ELSEVIER

Atherosclerosis Supplements 5 (2004) 125–130

ATHEROSCLEROSIS
SUPPLEMENTS

www.elsevier.com/locate/atherosclerosis

ELSEVIER

International Congress Series 1262 (2004) 3–8

www.ics-elsevier.com

The origin of the statins

Akira Endo*

Biopharm Research Laboratories, Main Office, 2-1-31, Minamicho, Kokubunji, Tokyo 185-0021, Japan

Abstract. In the early 1970s we isolated the first statin, mevastatin (formerly called compactin or ML-236B), from *Penicillium citrinum*, as a potent inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-controlling enzyme in cholesterol synthetic pathway. By the end of the 1970s we had demonstrated that mevastatin was highly effective in lowering serum total and low-density lipoprotein (LDL) cholesterol in both experimental animals and patients with primary hypercholesterolemia. The discovery of mevastatin paved the way for the worldwide development of its analogues (statins), and since then several statins—lovastatin, simvastatin, pravastatin, fluvastatin and atorvastatin—have been approved in many countries and are currently used by millions of patients.

Keywords: Cholesterol; HMG-CoA reductase inhibitors; Statins; Mevastatin; Lovastatin

1. Introduction

Coronary heart disease is the main clinical manifestation of atherosclerosis and is the major cause of death in modern, industrialized countries and increasingly a cause in developing countries; atherosclerosis might also affect the peripheral arteries and the cerebral circulation, leading to other debilitating or life-threatening conditions. One of the major risk factors for atherosclerosis and coronary heart disease is hypercholesterolemia.

2. Background

In the mid-1960s fascinated with several excellent reviews on cholesterol biosynthesis by Konrad Bloch of Harvard University (Boston, MA) [1,2], who received the Nobel Prize in 1964, I became interested in the biochemistry of cholesterol and other lipids. In early December 1965, I wrote to Bloch, inquiring about postdoctoral research in his laboratory.

Unfortunately, the postdoctoral fellowship was no longer available for the year 1966–1967. Soon after, I wrote to P. Roy Vagelos at the National Institute of Health (Bethesda, MD), inquiring about research into fatty acid synthesis in his laboratory. But after a month I still had no answer, so, in late January 1966, I determined to study phospholipids at the

* Tel.: +81-42-329-1070; fax: +81-42-329-8070.
E-mail address: aendo@biopharm.co.jp (A. Endo).

0531-5131/
doi:10.1016/j.ics.2003.12.099

1567-5688/$ – see front matter
doi:10.1016/j.atherosclerosissup.2004.08.033

Department of Molecular Biology (Bernard Horecker, chair), at the Albert Einstein College of Medicine (AECM), New York for 2 years (1966–1968) (soon after this appointment had been made, Vagelos answered me that he would grant my request).

In AECM, under the guidance of L. Rothfield, I isolated, purified, and worked out the mechanism of action of a bacterial phospholipid-requiring enzyme involved in the biosynthesis of cell wall lipopolysaccharide [3,4]. Rothfield had worked as a physician for nearly 10 years in the New York University Hospital before he joined AECM. I learnt from him that hypercholesterolemia was a major risk factor for coronary heart disease, the No. 1 killer in Western countries. In addition, my stay in the U.S. gave me the opportunity to learn about the regulation of the cholesterol metabolism. At that time, experiments with animals and humans by other groups had shown that cholesterol could either be absorbed from diet, or if diet was lacking sufficient cholesterol to meet the body's needs, then it could be synthesized mainly in the liver. However, if a diet was rich in cholesterol then synthesis within the body virtually stopped. HMG-CoA reductase, an enzyme that catalyzed conversion of HMG-CoA into mevalonate, was shown to be the rate controlling enzyme in cholesterol synthesis [5]. My experiences from those days aroused my curiosity about developing a cholesterol-lowering agent.

3. Discovery of mevastatin

In 1968, I returned to Sankyo, and in 1970, I hypothesized that levels of plasma cholesterol could be lowered more effectively by inhibiting hepatic HMG-CoA reductase than by decreasing absorption from the diet; and postulated that some microbes would produce secondary metabolites that inhibited HMG-CoA reductase, possibly as a defense mechanism against other microbes that required sterols and/or other mevalonate-derived isoprenoid compounds for their growth.

In April 1971, we created a research unit to isolate such products. We focused on fungi and mushrooms as a source of these metabolites. The search for a suitable compound took 2 years and involved 6000 microbes. As a result, the fungus, *Penicillim citrinum* was shown to inhibit lipid synthesis. The major inhibiting compound from *P. citrinum* broths, ML-236B (now known as mevastatin), was isolated by solvent extraction, silica gel chromatography and crystallization [6].

The lactone ring of the mevastatin molecule was reversibly converted to an open acid that resembled the HMG portion of HMG-CoA, the substrate of the reaction catalyzed by HMG-CoA reductase. In the reaction HMG-CoA is reduced to mevalonate, a key precursor to cholesterol. Mevastatin was shown to be an ideal potent competitive inhibitor to the enzyme reaction [7]. Although some early experiments carried out in rats indicated that mevastatin would only work in the very short term, in 1976–1977, we were able to show that mevastatin dramatically lowered plasma cholesterol levels in hens, dogs and monkeys. In early 1978, Akira Yamamoto (Osaka, Japan) and I showed that mevastatin dramatically lowered plasma cholesterol of patients with serious hypercholesterolemia [8]. In November 1978, based on these results, formal clinical trials of mevastatin were started in Japan. By mid 1980, these trials had shown that mevastatin lowered plasma total and LDL-cholesterol by 20–40% at 15–60 mg/day; no serious side effects were noticed [9].

4. Discovery of lovastatin

At the end of December 1978, I left Sankyo and joined the School of Agriculture at Tokyo Noko University in Tokyo. In February 1979, I isolated three mevastatin analogues—monacolin J, monacolin K, monacolin L—from *Monascus ruber* M1005 (Monacolin K was filed for a patent in February 1979 [10][1]; monacolin J in April 1979 [11]; and monacolin L in October 1979 [12])[1]. Of these three compounds, monacolin K, the major product, was slightly more effective in inhibiting HMG-CoA reductase than mevastatin.

Back in 1966, P. Roy Vagelos and Alfred Alberts from the NIH had moved to Washington University School of Medicine (St. Louis, MO), where they showed that animal cells required cholesterol to maintain their normal membrane structure. In 1975, taking Alberts with him, Vagelos joined Merck as senior vice-president in charge of research (President of the Merck Sharp and Dohme Research Laboratories-MSDRL-Division) [13]. In April 1976, H. Boyd Woodruff (Executive Administrator of MSDRL) who had noted the Sankyo patent application covering mevastatin, inquired of Sankyo about the possibility to obtain a sample of mevastatin for evaluation at MSDRL under a confidentiality agreement. Sankyo accepted Merck's request[2] and in July 1976, it provided MSDRL with samples (crystals) of mevastatin and confidential data of our biochemical, pharmacological and toxicological studies of mevastatin[2].

During the next two or so years, researchers at MSDRL studied mevastatin in cultured mammalian cells, with a cell-free enzyme system; and in vivo in rats and dogs, and by October 1978 had obtained results that corresponded closely to those obtained by my group[3]. On April 6, 1979, I gave a lecture at MSDRL in Rahway, NJ on the development of mevastatin; after which, Vagelos, Alberts and I discussed the experimental results of mevastatin obtained by both Alberts' and my groups. However, the discovery of other statins never came up in our discussion.

At the end of August, 1979, the discovery of monacolin K was published in a scientific journal [14], and soon after Alberts and Woodruff who had noticed the article flew to Tokyo and said: In September 1978, Alberts and his assistant Julie Chen started searching for an HMG-CoA reductase inhibitor, and on 16 November 1978, found that the extract of a soil fungus, *Aspergillus terreus*, strongly inhibited HMG-CoA reductase. In February 1979, Carl Hoffman and his associates at MSDRL isolated the pure inhibitor, called mevinolin (now called lovastatin), from cultured broths of *A. terreus*. In June 1979, Merck filed mevinolin for patent. Merck was anxious to know if it was distinct from monacolin K or not [13,15].

In October 1979, it was accepted that monacolin K and mevinolin were the same compound. Mevinolin had been discovered three months before monacolin K (November

[1] This patent was also applied in the following 30 countries: USA, UK, FRG, France, Italy, Switzerland, Spain, Belgium, The Netherlands, Canada, Sweden, South Korea, USSR, Ireland, Austria, Mexico, Australia, Argentina, The Philippines, South Africa, East Germany, Hungary, Poland, Finland, Norway, India, Pakistan, Formosa, New Zealand and Yugoslavia.

[2] A disclosure agreement on this matter was reached between B. Woodruff (Merck) and H. Okazaki (Sankyo) on July 21, 1976.

[3] A report covering these data was sent by mail from B. Woodruff (Merck) to R. Yamamoto (Sankyo) on October 19, 1978.

1978 vs. February 1979), but its patent application came four months after monacolin K (June 1979 [16] vs. February 1979 [10]). As a result, it became clear that patents were to be approved for mevinolin but not for monacolin K in the U.S. and several countries that gave priority to 'time of invention'. In some other thirty countries[1] that gave priority to 'time of application', patents were to be granted for monacolin K but not for mevinolin.

In November 1979, the patent right of monacolin K was passed to Sankyo; who paid Tokyo Noko University 35 million yen as an equivalent for the patent right[4]. This transfer made Merck unable to commercialize mevinolin (lovastatin) in the above-mentioned thirty countries without getting a license from Sankyo. Although Sankyo neither gave Merck the license nor commercialized monacolin K itself, it would get billions of dollars by blocking Merck's commercialization of mevinolin in the major countries except the U.S. (In 1981, Merck synthesized another statin-simvastatin and commercialized it world-wide in the 1990s [17].)

Meanwhile, rumors appeared from time to time that the work on the discovery of monacolin K from *M. ruber* M1005 had been done at Sankyo when I was there; that is pure invention. It is clear that the work on the isolation of monacolin K, together with its two analogues, monacolin J and monacolin L, from *M. ruber* M1005 was started and completed at Tokyo Noko University [18][4]. That work, then, had no legal connection with Sankyo.

In April 1980, Merck began clinical studies of lovastatin (mevinolin) [13]. Several months later, Sankyo discontinued clinical trials of mevastatin, which had been carried out since 1978, apparently because intestinal tumors were found in dogs. Although there was some question that the company's decision was right, on learning this in September 1980, Merck halted its clinical studies of lovastatin and undertook further pharmacological and toxicological studies.

In 1982, several clinicians, including Roger Illingworth (Portland, OR), David Bilheimer and Scott Grundy (Dallas, TX) began to give lovastatin to patients with serious hypercholesterolemia who were unresponsive to available agents [13]. These trials showed that lovastatin dramatically lowered plasma cholesterol, and very few side effects were noted.

In November 1983, based on these results as well as the recommendation of Daniel Steinberg (San Diego, CA) and Jean Wilson (Dallas, TX), formal clinical trials of lovastatin were started [13]. Large-scale clinical studies began in May 1984. In the 2 years that followed, the clinical trials and long-term safety studies were completed; and in November 1986, Merck submitted a new drug application for lovastatin to the Food and Drug Administration (FDA). The FDA moved rapidly and in late February 1987, an FDA

[4] On November 30, 1979, an agreement confirming the following terms under which Endo shall grant to Sankyo patent right of the Japanese Patent Application No. 54-17, 856, filed by Endo on February 20, 1979, with the title of 'Novel physiologically active substance monacolin K and process for producing the same' was entered into between both parties: ① Endo shall grant to Sankyo the worldwide patent right of this application. ② Sankyo shall pay 35,000,000 yen to the organization designated by Endo (Tokyo Noko University) as an equivalent to the patent right. ③ Sankyo shall file worldwide applications for this patent at its own expense. ④ Sankyo shall grant to Merck a non-exclusive license to develop and commercialize monacolin K worldwide, except for Japan. In case the license is granted to Merck, Sankyo shall pay to Tokyo Noko University a royalty of 0.75% of Merck's net sales of the monacolin K products.

advisory committee reviewed the company's application. The committee recommended approval of the drug for certain patients whose elevated cholesterol levels cannot be lowered through diet or other non-pharmacological methods. On September 1, 1987, the FDA announced that it had approved lovastatin (Mevacor®) [13].

5. Other statins

The following years witnessed the development in many countries of other statins based on mevastatin and lovastatin. Three main approaches have been utilized. First, chemical alteration of fungal products created statin drugs such as simvastatin. Second, microbial alteration of fungal compounds led to drugs such as pravastatin. Finally, synthetic compounds such as fluvastatin and atorovastatin were produced. Research in this area has concentrated on replacing the decaline ring of the fungal compounds with an aromatic ring [17]. These statins have been all well established as effective and safe cholesterol-lowering agents and have been used by tens of millions of patients. Several landmark clinical trials with statins demonstrated that the lipid-lowering therapy reduces cardiovascular morbidity and mortality in both primary and secondary prevention [19].

References

[1] K. Bloch, The biological synthesis of cholesterol, Science 150 (1965) 19–28.
[2] K. Bloch, Biogenesis and transformations of squalene, in: G. Wolftenholme, M. O'Connor (Eds.), Biosynthesis of Terpenes and Sterols, J. and A. Churchill, London, 1959, pp. 4–9.
[3] A. Endo, L. Rothfield, Studies of a phospholipid-requiring bacterial enzyme: 1. Purification and properties of uridine diphosphate galactose: lipopolysaccharide α-3-galactosly transferase, Biochemistry 8 (1969) 3500–3507.
[4] A. Endo, L. Rothfield, Studies of a phopholipid-requiring bacterial enzyme: 2. The role of phosholipid in the uridine diphosphate galactose: lipopolysaccharide α-3-galactosly transferase reaction, Biochemistry 8 (1969) 3508–3515.
[5] J. Dietschy, J. Wilson, Requlation of cholesterol metabolism, N. Engl. J. Med. 282 (1970) 1128–1138; 1179–1183; 1241–1249.
[6] A. Endo, M. Kuroda, Y. Tsujita, ML-236A, ML-236B, and ML-236C, new inhibitors of cholesterogenesis produced by *Penicillium citrinum*, J. Antibiot. 29 (1976) 1346–1348.
[7] A. Endo, K. Tanzawa, M. Kuroda, Competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase by ML-236A and ML-236B, fungal metabolites, having hypocholesterolemic activity, FEBS Lett. 72 (1976) 323–326.
[8] A. Yamamoto, H. Sudo, A. Endo, Therapeutic effects of ML-236B in primary hypercholesterolemia, Atherosclerosis 35 (1980) 259–266.
[9] Abstract Book of the 7th International Symposium on Drugs Affecting Lipid Metabolism, The Lorenzini Foundation, Milan, 1980 (May 28–31).
[10] A. Endo, Novel physiologically active substance monacolin K and process for producing the same, Japanese Patent Application 54-17856, filed on February 20, 1979.
[11] A. Endo, Novel physiologically active substance monacolin J and process for producing the same, Japanese Patent Application 54-44249, filed on April 13, 1979.
[12] A. Endo, Novel physiologically active substance monacolin L and process for producing the same, Japanese Patent Application 54-133991, filed on October 17, 1979.
[13] Merck and Co. (Rahway, NJ), The scientific search for an effective weapon against cholesterol, Press Release, November, 1987.
[14] A. Endo, K. Monacolin, A new hypocholesterolemic agent produced by a *Monascus* species, J. Antibiot. 32 (1979) 852–854.
[15] R. Vagelos, Are prescription drug prices high? Science 252 (1991) 1080–1084.

[16] R. Monaghan, A. Alberts, C. Hoffman, G. Albers-Schonberg, Hypocholesterolemic fermentation products and process of preparation, US Patent No. 4, 231, 938, 11/1980(549/292), filed on June 15, 1979.
[17] A. Endo, Discovery and development of the statins, in: A. Gaw, C. Packard, J. Shephered (Eds.), Statins The HMG-CoA Reductase Inhibitors in Perspective, Martin Dunitz, London, 2000, pp. 35–47.
[18] A. Endo, The story of HMG-CoA reductase inhibitor: 1. Details of the discovery of lovastatin (monacolin K), Igaku No Ayumi 172 (1995) 131–133.
[19] D. Steinberg, A. Gotto Jr., Preventing coronary artery disase by lowering cholesterol levels, JAMA 282 (1999) 2043–2050.