmaceuticals, was launched in 2008. These ARBs were marketed as being suitable for diabetes and heart failure as well. They were reported as having a powerful PPARγ bonding effect and an insulin-resistance improving effect, similar to thiazolidine-analog diabetes drugs (see Section 4.1.6. Thiazolidine Derivatives). They bond with thiazolidine transcription factor PPARγ to suppress the production of several proteins that set the stage for insulin resistance and have accordingly been used as diabetes drugs, while showing promise for using this effect combined with other effects. Basic research has also shown them to bond with PPARα, although fibrates also have this mechanism of action; this inhibits the growth of triglycerides. (See 4.3.3. Hyperlipidemia – Fibrate Lipid-Lowering Drugs.)

The discovery of drugs with a new effect among the second-generation AT1-blocking ARB anti-hypertensives is also very interesting from the perspective of the causes of metabolic syndrome, combining hypertension, hyperlipidemia and diabetes.

Sankyo launched olmesartan in 2004, with a powerful AT1 receptor bond. Azilsartan, known as a super ARB, was released by Takeda Pharmaceutical in 2012.

Besides inhibiting ACE, these ARBs have also been reported to have a direct protective effect on the kidneys by expanding the efferent arterioles of the kidneys, thereby lowering the pressure in the glomeruli. Fig. 4.12 shows the chemical structures of representative ARBs.

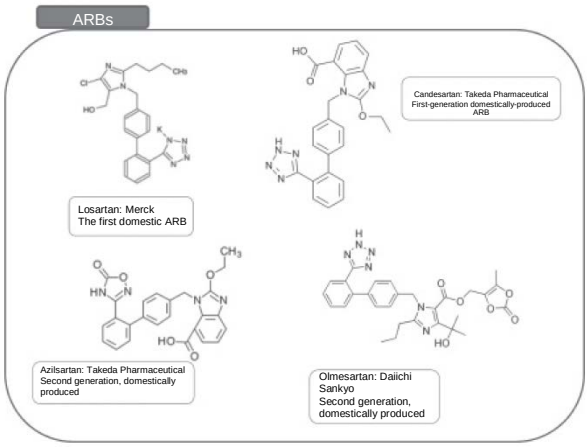

Fig. 4.12 Chemical Structures of Representative ARBs

Renin Inhibitors

In 2009, nearly two decades after the first ARB, losartan, was approved, Japan gave the approval for aliskiren, a new renin-angiotensin system anti-hypertensive. It was adapted for hypertension and demonstrated a hypotensive effect when taken once daily. It was produced by Ciba-Geigy (now Novartis Pharma) and marketed widely around the world. As a renin inhibitor, aliskiren acted farther upstream than ACE inhibitors and ARBs to suppress the production of angiotensin I; accordingly it was not accompanied by the increase in angiotensin II as found with the use of ARBs. Further, since it suppressed the production of angiotensin I, it was expected to also reduce the amount of "angiotensin II synthesized by non-ACE enzymes, which ACE inhibitors were not able to suppress" (Note 8).

It was also thought not to incur dry coughing and other side effects, since it did not inhibit the breakdown of bradykinin as the ACE inhibitors did (23).

4.2.5. Anti-Hypertensives and Combination Drugs

While drug combinations were found in Chinese medicine and cold remedies, government regulation was placed on prescription drugs in the 1990s, meaning that combination drugs were expressly not approved for patients with chronic lifestyle-related diseases. Where a patient needed to take two or more drugs with similar efficacy for a disease, they had to take multiple single drugs together. However, it was found that greater compliance was achieved when patients took combination drugs than multiple single drugs; with many combination drugs being used overseas in the 21st century, they began to draw attention in Japan as well. As a result, the Ministry of Health, Labour and Welfare issued a deregulation notice in 2005.

Many anti-hypertensives in particular used several different drugs in combination. Approval was given for combination drugs made up of the highest-selling single-drug ARBs and diuretics (hydrochlorothiazide HCT and trichlormethiazide TCM), as well as ARBs and calcium channel blockers. In 2006, MSD released Preminent®, a combination of ARB and HTC.

Synergizing anti-hypertensives with different mechanisms of action made it possible to not only reduce the amount of ingredients used and increase the efficacy of the drug, but also to reduce the side effects as well. This also led to extended life expectancy for basic drugs, which became a new strategy for drug discovery companies.

Combination drugs made up of anti-hypertensives and statins (lipid-lowering agents) began to emerge, as did combination drugs made up of multiple diabetes drugs with different mechanisms of action. Further attention will be given to combination drugs for lifestyle-related diseases in the future.

4.2.6. Hypertension Guidelines

Although the medicinal efficacy of anti-hypertensive drugs is evaluated by clinical study (clinical trial) before application is made for approval, after a drug goes to market, many large-scale clinical trials are conducted over long periods of time on large numbers of patients in order to carefully examine the efficacy (indicated diseases, concomitant drugs, dosage) and the side effects. The clinical trials essentially look for: (i) whether the mortality rate decreases; (ii) whether the incidence rate of the main disease decreases; (iii) whether the side effects decrease; (iv) whether the quality of life (QOL) improves. The criteria on which these are determined are called true endpoints. However, since in reality it is difficult to determine the actual mortality rate or disease onset within a short space of time, surrogate endpoints that can be assessed in the short term are used instead. While the surrogate endpoints make it possible to gather a lot of data, in many cases they do not represent the true medicinal efficacy. Many clinical studies have been undertaken using these surrogate endpoints, with the data being used to draft national guidelines.

There are countless hypertension sufferers in the developed nations and each country has its own guidelines. In Japan, the first *Guidelines for the Management of Hypertension* were issued in 1990 through collaboration between the Ministry of Health, Labour and Welfare and the Japan Medical Association (19). After that, the Japanese Society of Hypertension (JSH) spearheaded the *Hypertension Treatment Guidelines* published in 2000, with revised editions released in 2004, 2009 and 2014.

In the United States, the guidelines came in 1977 in the form of JNC-1, or the *First Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure*. This document mentions the definitions of normal blood pressure and hypertension, the risk factors for hypertensive patients, assessment for organ dysfunction and other complications and specific treatment guidelines.

In terms of the changes in first-line treatment for hypertension, JNC-1 and JNC-2 (1980) only mentioned

thiazide diuretics, while JNC-3 (1984) included both thiazide diuretics and β-blockers. In addition to these, JNC-4 (1988) also included calcium channel blockers and ACE inhibitors. However, JNC-5 (1992) went back to thiazide diuretics and β-blockers as first-line treatments, with ACE inhibitors, calcium channel blockers and α-blocker as substitutes for patients unable to undergo the first-line treatments. In JNC-6 (1997) the policy changed to select the anti-hypertensive treatment most expected to be beneficial, while limiting the use of diuretics and β-blockers as first-line treatment to patients with no complications.

The WHO guidelines WHO/ISH 1989 and WHO/ISH 1993 were published the year after JNC-1 and were limited to mild hypertensive patients. This was due to the idea that 80% of patients were mild hypertensive patients for whom the course of medication was significant.

According to these guidelines, there were five first-line treatments: diuretics, β-blockers, ACE inhibitors, calcium channel blockers and α-blockers [(21)]. This was quite different from the American guidelines.

According to Kikuo Arakawa, "In the 5th report of the JNC, the United States suddenly started to demand evidence [(Note 9)] for each drug, giving priority approval to diuretics and β-blockers as having a sufficient accumulation of evidence, while other drugs were limited to cases where these could not be used. This gave rise to a great deal of discussion worldwide" [(22)]. Thus, there was a significant difference from the WHO guidelines that made use of six types of drugs. Unclarified issues and inconsistencies were also identified in terms of whether adequate controls had been in place in past trials of the diuretics and β-blockers mentioned in JNC, leading to the idea of reconsidering evidence.

Discussion arose in 1995 over the safety of calcium channel blockers, with reports from several clinical trials identifying risks. Despite many reported results to the contrary, JNC-6 still approved the use of calcium channel blockers, although it did restrict the use of short-acting calcium channel blockers, recommending their long-acting counterparts instead.

JNC-7 (2003) emphasized cost effectiveness, deeming diuretics to be the best form of anti-hypertensive and presenting these as first-line treatment. JNC-8 (2014) offers calcium channel blockers, ACE inhibitors, ARBs and diuretics as first-line treatments, although priority is given to diuretics from the perspective of cost.

WHO/ISH 2003 clearly places diuretics as first-line treatment in all risk categories for the same reason. However, given the reality that in many cases hypertensive patients need to take a number of different drugs concomitantly, these guidelines adopt the stance that "it is meaningless to designate or emphasize a first-line treatment".

The Japanese guidelines fundamentally support the WHO idea, with JSH-2004 offering no fixed order of precedence for first-line treatment for hypertensive patients without complications, instead listing the names of anti-hypertensives in order of usage frequency in Japan and opting for the most appropriate drug for the age and gender of the patient.

JSH-2009 designates five first-line treatments, namely diuretics, β-blockers (including α/β-blockers), calcium channel blockers, ACE blockers and ARBs, and does not recommend α-blockers. Beta-blockers are listed as first-line treatment with the condition that they are "not necessarily the first-line treatment of choice for elderly patients without complications or patients with glycolipid metabolism abnormalities". While diuretics are the cheapest of these drugs in Japan, they are emphasized as concomitant drugs due to concerns over hypokalemia, abnormal glucose tolerance, hyperuricemia and the impact on fat that accompanies thiazide diuretics, as well as the fact that they lose more of their hypotensive effect as the dosage decreases. JSH-2014 recommends four first-line treatments: diuretics, calcium channel blockers, ACE inhibitors and ARBs. Although β-blockers are still one of the main anti-hypertensives, they have been deemed less effective than other drugs in terms of preventing cerebral hemorrhage, particularly in light of evidence from overseas, and have been excluded from first-line treatments for patients without complications. However, they are "actively applied" for patients with heart disease complications. The stance taken by the Japanese guidelines seems to be the right choice.

Evidence is confirmed by large-scale clinical studies. In relation to hypertension, research materials by Hisaichiro Tsukiyama and Keiko Otsuka et al. [(4:113-142)] include reports worldwide from 80 large-scale clinical trials on hypertension in the 1990s alone.

There are very few cases featuring the true endpoints of mortality rate and major disease onset; it is not easy to demonstrate whether there is a statistically significant difference between the administration group and the non-administration group (the group administered the placebo instead of the actual drug). Accordingly, it has not been uncommon for clinical trials to produce different results even when similar trials are repeated.

The protocols used in these trials vary for each trial. There are also inevitable differences between trials in terms of the backgrounds of the patients being administered the drugs, in other words, different genes, different disease histories and different attitudes toward taking medicine. In order to obtain repeatable results, the conditions have to be as identical as possible with the parameters increased. Although meta-analyses are conducted for that purpose, it is difficult to include the results of all clinical trials carried out under different protocols and points of doubt still remain. It may be time to reconsider the sense in conducting large-scale trials to obtain evidence. Despite this evident difficulty with clinical trials, the number of large-scale clinical intervention

trials has still continued to grow since the year 2000.

Non-clinical studies determine medicinal efficacy using animal experiments. Rodents (rats and mice) used in experiments are germ-free animals from pure strains in order to prevent infection. However, the reality is that biases (standard deviations) still emerge even when examining simple surrogate markers under specific genetic and environmental conditions. Although there are difficulties enough experiments on live organisms, it is even more difficult to align conditions for pathological animals.

One can easily guess that this difficulty is multiplied in the case of humans, comparing one group with another with different genes, different disease histories, different eating habits and different lifestyles. There is a need to explore more theoretical testing methods in future. Perhaps it is unreasonable to make one-size-fits-all evaluations for all of humanity, scattered as we are around the globe.

Note 1: President Roosevelt, Prime Minister Churchill and General-Secretary Stalin attended the Yalta Conference of February 1945; all three suffered from hypertension and died of cerebral hemorrhage. Churchill was 91 when he died; Stalin was 75 and Roosevelt 63.

Note 2: Cohort study – a research method in epidemiology that tracks the long-term progress of a particular group (cohort). A particularly well-known study is the Hisayama Study (*) in Fukuoka by Kyushu University.
*: The Hisayama Study -- A highly-detailed, non-interventional epidemiological study on lifestyle-related diseases conducted by Kyushu University Graduate School of Medical Sciences (Prof. Yutaka Kiyohara) and the people of Hisayama-cho in Fukuoka (population 8,400) since 1961, gathered significant amounts of epidemiological data. A high postmortem autopsy rate has ensured very accurate data, with 99% of residents tracked. Source: Website of the Department of Environmental Medicine, Kyushu University Graduate School of Medical Sciences.

Note 3: Clinical intervention trial -- Deliberately setting and controlling trial conditions relating to the treatment, lifestyle and behavior of study participants.

Note 4: The loop of Henle is the name of a portion of the uriniferous tubules at the point where the uriniferous tubules passing through the kidneys form long loops. See Fig. 4.8.

Note 5: While some are clearly distinguished by their mechanism of action, others were named according to a marketing strategy. Generally speaking, not all first-generation or second-generation drugs can necessarily be clearly defined.

Note 6: World Health Organization (WHO); International Society of Hypertension (ISH)

Note 7: The Joint National Committee (JNC) on Hypertension

Note 8: Angiotensin I is converted into angiotensin II not only by ACE, but also by chymase, an enzyme secreted from the mastocytes in the event of tissue damage. There are also other routes to angiotensin II production. See Fig. 4.10.
Note 9: Theory-based clinical trial results

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4.3 Medications for Hyperlipidemia (Hyperlipidemic Drugs)

According to the 2011 *Patient Survey* conducted by the Ministry of Health, Labour and Welfare, the total number of hyperlipidemia sufferers (patients assumed to be receiving ongoing treatment) was 1.89 million (530,000 males and 1.36 million females). The actual number of people with hyperlipidemia but not receiving ongoing treatment is assumed to be several times higher. As one of the causes of metabolic syndrome, hyperlipidemia has even frequently been featured on television; there is now very high public knowledge and awareness of this disease, including good and bad cholesterol. However, hyperlipidemic drugs described as having a hyperlipidemic effect started being used in the 1990s; and new drugs that actually lowered cholesterol levels emerged around 1990, as did those for other lifestyle-related diseases.

This section begins by tracing the history of hyperlipidemia, including cholesterol and triglycerides.

In 1908, Russian pathologist Nikolay Nikolaevich Anichkov observed atherosclerosis in the arterial walls of rabbits that had been given cholesterol. Examination of the lesion sites confirmed the accumulation of macrophages, lymphocytes and smooth muscle myocytes. Anichkov also reported on the relationship between cholesterol and atherosclerosis, stating that "there is no atherosclerosis without cholesterol". In 1930, he came to Japan and even lectured at the International Academy of Pathology in Osaka. However, the prevailing idea at the time was that arteriosclerosis occurred spontaneously with age. Anichkov's theory was not well received by the American medical circles and the rest of the academic world, since humans and rabbits were different species [1, 2].

Forty years on, in the 1950s, it was found that almost half of the deaths in the United States were due to coronary artery disease, particularly angina pectoris and myocardial infarction. John William Gofman of the University of California at Berkeley re-examined Anichkov's theory using the most advanced technology available at the time and reported that cholesterol exists in the blood as very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL), that LDL increases with a high fat diet and that VLDL increases with a high carbohydrate diet [3].

While the relationship between cholesterol and arteriosclerosis started gaining some attention at this time,

most of the research was to do with cholesterol in the diet. American dietician Ancel Keys noted a relationship between heart disease and lifestyle and began researching this from the 1940s onwards. In 1955, he made the novel proposal to conduct a scientific, continuous study on the relationship between cholesterol and heart disease by means of a large-scale epidemiological study focusing on the relationship between diet, lifestyle and heart disease. Consequently, the "Seven Countries Study" began in 1958, with seven countries taking part, including Japan, to clarify the relationship between cholesterol and heart disease. As part of this research, the "Mediterranean diet", which is low in animal fat, was reported to curb heart disease, while diets high in animal fat were reported to increase heart disease. Although this study had many inadequacies by today's standards, it has merit as an epidemiological study in being the first to investigate the relationship between lifestyle and heart disease. The study also examined the relationship between obesity and heart disease, the importance of exercise and the relationship between hypertension and cerebral hemorrhage [4, 5] [Note 1].

In 1948, the USPHS National Heart Institute selected Framingham, Massachusetts, a town of 28,000 people, as the target for a large-scale prospective epidemiological cohort study. Although basic research was being conducted in the United States at the time to examine the rise of coronary artery disease (ischemic heart disease) and how to deal with it, this was the Framingham Heart Study, which has continued to this day [6]. Since the risk factors for heart disease were not known at the time, the aim was to examine which risk factors were involved in coronary artery disease and to what extent. The risk factors selected were smoking, hypertension, cholesterol and glycosuria, as well as ageing. Bloods taken were cryopreserved for future research.

This study is said to have be initiated to clarify the cause of President Roosevelt's death, as he had died of cerebral hemorrhage despite having used a dietary treatment for his hypertension (see Section 4.2. Medications for Hypertension above).

More than 1,000 reports have been published from the Framingham Heart Study. Major progress reports from the 1960s report that "smoking, hypertension, high cholesterol and obesity are risk factors for heart disease" and that "exercise reduces the risk of disease". In the 1970s, it was reported that "hypertension increases the risk of cerebral hemorrhage" and that "stress increases the risk of heart disease". In the 1980s, it was found that "HDL curbs heart disease", while in the 1990s it was reported that "hypertension, smoking, glucose intolerance and high cholesterol combine to increase the risk of heart disease". In the 2000s, advances in genetic research resulted in reports on the relationship between genes and heart diseases, as well as the relationship between heart disease and various biomarkers. Many of the results from the Framingham Heart Study have matched the experience-based opinions of clinicians. With these momentous results providing a wealth of knowledge on analyzing and managing heart disease, the Framingham Heart Study has had a very significant role to play.

Cholesterol and triglycerides in the blood do not exist in isolation; they bond with proteins to form lipoprotein. Donald S. Frederickson applied the idea of lipoprotein clinically and discovered a number of apoproteins. He was able to categorize abnormal cholesterol levels by the apoprotein level. Frederickson's categorization of hypercholesterolemia is known as the Frederickson Classification; it was adopted by the WHO in 1971 to become the international classification system [7].

Meanwhile, Joseph Leonard Goldstein and Michael Stuart Brown were working on clarifying cholesterol metabolism. In addition to discovering that hepatocytes have LDL receptors, Goldstein and Brown also examined their relationship to genetic diseases and proved that in the event of familial hypercholesterolemia (FH), cholesterol is not absorbed into the liver and increases in the blood due to a lack of LDL receptors. They were jointly awarded the Nobel Prize in Physiology or Medicine in 1985 "for their discoveries concerning the regulation of cholesterol metabolism".

Goldstein and Brown's hypothesis that heart disease is caused by high cholesterol required evidence that reducing LDL could prevent arteriosclerosis, thereby preventing heart disease and cerebral hemorrhage. Cholesterol-lowering medications in clinical use at the time included cholestyramine to lower cholesterol levels, fibrates to lower triglyceride levels and niacin to lower HDL levels. No medications had yet been developed to significantly reduce the level of cholesterol most commonly observed in arteriosclerosis lesions. Cholestyramine was selected for this purpose and the Coronary Primary Prevention Trial (CPPT) was commenced in 1971 by the Lipid Research Clinics of the National Heart, Lung, and Blood Institute (NHLBI). The trial was a double-blind primary prevention trial (a clinical trial examining the extent to which initial event occurrence is suppressed in people with no medical history of ischemic heart disease). Coronary heart disease was clearly confirmed to have decreased in the patient group whose blood cholesterol levels had been lowered with the use of cholestyramine [8]. This result was published in 1980, causing the medical world to acknowledge that high blood cholesterol increases the risk of ischemic heart disease.

However, although cholestyramine lowered the risk of coronary heart disease in patients with arteriosclerosis, no significant difference was found between that group and the placebo group in terms of the overall mortality rate. A number of large-scale interventional secondary prevention

trials (clinical trials examining the extent to which repeat event occurrence is suppressed in people with a medical history of ischemic heart disease) were conducted in the 1970s and 1980s using clofibrate, niacin and EPA, but no significant difference in overall mortality rate was found despite lower total cholesterol levels (See Table 4.1).

Table 4.1 Pre-Statin Interventional Trials

	Name of trial	Drug	No. of cases (capita) control/ administration	Duration (years)	TC (pretreatment) mg/dl	TC (reduction rate) %	Heart disease onset rate (%)	Mortality rate (%)	Year published
Primary prevention trial	WHO	Clofibrate	5,331/5,296	5.3	250	Δ9	Δ20	+22	1978
	LRC-CPPT	Cholestyramine	1,906/1,900	7.4	292	Δ13	Δ19	NS	1984
	Helsinki Heart Study	Gemfibrozil	2,051/2,030	5	270	Δ9	Δ34	NS	1987
	VA Drug	Niacin	45/83	5	246	Δ4	NA		1974
Secondary prevention trial	CDP	Niacin	1,119/2,789	6.2	250	Δ10	Δ34	NS	1975
		Clofibrate	1,103/2,789	6.2	250	Δ6.5	NS	NS	1975
	DART	EPA	1,015/1,018	2	NA	NA	NS	Δ29	1989

Source: The Journal of the Japanese Society of Internal Medicine 98 (9): 281-290 (2009). NS: Not Significant

After around a decade of inactivity, large-scale interventional trials on hypercholesterolemia were resumed. The leading role of this new generation was taken up by statins, which have a powerful cholesterol-lowering effect. Many of the secondary prevention trials showed two-digit decreases in total cholesterol levels, which had not been achieved with previous drugs, as well as a significant improvement in major blood vessel events occurrence and overall mortality rate. With the powerful cholesterol-lowering effect of statins demonstrated to lower the overall mortality rate, statins gained a very firm footing as cholesterol-lowering medications.

Table 4.2 Statin Interventional Trials

Name of trial	Drug	No. of cases (capita) control/ administration	Duration (years)	TC (pretreatment) mg/dl	TC (reduction rate) %	Heart disease onset rate (%)	Mortality rate (%)	Year published
Secondary prevention trials								
4S	Simvastatin	2,221/2,223	5.4	261	Δ25	Δ34	Δ30	1994
CARE	Pravastatin	2,081/2,078	5	209	Δ20	Δ24	NS	1996
LIPID	Pravastatin	4,512/4,502	6.1	218	Δ18	Δ24	Δ22	1998
Statin meta-analysis	Statins	45,054/45,002	4.7			Δ23	Δ12	2005

Source: The Journal of the Japanese Society of Internal Medicine 98 (9): 281-290 (2009). NS: Not Significant

In the 1990s, further clarification was made on the formation mechanism of arteriosclerosis; further clarification was also made on the function of oxidized LDL and macrophages. Fig. 4.13 shows how arteriosclerosis occurs. The initial risk factors for vascular endothelial cell damage are known to include hypertension, hyperglycemia, ageing, smoking and oxidation; once damaged, LDL accumulates in the intima and is degenerated by reactive oxygen (superoxide, hydroxy radicals, hydrogen peroxide and singlet oxygen) produced by the endothelial cells. Monocytes then penetrate the intima and are activated to become macrophages, which consume the oxidized and degenerated LDL. These macrophages have scavenger receptors, which absorb oxidized LDL indefinitely, eventually turning into foam cells laden with cholesterol ester. Cholesterol ester accumulates in the lining membrane, where it forms plaque.

The platelets eventually adheres to the plaque, after which thrombi are formed by the action of the blood coagulation system, leading to infarction.

Although hyperlipidemia encompasses hypertriglyceridemia as well as high cholesterol, the focus has historically been on hypercholesterolemia in relation to arteriosclerosis. In recent years, some attention has begun to be paid to research examining the impact of triglycerides on arteriosclerosis. HDL is known to be capable of extracting the cholesterol in atherosclerotic plaque and sending it to the liver; however, increased VLDL including triglycerides reduces HDL. Increased VLDL is also known to convert LDL into small LDL and increase the amount of remnant degraded lipoproteins. Small LDL is also easily phagocytized by remnant lipoproteins and macrophages, which then advances the atherosclerotic effect. Reports also indicate that triglycerides increase the viscosity of the blood and aggravate inflammation.

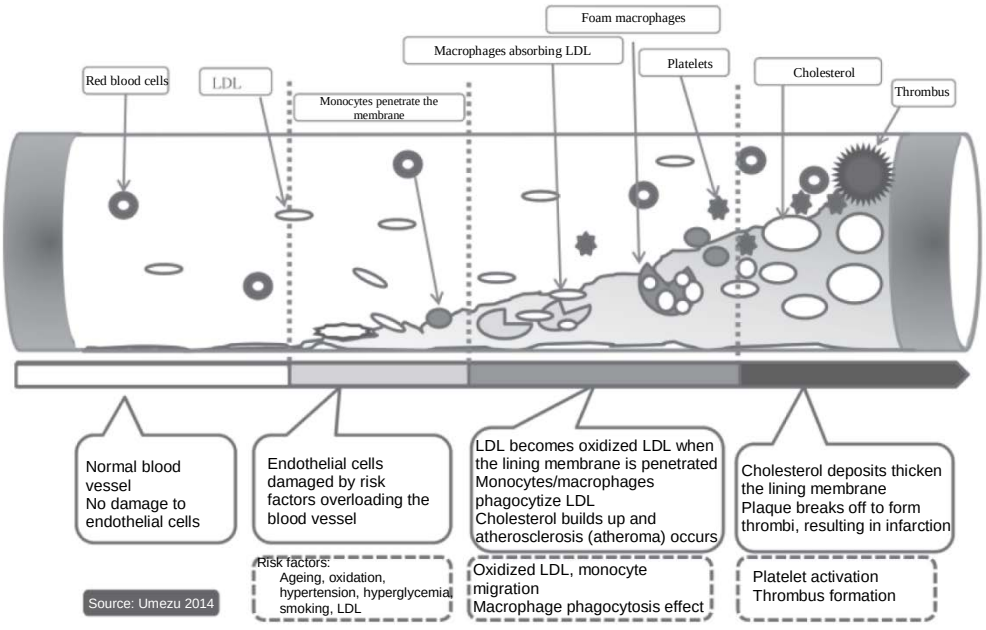

Fig. 4.13 Causes of Arteriosclerosis

The following sections provide an outline of the history of hyperlipidemia drugs discovered in the course of clarifying the relationship between arteriosclerosis and hyperlipidemia.

4.3.1. Niacin

Niacin is the generic term for nicotinic acid and nicotinamides. It is a water-soluble vitamin-B complex, is also known as vitamin B_3, and is a nutrient essential to the metabolism of carbohydrates, lipids and proteins. Its lipid-lowering effect has been known since the 1950s and it has been used clinically in the United States. With the idea emerging at the time that LDL is bad and HDL is good, Coronary Drug Trials were carried out between 1965 and 1974 to prove that HDL is good. Niacin was approved by the FDA as the best HDL-increasing agent available at the time; however, its most potent beneficial factors could not be identified, while the results found that it lowered both LDL and triglycerides [(9)].

Niacin lowers the LDL-cholesterol and VLDL levels in patients with hyperlipidemia, while also increasing the HDL-cholesterol, thereby allowing good control over blood lipid levels, although its effects are mild.

The mechanism of action of niacin was unclear at the time. Recent basic research has reported that niacin inhibits lipolysis in adipose tissue, the main source of free fatty acids in the blood. Although the liver produces triglycerides (TG) from free fatty acids, nicotinic acid lowers the triglyceride levels, which in turn lowers the VLDL concentration, thereby lowering the LDL concentration in the blood.

Later research also reported that niacin increases the secretion of plasminogen activators (t-PA), lowers the concentration of plasma fibrinogen and restores the endothelial function of endothelial cells damaged from atherosclerosis. However, none of these effects are very powerful.

In 1968, while researching nicotinate ester, researchers at Swedish company Bofors discovered the prodrug niceritrol, which is absorbed into the body and hydrolyzed into nicotinic acid. In 1970, Sanwa Kagaku Kenkyusho licensed the technology from Bofors and adapted it for hyperlipidemia, launching it on the market in 1982.

An entirely-Japanese niacin prodrug, nicomol (Kyorin Pharmaceutical), was also launched on the market; it was selected for manufacturing approval in 1971 from among 65 nicotinic acid derivative products. Yoshitomi Pharmaceutical also launched its own nicotinic acid derivative product pronicat in 1972, although it was indicated for arteriosclerosis obliterans rather than as a hyperlipidemic drug.

4.3.2. Natural Hyperlipidemia Medications

The first hyperlipidemic drug to be developed in Japan after the Second World War was polysaccharide dextran sulfate ester (MDS Kowa®) by Kowa Kagaku (now Kowa Pharmaceutical). According to the centennial history of Kowa, “until then, heparin had been used as a hyperlipidemic drug, but it had the drawback of being ineffective when taken orally and tended to cause bleeding with prolonged administration. To combat this, Professor Kozo Yamada of the Nagoya University School of Medicine proposed and developed polysaccharide dextran, which is structurally similar to heparin”. It was confirmed to have low toxicity and a cholesterol-lowering effect. It was launched on the market in 1963 [(10)]. It is still being used in hypertriglyceridemia treatment, with recent research showing that it activates serum lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) and reduces triglycerides in the blood.

In 1974, Ajinomoto and Morishita Pharmaceutical launched the hyperlipidemic drug soysterol. Soysterol is an extract containing 40-50% plant sterols and 18-22% natural tocopherols. Sterols have a cholesterol-lowering effect by integrating into the biliary micelles in enterohepatic circulation and competitively inhibit the ingestion of animal fat cholesterol in the digestive tract, thereby lowering the cholesterol level, as well as promoting the catabolic excretion of cholesterol. Currently, they are also utilized as food for specified health uses (FOSHU).

Epidemiological studies in the 1970s found that the indigenous Inuit of Greenland, with a staple diet of seals and other marine life, had a far lower heart disease mortality rate than Danish Caucasians. The blood of these indigenous people was found to contain high concentrations of eicosapentaenoic acid (EPA), which is found in ample supply in the blubber of seals and other animals. EPA began to gain attention. In Japan, epidemiological studies were carried out in places such as Chiba Prefecture and the township of Kumihama in Kyoto Prefecture, confirming that EPA has a secondary prevention effect for ischemic heart disease. Mochida Pharmaceutical eventually obtained approval for EPA as a pharmaceutical product due to its efficacy in “alleviating ulcers, pain and cold sensation accompanying arteriosclerosis obliterans”. In 1994, this was appended to include its efficacy against hyperlipidemia (see 4.4. Drugs that Act on the Blood System). A large-scale randomized controlled trial, the Japan EPA Lipid Intervention Study (JELIS), was conducted in 1996 to examine the coronary

event inhibitory effect of prolonged administration of high-purity EPA to Japanese hyperlipidemia patients. The results were published in 2007 and indicated that coronary artery disease is preventable [(Note 2)]. After that, other foods for specified health uses (FOSHU) with similar ingredients were also launched on the market.

The results of the DART Study also demonstrate a significant improvement in overall mortality rate among the EPA administration group (see Table 4.3.1).

A report by Studer et al. states that the only two drugs to have demonstrated a difference in improving overall mortality rate are statins and EPA [(11)].

4.3.3. Fibrate Lipid-Lowering Drugs

Fibrates have a long history as hyperlipidemic drugs. In France in 1953, a candidate compound was found that had a confirmed effect both on rats and clinically. This drew the attention of British company Imperial Chemical Industries (ICI), whose researchers used screening on rats to produce a compound with a powerful anti-cholesterol effect and low toxicity in 1962. This compound was clofibrate (ethyl-α-4-chlorophenoxyisobutyrate) [(12)]. While its mechanism of action was initially unknown, experiments on rats and clinical trials confirmed its VLDL and LDL lowering effects. It was reported as acting strongly on VLDL and less strongly on LDL. There were later concerns about side effects as it was found that prolonged administration to rats caused hypertrophy of the liver and peroxisome proliferation. However, it was deemed that the peroxisome proliferation and the lipid-lowering effect were due to different mechanisms; the drug was approved by the FDA in 1967 and was used in the United States as a hyperlipidemic drug. Clofibrate was approved in Japan in 1965.

Attention was again drawn to the cancer risk of fibrates as a result of chronic toxicity testing in rats. Further experiments confirmed that the onset of cancer was a phenomenon unique to rats and would not occur in primates [(13, 14)].

Gemfibrozil and fenofibrate were later used in the United States, while bezafibrate and ciprofibrate began to be prescribed in Europe.

These fibrates were also confirmed to be accompanied by a similar peroxisome proliferation phenomenon; this was later found to be a triglyceride-lowering mechanism related to the PPAR receptors [(15, 16)].

Progress was made on fibrate research and development in Japan, with Yoshitomi Pharmaceutical (now Mitsubishi Tanabe Pharma) obtaining approval for its own product, simfibrate, in 1971. Whereas clofibrate was oil soluble, this was produced in easily-administered solid formulations (capsules, granules), marking the beginning of easily-ingested prodrugs. Around 1980, it was deemed to have a HDL-cholesterol increasing effect and began to be introduced overseas [(17)].

In 1981, Sumitomo Chemical (now Dainippon Sumitomo Pharma) developed clinofibrate. However, fibrate sales dropped away due to poor cholesterol-lowering efficacy and issues with side effects. After that, however, products began to emerge with powerful TG-lowering effects as well as HDL boosting effects, the so-called second generation. Backed by basic research on the involvement of TG in arteriosclerosis, bezafibrate (developed by Kissei Pharmaceutical, discovered by Boehringer Ingelheim, now Roche) was introduced in Japan in 1991. In 1999, approval was granted for fenofibrate (Grelan Pharmaceutical, now ASKA Pharmaceutical; licensed from Fournier Pharmaceuticals and jointly marketed with Kaken Pharmaceutical) due to its benefit of once-a-day administration in addition to its TG-lowering effect.

The liver contains peroxisome proliferator-activated receptors (PPAR) that regulate lipid metabolism and belong to the nuclear receptor superfamily. PPAR-α regulates the expression of genes involved in the structure and function of lipoproteins, but when ligand fibrates bond with PPAR-α, transcription is initiated. This promotes the expression of lipoprotein lipase (LPL) and accelerates the catabolism of VLDL and chylomicrons. This also inhibits the expression of apolipoprotein C-II, the TG core, lowering its concentration and thereby lowering the TG levels. Conversely, apolipoprotein A-I and A-II expression increases, while HDL also increases. This effect is presumed to lower the triglyceride levels and cause a reduction in VLDL concentration.

Meanwhile, Japanese researchers have found fibrates to have another mechanism than lowering lipids. In 1975, researchers at Takeda Pharmaceutical found during screening that when a clofibrate derivative was administered to a diabetic mouse model, it not only lowered the lipid levels, but also lowered the blood glucose levels. This was further refined to produce the diabetes drug ciglitazone [(18, 19)].

Ciglitazone was found to act on PPAR-γ in the PPAR family, demonstrating a hypoglycemic effect, and gained much attention as a diabetes drug (see 4.1.6. Thiazolidine Derivatives).

Bezafibrate was also found to act on PPAR-γ, which is involved in insulin resistance, unlike other fibrates. This suggests the possibility of using PPAR-γ stimulation to directly improve insulin resistance. Several controlled clinical trials have been conducted on insulin resistance using bezafibrate and a placebo, with the administration groups showing greater suppression of increases in insulin resistance than the placebo groups, as well as significantly

lower primary endpoints of fatal and nonfatal myocardial infarction and sudden death [20].

More side effects have been reported from fibrates than from statins, including the breakdown of skeletal muscle tissue and rhabdomyolysis, in which necrosis causes the release of cell components, as well as renal dysfunction, headaches and nausea. Fibrates also have a less potent cholesterol-lowering effect than statins, which have a very powerful cholesterol-lowering effect. Accordingly, when statins appeared, they were expected to take over the market. However, the impact of triglycerides aside from cholesterol in arteriosclerosis has also drawn some attention, allowing fibrates to begin to be differentiated from statins due to their superior triglyceride-lowering effect.

Fig. 4.14 shows the chemical structures of representative hyperlipidemic drugs with different mechanisms of action. The distinguishing characteristics of the efficacy of each drug are given in Table 4.3.

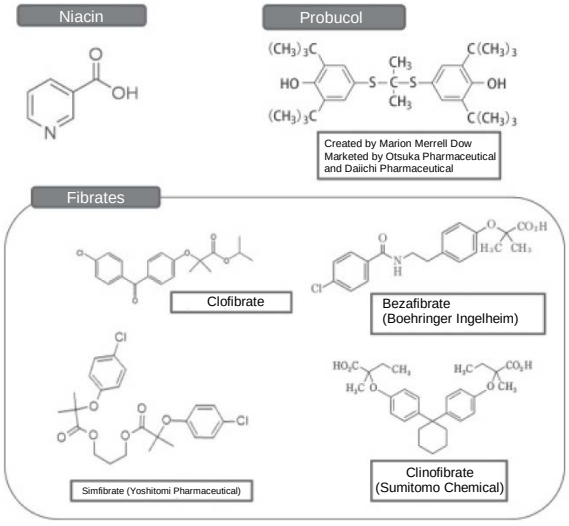

Fig. 4.14 Chemical Structures of Hyperlipidemic Drugs

Table 4.3 Efficacy of Hyperlipidemic Drugs

	LDL cholesterol	Total cholesterol	Triglycerides	HDL cholesterol	Main drugs (generic name)
Statins	↓↓↓	↓↓	↓	↑	Pravastatin Rosuvastatin
Ion-exchange resin	↓↓	↓	-	↑	Cholestyramine Colestimide
Fibrates	↓	↓	↓↓↓	↑↑	Bezafibrate Fenofibrate
Nicotinics	↓	↓	↓↓	↑	Tocophenol nicotinate
Probucol	↓	↓	-	↓↓	Probucol
EPA	-	-	↓	-	Eicosapentaenoic acid ester

↓↓↓: lowered 25% or more ↓↓↓: lowered 20-25% ↓: lowered 10-20%
↑: increased 10-20% ↑↑: increased 20-30%
-: 10 to -10%
Source: *Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases 2017*, Japan Atherosclerosis Society

4.3.4. Digestive Tract Lipid Absorption Inhibitors (Anion-Exchange Resin)

Cholestyramine is an anion-exchange resin developed by Merck in the 1960s. Merck initially developed the drug under the brand name Cumid® as a treatment for pruritus associated with biliary cirrhosis. This research was later taken over by Mead Johnson Nutrition, which developed it as a cholesterol-lowering drug under the brand name Questran® [21:140].

As a polymer, it is not absorbed into the body even when administered orally; rather, it binds with negatively-charged bile acid in the small intestine. The bound bile acid is then excreted with the feces. Bile acid is naturally repeatedly reabsorbed into the liver through enterohepatic circulation. When it is excreted from the body with the anionic-exchange resin, less bile acid is reabsorbed by the liver. This is detected by the liver cells and more bile acid is produced. Since bile acid is synthesized from cholesterol, increased bile acid production means lower cholesterol levels in the liver cells. To compensate for the reduced amount of cholesterol, the LDL receptors on the surface of the liver cells increase; the LDL in the blood then binds to the LDL receptors and is absorbed by the liver cells, resulting in a reduction of LDL-cholesterol in the blood. For details, see Fig. 4.15.

Even Goldstein and Brown used ion-exchange resin in their cholesterol metabolism experiments. The drug was also used in the Lipid Research Clinics Coronary Primary Prevention Trial (LRC-CTTP) [8], a well-known clinical trial that first proved that lipid-lowering treatments prevented ischemic heart disease. In this trial, the cholestyramine-administered group experienced a 13.4% reduction in total cholesterol and a 20.3% reduction in LDL-cholesterol. The primary endpoints of death from heart disease and onset of ischemic heart disease were curbed by 19%.

Cholestyramine was expected to have no serious side effects and a clear cholesterol-lowering effect. It was launched in Japan in 1985 by Bristol-Myers, but was met with low patient compliance due to the high dosage of 8-12g per day and the presentation of gastrointestinal side effects, including constipation and bloating. There was demand for a new drug with a lower dosage amount.

Mitsubishi-Tokyo Pharmaceuticals (Current Mitsubishi Tanabe Pharma) launched the improved drug colestimide in Japan in 1999. A copolymer of 2-methylimidazole and epichlorohydrin, it demonstrated superior bile acid absorption activity. It was easily ingested in the form of a pill coated with a cellulose base and did not need to be dissolved

in water as did cholestyramine. It also had a low dosage at 3-4g per day.

Since it had a different mechanism of action from other drugs, it could be taken concomitantly by patients showing poor cholesterol-lowering effects, although many patients were still concerned about the dosage.

In 1983, Sumitomo Chemical launched melinamide, a drug that inhibits the absorption of dietary cholesterol by inhibiting the function of cholesterol esterase, which hydrolyzes cholesterol ester in the digestive tract, and inhibiting acyl-CoA cholesterol acyl transferase (ACAT), which esterifies cholesterol in the intestinal mucosal cells.

Later, American company MSD developed ezetimibe, a drug that inhibits the absorption of cholesterol and plant sterol from the digestive tract, and launched it in Japan in 2007. The drug is reported as inhibiting 58% of cholesterol absorption compared to a placebo group. While there were issues with the high dosage of anionic-exchange resin, ezetimibe in pill form could offer a synergistic effect together with statins; accordingly, combination drugs were also developed. The mechanism of action is due to selectively inhibiting the absorption of biliary and dietary cholesterol by binding to the small intestine cholesterol transporter NPC1L1, which is responsible for cholesterol absorption. This cholesterol-lowering mechanism of action is completely different from that of ion-exchange resin.

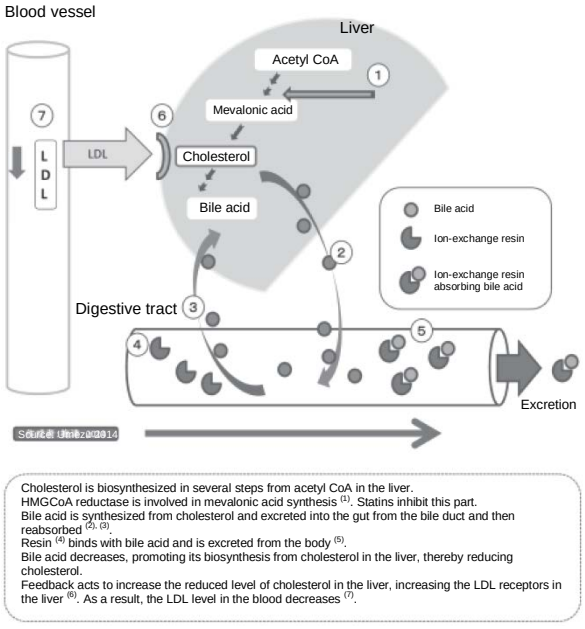

Fig. 4.15 Mechanisms of Action of Statins and Ion-Exchange Resin

4.3.5. Probucol

Probucol is a bisphenol compound synthesized by the Consolidation Coal Company of the United States as an antioxidant and later researched and developed by the Dow Chemical Company. It has an antioxidant effect and a specific total serum cholesterol lowering effect. Clinical trials of it began in the United States in 1968 and it was released on the market in 1977 following FDA approval. In Japan, Dow Chemical Japan started working on joint developments with Otsuka Pharmaceutical and Daiichi Pharmaceutical in 1979, with Daiichi launching Sinlestal® in 1985 and Otsuka launching Lorelco® the same year.

These drugs slightly lower the LDL level, which in turn lowers the cholesterol level in the blood. While some areas of this mechanism of action are still unclear, these drugs act to promote the catabolism and excretion of cholesterol into bile acid, as well as inhibiting cholesterol biosynthesis. Particular attention has been drawn to the antioxidant effect, with the theory that since arteriosclerosis can be caused by the oxidation of LDL in the blood and its absorption into foam cells, the antioxidant effect of Probucol inhibits that process, thereby inhibiting the progress of arteriosclerosis (30).

An early known issue with Probucol is that it reduces HDL-cholesterol. Recent reports have indicated that this decrease in HDL is due to increased expression of scavenger receptor class B type I (SR-BI), a scavenger receptor involved in the uptake of HDL, thus promoting HDL catabolism, thereby activating reverse cholesterol transport.

Use of Probucol remains low due to the fact that its attractive antioxidant effect has not been epidemiologically proven and the fact that it is less effective than statins at lowering cholesterol, despite its high dosage at 500-1,000mg per day.

4.3.6. Statins

Statins (named for the basic structure of the HMGCoA reductase inhibitors) emerged as innovative new hyperlipidemic drugs that actually lower the cholesterol level in the blood. They are no doubt one of the most effective drugs humans have ever developed.

Lovastatin was launched in Japan in 1987 by Merck Banyu, followed by pravastatin by Sankyo in 1989. In 2005, Shionogi released rosuvastatin, the seventh statin on the market. In 2011, the patent for lovastatin (Merck) expired, although out-of-patent products continued after that. The average sales for the first statins in the decade between 2003 and 2012 was ¥238.3 billion in Japan (based on shipments: Pharma Japan Handbook published by Jiho, various years). The world's highest-selling drug in 2005 was atorvastatin by Pfizer, at $13 billion.

The Japanese drug discovery company Sankyo and its researcher Akira Endo (now Professor Emeritus of Tokyo University of Agriculture and Technology and Representative Director of Biopharm Research Laboratories) made significant contributions in the discovery and development of statins as medication. Several sources are cited in tracing the history of statin development, which was fraught with difficulties [21, 22, 23, 24].

Endo joined Sankyo in 1971 and embarked on a project investigating cholesterol synthesis inhibitors. While a number of methods were being used at the time in Japan and overseas to treat hypercholesterolemia, including plant sterols and other cholesterol absorption inhibitors, bile acid adsorbent ion-exchange resin and fibrates to lower triglyceride levels, there were no drugs with a clear mechanism of action that powerfully lowered cholesterol levels.

Endo states, “At the time, the view was prevalent within the company that lowering your cholesterol would put you at risk, since cholesterol is a structural component of animal cell membrane and synthesizes bile acid and steroid hormones” [22]. However, Endo, believing that there would be no issue if the aim was to reduce abnormal levels back to normal levels, set the target for hydroxymethylglutaryl-CoA (HMGCoA) reductase, a rate-limiting enzyme in cholesterol biosynthesis. The idea was that since the majority of the cholesterol in human blood is endogenous rather than exogenous, it is logical to prevent its synthesis there.

Endo also writes that he had an interest in mold and mushrooms since childhood and that he thought that “some mold and mushrooms produced a cholesterol synthesis inhibitory substance as a means of competing against other microorganisms for survival” [22:17]. Since he was using very expensive radioisotopes to assess this, he devised a screening system that used only small amounts and was capable of screening large numbers of subjects. Using this, he conducted random screening on mold and mushroom culture filtrates. This was carried out *in vitro*. After screening 6,000 strains in one year, he came up with a hit from a variety of blue mold in 1973 [Note 3]. He refined this filtrate to isolate and identify a substance known as ML-236B.

ML-236B was a powerful inhibitor with inhibitory activity of 0.07mg/ml *in vitro*. This could be used as a lead compound to synthesize analogs and obtain further candidate compounds; however, it first had to be validated as being non-toxic to mice and having a cholesterol-lowering effect *in vivo*.

An initial crisis emerged at this point. Despite clearing the acute toxicity criteria, no change in serum cholesterol level was observed in young male rats after one week of dietary administration. The control group (comparison with a group administered a drug with known effects in order to objectively observe the effect of the trial drug) was administered clofibrate, already in clinical use, and saw a 21% reduction in cholesterol and a 32% reduction in triglycerides. Since initial cholesterol-lowering in rats was the global standard for assessing medicinal efficacy at the time, this result was poorly received, both within the company and externally.

Endo had doubts about testing medicinal efficacy on young rats with fast metabolisms; accordingly, he examined the efficacy of ML-236B in mature rats. He found that although there was a 20-30% reduction in cholesterol within eight hours after administration, the cholesterol level returned to its former state after that time. Discouragingly, supplementary administration offered no further lowering effects. It was not until a little later that it was found that inhibiting HMGCoA in rats activates a compensatory mechanism to increase the amount of enzymes [23].

The rat data alone was not compelling enough to make Endo drop ML-236B. He sought assistance from a colleague in another department at Sankyo, who was testing veterinary drugs on chickens and successfully convinced him to administer ML-236B to female chickens that were no longer being experimented on and were designated to be slaughtered. The results were surprising. The cholesterol level in female chickens administered with a diet containing 0.2% ML-236B dropped to two-thirds within two weeks of administration and to one half within one month (1976). Female chickens require large amounts of cholesterol to produce eggs and have high levels of endogenous cholesterol. Combined with the data from rats, it was surmised that there are differences between species in the compensatory mechanism at the time cholesterol synthesis is inhibited. Statins were confirmed to have extremely potent effects, depending on species and conditions.

After this, ML-236B was confirmed to lower the cholesterol levels in dogs as well and was used as a lead compound. While medicinal chemists worked on synthesizing and refining peripheral compounds of ML-236B, no further compounds were discovered and ML-236B itself was ultimately selected as a candidate compound for development [25].

However, a moment of tension arose at this point. ML-236B appeared in an article published in a scientific journal by British company Beecham as a new antibiotic (ML-236B was known as “compactin” at the time, thus referred to hereinafter for simplicity). Fortunately, since the

article did not mention the compound's cholesterol-lowering effect, Sankyo's utility patent had priority.

Safety testing was carried out on animals in accordance with GLP standards during preclinical testing to ensure drug safety in humans. This testing revealed a second crisis. In 1977, detailed pathological microscopy of lysosome organelles in the livers of rats administered compactin for five weeks revealed the presence of minute crystals. Discussion ensued between the group in charge of safety, who were cautious, and the drug discovery group, who favored development progress, as to whether these crystals were beneficial or harmful, with opinions divided. The company took the side of caution and safety and suspended the clinical application of compactin for the time being. At this point, compactin samples were offered to drug development companies overseas to gauge their interest, but neither Sandoz (now Aventis), Eli Lilly nor Warner-Lambert (now Pfizer) were interested, noting that it had been ineffective in rats.

Meanwhile, Prof. Akira Yamamoto of the Osaka University Faculty of Medicine sought to administer the drug to patients with serious familial hypercholesterolemia (individuals with genetically high levels of cholesterol) and no drug remedies. In 1978, a sample was provided for a physician-led clinical trial, with dramatic results obtained for patients with type IIa hyperlipidemia (heterozygous FH) (although cholesterol levels also initially dropped significantly in patients with homozygous FH, there are also reports of myopathy (rhabdomyolysis) occurring) [21, 22].

Despite that the minute crystals in the rat livers were assumed to be cholesterol ester, the safety group took a cautious stance and requested further long-term safety testing. However, backed by the fact that a number of patients in preliminary clinical trials saw actual results, full-scale phase 2 clinical trials were commenced in 1979. The results of the preliminary clinical trials and news of the commencement of clinical trials began to gain global attention [26].

When the results of the preliminary clinical trials were reported in May 1980 at the Eighth International Symposium on Drugs Affecting Lipid Metabolism (DALM) in Milan, the reaction to statins was significant. However, the euphoria was short-lived, as a third crisis emerged only three months later.

In August 1980, during clinical trials, a concurrently-conducted repeated toxicity test in dogs (one-year administration) found what was believed to be Hodgkin's lymphoma in the high administration group. Despite the admittedly high dosage (100 and 200mg/kg), Sankyo again decided to suspend development (NB: effective dosage in humans is 1mg/kg). Later, Sankyo worked on developing a back-up compactin compound, pravastatin which went on to become a cash cow.

In January 1979, Endo left Sankyo to take up a professorship at Tokyo University of Agriculture and Technology. The following month, having cultivated ten strains of *Monascus purpureus* mold in two types of culture medium at his new laboratory, he found one of the strains to be producing a compactin substance. This had to be either good intuition or good luck. He named the powerfully active substance monacolin K and applied for a Japanese patent in February 1979.

(Note: Sankyo's company history states that MB-530B (Monacolin K) was discovered by the Institute for Fermentation in 1974 from *Monascus ruber* mold as a related compound to ML-236B. [18])

However, fate works in mysterious ways. Although it has one more methyl group than compactin, monacolin K is identical to MK-803, a substance that Merck produced at almost exactly the same time from a different bacteria. Merck later marketed this in North American as lovastatin. Merck applied for patent in June 1979, having discovered the substance in December 1978. Endo applied for the monacolin K patent in February 1979.

As a result, Merck's patent was recognized in countries abiding by the first-to-invent system (such as the United States and Canada), while Endo's patent was recognized in countries abiding by the first-to-file system (such as Japan, Europe and Australia). Since Endo had surrendered the patent rights for monacolin K to Sankyo, Merck could not market its product in Japan, Europe or Australia without obtaining a license from Sankyo, but records suggest that "Merck made no request to Sankyo for a transfer of license" [22:68].

In April 1980, Merck put lovastatin (MK-803, a substance identical to monacolin K) to clinical trial; however, the trial was discontinued in September that year with the emergence of information that development of compactin had been suspended due to the results of repeated dose testing to indicate suspected carcinogenesis. Nevertheless, Merck began developing lovastatin again four years later, in 1984. Apparently, toxicity testing on animals was completed satisfactorily during this interval, as well as adequate discussion and consideration on carcinogenesis, and was deemed to be of guaranteed safety.

In the meantime, Hiroshi Mabuchi et al. of the Kanazawa University Faculty of Medicine published the results of a preliminary clinical trial (physician-led clinical trial) on compactin, drawing the attention of the world again to statins. This probably also supported Merck's development of lovastatin. In November 1986, Merck applied to the FDA for approval of lovastatin. With uncharacteristic promptness, the FDA granted approval for lovastatin in September 1987.

According to *Shinyaku Sutachin no Hakken* (Discovery of a New Drug Statin), the New York Times published an article on March 10 that year stating that lovastatin was a new blockbuster drug in the treatment of cholesterol [(22)]. This prompt series of actions begs the question whether the future prospects of statins had been predicted in US government policy.

Later, Sankyo changed its development compound from compactin to pravastatin, while Merck, with an eye on the global market, also switched its main product to simvastatin and away from lovastatin, which was difficult to expand into Europe and Asia without license permission from Sankyo. Statins were sweeping the world market (see Fig. 4.17 Advances in Statin Research and Development).

If compactin and lovastatin could be called the dawning-era first generation of statins, then pravastatin and simvastatin would be the second generation. Where compactin and lovastatin are refined extracts from culture filtrates, pravastatin and simvastatin are strucutrally-modified, semi-synthetic extracts. Pravastatin has the structure of compactin with a hydroxyl group attached; Sankyo achieved this reaction through fermentation. Despite early difficulties in industrial-scale production due to low productivity, large-scale mass production of pravastatin has been made possible through a new and rare two-stage fermentation method, in which compactin produced from blue mold is hydroxylated using *Streptomyces carbophilus*, a new species of ray fungus found in Australia [(24)].

With the efficacy of statins made clear, sales began to increase rapidly, with global drug discovery companies entering the arena in succession. The third generation of statins comprised synthetic products with a different parent nucleus from the second generation. Later products were fully synthetic, such as fluvastatin by Novartis, atorvastatin by Warner-Lambert, rosuvastatin by Shionogi and pitavastatin by Nissan Chemical/Kowa. These products demonstrated far more potent activity in *in vitro* testing and animal experiments than the second generation.

While it had been validated prior to the emergence of statins that clofibrate and cholestyramine showed a trend of lowering the risk of coronary artery disease by lowering cholesterol levels, these were far from perfect in terms of lowering total cholesterol or reducing the disease onset rate or mortality rate. Meanwhile, the combined results of five large-scale clinical trials on statins, published up to 2000, demonstrate a 25-35% reduction in LDL-cholesterol and a 20-40% reduction in the heart disease mortality rate, leaving nobody in doubt as to the cholesterol-lowering effects of modern statins. The principle was found to be that inhibiting HMG-CoA reductase to reduce the cholesterol in the liver would cause an increase in LDL-receptors, causing LDL to be absorbed into the liver from the blood, while lowering the cholesterol level in the blood (see Fig. 4.15).

Advances have also been made in research on the binding of statins to the active sites of HMG-CoA reductase. Deisenhofer et al., awarded the Nobel Prize in Chemistry "for the determination of the three-dimensional structure of a photosynthetic reaction centre", reported results from X-ray diffraction of enzyme protein and statin bonded crystals [(27)].

Mevalonic acid is synthesized using a substrate of HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) and NADPH. The enzyme is HMG-CoA reductase. While cholesterol is biosynthesized after several further steps, HMG-CoA reductase is the rate-limiting enzyme. Pravastatin and simvastatin have an R-lactone ring (a) that is cleaved (b), competing with HMG-CoA in the active center of the enzyme. Although there are two asymmetric carbons (3, 5) in the mevalonic acid equivalent portion of the statins, they must have three-dimensional structures (3-R, 5-R). Fully synthetic third-generation statins (flavastatin, atorvastatin, pitavastatin) have the butyl group portion of the second generation replaced by a fluorinated phenyl group to increase the affinity. The decalin ring portion of the second generation is also replaced by a larger hydrophobic group, which characteristically increases the affinity to the active center (Fig. 4.16) [(28)].

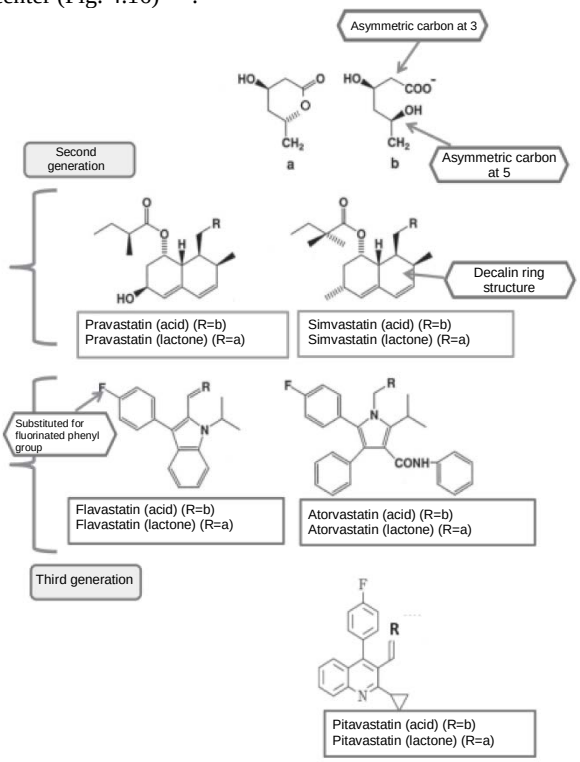

Fig. 4.16 Chemical Structures of Statins

A succession of new efficacies has been reported recently, both in basic research and clinically. Attention is being drawn to future research on the effect of statins on the brain and their relationship with type 4 apolipoprotein, thought to be connected with Alzheimer's disease.

Meanwhile, known side effects include rhabdomyolysis

(myopathy), which involves the breakdown and necrosis of skeletal muscle cells, causing muscular pain and weakness. In more serious cases, the high amounts of muscle protein (myoglobin) released into the bloodstream can damage the uriniferous tubules in the kidneys, causing acute renal failure. This is not unique to statins and has been observed in fibrates as well as new quinolone antibacterials. In 2011, the FDA recommended the maximum 80mg dose of simvastatin only to be used on patients who have taken the drug for 12 months or more without myopathy.

While Brown and Goldstein were awarded the Nobel Prize for their research on cholesterol metabolism, the award was not offered to Endo. It is common knowledge that their Nobel Prize was strongly backed by the discovery of statins and they even expressed their compliments and aspirations to Endo. Statins are among the top ten best drugs created by humanity and attention should once more be drawn to the fact that a Japanese researcher and a Japanese drug discovery company were very much involved in this. Fig. 4.17 shows the advances in statin research and development to date.

4.3.7. Other Cholesterol-Lowering Medications

Research and development has progressed on other cholesterol-lowering drugs with different mechanisms of action. These include acetyl-coenzyme A acetyltransferase (ACAT) inhibitors and cholesterol ester transfer protein (CETP) inhibitors, aimed at increasing HDL-cholesterol, as well as squalene synthase inhibitors, which target another rate-limiting enzyme that is present downstream from HMG-CoA in the cholesterol synthesis system.

Mention must be made of improved new drugs and follow-on drugs. Suppose that a pioneering drug discovery company produces an innovative new drug. Suppose also that there is no patent conflict and another drug discovery company then produces a second and third drug that are structurally similar with greater activity, fewer side effects or are more easily administered. These are improved new drugs. Sometimes as many as ten such drugs emerge on the market; these are called follow-on drugs. While improved new drugs are the characteristic forte of Japanese drug discovery companies, they are purportedly less competent in discovering innovative new drugs. However, when it comes to statins, both lovastatin by Merck and atorvastatin (Parke Davis, a division of Warner-Lambert; marketed by Pfizer), which makes more than the equivalent of ¥1 trillion in annual sales, are improved new drugs.

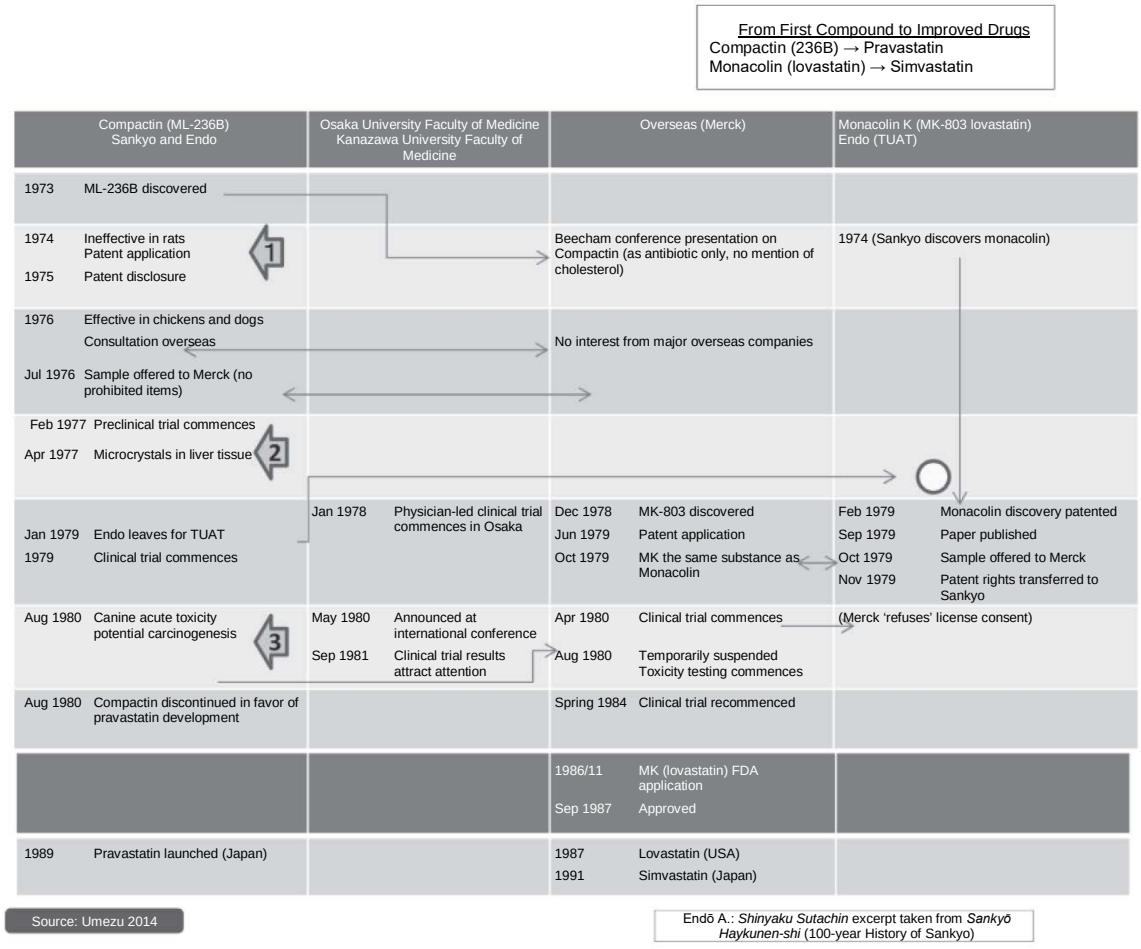

Fig. 4.17 Innovative New Drugs: Advances in Statin Research and Development

Roy Vagelos, Merck's Senior Vice-President for Research, took an interest in compactin and made contact with Sankyo

in 1973. The discovery of lovastatin came five years later, in December 1978 [29].

Parke-Davis researchers Bruce Roth et al. started investigating the publically-available date on copmactin in 1982, discovering a candidate compound six years later. However, it was found that the patent for this had already been published by Sandoz. The Parke-Davis group started investigating again and discovered atorvastatin five years later, in 1993.

It must be emphasized that improved new drugs need to be highly evaluated. The mission is not to create drugs with new mechanisms of action, but to provide better drugs to humanity. Therefore, it is vital for drug discovery companies to have ways of using certain information to create better drugs faster.

In the above example of statins, it is a fact that there are drug discovery companies with the capabilities/mechanisms (systems science) to be able to create new candidate compounds within five to six years given "certain powerful information". This creates a significant difference between companies with "mechanisms and policies" and those without.

Note 1: Following a number of trials, the American Heart Association announced in 1976 that the consumption of high amounts of butter, eggs, lard and beef was highly likely to cause coronary heart disease. Later, the US government also announced its recommendation of a low-fat diet.

Note 2: The Japan EPA Lipid Intervention Study (JELIS) was conducted on 18,645 Japanese hyperlipidemia patients (14,981 coronary heart disease primary prevention patients and 3,664 secondary prevention patients). This was the world's first prospective randomized open-label trial, tracking the cumulative incidence of major coronary events in an EPA+statin administration group and a statin-only, EPA-non-administration group for five years (4.6 years on average). There were 324 instances (3.5%) of major coronary artery disease in the control group and 262 instances (2.8%) in the administration group.

Note 3: Details on the drug discovery screening process are given in Chapter 3: Technological Advancement of Pharmaceutical Drug Discovery.

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4.4 Drugs that Act on the Blood System

This section provides an overview of drugs used to treat clots, hemorrhages and other cardiovascular abnormalities that interfere with the natural activity of the blood, causing serious functional impairment.

Thrombotic disorders caused by coagulation of blood that normally flows within the blood vessels include acute myocardial infarction, deep vein thrombosis, pulmonary embolism and acute ischemic stroke. These disorders are treated using anticoagulants and thrombolytics. According to the *2008 Patient Survey Overview* by the Ministry of Health, Labour and Welfare, the total number of patients receiving ongoing treatment for ischemic heart disease was 810,000, while the total number of patients receiving ongoing treatment for strokes, including cerebral infarction and cerebral hemorrhage, was 1.34 million. These figures are predicted to continue to increase as ageing society advances. Increasing incidences of thrombosis and pulmonary embolism have gained attention in recent years, along with the term “economy class syndrome” or “long flight syndrome”.

While hemorrhagic disorders can be hereditary, such as hemophilia, or occur pathologically, such as postoperative fibrinolysis, hemorrhaging can also occur after using anticoagulants.

Currently, there are relatively few patients with hemorrhagic disorders compared to those with thrombolytic

or embolic disorders, but it goes without saying that preventing hemorrhage is crucial to preserving life.

Hematological drugs can be classified as (i) antiplatelets, (ii) anticoagulants, (iii) thrombolytics and (iv) hemostatics.

Thrombosis is mainly caused by the generation of fibrins with the stimulation of the blood coagulation system resulting from vascular wall damage due to arteriosclerosis, changes in blood components or abnormal blood flow, as well as the formation of platelet aggregates, resulting in vascular stenosis (narrowing of blood vessels, making it difficult for blood to pass through) or occlusion (blood vessel blockage).

Thrombus formation is mainly due to the coagulation system and the platelets involved in fibrin production. Platelet and the coagulation cascade work together in the body to cause the blood to clot. When a blood vessel is damaged, platelets first adhere to the vascular endothelium and then aggregate together to form a primary hemostatic plug around the opening of the wound. After that, various clotting factors are released to coagulate the fibrins in the blood, forming a secondary hemostatic plug.

Fibrin coagulation is a continuous reaction that takes place by means of the mechanism shown in Fig. 4.18. This is called the blood coagulation cascade. The intrinsic coagulation pathway and the extrinsic coagulation pathway are both transmitted through several continuous chain reactions involving the gradual conversion from plasma factors (enzyme precursors) to activated enzymes (mainly serine proteases). (This cascade has 12 types of coagulation factors, which have been numbered by Roman numerals in order of discovery.) The final step in the cascade involves activated Factor Xa (FXa), an enzyme that converts prothrombin into thrombin, and activated Factor V. Thrombin activated by the enzyme action of FXa acts on the mesh-shaped fibrin precursor fibrinogen to produce fibrin monomers; these fibrin monomers link together through covalent bonds to form fibrin polymers. Finally, the enzyme action of FXIIIa forms a stable fibrin clot and coagulation is complete. Thrombin is activated Factor II.

The body also has mechanisms to prevent excessive stimulation of the coagulation system. One mechanism is antithrombin (AT), which controls excessive thrombus formation by binding to thrombin, FXa and FIXa to inhibit their action, thereby localizing coagulation. Another mechanism is the thrombomodulin (TM) and protein C control system. TM binds with thrombin to convert the protein C in the blood into activated protein C (APC). APC breaks down VIIIa and Va in the coagulation system, thus avoiding the generation of fibrins and controlling thrombus formation.

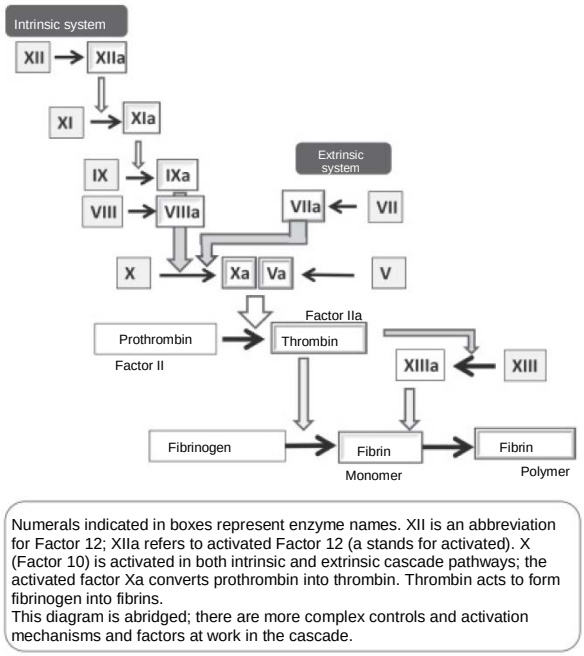


Fig. 4.18. Blood Coagulation Cascade

- An antithrombin formulation (Behring) was developed and marketed in Japan by Hoechst Japan in 1987. An activated protein C formulation was also released as a hematological drug in 2000. -

The fibrins that are produced are broken down in the fibrinolytic (fibrinolysis) system. The key component in this system is plasmin. Plasminogen is present in the blood as a precursor; it breaks down fibrin clots after being converted into the active enzyme plasmin by the action of tissue plasminogen activator (t-PA). The blood in the body is controlled by the balance between the coagulation and fibrinolytic systems (see Figure 4.19).

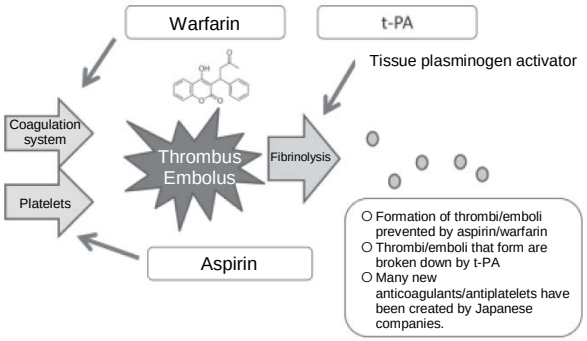


Fig. 4.19 Coagulation/Fibrinolysis System and Drugs

Platelets are blood cells that are fragments of megakaryocyte cytoplasm protrusions. They are amorphous in shape and have no nucleus. The platelet membrane has receptors that bind to thrombin, serotonin (5HT), ADP and

exposed collagen. In the absence of damage to a blood vessel, platelets circulate freely in the blood in a regulated state. When the receptors are activated by attaching to ligands, granules within the platelet cells are released into the blood; the ADP, serotonin and other substances within these granules act on the receptors on other platelets, causing a reaction chain phenomenon. The platelets interact with each other, resulting in aggregation (primary hemostatic plug) [(Note 2)]. The activated platelets also activate the coagulation system, triggering the polymerization of fibrins to form clots (secondary hemostatic plug).

Although the number of cerebral infarction and myocardial infarction patients has grown recently, attention is now being given to secondary prevention following the acute phase. There are growing hopes for anticoagulants and antiplatelets to achieve this. An increase in pulmonary thromboembolism and deep vein thrombosis, known as long flight syndrome or economy class syndrome, has also been noted. Even in everyday life, modern humans are suffering from disorders caused by coagulation or clotting of the blood. While basic research has long been carried out on the coagulation system, there has been a rapid increase in recent years of basic research on the mechanisms leading from platelet receptors to aggregation, with successive drugs emerging to inhibit various different parts of the signal transduction pathway leading to thrombosis [(1, 2)].

In the area of hemostatics, Japanese universities and private companies collaborated in a modern drug discovery approach to produce the world's first low-molecular-weight hemostatic drug at a time when drug discovery systems were far from complete, still suffering the effects of the Second World War. The following section outlines some of these drugs.

4.4.1. Hemostatics

Natural extract protamine sulfate (extracted from the testes and semen of fish; it is positively charged due to its high arginine content; used to antagonize the anticoagulant effect of heparin) has been used to neutralize the effects of heparin [(Note 3)] in patients undergoing anticoagulation therapy. While vitamin K as well as aprotinin (a bovine lung protein that has a serine protease catabolic enzyme inhibitory effect and inhibits plasmin) have also been used to prevent bleeding, low-molecular-weight synthetic drugs emerged in the 1950s and 1960s, including aminocaproic acid and tranexamic acid.

Noteworthy in the history of Japanese drug discovery, these synthetic hemostatics were researched and developed independently in Japan. Although they were discovered by a team working under Prof. Shosuke Okamoto of the Kobe University School of Medicine, joint research began between Hayashi Kenkyusho (a private institute founded by writer Takashi Hayashi) and Mitsubishi Chemical in 1947, when battle scars were still fresh from the war. Okamoto was at that time working at the Keio University Faculty Medicine as the Hayashi Kenkyusho representative, while Fujio Nagasawa, the institute's deputy director and representative from Mitsubishi Chemical, was in charge of planning and direction. Research began in 1947 at the Mitsubishi Chemical laboratory at Mizonokuchi in Kawasaki.

According to *Sekai wo Ugokasu Nihon no Kusuri* (Japanese Medicine to Move the World) (Okamoto S. (ed.), Tsukiji Shokan, 2001) [(3)], this concept of a joint laboratory was unique. The concept was to "firstly, delineate international standards, secondly, to target fields that were still untouched and unexplored by researchers, and thirdly, to produce drugs that were useful in the treatment of diseases".

While the postwar drug production industry was concentrating on sulfa drugs, penicillin and other antibiotics and antihistamines, the research question selected by this project was antiplasmins, a highly original field with no competition anywhere in the world.

It has long been known that there are specific enzymes that break down fibrins in the blood. Oxford University researchers Robert Macfarlane and Rosemary Biggs discovered a protease that rapidly and selectively broke down fibrins in the blood, naming it plasmin soon afterwards.

Okamoto wrote, "For screening, the *in vitro* system was selected, allowing highly efficient experimentation without using animals. A fibrin solution was created in a test tube, while equine plasma was treated with low acidity to produce a precipitate containing plasmin. This was added to the fibrin solution and the decomposition rate measured. Available compounds were sequentially screened. Negatively-charged glutamic acid and aspartic acid appeared to promote the action of the plasmin, albeit slightly, while positively-charged arginine and histidine appeared to inhibit the action of the plasmin; thus, the focus fell on lysine, an amino acid that is similarly positively charged. However, it was almost impossible to obtain amino acids such as lysine in that postwar period" [(3:14)].

They were eventually able to obtain lysine and examine its activity to confirm its powerful effect. Not long after discovering the inhibitory activity of lysine, the focus fell on nylon as a lysine analog. Nylon stockings were hydrolyzed in hydrochloric acid to produce needle crystals. When these were tested, they were found to have an even more powerful inhibitory activity than lysine. Thus, the antiplasmin epsilon (ε-aminocaproic acid) came into being. While lysine has a caproic acid structure with amino groups in the alpha and epsilon positions, epsilon has no amino group at the alpha position; it was found to have an antiplasmin effect around

ten times more powerful than that of lysine. Although epsilon has been considered possible to implement clinically due to its low toxicity, its clinical application has been non-committal as there is no precedent concept for such a drug.

Around this time, it started becoming known that plasmin increases in the event of allergy or functional uterine bleeding. Clinical trials were conducted by the Departments of Pediatrics and Obstetrics & Gynecology at Keio University; the results were confirmed for chronic allergic eczema in children and for functional uterine bleeding. Full-scale clinical testing was undertaken by Daiichi Pharmaceutical [(4:81-85)].

At the time, pharmaceutical product approval in Japan mainly related to safety. This was a time when dozens and even hundreds of patients were approved if they did not present with side effects. The United States Patent and Trademark Office even has a record stating that when an application was made for an American patent for epsilon in the 1950s, "a complete review was called for, as there was insufficient data on the medicinal efficacy; the project team, which comprised a number of physicians from various departments of the Keio University School of Medicine, responded with their results" [(3:19-21)].

Although the hypothesis that the inhibition of plasmin would be therapeutic was proven, epsilon lacked sufficient medicinal efficacy to be able to treat plasmin-related severe bleeding events or serious bleeding such as that accompanying lung surgery. Accordingly, efforts continued in order to further increase its activity.

Epsilon has a simple structure with five carbon atoms and a five-ångström hydrocarbon with positive and negative charges at each end. Several compounds were synthesized replacing the straight-chain hydrocarbon with a cyclic hydrocarbon to make this distance fixed; one such compound was aminomethyl cyclohexanecarboxylic acid (tranexamic acid). Tranexamic acid is a stereoisomer; it was successfully broken down by Masashi Shimizu et al. of Daiichi Pharmaceutical, no simple task for the time [(3:25)]. The result was powerful efficacy for the chair-type (trans-) isomer and hardly any activity for the board-type (cis-) isomer. Thus created, tranexamic acid was ten times more potent than epsilon.

Epsilon-aminocaproic acid was released on the market in 1954, followed by tranexamic acid in 1965. These are still widely used today as oral drugs and injected drugs, not only in Japan, but internationally as well. The 2012 edition of the American textbook Pharmacology in the Lippincott Illustrated Review Series states that "fibrinolysis can be controlled by the administration of ε-aminocaproic acid or tranexamic acid. Both products are synthetic compounds, are effective when administered orally, are excreted in the urine and inhibit the activation of plasminogen" [(1)] (see Fig. 4.20).

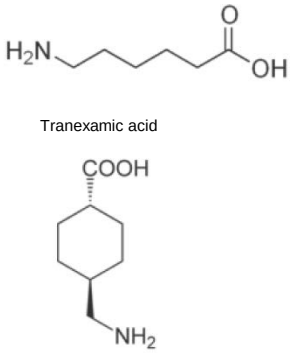


Fig. 4.20 World-First Synthetic Hemostatics

There is much to be learnt from the attitude of drug discovery companies in the difficult postwar era, successfully undertaking industry-academy collaborative projects and setting targets irrespective of current trends, with high aims of discovering drugs worthy of evaluation at international level. "Target exploration" in the age of 21st century genomic drug discovery has incorporated the idea of first creating a target enzyme or receptor inhibitor and then considering the applicable diseases for treatment (see Chapter 3), although, surprisingly, a similar idea has already existed in Japan for over 68 years. Optical resolution of active substances is also being carried out in this era.

These hemostatics were launched on the market by Daiichi Pharmaceutical. In 1969, approval was given for an additional indication for allergic inflammation. Sales are said to have peaked at ¥4 billion [(4:130, 5)]. With drug production volumes in Japan at around ¥450 billion in 1965, it is clear that epsilon and tranexamic acid were significant discoveries both for academia and for business.

4.4.2. Anticoagulants

Bleeding and clotting are opposites, but it is essential for the body to have a balance between the two. Okamoto shifted focus to the discovery of anticoagulants (mentioned later). In the 1970s, lifestyle-related diseases were rare in Japan and thrombosis was not as problematic as it was in the West. Instead, hemorrhagic disorders were the focus of attention. However, as eating habits and lifestyles changed to a more Western style with the rapid growth of the economy, the number of thrombosis patients also increased and Japan began initiatives to combat thrombosis.

Meanwhile, in the West, there were a number of studies being conducted in the 1970s on the treatment of thrombosis. While this research laid the foundation for heparin treatment and warfarin treatment to regulate the blood coagulation cascade and fibrinolytic treatment to break down clots, each had their own problem areas. The following is an outline of this history, with details of anticoagulant drug discovery begun in Japan.

Heparin

The history of anticoagulants began with the discovery of the anticoagulant effect of heparin by medical student Jay McLean in 1916 using canine liver.

Heparin is a mixture of linear acidic mucopolysaccharides made up of repeating units of uronic acid and glucosamine, also present in human cells. Heparin molecules contain a high amount of sulfate groups and are negatively charged. Heparin from porcine intestinal mucosa has long been used as a treatment drug.

Heparin binds with heparin cofactor (antithrombin III [Note 4)]) and changes its structure to increase the efficacy of antithrombin III one thousand fold. Antithrombin III binds with thrombin, FXa (activated Factor X) and serine protease (a protease with serine at the active center; many enzymes in the coagulation cascade are of this type) active sites to inhibit the action of those enzymes. In other words, antithrombin III regulates the coagulation cascade. Heparin binds with antithrombin III and changes its structure to enhance its inhibitory effect.

Heparin was developed as a pharmaceutical product in Canada (1937) and in Sweden (1939). In Japan, sodium salt began to be marketed in 1962 as a treatment and prevention for arterial/venous thrombosis.

Heparin has long been used clinically as an injected drug; however, side effects have included: (i) the possibility of antigenicity, since it is produced by non-human animals (processed from bovine or porcine intestinal mucosa, with a molecular weight range of around 30,000 to 35,000, it is not a single substance) and (ii) hemorrhagic complications despite the original effect. Other side effects observed included (iii) thrombocytopenia and (iv) thrombosis. While caution was necessary because of these side effects, bleeding was a particularly major drawback. Since the action of heparin was significantly affected by the amount of antithrombin III, there were also issues with the level of side effects differing between individual patients.

With the aim of reducing these side effects, low-molecular-weight heparin (LMWH), with a molecular weight of 6,000 or below, was produced from unfractionated heparin by enzymatic and chemical depolymerization. LMWH did not require such focused monitoring as heparin and began to replace heparin in clinical application. However, little difference was observed in side effects other than thrombocytopenia. In Japan, dalteparin sodium (approved in 1992), parnaparin sodium (approved in 1994), reviparin sodium (approved in 1999) and other similar drugs were developed, with approval limited to preventing coagulation during hemodialysis. All of the other drugs were imported from overseas, with very little domestic research in Japan [(18)].

Warfarin

Warfarin comes from a coumarin compound isolated from decomposed sweet clover by Karl Paul Gerhard Link et al. of the University of Wisconsin as part of a causal investigation on a phenomenon occurring on ranches in the United States and Canada in the 1920s, in which cattle that had eaten decomposing sweet clover were bleeding to death. This compound was found to be the cause of the hemorrhagic disorder. An analog, dicumerol, was synthesized and used as a rodenticide. While dicumerol was later even used clinically as an anticoagulant, a later derivative, wafarin, offered better gastrointestinal absorption and an application was made to patent it as a coagulation inhibitor in 1945. Its efficacy was validated and it began to be used as well as heparin as an anticoagulant in the United States in the 1950s.

Biosynthesis of blood coagulation factors prothrombin (Factor II), Factor VII, Factor IX and Factor X occurs in the liver. This involves vitamin K as a coenzyme to these amino-terminal converting enzymes. Research has shown that warfarin acts to antagonize vitamin K and inhibit the biosynthesis of these enzymes, as well as indirectly inhibiting blood coagulation. As mentioned previously, prothrombin is a vitamin K dependent factor that is particularly important in the blood coagulation cascade. Where other coagulation factors have short half-lives of 6-36 hours, prothrombin has a long half-life of 60-72 hours. Warfarin is thought to demonstrate its anticoagulation effect mainly by reducing the levels of prothrombin due to it being unable to biosynthesize. Consequently, it can take 3-4 days from administration for the effects of the drug to manifest; conversely, the effects can continue for 4-5 days after administration has been ceased.

Warfarin has become widely used around the world due to its low cost and reliable antithrombotic effect. However, like heparin, warfarin has long had issues with its user-friendliness, as the optimal dosage varies between individuals. Even though it is the best drug in use today, its blood concentration levels still require monitoring.

Antithrombotics: Argatroban

Prof. Shosuke Okamoto (mentioned previously) examined the available information on antithrombotics in the West at the time and wrote that “given the circumstances, there

should be several antithrombotic substances or other thrombotic treatments prepared for clinical use" [(3:38)]. Thrombin was selected as the target for this [(Note 5)]. Research commenced in 1970 with the hypothesis that while plasmin cleaves the C-terminal region of the amino acid lysine, thrombin cleaves the C-terminal region of arginine, meaning that there should be inhibitors in the periphery of arginine. Unfortunately, no candidate compounds were discovered in the periphery of arginine; however, extended screening resulted in the discovery of low-level activity by tosylarginine methyl ester (TAME), a quantity reagent for thrombin activity. This was selected as a lead compound.

Okamoto again collaborated with Mitsubishi Chemical to draw from the company's success with hemostatics. A research team was established in Mitsubishi Chemical's pharmaceuticals department, led by synthetic organic chemist Ryoji Kikumoto. Kikumoto demonstrated his worth as a medicinal chemist on this project, producing a thrombin-specific inhibitor. Based on the information available in 1970, Kikumoto proposed a molecular design concept for the project. This comprised a tripod structure, with the idea that thrombin has three pockets (see Fig. 4.21).

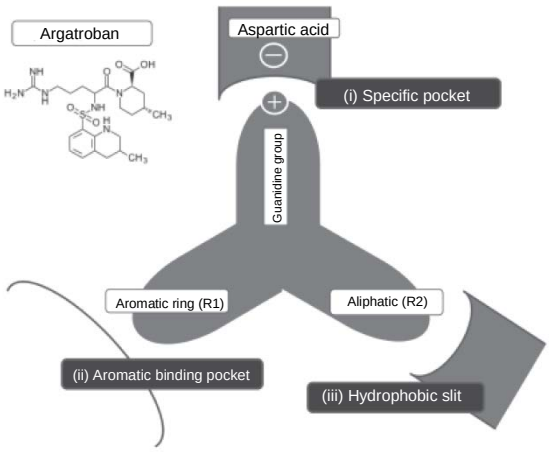


Fig. 4.21 Tripod Hypothesis

Thrombin selectively cleaves the C-terminal region of arginine. In other words, it causes limited proteolysis of the arginine-glycine bonds between A_α chains 16-17, B_β chains 14-15 and Factor XIII 36-37 in the fibrinogen substrate. This means that there are areas with confirmed arginine residue with specific C-terminal and N-terminal structures near thrombin active sites.

Further, since ℓ-arginine enters thrombin active sites, lysine and d-arginine demonstrated no inhibitory effect.

Since ℓ-arginine is characterized by a having basic guanidino group, it was assumed that this could be counteracted by aspartic acid, which has a highly-acidic carboxyl group at the enzyme site (see (i)) (specific pocket). Amino groups, imino groups and guanidino groups with organic bases to counteract the aspartic acid residue at (i) were investigated, with the guanidine group eventuating as the best. Site (ii) of the tripod structure was envisaged as a hydrophobic substituent pocket. Since the ninth position from the N-terminus of the A chain of fibrinogen hydrolyzed by thrombin is always phenylalanine in mammals of all species, an aromatic hydrophobic amino acid residue is present near the active center, which is thought to play an important role in substrate specificity. Thus, an aromatic hydrophobic substituent was selected (aromatic binding pocket). (iii) is the region corresponding to the C-terminus of arginine; the wall on the enzyme side is presumed to have a narrow slit-shaped hydrophobic structure (hydrophobic slit).

Based on this hypothesis, synthesis was undertaken systematically to produce one pocket and then the others by changing the group for each pocket. Upon examination of the structure-activity relationship, the optimum specific pocket for (i) was found to have a length of 4-5 carbons, while the 4-methylpiperidine was found to be optimum for (ii). With these established, (iii) was modified, with *in vitro* analysis showing a gradual increase in inhibitory activity compared to the lead compound TAME. No. 205 showed I50 (representing the concentration at which the coagulation time doubled using the thrombin activity evaluation method; the lower the value, the more powerful the effect) that was 10,000 times more powerful than TAME.

Examination of inhibitory action on serine proteases other than thrombin revealed very high thrombin specificity, indicating that this was a thrombin-specific inhibitor. No. 205 was presumed to bind to thrombin active sites at the third point on the conjectured tripod. The tripod hypothesis turned out to be correct [(6)] (see Table 4.4 Argatoban Enzyme Selectivity).

Table 4.4 Argatoban Enzyme Selectivity

Enzyme	Ki (μM)
Thrombin	0.039
FXa	210
Plasmin	800
Kallikrein	1,500
Trypsin	5.0
t-PA	210
U-PA	>1,700

Argatroban had high selectivity for thrombin.

However, experiments on rats showed this compound to have high acute toxicity. A number other compounds were synthesized to avoid this. One of these compounds, No. 407, was obtained by introducing a carboxyl group to the C-terminus, reducing the acute toxicity to one-tenth that of No. 205. Kikumoto conjectured that “these compounds are thrombin-specific, with the exception that they have very high affinity to pseudocholinesterase. Introducing the carboxyl group would make No. 407 lose its pseudocholinesterase inhibitory effect, which would be the key point of reducing the acute toxicity.”

To optimize the compound further, the (R1) structure affinity at (ii) was modified to produce the final compound. This was the 700th compound to be synthesized since TAME; it was a 4-methylpipecolin-2-carboxylic acid with four isomers. Kikumoto conjectured that, of the four isomers, 2R and 4R would demonstrate the strongest activity, and he developed a new synthetic method of 4R-methylpipecolin-2R-carboxylic acid: four stereoisomers were split, with isomers synthesized for each of them. This resulted in a surprisingly significant difference between the strongest (2R, 4R) isomer and the weakest (2S, 4S) isomer, with the strongest being 15,000 times more powerful than the weakest. The 2R, 4R isomer became the pharmaceutical product argatroban (No. 805) [7] (see Figures 4.22 and 4.23).

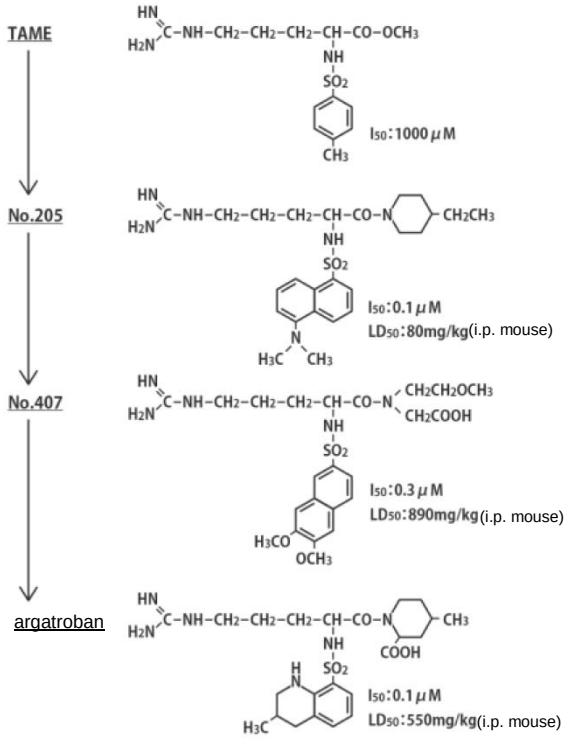


Fig. 4.22 Antithrombotic Optimization

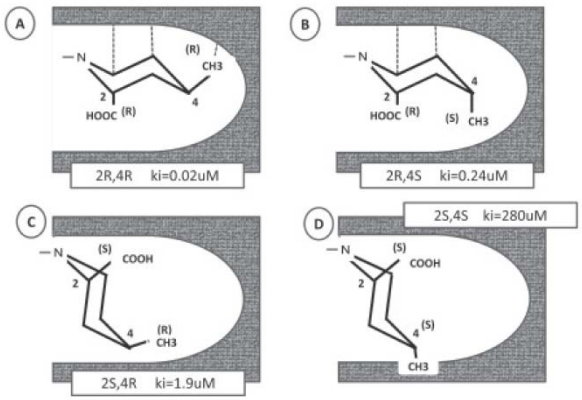


Fig. 4.23 Schematic Diagram of Argatroban Enantiomer Binding to Thrombin Active Site

The laboratory later conducted X-ray analysis on an argatroban-thrombin complex to clarify the structure of the binding site in detail. It was the same as the relationship between argatroban and the active center hypothesized by Kikumoto in the exploratory research.

Developing No. 805 as an antithrombotic required validating its efficacy in animal experiments. Yoshikuni Tamao et al., in charge of pharmacology, came up with a new model involving exposing the carotid artery of a rabbit and immersing the outside of it in acetic acid. The acetic acid penetrated into the arterial intima cells, damaging the vascular endothelium and causing a blood clot within 2-3 hours. Viewing under scanning electron microscopy revealed that the clot was a platelet thrombus. The opinion at the time was that antiplatelets such as aspirin were effective against platelet thrombi, while anticoagulants such as heparin and warfarin were not; this study drew the same conclusion. However, argatroban administered by intravenous injection demonstrated a powerful efficacy; unlike heparin, which worked its effect through antithrombin III, this drug was found to work its antithrombotic effect by directly inhibiting thrombin, showing great promise for clinical application [8].

As well as suppressing fibrin production by inhibiting thrombin, argatroban also inhibited the fibrin stabilizing effect of Factor XIII and demonstrated a platelet aggregation inhibitory effect.

The key players of the 1970s were the medicinal chemists. The history of argatroban research is a typical example of their experience and intuition being put to use in clarifying structure-activity relationships in drug discovery. Based on the initial hypothesis, more than 800 compounds were organically synthesized and tested by trial and error until a target was reached. This commendable approach resulted in the release of the world’s first thrombin inhibitor onto the market. However, argatroban does not readily dissolve in water, making oral administration difficult. The drug was launched in 1990 as an injected drug indicated for chronic arterial occlusion. In 2000, it was approved for heparin-induced thrombocytopenia (HIT) in the United States. Approval was granted in Japan for the same application in 2008.

Later, antithrombin antagonistic FXa inhibitor fondaparinux appeared as an injected drug in 2007. While researched and developed by Sanofi Sante, clinical development in Japan was consigned to GSK. Since this drug enabled greater antithrombin III FXa inhibiting selectivity, it was used in the prevention and treatment of venous thromboembloism (VTE). The emergence of fondaparinux also proved that even with poor thrombin inhibition, thrombosis could be prevented and treated by inhibiting FXa further upstream in the coagulation cascade.

Oral Anticoagulants

With long-term administration of injected drugs proving problematic to patients, companies began to compete over the research and development of orally-administered antithrombotics and FXa inhibitors. As explained by the structure-activity relationship of argatroban, the idea was to inhibit thrombin and FXa by the entry of a guanidino group into the active center; however, it was found that the guanidino group also caused a decline in absorption by the body.

Consequently, new compounds emerged with pyridine rings and with chlorothiophene structures in place of arginine-analogous structures. In 2011, Nippon Boehringer Ingelheim launched the oral antithrombotic daibigatran, indicated for “inhibiting the onset of ischemic stroke and systemic embolism in patients with non-valvular atrial fibrillation”. The same year, Sankyo released Japan’s first oral FXa inhibitor edoxaban, indicated for “inhibiting the onset of venous thromboembolism (VTE) in patients undergoing or having undergone orthopedic surgery on the lower limbs”. In 2012, FXa inhibiting rivaroxaban (Bayer) was released on the market with similar indications.

Apixaban is an orally-administered anticoagulant jointly developed by Bristol-Myers Squibb (BMS) and Pfizer to reversibly inhibit FXa. This drug was approved and marketed in Japan in 2013 for its efficacy in “inhibiting the onset of ischemic stroke and systemic embolism in patients with non-valvular atrial fibrillation”. A recently published meta-analysis of comparative studies between warfarin and these oral anticoagulants showed them to be significantly better in terms of the relative risk of intracranial hemorrhage [(9)]. With this side effect having been deemed to be of particular concern to Asians, who are more susceptible to intracranial hemorrhage, these oral anticoagulants are expected to reduce that risk.

There is also ongoing focus on the efficacy of these new oral anticoagulants in preventing cerebral hemorrhage in patients with atrial fibrillation [(10)].

Macromolecular Anticoagulants

While it has long been known that certain types of snake venom contain substances that affect blood clotting, it was not until 1960 that Pentapharm (Switzerland) isolated a thrombin-like enzyme from the venom of the common lance-head pit viper *Bothrops atrox*, found in a wide-ranging habitat throughout Central and South America. A comparative examination of snake venoms found this venom to contain a protein that lowers the fibrinogen levels in the blood. This substance, known as batroxobin, was clinically confirmed to have a fibrinogen-lowering effect and was approved for use on thrombotic diseases in many countries. In Japan, Tobishi Seiyaku noted a peripheral circulation improving effect and developed this, gaining approval in 1993 to use this as an antithrombotic peripheral circulation improvement agent.

Thrombomodulin alpha (TM-α), developed by Asahi Kasei Pharma in collaboration with Prof. Maruyama of Kagoshima University and Prof. Suzuki et al. of Mie University using gene recombination technology, is the world’s first human thrombomodulin. It was released on the market in 2008 as an injected drug indicated for disseminated intravascular coagulation (DIC) [(Note 6)].

Thrombomodulin is a glycoprotein present on the surface of vascular endothelial cells that demonstrates an anticoagulation effect by forms a complex with thrombin in the blood. It has the effect of suppressing abnormal thrombin activation. While injecting this could be expected to have an anticoagulation effect, natural thrombomodulin is a membrane protein that is present on the vascular endothelial cells and is insoluble. Since it is preferable for pharmaceutical products to be soluble, researchers at Asahi Kasei Pharma used biotechnology to successfully convert the extracellular section containing the thrombomodulin active sites into soluble molecules, thus developing thrombomodulin-α, or recombinant human soluble thrombomodulin (rsTM). rsTM is the world’s first drug to be more effective than heparin, used in a prospective comparative clinical study on DIC patients. rsTM was proven to inhibit the onset of DIC by promoting the activation of protein C to prevent the activation of thrombin; it was also proven to have a lower hemorrhagic effect than heparin [(11)].

4.4.3. Platelet Aggregation Inhibitors

Platelet aggregation is suppressed by aspirin. While aspirin was originally used as an anti-inflammatory for its antipyretic and analgesic effects, since the discovery by H. J. Weiss et al. in 1967 that aspirin at low doses had a platelet aggregation inhibitory effect [(12)], multiple large-scale clinical studies have proven the utility of aspirin as an antiplatelet drug. In 1998, all aspirin formulations (non-prescription

drugs) were confirmed by the FDA for suppressing thromboembolism in atherosclerotic diseases. Aspirin is now used as an antiplatelet drug in countries all over the world. In Japan, enteric coated aspirin tablets have been marketed by Bayer as antiplatelet drugs since 2001, indicated for suppressing thromboembolism in myocardial infarction and ischemic cerebrovascular diseases (transient ischemic attack (TIA) and cerebral infarction) [13].

The pharmacological mechanism of action of aspirin was found to be due to inhibiting COX-1 by irreversibly acetylating serine residue at cyclooxygenase-I (COX-I) active centers. As a result, the production of thromboxane A_2 (TXA_2: promotes the cluster formation necessary to rapid thrombus formation) downstream from COX-1 is inhibited, thereby suppressing the aggregation of platelets. Since platelets have no nucleus and new protein cannot be synthesized within the lifespan of a platelet (seven to ten days), acetylated COX-I remains inactive, thus continuing the antiplatelet effect. Accordingly, excessive aspirin treatment for inflammatory diseases or rheumatism can prolong bleeding time and at high concentrations has even caused gastrointestinal bleeding and cerebral hemorrhage.

The antiplatelet action of aspirin varies between individuals. In some patients, known as aspirin non responders, it does not inhibit the biosynthesis of TXA_2. These patients are known to have a significantly higher risk of vascular death; in refractory cases, the precaution is taken to change to other antiplatelets [14].

In 1971, Daiichi Pharmaceutical, which had worked on a joint project with Prof. Shosuke Okamoto and Mitsubishi Chemical in researching and developing the world's first antiplasmin (see 4.4.1. Hemostatics), embarked on another blood-related project: platelet aggregation inhibitors. It was predicted that as time went on, there would be increasing demand for antithrombotics.

Daiichi Pharmaceutical devised a platelet measuring device and started screening, but no lead compound was readily forthcoming. Instead, the company turned its attention to ticlopidine, discovered by French company Parcor (now Sanofi-Aventis) a short time before, in 1974, and decided to introduce it. Using animal experiments, the research team at Daiichi Pharmaceutical found that rats administered ticlopidine had elevated platelet cAMP levels, but no changes in other organs. Later research showed that ticlopidine in the body metabolizes into an active substance that has an inhibitory effect on the platelet ADP receptors (ADP enhances inhibitory G-protein activity, which inhibits adenylyl cyclase) and also prevents platelet aggregation by maintaining cAMP at high concentration levels.

The company history *Daiichi Seiyaku Kyūjū-nen-shi* (90-year History of Daiichi Pharmaceutical) records that while the initial question was to determine whether "inhibiting platelet aggregation actually produces an antithrombotic effect", this became more complicated with other opinions that "inhibiting platelet aggregation can prevent thrombi from forming but cannot break down existing thrombi" and that "it makes sense to prevent the formation of new thrombi, since there must be some kind of thrombotic turn-over at the thrombus site" [4:202-205].

With clinical trials using aspirin as a control drug confirming the superior platelet aggregation inhibitory effect of ticlopidine as well as its utility against peripheral arterial occlusive diseases, the drug was put to market in 1981. Later clinical trials also demonstrated its utility on ischemic brain injuries, including transient cerebral ischemia, cerebral infarction and cerebral ischemia in subarachnoid hemorrhage. While it was France that discovered the medicinal efficacy of ticlopidine, it was Japan and its research on antiplatelet therapy that played the lead role in making the world aware of its utility in this market. The term "antiplatelet", coined by Daiichi Pharmaceutical, is now a term commonly used the world over. This concept is hugely significant in the field of antithrombotics.

However, after some time in clinical use, despite its potent efficacy, ticlopidine was found to have some side effects, including thrombotic thrombocytopenic purpura (TTP), agranulocytosis and hepatic dysfunction. Thus, the need arose for new antiplatelet drugs.

A considerable number of Japanese antiplatelets then emerged in this field, with different mechanisms of action.

Dipyridamole is an antiplatelet that has long been used alongside aspirin. Researched and reported in 1951 by Fisher and Roch, dipyridamole was marketed as an antianginal by a German pharmaceutical company, Dr. Karl Thomae. It was later found to suppress platelet aggregation and for over half a century it has been widely used around the world to treat cerebral infarction and the like. Its effect on platelets is to prevent the cAMP catabolic enzyme phosphodiesterase (PDE), thereby elevating the platelet cAMP level and suppressing aggregation. In Japan, it was released on the market by Nippon Boehringer Ingelheim as a treatment for coronary artery disease in 1960.

However, the results of recent large-scale clinical trials in the West have disproven the efficacy of dipyridamole as an antiplatelet. As a result, the discussion needs to be had as to whether it can be adapted for the Japanese [14, 15].

Cilostazol (Otsuka Pharmaceutical) is a domestic product marketed in 1988. Like dipyridamole, it prevents the cAMP catabolic enzyme phosphodiesterase (PDE), thereby elevating the platelet cAMP level and suppressing aggregation. It was approved for its improvement in ischemic symptoms from chronic arterial occlusion. Randomized controlled clinical trials have also confirmed its utility in the secondary prevention of cerebral infarction.

Limaprost alfadex (PGE_1 derivative) (Ono Pharmaceutical and Dainippon Pharmaceutical) was also launched in 1988.

This unique antiplatelet was researched and developed jointly by two Japanese companies, with research carried out by Ono Pharmaceutical, with its track record in prostaglandin research, and development carried out by Dainippon Pharmaceutical. When administered orally, it demonstrates a vasodilation effect and an antiplatelet effect by binding to the PGE receptors to activate adenylyl cyclase and elevate the cAMP levels. It is more active in the body than the ligand PGE_1 and has been clinically adapted for peripheral arterial occlusive disease.

Another drug with a similar mechanism of action but is classified as a vasodilator is alprostadil (PGE_1 intravenous injection), developed by Green Cross Corporation (now Mitsubishi Tanabe Pharma) and Taisho Pharmaceutical and released on the market in 1988 for peripheral arterial occlusive disease. Devised by Prof. Yutaka Mizushima of the University of Tokyo's Faculty of Medicine, the drug comprises a lipoated (lipo PGE_1) formulation of PGE_1 dissolved in fine fat emulsion particles, where the fat emulsion particles are used as carriers for the PGE_1. Since the fine emulsion particles can be easily distributed, particularly by damaged blood vessels, PGE_1 can be accumulated efficiently at lesion sites. Being difficult to inactivate in the body, it demonstrates superior efficacy in small doses. This formulation was developed using the drug delivery system (DDS) idea. It has proven to be effective at ¼ to ⅛ the dosage of conventional PGE_1 formulations.

Prostacyclin (PGI_2) is an endogenous biologically active substance with a strong platelet aggregation inhibitory effect and a vasodilation effect, discovered by John R. Vane et al. of the Wellcome Research Laboratories (now GlaxoSmithKline) in the United Kingdom in 1976 [(17)]. His achievements with prostaglandin earned Vane the Nobel Prize in Physiology or Medicine in 1982, along with Bengt Samuelsson, discoverer of leukotrienes, and Sune Bergström, who determined the structures of prostaglandins E_1 and F_1, for "their discoveries concerning prostaglandins and related biologically active substances".

Wellcome (now GlaxoSmithKline) trialed prostacyclin as a drug in the hope that systemic administration would prevent thrombus formation; however, this was not successful. In 1981, Wellcome obtained approval in the United Kingdom to market epoprostenol (intravenously injected prostacyclin) with efficacy limited to use as an anticoagulant in extracorporeal circulation during renal dialysis. Approval was given in Japan for its use on primary pulmonary hypertension in 1999 and on pulmonary arterial hypertension in 2004.

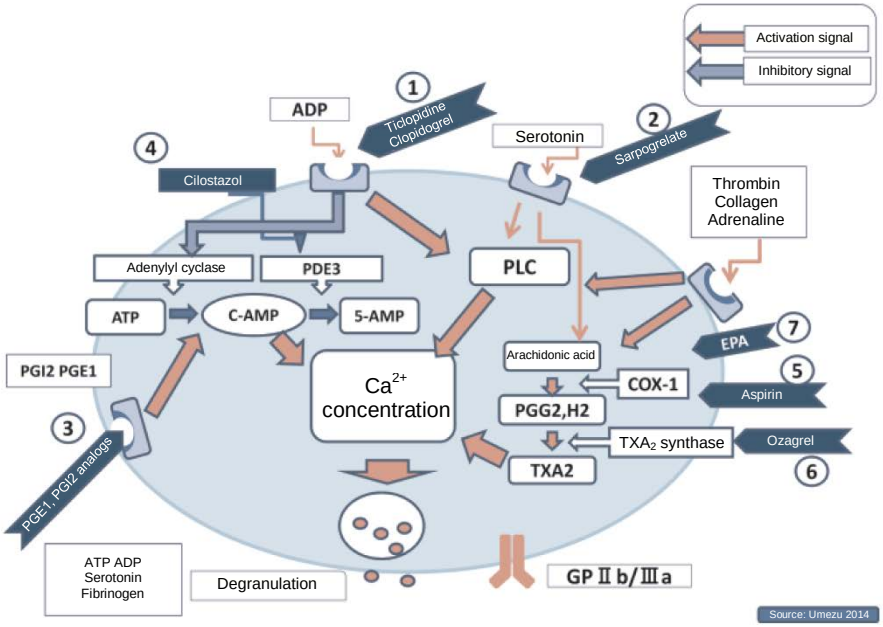
Meanwhile, Japanese research on PGI_2 derivatives continued. PGI_2 derivative beraprost, research and developed by Japanese companies (Toray / Kaken Pharmaceutical) was released on the market in 1992. This antiplatelet demonstrates a PGI_2 effect, inhibiting the aggregation of platelets by activating adenylyl cyclase on the membrane to elevate cAMP levels. It was approved, indicated for "improving ischemic symptoms accompanying chronic arterial occlusion".

Ozagrel is a selective TXA_2 synthase inhibitor researched and developed jointly by Ono Pharmaceutical [(Note 7)], with its strong track record in the area of prostaglandins, and Kissei Pharmaceutical. While inhibiting TXA_2 inhibits platelet aggregation, it does not inhibit PG-endoperoxide, which has a powerful aggregation action. As a result, it does not completely inhibit arachidonic acid aggregation and collagen aggregation, but it does inhibit spasms caused by TXA_2. Approval was granted in 1988 for improving cerebral vasospasms following surgery for subarachnoid hemorrhage and accompanying symptoms of cerebral ischemia. In 1992, additional adaptation approval was given for this injected drug for improving dyskinesia accompanying cerebral thrombosis. An orally-administered version of the drug was also developed, but it was more trouble to find a target disease for this than the injected drug. Experiments on dogs demonstrated efficacy against bronchial hyperreactivity; following clinical trials, the drug was marketed as a treatment for bronchial asthma in 1992 under the brand name of Vega®.

The antiplatelet Sarpogrelate, marketed by Mitsubishi Chemical in 1993, used a new mechanism to inhibit platelet aggregation: that of antagonizing serotonin at the serotonin II receptors on the platelet membrane. Serotonin also causes vasoconstriction, so it is considered clinically advantageous to inhibit it. It is being adapted for use against peripheral arterial occlusive disease, although further evidence is deemed necessary.

Clopidogrel, researched and developed overseas in 1988 by Sanofi Sante and Organon, has achieved major commercial success, valued second only to aspirin worldwide. Clinical development began in Japan in 1996 by Sanofi Aventis, with approval granted in 2006 for efficacy in "preventing recurrence of ischemic cardiovascular disease (except cardiogenic embolism)". In 2012, addition approval was given for efficacy in "preventing thromboembolism in peripheral arterial disease". This drug was created for the purpose of alleviating the side effects from ticlopidine; clinically, it has "significantly lower incidence of side effects such as liver damage and lower neutrophil count" than ticlopidine.

Another substance involved in the inhibition of platelet aggregation is cAMP. The higher the cAMP level, the greater the inhibition of platelet aggregation. cAMP is biosynthesized by adenylyl cyclase (AC), while the ADP receptors work to inhibit this enzyme. Clopidogrel has been found to bind irreversibly to the ADP receptors, thereby preventing the inhibition of adenylyl cyclase and increasing the cAMP level [(15)]. (see Figure 4.24 Platelet Aggregation and Mechanism of Action of Antiplatelets and Figure 4.25 Chemical Structures of Antiplatelets)



Platelet aggregation mechanism

○ When the vascular wall is damaged, collagen is released from the vascular wall and binds to vWF factor produced by the endothelial cells. Platelets bind to this by means of their GPIb receptors. When this occurs, the platelets adhere to the endothelial tissue and stasis occurs. This is called primary hemostasis or primary aggregation.

○ Where platelets adhere, the signal transduction system is activated. Intracellular Ca^{2+} levels elevate due to increases in TXA_2 and decreases in cAMP. As a result, degranulation occurs, with extracellular release of ADP, serotonin and thrombin contained in the granules. These activators secondarily active the surrounding platelets, causing the GPIIb/IIIa receptors to be expressed on the surface of the platelets. These receptors connect with fibrinogen and the aforementioned vWF factor, and secondary platelet aggregation occurs.

○ Meanwhile, extrinsic coagulation initiator tissue factor (TF) binds with VIIa to activate the blood coagulation cascade.

Mechanism of Action of Antiplatelets

[Receptor Antagonists]

As receptor antagonists, ticlopidine acts on ADP receptors (①) and sarpogrelate acts on serotonin receptors (②).

[cAMP Agonists]

PGI2 and PGE1 (③) elevate intracellular cAMP levels. Cilostazol elevates cAMP levels by inhibiting cAMP catabolic enzymes (④).

[Arachidonate Metabolism Inhibitors]

Aspirin inhibits COX (⑤); ozagrel inhibits TXA_2 synthesis (⑥); EPA activates the ω3 system and inhibits the ω6 system, thereby suppressing signal transduction and the increase of Ca, thus inhibiting platelet aggregation (⑦).

Fig. 4.24 Platelet Aggregation and Mechanism of Action of Antiplatelets

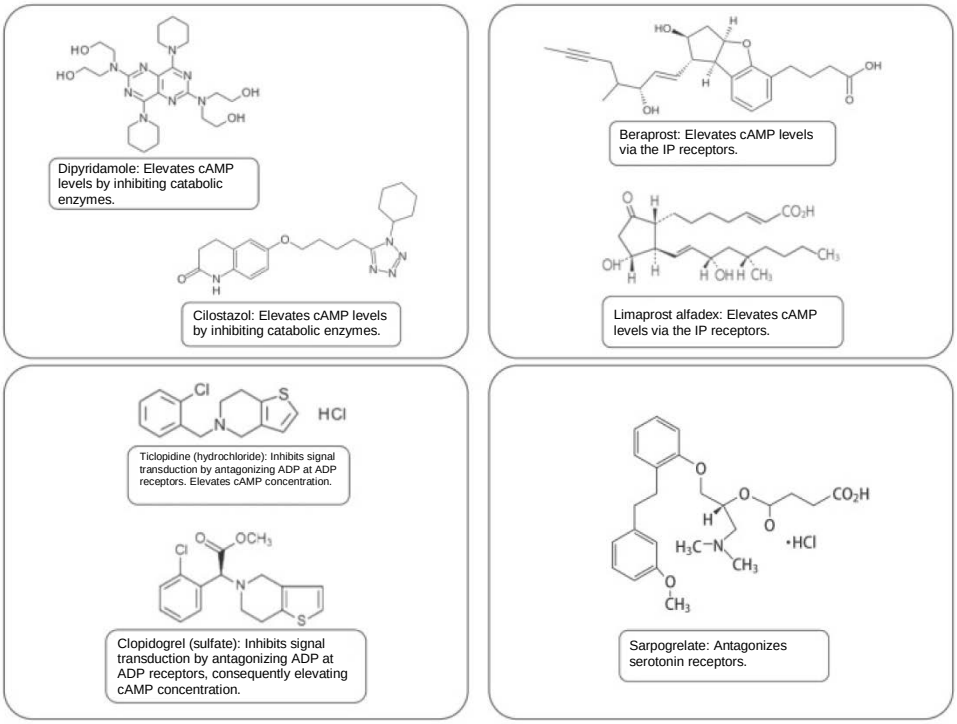


Fig. 4.25 Chemical Structures of Antiplatelets

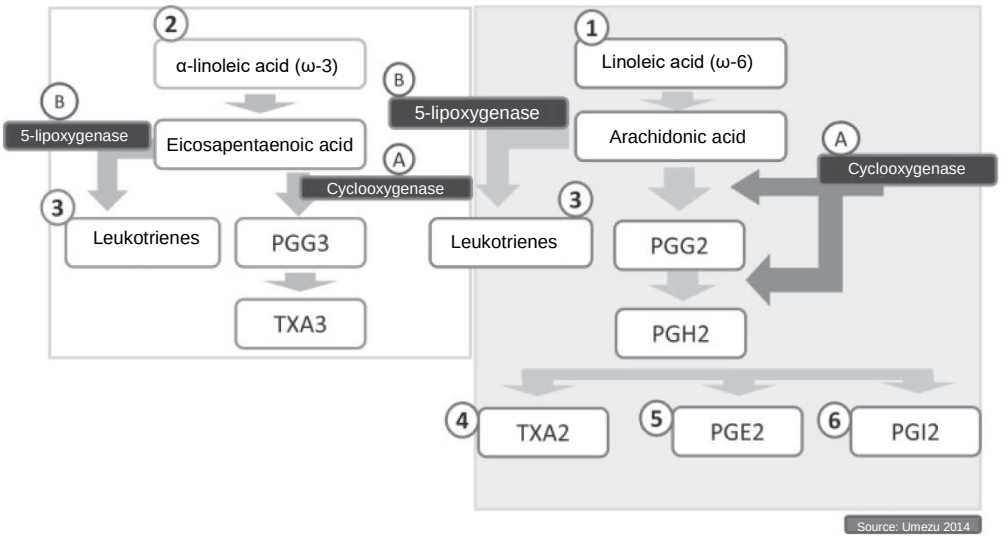
Epidemiological studies in the 1970s found that the indigenous population of Greenland, whose staple food is the seal, were found to have a lower mortality rate from cardiovascular diseases than Danish Caucasians, as mentioned in 4.3.2 (Natural Hyperlipidemia Medications). Research into the cause of this concluded that eicosapentaenoic acid (EPA), which is found in ample supply in the blubber of seals and fish, suppresses cardiovascular diseases through an antiplatelet effect.

In 1980, Nippon Suisan Kaisha became the first in the world to successfully refine eicosapentaenoic acid from fish oil to a high level of purity (over 85%) by ethyl esterification. In 1981, the company began developing eicosapentaenoic acid ester (EPA-E) into a pharmaceutical product in capsule form, in collaboration with Mochida Pharmaceutical. EPA-E was approved for “alleviating ulcers, pain and cold sensation accompanying arteriosclerosis obliterans”. This unique idea of creating a drug from fish oil deserves recognition.

Basic research was conducted to analyze the antiplatelet effect of EPA-E. It was found that the ω-6 in linoleic acid and the like produces arachidonic acid and also biosynthesizes prostaglandin (PG) and thromboxane (TXA), substances that cause or are involved in inflammation, while the ω-3 in α-linoleic acid also synthesizes PG and TXA. Prolonged ingestion of EPA-E replaces the phospholipid arachidonic acid (AA) on the platelet membrane with EPA. While EPA, like AA, forms a phospholipase A2 substrate and joins the prostaglandin cascade, EPA produces PGI_3 and TXA_3, rather than the PG_2 and TXA_2 produced by AA. Although PGI_2 and PGI_3 have similar effects, TXA_3 lacks the powerful platelet aggregation effect of TXA_2. Accordingly, people taking EPA will have slightly inhibited platelet aggregation (see Figure 4.26).

This moderate effect and the lack of side effects are significant characteristics of this drug. In 1994, approval was extended, indicated for hyperlipidemia.

Research and development has also been carried out on GP IIb/IIIa inhibitors and plasminogen activator inhibitor-1 (PAI-1), necessary for platelet adhesion, as anticoagulants.



- ○ Production of prostaglandin (PG) and leukotrienes (LT) from linoleic acid ① by means of arachidonic acid is the route taken by ω-6, while ω-3 produces PG and leukotrienes from α-linoleic acid ② by means of eicosapentaenoic acid.
- ○ In A, arachidonic acid and eicosapentaenoic acid are converted to PGG2 and PGG3 by cyclooxygenase (COX). In B, 5-lipoxygenase converts arachidonic acid and eicosapentaenoic acid into (several kinds of) leukotrienes ③.
- ○ TXA2, PGE2 and PGI2 are present downstream from PGH2 (④/⑤/⑥) and are closely involved in inflammation, clotting and vasodilation. Leukotrienes are closely involved in allergy symptoms.
- ○ ω-3 PG and TXA differ from their ω-6 counterparts in that they have no inflammation-inducing or thrombus-promoting effects.

Fig. 4.26 Arachidonic Acid Cascade and EPA Cascade

4.4.4. Fibrinolytic Drugs

Coagulation involves the coagulation system and the fibrinolytic system. Since activation of the fibrinolytic system breaks down thrombi, inhibiting the coagulation system not only inhibits thrombus formation, but is also believed to activate the fibrinolytic system, which promotes the breaking down of thrombi.

In 1933, William S. Tillett discovered the effects of streptokinase by chance. This was put to use as early as 1958 on patients with acute myocardial infarction. Streptokinase is a protein excreted from the cells of the hemolytic streptococcus *pyogens*. When administered to humans *in vivo*, it demonstrates a specific serine protease action, forming a complex with plasminogen. This acts on plasminogen to convert it into plasmin, which stimulates the fibrinolytic system. Due to its low cost, it was used in the West for myocardial infarction and pulmonary embolism (Note 8), although it has largely fallen out of use due to significant issues with side effects from antigens, with human t-PA and other drugs being developed in its place.

Fibrinolytic system activating factors include urokinase-type plasminogen activator (u-PA), discovered in urine in 1947, and tissue-type plasminogen activator (t-PA). Both stimulate fibrinolysis by activating plasminogen and converting it into plasmin. The major difference between these two enzymes is their fibrin specificity. t-PA activates plasminogen on fibrin thrombi by binding with fibrin molecules, while the plasmin produced efficiently breaks down the fibrin.

By contrast, u-PA has little fibrin affinity, instead breaking down thrombi by activating blood plasminogen into plasmin. Consequently, u-PA has limited use in treatment. As a product, urokinase refined from human urine was developed and marketed by Green Cross and Mochida Pharmaceutical in 1983 (Note 9). Urokinase refined from human renal cell tissue culture was developed by Dainippon Pharmaceutical and Mitsubishi Petrochemical under license from Abbott and launched on the market in 1983. Green Cross developed nasaruplase, a urokinase precursor with greater fibrin affinity, and obtained approval for it in 1991 (18).

Unlike u-PA, t-PA can be mass-produced through genetic engineering. A more stable second-generation t-PA has been produced by altering some of the genes.

Human t-PA was refined by Collen in 1981. The following year, U.S. company Genentech successfully mass produced it using genetic engineering technology. In Japan, it was licensed to Mitsubishi Chemical and clinically developed in collaboration with Kyowa Hakko, gaining approval in 1991 as a “thrombolytic for acute myocardial infarction”. In 2005, approval was extended to include indications for “alleviating functional disorders accompanying acute-phase ischemic cerebrovascular disease (within three hours of onset)”. This was the drug known as alteplase. In 1996, Mitsui Toatsu, Mitsui Chemicals and Mochida Pharmaceutical expressed human fibroblast t-PA from mouse cells and developed this as nateplase.

Asahi Kasei and Kowa produced t-PA by tissue cultivation and marketed this in 1991 as tisokinase.

In 1986, Eisai started researching t-PA derivatives with a longer biological half-life that could be administered as a single intravenous injection. After investigating converting t-PA into an amino acid sequence using gene recombination technology, the company selected a chimpanzee derivative known to produce no antibodies (with the amino acid residue Cys in 84th position from the N-terminus of t-PA replaced with Ser) as a candidate compound for clinical development.

Clinical trials confirmed this t-PA derivative to have fibrin bonding properties and a sustained thrombolytic effect. In 1998, approval was granted for “breakdown of coronary thrombi associated with acute myocardial infarction (within six hours of onset)”. The drug was released on the market in June the same year, nine years after clinical trials began. This was the thrombolytic drug known as monteplase.

In 1999, Yamanouchi Pharmaceutical started marketing pamiteplase. Pamiteplase is an improved drug with a longer than natural acting time, with the t-PA kringle-1 removed by gene manipulation and Arg in 275th position substituted for Glu.

Note 1: “Intrinsic coagulation” refers to coagulation that occurs by the activation of coagulation factors due to causes within a blood vessel. “Extrinsic coagulation” refers to coagulation that occurs when thromboplastin from externally damaged tissue binds to Factor VII and activates it (FVIIa). FVIIa then directly activates Factor X (FX) into (FXa) in the presence of Ca^{2+}. After that, fibrin formation occurs as it does for intrinsic coagulation.

Note 2: There are two phases of platelet aggregation: primary aggregation, in which platelets adhere to vascular subendothelial tissue to cause stasis, and secondary aggregation, which occurs when various biologically active substances released from the activated platelets activate surrounding platelets. Primary hemostasis and secondary hemostasis are hemostatic effects in the coagulation cascade.

Note 3: Heparin is a mucopolysaccharide sulfate with an anticoagulation effect in the body. For details, see 4.4.2.

Note 4: Antithrombin III was termed antithrombin by the International Society on Thrombosis and Haemostasis in 1994. It was originally thought that antithrombins I to VI were present in the body, but in fact only III is present.

Note 5: Drugs to inhibit thrombin include gabexate mesilate and nafamostat mesilate. These were approved in 1977 and 1986 as treatments for acute pancreatitis; their indications were extended in 1989 for disseminated intravascular coagulation (DIC). However, with a mechanism of action due to inhibiting a wide range of "serine proteases", including trypsin, kallikrein, plasmin and thrombin, they were not specific inhibitors.

Note 6: Disseminated intravascular coagulation (DIC) is a disorder in which endotoxins and the like produce abnormal stimulation of the coagulation system, thereby activating the proinflammatory cytokine network, resulting in the overproduction of thrombin and the repeated formation of thrombi in microvascular endothelial cells throughout the body. This leads to ischemic organ dysfunction.

Note 7: Ono Pharmaceutical has worked entirely on "prostaglandin (PG) research and development" since 1965, at the discretion of the company president at the time, Yuzo Ono. This was five to ten years before prostaglandin gained attention due to work by Nobel laureates Vane, on inflammation and PG, and Samuelsson, on TXA_2 and leukotrienes. Ono Pharmaceutical later went on to market 12 new products related to PG. Between 1974 and 1979, the company commercialized pharmaceutical products from natural PG, marketing the world's first PG oxytocic $PGF_2\alpha$ in 1974 and PGE_2 as a uterotonic in 1976. Between 1984 and 1988, the company commercialized derivative pharmaceutical products, marketing PGE_1 derivative limaprost alfadex for peripheral arterial occlusive disease in 1984 and TXA_2 synthesis inhibitor ozagrel and leukotriene receptor inhibitor pranlukast between 1988 and 1995. Much can be learned from this company's conviction and endeavor in repeatedly taking on new, uncharted fields and creating products in all kinds of areas.

Note 8: Although streptokinase is indicated for removing hematoma in Japan, it is not sold for its indication as a thrombolytic.

Note 9: Urokinase from urine was approved in 1965 indicated for thromboembolism, but it has come under review as a pharmaceutical product because the dosage differs significantly from the West and there is doubt as to its efficacy.

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4.5 Medications for Gout/Hyperuricemia

According to the 2004 *Comprehensive Survey of Living Conditions* by the Ministry of Health, Labour and Welfare, the total number of patients receiving treatment for gout was 870,000, around double the 1995 figure. The number of people with hyperuricemia, which could be termed as pre-gout, could be as high as 10 million [(1)].

According to Hippocrates of ancient Greece, gout is a disease accompanied by intense pain even when the wind blows. He observed and recorded a number of accurate facts as a physician, including that castrated men do not suffer from gout, that it does not occur in women until after menopause, that even in men, it does not occur until a more mature age, that the inflammation subsides within 40 days and that gout is more active in spring and fall [(2)].

Dioscorides (mentioned previously), a military physician under the Roman emperor Nero, also recorded 2000 years ago in his work *De Materia Medica* that willow bark [(Note 1)] and meadow saffron (*Colchicum autumnale*) were effective against gout.

Many notable people in Western history suffered from gout. According to the Gout Research Foundation, these include Alexander the Great, Louis XIV of France, Martin Luther, Francis Bacon, Isaac Newton, Charles Darwin, Goethe, Stendhal and Guy de Maupassant [(2:5, 3)]. By contrast, the disease was unknown in Japan until the Meiji Period. Luís Fróis, a Portuguese missionary during the Azuchi-Momoyama Period, wrote that “there is no gout among the Japanese”. It seems that the disease suddenly appeared in the Meiji Period. Gout sufferers began to rapidly increase in number in the 1960s, presumably as a result of changes in diet.

When the level of uric acid in the blood and tissue fluid rises above the saturation point (7.0mg/dl), monosodium urate is deposited into the tissue in crystalline form. While the kidneys and joints are susceptible to a build-up of these crystals, the base joint of the big toe is particularly susceptible to this build-up, with initial onset occurring at this site in 70% of cases. The deposited crystals eventually leak into the synovial fluid and are attacked by inflammatory leukocytes as foreign contaminants. Although leukocytes ingest uric acid through phagocytosis, they have no mechanism for breaking down the crystals; thus, the lysosomes (intracellular organelles, including catabolic enzymes) become damaged and the enzymes leak out. At the same time, the leukocytes also release cytokines and other inflammatory biologically active substances. These released substances (prostaglandin, proteinase and reactive oxygen) act as attractants, causing an inflammatory reaction the same as in other tissues. Gout-related inflammation is characterized in that it becomes extremely painful within a short period from onset.

Uric acid, which causes gout, is synthesized from the metabolism of DNA/RNA structural components adenine and guanine (called purine bodies due to their chemical structures) into hypoxanthine and xanthine, which are then converted into uric acid through the action of oxidoreductase xanthine oxidase.

Hypoxanthine-guanine phosphoribosyl transferase (HGPRT) is a reverse metabolic pathway enzyme that prevents the overproduction of uric acid. Patients with Lesch-Nyhan syndrome hereditarily lack this enzyme and have abnormally high levels of uric acid (see Figure 4.27 Uric Acid Biosynthesis Pathway and Synthesis Inhibitors).

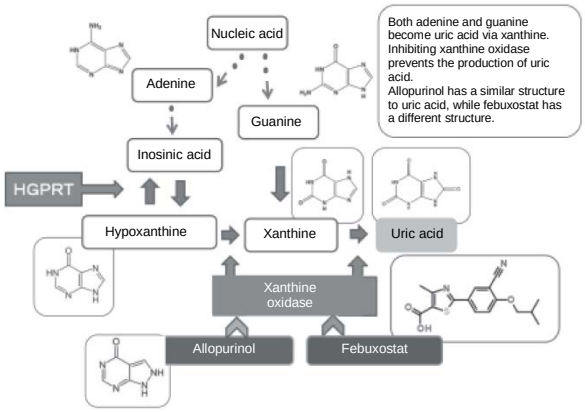

Fig. 4.27 Uric Acid Biosynthesis Pathway and Synthesis Inhibitors

Uric acid is synthesized in the body and also derived from food; it is excreted from the body with the urine and feces, although some is retained to maintain steady uric acid levels in the body. Around 80-90% of uric acid in humans is synthesized in the body, while the remaining 10-20% is derived from food.

Since such a small amount of uric acid is food-derived, it is difficult to explain why there was no gout in Japan until the Meiji Period. Perhaps it was not only the Japanese diet that changed, but also the internal metabolism due to changes in living environment. Perhaps the Japanese started secreting more male hormones and adrenaline in the Meiji Period of wealth, military strength and industry promotion?

Most gout sufferers are males in their 30s and 40s, although recent years have seen an increase in younger patients. There are far fewer female patients due to the fact that female hormones help to excrete uric acid from the body; accordingly, there are higher uric acid levels among postmenopausal women. Uric acid levels in males are known in to increase with puberty.

If the uric acid metabolism becomes unbalanced for some reason, the uric acid level in the blood increases, resulting in hyperuricemia. There are two causes for this: (i) excessive production of uric acid; (ii) reduced excretion of uric acid. Around 60% of Japanese patients fall into the second category. Uric acid excretion reduces with deterioration in kidney function due to arteriosclerosis, hypertension, diabetes or nephritis. However, it has also been suggested that Japanese have hereditarily low uric acid excretion. Vigorous exercise, stress, excessive drinking, obesity, medications (diuretics or immunosuppressants) and hereditary factors can all cause excessive uric acid biosynthesis and can also cause reduced uric acid excretion.

Since hyperuricemia, a pre-symptom for gout, is not accompanied by pain or other subjective symptoms, its detection is often delayed. This is also true of other lifestyle-related diseases, such as hypertension, hyperlipidemia and hyperglycemia, which also show no subjective symptoms.

The disease progresses from asymptomatic hyperuricemia to a period of intermittent gout (a stage of recurring gout attacks), then followed by chronic gout accompanied by constant pain and the formation of tophi. Various complications occur, including gouty kidneys, hypertension and hyperlipidemia. Accordingly, it is important to treat hyperuricemia before it gets to that stage. For example, a comparison between treated and untreated groups of asymptomatic hyperuricemia patients found a 0% incidence rate of urinary tract stones during 44 weeks of observation among the treated group compared to a 7.6% incidence rate among the untreated group [(4)]. It has recently been found that obese people have a higher incidence rate of gout. There is ongoing discussion on the progression of hyperuricemia due to metabolic abnormalities caused by visceral fat accumulation. Gout is now included among the lifestyle-related diseases collectively termed as metabolic syndrome [(1)]. There is another theory that uric acid levels in the blood can be used as a biomarker for early diagnosis of metabolic syndrome, since this is affected earlier than other metabolic syndrome indicators.

4.5.1. Medications for Gout

The seeds and bulbs of meadow saffron *Colchicum autumnale* have been used to treat gout since ancient Greece. Its active ingredient colchicine was first isolated in 1820 by French chemists Pierre Joseph Pelletier and Jean Bienaimé Caventou (mentioned previously: 2.2.1.).

While colchicine has demonstrated superior efficacy as a medication for gout, in 1937, American botanist Albert Blakeslee found the substance to induce the doubling of chromosomes in plant cells. Highly toxic, it was reported by the United States Pharmacopeia as being “extremely toxic” [(5)]. However, colchicine is a specific medicine for gout pain and is still used in the West as a first-line drug. The pharmacological effect of colchicine is known to be due to preventing the formation of microtubules by binding to tubulin, the main protein in microtubules. It is also known to have an anti-inflammatory effect by preventing neutrophil activity (migration and activation). This anti-inflammatory effect is specific to gout and has no effect on arthritis or rheumatism; its mechanism of action is not yet clearly known.

In Japan, corticosteroids and colchicine are also used as the main non-steroidal anti-inflammatory drugs for gout attacks. Non-steroidal drugs include indomethacin, naproxen, oxaprozin and pranoprofen [(6)].

4.5.2. Medications for Hyperuricemia

The intermittent gout stage involves recurring gout attacks with intervening remission. Many patients stop taking their medication when the pain stops. There has been little awareness of the need to treat hyperuricemia and very few treatment options for it. However, with growing awareness that hyperuricemia is a pre-symptom for gout and that uric acid levels need to be lowered at this stage, active measures have been taken in the 21st century for drug treatment as well as improved lifestyles and dietary cures. Japanese drug discovery companies have contributed to research and development of new hyperuricemia drugs that either inhibit the biosynthesis of uric acid or promote its excretion.

Uric Acid Production Inhibitors

The cause of gout is clear. Allopurinol, which inhibits xanthine oxidase, essential for uric acid production, has long been used to treat gout. Allopurinol was discovered by Gertrude Belle Elion [(Note 2)] and George Herbert Hitchings of British company Wellcome Research Laboratories. In 1957, it was found to have a xanthine oxidase inhibitory effect *in vitro* [(7)].

Allopurinol was trialed in concomitant administration with anti-tumor agent 6-mercaptopurine, which was known to lose its activity due to the effects of xanthine oxidase. Rundles et al. then discovered that it lowered the uric acid level in the blood and urine [(8)]. Since then, it has been administered for gout and hyperuricemia. In Japan, Tanabe Seiyaku licensed it from Wellcome and started selling it in 1969 under the name of Zyloric® [(9, 10)].

With little sales of gout medications in Japan, there were few drug discovery companies getting involved in the market. With no successor to allopurinol for 40 years, treatment options were limited. In 2011, Teijin Pharma launched domestically-produced febuxostat. Where allopurinol has a

purine analog structure, febuxostat is a non-purine compound with a characteristically powerful inhibitory effect on xanthine oxidase [11]. Another feature of this compound is that it is almost completely excreted in the feces and urine once metabolized by the liver, meaning that it can be administered at full dosage even for patients with impaired renal function. Although it was marketed in the United States in 2009, earlier than in Japan, even there it was the first uric acid production inhibitor in 40 years. It was released in Europe in 2010.

Teijin (now Teijin Pharma) embarked on its own drug discovery research in 1988 and discovered the new non-purine drug febuxostat in 1991. According to *Shinyaku ni idonda Nihonjin Kagakushatachi* (Japanese Scientists who Strove for New Medicine) by Asako Tsukasaki, "the synthesist Shira Kondo retreated from the field in which the major players were competing with topic setting and opted for the medium-scale market. However, there were a lot of patents in the area of gout, more than 50 of them better than allopurinol, but none of them had been developed into drugs. Kondo et al. knew that allopurinol inhibited xanthine oxidase through a more complex mechanism of action than Elion and Hitchings had envisaged, so they steered away from the basic purine structure and created a mother nucleus that formed a non-purine consensus structure. Eventually, febuxostat was synthesized and was found to firmly embed itself in the pocket of the enzyme usually occupied by hypoxanthine".

While allopurinol is excreted from the kidneys, febuxostat is metabolized by the liver and excreted in the feces and urine, requiring less work by the kidneys. The drug was marketed in the United States in 2009, Europe in 2010 and Japan in 2011. This was Japan's first drug to be indicated for hyperuricemia.

Although clinical trials of febuxostat began in 1995, it was not until 2011 that the clinical trials came to a close and the drug was put to market. It had been 23 years since research began and 16 years since clinical trials began. While it was not rare for clinical trials to take more than ten years in Japan, with the advent of the age of internationalization in the latter half of the 20th century, doubts began to arise over the way clinical trials and approval reviews were being done.

Fuji Yakuhin came up with a new drug to inhibit xanthine oxidase selectively and reversibly: topiroxostat. Clinical development started in 2004, with Sanwa Kagaku collaborating from phase III trials onwards. The drug was approved in 2013 and sales commenced.

Figure 4.28 shows the chemical structures of representative hyperuricemia drugs.

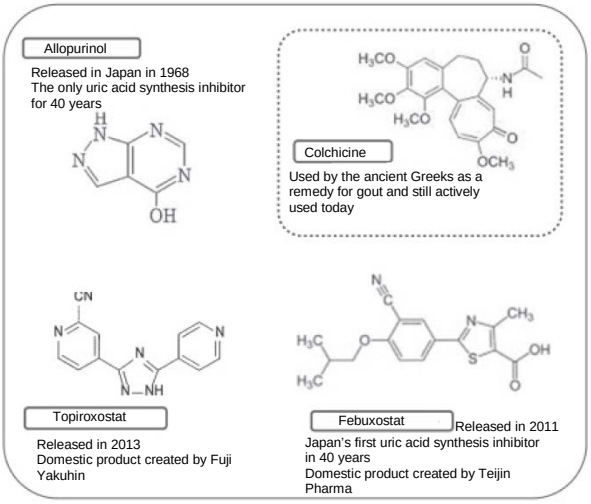

Fig. 4.28 Colchicine and Uric Acid Production Inhibitors

Uricosurics

Uric acid in the blood is 100% filtered by the glomeruli in the kidneys. Once it has been reabsorbed by the uriniferous tubule, it is again secreted to the uriniferous tubule and then excreted into the urine after being absorbed once more (the four-component model). The uric acid that is finally excreted into the urine is around 6-10% of the volume that was filtered.

The epithelial cells of the proximal renal tubule near the glomeruli have a transporter for uric acid called uric acid transporter 1 (URAT1). This transports uric acid from the uriniferous tubule into the blood vessels in exchange for organic anions (such as negatively-charged amino acids, lactic acid and nicotinic acid). This constitutes the reuptake of uric acid from the urine into the blood. Inhibiting URAT1 suppresses the reuptake of uric acid, thereby promoting its excretion from the blood and reducing the amount of uric acid. URAT1 is a target for uricosurics.

Uric acid is again secreted from the blood into the urine by the uriniferous tubule. After that, it is reabsorbed into the blood by the uric acid transporter. The reuptake of uric acid is inhibited if the transporter is suppressed.

Probenecid was originally used as a drug to inhibit the excretion of penicillin. This drug contributed to research on the activity duration of the new antibiotic penicillin during the Second World War. Interestingly, the main uric acid production inhibitor allopurinol, mentioned above, was also used for maintaining the efficacy of antitumor drugs. The indications for probenecid were later expanded to include hyperuricemia.

The main mechanism of action of probenecid is known to be due to inhibiting the initial reuptake of uric acid secreted from the uriniferous tubule. Due to its pharmacological effect, probenecid is known to affect the metabolism of a number of

drugs, including antibacterials, and concomitant administration needs to be undertaken with care. It has also long been used as a basic pharmacological research tool for its properties.

Most other uricosurics developed later inhibit the reuptake of uric acid after secondary secretion. In terms of medicinal efficacy, the latter reuptake inhibitors are more potent.

Bucolome is a drug researched and developed in Japan as a non-steroidal anti-inflammatory drug (NSAID) and also has a uricosuric effect. In a collaborative study with Hajime Fujimura of Gifu University and Shigeo Senda of Gifu Pharmaceutical University, Takeda Pharmaceutical investigated pyrazolidenedione derivatives and peripheral compounds and discovered this compound, which has both anti-inflammatory and uricosuric effects. With few of the usual side effects of anti-inflammatories and no effects on the kidneys, the drug was launched in 1967 as a uricosuric suitable for prolonged administration.

Benzbromarone is a uricosuric developed by Torii Pharmaceutical under license from French company Labaz. The most potent of all uricosurics, it is the most widely used today, as its long half-life of 18 hours allows it to retain its uricosuric effect with once-a-day administration.

Known to inhibit uric acid transporter URAT1 [(13)], it is also believed to strongly inhibit secondary uric acid reuptake.

Not only is benzbromarone metabolized by drug-metabolizing enzyme CYP2C9 to become an active metabolite, it also has a CYP2C9 inhibiting effect. Accordingly, care must be taken when used concomitantly with other drugs, as the metabolism of other drugs metabolized by CYP2C9 will be delayed, causing blood concentrations to elevate.

For example, blood concentrations are monitored when using the blood thinner warfarin and care must be taken with the concomitant administration of benzbromarone due to concerns over elevated blood concentration.

Since uricosurics increase the amount of uric acid excreted into the urine, care must also be taken due to greater susceptibility to uric acid stones in the kidneys, urinary bladder and urethra.

Figure 4.29 shows the chemical structures of representative uricosurics.

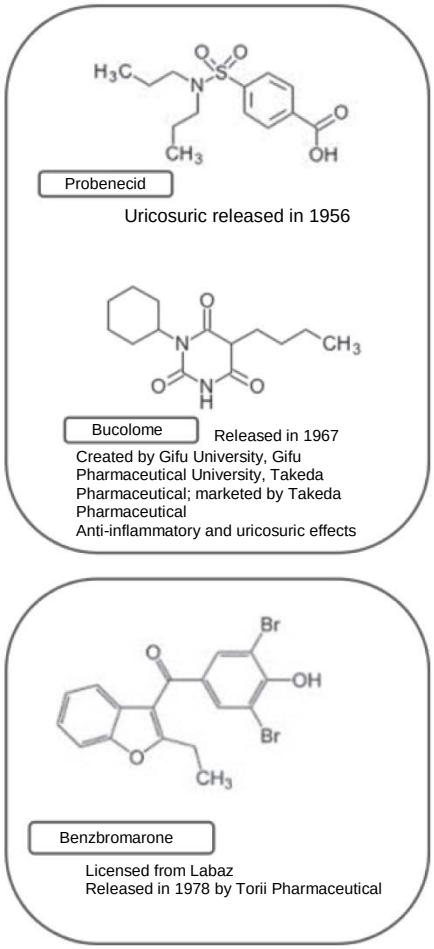

Fig. 4.29 Uricosurics

Interestingly, Japan has created many new drugs for gout and hyperuricemia.

Note 1: Strictly speaking, recent investigation has found that salicin was isolated from meadowsweet *Filipendula ulmaria* rather than willow bark. This is now presumed to have been ineffective against gout, although it did have an analgesic effect.

Note 2: Gertrude Elion was awarded the Nobel Prize in Physiology or Medicine in 1988, together with George Hitchings and James Black (see 2.4.3), “for their discoveries of important principles for drug treatment”. As well as allopurinol, Elion invented at least five other types of world-renowned new drugs, including 6-mercaptopurine, azathioprine and aciclovir.

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5 Conclusion/Acknowledgements

Drugs created through modern science have been of huge benefit to humanity. Antibiotics and antibacterials have achieved remarkable success in overcoming infectious diseases, our greatest enemy since the dawn of humankind, as well as in reducing infant mortality and significantly extending our average life expectancy. This paper has examined in detail how drug discovery has turned in focus from fighting pathogens to addressing lifestyle-related diseases. The discussion has outlined how drugs with different mechanisms of action have been adapted for patients, for example, antihypertensives for hypertension patients, such as beta-blockers, calcium channel blockers, ACE inhibitors and angiotensin II receptor blockers. The results are evident in the rapid decline in the cerebrovascular disease mortality rate.

The discussion on diabetes drugs has examined the competition to create new drugs, such as pancreatic β-cell stimulants, insulin sensitizers, small intestine glucose absorption inhibitors, incretin breakdown inhibitors and urinary glucose reuptake inhibitors, with successive research and development of drugs with new mechanisms of action. These drugs have significantly contributed to the treatment of diabetes and it has been Japanese researchers who have made outstanding achievements at the global forefront of the field, discovering new drugs with new mechanisms of action, including insulin sensitizers and urinary glucose reuptake inhibitors.

Anticholesteremic HMG-CoA reductase inhibitors (statins) have had ground-breaking results in lowering the mortality rate of patients with ischemic heart disease, thanks to Japanese scientists. Japanese scientists also developed the idea of antiplatelets, developed hemostatics in the wake of the Second World War and discovered antithrombins. While some of these achievements have been by chance, the Japanese have also thought outside the box to come up with ideas that other researchers did not and have had the resilience to surmount repeated failures, which has played significant role in their success. May future generations of researchers draw from this.

As well as world-first innovative drugs, this paper has also examined how Japanese drug discovery companies have discovered many improved new drugs. While improved new drugs are somewhat critically regarded as Japan's forte, the ability of medicinal chemists to produce drugs that are better than preceding products at low cost and in a compact manner by means of experience, intuition and luck simply demonstrates their superior competence as researchers. Major drug discovery companies in the West have similarly produced and marketed improved new drugs, many of which have gained worldwide use. Since the aim of drug discovery is to discover drugs that will contribute to humanity rather than to be the first in the pack, it is worth far more to be best in class than first in class. Japanese drug discovery has a unique history in that quite a number of companies, not all of them specialized, have cleverly maximized their limited resources to create improved new drugs. Drug discovery in the future will involve companies studying what has gone before and developing new improved drug technologies, with confidence in their conventional improved-drug-oriented approach.

The history of modern drug development involves large numbers of patients and progress in determining the causes of diseases. When examining diseases by their cause, pathogens and viral infections are obvious targets. Stomach ulcers have been brought under control by preventing excessive secretion of gastric acid. Anti-inflammatories have been effective against inflammatory pain. The mechanisms of hypertension and hyperlipidemia have been determined and effective new drugs have emerged. However, there are still many cases of diseases without one simple cause, or niche diseases; these will become the targets of new fields of drugs in the future.

Nevertheless, current competition in the field of drug discovery is not limited to these new areas. Even in fields where the repertoire of drugs is near complete, there is sometimes an ongoing quest to discover new blockbuster drugs with new mechanisms of action. Is it truly necessary to discover new drugs with new mechanisms of action or try to find blockbuster drugs for diseases that are already adequately treated with existing drugs? It does seem reasonable to consider ways to make existing drugs more effective.

Aspirin is known and used all around the world as an analgesic and antipyretic and is also a very good platelet aggregation inhibitor. However, there are patients who are unresponsive to aspirin, while other patients suffer from severe side effects with even the slightest amount. The effect of the drug is made more accurate by identifying aspirin-unresponsive patients through advance gene expression testing and then administering accordingly. While some endeavors have been made to that end, it is important to use such means more widely in consideration of other drugs as well.

How effective are scientifically-created drugs in this modern era? The efficacy of drugs is determined in clinical drug trials by measuring subjective or objective indicators using double blind testing under GCP-compliant protocols. With neither doctors nor patients knowing if is the drug or the placebo being taken, such conditions should clearly reveal whether the drug actually is effective. However, in many cases, there is little discernible difference between the control group and the treatment group. Table 5.1 reveals that drug efficacy varies considerably between clinical trials (except for anti-pathogenic drugs). Even properly controlled

clinical trials yield these results, indicating variations in efficacy on administration to ordinary patients after the drug has gone to market.

Table 5.1 How Effective are Drugs?

Disease	Drug	Efficacy
Rheumatism	NSAID COX-2 inhibitors	50-80%
Cancer	Various anti-cancer drugs	0-30
Depression	SSRI inhibitors	60-80
Diabetes	Sulfonylurea	50-70
Gastric ulcer Duodenal ulcer	Proton pump inhibitors	10-80
Hyperlipidemia	Statins	25-70
Hypertension	Diuretics	25-50
"	β-blockers	70-80
"	ACE inhibitors	70-90
"	ARBs	70-90
Migraine	Serotonin antagonists	50-75

Source: BM Silber, "Pharmacogenomics, Biomarkers, and the Promise of Personalized Medicine," Pharmacogenomics, W. Kalow and U. Meyer, editors, Marcel Dekker Publishers, New York, 2001; Perlegen anlysis.

Placebos have been discussed in this paper, but there are other differences in environment and lifestyle. The other major cause of variation is thought to be due to individual and racial differences between patients, rather than due to the inadequacy of the efficacy of the drug. There are some drugs that have no effect on certain patients. The extent to which a drug is effective depends on differences in disease-related gene expression in different patients, as well as differences in drug metabolism in different individuals.

The concept of personalized medicine is that of using individual gene expression testing and biomarker testing to predict the effects and side effects when administering a particular drug. The ideal treatment is that which uses the optimal drugs for the individual, as also mentioned in this paper. Research in this area has been ongoing since the end of the 20th century, with a view to integrating diagnosis and drug administration in treatment. However, there is no easy way forward. The more diagnostic methods and types of drugs there are, the higher the medical expenses are going to be. This will also add to the load on doctors and other medical personnel as well as increasing the cost of drug manufacturing. Research has to begin with genetic diagnosis to identify the optimal biomarkers. At this point, there are a number of issues needing to be resolved, requiring close collaboration and joint initiatives between the pharmaceutical industry and the diagnostic equipment industry, the medical examination industry and university researchers. However, this is a road that must be travelled. It will require companies that have produced successful products to get involved in increasing the efficacy and reducing the side effects of their products.

Active drug discovery research is continuing in fields in which there are no adequate drugs as yet. While one strategy is to try to find a blockbuster drug in fields where solutions can be achieved by extending past experience, there are many diseases that have complex causes relating to many different genes. While we are entering an age of genomic drug discovery, many candidate genes are being discovered that are potential causes for each disease. If there are over 100 candidate genes that could cause schizophrenia, it is difficult to determine which gene is actually the cause. Conversely, diseases are also thought to occur due to the involvement of multiple genes. Accordingly, the fact that multiple genes are involved in diseases in a complex manner really implies that no perfect drug with one mechanism of action will be forthcoming.

These fields must be approached with new treatment ideas that are distinct from conventional treatment. Drug discovery also needs to be undertaken in line with such policies. The correlation between the disease suffered by a patient and that patient's genetic makeup in relation to that disease must be clearly understood. This will require drugs with multiple mechanisms of action as well as patient profiling. This is also part of personalized medicine.

There are various different cases to be considered in relation to the future direction of drug discovery. The hope is that Japanese drug discovery companies will clearly recognize their past achievements and move forward with new concepts and creativity without getting stuck on the conventional methods of drug discovery.

How long can human life be extended?

The introduction of this paper stated that the average life expectancy steadily increased from the Meiji Period onwards and then rapidly increased after the Second World War. One theory suggests that simply eliminating death from various infectious diseases in infancy and childhood would dramatically increase the average life expectancy in adulthood.

As there are no official death records dating prior to the Meiji Period, no simple comparison can be made with the post-Meiji era. However, according to *Hida O Jiin Kako-chō no Kenkyū* (Study of the Death Records in Hida O Temple) (Note 1) (1973), a work by Gifu doctor Keizo Suda, the average age at death in that region between 1771 and 1870 was 28.7 years for males and 28.6 years for females, while the average age at death among adults over the age of 21 years was 61.4 years for males and 60.3 years for females. Historian Shōji Tachikawa found that the average age of death of notable people in Japanese history was 60.4 years during the Warring States Period of the mid-15th to mid-16th century, 66 years during the Edo Period of the 17th to 19th centuries and 60.6 years in the Meiji and Taisho Periods of the late 19th century to early 20th century (*Nihonjin no Byōreki*, Chuko Shinsho, 62-67, 1988).

As the Edo Meiji and Periods seem to have lacked any policies to use treatment to extend the lives of sick people, the average life expectancy of adults would have been the healthy life expectancy (Note 2). According to the 2014 edition of the Ministry of Health, Labour and Welfare white paper *Towards Achieving a Healthy and Long-Living Society*, the healthy life expectancy in 2010 was 70.4 years for males and 73.6 years for females. Compared to Suda's data, the healthy life expectancy has only increased by 9 years for males and 13.3 years for females in 143-243 years.

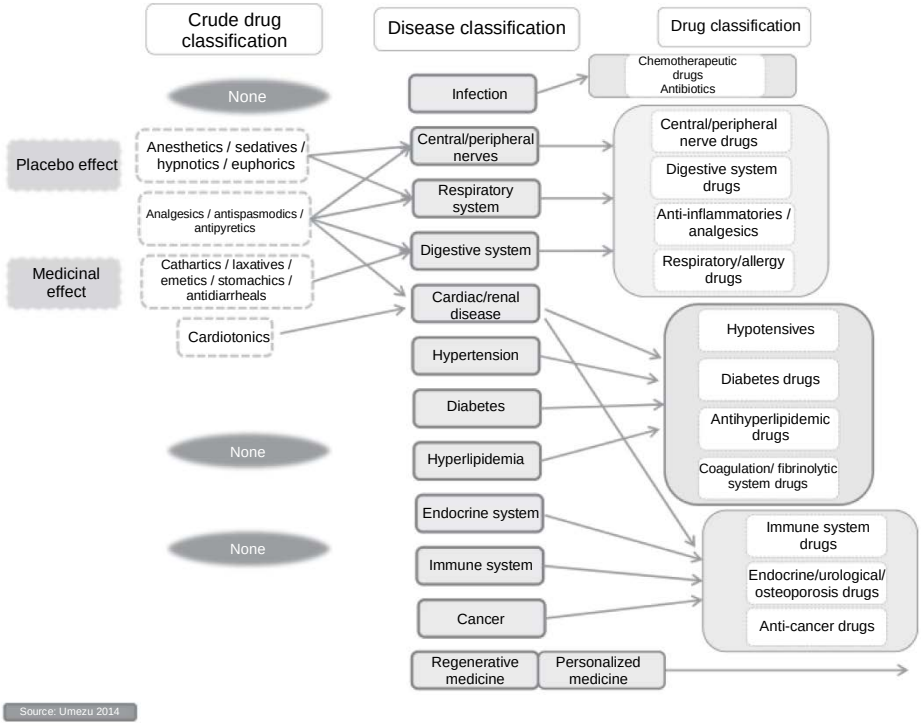

Fig. 5.1 Progression from Crude Drugs to Modern Drugs

It would also seem that the lifestyle-related disease medications mentioned earlier have had surprisingly little effect. Up until the Meiji Period, essentially genetically healthy people survived being eradicated by pathogens and had a high probability of reaching adulthood or old age, while modern-day adults have reached adulthood protected from infection and other diseases by the power of medicine. Accordingly, a simple comparison does not work. However, perhaps we are reaching the limits of how far drugs can increase the healthy life expectancy.

A significant challenge for the future will be how to extend the healthy life expectancy for all people, genetic differences aside. Although there are new drugs to prevent arteriosclerosis and treat diabetes, these are not the only diseases that threaten our day-to-day health. There are many other diseases and factors that can put an end to a healthy life, such as becoming bed-ridden with an accidental broken bone as a result of weakened bones and muscles and deteriorated senses, loss of quality of life due to constant pain, diseases resulting from a poor immune system, loss of mental energy and dementia. If a tissue or organ ceases to function properly, this can affect other parts of the body as well, thus impairing the functionality of the entire body. Extending the healthy life expectancy requires a balance of physical and mental ageing. This will not be achieved by drugs alone; it will require a full arsenal combining medical treatment and health maintenance.

The goal is to extend the healthy life expectancy not just for the genetically healthy, but for all people regardless of genetic differences. Although this will take some very in-depth basic research and may seem out of reach, it is a goal that is worthwhile.

In this era of regenerative medicine becoming a reality, there are still many areas of active drug discovery. The hope is that armed with the knowledge of the drug discoveries achieved by our forebears, we would not tread the same paths they took to seek out new drugs, but rather take on new concepts and incorporate collaborative, holistic treatment that spans areas outside of drugs as well in order to battle against diseases and ageing and extend the healthy life expectancy of humankind.
(Figure 5.1 outlines the flow of this paper. Crude drugs have been broadly categorized four ways according to disease; lifestyle-related diseases have been examined as separate topics in this paper.)

Note 1: Many Japanese people in the Edo period were the parishioners of temples, and the temples have records of their death period.

Note 2: The healthy life expectancy refers to the life span that can be expected for individuals that are not limited by health issues in their day-to-day life.

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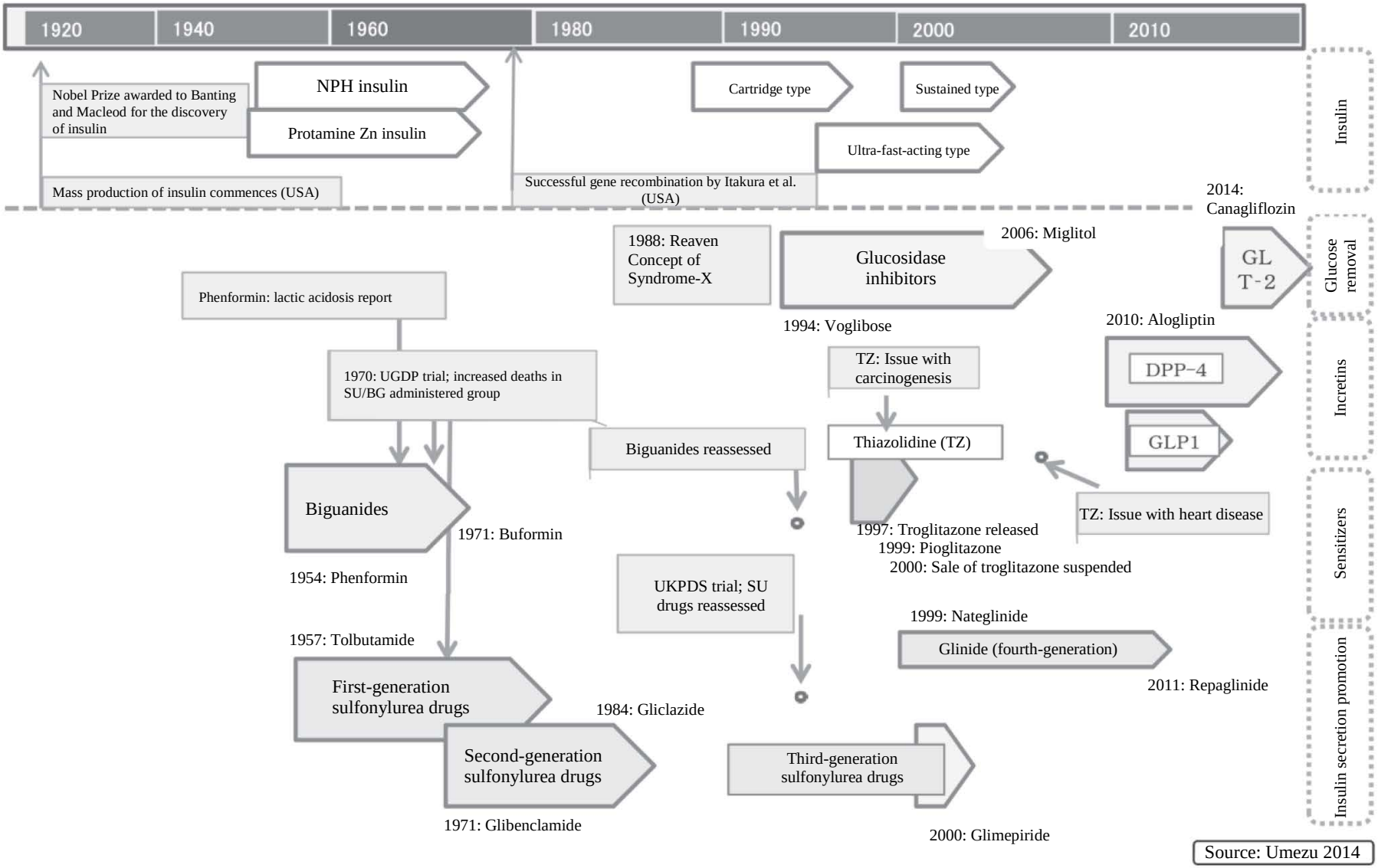
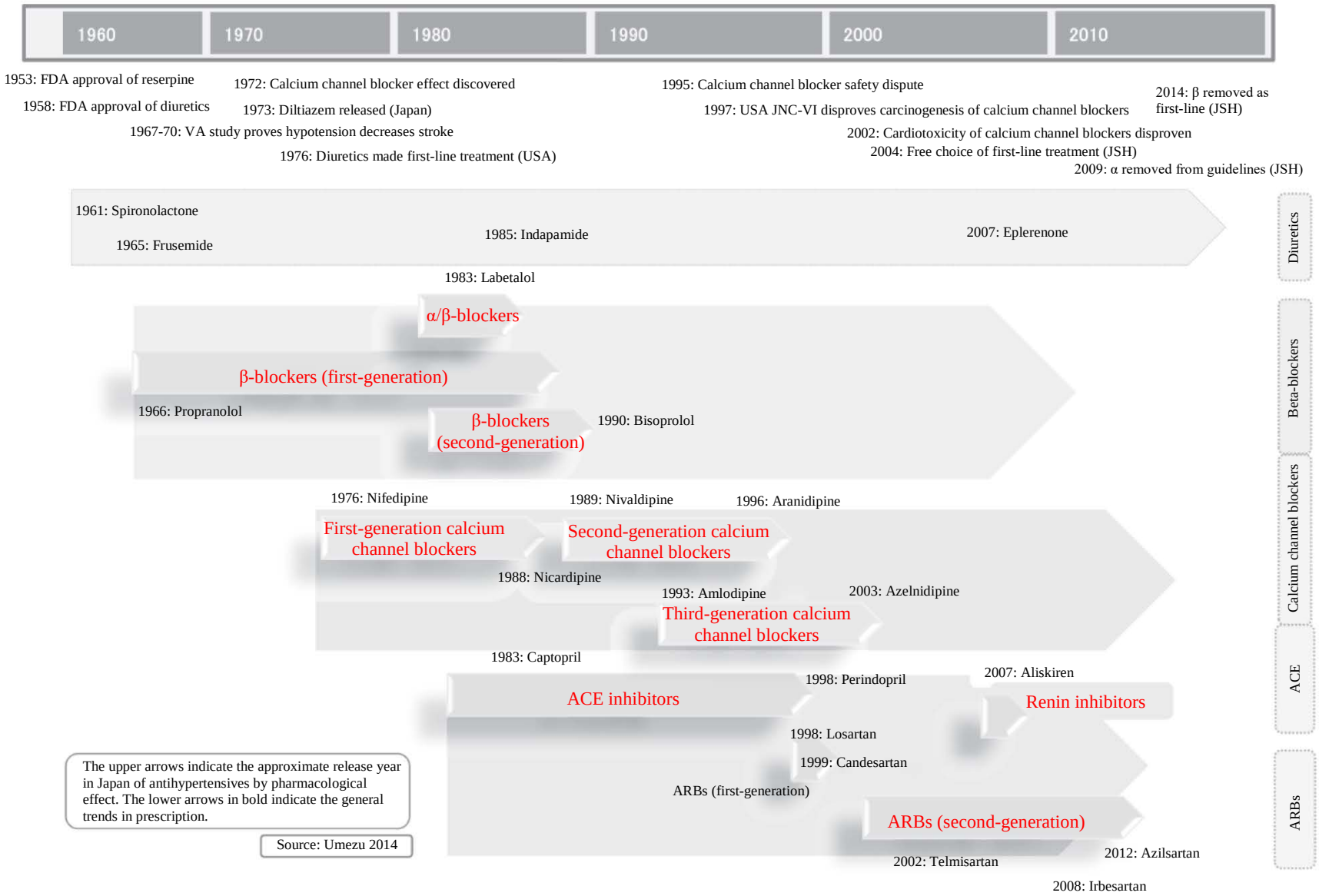
Sincere thanks also to all of the various company personnel for generously providing company history information.

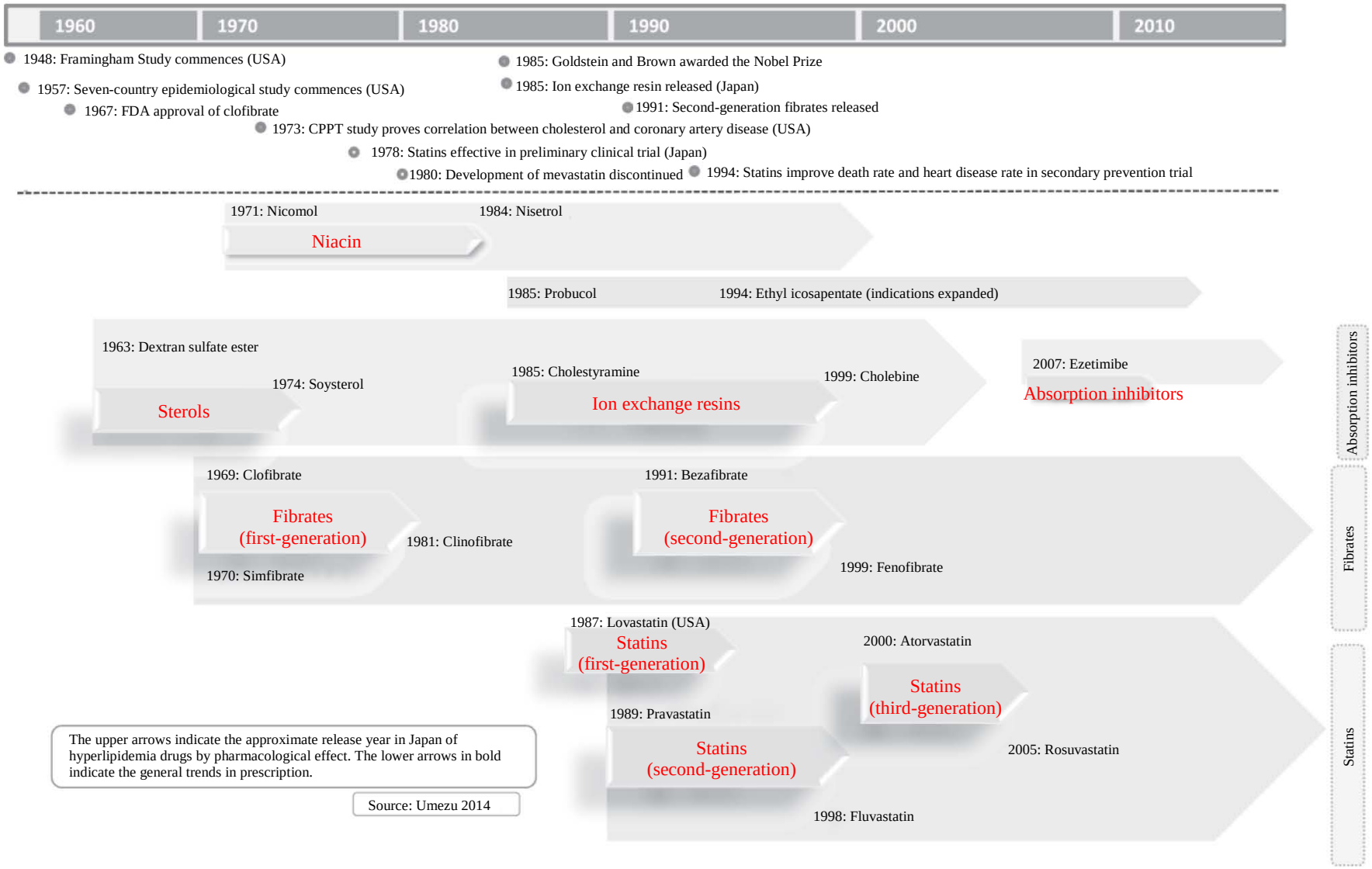
Systematization Diagram of Drug Discovery Technology in Japan (market release history of main diabetes drugs by mechanism of action)



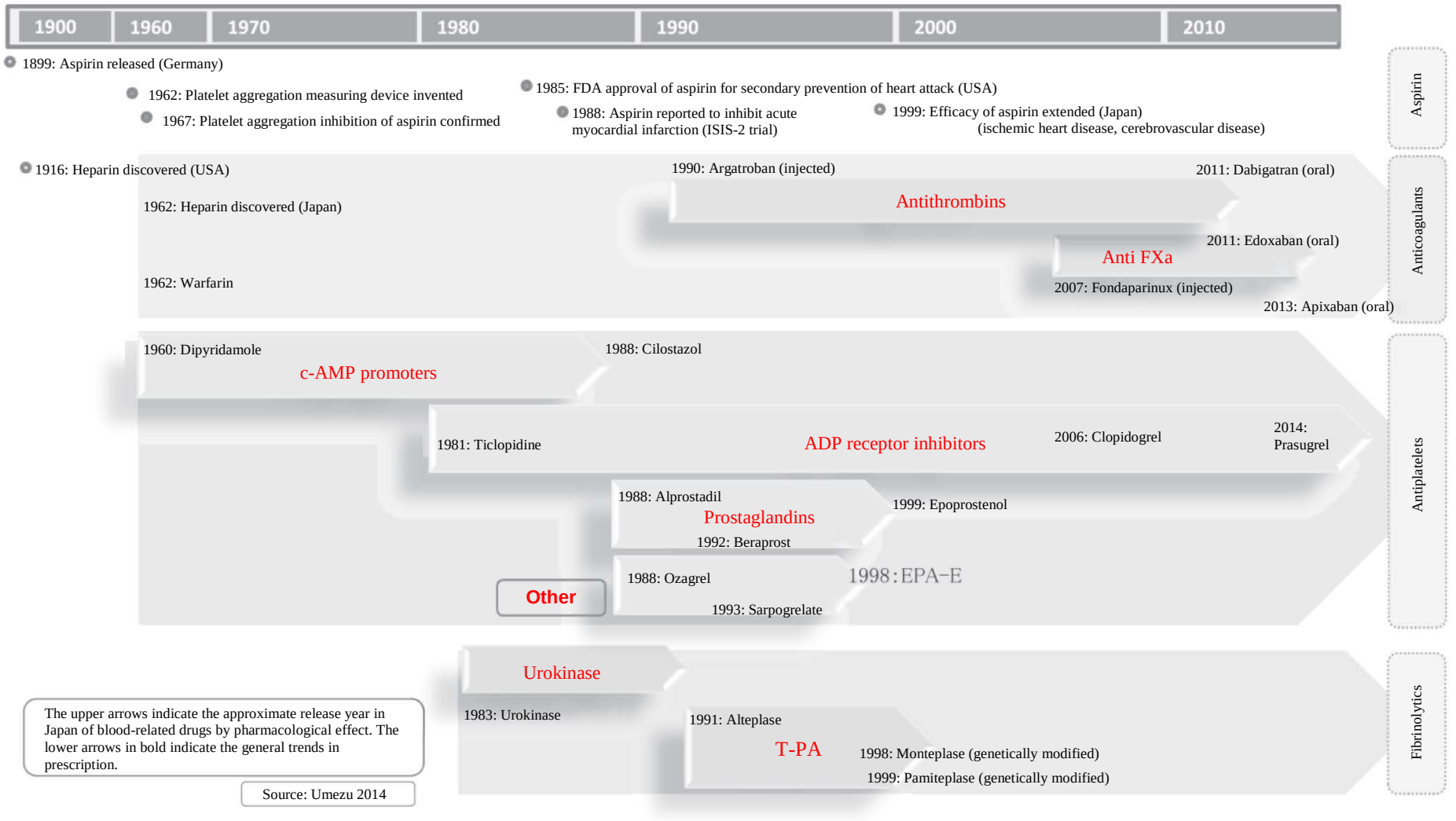
Systematization Diagram of Drug Discovery Technology in Japan (market release history of main hypertension drugs by mechanism of action)



Systematization Diagram of Drug Discovery Technology in Japan (market release history of main hyperlipidemia drugs by mechanism of action)



Systematization Diagram of Drug Discovery Technology in Japan (market release history of main blood-related drugs by mechanism of action)



Pharmaceutical Industry Technology History Resources　Location Confirmed

No.	Item	Year	Manufacturer	Type of resource	Status of resource	Location	Reason for selection
1	Experiment book and monthly report recording the isolation and purification of component ML-236 from culture filtrate (the world's first HMG-CoA reductase inhibitor)	1973	Akia Endo	Notes	TUAT Nature and Science Museum	2-24-16 Nakachō, Koganei-shi, Tokyo	A record of the discovery of the world's first HMG-CoA reductase inhibitor ML-236. This drug formed the basis for many statins and saved many patients from diseases caused by arteriosclerosis.
2	Takadiastase vial	1908-1913	Sankyo	Vial	Dr. Jokichi Takamine Memorial Room, Daiichi Sankyo Co., Ltd.	1-2-58 Hiromachi, Shinagawa-ku, Tokyo	A digestive system drug researched and developed by Jokichi Takamine. The oldest domestic pharmaceutical product vial in Japan.
3	Adrenaline chloride vial and packaging	1920-1932	Sankyo	Vial and packaging	Dr. Jokichi Takamine Memorial Room, Daiichi Sankyo Co., Ltd.	1-2-58 Hiromachi, Shinagawa-ku, Tokyo	Jokichi Takamine and Keizo Uenaka's discovery of adrenalin made into a pharmaceutical product.

Chronological Table of Drug Development (Antidiabetics)

Classification	Mechanism of Action Category		Year released	Generic name	Researched/developed by (Note 1)	Domestic product
Insulin secretagogues	Sulfonylureas	first-generation	1957	Tolbutamide	Discovered by Upjohn	
			1959	Chlorpropamide		
			1964	Acetohexamide	Eli Lilly / Shionogi	
			1965	Glyclopyramide	Kyorin Pharmaceutical	
		second-generation	1971	Glibenclamide	Discovered by B. Mannheim, F. Hoechst /	
					Developed by Chugai Pharmaceutical	
			1984	Gliclazide	Discovered by Servier / Dainippon Pharma	
		third-generation	2000	Glimepiride	Sanofi Aventis	
	Fast-acting insulin Secretagogues (Glinides)	(fourth-generation)	1999	Nateglinide	Ajinomoto	Yes
			2004	Mitiglinide	Kissei Pharmaceutical	Yes
			2011	Repaglinide	Sumitomo Dainippon Pharma	Yes
Glucose removals	Glucose absorption inhibitors (Alpha-glucosidase inhibitors)		1993	Acarbose	Bayer	
			1994	Voglibose	Takeda Pharmaceutical	Yes
			2006	Miglitol	Sanwa Kagaku	
	Glucose reuptake inhibitors (GLT-2 inhibitors)		2014	Canagliflozin	Mitsubishi Tanabe Pharma	Yes
			2014	Ipragliflozin	Kotobuki Pharmaceutical & Astellas	Yes
			2014	Dapagliflozin	AstraZeneca, BMS	
			2014	Luseogliflozin	Taisho Toyama Pharmaceutical	Yes
			2014	Tofogliflozin	Chugai Pharmaceutical / Sanofi, Kowa & Chugai Pharmaceutical	Yes
Insulin sensitizers	Biguanides		1954	Phenformin		
			1961	Metformin	Aron Laboratories	
			1971	Buformin		
	Thiazolidines		1997	Troglitazone	Sankyo	Yes
			1999	Pioglitazone	Takeda Pharmaceutical	Yes
Incretins	GLP-1 analogs (Incretins)		2010	Liraglutide	Novo Nordisk	
			2013	Exenatide	AstraZeneca	
	DPP-4 inhibitors		2009	Sitagliptin	Merck	
			2010	Vildagliptin	Novartis	
			2010	Alogliptin	Takeda Pharmaceutical	Yes
			2010	Linagliptin	Boehringer Ingelheim	
			2012	Teneligliptin	Mitsubishi Tanabe Pharma	Yes
			2012	Anagliptin	Sanwa Kagaku	Yes
			2013	Saxagliptin	Kyowa Hakko Kirin	Yes

Chronological Table of Drug Development (Antihypertensives)

Classification	Mechanism of Action Category		Year released	Generic name	Researched/developed by (Note 1)	Domestic product
Diuretics	Loop diuretics		1965	Furosemide	Hoechst	
	Thiazides		1959	Hydrochlorothiazide	Merck / Banyu	
			1960	Trichloromethiazide	Schering-Plough / Shionogi	
	Thiazide analogs		1975	Mefruside	Bayer / Yoshitomi	
			1982	Tripamide	Eisai	
			1985	Indapamide	Servier (Fr.) / Kyoto & Sumitomo	
	Potassium retaining		1961	Spironolactone	G.D. Searle / Pfizer	
			1962	Triamterene	SKF / Kyoto & Sumitomo	
	(selective aldosterone inhibitor)		2007	Eplerenone	Pfizer	
β-blockers	β1 non-selective	first-generation	1966	Propranolol	AstraZeneca	
			1973	Pindolol	Novartis	
			1974	Bufetrol	Yoshitomi	Yes
			1980	Indenolol	Yamanouchi	Yes
			1981	Carteolol	Otsuka	Yes
			1986	Nadolol	Squibb / Dainippon	
			1988	Nipradilol	Kowa	Yes
			1992	Tilisolol	Nisshin / Tomiyama	Yes
	β1 selective	second-generation	1981	Acebutolol	Aventis	
			1983	Metoprolol	AstraZeneca	
			1984	Atenolol	AstraZeneca	
			1990	Bisoprolol	E Merck / Tanabe	
			1993	Betaxolol	Santelabo / Mitsubishi Kasei	
	α1 + β blocking		1983	Labetalol	GlaxoSmithKline	
			1985	Arotinolol	Sumitomo & Ono	Yes
			1988	Amosuralol	Yamanouchi	Yes
			1993	Carvedilol	Boehringer Mannheim / Daiichi	
α-blockers			1981	Prazosin	Pfizer	
			1985	Bunazosin	Eisai	Yes

Chronological Table of Drug Development (Antihypertensives)

Classification	Mechanism of Action Category		Year released	Generic name	Researched/developed by (Note 1)	Domestic product
Calcium channel blockers	Dihydropiridine	first-generation	1976	Nifedipine	Bayer	
			1988	Nicardipine	Yamanouchi	Yes
		second-generation	1989	Nilvadipine	Fujisawa	Yes
			1990	Nisoldipine	Bayer	
			1990	Manidipine	Takeda Pharmaceutical	Yes
			1990	Nitrendipine	Bayer / Tanabe	
			1991	Benidipine	Kyowa Hakko	Yes
			1992	Barnidipine	Yamanouchi	Yes
			1994	Efonidipine	Nissan Chemical / Zeria	Yes
			1995	Felodipine	Astra & Hässle / Chiba-Geigy Japan & Hoescht Japan	
			1995	Cilnidipine	Fujirebio & Ajinomoto	Yes
			1996	Aranidipine	Maruko & Taiho	Yes
		third-generation	1993	Amlodipine	Pfizer	
			2003	Azelnidipine	Ube / Daiichi Sankyo	Yes
	Diltiazem		1973	Diltiazem	Tanabe	Yes
ACE inhibitors	SH-type	first-generation	1983	Captopril	Squibb / Sankyo	
			1988	Alacepril	Dainippon	Yes
	COOH-type	second-generation	1986	Enalapril	Merck / Banyu	
			1989	Delapril	Takeda Pharmaceutical	Yes
			1990	Cilazapril	Eisai	
			1991	Lisinopril	Merck & ICI / AstraZeneca	
			1993	Benazepril	Novartis	
			1993	Imidapril	Tanabe / Nihon Schering	Yes
			1994	Temocapril	Sankyo	Yes
			1995	Quinapril	Warner-Lambert / Tanabe	
			1996	Trandolapril	Discovered by Hoescht / Hoescht Japan & Roussel Japan	
			1998	Perindopril	Servier / Daiichi	
Angiotensin 1 receptor inhibitors		first-generation	1998	Losartan	Merck / Banyu	
			1999	Candesartan	Takeda Pharmaceutical	Yes
			2000	Valsartan	Chiba-Geigy Japan	
		second-generation	2002	Telmisartan	Boehringer Ingelheim Japan	
			2004	Olmesartan	Sankyo	Yes
			2008	Irbesartan	Sanofi / Dainippon Sumitomo	
			2012	Azilsartan	Takeda Pharmaceutical	Yes
Renin inhibitors			2007	Aliskiren	Novartis Pharma	

Chronological Table of Drug Development (Hyperlipidemia Drugs)

Classification	Mechanism of Action Category	Year released	Generic name	Researched/developed by (Note 1)	Domestic product
Natural products		1963	Dextran sulfate ester	Kowa	Yes
		1966	Tocopherol nicotinate	Eisai	Yes
		1974	Soysterol	Ajinomoto & Morishita	Yes
		1994	Ethyl icosapentate	Mochida / Dainippon	Yes
Niacin		1971	Nicomol	Kyorin	Yes
		1984	Niceritrol	Bofors / SKK	
Probucol		1985	Probucol	Dow Chemical	
Absorption inhibitors	Ion exchange resins	1985	Cholestyramine	BMS	
		1999	Cholebine	Mitsubishi Chemical	Yes
	Cholesterol absorption inhibitors	1983	Melinamide	Sumitomo	Yes
		2007	Ezetimibe	MSD	
Fibrates	first-generation	1965	Clofibrate	Discovered by ICI / Sumitomo	
		1970	Simfibrate	Yoshitomi	Yes
		1981	Clinofibrate	Sumitomo	Yes
	second-generation	1991	Bezafibrate	Boehringer Mannheim / Kissei	
		1999	Fenofibrate	Discovered by Fournier / Grelan	
Statins	first-generation (isolated from culture solution)		Mevastatin	Sankyo (development discontinued in 1980)	Yes
		1987 (USA)	Lovastatin	Merck (USA and elsewhere; not sold in Japan)	
	second-generation (semi-synthesized)	1989	Pravastatin	Sankyo	Yes
		1991	Simvastatin	Merck	
		1998	Fluvastatin	Novartis Pharma	
	third-generation (fully synthesized)	2000	Atorvastatin	Pfizer	
		2003	Pitavastatin	Nissan Chemical & Kowa	Yes
		2005	Rosuvastatin	Shionogi	Yes

Chronological Table of Drug Development (Circulatory System Drugs)

Classification	Mechanism of Action Category	Year released	Generic name	Researched/developed by (Note 1)	Domestic product
Hemostatics	Plasmin inhibitors	1954	Aminocaproic acid	Hayashi Kenkyusho & Mitsubishi Chemical / Daiichi	Yes
		1965	Tranexamic acid	Kobe University, Mitsubishi Chemical & Daiichi / Daiichi	Yes
Antiplatelets	COX I inhibitors	1900	Aspirin	Bayer (trademark registered; activity started by MR in 1908)	
	PDE inhibitors	1960	Dipyridamole	Karl Thomae / Boehringer Ingelheim Japan	
		1988	Cilostazol	Otsuka	Yes
	Prostaglandins	1988	Alprostadil	Green Cross & Taisho	Yes
		1988	Limaprost alfadex	Ono & Dainippon	Yes
		1992	Beraprost	Toray & Kaken	Yes
		1999	Epoprostenol	GSK	
	TXA2 synthase inhibitors	1988	Ozagrel	Ono & Kissei	Yes
	5HT2 antagonists	1993	Sarpogrelate	Mitsubishi Chemical	Yes
	Eicosapentaenoic acid	1998	EPA-E (eicosapentaenoic acid ester)	Nissui & Mochida	Yes
	ADP receptor antagonists	1981	Ticlopidine	Parcor / Daiichi	
		2006	Clopidogrel	Sanofi Aventis	
		2014	Prasugrel	Ube / Sankyo	Yes
Anticoagulants	Vitamin K blockers	1962	Warfarin		
	Antithrombin (injected)	1990	Argatroban	Mitsubishi Chemical / Daiichi	Yes
	Antithrombin (oral)	2011	Dabigatran	Boehringer Ingelheim Japan	
	Anti-FXa (injected)	2007	Fondaparinux	Discovered by Sanofi-Sante / GSK (developed)	
	Anti-FXa (oral)	2011	Edoxaban	Daiichi Sankyo	Yes
		2012	Rivaroxaban	Bayer	
		2013	Apixaban	BMS & Pfizer	
	Thrombomodulin	2009	Thrombomodulin α (recombinant modification)	Asahi Kasei Pharma	Yes
Fibrinolytics (thrombolytics)	Bacterial fibrinolysis		Streptokinase		
	Urokinase	1983	Urokinase (extracted and refined)	Green Cross & Mochida	Yes
		1983	Urokinase (tissue culture)	Abbott / Dainippon & Mitsubishi Petrochemical	
		1992	Nasaruplase (tissue culture)	Green Cross	Yes
	t-PA (natural)	1991	Alteplase (genetically modified)	Genentech / Mitsubishi Chemical & Kyowa Hakko	
		1991	Tisokinase (tissue culture)	Asahi Kasei & Kowa	Yes
		1996	Nateplase (genetically modified)	Mitsui Toatsu, Mitsui & Mochida	Yes
	t-PA, genetically modified (second-generation)	1998	Monteplase (genetically modified)	Eisai	Yes
		1999	Pamiteplase (genetically modified)	Yamanouchi	Yes

Chronological Table of Drug Development (Gout/Hyperuricemia Drugs)

Classification	Mechanism of Action Category		Year released	Generic name	Researched/developed by (Note 1)	Domestic product
Gout Hyperuricemia	Uricosurics	Primary tubular reabsorption inhibitor	1956	Probenecid	Developed by Kaken	
		Secondary tubular reabsorption inhibitor, anti-inflammatory effect	1967	Bucolome	Gifu University, Gifu Pharmaceutical University & Takeda / Takeda	Yes
		Secondary tubular reabsorption inhibitor	1978	Benzbromarone	Discovered by Labaz (Fr.) / Torii	
	Uric acid production inhibitors	Xanthine oxidase inhibitor	1968	Allopurinol	Wellcome	
		Xanthine oxidase inhibitor	2011	Febuxostat	Teijin Pharma	Yes
		Xanthine oxidase inhibitor	2013	Topiroxostat	Fuji	Yes
Gout	Gout-specific anti-inflammatory		1964	Colchicine		
	Additional indications for gout drugs	Anti-inflammatory (expanded indications as gout drugs)		Indomethacin		
				Naproxen		
				Oxaprozin		
				Pranoprofen, etc.		
		Corticosteroids		omitted		

Note 1
Where (/) is indicated, the main companies engaged in researching the new drug are given to the left of the (/).
Companies involved from clinical development onwards or mainly involved in clinical development are given to the right of the (/).
Here, “discovered by” refers to new drug research. Joint research or joint development is indicated with (&).