# HMG-CoA Reductase Inhibitors

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# Preface

The discovery of drugs is still an unpredictable process. Breakthroughs are often the result of a combination of factors, including serendipidity, rational strategies and a few individuals with novel ideas. An encouraging development in the treatment of hypercholesterolemia has been the introduction of a new class of fungal-derived compounds that are potent competitive inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-controlling enzyme in the biosynthetic pathway for cholesterol. HMG-CoA reductase (HMGR) inhibitors (statins) are established drugs for the treatment of hypercholesterolemia, and have been shown to induce regression of vascular atherosclerosis as well as reduction of cardiovascular-related morbidity and death in patients with and without coronary artery disease.

This book deals with statins which have substantially altered the approach to therapy of atherosclerosis and its sequelae. Emphasis is placed on the scientific background to the discoveries and the development of the therapy, with an overview of the current state of knowledge of the drugs by experts in the field. We are happy to say that invitations to these authors were gratefully accepted and each contribution constitutes an important part of this book. Each of the chapters has been designed in such a way that it can be read independently of the others, but has been written with a uniformity of theme and style that should allow smooth transitions. In the first chapter, an overview of the history and development of HMG-CoA reductase inhibitors is provided by *Stefano Bellosta*, *Rodolpho Paoletti* and *Alberto Corsini*. Although the cholesterol-lowering ability of this class of drugs is irrefutable, the mechanisms responsible for their hypocholesterolemic effects are yet to be completely defined. The goal of the following chapter written by *Margaret E. Brousseau* and *Ernst J. Schaefer* is to review the results of recent *in vitro* and *in vivo* studies that have investigated the mechanisms by which statins reduce plasma LDL concentrations, with particular emphasis on the metabolism of apoB-containing lipoproteins in humans.

Inhibition of the HMG-CoA reductase and therefore of cholesterol biosynthesis leads to an alteration of intracellular signaling cascades by modifying subcellular localisation of small G-proteins via prenylation. Other mechanisms involve the regulation of cholesterol-regulated transcription factors, as described in the third chapter (*Jörg Kotzka*, *Wilhelm Krone* and *Dirk Müller-Wieland*).

Independent of their ability to reduce plasma cholesterol, several other potential targets for statins are emerging. These targets comprise blood cells

(*Gerd Schmitz* and *Michael Torzewski*) as well as cells of the vascular wall (*Koichi Node* and *James K. Liao*). Indications and contraindications for statin treatment (primary and secondary prevention of hypercholesterolemia) are then described by *Hans-P. Thomas* and *Elisabeth Steinhagen-Thiessen*. An overview of the five large clinical trials of the beneficial effect of statins on coronary disease, which have been published since 1994, is given in the following chapter by *Helena K. Gylling* and *Tatu A. Miettinen*. The final chapter written by *Colin Berry*, *Andrew Davie* and *John McMurray* deals with the economic impact of statin therapies.

It is our hope that this book provides the reader not only with information but also stimulates further research into the pathogenesis of atherosclerosis and the mechanisms behind the action of effective statins.

Gerd Schmitz and Michael Torzewski
Regensburg, February 2002

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# History and development of HMG-CoA reductase inhibitors

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## Introduction

Coronary heart disease (CHD) is the leading cause of mortality in Western countries [1, 2]. A strong, positive correlation between high levels of plasma total and low-density lipoprotein (LDL) cholesterol and CHD is well established [3]. Dietary and/or pharmacological approaches aimed at lowering elevated plasma LDL appears therefore to be a logical intervention to reduce incidence of CHD or even reversing the development of coronary atherosclerosis [1, 4–8]. A number of cholesterol-lowering drugs are currently available for human use [1, 2, 9]. Among these, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, the so-called statins: atorvastatin, cerivastatin[1], fluvastatin, pitavastatin, pravastatin, lovastatin, rosuvastatin and simvastatin, can achieve relatively large reductions in plasma cholesterol levels and are a well-established class of drugs for the treatment of hypercholesterolemia [10]. Clinical trials have demonstrated that they can induce regression of vascular atherosclerosis, as well as reduction of cardiovascular-related morbidity and mortality, in patients with and without coronary artery disease CAD [11–21]. These trials provide a powerful endorsement of the value of lipid-lowering therapy with a statin in patients who are at risk for CAD.

The beneficial effects of HMG-CoA reductase (HMGR) inhibitors are usually assumed to result from their ability to reduce cholesterol synthesis [22]. However, since mevalonic acid, the product of the effect of HMGR on HMG-CoA, is the precursor not only of cholesterol but also of numerous metabolites (Fig. 1) [23, 24], inhibition of HMGR has the potential to result in pleiotropic effects [25–28]. The beneficial effect of statins on clinical events may therefore involve non-lipid-related mechanisms that modify endothelial function, inflammatory responses, plaque stability and thrombus formation [25–30].

The purpose of this review is to summarize and discuss the steps that led to the discovery and development of the inhibitors of HMGR.

[1] In August 2001, Cerivastatin has been withdrawn from worldwide sale in the light of drug-interactions concern.

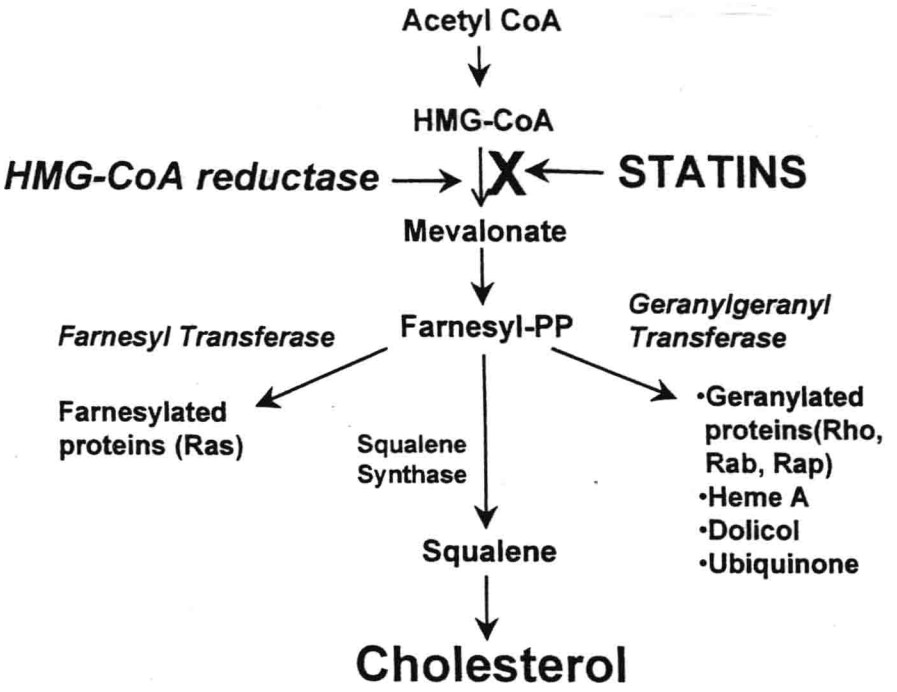


Figure 1. The mammalian cell mevalonate pathway. Legend: CoA, coenzyme A; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; PP, pyrophosphate.

## History and discovery of HMG-CoA reductase inhibitors

An encouraging development in the treatment of hypercholesterolemia has been the introduction of a new class of fungal-derived compounds that are potent competitive inhibitors of HMGR, the rate-controlling enzyme in the biosynthetic pathway for cholesterol (Fig. 2).

In the early '70s, Endo and Kuroda, while searching for HMGR inhibitors of microbial origin, assayed over 6,000 microbial strains for their ability to block lipid synthesis. In particular, culture broths effective in inhibiting early stages, between acetate and mevalonate, of the cholesterol synthetic pathway were further investigated in order to identify the main active component(s). In 1976 Endo and Kuroda [31] reported that citrinin, an antibiotic isolated from *Phytium ultimum,* acted as an inhibitor of cholesterol synthesis in rat liver. Further work from the same authors [32] led to the isolation of three fungal metabolites (ML-236 A, B and C) produced by *Penicillium citrinum.* ML-236B (compactin, mevastatin), was also isolated by Brown et al. from *Penicillium brevicompactum* [33].

The structure of mevastatin was determined by a combination of spectroscopic, chemical, and X-ray crystallographic methods [32]. Mevastatin has a hexahydronaphthalene skeleton substituted with a β-hydroxy-δ-lactone moi-

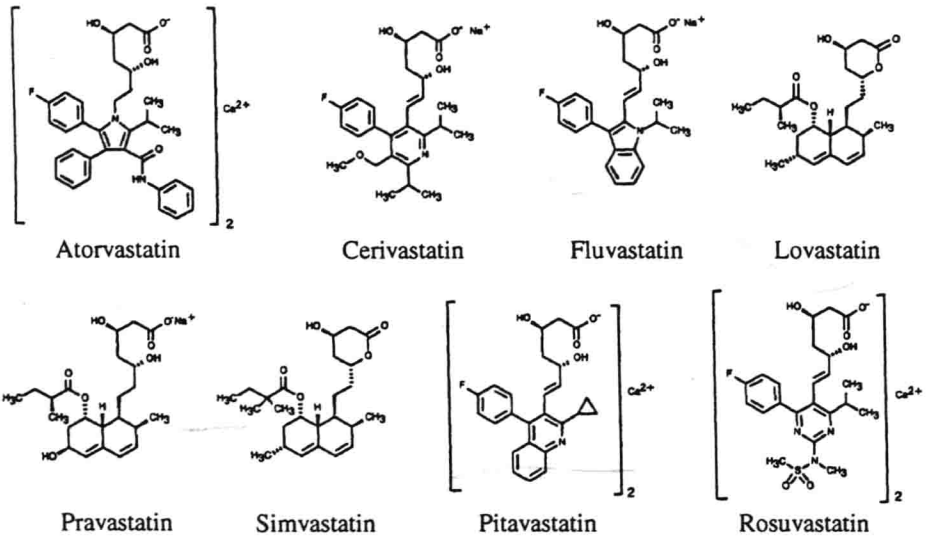


Figure 2. Chemical structure of statins.

ety, which can be converted into the water-soluble open acid by treatment with alkali [34].

ML-236 B, when administered to rats, at 5 and 20 mg $kg^{-1}$ as a single dose, effectively reduced plasma cholesterol and [$^{14}$C]acetate incorporation into digitonin-precipitable sterols [32]. Endo, Kuroda and Tanzawa [34] further investigated the action of ML-236 B: this compound inhibited cholesterol synthesis from acetate, acetyl-CoA and HMG-CoA, but failed to affect the conversion of mevalonate into sterols, thus suggesting a specificity of action on HMGR. Enzyme kinetic studies showed that the compound acted as a competitive inhibitor of the HMGR due to the similarity of the open lactone acid form to 3-hydroxy-3-methylglutarate (Fig. 2). This hypothesis is further stressed by the observation that, *in vitro,* the lactone form is much less effective than the open acid form of the molecule [34].

Since this original discovery, a number of compounds that are competitive inhibitors of HMGR have appeared. In 1979 Endo isolated monacolin K (lovastatin) from a culture of *Monascus ruber* [35] and the same compound was also isolated from *Aspergillus terreus* in 1980 by Alberts and coworkers [36]. The structure of this compound differs from compactin in the presence of a methyl group at position 6 (Fig. 2).

Subsequently, the search for additional HMGR inhibitors was continued for several years, leading to the isolation of several compounds of the mevastatin family [34]. Among them, pravastatin is a ring hydroxylated metabolite of mevastatin, originally isolated from the urine of mevastatin-treated dogs. This drug is now obtained by microbial hydroxylation of mevastatin [37]. Simvastatin is synthesized directly from lovastatin by replacement of the

2-methylbutanoyl side chain with a 2,2-dimethylbutanoyl group [38]. Lovastatin and simvastatin are prodrugs that are enzymatically hydrolyzed *in vivo* to the open ring form, while pravastatin is in the active, open ring, form. Fluvastatin, which is a racemate, is the first entirely synthetic HMGR inhibitor, which differs from the original structure in its lipophilic moiety [39]. More recently, several fully HMGR synthetic enantiomeric inhibitors (Fig. 2) are being developed or are already in clinical testing.

## HMG-CoA reductase

The enzyme HMGR catalyzes the NADPH-dependent reduction of HMG-CoA to mevalonate. This reaction is the committed step in cholesterol biosynthesis and is a target for intervention in the treatment of hypercholesterolemia [23].

HMG-CoA binds the reductase first to form a binary complex which in turn, upon NADPH binding, evolved into a ternary complex [40].

The overall reaction can be divided into three steps. The first reduction results in the formation of a mevaldyl-CoA hemy-thioacetal intermediate. This intermediate decomposes to mevaldehyde and $CoAS^-$ and is subsequently protonated. A second NADPH molecule then replaces $NADP^+$ and reduces mevaldehyde to mevalonate [41].

HMG-CoA reductase is a 97 kDa integral membrane protein of the endoplasmic reticulum with 8 transmembrane spans [42]. Previous studies demonstrated that the membrane-spanning regions of this enzyme are necessary for the regulated degradation of the enzyme [43–45].

Human HMGR consists of a single polypeptide chain of 888 amino acids. The aminoterminal 339 residues are membrane-bound and reside in the endoplasmic reticulum membrane projecting into the cytoplasm. The catalytic activity of the protein resides in its cytoplasmic, soluble C-terminal portion (residues 460-888). A linker region (residues 340–459) connects the two portions of the protein [46].

The crystal structure of the catalytic portion of HMGR has been determined recently with bound reaction substrates and products. The structure illustrates how HMG-CoA and NADPH are recognized and suggests a catalytic mechanism. Catalytic portions of human HMGR form tight tetramers, explaining the influence of the enzyme's oligomeric state on the activity and suggesting a mechanism for cholesterol sensing [46].

The enzyme forms tetramers with approximate D2 symmetry. The individual monomers wind around each other in an intricate fashion. The monomers are arranged in two dimers, each of which has two active sites. Residues from both monomers (called $\alpha$ and $\beta$) form the active sites [46]. At each active site, the HMG moiety of one HMG-CoA molecule, which is bound predominantly to a single monomer, comes into the proximity of the nicotinamide ring of an NADPH molecule, whose binding pocket is located in the neighboring monomer [41]. Thus, the active sites are positioned at the interface of the two

monomers of a dimer. The formation of the tetramer does not appear to be involved in substrate binding.

HMGR is among the most highly regulated enzymes known [23]. Transcription and translation of HMGR increase when the concentrations of products of the mevalonate pathway are low. Conversely, when sterol concentrations are high, the intracellular HMGR concentration decreases rapidly [47]. A third level of regulation is achieved by phosphorylation of S872 (human enzyme) by AMP-activated protein kinase, which decreases the enzyme's activity [48]. Finally, Simoni and colleagues [49] have recently shown that HMGR is degraded in endoplasmic reticulum membranes prepared from sterol pretreated cells and that such degradation is catalyzed by a cathepsin L-type cysteine protease within the reductase membrane domain. Cathepsin L-dependent proteolysis was observed to occur preferentially in sterol-pretreated cells, suggesting that sterol treatment results in conformational changes in HMGR that make it more susceptible to such cleavage. Therefore, this enzyme is highly regulated at the level of synthesis, as well as at the level of degradation, of the protein [50, 51].

For the above-mentioned reasons HMGR appeared to be an ideal target for agents affecting cholesterol synthesis to be used in therapy.

## Inhibition of HMG-CoA reductase by statins

All statins share an HMG-like moiety, which may be present in an inactive lactone form (Fig. 2). *In vivo*, these prodrugs are enzymatically hydrolyzed to their active hydroxy-acid forms [52]. The statins share rigid, hydrophobic groups that are covalently linked to the HMG-like moiety.

Relative lipophilicity estimated for the dihydroxy acid form of all statins clearly show that pravastatin and rosuvastatin are very hydrophilic as compared to other statins (Tab. 1).

### In vitro *activity of HMGR inhibitors: effects on cell-free systems*

The activity of HMGR inhibitors on enzymatic activity was first shown on microsomal extracts from rat liver [34]. This study demonstrated that the inhibitory effect is competitive with respect to HMG-CoA, but is not competitive with respect to NADPH, indicating that the two substrates bind the enzyme in a coordinated fashion. The binding is reversible, therefore the drug detaches from the enzyme upon dilution. The potency of HMGR competitive inhibitors is related to a slow binding step to the enzyme [53], which triggers the formation of the final complex.

Endo et al. investigated the specificity of mevastatin using rat liver cytosol [34]. Mevastatin (5 and 50 μM), sharply reduced the incorporation of acetate, acetyl CoA and HMG-CoA into sterols by 25 and 70% respectively; meval-

Table 1. Inhibition of rat microsomal HMG-CoA reductase activity by statins and their relative lipophilicity

| Inhibitor | *Ki* (M) | *ClogP* |
|---|---|---|
| Compactin | $1.4 \times 10^{-9}$ | 3.85 |
| Atorvastatin | $7.5 \times 10^{-9}$ | 4.06 |
| Cerivastatin | [2] $1.3 \times 10^{-9}$ | 1.47 |
| Fluvastatin | $0.3 \times 10^{-9}$ | 3.24 |
| Lovastatin | $0.6 \times 10^{-9}$ | 4.27 |
| Pitavastatin | $1.7 \times 10^{-9}$ | 1.50 |
| Pravastatin | $2.3 \times 10^{-9}$ | −0.22 |
| Rosuvastatin | $12 \times 10^{-9}$ | [3] −0.33 |
| Simvastatin | $0.12 \times 10^{-9}$ | 4.68 |
| HMG-CoA | *Km* $1.4 \times 10^{-6}$ | |

[2] In the same experimental condition lovastatin has been shown to be 100-fold less potent [57].
[3] Log D [58].

onate incorporation into sterols, however, was not affected [34]. This is consistent with a specific inhibition of HMGR without any effect on later steps of cholesterol biosynthesis. Similar data have been obtained with other HMGR inhibitors [36, 39, 54–58].

The $K_i$ (inhibition constant) values for the statin-enzyme complexes using a rat microsomal preparation range between 0.1 to 12 nM (Tab. 1) [36, 39, 52, 54–58], whereas the Michaelis constant, $K_m$, for HMG-CoA is 4 μM [59]. Similar potencies have been obtained using microsomal HMG-CoA reductase from hamster liver, dog liver, human hepatoma HepG2 cells and purified human catalytic domain [56–58, 60, 61].

More recently, Istvan and Deisenhofer have determined the structures of the catalytic portion of human HMGR complexed with six different statins [62]. To determine how statins prevent the binding of HMG-CoA, they solved six crystal structures of the catalytic portion of human HMGR bound to six different statin inhibitors. The structures illustrate that statins inhibit HMGR by binding to the active site of the enzyme, thus sterically preventing substrate from binding. This agrees well with kinetic studies that indicate that statins competitively inhibit HMG-CoA, but do not affect NADPH binding [34]. The study by Istvan and Deisenhofer [62] reveals also how the bulky, hydrophobic compounds of statins occupy the HMG-binding pocket and part of the binding surface for CoA. Thus, access of the substrate HMG-CoA to HMGR is blocked when statins are bound. The tight binding of statins is probably due to the large number of van der Waals interactions between inhibitors and with HMGR. The structurally diverse, rigid hydrophobic groups of the statins are accommodated in a shallow non-polar groove that is present only when COOH-residues of HMGR are disordered [62].

*Effect on cultured cells*

Statins effectively inhibit cholesterol synthesis in a number of cultured mammalian cells. All statins, except pravastatin and rosuvastatin, inhibit cholesterol synthesis to a similar degree *in vitro* in a number of peripheral cell lines. Much higher doses of pravastatin and rosuvastatin are required, indicating that in extra-hepatic tissues the cellular uptake of these drugs is severely impaired by their hydrophilicity. The efficacy of pravastatin and rosuvastatin in inhibiting cholesterol synthesis in human and rat hepatocytes to a degree comparable to other statins, however, suggests that specific uptake occurs in these cells. Several authors [58, 63, 64] have demonstrated the presence of a carrier that facilitates the entry of statins into hepatocytes. It is important to note that the human hepatoma HepG2 cell line does not possess a specific transporter for statins. Indeed, it has been reported that higher concentrations of hydrophilic statins, comparable to those used in peripheral cells, are required to inhibit cholesterol synthesis in HepG2 cells compared with those utilized in rat hepatocytes [56].

Surprisingly, incubation of the cells with HMGR inhibitors causes a several-fold increase of HMGR mass [65]. The competitive block of enzymatic activity, in fact, stimulates the *de novo* synthesis of HMGR, thus leading to an accumulation of the enzyme in cells [66, 67]. The build-up of HMGR in the cells is also due to a stabilization of the enzyme by statins. Edwards et al. [68] demonstrated that lovastatin increases the enzyme half-life from 2 to more than 10 h.

These results implicated a nonsterol mevalonate metabolite as having an important role in the degradation of HMGR. Studies that used either inhibitors that acted on more distal enzymatic steps in the isoprenoid/cholesterol pathway, or those in which analogs of farnesyl diphosphate were added to cells, were consistent with the proposal that the nonsterol component was derived from farnesyl diphosphate. This mechanism contributes significantly to the accumulation of the enzyme within the cell and can be regarded as an attempt by the cells to overcome the shortage of mevalonate-derived compounds that are essential for cellular homeostasis and growth [23].

## *In vivo* activity of HMGR inhibitors

*Inhibitors of cholesterol synthesis*

The *in vivo* inhibition of HMGR by statins has been demonstrated in a number of animal species from mouse to monkey. The effect can be detected by determining the *in vivo* rate of incorporation of precursors into cholesterol [52].

The dose required to achieve significant reduction of cholesterol synthesis with statins is in the range of 0.01–20 mg $Kg^{-1}$ $day^{-1}$ both in acute and sub-

chronic studies. Not every species, however, will respond to the administration of statins with a reduction of plasma cholesterol levels. The rat, for instance, can up-regulate cholesterol synthesis to a point at which the hypocholesterolemic effect of these drugs is overcome by the high amount of HMGR synthesized [52], thus keeping a "normal" rate of cholesterol synthesis also in the presence of competitive inhibitors [52]. On the other hand, several animal species cannot modulate their cholesterol synthesis as effectively as the rat [69]; this will result in the lowering of plasma cholesterol.

*Hypolipidemic activity*

Statins are effective hypocholesterolemic compounds in several animal species. The hypocholesterolemic activity has been reported in rat, hamsters, hen, rabbits, minipigs, dogs, guinea pigs, and monkeys. Doses ranging from 5 to 50 mg $Kg^{-1}$ $day^{-1}$ elicit reductions of plasma cholesterol from 15 to 60% [52, 70]. Among lipoprotein classes apoB- and apoE-containing lipoproteins, mainly LDL and HDL subclasses, are preferentially modulated.

The hypotriglyceridemic effect of statins can be also detected in animal species [57, 70, 71].

## Pleiotropic effects of HMGR inhibitors

Clinical trials have firmly established that HMGR inhibitors can induce regression of vascular atherosclerosis, as well as reduction of cardiovascular-related morbidity and death, in patients with and without CAD. These beneficial effects on coronary events have generally been attributed to the hypocholesterolemic properties of statins. However, because mevalonate, the product of the enzyme reaction, is the precursor not only of cholesterol but also of many nonsteroidal isoprenoid compounds, inhibition of HMGR may result in pleiotropic effects [10, 23, 24, 27]. Indeed, the mevalonate pathway yields a series of isoprenoids (Fig. 1) that are vital for diverse cellular functions. These isoprenoids include: isopentenyl adenosine, present in some types of transfer RNA; dolichols, required for glycoprotein synthesis; polyisoprenoid side chains of ubiquinone and heme A, involved in electron transport [23, 24]. Several proteins post-translationally modified by the covalent attachment of mevalonate-derived isoprenoid groups, either farnesyl- or geranylgeranylpyrophosphate, have been identified [23, 72, 73]. These proteins must be prenylated as a prerequisite for membrane association, which is required for their function [72, 73]. Members of this family are involved in a number of cellular processes including cell signaling, cell differentiation and proliferation, myelination, cytoskeleton dynamics and endocytotic/exocytotic transport (Tab. 2). Hence, through the inhibition of HMGR, statins could affect several