A gift from nature: the birth of the statins

Akira Endo

I was born into a rural farming family in northern Japan, near Akita, where I lived for 17 years with my extended family, including grandparents, parents, three brothers and two sisters. My grandfather, who had an interest in medicine and science, was a great home teacher to me. Thanks to his influence, at the age of 8, I dreamt of becoming a scientist, much like the renowned Japanese scientist Hideyo Noguchi, who, in 1900 at the age of 24, went to the United States and studied syphilis and yellow fever at the Rockefeller Institute in New York.

After finishing high school in Akita, I entered Tohoku University's College of Agriculture in Sendai in 1953. I excelled in organic chemistry at high school. So, in addition to studying organic chemistry at the university, I also chose biochemistry and applied microbiology as my preferred subjects. At that time, many drug companies and universities in Japan were conducting active research and development in finding effective antibiotics. As a student, I was deeply impressed by the knowledge that antibiotics had saved the lives of many patients with infectious diseases.

Upon graduating in 1957, I joined the pharmaceutical company Sankyo in Tokyo, where I was assigned to one of the applied microbiology groups. I worked toward developing a new pectinase that hydrolyzes viscid pectin contaminating wines and ciders. In 1958 I found a grape-parasitic fungus, *Coniothyrium diplodiella*, to be a potent producer of such an enzyme. I then purified it and elucidated its properties. For these studies, I received a PhD degree from Tohoku University in 1966.

At this point, I became interested in cholesterol biosynthesis. Toward the end of 1965 I wrote a letter to Konrad Bloch, who had received the Nobel Prize for his research on cholesterol biosynthesis in 1964, expressing my wish to study under him. Unfortunately, the autumn 1966 class for which I was applying was already full. So I eventually studied from September 1966 to August 1968 at the Department of Molecular Biology at the Albert Einstein College of Medicine in New York. Under the guidance of Bernard Horecker and Lawrence Rothfield, I studied an enzyme that was involved in the biosynthesis of bacterial cell wall lipopolysaccharide. The outcome of my research depended upon purifying the unstable enzyme. A year into the project, I succeeded and showed that the formation of an enzyme–phospholipid complex was crucial for its action.

While living in New York, I was very surprised by the large number of elderly and overweight people, and by the rather rich dietary habits of Americans compared to those of the Japanese. In the residential area of the Bronx where I lived, there were many elderly couples living by themselves, and I often saw ambulances coming to take an elderly person who had suffered a heart attack to the hospital. At that time, coronary heart disease was the main cause of death in the United States. The number of people with hypercholesterolemia, a precursor to coronary heart disease, was said to exceed 10 million.

Cholesterol in the body comes from what is absorbed from diet and from what is synthesized in the body, mainly by the liver. In the 1960s it was shown that, in humans, cholesterol produced in the liver exceeds what is absorbed from the diet. 3-Hydroxy-3-methylglutaryl (HMG)-CoA reductase, an enzyme that catalyzes the conversion of HMG-CoA into mevalonate, proved to be the rate-controlling enzyme in cholesterol synthesis. On the basis of these facts, I speculated that a cholesterol-synthesis inhibitor, particularly a HMG-CoA reductase inhibitor, would be an effective cholesterol-lowering agent.

In the 1960s, a number of lipid-lowering agents, such as clofibrate, niacin and cholestylamine, were available, but none of them were considered safe and effective. Moreover, a HMG-CoA reductase inhibitor had not yet been developed. My experience of living in New York made me realize the importance of developing a cholesterol-lowering drug. In August 1968, I finished my studies in the United States and returned to Sankyo to continue to work on this problem.

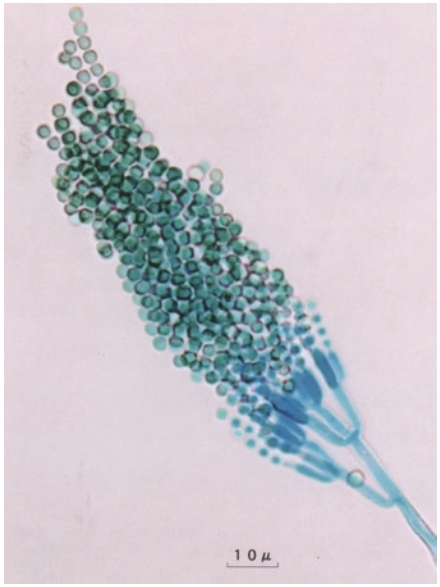

Figure 1 Micrograph of *Penicillium citrinum* Pen-51, the fungus that produces compactin. Scale bar, 10μm.

Discovering compactin from blue-green mold

In 1971, I speculated that microbes would produce antibiotics that inhibited HMG-CoA reductase as a defense mechanism against other microbes that require sterols and/or other mevalonate-derived isoprenoids for their growth, and we created a research unit to isolate such products, focusing on fungi as a source of these metabolites.

The search for a suitable compound took 2 years and involved 6,000 strains of microbe. Initially, we looked for microbial culture broths that would inhibit the incorporation of ^{14}C-acetate into sterols. We then tested culture broths that had shown inhibitory activity

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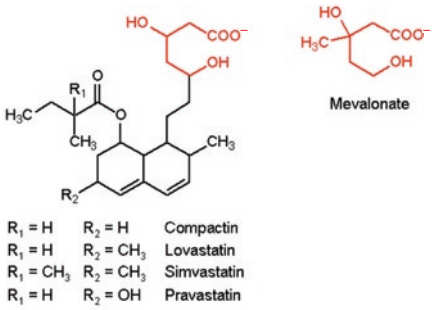

Figure 2 A comparison of the structures of four statins and mevalonate.

in that assay to see if they would inhibit the production of sterols from ^{3}H-mevalonate. Broths active in the first screen but not in the second were deemed to be inhibitors in the early part of cholesterol synthesis. Broths from two fungi met our requirements. The first fungus, *Pythium ultimum* ML-145, produced citrinin (a known antifungal agent) as the active principle. The second fungus, *Penicillium citrinum* Pen-51, was a blue-green mold isolated from the rice of a vendor in Kyoto in the 1960s (**Fig. 1**). We isolated the inhibitory compound from *P. citrinum* Pen-51 broths—ML-236B (now known as compactin)—by solvent extraction, silica gel chromatography and crystallization.

We soon realized the structural similarities between compactin and mevalonate, the product of the HMG-CoA reductase reaction. The structure compactin was exactly as we had previously envisioned (**Fig. 2**)[1]. Compactin was a potent inhibitor of HMG-CoA reductase, and its mechanism of action, suggested by its structure, was that of a competitive inhibitor[2]. I had had my sights set on finding a competitive inhibitor of HMG-CoA reductase, and compactin seemed to be a wonderful gift from nature.

A series of critical points

In 1974, biologists at Sankyo evaluated the efficacy of compactin by feeding rats a diet supplemented with compactin for 7 days. Unfortunately, there was no reduction in plasma cholesterol. With these results, there was no hope of convincing the biologists of evaluating compactin, which did not work on rats, in dogs and monkeys. We therefore set out to try to elucidate the mechanism of action of compactin and the reason why it was not effective in rats. These experiments showed that the amount of HMG-CoA reductase in the liver, the main organ for cholesterol synthesis, rapidly increased after administrating compactin, suggesting that the main reason for compactin's failure to work in rats was the abnormal induction of HMG-CoA reductase[3].

In the early spring of 1976, Noritoshi Kitano, a pathologist at Sankyo who was keeping laying hens for research purposes, kindly agreed to a joint research project to evaluate compactin using his hens. The experiments were a great success. The plasma cholesterol of laying hens that received compactin decreased by 34% after 2 weeks. Soon after, we were able to confirm the profound cholesterol-lowering effects of compactin in dogs and monkeys[4,5]. These results defined compactin as a candidate for a new type of drug. So, the 'Compactin Development Project'—headed by myself and including pharmacologists, pathologists, toxicologists, organic chemists and applied microbiologists—was launched in August 1976.

Interestingly, at around the same period, researchers from England's Beecham Laboratories had also discovered compactin as an antibacterial agent from another blue-green mold (*Penicillium brevicompactum*). However, they were unable to develop the HMG-CoA reductase inhibitor as a cholesterol-lowering agent due to the inability of compactin to lower the blood cholesterol of rats and mice as, consistent with our findings, it strongly induced the HMG-CoA reductase of the liver[6].

We encountered a second challenge in April 1977. The issue was the detection of microcrystalline structures in the liver cells of rats that had been fed extremely large amounts of compactin (more than 500 milligrams per kilogram body weight per day (mg/kg/d)) for 5 weeks. The toxicologists insisted that these structures were toxic substances. It took us 9 months to identify these microcrystalline structures as nontoxic cholesterol.

In August 1977, Akira Yamamoto from Osaka University Hospital inquired about using compactin in the treatment of a homozygous patient with severe familial hypercholesterolemia. This patient (**Fig. 3**) was an 18-year old woman with a serum cholesterol of 1,000 mg per deciliter. Yamamoto started treating her with compactin in February 1978, and her serum cholesterol temporarily dropped to ~700 mg per deciliter (ref. 7). However, we realized that, to lower it any further, an increased dosage would be required, thus resulting in adverse effects. In the following 6 months, Yamamoto treated five heterozygous patients with familial hypercholesterolemia and four patients with combined hyperlipidemia with compactin, and their cholesterol declined by roughly 30% on average; no severe side effects were noted[7]. In November 1978, Sankyo started a phase 1 clinical trial for compactin, followed by a phase 2 trial in the summer of 1979. In the phase 2 trial, compactin was administered to subjects with serious cases of hypercholesterolemia at more than ten hospitals. All of the participating hospitals reported positively on the remarkable efficacy and excellent safety profile of compactin.

In August 1980, Sankyo held a meeting with the physicians involved in the trials to announce the suspension of the clinical development of compactin, which had been progressing smoothly. Although the detailed reasons for this decision were not revealed, we all considered it to be the result of compactin experiments in dogs. In these long-term toxicity experiments, in which dogs were given compactin for 2 years, no abnormalities were noted in the group receiving 25 mg/kg/d. However, lymphomas were detected in the group receiving 100 or 200 mg/kg/d. In long-term toxicity experiments on dogs for pravastatin (a drug developed later and discussed below), Sankyo lowered the maximum dose to 25 mg/kg/d; there was no mention of any 100 or 200 mg/kg/d group[5]. So, it seems that, regardless of whether lymphomas were actually detected or not, Sankyo could have continued the development of compactin by limiting its maximum dose to 25 mg/kg/d. (I retired from Sankyo at the end of 1978 and joined Tokyo Noko University in January 1979.)

The arrival of Merck

In the summer of 1976, Sankyo and Merck entered into a contract for the disclosure of information on compactin. Until the autumn of 1978, Sankyo continued to provide experimental data on biochemistry, drug efficacy, pharmacology and pathology, along with compactin crystals, to Merck. We were, in fact, under the impression that both companies were jointly developing

Figure 3 Akira Yamamoto's homozygous patient with familial hypercholesterolemia who first received compactin in 1978. Her treatment paved the way to the clinical development of compactin. Here she is holding her baby 7 years after the treatment.

compactin. However, in the fall of 1978, Merck independently discovered lovastatin from another fungus, *Aspergillus terreus*. Lovastatin differed from compactin by only one methyl group (**Fig. 2**), and it had very similar biological properties[6,8]. By the spring of 1980, Merck had progressed to phase 1 of their clinical trial of lovastatin. But, as I mentioned above, in August 1980 the development of compactin was suddenly suspended, leading Merck to halt its clinical development of lovastatin also.

In 1981, Hiroshi Mabuchi's group at Kanazawa University reported the results of their highly successful compactin treatment of seven heterozygous patients with familial hypercholesterolemia. The LDL-cholesterol of these patients, who underwent a 24-week course of 30–60 mg/d of compactin, declined by an average of 29% with no fall in high-density lipoprotein–cholesterol; rather, a slight increase was noted[9]. The following year, several US physicians started using lovastatin to treat serious cases of hypercholesterolemia and obtained results as impressive as Mabuchi's. This led Merck to give the green light for resuming the clinical development of lovastatin in 1984, with the drug receiving US Food and Drug Administration approval in 1987 to become the first commercial statin[6].

Lovastatin was followed by a new statin with an extra methyl group—simvastatin (**Fig. 2**). Sankyo, in turn, developed pravastatin—compactin with an extra hydroxyl group (**Fig. 2**)—and launched it in 1989. Five other types of synthetic statin—fluvastatin, cerivastatin, atorvastatin, rosvastatin and pitavastatin—were subsequently developed; however, cerivastatin was eventually discontinued due to reports of myopathy as a side effect.

More than ten large-scale clinical trials on the statins reported since the mid-1990s have shown decreases in LDL-cholesterol, in the incidence of coronary artery disease, and in general mortality rates of 25–35%, 25–30% and ~30%, respectively. Statins have also been shown to reduce the incidence of stroke by 25–30%. At present, statins are being administered daily to more than an estimated 30 million patients for the prevention of coronary heart diseases and stroke.

ACKNOWLEDGMENTS

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