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Blood Cholesterol and Mitochondrial Toxicity of Simvastatin

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Abstract—Some adverse effects of simvastatin on biochemical reactions of cells and organs and the molecular mechanisms of simvastatin cytotoxicity are reviewed. Special attention is paid to the simvastatin-induced disturbance of mitochondrial functions both in vivo and in vitro.

Keywords: simvastatin, mitochondria, toxicity.

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Statins are widely used as cholesterol-lowering drugs, and they are well tolerated by patients. Statin therapy reduces the morbidity of coronary heart disease. Simvastatin is one of nine well-known statins, a specific and competitive inhibitor of the enzyme 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG CoA reductase) that limits the rate of cholesterol biosynthesis. It is suggested that simvastatin and other statins reduce cholesterol concentration in blood plasma via inhibiting the activity of that enzyme in the liver.

Along with the general safety, high efficiency, and popularity of statins, their side effects and complications after administration are also well-known in medicine. The molecular-cellular mechanisms of the toxic action of statins have not been revealed.

The materials discussed in this work were presented at a conference on mitochondrial biology in cardiovascular diseases [27].

CHOLESTEROL IN THE BLOOD

In medicine, word *cholesterol* is generally associated with the dangerous consequences of its accumulation within an organism. The abundance of cholesterol in the blood plasma represents a prognostic factor for the development of hypercholesteremia with natural consequences in the form of atherosclerosis and coronary heart disease. Pure cholesterol is an oncogene and mutagen, and it influences reproductive ability. Although cholesterol was isolated in pure form 220 years ago, it is not widely used in daily life. However, recommendations for cholesterol management based on indirect evidence have been developed. Administration of high doses of cholesterol may provoke irritation of the gastrointestinal tract and airways, skin, and eyes; it is recommended to avoid aspiration of the dust, vapors, and gases of cholesterol; its contact with skin and eyes; administration with food; and inhalation [63]. The features of cholesterol that allow it to act in such a way have not yet been investigated. A priori, it is accepted to consider cholesterol an adversary to human health.

Theoretically, life is not possible without cholesterol. It is a necessary and indispensable component of all cell membranes in the animal world; plants do not produce cholesterol at all. In the organism of mammals, cholesterol and its direct precursors form via metabolic pathways steroid hormones, bile acids, coenzyme Q (CoQ), and vitamins of the D group. Invisible and essential cholesterol is synthesized predominantly in the liver in a very complex way from products of fatty acid degradation (Fig. 1), although cholesterol itself does not belong to the lipid group. For 200 years, all chemists have known that cholesterol is a special spirit; however, modern biologists and physicians obstinately (and erroneously) refer it to the chemical class of lipids.

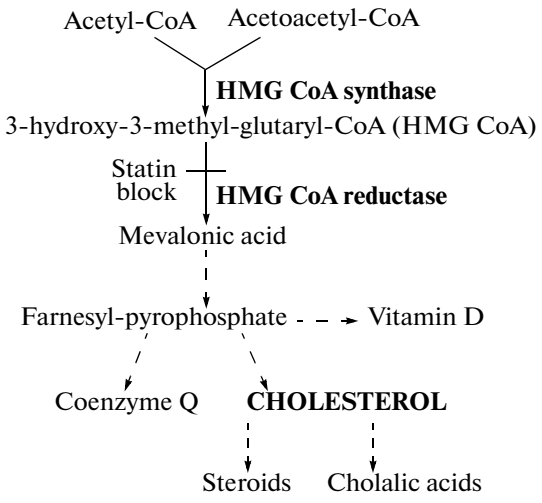

Fig. 1. Metabolic pathways for cholesterol. Multienzymatic reactions are indicated with dashed-line.

In atrabiliary capsules and ovaries, cholesterol is transformed into steroid hormones, while in liver cells it is transformed into bile acids participating in digestion.

Endogenous cholesterol in usual concentrations does not present any hazard to human health; it does not show any effect. Recently, the cytotoxicity of pure cholesterol was described for model cells [31]. Etherified cholesterol changed as a result of the attack of free oxygen radicals and other oxygen intermediators is dangerous; it is oxycholesterol and may be harmful in the content of lipoproteins also enriched with triglycerides and phospholipids. Lipoproteins of low density (LLD) are the main source of cholesterol and its ethers in the blood. Each spherical LLD particle has a diameter of 19–21 nm, mass of 4×10^{-18} g, molecular weight of 2.3–3 million and contains 600–900 molecules of cholesterol (8% of total particle mass), 1500–1900 molecules of cholesterol ethers (37%), 100–200 molecules of triglycerides (10%), 600–800 molecules of phospholipids (20%), and one molecule of ApoB100 protein (25%) [12]. It is not still known how these molecules combine into such a particle; it remains to be elucidated whether it a spontaneous or genetically programmed event.

It was reasonably suggested that the prevention and reduction of hypercholesteremia may be connected with the inhibition of cholesterol synthesis, which probably increased in pathologic conditions. Why "probably"? Because the rate of cholesterol synthesis in the liver has not been determined yet, and it is measured based on changes in concentration within a definite period of time. Thereby, the possibility of decreased utilization of existing and newly formed cholesterol is not taken into account.

Metabolic pathways of cholesterol consist of a few enzymatic stages and include modification of a dozen intermediate products (Fig. 1). HMG CoA reductase is a speed-limiting enzyme in the synthesis of cholesterol. This key enzyme is located in the liver in membranes of the endoplasmic reticulum and catalyzes the formation of HMG CoA, the first cholesterol precursor, from two products of beta-oxidation of fatty acids: the final product of acetoacetyl-CoA and the transitional product acetyl-CoA. The last point seems very important, because the role of acetoacetyl-CoA in cholesterol synthesis is completely ignored in the literature (see, for example, [61]); at the same time, the number of formed HMG CoA molecules and acetoacetyl-CoA molecules used for its synthesis is correlated by a stoichiometry of 1 : 1, but much less and nonproportional to the quantity of acetyl-CoA molecules.

STATINS

Inhibitors of HMG CoA reductase were named statins, and nine types are known at present: atorvastatin, lovastatin, mevastatin, pravastatin, pitavastatin, rosuvastatin, simvastatin, fluvastatin, and cerivastatin. In theory, statins reduce the concentration of cholesterol in blood plasma via inhibition of HMG CoA reductase in the liver. For these reasons, statins were successfully tested and used in medicine for the treatment of hypercholesteremia, hypertension, and heart ischemia and became very popular.

Investigations showed that statin therapy is very efficient where stroke prevention is concerned [19]. Besides decreasing LLD in blood plasma, statins act favorably on the stability of atheromatous plaques and the function of endotheliocytes; it also has anticoagulative and anti-inflammatory effects. Statins are used to treat other diseases such as ventricular arrhythmia, dilatation idiopathic cardiomyopathy, cancer, osteoporosis, and diabetes [16].

Sometimes, statin therapy lowers the mortality rate of coronary heart disease. In some clinical studies, statins reduced the mortality rate in 24–42% of cases [38, 47], thereby indicating the necessity for clarification of the molecular mechanisms of statin action.

TOXICITY OF STATINS

Statins do not form in the animal or human organism. Lovastatin, mevastatin (compactin), and pravastatin (a derivative of mevastatin) are natural metabolites in lower fungi, while simvastatin is a semisynthetic derivative of lovastatin. Atorvastatin, pitavastatin, rosuvastatin, fluvastatin, and cerivastatin are of entirely synthetic origin [35, 53]. It would be surprising to expect their nontoxic action within the human organism, since all of these compounds are xenobiotics.

Along with the general safety of statins, numerous side effects, some serious, and complications connected with their administration are known. Statins may provoke myopathy and other conditions [56]. Statins act on blood vessels and hemodynamics and on the liver, kidney, and muscle systems, including the heart. The most frequent side effect of statins is directed at skeletal muscles and appears as damage of various degrees: from myopathy of a moderate seriousness (myalgia, convulsions, exercise intolerance, rapid fatigability) to myositis or rhabdomyositis [59]. Rhabdomyolysis is a syndrome characterized by muscle necrosis and the release of toxic products of intracellular metabolism from the muscles to the blood; it may be a potentially fatal side effect of statin therapy. The irreversible damage to skeletal muscles may result in fatal kidney damage via a range of biochemical reactions [65].

According to the literature data, side effects of statins are rarely seen: the frequency of unfavorable consequences is 0.5%, and the frequency of myotoxicity is less than 0.1% [19]. However, there are other more disturbing data: the frequency of myotoxicity in patients receiving statin therapy varied from 1 to 5–7% [16, 22, 64]. Some investigators in their multiyear clinical practice reported that about 10% of patients developed various forms of myopathy after statin therapy [70]. According to the US Food and Drug Administration, the total number of

rhabdomyolysis cases due to administration of statins reached 601 over 29 months of observation [45] and 3339 over 12 years [64]. Seventy-three or 260 patients, according to one [60] or another [64] observation, respectively, died from rhabdomyolysis over 15 years of clinical statin use (from 1987 to 2001).

Due to the high mortality from rhabdomyolysis connected with cerivastatin administration, clinical utilization of that drug was totally prohibited in 2001 [60]; this caused the Bayer company to voluntarily withdraw from north American statin market. Despite this fact, cerivastatin is still used in Europe [54].

Statins were discovered by Japanese scientist Endo and his colleagues [17] during an investigation of 6000 fungal extracts; a single chemical compound, ML-236B, was revealed and later named compactin and then mevastatin [61]. A historical review by Steinberg on statin application in preventive cardiology resembles a heroic ode devoted to the author of discovery of statins [61]. In reality, statins represent a class of drugs used successfully in the treatment of hypercholesteremia, atherosclerosis, and coronary heart disease. The fact that from 1987 to 2001 US citizens were given 484 million prescriptions for statins [60]—32 million per year with a population of less than 300 million—attests to the popularity of statins.

However, the above-mentioned numbers also mean that the "wonderful picture on statin safety" (according to phrase by Glynn [21]) transformed to an optimistic drama and sometimes tragedy for 484 thousand (at a frequency of unfavorable conditions of 0.1%) or 48 million (at a frequency of 10%) holders of these prescriptions. It should be mentioned that only 20 cases of human death from prion diseases in the world by 1997 prompted scientists to start investigating Creutzfeldt-Jakob disease, which was supposedly not detected previously [1]. Statins are not prion proteins and are generally nontoxic compounds; however, 3339 cases of health hazard and the death of 260 patients as a result of treatment [64] are not very usual events in medicine. "Rare" cases of side effects amount to many hundreds of thousands of unfavorable outcomes and many hundreds of deaths in the United States (all mentioned cases are concerning only Americans and medical practice in the United States); probably, the level of statin damage in other countries is much higher.

The presented data are from published and official sources, which sometimes do not reflect the reality. Taking into account that physicians do not advertise their work and only in 1% of cases report on serious health problems in their patients [60], the risk of unfavorable consequences after statin therapy may be many times higher.

TOXICITY OF SIMVASTATIN

Simvastatin is the most frequently and successfully used statin. What are its toxic features? Out of 601 cases of rhabdomyolysis, 215 cases (or 36%) were registered for simvastatin [45]; out of 260 cases of fatal rhabdomyolysis, 49 cases (19%) were caused by simvastatin [64]. Considering this feature, simvastatin is less toxic (causes rhabdomyolysis and death less frequently) than cerivastatin, which was taken out of production.

The toxicity of simvastatin concerns various organs, tissues, and functions. In 5% of patients (in 5 out of 100 ambulant patients) taking simvastatin for 6 months, there was liver toxicity, with restoration only after stopping its administration [4]. Cases of rhabdomyolysis complicated with acute liver failure and requiring urgent hemodialysis were described for simvastatin [49, 71]. Administration of simvastatin was frequently accompanied by tendon pain [37]. The drug causes serious renal failure and acts unfavorably on trophoblasts when it is taken from women in the first trimester of pregnancy. Statins were frequently prescribed to recipients of transplanted organs with hyperlipidemia; however, a case of complicated rhabdomyolysis and acute renal failure in a patient with a transplanted heart was described after replacement of pravastatin by simvastatin [3]. Owing to the systematic administration of simvastatin, muscle necrosis developed in one patient [18]. It was reported that a combination of simvastatin with ezetimibe provoked liver failure that then required renal transplantation [67]. Simvastatin provokes lactic acidosis [22, 28] and pancreatitis [25, 57]. Rhabdomyolysis with cholangiolitic hepatitis developed in a patient receiving simvastatin with chlorzoxazone [6]. Rhabdomyolysis episodes were also provoked by simultaneous administration of ranolazine, carvedilol, and diltiazem together with cyclosporine and simvastatin [50]. Fatal rhabdomyolysis was detected after administration of usually safe propofol, methylprednisolone, and cyclosporin in combination with simvastatin [20]. More careful analysis revealed that 60% of all rhabdomyolysis cases occurred after simultaneous administration of statins with other drugs [8].

The above-mentioned facts dictate the necessity for clarification of mechanisms of simvastatin toxicity, and some investigations have been performed in animals. In rabbits receiving simvastatin via stomach intubation for 4 weeks, necrosis and degeneration of muscle fibrils were detected according to light microscopy, and damage and hypercontractility of myofibrils were found according to data of electron microscopy [42]. After 15–16 days of daily simvastatin introduction to rats, necrosis developed in 17 cyclosporine muscles under study (Latin names: extensor digitorum longus, gastrocnemius, biceps femoris, semitendinosus, semimembranosus, tibialis cranialis, vastus medialis, supraspinatus, triceps brachii caput longum, triceps brachii caput laterale, biceps brachii, extensor carpi radialis longus, trapezius, longissimus lumborum, diaphragm, abdominal peritoneal, panniculus carnosus from skin), which indicates the commonness of the effect [72]. Weakened three-dimensional memory was detected in rats of the Spreg-Douli line receiving simvastatin for a long time, in comparison with

Table 1. Toxicity of simvastatin

Object	Unfavorable action of simvastatin	Literature source
Patients	Rhabdomyolysis	[45, 60, 64]
Patients	Fatal rhabdomyolysis	[64]
Patients	Lactic acidosis	[22, 28]
Patient	Liver failure resulting in transplantation	[67]
Patient	Rhabdomyolysis with cholangiolitic hepatitis	[6]
Patient	Rhabdomyolysis and acute kidney failure	[49, 71]
Patient	Hepatotoxicity	[4]
Patient	Pains in tendons	[37]
Patient	Distortion of trophoblast proliferation in the first trimester of pregnancy	[32]
Recipient of transplanted heart	Rhabdomyolysis, acute kidney failure	[3]
Rabbits	Necrosis and degeneration, damage and hypercontractility of muscular fibrils	[42]
Rats	Necrosis of muscular fibrils in 17 different muscles	[72]
Rats	Cataract	[11]

control rats [5]. Introduction of simvastatin in therapeutic doses to the stomach of hamsters was lethal for them, and it caused hepatotoxic and nephrotoxic action [46]. Oral simvastatin became an inductor of cataract in rats of the Chbb:Thom line [11]. Side effects are briefly listed in Table 1.

CYTOTOXICITY OF SIMVASTATIN

Human cells. Simvastatin caused apoptosis in human differentiated skeletal muscular cells [51], heart myocytes [14], T- and B- tumor cells and myeloma [10], the TR-PCT1 cell line of pericytes and freshly isolated pericytes (cells surrounding endothelial cells in metarteriole, capillary tubes, and venous capillaries) [9], fibroblast-like synoviocytes obtained from patients with rheumatoid arthritis [74], and three cell lines of prostate tumor (PC3, DU145, and LnCap) [23]. In cells of the PC3 line, simvastatin causes apoptosis or necrosis depending on its concentration [44]. Incubation of HepG2 cells of human hepatocellular carcinoma with simvastatin is accompanied by their death [62]. Statin acts unfavorably on trophoblasts obtained from females during the first trimester of pregnancy: it inhibits the migration of extravilliferous trophoblast cells and proliferative events in villi, and it prevents secretion of progesterone in explants of the placenta [32].

Simvastatin inhibits the proliferation in culture of myoblasts of human striated muscle [68]. Among four studied statins (simvastatin, atorvastatin, cerivastatin, and fluvastatin), simvastatin was characterized by the highest cytotoxic activity against a culture of human leukemia cells (HL-60) [66].

Animal cells. Using simvastatin to treat the proliferating cells of mice uriniferous tubules [7], a culture of endothelial cells of rat pulmonary vein [29], and skeletal muscular cells of the rat L6 line [30] is connected with cell death. In an astrocyte culture from new-fallen rats, simvastatin caused the time- and dose-dependent formation of cells with starlike morphology and then apoptosis [40]. Similarly, simvastatin caused programmed cell death in a culture of granular neurons of rat cerebellum [40], a K562 cell culture of human myeloleukemia [73], a VOT-33 neuroblast culture of mice cochlea [48], a culture of nonstriated muscular cells of rabbit aorta [39], and OE33 and BIC-1 cell lines of gullet adenocarcinoma [43].

Cultured heart myocytes of mice treated with simvastatin lose their ability to contract and their resistance to oxidative stress [33].

Simvastatin reduces the growth, migration, and invasion of HT144, M14, and SK-MEL-28 melanoma cells [21]. Data on simvastatin cytotoxicity are summarized in Table 2.

Thus, simvastatin in pharmaceutical doses was toxic for a majority of human and animal cells and cell cultures, not only for tumor but also for normal cells. Generally, its toxicity results in apoptosis and is consequently connected with mitochondria damage.

MECHANISMS OF SIMVASTATIN CYTOTOXICITY

Some effects of statins are clearly not connected with inhibition of HMG CoA reductase [13] and are not considered here. The probable mechanisms of statin-induced myotoxicity are complex and not clear. They include the depletion of important intercellular metabolites and destabilization of cell membranes, resulting in increased cytotoxicity [19].

Simvastatin reduces synthesis of nuclear DNA in myoblasts of human skeletal muscles by more than 80% [68]. Treatment of human hepatocellular carcinoma HepG2 cells, a culture of endothelial cells of rat

Table 2. Cytotoxicity of simvastatin

Object	Action of simvastatin	Literature source
Cells of human skeletal muscle	Apoptosis	[51]
L6 cells of rat skeletal muscle	Cell death and DNA fragmentation	[30]
Mice cardiomyocytes	Reduction of retractive activity	[33]
T-cells, B-cells, cancer cells of human myeloma	Apoptosis	[10]
HL-60 cells of human leukemia	Apoptosis	[66]
HepG2 cells of human hepatoma	Cell death and oxidative damage of DNA	[62]
PC3 cells of human prostate cancer	Apoptosis or necrosis	[44]
TR-PCT1 cell line of pericytes and freshly isolated pericytes	Apoptosis	[9]
Myoblasts from human skeletal muscle	Inhibition of proliferation and synthesis of DNA	[68]
Cells of mice kidney tubules	Apoptosis	[7]
Astrocytes and granular neurons of rat cerebellum in culture	Apoptosis	[40]
Endotheliocytes of rat pulmonary vein in culture	Reduced viability, DNA fragmentation, apoptosis	[29]
Cell lines of HT144, M14, and SK-MEL-28 melanoma	Apoptosis and growth inhibition, migration and invasions	[21]

pulmonary vein [29], and skeletal muscular cells of the rat L6 line [30] with simvastatin resulted in an increase in oxidative damage and fragmentation of DNA. Thus, there is a cause-and-effect relationship between simvastatin action and damage to nuclear DNA.

After rats were treated for more than 10 days with simvastatin, the only change in muscular cells, in which the necrosis of individual fibrils was observed, was the morphologic alteration of mitochondria [72]. This experimental fact proves the primary role of mitochondrial dysfunction in simvastatin-induced myotoxicity.

Simvastatin has a toxic action on mitochondria of the heart, skeletal muscles, and liver, as well as the mitochondria of a range of cell cultures both in vivo and in vitro. Although simvastatin prevents apoptosis in cells caused by other chemicals (see for example [26]), data on the favorable action of simvastatin on intact mitochondria are still absent. The majority of toxic effects of simvastatin are connected with mitotoxicity, with numerous alterations of the structure and functions of mitochondria.

Influence of simvastatin on mitochondrial functions in vivo. In patients with hypercholesteremia, simvastatin therapy is accompanied by an increase in the lactate : pyruvate ratio in the blood, which indicates the distortion of mitochondrial functions [15]. Due to simvastatin therapy, the amount of mitochondrial DNA is reduced in bioptates of skeletal muscles of a patient [55], which suggests the participation of simvastatin in the pathological process connected with fragmentation of mitochondrial DNA in the muscle.

CoQ depletion resulting in the abruption of mitochondrial functions, the traditional explanation for simvastatin cytotoxicity, may be one of the possible mechanisms of statin-induced myopathy. Statins inhibit the enzymatic restoration of HMG CoA to mevalonic acid, a precursor of CoQ (Fig. 1), and inhibit CoQ biosynthesis indirectly.

CoQ is synthesized in cell cytoplasm but carries out its electron-transport function only in mitochondria. CoQ is a key and indispensable component of the mitochondrial respiratory chain. It is CoQ that accepts electrons from dehydrogenases of respiratory substrates and transfers it to the cytochrome part of the respiratory chain; i.e., it is a link between complex I and complex III, and between complex II and complex III.

Mitochondrial swelling and reduction of CoQ content in skeletal muscles are detected after chronic administration of simvastatin in rabbits [42]. The 3-week introduction of simvastatin to dogs resulted in a reduction of CoQ content in the myocardium and deteriorated mitochondrial respiration in heart muscle [52]. Oral administration of simvastatin to mice resulted in decreasing CoQ concentration in the liver and heart, while there was a significant increase in compounds reacting with thiobarbiturates [33]. The latter fact points to the activation of oxidation stress in mitochondria. Treatment of rats of the Spreg-Douli and Chbb::Thom lines with simvastatin reduced the concentration of CoQ in the crystalline lens [11] and, therefore, altered mitochondrial functions in the filamentous cells of the crystalline lens.

In patients with myalgia related to simvastatin therapy, the administration of CoQ does not influence myalgia [75]. These results allow one to suggest that CoQ taken orally does not reach the mitochondria (for example, it penetrates cellular and mitochondrial membranes poorly, it is not included in the respiratory chain, or it may be damaged on the way to mitochondria), or it is possible to think that CoQ deficiency is not the only reason for mitochondrial dysfunction. In

Table 3. Mitotoxicity of simvastatin in vivo

Object	Action of simvastatin	Literature source
Human skeletal muscle	Fragmentation of mitochondrial DNA	[55]
Rat skeletal muscle	Alteration of mitochondrial morphology	[72]
Rabbit skeletal muscle	Mitochondrial swelling and reduction of CoQ content	[42]
Dog myocardium	Reduction of CoQ content and rate of mitochondrial respiration	[52]
Mitochondria of mice liver and heart	Reduction of CoQ content, increase in products of peroxide lipid oxidation	[33]
Crystalline lens of rat	Reduction of CoQ content	[11]

an analysis of the relationship between statin therapy and CoQ content, it was concluded that it is not currently possible to state the etiologic role of CoQ deficiency in statin-induced myopathy [36].

Mitochondria isolated from the liver of rats with hypercholesteremia, knocked out on the receptor for LLD and treated with therapeutic doses of simvastatin for 15 days, are more sensitive (in comparison with liver mitochondria of normal rats) to the development of mitochondrial stoma of nonspecific permeability (MSP). In in vitro experiments, simvastatin induced MSP on the mechanism sensitive to cyclosporine (blocker of permeability stoma), dithiothreitol (deoxidizer of sulfhydryl groups in proteins), the inhibitor of transmembrane transport of adenine-nucleotides, catalase (neutralizing the toxic action of H_2O_2), and EGTA (ethylene glycol tetraacetic acid, a calcium chelator) [69], and it reduced the content of SH-groups in proteins of the mitochondrial membrane. Thus, statin may act on mitochondria in vivo via induction of MSP, a process involved in cell death. A brief list of simvastatin's toxic effects on mitochondria in vivo is presented in Table 3.

Simvastatin influence on mitochondrial function in vitro. The important role of CoQ deficiency in simvastatin-induced cytotoxicity was demonstrated for hepatoma cell culture HepG2 [62]. Simvastatin reduced the content of CoQ in mitochondria; at higher concentrations, it increased the proportion of dead cells, enhanced oxidative damage to DNA, and decelerated ATP synthesis. Addition of CoQ to the culture resulted in prevention of the above-mentioned damage. These results suggest that CoQ deficiency plays an important role in statin-induced hepatopathy in vitro and that supplementation with CoQ protects HepG2 cells from this complication.

With the help of fluorescence imaging analysis and oxigraphy on skeletal muscle samples of humans and rats, it was shown that simvastatin-induced mitochondrial damage occurs as a result of inhibition of the respiratory chain complex I [58]. Similar alterations in mitochondria and changes in Ca^{2+} homeostasis, caused by simvastatin, were also detected in cardiomyocytes of rat myocardium.

Acute application of simvastatin to fibrils of human skeletal muscle includes a wave of intracellular calcium as a result of Ca^{2+} release from the sarcoplasmic reticulum, and it increases NADN content in mitochondria and provokes depolarization of the mitochondrial inner membrane [59].

Simvastatin in the dose of 10 μM induces apoptosis in PC3 cells, which cannot be prevented by cyclosporine A, an inhibitor of calcineurin and MSP blocker. In the concentration of 60 μM, simvastatin provokes necrosis with a precedential 3-fold increase in free Ca^{2+} in the cytoplasm and a significant reduction of the respiration rate in mitochondria and mitochondrial membrane potential. Mitochondrial dysfunction and cell necrosis are sensitive to cyclosporine A and bongkrekic acid, an inhibitor of adenine nucleotide translocase, and also to calcineurin inhibitor FK506. Thus, simvastatin-induced apoptosis in PC3 cells depends on inhibition of HMG CoA reductase and not on MSP, while simvastatin-induced necrosis is mediated by dysfunctions in mitochondria and calcineurin [44].

Simvastatin worsens mitochondrial respiration and inhibits complexes I and II + III of oxidative phosphorylation in isolated mitochondria of rat liver [41]. This means that simvastatin directly changes mitochondrial functions in the absence of a cell membrane and cytoplasmic HMG CoA reductase.

MSP induction is one of the apoptotic markers. However, in the PC3 cell line of human prostate cancer, simvastatin predominantly induces apoptosis, which cannot be prevented with cyclosporine A and does not depend on MSP [44]: this indicates that simvastatin is responsible for apoptotic induction, when significant mitochondrial damage occurs.

Mitochondrial damage may result in induction of cytoplasmic cysteine protease: caspase-9 and caspase-3. Together with eight other caspases detected in the brain, both enzymes participate in the caspase cascade, which is responsible for the late stages of apop-

tosis [69]. After binding with Apaf-1 (factor 1 for activation of apoptotic proteases), which requires the presence of ATP (or dATP) and cytochrome *c* in cytosol, procaspase-9 is activated up to caspase-9, and the latter is able to destroy and activate caspase-3. Caspase-3 may directly participate in DNA fragmentation [24] or activate endonucleases (for example, caspase-activated DNAse [2], which damages chromatin), thereby finishing apoptosis.

Cytochrome *c*, usually located in the intermembrane space of mitochondria, is one of the proteins activating the caspase cascade. It is released from mitochondria through the external membrane into cytosol at the early stages of apoptosis [34]. After that, cytochrome *c* starts the proteolytic maturation of caspases inside the apoptosome: a complex of Apaf-1, procaspase-9, ATP (or dATP), and cytochrome *c* [34].

The ability of statins to activate the caspase cascade was assessed in vitro, and stringent evidence was obtained for their ability to act on this manner of cell death. Simvastatin caused apoptosis in a primary culture of human skeletal muscle cells [51]. These studies showed that simvastatin triggers incessant intracellular Ca^{2+} transitions, resulting in activation of calpain and translocation of Bax factor to mitochondria via a pathway independent of caspase-8. The subsequent activation of caspase-9 and caspase-3 terminates apoptotic cell death. Boucher et al. [9] showed that simvastatin induces apoptosis in the TR-PCT1 cell line of pericytes and in freshly isolated pericytes in a process dependent on cholesterol, caspase-3, and caspase-7. Simvastatin treatment of fibroblast-like synoviocytes obtained from patients with rheumatoid arthritis reduces their viability and provokes apoptosis dependent on the dose of simvastatin, with the participation of caspase-3 and caspase-9 [74].

Simvastatin effectively reduces the viability of three cell lines of prostate cancer (PC3, DU145, and LnCap) via induction of apoptosis and blockade of cell growth at the G_1 stage, as well as activation of caspase-6, caspase-3, and caspase-9 [23]. In the primary culture of human skeletal muscle cells, simvastatin causes apoptosis after 24 h; there are fewer than 20 % viable cells after 72 h, and there is 2- to 3-fold increase in the activity of cytoplasmic caspase-3 and caspase-9 [51]. In HL-60 cells, simvastatin directly and rapidly destroys mitochondria with the loss of membrane potential; it accelerates the generation of active oxygen intermediators and the subsequent irreversible damage with the release of cytochrome *c* and apoptosis via activation of caspase-9 [66]. Simvastatin provokes the release of the second caspase activator of mitochondrial origin into cytosol in T and B cancer cells and human myeloma [10]. Taken together, these data indicate that numerous caspases may participate in simvastatin-induced apoptosis in various cells in vitro.

HMG CoA reductase is a rate-limiting enzyme in the biosynthesis of cholesterol and other products participating in prenylation and farnesylation of some important membrane proteins. Simvastatin treatment of proliferating cells from the proximal tubule of mice is accompanied by cytochrome *c* release from mitochondria to cytosol, and activation of caspase-9 and caspase-3 [7]. These changes may be prevented by mevalonic acid, farnesyl-pyrophosphate, and geranyl-geranyl-pyrophosphate. In fibroblast-like synoviocytes from patients with rheumatoid arthritis and in the VOT-33 culture of neuroblasts from mice cochlea, simvastatin increases the activity of caspase-3, and mevalonic acid prevents this action. These data allow one to suggest that protein prenylation is involved in simvastatin cytotoxicity. A brief list of simvastatin toxic effects in vitro on mitochondria is presented in Table 4.

One additional possible mechanism. Alteration of mitochondrial beta-oxidation of fatty acids may be one of the mechanisms of simvastatin cytotoxicity. However, it is not yet described in the literature.

It is known that the activity of beta-oxidation of fatty acids in isolated mitochondria from the L6 cell culture of rat skeletal muscle decreases by 88–96 % at a simvastatin concentration causing death in 27–49% of cells [30]. These dramatic changes in the oxidation of fatty acids influence mitochondrial functions, resulting in mitochondrial swelling, cytochrome *c* release to cytosol, and fragmentation of mitochondrial DNA. It is widely accepted that alteration of fatty acid metabolism in mitochondria is directly connected with the inhibiting action of simvastatin (and other statins) on the microsomal enzyme HMG CoA reductase.

Another enzyme, HMG CoA synthase, catalyzes HMG CoA biosynthesis from two substrates: acetyl-CoA, the substrate for synthesis of fatty acids and lipids, and acetoacetyl-CoA, the final product of beta-oxidation of fatty acids (Fig. 1). Therefore, statins should be considered not only as inhibitors of HMG CoA reductase but also as compounds distorting the balance in HMG CoA metabolism. Statins block the pathway of cholesterol biosynthesis at the stage of HMG CoA reductase, which does not allow HMG CoA to change at the necessary rate and favors its rapid accumulation. This block results in the depletion of intermediators formed after this block and the accumulation of intermediators preceding this block in the metabolic pathway. HMG CoA synthase is localized in mitochondria; therefore, inhibition of microsomal HMG CoA reductase should result in accumulation of HMG CoA, acetyl-CoA, and acetoacetyl-CoA and in distortion of the metabolism of fatty acids and lipids in mitochondria. At present, it is not known whether these abnormalities contribute to simvastatin-induced cell death and myopathy. A brief list of the toxic effects of simvastatin on mitochondria is presented in Fig. 2.

CONCLUSIONS

Besides its ability to reduce levels of cholesterol in blood plasma and protect from cardiovascular disease, simvastatin may induce myopathy and other side and

Table 4. Mitotoxicity of simvastatin in vitro

Object	Action of simvastatin	Literature source
Fibrils of human skeletal muscle	Depolarization of mitochondrial membrane, increase in NADN content in mitochondria	[59]
Skeletal muscle of humans and rats, cardiomyocytes of rat	Inhibition of complex I of the respiratory chain, distortion of Ca^{2+} homeostasis	[58]
L6 cells of mice skeletal muscle	Depolarization of mitochondrial membrane	[30]
T-cells, B-cells, cancer cells of human myeloma	Depolarization of mitochondrial membrane, release of secondary activator of caspase from mitochondria to cytosol	[10]
Cells of mice kidney tubules	Release of cytochrome *c* from mitochondria, activation of caspase-3	[7]
Mitochondria from L6 cells	Reduction of respiration rate and coefficient of respiration control, activity of beta-oxidation, mitochondrial swelling, loss of cytochrome *c*	[30]
HepG2 cells	Reduction of CoQ content and rate of ATP synthesis	[62]
HL-60 cells of human leukemia	Depolarization of mitochondrial membrane, stimulation of oxygen radical generation, loss of cytochrome *c*	[66]
Cancer cells of human prostate PC3 line	Reduction of respiration rate, depolarization of mitochondrial membrane	[44]
Mitochondria from rat liver	Inhibition of respiration and oxidative phosphorylation	[41]
Mitochondria from mice liver (gene for LLD receptor)	Increase in sensitivity to development of nonspecific membrane permeability	[69]

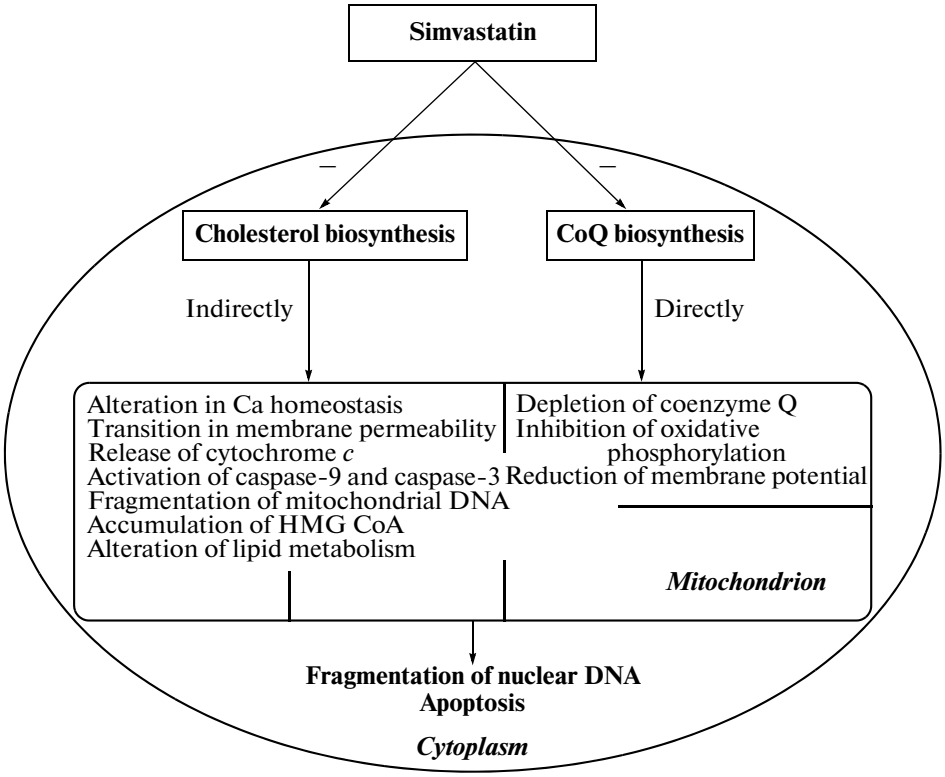

Fig. 2. Mitotoxic effects of simvastatin.

algetic effects in tissues and cells of humans and other mammals. The main part of simvastatin toxic effects is related to mitochondrial dysfunctions. Administration of simvastatin in vivo in humans and animals and its addition in vitro to various cells provoke the majority of mitochondrial alterations; no favorable effect of simvastatin on intact mitochondria has been found to date. Simvastatin may directly influence mitochondria independently of its action on HMG CoA reductase. Mechanisms of simvastatin cytotoxicity include a cascade of reactions starting in mitochondria and resulting in cell death. One of these events may be connected with the accumulation of HMG CoA, acetyl-CoA, and acetoacetyl-CoA in mitochondria. Clarification of the mechanisms for simvastatin cytotoxicity and myotoxicity may be useful for understanding the molecular mechanisms of statin side effects, in revealing why only 0.1 to 10% of patients taking simvastatin are subject to the risk of secondary pathology and in prevention of neopathy.

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