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Review

Statins: mechanism of action and effects

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Abstract

The beneficial effects of statins are the result of their capacity to reduce cholesterol biosyntesis, mainly in the liver, where they are selectively distributed, as well as to the modulation of lipid metabolism, derived from their effect of inhibition upon HMG-CoA reductase. Statins have antiatherosclerotic effects, that positively correlate with the percent decrease in LDL cholesterol. In addition, they can exert antiatherosclerotic effects independently of their hypolipidemic action. Because the mevalonate metabolism generates a series of isoprenoids vital for different cellular functions, from cholesterol synthesis to the control of cell growth and differentiation, HMG-CoA reductase inhibition has beneficial pleiotropic effects. Consequently, statins reduce significantly the incidence of coronary events, both in primary and secondary prevention, being the most efficient hypolipidemic compounds that have reduced the rate of mortality in coronary patients. Independent of their hypolipidemic properties, statins interfere with events involved in bone formation and impede tumor cell growth.

Keywords: HMG CoA reductase - cell signaling - LDL oxidation - atherosclerosis - cancer - osteoporosis - endothelial dysfunction - macrophage - smooth muscle cell

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Introduction

Statins, inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, have revolutionized the treatment of hypercholesterolemia. They are the most efficient agents for reducing plasma cholesterol, being also appreciated for their good tolerance. Angiographic studies have demonstrated that these compounds reduce the progression and may induce the regression of atherosclerosis. These effects were translated in significant cardiovascular morbidity and mortality reductions in many clinical trials (WOSCOPS, AFCAPS/TexCAPS, HS, CARE, LIPID, HPS) [1]. The beneficial effects of the HMG-CoA reductase inhibitors are usually attributed to their capacity to reduce the endogenous cholesterol synthesis, by competingly inhibiting the principal enzyme involved [2]. Since mevalonate, the product of HMG CoA reductase reaction, is the precursor not only for cholesterol, but also for many other non-steroidal isoprenoidic compounds, inhibition of this key enzyme may result in pleiotropic effects. They have been divided into two categories, involving: directly lipids, or intracellular signaling pathways. The first category includes: inhibition of cholesterol biosynthesis, increased uptake and degradation of low density lipoproteins (LDL), inhibition of the secretion of lipoproteins, inhibition of LDL oxidation, and inhibition of the scavenger receptors expression [3]. Statins modulate a series of processes leading to reduction of the accumulation of esterified cholesterol into macrophages, increase of endothelial NO synthetase, reduction of the inflammatory process, increased stability of the atherosclerotic plaques, restoration of platelets activity and of the coagulation process [3].

In addition, statins can inhibit tumor cells growth and enhance intracellular calcium mobilization. It

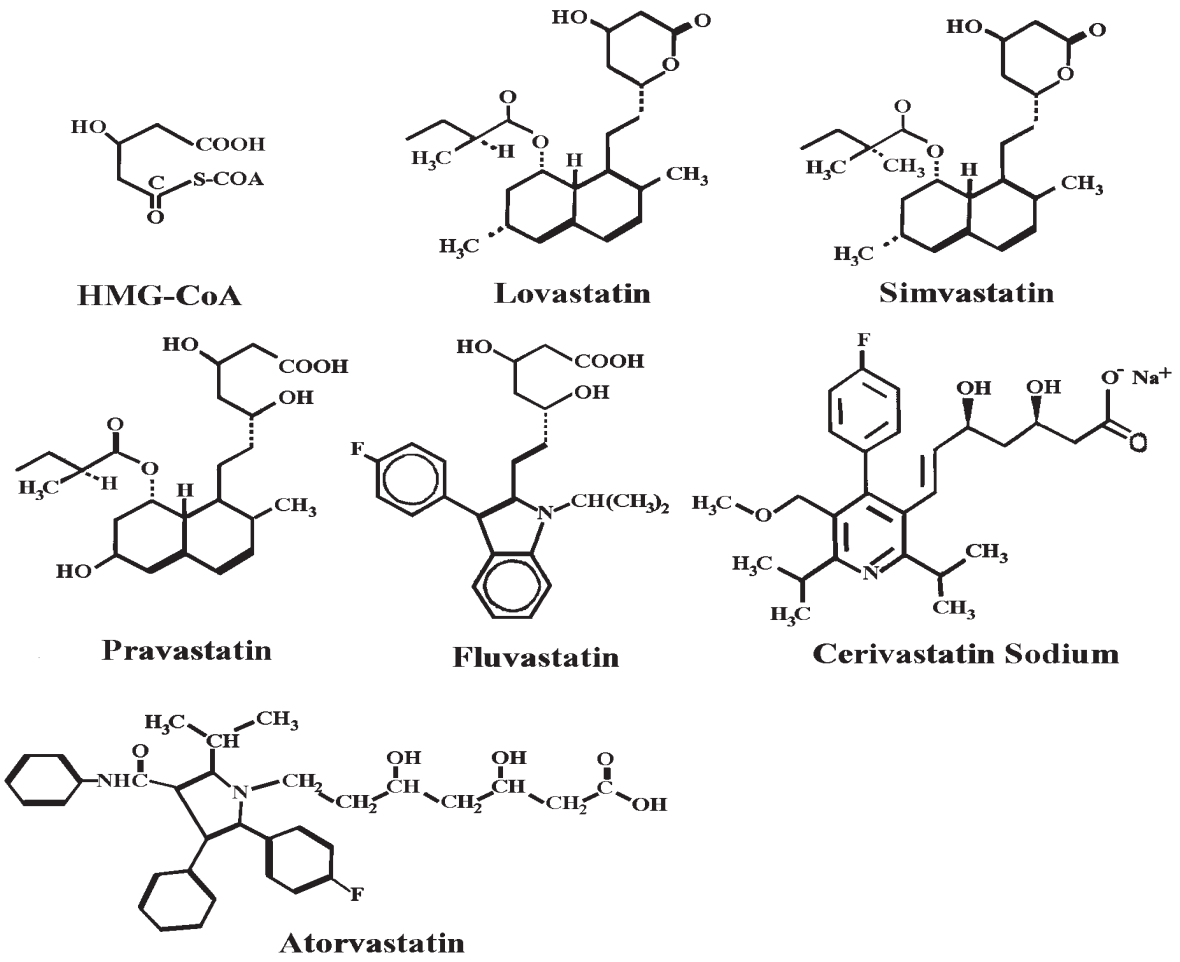

Fig. 1 Chemical structure of the statins and HMG-CoA.

was observed that inhibitors of HMG-CoA reductase induce a reduction of the formation of osteoclasts in rodents [3]. Human subjects treated with statins have shown a reduction in the number of bone fractures [4].

The discovery of statins has led to an important progress in the primary and secondary prevention of coronary heart disease. Although angiographic modifications following statin therapy were modest, clinical benefits that accompanied the therapy have been significant. Numerous clinical studies have correlated the reduction of blood cholesterol induced by these compounds with the reduction of the number of major coronary events, as well as general mortality in coronary patients [1].

Classification of statins

There are a number of classification criteria for statins, including: 1) how they are obtained, 2) liver metabolism, 3) physico-chimical properties, 4) specific activity.

How they are obtained

Some of the statins are obtained after fungal fermentation: lovastatin (Mevacor), pravastatin (Lipostat, Pravachol) and simvastatin (Zocor), others by synthesis: fluvastatin (Lescol), atorvastatin (Sortis, Lipitor), and cerivastatin (Baycol, Lipobay).

It must be stressed that on August 8, 2001, Bayer AG voluntarily withdrew cerivastatin (Baycol, Lipobay) from the world pharmaceutical market, after 31 patients died by acute renal failure caused by rhabdomyolysis. FDA supported this decision. As a result, only five statins are, at this moment, in clinical use: lovastatin, simvastatin, pravastatin, atorvastatin and fluvastatin.

Liver metabolism

All statins have the liver as target organ. The percentage of the dose retained by the liver is as follows: > 70% for fluvastatin and lovastatin, >80% for simvastatin and 46% for pravastatin [5]. There are no available data for atorvastatin and cerivastatin. For their liver metabolism, lovastatin, simvastatin, atorvastatin and cerivastatin follow the citocrom P450 (CYP 3A4) pathway. Fluvastatin follows the CYP 2C9 pathway, and pravastatin is metabolized differently [6]. The majority of the statins have a low circulation concentration: 12% for atorvastatin, 17% for pravastatin, 20-30 % for fluvastatin, 5% for simvastatin and lovastatin. Cerivastatin has a circulation distribution of more than 60%.

Physico-chemical properties

Pravastatin is extremely hydrophilic, fluvastatin has intermediar characteristics, lovastatin, simvastatin, atorvastatin and cerivastatin are hydrofobic [7].

Specific activity

Atorvastatin, cerivastatin, fluvastatin and pravastatin are administered as active compounds (acid form). Lovastatin and simvastatin are administered as inactive forms (lactone), which have to be enzymatically hydrolized to generate active forms [7].

Mechanisms for the action of statins

Mechanisms involving lipids

Dyslipidemia and hypercholesterolemia are controled by the liver. Hepatocytes take up from the circulation ~ 50% of LDL cholesterol. An increase in the activity of LDL receptor in hepatocytes could be an efficient method to decrease plasma LDL cholesterol level.

Inhibition of HMG CoA reductase

Statins target hepatocytes and inhibit HMG-CoA reductase, the enzyme that converts HMG-CoA into mevalonic acid, a cholesterol precursor. The statins do more than just compete with the normal substrate in the enzymes active site. They alter the conformation of the enzyme when they bind to its active site. This prevents HMG-CoA reductase from attaining a functional structure. The change in conformation at the active site makes these drugs very effective and specific. Binding of statins to HMG-CoA reductase is reversible, and their affinity for the enzyme is in the nanomolar range, as compared to the natural substrate, which has micromolar affinity [8]. The inhibition of HMG-CoA reductase determines

Table 1 Role of prenylated proteins in cellular functioning [3].

Molecular weight (kDa)	**Comments**
Farnesylated	
66-72	Nuclear laminin family
53-55	Unidentified proteins
41-46	Inositol triphosphate 5-phosphatase 2', 3'-cyclic nucleotide 3"-phosphatase
37	Peroxisomal protein
21-28	Ras, involved in cell proliferation and differentiation
Geranylgeranylated*	
21-28	Rho/Rac/Cdc42, involved in cytoskeletal assembly, superoxide generation and cell cycle progression
	Rab, involved in transport of vesicles
	Rap, involved in cellular replication, platelets activation and generation oxygen radicals
5-8	G proteins (γ-subunit), involved in signal transduction

* in mammalian cells, 0.5-1% of total cellular proteins are geranyl-geranylated.

the reduction of intracellular cholesterol, inducing the activation of a protease which slices the sterol regulatory element binding proteins (SREBPs) from the endoplasmic reticulum. SREBPs are translocated at the level of the nucleus, where they increase the gene expression for LDL receptor. The reduction of cholesterol in hepatocytes leads to the increase of hepatic LDL receptors, that determines the reduction of circulating LDL and of its precursors (intermediate density - IDL and very low density- VLDL lipoproteins) [9]. All statins reduce LDL cholesterol non-liniarly, dose-dependent, and after administration of a single daily dose [5]. Efficacy on triglyceride reduction parallels LDL cholesterol reduction [10].

Direct effects of HMG CoA reductase inhibition
Statins inhibit hepatic syntesis of apolipoprotein B-100, determining a reduction of the synthesis and secretion of triglyceride rich lipoproteins [11] and an increase of receptors production for apolipoproteins B/E [12]. This can explain why atorvastatin and simvastatin are capable of reducing LDL in patients with homozigous family hypercholesterolemia, where LDL receptors are not functional [13, 14]. Statins have a modest effect on HDL increase, and no influence on lipoprotein(s) concentration [15].

Reduction of LDL susceptibility towards oxidation
At least 4 mechanisms were proposed to explain statins' antioxidant properties [16]. (1) The hypocholesterolemic effect, resulting in reduced lipoprotein cholesterol, and thus, reduced level of oxidation substrate [17]. (2) The decrease of cell oxygen production, by inhibiting the generation of superoxide by macrophages. Recently, it was demonstrated that statins can attenuate the formation of superoxide anion in endothelial cells, by preventing the prenylation of p21 Rac protein [18]. Statins can also prevent LDL oxidation by preserving the activity of the endogenous antioxidant system, like superoxide dismutase [19]. (3) The binding of statins to phospholipids on the surface of lipoproteins (fluvastatin and lovastatin bind to LDL phospholipids) preventing the diffusion towards the lipoprotein core of free radicals generated during oxidative stress [16]. (4) The potent antioxidative potential of the metabolites (*i.e.* atorvastatin and fluvastatin metabolites) also results in lipoproteins protection from oxidation.

Inhibition of the expression of type A scavenger receptor in THP-1 cells and in human monocytes [20], which decrease the receptor-mediated degradation of oxidised LDL. Statins can also reduce mRNA level

and CD36 expression on the cell surface, as well as LDL binding to human U937 monocytes [21].

Mechanisms involving intracellular signaling pathways

Prenylated proteins and their role in cellular signaling
A variety of proteins have covalently attached isoprenoid groups, mainly the C_{15} farnesyl and C_{20} geranylgeranyl residues. The most common isoprenylation site in proteins is the C-terminal "CaaX", where "C" is Cys, "a" is often an aliphatic aminoacid residue, and "X" is any aminoacid. Proteins are farnesylated when "X" is Ala, Met or Ser and geranylgeranylated when "X" is Leu. In both cases the prenyl group is enzymatically linked to the Cys sulfur atom via a thioether linkage. The "aaX" tripeptide is then proteolytically excized and the newly exposed terminal carboxyl group is esterified with a methyl group [22]. Many prenylated proteins are associated with intracellular membranes and mutating their Cys prenylation sites blocks their membrane localization. The hydrophobic prenyl group can act to anchor its attached protein to a membrane. Prenylated proteins may interact with specific membrane- bound receptor proteins and hence prenylation also mediates protein-protein interactions [22].

The complex process of cell signaling is very important for intercellular communication. Extracellular signaling molecules, which are water soluble and have high molecular weight need to bind to specific receptors on the cell surface, which transduce the extracellular signals into the cell by intracellular signaling pathways (cascades). Many intracellular signaling molecules are prenylated proteins. The specific receptors on the cell surface are associated with trimeric G protein, or have Ser/Thr/Tyr kinases activities. The trimeric G protein has a geranylgeranylated subunit (gamma), allowing this signaling protein to be inserted in the cell membrane near specific membrane receptors and to receive extracellular signals, which are then transfered to the secondary signaling molecules in the cell. Another important class of prenylated signaling molecules are the components of Ras family, which are farnesylated and intermediate the Ser/Thr/Tyr kinases activities of membrane receptors from the cell surface. The important role of all these prenylated signaling molecules for the living cell is presented in Table 1.

The mevalonate pathway yields a series of isoprenoids which are vital for diverse cellular functions. These isoprenoids include: isopentenyl adenosine, present in some types of transfer RNA, dolichols required for glycoprotein synthesis, and poly-isoprenoid side chains of ubiquinone and hemeA, involved in electron transport [23].

Beneficial effects of statins

Effects on cholesterol esterification and its accumulation in macrophages
Studies done in mouse peritoneal macrophages have shown that fluvastatin and simvastatin, but not pravastatin, inhibit cholesterol esterification induced in cells by acetyl LDL [23]. The efficacy of fluvastatin in inhibiting cholesterol esterification is more increased in cholesterol loaded cells than in normal ones, effect that might be explained by the fact that the HMG CoA reductase is already inhibited in lipid-loaded cells, as compared with unloaded ones [24].

Effects on endothelial cell function
Endothelial dysfunction represents an early event in the initiation of atherosclerotic lesion, induced by hypercholesterolemia. Nitric oxide (NO) regulates the anti-atherosclerotic function of the endothelium [1]. Hypercholesterolemia reduces the capacity of endothelial cells to produce NO, probably due to the reduced availability of L-arginine, the physiologic substrate of NO syntase, and determins an increased degradation of NO. Cholesterol reduction by statins leads to a significant increase of the endothelial function. The effect of statins on the endothelial function can be partially independent of the reduction of the lipid level. Simvastatin, as well as lovastatin, induce the transcriptional activation of eNOS gene in human endothelial cells *in vitro* [25]. Activation of eNOS by statins takes place post-translationally and is prevented by isoprenoid derivatives, mevalonate and geranylgeraniol [25]. The endothelial function was increased in primates treated with pravastatin, without the reduction of LDL cholesterol [25].

Simvastatin administration to hyperlipemic hamsters (HH) restores the antioxidant potential of the serum [26]. Although the exact mechanism is not known, this effect may be the result of a

decrease in cell oxygen production, binding of LDL to surface phospholipids or due to the antioxidant action of simvastatin metabolites [16]. In addition, experiments done by Simionescu et al., 2001 [26] showed that simvastatin reduced transcytosis of LDL and was able to restore the endothelial dependent relaxation, probably due to an increase in NO-synthesis. This latter assumption was based on two facts: (i) NO-synthase inhibitor, L-NAME, inhibited the response of the arteries and (ii) serum NO level increased in simvastatin-treated HH. These data confirmed and extented previous reports indicating that statins upregulate eNOS expression, and prevent native LDL-mediated down-regulation of eNOS expression.

Simvastatin, as well as lovastatin, exerted a protective, dose-dependent effect in an experimental model of cerebral infarction, a neuroprotective effect mediated rather by the increased production of eNOS, than by the reduced level of cholesterol [27]. The neuroprotective effect of statins is completly absent in mice defficient in eNOS, indicating that the increased activity of eNOS induced by statins is the mechanism by which these compounds protect against cerebral lesions [27]. Recent results obtained by Wagner et al. (2000) [18] suggest that statins inhibit the formation of O^{2-} by endothelial cells, producing modifications in the NO-/ O^{2-} balance, modification leading to the restoration of the endothelial cell function.

Effects on the inflammatory process

Adhesion to the endothelium and transendothelial diapedesis of circulating monocytes and of T lymphocytes represent key events in the atherosclerotic lesion formation [28]. Cytokines secreted by macrophages and lymphocytes can modify endothelial function, smooth muscle cells (SMC) proliferation, collagen degradation and thrombosis [3]. Statins can reduce the expression and function of molecules on the leukocytes surface [29]. Atorvastatin reduces the number of intimal macrophages, monocyte-chemoattractant protein-1 (MCP-1) and the activation of nuclear factor NFkB in hypercholesterolemic rabbits [30]. Cytokines receptors are coupled to GTP-bound proteins, and the binding of leukocytes to the endothelium is regulated by G protein. Statins can affect small GTP-ases or trimeric G proteins, by preventing their prenylation and thus reducing the inflammatory response. Statins diminish leukocytes recruitment in postcapillary venules, stimulated by a lipid mediator (platelets activation factor-PAF or leukotriene B4) in hypercholesterolemic rats [31]. In addition, statins are capable to inhibit transendothelial migration and chemotaxisis of neutrophiles, which can explain the antiinflammatory effect of these compounds. Another antiinflammatory effect of statins on monocytes and macrophages was the decrease of the expression of intercellular adhesion molecule -1 and the secretion of interleukine-6 (IL-6), induced by lipopolysacharides (LPS) [3].

Effects on proliferation, migration and apoptosis of arterial SMC

All statins, except for pravastatin, reduce aortic SMC proliferation [8]. Mevalonate, trans-farnesol and trans-geranylgeraniol prevent the inhibitory effect of statins on SMC proliferation, suggesting that this effect derives from the inhibition of the mevalonate pathway [8]. Fluvastatin, simvastatin and cerivastatin, but not pravastatin, inhibit in a dose dependent manner, arterial SMC migration induced by fibrinogen [8].

Preclinical observations and *in vitro* studies suggest that apoptosis can modulate the arterial wall in restenotic or proliferative lesions, where SMC are dominant [3]. It was reported that statins can induce apoptosis of vascular SMC in culture. Lesioned carotid arteries from rabbits that received fluvastatin or atorvastatin, 5 days prior to lesion induction, presented an increased number of apoptotic SMC [32].

Effects on the stability of the atherosclerotic plaque

Coronary events are the result of unstable atherosclerotic lesion rupture and thrombus formation [33]. The plaque instability, manifested as an ulceration of the fibrous cap, the rupture of the plaque and internal hemorrhage, are characteristics of the plaques with numerous lipid deposits and macrophages in the cap. Recently, it was demonstrated that statins (fluvastatin, simvastatin) can inhibit the gelatinolytic activity of metalloproteases, as well as their secretion by human macrophages in culture [34]. Angiographic studies showed that statins reduce the progression

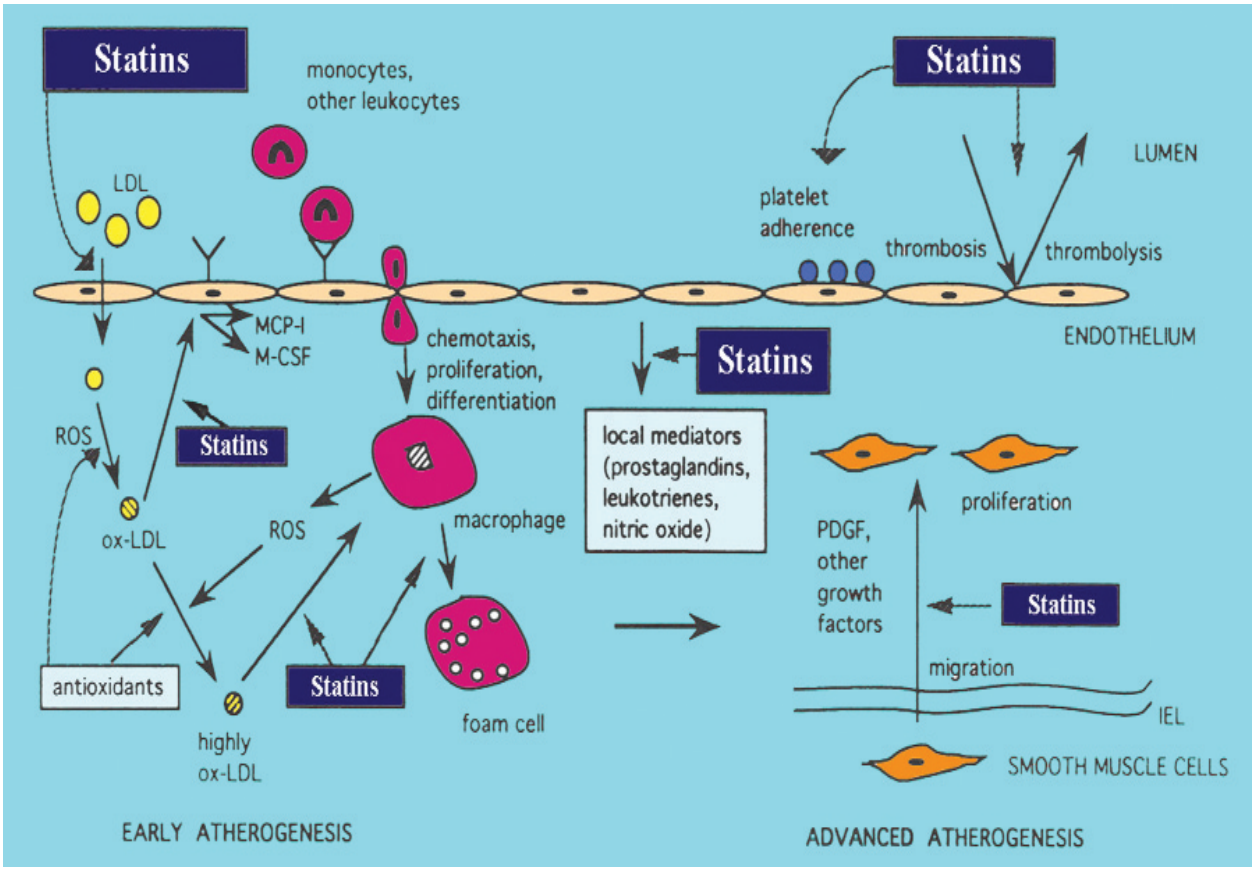

Fig. 2 Hypercholesterolemia favors entry of LDL particles into subendothelial space at lesion-prone arterial sites. Monocyte chemotactic protein-1 (MCP-1) and oxidized-LDL act as chemoattractants to direct accumulation of monocytes and their migration to the subendothelial space, where monocytes undergo phenotypic transformation into macrophages. Concurrently, oxigen free radicals modify LDL. Oxidatively modified LDL is taken up by nondownregulating macrophage receptors to form lipid-rich foam cells. Foam cells develop into fatty streaks, precursor of atherosclerotic plaques. Statins exihibit pleiotropic effects on many components of atherosclerosis that accompany hypercholesterolemia, including platelet coagulation abnormalities, abnormal endothelial function, and determinants of plaque thrombogenicity such as plaque inflammation and proliferation.

and induce the regression of coronary atherosclerosis, reduce the formation of new lesions and the incidence of coronary events [35]. Computer tomography provided evidence of the reduction in the volume of coronary calcified plaques after 12 months of statin treatment. It was concluded that alterations in the composition of lesions confers an increased stability [1].

Other reports show that simvastatin treatment determines an increase of the antioxidant potential in sera from stable (SA) and unstable angina (UA) patients. Incubation of U937 macrophages and human aortic SMC with sera from simvastatin treated SA or UA patients led to a decreased accumulation of esterified cholesterol as a function of the duration of simvastatin administration [36]. Although the duration of treatment (6 weeks) was short as compared with other clinical trials, simvastatin acted efficiently as a scavenger for peroxyl radicals.

Effects on platelet activation

Hypercholesterolemia is associated with hypercoagulability, as well as with increased platelet activation [1]. An increased level of LDL determines an increased platelets reactivity, associated with an increased thromboxan A_2 biosyntesis. Recently it was elucidated a new mechanism by which platelets activity is increased in hypercholesterolemia, due to LDL

inhibiting the platelet antiport Na^+/H^+ [37]. In addition, platelet dependent thrombin generation is increased in hypercholesterolemic subjects, and pravastatin treatment determines a restoration of thrombin formation. Statin therapy was accompanied by a reduction of platelet aggregation induced by ADP, collagen or fibrinogen, as well as of thromboxan production, in parallel with LDL cholesterol reduction.

Effects on the coagulation process

Primary and secondary prevention studies demonstrate that statin therapy reduces significantly thrombus formation. Recently, a link between the enhancement of endothelial fibrinolytic system and the inhibition of mevalonate pathway was reported [38]. Statins action involves an increase in tPA activity, as well as a decrease in PAI-1 activity [38]. The tissue factor plays an important role in the initiation of the extrinsec coagulation pathway and it was localized in lipid-loaded macrophages from atherosclerotic plaque. Recently, Colli *et al.* [39] have shown that lipophylic statins (simvastatin and fluvastatin) reduce the expression and activity of the tissue factor in human monocyte-derived macrophages in culture, effect prevented by the addition of mevalonate and trans-geranylgeraniol.

Other beneficial effects of statins

The fact that mevalonate plays a key role in cell proliferation and that many malignant cells present an increased HMG-CoA reductase activity, suggests that a selective inhibition of this enzyme could lead to a new chemotherapy for cancer disease [3].

Results obtained *in vitro* have shown that statins can inhibit tumor cell growth, a fact confirmed by some *in vivo* experiments also. The obtained reduction of sterols synthesis by statins, suggests that inhibition of tumor cell growth can be related to the reduction of nonsteroidal isoprenoid compounds. This effect can influence Ras protein farnesylation, thus inhibiting Ras dependent tumor cell growth [3].

Recent experimental evidence support a role for mevalonate pathway in murine and rabbit osteoclast formation and bone resorption. Lovastatin inhibited both processes, while mevalonate and geranylgeraniol prevented the effects, suggesting that a nonsterol intermediate is probably required for the prenylation of GTP-binding proteins that control cytoskeletal reorganization, vesicle fusion and apoptosis, processes involved in osteoclasts activation and survival. In addition, it was demonstrated *in vitro* and *in vivo* in rodents, that statins enhance new bone formation [3]. Statins administration is associated with a decrease of bone fracture risk in subjects over 50 years, probably because of the increase of the mineral density of the bones [4]. Thus, subjects with hyperlipidemia known to present increased risk for osteoporosis (mostly post-menopausal women) could benefit from statin therapy.

Adverse effects of statin therapy

Statins are generally well tolerated. The most important adverse effects are liver and muscle toxicity. Myopathy can happen if inhibitors of cytochrom P450 or other inhibitors of statins metabolism are administered together with statins, determining the increase of their blood concentration. Such are the azole antifungals [40]. Fibrates and niacin enhance myopathy risk by a mechanism not involving the increased statins blood concentration. Other risk factors are: hepatic dysfunction, renal insufficiency, hypothyroidism, advanced age and serious infections.

The already discussed suspension of cerivastatin from the clinical use, because of fatal rhabdomyolysis in a number of patients confirms the muscle toxicity of statins. On the other hand, it must be stressed that cerivastatin is at least 10 times more myotoxic than other statins and it was used in unusual high doses.

Conclusions

Statins are widely used for the treatment of hypercholesterolemia. They inhibit HMG-CoA reductase competitively, reduce LDL levels more than other cholesterol-lowering drugs, and lower triglycerides levels in hypertriglyceridemic patients. Statins have antiatherosclerotic effects, that correlate positively with the percent decrease in LDL

cholesterol. In addition, they can exert antiatherosclerotic effects independently of their hypolipidemic action (fig. 2). Because the mevalonate metabolism generates a series of vital isoprenoids for different cellular functions, from cholesterol synthesis to the control of cell growth and differentiation, HMG-CoA reductase inhibition has beneficial pleiotropic effects. Consequently, statins significantly reduce the incidence of coronary events, both in primary and secondary prevention, being the most efficient hypolipidemic compounds that have reduced the rate of mortality in coronary patients. Statins are well tolerated and have an excellent safety record. Independent of their hypolipidemic properties, statins interfere with events involved in bone formation. In addition, it was demonstrated that HMG-CoA reductase inhibitors impede tumor cell growth.

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