## Development of pravastatin and its characteristics

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### 1. History of the development of pravastatin

Coronary heart diseases (CHD) are one of the major causes of death in not only Western countries but also other industrialized countries. Among CHD, ischemic heart diseases (IHD) lead to the highest rate of death. It is well known that the three major risk factors of IHD are hypercholesterolemia, hypertension and smoking (1). Among them, hypercholesterolemia has long been considered to be the most important risk factor. In order to reduce the risk associated with high plasma cholesterol levels, the development of several hypolipidemic drugs and therapies have been explored in many countries.
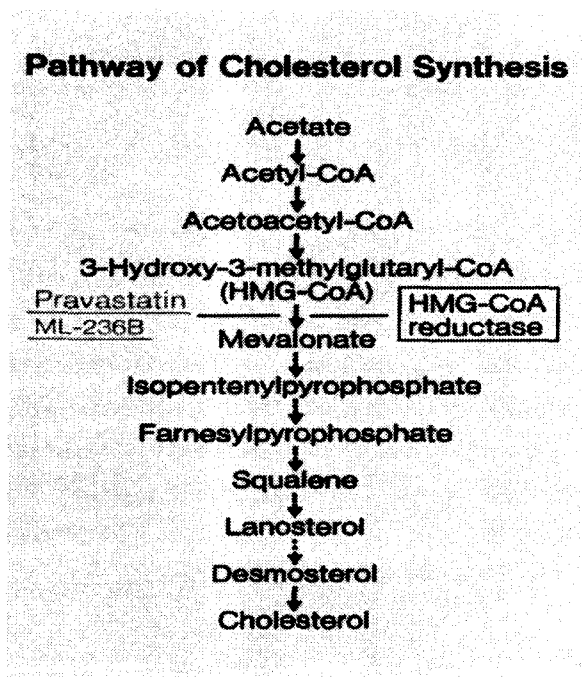
Fig.1



How to reduce body cholesterol
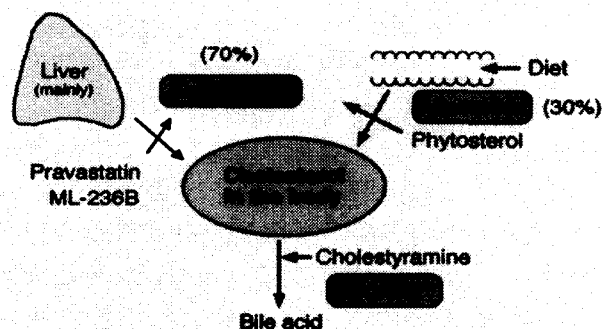
As shown in Fig. 1, the body cholesterol is supplied by absorption from diet (0.3-0.5 g/day in human) and biosynthesis (1.0-1.2 g/day) and is excreted mainly as bile acids into feces (0.8-1.3 g/day). In order to reduce body cholesterol, three major strategies can be considered: (i) inhibition of cholesterol absorption by a compound such as $\beta$-sitosterol, a plant sterol, (ii) inhibition of bile acids reabsorption by a compound such as cholestyramine, anion exchange resin, and (iii) inhibition of cholesterol biosynthesis. Since more than 70% of the total input of body cholesterol in humans is thought to be derived from *de nove* synthesis(2), it is expected that plasma cholesterol levels could be reduced by inhibition of cholesterol biosynthesis.

Cholesterol is biosynthesized from acetyl-coenzyme A (Co-A) in a process which includes more than twenty steps (Fig. 2).
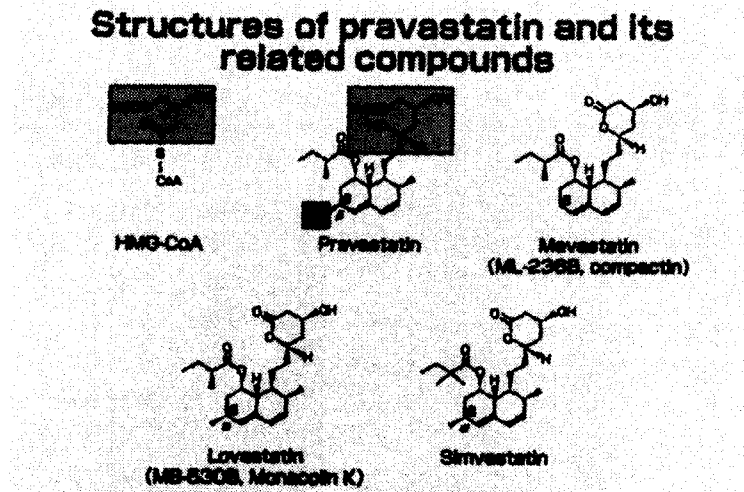
Fig.2



Pathway of Cholesterol Synthesis

Acetate
↓
Acetyl-CoA
↓
Acetoacetyl-CoA
↓
3-Hydroxy-3-methylglutaryl-CoA
Pravastatin      (HMG-CoA)      [HMG-CoA reductase]
ML-236B          ↓
Mevalonate
↓
Isopentenylpyrophosphate
↓
Farnesylpyrophosphate
↓
Squalene
↓
Lanosterol
↓
Desmosterol
↓
Cholesterol

The rate limiting enzyme of this pathway is 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase which catalyzes the reduction of HMG-CoA to mevalonate. In general, later steps of biosynthesis, those close to the target substances are the most suitable sites of inhibition, because the production of other substances is minimally disturbed. Hydrophobic substances such as cholesterol have shown to be an exception to this idea due to the accumlation of hydrophobic intermediates. Attempt to reduce plasma cholesterol levels by inhibiting cholesterol synthesis were first reported in 1958. Two drugs, triparanol (3) and AY-9944 (4), inhibited the last step of cholesterol synthesis which is catalyzed by desmosterol reductase. Desmosterol, a sterol intermediate, was found to accumulate, causing serious adverse effects which resulted in cessation of the use of these drugs.

In 1971, we started screening for inhibitors of cholesterol biosynthesis from the culture broth of microorganisms using a cell-free enzyme system from rat liver. After screening 6,000 strains of microorganims, ML-236B (mevastatin, compactin, Fig. 3) was discovered in the culture broth of *Penicillium citrinum* in 1973 (5). It is noteworthy that compactin, a compound identical to ML-236B, was independently isolated by Beecham Pharmaceuticals as a weak antifungal antibiotic two years after Sankyo's patent filing.

Fig.3

## Structures of pravastatin and its related compounds



HMG-CoA          Pravastatin          Mevastatin
                                       (ML-236B, compactin)

Lovastatin                           Simvastatin
(MB-530B, Monacolin K)

As shown in Fig. 3, a portion of the ML-236B structure resembles that of HMG, the part of HMG-CoA that serves as the substrate of HMG-CoA reductase. Accordingly, ML-236B and the related compounds shown in Fig. 3 inhibit the enzyme in a competitive manner with respect to HMG-CoA. Despite having a marked inhibitory activity on cholesterol synthesis *in vitro* and *in vivo*, ML-236B did not show hypocholesterolemic activity in rats and mice (Fig. 4), commonly used in the initial

Fig.4

### Effect of ML-236B on serum lipids in rats and mice
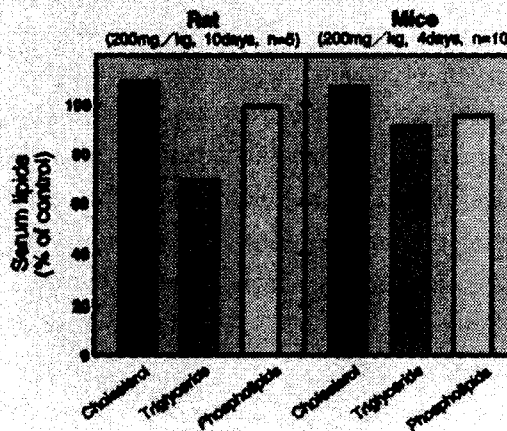


stage of evaluation of efficacy in animals. After many attempts for the experiments using rats and mice, we had a chance for administration of ML-26B to hens. Surprisingly, ML-236B markedly reduced serum cholesterol levels in hens as shown Fig. 5. Almost three years had passed before we found that ML-236B showed strict species specificity in its efficacy.

Fig.5

**Effect of ML-236B sodium
salt on serum lipids in hens**

(Dose : 0.2% in diet)
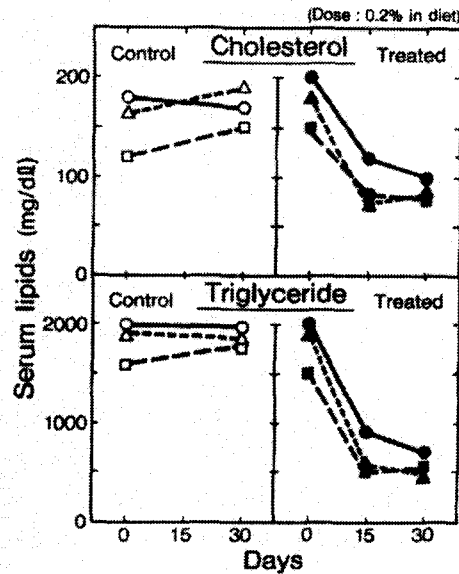


It is well known that the liver and intestine are the major organs involved in *de novo* cholesterogenesis. We focused on finding a drug having enhanced target-organ directed characteristics, because a target-organ directed inhibitor would be expected to disturb cholesterol metabolism minimally in other organs, including hormone producing ones. After screening microbial products as well as chemically and biologically modified derivatives of ML-236B, pravastatin sodium (hereafter pravastatin) was finally chosen as the candidate for the development.

Pravastatin contains a hydroxyl group at the 6 $\beta$ position of its decaline structure (Fig. 3). This drug was first found as a minor urinary metabolite of ML-236B in dogs in 1979. For the industrial hydroxylation of ML-236B, chemical syntheses had been initially attempted, but the cost made this method unfeasible. For this reason, microbial hydroxylation was chosen for the production of pravastatin. After screening for microorganisms capable of converting ML-236B to pravastatin, *Streptomyces carbophilus* was selected for the second step of the fermentation process(6, 7).

In 1981, pravastatin was chosen for the development as a hypolipidemic drug, and clinical trails were started in 1984. Pravastatin was approved for production in 1989 and launched in the same year as "Mevalotin" in Japan. The drug was licensed to Bristol-Myers Squibb Company and has been developed worldwide.

Pravastatin showed anti-atherosclerotic activity in Watanabe-heritable hyperlipidemic (WHHL) rabbits, an animal model for familial hypercholesterolemia (FH) in man (8-10).

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Pravastatin is currently medicated for more than 4.5 million hyperlipidemic patients in 76 countries. Several large scale clinical trials have been carried out, such as the West of Scotland Coronary Prevention Study (WOSCOPS, 11), the Cholesterol and Recurrent Events trial (CARE, 12), the Long-term Intervention with Pravastatin in Ischemic Disease (LIPID, 13). From the results of these studies, it has been proved that pravastatin has anti-atherosclerotic effect in patients with hyperlipidemia. On the basis of these evidences, the indication of pravastatin for the treatment of atherosclerosis has been added in United States and some European countries.

HMG-CoA reductase inhibitors, statins, including pravastatin, showed several beneficial activities, so-called "pleiotropic effects", other than hypolipidimic effects. Some of these effects thought to contribute for anti-atherosclerotic effect of statins.

2. Tissue selective inhibition of cholesterol synthesis

As shown in Table 1, three statins(pravastatin, lovastatin and simvastatin) demonstrated inhibitory activities on sterol synthesis in freshly-isolated or cultured cells from animals(14) and humans(15).

Table.1  **Comparison of inhibitory activity of HMG-CoA reductase inhibitors on sterol syntgesis in various animal and human cells**

| | Inhibitory activity ( I₅₀ ) | | |
|---|---|---|---|
| | Pravastatin | Lovastatin | Simvastatin |
| **Animal cells** | | | (ng/ml) |
| Freshly-isolated rat hepatocytes | 2.0 | 1.9 | 1.4 |
| Freshly-isolated rat spleen cells | 70 | 1.4 | 2.2 |
| Mouse L cells | 800 | 0.8 | 1.6 |
| **Human cells*** | | | (nM) |
| Freshly-isolated hepatocytes | 2.0 | 4.1 | 8.1 |
| Endothilal cells | 1172 | 2.4 | 5.5 |
| Granulosa cells | 1539 | 27.0 | 16.3 |

\* Data of TNO Institute (The Netherlands )

In freshly-isolated rat hepatocytes, these drugs strongly inhibited sterol synthesis and the concentrations required for 50% inhibition of cholesterol synthesis, $I_{50}$, were similar among them. On the other hand, in the cells from the extra-hepatic tissues, freshly-isolated rat spleen cells and mouse L cells, the inhibitory activities of lovastatin and simvastatin were as potent as in rat hepatocytes. In contrast, the inhibitory activity of pravastatin was much less potent than that in rat hepatocytes. Almost the same results were obtained from human cells. Using radio-labeled drugs, pravastatin incorporated into hepatocytes as same as other statins, but only weakly incorporated
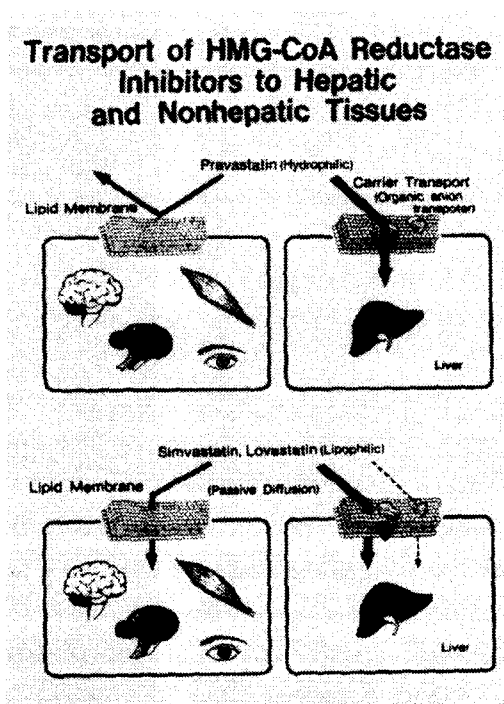
into the cells from extra-hepatic tissues. In contrast, lovastatin and simvastatin incorporated into the cells from extra-hepatic tissues as almost the same degree as hepatocytes(14).

In the animal studies using rats and mice, pravastatin strongly inhibited the sterol synthesis in liver and intestine, the major sites of cholesterogenesis, but only weakly inhibited that in extra-hepatic tissues including hormone producing ones. Regarding lovastatin and simvastatin, these statins inhibited the sterol synthesis not only liver and intestine but also other tissues(14, 16, 17). These results are consistent with those obtained in the cellular experiments.

The mechanism of tissue selectivity of pravastatin is attributed its hydrophilic property. In general, the cytoplasmic membrane is permeable to hydrophobic compounds but impermeable to hydrophilic ones. Partition coefficient of lovastatin and simvastatin (both of them are used open acid type) were 32.4 and 75.8, respectively, whereas that of pravastatin was 0.34(18). In the case of liver, pravastatin was incorporated via organic anion transporter.

The mechanism of cellular uptake of statins is summarized in Fig. 6. Incorporation of pravastatin into the cells from extra-hepatic tissues is virtually nonexistent due to the hydrophilic nature of this drug, but the drug is actively transported into hepatocytes.

Fig.6



Transport of HMG-CoA Reductase
Inhibitors to Hepatic
and Nonhepatic Tissues

Hydrophobic lovastatin and simvastatin, on the other hand, are incorporated not only hepatocytes but also the cells from extra-hepatic tissues mainly by passive diffusion mechanism.

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3. Hypolipidemic activity of pravastatin

Pravastatin decreased serum cholesterol levels dose dependently in beagle dogs, cynomolgus monkeys, Japanese white rabbits and WHHL rabbits at the doses of 0.625-50 mg/kg/day(14). It should be emphasized that pravastatin exhibited a cholesterol-lowering effect in LDL-receptor defective animals, WHHL rabbits. Atherogenic lipoprotein cholesterol such as VLDL-, IDL- and LDL-cholesterol in these animals tested except rats, were preferentially reduced by pravastatin treatment.
On the other hand, pravastatin, like ML-236B, showed no hypocholesterolemic effect in rats, even at high dose, 500 mg/kg/day(14). The mechnism of lack of hypolimidemic activity of ML-236B and pravastatin in rats has been extensively studied (19-21 ).

4. Clinical study

Clinical studies of pravastatin had been conducted by the late Yuichiro Goto, professor emeritus Tokai University, School of Medicine. The clinical dosage of pravastatin is up to 20 mg/day in Japan and 40 mg/day in other countries. According to the 8-years consecutive treatment of pravastatin at the doses of 10-20 mg/day in FH (hetero zygote)(22) and non-FH patients(23), LDL-cholesterol levels were maintained about 20% lower levels than that of pretreatment levels throughout of the study period . Concerning the side effect of pravastatin, the incidence of side effects was only 2.93% among 11,224 patients. Gastric discomfort and rash were mainly noted but no serious adverse effect was reported. The major abnormal values were increases of transaminases, $\gamma$ -GTP and Creatine kinase, but they were mild, transient and not serious in most cases.

5. Anti-atherosclerotic effect of pravastatin

The ultimate purpose of hypolipidemic drugs is to prevent the progression or regression of atherosclerosis. Firstly, we examined whether pravastatin showed anti-atherosclerotic activity in WHHL rabbits.
1) WHHL rabbits
Ex-professor Watanabe, Kobe University, School of Medicine, discovered WHHL rabbit as an animal model for FH in 1973(24). As a consequence of extremely hyperlipidemia from bath, all of the rabbits onset atherosclerosis at 5-month-old.

We tried whether the progression of aortic or coronary atherosclerosis could be suppressed by reduction of serum cholesterol levels using pravastatin in WHHL rabbits. Therefore, we carried out three lines of experiment: the first, pravastatin (50 mg/kg/day) was administered to young WHHL rabbits with minimum atherosclerosis in their arteries for 6 months. Pravastatin demonstrated suppression of progression of coronary atherosclerosis and xanthomas in digital joints by maintaining approximately 25% reduction of serum cholesterol levels(8).

The second, pravastatin (50 mg/kg/day) was administered in combination with cholestyramine (2% in diet), a bile acid sequestrant, to mature WHHL rabbits bearing established atherosclerosis(9). Serum cholesterol levels in the treated group were reduced approximately 60% lower than those in the placebo group at 1 month after initiation of the drug treatment and the low levels were maintained throughout of the experimental period, 8 months. As the result, the progression of aortic and coronary atherosclerosis was significantly suppressed, and histopathological findings supported this observation.

The third, we tried administration of pravastatin (50 mg/kg/day) alone to mature WHHL rabbits for 12 months (10). LDL-cholesterol levels were reduced approximately 22% lower than those in the placebo group at 1 month after initiation of the drug treatment and the levels were maintained throughout of the experimental period. Data for atherosclerosis indicated a significant decrease in percent of surface lesion area (26% reduction) and in intimal thickening (30% reduction) in the abdominal aorta, as well as in coronary stenosis (29% reduction). As shown Fig. 7, data for lesional composition indicated a significant decrease in the percent area of macrophage plus extracellular lipid deposits in aortic lesion (32% reduction) and coronary lesions (45% reduction). Moreover, a significant increase was observed in the percent area of

Fig.7



Percent of lesional components in atherosclerotic lesion of mature WHHL rabbits treated with pravastatin
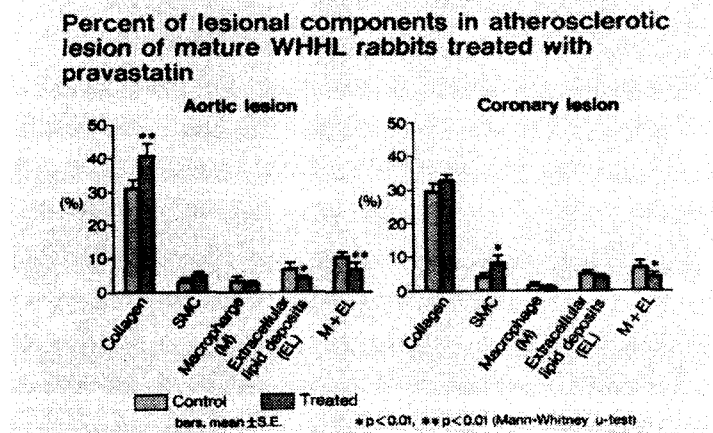
collagen in aortic lesions and that of smooth muscle cells in coronary lesions. It is considered that the increase of collagen fiber and smooth muscle cells, and the decrease of macrophage plus extracellular lipids deposit may have positive effects in stabilization of plaques to avoid rupture of them followed by thrombosis. These results suggest that

pravastatin stabilizes the plaques in WHHL rabbits.

2) Clinical study

Anti-atherosclerotic effects of pravastatin and other statins have been extensively carried out in patients with hyperlipidemia. Table 2 shows comparison of secondary prevention studies with various statins.

Table.2

## Secondary prevention studies with various HMG-CoA reductase inhibitors

| Study | Drug (mg/day) | n | Term (Year) | TC reduction (%) | Suppression of C. events (%) | Change in min. dia. of coronary (mm) | |
|---|---|---|---|---|---|---|---|
| PLAC-I | Pravastatin (40) | 408 | 3 | 18 | 61* | C T DM/Y | 0.05 0.03 0.02 |
| REGRESS | Pravastatin (40) | 885 | 2 | 20 | 39** | C T DM/2Y | 0.09 0.03 0.06 |
| MARS | Lovastatin (80) | 270 | 2 | 32 | 28 | C T DM/2Y | 0.06 0.03 0.03 |
| MAAS | Simvastatin (20) | 381 | 4 | 23 | 25 | C T DM/4Y | 0.13 0.04 0.08 |

* P<0.02, ** P<0.002, C. Control ; T. Treated

PLAC-I and REGRESS are pravastatin's studies, MARS is lovastatin's study and MAAS is simvastatin's study. Although the reduction of plasma total cholesterol levels by lovastatin and simvastatin were higher than that by pravastatin, the suppression of coronary events by pravastatin was much higher than that by lovastatin and simvastatin. One of the possible explanations of these results is that pravastatin has stronger plaque stabilization activity than lovastatin and simvastatin.

The large scale clinical trials of pravastatin for anti-atherosclerotic effect have been done, such as WOSPOPS(11), CARE(12) and LIPID(13). Among them, WOSCOPS was the first primary prevention trial for atherosclerosis of statins. The study was carried out in the west of Scotland using more than 6.5 thousand hyperlipidemic patients without CHD. Pravastatin was administered at the dose of 40 mg/day for about 5 years. Reduction of plasma cholesterol and LDL cholesterol were 20% and 26%, respectively. As shown Fig. 8, nonfatal myocardial infarcion (MI) and CHD death was significantly reduced by 31% treated with pravastatin as compared to that of the placebo group.

Fig.8



Nonfatal MI or CHD Death
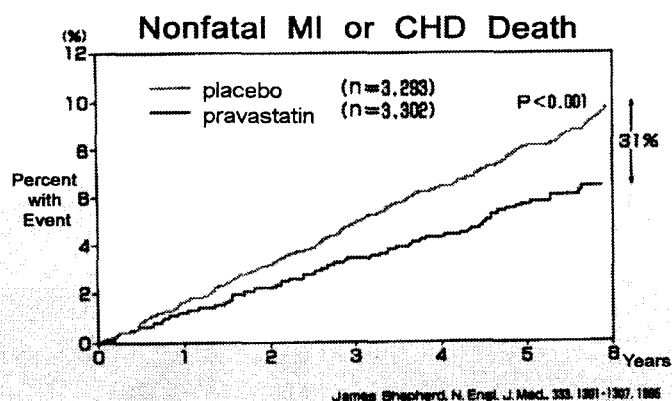
On the other hand, there was no difference on non-cardiovascular death, such as caused by other diseases, suicide or accident, between the pravastatin group and the placebo group. These data suggest that pravastatin can prevent coronary events on the patients who have no history of MI or angina pectoris. Base on these evidences, the risk reduction of recurrent MI and the risk reduction of undergoing revascularization procedures have been added to the indication of pravastatin in United States and some European countries.

6. Chemically synthesized statins and inhibitors acting other steps of cholesterol synthesis

In contrast to pravastatin, lovastatin and simvastatin, which are natural products or some modifications of them, chemically synthesized statins have been widely developed. The structures of chemically synthesized statins that have already launched are shown in Fig. 9. Rosuvastatin have not been launched yet in Japan.

Chemically synthesized HMG-CoA reductase inhibitors

Fig.9



Fluvastatin (Novartis)

Atorvastatin (Pfizer)

Pitavastatin (Kowa)

Rosuvastatin (AstraZeneca)
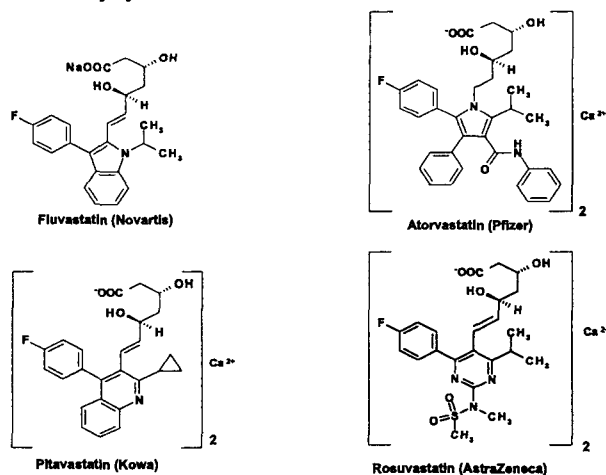
The most of them exhibit more potent inhibitory activity of HMG-CoA reductase as compare to naturally available ones. However, the most important matter in these types of drug is safety. The daily dose of pravastatin is 10 to 40 mg, which is already very small amount.

At the beginning of our research, it was reported that HMG-CoA reductase was only enzyme that received the feedback regulation by cholesterol. Later, it has been found that several enzymes also received the feedback regulation by cholesterol, such as HMG-CoA synthase, squalene synthase and squalene epoxidase (Fig. 10). Attempt to find inhibitors for these enzymes have been carried out, but none of them succeeded to become hypolipidemic drug at this time.

Fig.10



Pathway of cholesterol synthesis
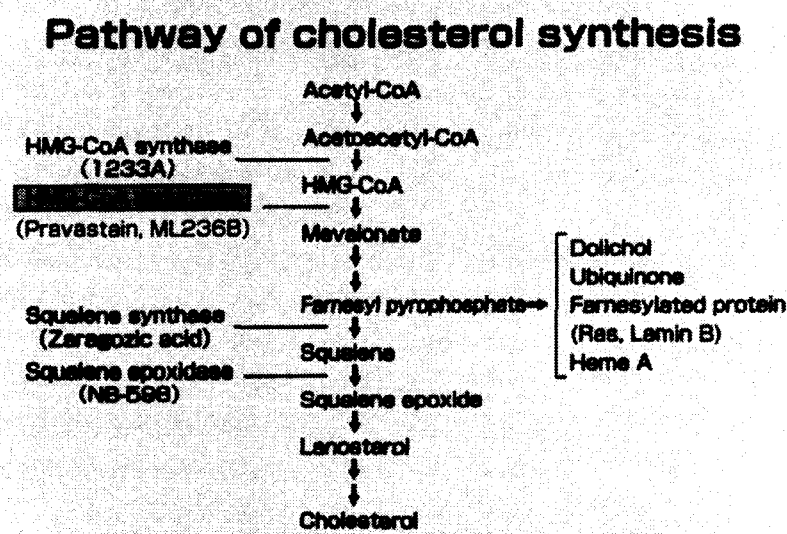
Among them, squalene synthase inhibitor, zaragozic acid(25) had quitted to develop by acidosis in animal experiment. Although some other squalene synthase inhibitors are still now under development, statins might be used continuously as the hypolipidemic drug with the mechanism of inhibition of cholesterol synthesis even in future.

7. Pleiotropic effect of statins

Besides cholesterol lowering effect of statins, it has been reported that statins show many beneficial effects containing stabilizing plaque(26), stabilizing endothelial cells functions(27), anti-thrombosis(28), anti-diabetes(29), anti-alzheimer disease(30) and so on as shown in Fig. 11. Most of these effects are prevented by addition of mevalonate suggesting that the effects are mediated by the inhibition of mevalonate synthesis.

Fig.11                 **P le lotropic effect of statins**
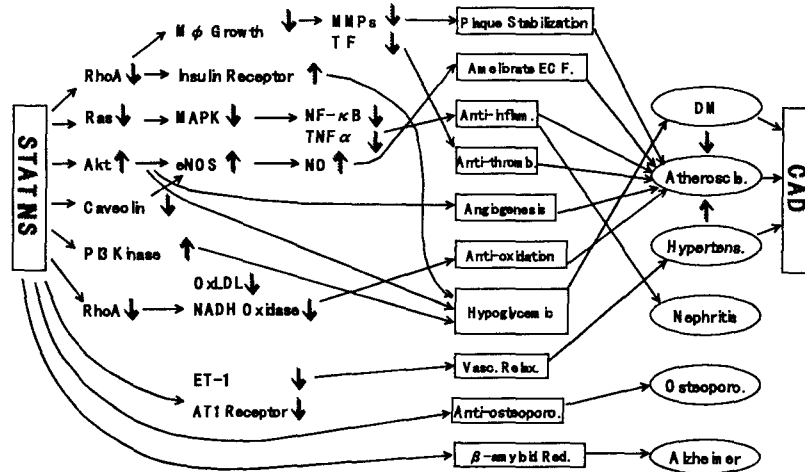


8. Summary

ML-236B is the first statin and pravastatin was found at first as urinary metabolite of ML-236B in dogs. Eighteen years had passed for the development of pravastatin. Pravastatin showed tissue-selective inhibition of cholesterol synthesis; the drug strongly inhibited the sterol synthesis in liver and intestine, the major organs of cholesterogenesis, but only weakly inhibited in other tissues including hormone producing ones. This property is sought to be beneficial for safety of the drug because of lesser effect on cholesterol synthesis in hormone producing tissues. Pravastatin preferentially reduced atherogenic lipoprotein cholesterol in several animal species and humans except rodents. Pravastatin demonstrated anti-atherosclerotic activity not only WHHL rabbits but also hyperlipidemic patients with lesser side effects. In addition, pravastatin showed several pleiotropic effects including stabilization of plaque, anti-diabetic effect and so on.

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