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MINI-REVIEW

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Biosynthesis and biotechnological production of statins by filamentous fungi and application of these cholesterol-lowering drugs

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Abstract Hypercholesterolemia is considered an important risk factor in coronary artery disease. Thus the possibility of controlling de novo synthesis of endogenous cholesterol, which is nearly two-thirds of total body cholesterol, represents an effective way of lowering plasma cholesterol levels. Statins, fungal secondary metabolites, selectively inhibit hydroxymethyl glutaryl-coenzyme A (HMG-CoA) reductase, the first enzyme in cholesterol biosynthesis. The mechanism involved in controlling plasma cholesterol levels is the reversible inhibition of HMG-CoA reductase by statins, related to the structural similarity of the acid form of the statins to HMG-CoA, the natural substrate of the enzymatic reaction. Currently there are five statins in clinical use. Lovastatin and pravastatin (mevastatin derived) are natural statins of fungal origin, while symvastatin is a semi-synthetic lovastatin derivative. Atorvastatin and fluvastatin are fully synthetic statins, derived from mevalonate and pyridine, respectively. In addition to the principal natural statins, several related compounds, monacolins and dihydromonacolins, isolated fungal intermediate metabolites, have also been characterized. All natural statins possess a common polyketide portion, a hydroxy-hexahydro naphthalene ring system, to which different side chains are linked. The biosynthetic pathway involved in statin production, starting from acetate units linked to each other in head-to-tail fashion to form polyketide chains, has been elucidated by both early biogenetic investigations and recent advances in gene studies. Natural statins can be obtained from different genera and species of filamentous fungi. Lovastatin is mainly produced by *Aspergillus terreus* strains, and mevastatin by *Penicillium citrinum*. Pravastatin can be obtained by the biotransformation of mevastatin by *Streptomyces carbophilus* and simvastatin by a semi-synthetic process, involving the chemical modification of the lovastatin side chain. The hypocholesterolemic effect of statins lies in the reduction of the very low-density lipoproteins (VLDL) and LDL involved in the translocation of cholesterol, and in the increase in the high-density lipoproteins (HDL), with a subsequent reduction of the LDL- to HDL-cholesterol ratio, the best predictor of atherogenic risk. The use of statins can lead to a reduction in coronary events related to hypercholesterolemia, but the relationship between benefit and risk, and any possible interaction with other drugs, must be taken into account.

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Introduction

In western countries coronary artery disease and progression of atherosclerotic lesions, related to the primary risk factor of hypercholesterolemia, represent the most-important causes of death. In subjects with a regular lipid metabolism, only one-third of the total body cholesterol is diet derived, two-thirds being synthesized directly from intracellular precursors by various organs of the body (Alberts et al. 1980; Demain 1999; Furberg 1999). For this reason the control of cholesterogenesis by inhibiting its biosynthesis is an important means of lowering plasma cholesterol levels. Cholesterol, a steroid molecule, is an essential component of cell membranes and represents the substrate for the biosynthesis of bile acids essential to adsorption from the intestine of fats and fat-soluble vitamins (Alberts 1988). Cholesterol is also involved in steroid hormone biosynthesis and is required for the production, in hepatocytes, of very low-density lipoproteins (VLDL), which are responsible for the transport of fats to peripheral tissues, for subsequent metabolism or storage (Alberts 1988; Alberts et al. 1980; Tobert 1987).

The cholesterol biosynthetic pathway, starting from acetyl-CoA units, involves more than 25 enzymes, but the rate-limiting step is the conversion of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate

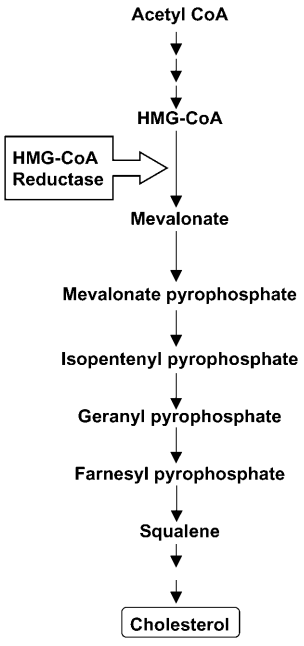

Fig. 1 Cholesterol biosynthetic pathway

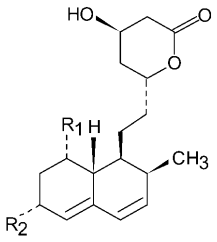

Fig. 2 Base structure of statins (naphthalene ring and β-hydroxylactone)

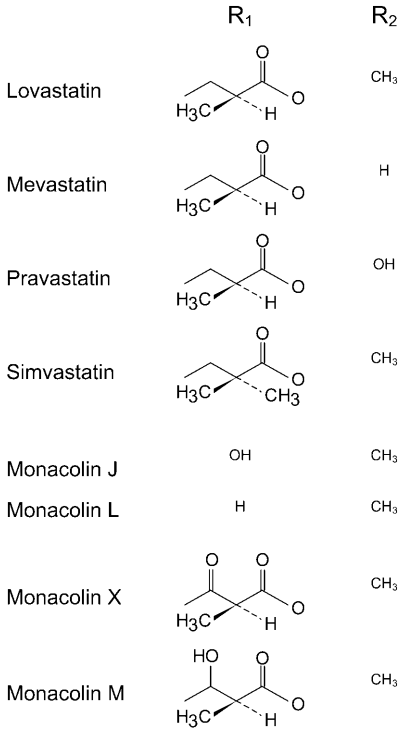

Fig. 3 Statin side chains linked at C8 (R_1) and C6 (R_2) of the base structure

by HMG-CoA reductase (EC. 1.1.1.34) (Fig. 1). Early studies on cholesterol synthesis inhibitors focused on the late stages of the pathway. Triparanol, one of the developed compounds that blocks the penultimate biosynthetic step, produces sterol accumulation in tissues with serious side effects (Kirby 1967; Steinberg et al. 1961).

Squalene synthase is the first pathway-specific enzyme and catalyzes head-to-head condensation of two molecules of farnesyl pyrophosphate (FPP) to form squalene. The early FPP analogues, the major class of inhibitors of squalene synthase, lack specificity and can potentially interfere with other FPP-consuming transferases. To increase enzyme specificity, FPP analogues and other mechanism-based enzyme inhibitors have been synthesized, namely BMS-188494, a potent synthase inhibitor that is effective as an anticholesterol drug (Biller and Magnin 1995; Lawrence et al. 1995; Patel 2000).

However, the inhibition of HMG-CoA reductase, at an earlier stage of cholesterol biosynthesis, results in accumulation of HMG-CoA, which can be metabolized to simpler compounds, without any build up of lipophilic intermediates with a sterol ring. Statins are a class of molecules with a polyketide structure, obtainable by secondary fungal metabolism, which can inhibit HMG-CoA reductase activity. Thus the mechanism involved in the control of endogenous cholesterol levels by statins makes these molecules suitable for therapeutic use (Alberts 1988; Alberts et al. 1980; Chong et al. 2001; Endo 1985a; Farnier and Davignon 1998; Furberg 1999; Maron et al. 2000; Stein et al. 1998; Tobert 1987).

Structure

Different types of statins are currently available: the natural statins (lovastatin and pravastatin), obtained directly by fermentation, and the semi-synthetic (simvastatin) and synthetic statins (atorvastatin and fluvastatin). Cerivastatin, a fully synthesized statin approved in the United States in 1997, has been employed until the recent withdrawal from the market (FDA Talk Paper 2001).

Natural statins are of very similar chemical structure. They possess a common main polyketide portion, a hydroxy-hexahydro naphthalene ring system (Fig. 2), to which different side chains are linked at C8 (R_1) and C6 (R_2) (Fig. 3). Lovastatin (or mevinolin, monacolin K, and Mevacor, Merck) contains a methylbutyric side chain (R_1) and a 6-α methyl group (R_2), which is lacking in mevastatin (or compactin, ML-236B, and CS-500). Pravastatin (or eptastatin and Pravachol, Bristol-Myers Squibb/Sankyo) has the β-hydroxylactone in the 6-hydroxy sodium salt form and is the C6-hydroxy analogue of mevastatin. Simvastatin (or Synvinolin and Zocor, Merck) contains an additional methyl group in the 2' position of the side chain.

In addition to these most-important compounds, several lovastatin- or mevastatin-related metabolites have

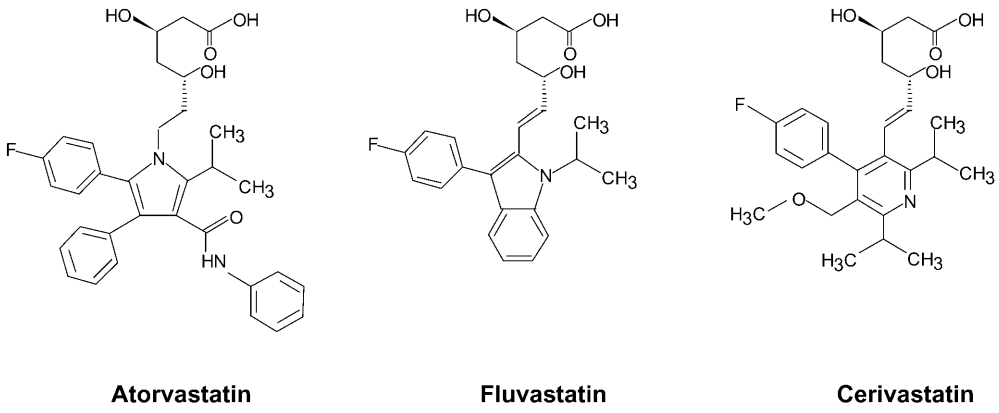

Fig. 4 Chemical structures of the synthetic statins atorvastatin, fluvastatin, and cerivastatin

been isolated and characterized. Monacolins X and M have a different composition at the C8 side chain. Monacolins J and L lack the lovastatin methylbutyric side chain. In monacolin J a hydroxyl group is present in the C8 position, while it is substituted by a hydrogen in monacolin L and dihydromonacolin L (Fig. 3) (Endo and Hasumi 1985; Endo et al. 1985a, 1986a; Juzlová et al. 1996; Kimura et al. 1990; Komagata et al. 1989).

The structures of the synthetic statins atorvastatin (Lipitor, Parke-Davis), fluvastatin (Lescol, Novartis), and cerivastatin (Baycol and Lipobay, Bayer) are dissimilar, and quite different from the natural statins (Fig. 4). Only the HMG CoA-like moiety, responsible for HMG-CoA reductase inhibition, is common to both natural and synthetic statins. Unlike lovastatin and simvastatin, synthetic statins are obtained in hydroxy acid form. Fluvastatin (Levy et al. 1993), derived from mevalolactone, was the first entirely synthetic statin available, while atorvastatin (Bakker-Arkema et al. 1996) and cerivastatin, pyridine derivatives, are a new generation of highly purified statins.

Biogenesis and biosynthesis

Early biogenetic investigations of statins carried out on ^{14}C-labelled monacolin J and L, employing a strain of *Monascus ruber*, suggested that these compounds are precursors of lovastatin and, consequently, can be classified as isolated intermediate metabolites in the lovastatin biosynthetic pathway (Endo et al. 1985b). Studies on the pathway involved in monacolin synthesis have demonstrated that monacolin L is the precursor of monacolin J. In fact in the hydroxylation reaction $^{18}O_2$ was incorporated into monacolin J through the action of a monooxygenase system involving cytochrome P-450, present in the cell-free extract of *M. ruber* (Komagata et al. 1989). Subsequent experiments again employing the cell-free extract of *M. ruber* and living cells of *Paecilomyces viridis* have demonstrated the transformation of monacolin J to lovastatin (Kimura et al. 1990). Moreover, a combination of physical techniques indicates monacolin M derivation from monacolin J, via a pathway that is quite distinct from that for the synthesis of lovastatin, the α-methylbutyryl ester of monacolin J (Endo et al. 1986a).

From an overview of the early biogenetic studies carried out on the monacolins, it has been possible to demonstrate that monacolin L is the first to be synthesized from nine molecules of acetate and is, in turn, converted to monacolin J by hydroxylation; monacolin K is then derived from monacolin J. The monacolin X, i.e., the α-methyl-β-ketobutyryl ester of monacolin J, is converted to lovastatin, while it is accumulated in cultures of mutant strains producing no detectable amounts of lovastatin (Endo et al. 1986a).

Earlier investigation of the biogenesis of lovastatin, carried out mainly in *Aspergillus terreus* strains employing labelled precursors (Chan et al. 1983; Greenspan and Yudrovitz 1985; Moore et al. 1985; Shiao and Don 1987), indicated that the lovastatin biosynthetic pathway starts from acetate units (4- and 8-carbons long) linked to each other in head-to-tail fashion to form two polyketide chains (Fig. 5). The methyl group present in some statins in the side chain or at C6 derives from methionine, as frequently occurs in fungal metabolism, and is inserted in the structure before the closure of the rings (Shiao and Don 1987). This mechanism demonstrates that mevastatin, which lacks the 6α-methyl group at C6, is not an intermediate in lovastatin biosynthesis. The main chain is then cyclized and in some statins esterified by a side chain at C8. The oxygen atoms present in the main chain are inserted later by aerobic oxidation using a deoxygenated precursor (Alberts et al. 1980; Greenspan and Yudrovitz 1985; Moore et al. 1985). Studies on the ^{13}C incorporation in lovastatin and mevastatin carried out with *Penicillium citrinum* and *M. ruber* strains indicated a similar pathway; enzymatic hydroxylation and subsequent esterification at C8 was also observed (Endo 1985b).

More-recent investigations have studied enzymatic kinetics together with gene regulation and expression involved in *A. terreus* statin biosynthesis. Earlier genetic research investigated the mechanisms involved in lovastatin biosynthesis, particularly with regard to the two polyketide chains. The results, including the characterization of *A. terreus* lovastatin-blocked mutants, showed that the multifunctional polyketide synthase system

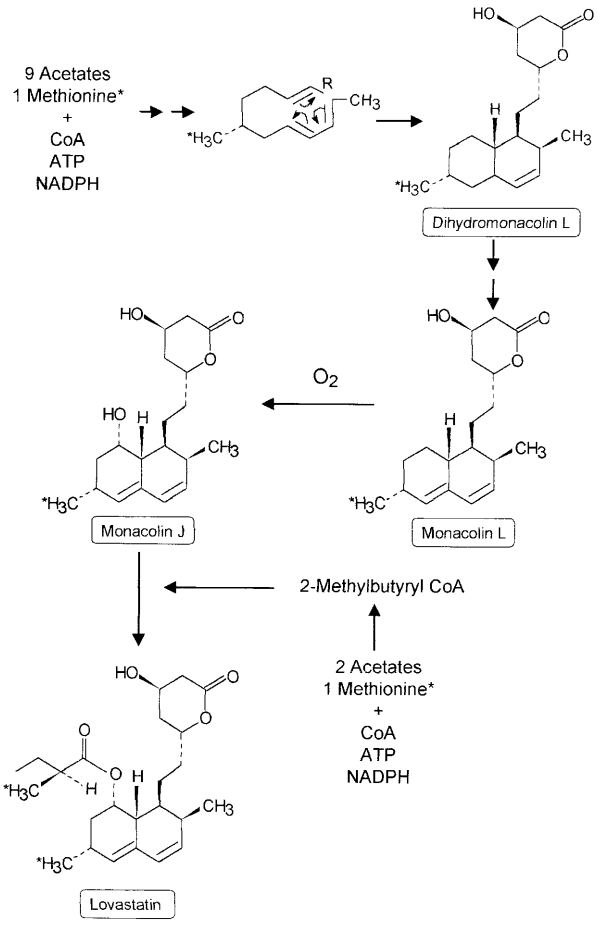

Fig. 5 Lovastatin biosynthetic pathway

(PKSs) comprises a lovastatin nonaketide synthase (LNKS) involved in the cyclization of the main polyketide chain, to form the hexahydro naphthalene ring system, and a diketide synthase (LDKS) involved in the transfer of the methylbutyryl side chain to monacolin J. Study of the primary structure of the PKS that forms the lovastatin nonaketide provided new details of lovastatin biosynthesis (Hendrickson et al. 1999). The characterization of the LNKS gene was of fundamental importance for understanding how the carbon skeletons of dihydromonacolin L and lovastatin are assembled. Other aspects of the biosynthesis of lovastatin related to PKSs have been investigated. The LNKS, product of *lovB* gene, interacts with *lovC* (a putative enoyl reductase), to catalyze the reactions in the first part of the biosynthetic pathway, leading to dihydromonacolin L (Fig. 6). In the final step of the lovastatin pathway, the LDKS, made by *lovF*, interacts with *lovD* (transesterase enzyme) that catalyzes the attachment of the 2-methylbutyric acid to monacolin J, derived from monacolin L. Key features of genes encoding these enzymes and regulatory factors in lovastatin production in *A. terreus* have been elucidated (Hutchinson et al. 2000; Kennedy et al. 1999). An intramolecular Diels-Alder *endo* closure of the hexaketide, to form a bicyclic system, with the same ring stereochemistry as dihydromonacolin L, catalyzed by LNKS purified from *A. nidulans* was recently demonstrated (Auclair et al. 2000). Finally in a strain of *A. terreus*, in which the *lovC* gene has been disrupted, the post-PKS (post-polyketide synthase) steps involved in the biosynthesis of lovastatin were investigated. The results demonstrated that the role of the *lovC* protein is to ensure correct assembly of the nonaketide chain in lovastatin by the *lovB* protein. In contrast, the construction of the methylbutyrate side chain by the LDKS (*lovF* protein) does not require *lovC* protein. The study also demonstrated that the *lovC* protein has no detectable function in post-PKS processing of dihydromonacolin L (Auclair et al. 2001).

In conclusion, the recent advances in gene cloning have allowed the identification of most of the enzymes involved in lovastatin biosynthesis and have confirmed the biosynthetic pathways hypothesized in earlier investigations (Sutherland et al. 2001).

Production

The investigations carried out since the 1970s have indicated the possibility of obtaining a wide range of statins as both the final products and intermediates of secondary microbial metabolism, or as products of biotransformation process. Large-scale processes have been developed only for a few of the statins described in the literature. For other molecules research is still ongoing and therefore greatly susceptible to future development. Nevertheless these studies have resulted in a deeper understand-

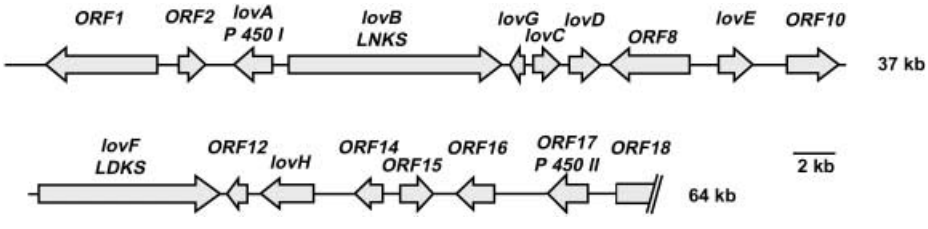

Fig. 6 Lovastatin biosynthesis gene cluster (*lovB* and *lovF* polyketide biosynthesis; *lovC* enoyl reductase; *lovD* transesterase; *lovE* and *lovH* regulatory genes; *ORF2* and *ORF17* cytochrome P-450 genes; *ORF1* and *ORF10* potential resistance genes; *ORF14* and *ORF16* transporter genes; *lovG*, *ORF2*, *ORF12*, *ORF15*, and *ORF18* unknown function) (Auclair et al. 2001; Hutchinson et al. 2000; Kennedy et al. 1999; Sutherland et al. 2001)

ing of the different aspects relative to the mechanisms involved in the production of statins, providing a necessary basis for the development of large-scale processes.

Natural statins are synthesized mainly by strains of *A. terreus* (Alberts et al. 1980; Buckland et al. 1989; Greenspan and Yudrovitz 1985) and by different species of the genus *Monascus* (Endo 1979, 1980; Endo et al. 1985a; Kimura et al. 1990; Komagata et al. 1989; Negishi et al. 1986; Shiao and Don 1987), but also by other genera of filamentous fungi such as *Penicillium* (Endo et al. 1976a, b; Hosobuchi et al. 1993a), *Doratomyces, Eupenicillium, Gymnoascus, Hypomyces, Paecilomyces, Phoma, Trichoderma* (Endo et al. 1986b), and *Pleurotus* (Gunde-Cimerman et al. 1993).

Lovastatin was the first statin to be approved by the United States Food and Drug Administration (1987) and made available on the pharmaceutical market as an anticholesterolemic drug (Tobert 1987). However, mevastatin was the first statin discovered. It was isolated in 1976 from a strain of *Penicillium citrinum* and named ML-236B (Endo et al. 1976a, b, c, 1977) and, at about the same time, during research on antifungal metabolites, from a strain of *Penicillium brevicompactum* and named compactin at the Beecham Laboratories (Brown et al. 1978).

Lovastatin (named mevinolin) was later obtained from a strain isolated from soil and classified as *A. terreus* at the CIBE Laboratories in Madrid (Spain) (Alberts et al. 1980) and from *M. ruber* (named monacolin K) by Endo (1979) (Albers-Shonberg et al. 1981, 1983; Monaghan et al. 1980).

A few years later lovastatin was also obtained from 17 strains of different species of 124 tested strains of the genus *Monascus,* in particular *M. ruber, M. purpureus, M pilosus, M. vitreus, and M. pubigerus* (Negishi et al. 1986). Note that the genus *Monascus,* particularly the species *M. anka* and *M. purpureus,* is traditionally employed in Asian countries as "red koji" for fermented food and beverage production, as well as for red dye. Lovastatin-producing strains are, instead, generally poor producers of red pigments and exhibit an optimum temperature for lovastatin production of around 25°C. Lovastatin productivity failed and no increase in red pigments was observed when the temperature range for koji production (30–37°C) was employed (Juzlová et al. 1996; Negishi et al. 1986).

Studies on the synthesis and characterization of the lovastatin-related compounds indicated that several monacolins were obtainable, mostly from *Monascus* strains. Monacolin J and L were isolated and characterized from cultures of an *M. ruber* strain (Endo et al. 1985a). Again in 1985 Endo reported dihydromonacolin L and monacolin X production and activity from a mutant strain of *M. ruber* (Endo and Hasumi 1985).

Investigations also focused on the development of new statins with improved biological activity, obtainable by the modification of already characterized statins such as lovastatin and mevastatin. After largely unproductive efforts to obtain new molecules by chemical hydroxylation, microbial biotransformation resulted in a more-practical and economical approach (Hirama and Uei 1982; Hsu et al. 1983).

Different active compounds were obtained by the biotransformation of lovastatin or mevastatin added directly to the cultures, and from among the resultant molecules pravastatin was found to be of particular interest (Arai et al. 1988; Endo et al. 1979; Terahara and Tanaka 1982). This new statin, more potent in inhibiting cholesterol synthesis in vitro than its precursor mevastatin, was first evidenced as a minor urinary metabolite during mevastatin experimentation in dogs (Haruyama et al. 1986; Serizawa et al. 1983a). Pravastatin can be obtained by a two-step fermentation process, the first step being the production of mevastatin and the second its biotranformation. The β-hydroxylation in the C6 position of mevastatin can be carried out by micro-organisms belonging to different genera and species of fungi and actinomycetes (Terahara and Tanaka 1982). From among the screened fungi, *Mucor hiemalis* strains proved good producers (conversion ratio 30–90%), but they were sensitive to the mevastatin concentration (substrate concentration 0.05% as lactone form), because of its antifungal activity. Similar conversion ratios were obtained among the bacteria, using different species of the genus *Nocardia,* with tolerance to greater amounts of mevastatin (substrate concentration 0.2% as sodium salt) (Serizawa et al. 1983a, b, c). Another screened actinomycetes, classified as a new species of the genus *Streptomyces* and named *S. carbophilus,* was found to be a potent mevastatin converter by a cytochrome P-450-containing enzyme system (Matsuoka et al. 1989), with only small amounts of by-products. This permitted the successful development of pravastatin production on an industrial scale.

Subsequent investigation resulted in the finding of a new hydrolase system present in a rare actinomycete *Actinomadura,* converting mevastatin to pravastatin (Peng and Demain 1998; Peng et al. 1997; Yashphe et al. 1997). By employing selected strains and suitable substrates, pravastatin was alternatively synthesized by *Aspergillus* and *Monascus,* through its direct accumulation in the cultures as a final fermentation product. The recovered pravastatin was found in the mycelium and the culture filtrate extracts in both strains (Manzoni et al. 1998, 1999).

A series of statins was also obtained by chemical modification of the C8 side chain in the lovastatin molecule and a systematic evaluation of the structure-activity relationships of the obtained compounds was also carried out. One of the obtained molecules, simvastatin, was found to be a semi-synthetic molecule with practical applications.

Through the analysis of the inhibitory effect on HMG-CoA reductase, it was possible to highlight the influence of the obtained structures on biological activity. The stereochemistry of the side chain ester moiety is not important for inhibitory binding to HMG-CoA reductase, as the spatial requirements of the acyl moiety are compatible with compact, branched-chain aliphatic acyl

groups, and additional branching at the α carbon of the acyl moiety increases potency.

From among the natural statins (or derived by a semi-synthetic process) isolated, characterized, and described in the literature, only lovastatin, pravastatin, and simvastatin are available on the market. However, there are other new lipid-lowering agents under development in the class of HMG-CoA reductase inhibitors, such as rosuvastatin (phase 3), pitavastatin (phase 2), NK-104, and itarvastatin (clinical trials) (Brown 2001).

The industrial process for the production of lovastatin was set up in 1980 using an *A. terreus* strain (Mevacor, Merck). The process development involved the analysis of different fermentation parameters such as culture homogeneity, effect of various carbon sources, pH, aeration, and agitator design. Producer strain reisolation together with pH control and slow use of the carbon source, in particular glycerol, yielded a fivefold increase (about 200 U/l, arbitrary units) with respect to the initial lovastatin productivity. Scaling-up of the process from a 800-l to a 19,000-l scale revealed that oxygen transfer, related to high viscosity of the fermentation broth, is a serious limiting factor in lovastatin productivity. This limitation was overcome by setting up a more-efficient impeller with increased hydrodynamic thrust and a reduction of power requirement, 66% of that of the Rushton standard turbine (Buckland et al. 1989).

Recently (Metkinen News February 1997; Metkinen Oy, Finland) the productivity of *A. terreus* ATCC 20542 strain, the original lovastatin producer, was increased by the Metkinen group, to reach 7–8 g/l, using mutagenesis procedures and experience acquired in the development program of process improvement.

Biocon (Biocon India, Bangalore, India) is one of the companies that has obtained United States FDA approval for lovastatin production (January 2001), which goes off patent in June 2001. Biocon is the first Indian company to get FDA approval for fermentation-derived molecules for pharmaceutical use. The company's lovastatin process is based on a proprietary fermentation technology, the Plafractor, a large-scale solid-matrix bioreactor (Suryanarayan and Mazumdar 2001). This new bioreactor has the advantages of solid substrate and submerged fermentation, and allows a reduction of downstream processing problems during product extraction.

Simvastatin (Zocor, Merck), the main semi-synthetic derived from lovastatin, is obtained by protection of the C13 group by preparation of the corresponding silyl ether. After acylation, deprotection is carried out under controlled conditions to avoid degradation of the lactone ring (Hoffman et al. 1986).

Pravastatin, approved for production in 1989, was developed by Sankyo and Squibb and sold worldwide (Pravachol licensed to Bristol-Myers Squibb). The large-scale production of microbial metabolites requires different approaches to attain strain and medium improvement and consequently increase the obtainable yields. The amount of mevastatin produced by the original *P. citrinum* strain was too small for industrial purposes, so a genetic approach involving a selective strategy was developed to set up a proper strain transformation system (Serizawa et al. 1997). Moreover, fermentation parameters, which control the morphology of the fungal pellets, greatly influence productivity, and include the use of a computer system using a fuzzy control. The developed software for fuzzy control is based on analysis of the experience of skilled operators, to control the sugar feed rate, for maintaining optimum pH (from 3.8 to 4.0). Use of fuzzy control resulted in mevastatin being produced at a nearly constant rate for 80 h of fermentation (about 120 U/ml, arbitrary units). Mevastatin production was 10% higher using fuzzy control than using manual control (Hosobuchi et al. 1993a, b, c).

The subsequent biotransformation step employing *S. carbophilus*, with a fully automated computer system control of the mevastatin feed rate, was set up, the conversion to pravastatin being inhibited by a high concentration of mevastatin (10 U/ml, arbitrary units). To overcome this limitation, an on-line monitoring system (cross-flow filtration module, HPLC analyzer, feed pump, and data processor) was set-up to maintain the mevastatin concentration at optimum levels (7.5±0.2 U/ml).

The biochemical and biotechnological approaches adopted to overcome the limitations were applied to the large-scale process. In a fed-batch culture, scaled up from a 30-l to a 6,000-l fermenter, morphology control and computer application increased the volumetric productivity of pravastatin threefold over that obtained with manual mevastatin feed control (Hosobuchi et al. 1993d; Serizawa et al. 1997).

Recently substantial progress has been reported for the pravastatin process. Mevastatin production by *P. citrinum* was increased from the original 40 mg/l to 5 g/l. A new *Streptomyces* mutant strain with resistance to 3 g/l of mevastatin with an 80% conversion yield was obtained by Metkinen (Metkinen News March 2000; Metkinen Oy, Finland).

Mechanism of action

Lovastatin and related compounds, with the exception of pravastatin, are produced as predrugs, being a mixture of the lactone and the β-hydroxyacid form. The lactone ring is converted into the corresponding β-hydroxyacid form in vivo (Alberts et al. 1980).

The mechanism involved in the hypocholesterolemic activity of statins is based on the competitive inhibition of HMG-CoA reductase, due to the structural homology between the β-hydroxyacid form of the statins and the HMG-CoA intermediate formed (Fig. 7). The affinity of the inhibitor (statins) is several times higher with respect to the intermediate. The K_m (Michaelis constant) for the substrate of the HMG-CoA reaction is 4×10^{-6} M, while a K_i (inhibition constant) of 6.4×10^{-10} M has been determined for lovastatin (Alberts 1988; Alberts et al. 1980). Comparative kinetic analysis of HMG-CoA reductase has shown that the methyl group in lovastatin in the 6α-

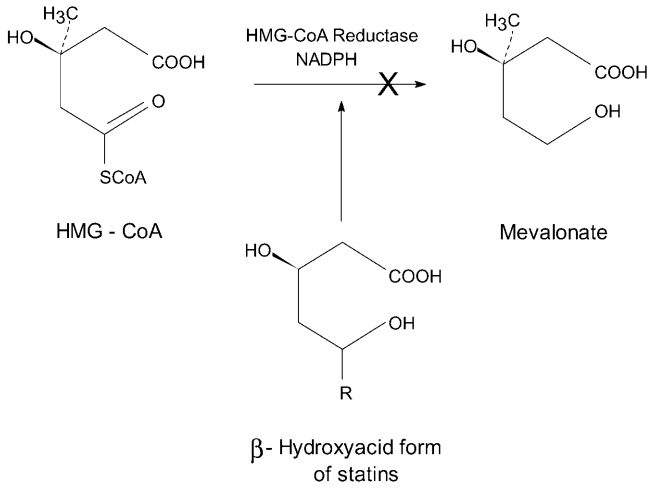

Fig. 7 Structural analogy between HMG-CoA and the β-hydroxy-acid form of statins and mechanism of inhibition

position confers a two- to threefold enhancement of the intrinsic inhibitory activity with respect to mevastatin (Alberts et al. 1980).

The crystal structures of the catalytic portion of human HMG-CoA reductase in complex with some substrates and products (HMG-CoA, HMG, CoA, NADPH) provide a detailed view of the enzyme active site (Istvan and Deisenhofer 2000; Istvan et al. 2000). The structures of the catalytic portion of HMG-CoA reductase complexed with six different statins [mevastatin, simvastatin, fluvastatin, atorvastatin, cerivastatin, (withdrawn in August 2001), and rosuvastatin (in the late stage of clinical development)] were recently described. The statins occupy a portion of the HMG-CoA binding site, thus blocking substrate access to the active site of the enzyme. The tight binding of statins is probably due to the large number of van der Waals interactions between inhibitors and HMG-CoA reductase (Istvan and Deisenhofer 2001).

The hypocholesterolemic effects of statins are evident after only a few days of therapy. Lovastatin, simvastatin, and pravastatin are well tolerated drugs; at 40 mg lovastatin b.i.d. a mean reduction of 30% in total plasma cholesterol, 40% in LDL- (low-density lipoprotein), 35% in VLDL- (very low-density lipoprotein) cholesterol, and 25% in triglycerides, and an increase of 10% high density lipoprotein (HDL)-cholesterol was observed (Tobert 1987). The Scandinavian Simvastatin Survival Study (1994) was the first major trial to show that lowering cholesterol decreases the incidence of death from coronary artery disease. In patients treated with simvastatin, a reduction of 25%–35% total cholesterol and LDL-cholesterol levels were observed. The reduction of cholesterol, especially VLDL and LDL, involved in the translocation of cholesterol, and the increase in the number of LDL-specific membrane receptors in extrahepatic tissues, were also observed for patients treated with simvastatin and pravastatin. In addition to reducing LDL-cholesterol, the clinical data showed that lovastatin, simvastatin, and pravastatin increase HDL, with a subsequent decrease, by almost 50%, of the LDL- to HDL-cholesterol ratio, considered the best predictor of atherogenic risk (Alberts 1988; Buckland et al. 1989; Eisenberg 1998; Tobert 1987).

Synthetic statins appear to be as efficacious as natural statins. In vivo statins have different effects on LDL-cholesterol reduction, with dose-dependent reduction of plasma levels of up to 60%, although reductions of 20%–25% are more typical with the dose commonly used in clinical practice. The greatest LDL-cholesterol reduction is obtained with atorvastatin and simvastatin (Chong et al. 2001; Farnier and Davignon 1998). It was also observed that statins may contribute to decrease triglyceride levels, especially in hypertriglyceridemic subjects (Bakker-Arkema et al. 1996; Stein et al. 1998).

Adverse side effects of statin use, which are generally dose related, are rare and transient. The appearance of myopathy, as rhabdomyolysis, with or without acute renal failure secondary to myogloburia, increases with concomitant use of some statins with other drugs, such as immunosuppressive drugs (i.e., ciclosporine) and some antibiotics (i.e., erythromycin, clarithomycin, ketoconazole, itraconazole). Also combined gemfibrozil (a fibric acid derivative) and statin therapy, employed for patients at very high risk of coronary artery disease who have evidence of atherosclerosis, may produce myopathy, rhabdomyolysis, myoglobinuria, and renal injury. Moreover statins should not be used with nicotinic acid, as this leads to increased risks not only of myopathy, but also of hepatotoxicity (Chong et al. 2001; Farnier and Davignon 1998; Horsmans 1999; Maron et al. 2000). All statins are metabolized in the liver, with different pharmacokinetics. Lovastatin, simvastatin, atorvastatin, and cerivastatin have a common metabolic pathway through the cytochrome P-450 3A4 enzyme system, fluvastatin has a metabolic pathway through the P-450 2C9 system, while pravastatin has multiple metabolic pathways (Chong et al. 2001; Maron et al. 2000; Tsujita et al. 1986). These differences are closely related to potential interactions with other drugs, which are metabolized through the same pathway (Chong et al. 2001; Horsmans 1999). Drug interactions may also depend on the plasma half-life of the statin, which, for all statins, is about 2–3 h, the exception being atorvastatin, which has a half-life of 14–20 h (Chong et al. 2001; Furberg 1999).

Roche Laboratories withdrew Posicor (Mibefradil) from the market in June 1998. Posicor, used in the treatment of patients with hypertension and chronic stable angina, gave serious adverse reactions after co-administration with other drugs, including lovastatin and simvastatin (United States FDA Talk Paper 1998). On 8 August 2001, the FDA announced that Bayer had removed the fully synthesized statin cerivastatin [Baycol (trade name in the United States) and Lipobay (worldwide)] from the market because of reports of fatal rhabdomyolysis (US FDA Talk paper 2001). The side effect was related to higher doses of cerivastatin, especially when combined with gemfribozil.

Trends and prospects

The results reported since 1987, the year of approval of lovastatin as a therapeutic drug by the FDA, indicate that statins can be employed successfully in the treatment of hypercholesterolemia. However, the benefit-risk relationship must always be taken into account. The marked lipid-lowering effects of statins have led to a substantial reduction in coronary events, as revealed by clinical, epidemiological, and pathological studies (Chong et al. 2001; Farnier and Davignon 1998; Furberg 1999; Maron et al. 2000).

In addition to reducing the risk of cardiovascular morbidity and mortality, statins can prevent stroke and reduce the development of peripheral vascular disease (Maron et al. 2000). Statins have biological effects beyond the level of LDL-cholesterol reduction, including antithrombotic and anti-inflammatory effects, which may offer protection against atherosclerotic plaque growth (Fenton and Shen 1999; Rosenson and Tangney 1998; Vaughan et al. 2000). Other potential uses of statins may include hypertension, osteoporotic fractures, ventricular arrhythmia, and immune response (Chong et al. 2001; De Sutter et al. 2000; Glorioso et al. 1999; Meier et al. 2000). Further thorough investigations are nevertheless required before any clinical applications of statins.

Currently there are five FDA-approved statins of proved effectiveness and safety (lovastatin, simvastatin, pravastatin, fluvastatin, and atorvastatin), and research into the pharmacological regulation of dyslipidemia is ongoing. Recently a New Drug Application was submitted to the US FDA by AstraZeneca (June 2001), for the new synthetic rosuvastatin (Crestor), based on the results obtained in preclinical and clinical trials (McTaggart et al. 2001; Olsson 2001). Moreover, Novartis (Switzerland) has acquired (Novartis media release 23 April 2001a), European rights to a "superstatin," pitavastatin, which is currently in phase 2 clinical development. Nevertheless further experiments are in progress, as for this statin new results have also indicated muscle side effects if employed at high doses (Novartis media release 30 October 2001b).

In conclusion, for each statin the benefit in relation to potential side effects, which in some subjects may be important, and any possible interaction with other drugs, have to be carefully considered. Notwithstanding, statins clearly represent the first-line drug therapy for cholesterol lowering in the prevention of coronary artery disease.

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