

# Molecular mechanisms of toxicity of simvastatin, widely used cholesterol-lowering drug. A review

Review Article

Yury G. Kaminsky\*, Elena A. Kosenko

*Institute of Theoretical and Experimental Biophysics,  
Russian Academy of Sciences, 142290 Pushchino, Russia*

Received 26 June 2009; Accepted 2 November 2009

**Abstract:** Statins are widely used and well tolerated cholesterol-lowering drugs, and when used for therapy purposes reduce morbidity and mortality from coronary heart disease. Simvastatin is one of nine known statins, specific inhibitors of hepatic enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase, the rate-limiting step of cholesterol biosynthesis, and is believed to reduce plasma cholesterol levels by decreasing the activity of this enzyme. Statin drugs represent the major improvement in the treatment of hypercholesterolemia that constitutes the main origin of atherosclerosis, leading to coronary heart disease. Although statins are generally safe, minor and severe adverse reactions are well known complications of statin use. Adverse events associated with simvastatin therapy are uncommon, but potentially serious. In this review some details about statins including their adverse effects in humans and animals, the effects of simvastatin on various intracellular and mitochondrial processes, and molecular mechanisms underlying simvastatin cytotoxicity are discussed.

**Keywords:** *Statins • Simvastatin • Mitochondria • Toxicity*

© Versita Sp. z o.o.

## 1. Introduction

In medicine, “cholesterol” has negative connotations associated with it is commonly linked to dangerous consequences of cholesterol accumulation in the body. Extra plasma cholesterol is a symptom prognostic of stable hypercholesterolemia with consequences of atherosclerosis and coronary heart disease.

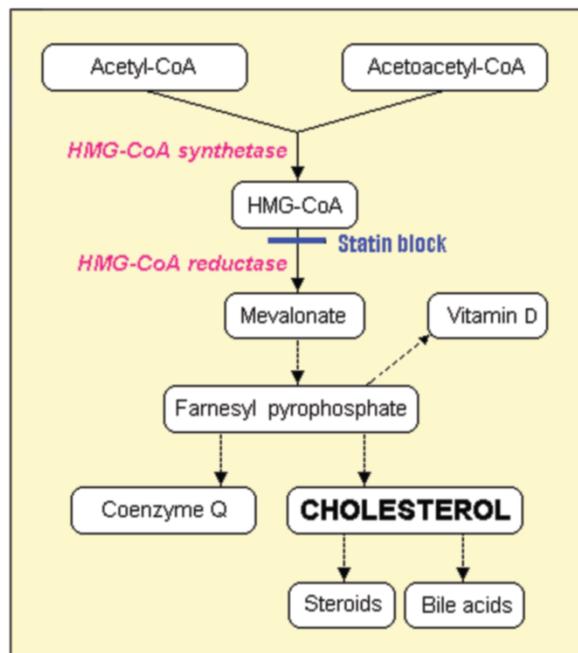
Cholesterol is essential to life. It is an absolutely indispensable component of any cellular membrane. Cholesterol and immediate upstream metabolites of its biosynthetic pathway are precursors of steroid hormones, bile acids, Coenzyme Q (CoQ) and vitamin D. It is synthesized mainly by the liver from fatty acid metabolites. It is supposed that a decrease in the reaction rate of cholesterol biosynthesis can be a mean for prevention or attenuation of hypercholesterolemia.

The biosynthetic pathway of cholesterol proceeds through several intermediates and involves a number of enzymes (Figure 1). The rate-limiting step of cholesterol synthesis from 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) is HMG-CoA reductase.

HMG-CoA reductase inhibitors are statins. There are nine Statin species today: atorvastatin, cerivastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin. In theory, statins reduce plasma cholesterol levels by decreasing the activity of HMG-CoA reductase and hence the rate of cholesterol biosynthesis in the liver. Along this line, statins were tested with great success, prescribed for hypercholesterolemia, hypertension and ischemia. Statin drugs represent a major improvement in the treatment of hypercholesterolemia that constitutes the main origin of atherosclerosis, which leads to coronary heart disease. Now, statins are widely used and well tolerated cholesterol-lowering drugs, and in addition to this, statin therapy reduces morbidity and mortality from coronary heart disease. Recent evidence has demonstrated that benefits of statin therapy may also extend into stroke prevention [1,2]. In addition to lowering low density lipoprotein (LDL) cholesterol [3,4], the benefit has been attributed to several other effects of statins, such as improved plaque stability [5,6], endothelial cell function [7] or antithrombotic and anti-inflammatory effects [2]. Statin use has expanded to the treatment of many other

\* E-mail: kaminsky@iteb.ru

**Figure 1.** Metabolic pathways of cholesterol. Dotted lines indicate multienzyme reactions.



conditions, including ventricular arrhythmias, idiopathic dilated cardiomyopathy, cancer, osteoporosis, and diabetes [8].

Statin therapy has been found to decrease coronary heart disease events and mortality, but not all-cause mortality. Several large clinical trials have documented that statins reduce death from coronary disease or stroke by 24–42% [9,10], underscoring the need for ascertainment of underlying mechanisms.

In this review some details about statins including their adverse effects in humans and animals, the effects of simvastatin on various intracellular and mitochondrial processes, and molecular mechanisms underlying simvastatin cytotoxicity are discussed. Although work in this area spans over a 20 year period, comprehensive understanding of these molecular mechanisms remains a non-trifling task.

The brief account of this work was recently considered (Y. Kaminsky and E. Kosenko, presented at The Mitochondrial Biology in Cardiovascular Health and Diseases Conference, Bethesda, ML, USA, October 6 and 7, 2008).

## 2. Toxicity of statins

Statin are not formed in the human or animal body. Lovastatin, mevastatin (compactin) and pravastatin (mevastatin derived) are natural statins of fungal origin,

while simvastatin is a semi-synthetic lovastatin derivative. Atorvastatin, cerivastatin, fluvastatin, pitavastatin and rosuvastatin are fully synthetic compounds [11,12].

Although statins are generally safe, minor and severe adverse reactions are well known complications of statin use [13]. Hepatic, renal and muscular systems, including myocardium, are affected during statin therapy. Adverse reactions affecting skeletal muscle are the most common, ranging from mild myopathy (myalgia, cramp, exercise intolerance, and fatigability) to myositis and rarely to serious rhabdomyolysis [14]. Rhabdomyolysis, a syndrome characterized by muscle necrosis with release of toxic intracellular muscle constituents, can be a potentially fatal side effect of statin therapy.

Side effects of statins are rare: an overall adverse event frequency is less than 0.5% and a myotoxicity event rate is less than 0.1% [1,15]. However, there are other and more inauspicious data. The incidence of myotoxic reactions in patients treated with statins ranges from 1% to 5%–7% [8,16,17]. Venero and Thompson [18] reported approximately 10% of patients treated with statins experienced some form of muscle-related side effects in clinical practice. The US Food and Drug Administration has registered 601 cases of statin-associated rhabdomyolysis in the 29-month time frame examined [19] and 3339 cases within the 12-years period [17]. For first 15 years of clinical use of statins in the USA (1987 to 2001), death was listed as the outcome in 73 cases of statin-associated rhabdomyolysis as reviewed by Staffa et al. [20] and even 260 cases by Thompson et al. [17]. Given that only about 1% of all serious events are directly reported by physicians [20], the risk of adverse events may be increased many times.

## 3. Toxicity of simvastatin

Simvastatin seems to be the most commonly prescribed statin. In 601 clinical cases of statin-associated rhabdomyolysis, 215 cases (36%) were associated with simvastatin [19], and in 260 cases of fatal rhabdomyolysis 49 cases (19%) were attributed to simvastatin [17]. To these appearances, myotoxicity of simvastatin (the incidence of rhabdomyolysis) is only 1/5 to 1/3 that of cerivastatin, the drug totally disused in 2001.

Simvastatin-induced toxicity expatiates upon different organs, tissues and functions. In 5% of simvastatin-treated patients significant liver toxicity was observed, which required drug discontinuation [21]. Waness et al. [22] and Qari [23] have described a case of simvastatin-induced rhabdomyolysis complicated by acute renal failure requiring urgent hemodialysis. Tendinopathy often occurred after simvastatin initiation [24]. Statins

**Table 1.** Adverse effects of simvastatin in humans and animals.

Objects	Adverse effects of simvastatin	Refs
Patients	Rhabdomyolysis	[17,19,20]
	Fatal rhabdomyolysis	[17]
	Lactic acidosis	[16,28]
	Liver toxicity	[21]
	Tendinopathy	[24]
Cases	Rhabdomyolysis complicated by acute renal failure	[22]
	Liver failure resulted in liver transplantation	[27]
	Rhabdomyolysis with cholestatic hepatitis	[31]
	Proliferation of first trimester trophoblast	[47]
A cardiac transplant recipient	Rhabdomyolysis, acute renal failure	[25]
Rabbits	Muscle fiber necrosis and degeneration, disruption and hypercontraction	[34]
Rats	Muscle fibre necrosis in 17 individual muscles	[35]
	Impairment of spatial memory	[36]
	Cataract	[38]
Hamsters	Hepatotoxic and nephrotoxic effects	[37]

were widely prescribed to organ transplant recipients with hyperlipidemia, however, a case was reported of a cardiac transplant recipient who developed severe rhabdomyolysis and acute renal failure after being switched from pravastatin to simvastatin [25]. A patient developed muscular necrosis while on simvastatin treatment [26]. There was the case report of simvastatin plus ezetimibe-induced liver failure that resulted in liver transplantation [27]. Simvastatin induced lactic acidosis [16,28] and pancreatitis [29,30]. Bielecki et al. [31] have reported the case of marked rhabdomyolysis with cholestatic hepatitis in a patient treated with simvastatin and chlorzoxazone. Ranolazine, carvedilol, and diltiazem, acting in concert with cyclosporin and simvastatin, caused the episode of rhabdomyolysis [32]. The combination of generally harmless propofol, methylprednisolone, and cyclosporin with simvastatin triggered rhabdomyolysis and death of the patient [33].

The above dictates the ascertainment of mechanisms underlying toxicity of simvastatin.

Simvastatin-induced myotoxicity and other adverse events are confirmed by experimental studies. In rabbits administered with simvastatin by gavage for 4 weeks, the pathological findings are muscle fiber necrosis and degeneration by light microscopy and disruption and hypercontraction of myofibrils by electron microscopy [34]. For 15–16 days of daily administration of simvastatin to rats the development of muscle fiber necrosis occurs in 17 particular individual muscles (extensor digitorum longus, gastrocnemius, biceps femoris, semitendinosus, semimembranosus, tibialis cranialis, vastus medialis, supraspinatus, triceps brachii caputlongum, triceps brachii caputlaterale, biceps brachii, extensor carpi radialis longus, trapezius, longissimus

lumborum, diaphragm, abdominal peritoneal, panniculus carnosus from skin) [35] indicating a communion of the effect. When studying the behavioral effects of long-term simvastatin application using Sprague-Dawley rats Baytan et al. [36] found poorer scores on spatial memory compared to the vehicle group. In hamsters, gastric administration of simvastatin was lethal and had hepatotoxic and nephrotoxic effects [37]. Oral simvastatin was an inducer of cataracts in Chhb:Thom rats [38].

The summary of adverse effects of simvastatin is given in Table 1.

## 4. Cytotoxicity of simvastatin

### 4.1. Human cells

Simvastatin induces apoptosis in human skeletal muscle cells [39] and cardiac myocytes [40], human T, B and myeloma tumor cells [41], the TR-PCT1 pericyte cell line and freshly isolated human pericytes, which surround endothelial cells in precapillary arterioles, capillaries, and postcapillary venules [42], fibroblast-like synoviocytes derived from patients with rheumatoid arthritis [43], three human prostate cancer cell lines (PC3, DU145, and LnCap) [44]. In the PC3 cell line, simvastatin induces apoptosis or necrosis depending on concentration [45]. Simvastatin application to human hepatocellular carcinoma HepG2 cells [46] is associated with cell death. This statin adversely affects human first trimester trophoblasts: inhibits migration of extravillous trophoblast cells and the proliferative events in the villi, decreases secretion of progesterone by placental explants [47]. In cultured myoblasts from human

**Table 2.** Cytotoxicity of simvastatin.

Object	Effects of simvastatin	Refs
Human skeletal muscle cells	Apoptosis	[39]
Human T, B and myeloma tumor cells	Apoptosis	[41]
Human leukemia cells HL-60	Apoptosis	[49]
Human hepatoma cells HepG2	Cell death and DNA oxidative damage	[46]
PC3 human prostate cancer cell line	Apoptosis or necrosis	[45]
Freshly isolated human pericytes, TR-PCT1 pericyte cell line	Apoptosis	[42]
Myoblasts from human striated muscle	Inhibition of proliferation and DNA synthesis	[48]
Mouse cardiac myocytes	Decreased responsiveness to electrical field stimulation	[58]
Murine tubular cells	Apoptosis	[50]
L6 rat skeletal muscle cell line	Cells death and DNA fragmentation	[52]
Cultured rat astrocytes, cultured rat cerebellar granule neurons	Stellation and apoptosis	[53]
Cultured rat pulmonary vein endothelial cells	Reduced cell viability, DNA fragmentation and apoptotic cell death	[51]
HT144, M14 and SK-MEL-28 melanoma cell lines	Apoptosis and inhibition of the cell growth, migration and invasion	[59]

striated muscle, simvastatin inhibits the proliferation of these cells [48]. Among four tested statins (simvastatin, atorvastatin, cerivastatin, fluvastatin), simvastatin has the most cytotoxic potency against cultured human leukemia cells HL-60 [49].

#### 4.2. Animal cells

Treatment of proliferating murine tubular cells [50], cultured rat pulmonary vein endothelial cells [51] and the L6 rat skeletal muscle cell line [52] with simvastatin is associated with cell death. In cultured astrocytes from newborn rats, simvastatin causes a time- and dose-dependent stellation, followed by apoptosis. Similarly, the statin elicits programmed cell death of cultured rat cerebellar granule neurons [53], cultured human myeloleukemia K562 cells [54], cultured mouse cochlear neuroblasts VOT-33 [55], cultured rabbit aorta smooth muscle cell [56], the OE33 and BIC-1 esophageal adenocarcinoma cell lines [57].

Cultured mouse cardiac myocytes treated with simvastatin lose in part the ability to contract and resistance to oxidative stress [58].

Simvastatin inhibits the growth, cell migration and invasion of HT144, M14 and SK-MEL-28 melanoma cells [59].

The summary of cytotoxicity of simvastatin is given in Table 2.

Thus, simvastatin appears to be toxic to a number of human, animal and cultured cells, not only tumorigenic but also normal ones. Its cytotoxicity is usually terminated by apoptosis and, therefore, associated with mitochondrial dysfunction.

## 5. Mechanisms of cytotoxicity of simvastatin

Some of the effects of the statins are not definitely related to the inhibition of the HMG-CoA reductase and need to be clarified [60].

The potential mechanisms underlying statin-induced myotoxicity are complex with no clear consensus of opinion. Candidate mechanisms include intracellular depletion of essential metabolites and destabilization of cell membranes, resulting in increased cytotoxicity [1].

In cultured myoblasts from human striated muscle, simvastatin decreases nuclear DNA synthesis by more than 80% [48]. Simvastatin application to human hepatocellular carcinoma HepG2 cells [46], cultured rat pulmonary vein endothelial cells [51] and the L6 rat skeletal muscle cell line [52] is associated with increased DNA oxidative damage and fragmentation. Causal connection between simvastatin effect and nuclear DNA damage is traceable.

After treatment of rats with simvastatin for more than 10 days, in some fibers from skeletal muscles showing early multifocal single fiber necrosis the only subcellular alterations are morphological alterations of mitochondria [35]. This experimental fact supports the primary role of mitochondrial dysfunction in simvastatin-induced myotoxicity.

Simvastatin exerts toxic influence on heart, skeletal muscle and liver mitochondria and on mitochondria of a number of cultured cells, both *in vivo* and *in vitro*. Although simvastatin antagonized cell apoptosis induced by other drugs (for example, see [61]), no beneficial effect of simvastatin on intact mitochondria was reported up to now.

**Table 3.** Effects of simvastatin on mitochondria *in vivo*.

Object	Effects of simvastatin	Refs
Human skeletal muscle	Mitochondrial DNA fragmentation	[62]
Rat skeletal muscle	Changes in mitochondrial morphology	[35]
Rabbit skeletal muscle	Mitochondrial swelling and a decrease in CoQ	[34]
Dog myocardium	Decrease in CoQ and mitochondrial respiration	[64]
Rat lens	Decrease in CoQ	[38]
Mouse liver and heart mitochondria	Decrease in CoQ, increase in lipid peroxidation products	[58]

### 5.1. Effects of simvastatin on mitochondrial function *in vivo*

During simvastatin therapy, mitochondrial DNA in patient's skeletal muscle biopsy specimens is reduced [62] suggesting that simvastatin is involved in pathological processes associated with muscle mitochondrial DNA fragmentation. In hypercholesterolaemic patients, simvastatin therapy is associated with high blood lactate/pyruvate ratio suggestive of mitochondrial dysfunction [63].

Mitochondrial dysfunction as a consequence of depletion of CoQ is considered one of possible mechanisms of simvastatin-induced myopathy and acquiring currency explanation of cytotoxicity of simvastatin. Statins inhibit the conversion of HMG-CoA to mevalonate, a precursor of CoQ (Figure 1), and inhibit CoQ biosynthesis indirectly but necessarily. CoQ is synthesized in the cytoplasm but functions only in mitochondria. It is a key and essential coenzyme of the mitochondrial electron transport chain. Just CoQ accepts electrons from dehydrogenases of respiratory substrates and transfers these electrons to the cytochrome part of the respiratory chain, i.e. is a link between oxidative phosphorylation Complexes I and Complex III, Complexes II and Complex III.

In rabbits administered chronically with simvastatin, skeletal muscle mitochondrial swelling and a decrease in skeletal muscle CoQ are observed [34]. Simvastatin administered to dogs for 3 weeks significantly decreases the myocardial level of CoQ and causes worsening of the myocardial mitochondrial respiration [64]. When simvastatin is administered orally to mice, the levels of CoQ in liver and heart decreases significantly while the levels of thiobarbituric acid reactive substances in liver and heart mitochondria increases significantly [58]. The latter indicates that simvastatin induces the oxidative stress in mitochondria. Treatment of Sprague-Dawley and Chbb:Thom rats with simvastatin decreases eye lens CoQ levels of both animal lines [38] suggesting the mitochondrial dysfunction in lens fiber cells.

In patients with myalgia associated with simvastatin therapy, CoQ supplementation did not improve myalgia [65]. There results suggest that either supplemented

CoQ does not attain mitochondria (e.g. it either penetrates cellular and mitochondrial membranes not good enough, is not incorporated into the respiratory chain, or is broken down on the way) or CoQ deficiency is not only reason of mitochondrial dysfunction. When analyzing the relationship between statin treatment and CoQ levels in patients, Marcoff and Thompson [66] have concluded that there was insufficient evidence to prove the etiologic role of CoQ deficiency in statin-associated myopathy.

The summary of mitotoxicity of simvastatin *in vivo* is given in Table 3.

### 5.2. Effects of simvastatin on mitochondrial function *in vitro*

An important role of CoQ deficiency in simvastatin-induced hepatopathy was demonstrated on HepG2 cells [46]. Simvastatin decreased mitochondrial CoQ<sub>10</sub> levels, and at higher concentrations was associated with a moderately higher degree of cell death, increased DNA oxidative damage and a reduction in ATP synthesis. Supplementation of CoQ<sub>10</sub> reduced cell death and DNA oxidative stress, and increased ATP synthesis. It is suggested that CoQ<sub>10</sub> deficiency plays an important role in statin-induced hepatopathy, and that CoQ<sub>10</sub> supplementation protects HepG2 cells from this complication.

Using fluorescence imaging analysis and oxygraphy on human and rat skinned skeletal muscle samples; Sirvent et al. [67] showed that the simvastatin-induced mitochondria impairment results from inhibition of the Complex I of respiratory chain. Similar simvastatin-induced mitochondria impairment and alteration of Ca<sup>2+</sup> homeostasis occur in permeabilized but not in intact ventricular rat cardiomyocytes.

Acute applications of simvastatin on human skeletal muscle fibers triggered a Ca<sup>2+</sup> wave of intra-cellular Ca<sup>2+</sup> that mostly originates from sarcoplasmic reticulum Ca<sup>2+</sup>-release, and increased mitochondrial NADH content and induced mitochondrial membrane depolarization suggesting an altered mitochondrial function [14].

In PC3 cells, simvastatin at 10 μM induced principally apoptosis, which was not prevented by cyclosporin A, the

inhibitor of calcineurin and mitochondrial permeability transition (MPT). At 60  $\mu\text{M}$ , simvastatin induced the necrosis preceded by a threefold increase in cytosolic free  $\text{Ca}^{2+}$  concentration and a significant decrease in both respiration rate and mitochondrial membrane potential. Both mitochondrial dysfunction and necrosis were sensitive to cyclosporin A, a MPT blockator, and bongkreikic acid, an inhibitor of adenine nucleotide translocator, as well as the calcineurin inhibitor FK506. Thus, simvastatin-induced PC3 cells apoptosis is dependent on HMG-CoA reductase inhibition and independent of MPT, whereas simvastatin-induced PC3 cells necrosis is dependent on mitochondrial dysfunction caused, at least in part, by calcineurin [45].

Simvastatin impairs mitochondrial respiration and inhibits oxidative phosphorylation Complexes I and II+III in isolated rat liver mitochondria [68]. These data suggest that simvastatin alters mitochondrial function directly, without the need for entering the cell and inhibiting HMG-CoA reductase.

Liver mitochondria isolated from hypercholesterolemic LDL receptor knockout mice treated during 15 days with therapeutic doses of simvastatin present a higher susceptibility to develop MPT. In experiments *in vitro*, simvastatin induces MPT in a dose-dependent manner by a mechanism sensitive to cyclosporin A (cyclophilin sequestrant), dithiothreitol (sulfhydryl reducing agent), ADP (adenine nucleotide translocator inhibitor), catalase ( $\text{H}_2\text{O}_2$  reductant) and EGTA (calcium chelator). Simvastatin, also, decreases the content of total mitochondrial membrane protein thiol groups. Thus, the statin can act directly on mitochondria *in vitro* as well *in vivo* inducing MPT, which is a process involved in cell death [69].

MPT induction is one of apoptosis markers. However, in the PC3 human prostate cancer cell line, simvastatin induces principally apoptosis which was not prevented by cyclosporin A and, therefore, independent of MPT [45] indicating that simvastatin is responsible for inducing apoptosis when significant damage occurs in the mitochondria. Mitochondrial disruption can result in induction of cytosolic cysteine proteases caspase 9 and caspase 3. Both enzymes are responsible for the later steps of apoptosis [70]. After binding with apoptosis protease activating factor-1 (Apaf-1) which requires the presence of cytochrome *c* and ATP (or dATP) in the cytosol (i.e., after apoptosome formation), pro-caspase 9 is activated to caspase 9, and the latter becomes capable of cleaving and activating caspase 3. Caspase 3 can participate in DNA fragmentation directly [71] or activate endonucleases such as caspase-activated DNase cleaving chromatin and, as a consequence, execute apoptosis. One of caspase cascade-activating

proteins is cytochrome *c* which is normally sequestered in the mitochondrial intermembrane space. It has been shown to be released through the mitochondrial outer membrane into the cytosol early during apoptosis [72-74], then released cytochrome *c* can trigger the proteolytic maturation of caspases within an apoptosome, the caspase activation complex [74-77].

The ability of statins to induce activation of the caspase cascade has been evaluated *in vitro* and provided strong evidence that statins can impact this cell-death pathway. Simvastatin induces cell death in human skeletal muscle cells [39]. These investigators showed that simvastatin triggered sustained intracellular  $\text{Ca}^{2+}$  transients, leading to calpain activation, to a translocation of Bax to mitochondria in a caspase 8-independent manner. Consecutive activation of caspases 9 and 3 execute apoptotic cell death. Boucher et al. [42] demonstrate that simvastatin induces dose-dependent apoptosis in the TR-PCT1 pericyte cell line and in freshly isolated pericytes, and that simvastatin-induced apoptosis in pericytes is cholesterol, caspase-3, and caspase-7 mediated. Treatment with simvastatin of fibroblast-like synoviocytes derived from patients with rheumatoid arthritis reduces cell viability and induces prominent apoptosis in cells in a dose-dependent manner and depended on caspase-3 and caspase-9 [43].

Simvastatin effectively decreases cell viability in three prostate cancer cell lines (PC3, DU145, and LnCap) by inducing apoptosis and cell growth arrest at  $G_1$  phase and inducing activation of caspase-8, caspase-3, and caspase-9 [44]. In primary cultures of human skeletal muscle cells simvastatin increases caspase-9 and caspase-3 activities up to 3-fold, induces cell apoptosis as soon as 24 h following application, and as many as 80% of cells are died 48 h later [39]. In HL-60 cells, simvastatin directly and rapidly disorders mitochondria with a loss of its membrane potential, elevates reactive oxygen species generation and subsequent irreversible damage with cytochrome *c* leakage and apoptosis through caspase 9 activation [49]. Simvastatin induces the cytosolic release of the second mitochondria-derived activator of caspases in human T, B and myeloma tumor cells [41]. Taken together, these findings indicate that multiple executioner caspases may be involved in neuronal apoptosis induced by simvastatin *in vitro*.

HMG-CoA reductase is the rate-limiting enzyme in the biosynthesis of cholesterol and of products involved in prenylation or farnesylation of several important membrane-bound proteins. Simvastatin treatment of proliferating murine tubular cells was associated with cytochrome *c* release from the mitochondria to the cytosol, the presence of active caspases 9 and 3. These effects

**Table 4.** Effects of simvastatin on mitochondria *in vitro*.

Object	Effects of simvastatin	Refs
Human skeletal muscle fibers	Mitochondrial membrane depolarization, increase in mitochondrial NADH	[14]
Human skeletal muscle; rat skeletal muscle; rat ventricular cardiomyocytes	Inhibition of the complex I of respiratory chain, disbalance of Ca <sup>2+</sup> homeostasis	[67]
Human T, B and myeloma tumor cells	Mitochondrial membrane depolarization, release of the second mitochondrial activator of caspases	[41]
HL-60 human leukemic cells	Mitochondrial membrane depolarization, reactive oxygen species generation, cytochrome c leakage	[49]
PC3 human prostate cancer cell line	Decrease in respiration rate, mitochondrial membrane depolarization	[45]
Murine tubular cells	Cytochrome c release from the mitochondria, activation of caspase 3	[50]
L6 rat skeletal muscle cell line; mitochondria from L6 cells	Mitochondrial membrane depolarization; decrease in respiratory rate and beta-oxidation, cytochrome c release	[52]
HepG2 cells	Decrease in mitochondrial CoQ levels, decrease in ATP synthesis	[46]
Isolated rat liver mitochondria	Impairment of mitochondrial respiration, inhibition of oxidative phosphorylation	[68]
Isolated mouse liver mitochondria	Higher susceptibility to develop membrane permeability transition	[69]

were reversed by mevalonate, farnesylpyrophosphate, and geranylgeranylpyrophosphate. In fibroblast-like synoviocytes derived from patients with rheumatoid arthritis and cultured mouse cochlear neuroblasts VOT-33 simvastatin treatment increases caspase 3 activity and this effect is abolished by mevalonate. These data suggest the involvement of protein prenylation in the toxic effects of simvastatin.

The summary of mitotoxicity of simvastatin *in vitro* is given in Table 4.

Damage to mitochondrial fatty acid beta-oxidation can be one of mechanisms underlying mitochondrial toxicity of simvastatin. However, it has not been considered in the literature.

It was reported that mitochondrial fatty acid beta-oxidation in the L6 rat skeletal muscle cell line was decreased by 88-96% in the presence of 100  $\mu\text{mol/l}$  simvastatin, concentration induced death in 27-49% of the cells [52]. Such dramatic alteration in fatty acid oxidation affects evidently the mitochondrial function resulting in mitochondrial swelling, cytochrome c release and DNA fragmentation. Disturbance of mitochondrial fatty acid metabolism is believed to be directly associated only with inhibitory effect of simvastatin (and other statins) on microsomal enzyme HMG-CoA reductase. HMG-CoA synthetase is the enzyme catalyzing biosynthesis of HMG-CoA from both acetyl-CoA, a substrate for both fatty acid and lipid syntheses, and acetoacetyl-CoA, the end product of beta-oxidation of fatty acids (Figure 1). Statins are more than inhibitors of HMG-CoA reductase, they also disturb HMG-CoA metabolism. Statins form a block in the cholesterol biosynthesis at the HMG-CoA reductase step, which forbids accumulated HMG-CoA to be readily catabolized, and conduce to its continual accumulation. This blocking leads to depletion of

downstream intermediates and accumulation of upstream intermediates. HMG-CoA synthetase is localized in mitochondria, so the inhibition of microsomal HMG-CoA reductase would result in the accumulation of HMG-CoA, acetyl-CoA and acetoacetyl-CoA in mitochondria and in disbalance of mitochondrial fatty acid and lipid metabolism. More research is needed to define whether and to what extent these abnormalities contribute to simvastatin-induced cell death and myopathy.

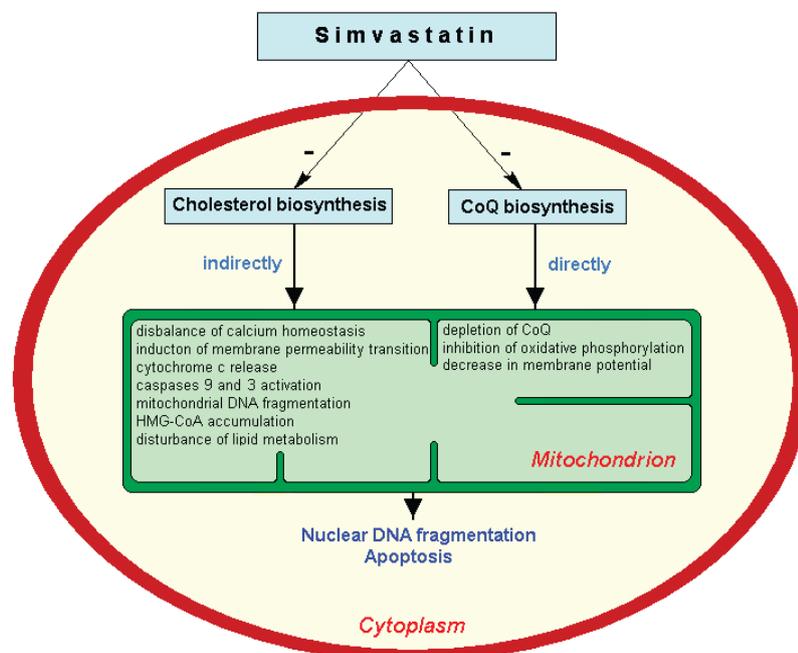
Summary of the effects of simvastatin on mitochondria is given in Figure 2.

The results above are evidence that allows to do only one conclusion: simvastatin induces the apoptotic program starting from mitochondrial dysfunction.

## 6. Conclusion

Simvastatin, apart from its capacity to lower cholesterol plasma levels and to protect against cardiovascular disease, can cause myopathy and other adverse events in humans, dogs, rabbits, rats, hamster, mice tissues and cells. The majority of toxic effects of simvastatin is associated with mitochondrial dysfunction. An application of simvastatin *in vitro* and addition *in vitro* to humans, animals and various cells appears to cause numerous mitochondrial disturbances with no beneficial effect on intact mitochondria. The mechanisms underlying cytotoxicity of simvastatin involve a cascade of cellular processes originating from mitochondria and resulting in cell death. One of the processes may be that based on mitochondrial accumulation of all HMG-CoA, acetoacetyl-CoA and acetyl-CoA. The ascertainment of mechanisms involved in simvastatin-induced

**Figure 2.** Summary of mitotoxic effects of simvastatin.



cytotoxicity and mitotoxicity can highlight abnormalities and therefore reduce potentially damaging effects, would help us to understand why only 0.1% and up to 10% of patients receiving simvastatin treatment for hypercholesterolemia were in danger of adverse attack and to prevent the neopathy.

### Conflict of Interest Statement

No potential conflict of interest and financial interests relevant to the subject of this manuscript was and will be published.

### References

- [1] Evans M., Rees A., Effects of HMG-CoA reductase inhibitors on skeletal muscle: are all statins the same? *Drug Saf.*, 2002, 25, 649-63
- [2] Kent D.M., Stroke — An Equal Opportunity for the Initiation of Statin Therapy, *N. Engl. J. Med.*, 2006, 355, 613-5
- [3] Illingworth D.R., An overview of lipid-lowering drugs, *Drugs*, 1988, 36 Suppl 3, 63-71
- [4] Walker J.F., HMG CoA reductase inhibitors, Current clinical experience, *Drugs*, 1988, 36 Suppl 3, 83-6
- [5] Qiao Z., Ren J., Chen H., Simvastatin reduces expression and activity of lipoprotein-associated phospholipase A(2) in the aorta of hypercholesterolaemic atherosclerotic rabbits, *J. Int. Med. Res.*, 2009, 37, 1029-37
- [6] Corti R., Osende J.I., Fallon J.T., Fuster V., Mizsei G., Jneid H., et al., The selective peroxisomal proliferator-activated receptor-gamma agonist has an additive effect on plaque regression in combination with simvastatin in experimental atherosclerosis: in vivo study by high-resolution magnetic resonance imaging, *J. Am. Coll. Cardiol.*, 2004, 43, 464-73
- [7] Werner N., Kosiol S., Schiegl T., Ahlers P., Walenta K., Link A., et al., Circulating Endothelial Progenitor Cells and Cardiovascular Outcomes, *N. Engl. J. Med.*, 2005, 353, 999-1007
- [8] Dirks A.J., Jones K.M., Statin-induced apoptosis and skeletal myopathy, *Am. J. Physiol. Cell Physiol.*, 2006, 291, C1208-12
- [9] Maron D.J., Fazio S., Linton M.F., Current perspectives on statins, *Circulation*, 2000, 101, 207-13
- [10] Palinski W., Napoli C., Unraveling pleiotropic effects of statins on plaque rupture, *Arterioscler. Thromb. Vasc. Biol.*, 2002, 22, 1745-50
- [11] Manzoni M., Rollini M., Biosynthesis and biotechnological production of statins by filamentous fungi and application of these cholesterol-lowering drugs, *Appl. Microbiol. Biotechnol.*, 2002, 58, 555-64
- [12] Schachter M., Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update, *Fundam. Clin. Pharmacol.*, 2005, 19, 117-25

- [13] Shepherd J., Who should receive a statin these days? Lessons from recent clinical trials, *J. Intern. Med.*, 2006, 260, 305-9
- [14] Sirvent P., Mercier J., Vassort G., Lacampagne A., Simvastatin triggers mitochondria-induced Ca<sup>2+</sup> signaling alteration in skeletal muscle, *Biochem. Biophys. Res. Commun.*, 2005, 329, 1067-75
- [15] MRC/BHF Heart Protection Study Collaborative Group, Armitage J., Bowman L., Collins R., Parish S., Tobert J., Effects of simvastatin 40 mg daily on muscle and liver adverse effects in a 5-year randomized placebo-controlled trial in 20,536 high-risk people, *BMC Clin. Pharmacol.* 2009, 9, 6
- [16] Goli A.K., Goli S.A., Byrd R.P., Roy T.M., Simvastatin-induced lactic acidosis: a rare adverse reaction? *Clin. Pharmacol. Ther.*, 2002, 72, 461-4
- [17] Thompson P.D., Clarkson P., Karas R.H., Statin-associated myopathy, *JAMA*, 2003, 289, 1681-90
- [18] Venero C.V., Thompson P.D., Managing statin myopathy, *Endocrinol. Metab. Clin. North. Am.*, 2009, 38, 121-36
- [19] Omar M.A., Wilson J.P., FDA adverse event reports on statin-associated rhabdomyolysis, *Ann. Pharmacother.*, 2002, 36, 288-95
- [20] Staffa J.A., Chang J., Green L., Cerivastatin and reports of fatal rhabdomyolysis, *N. Engl. J. Med.*, 2002, 346, 539-40
- [21] Ballarè M., Campanini M., Airoidi G., Zaccala G., Bertocelli M.C., Cornaglia G., et al., Hepatotoxicity of hydroxy-methyl-glutaryl-coenzyme A reductase inhibitors, *Minerva Gastroenterol. Dietol.*, 1992, 38, 41-4
- [22] Waness A., Bahlas S., Al Shohaib S., Simvastatin-induced rhabdomyolysis and acute renal injury, *Blood Purif.*, 2008, 26, 394-8
- [23] Qari F.A., Severe rhabdomyolysis and acute renal failure secondary to use of simvastatin in undiagnosed hypothyroidism, *Saudi J. Kidney Dis. Transpl.*, 2009, 20, 127-9
- [24] Marie I, Delafenêtre H, Massy N, Thuillez C, Noblet C; Network of the French Pharmacovigilance Centers, Tendinous disorders attributed to statins: a study on ninety-six spontaneous reports in the period 1990-2005 and review of the literature, *Arthritis Rheum.*, 2008, 59, 367-72
- [25] Bae J., Jarcho J.A., Denton M.D., Magee C.C., Statin specific toxicity in organ transplant recipients: case report and review of the literature, *J. Nephrol.*, 2002, 15, 317-9
- [26] Evangelista T., Ferro J., Pereira P., de Carvalho M., A case of asymptomatic cytoplasmic body myopathy revealed by simvastatin, *Neuromuscul. Disord.*, 2009, 19, 66-8
- [27] Tuteja S., Pyrsopoulos N.T., Wolowich W.R., Khanmoradi K., Levi D.M., Selvaggi G., et al., Simvastatin-ezetimibe-induced hepatic failure necessitating liver transplantation, *Pharmacotherapy*, 2008, 28, 1188-93
- [28] Kanathur N., Mathai M.G., Byrd R.P., Fields C.L., Roy T.M., Simvastatin-diltiazem drug interaction resulting in rhabdomyolysis and hepatitis, *Tenn. Med.*, 2001, 94, 339-41
- [29] Johnson J.L., Loomis I.B., A case of simvastatin-associated pancreatitis and review of statin-associated pancreatitis, *Pharmacotherapy*, 2006, 26, 414-22
- [30] Singh S., Loke Y.K., Statins and pancreatitis: a systematic review of observational studies and spontaneous case reports, *Drug Saf.*, 2006, 29, 1123-32
- [31] Bielecki J.W., Schraner C., Briner V., Kuhn M., Rhabdomyolysis and cholestatic hepatitis under treatment with simvastatin and chlorzoxazone, *Schweiz. Med. Wochenschr.*, 1999, 129, 514-8
- [32] Rifkin S.I., Multiple drug interactions in a renal transplant patient leading to simvastatin-induced rhabdomyolysis: a case report, *Medscape J. Med.*, 2008, 10, 264
- [33] Francis L., Bonilla E., Soforo E., Neupane H., Nakhla H., Fuller C., et al., Fatal toxic myopathy attributed to propofol, methylprednisolone, and cyclosporine after prior exposure to colchicine and simvastatin, *Clin. Rheumatol.*, 2008, 27, 129-31
- [34] Nakahara K., Kuriyama M., Sonoda Y., Yoshidome H., Nakagawa H., Fujiyama J., et al., Myopathy induced by HMG-CoA reductase inhibitors in rabbits: a pathological, electrophysiological, and biochemical study, *Toxicol. Appl. Pharmacol.*, 1998, 152, 99-106
- [35] Westwood F.R., Bigley A., Randall K., Marsden A.M., Scott R.C., Statin-induced muscle necrosis in the rat: distribution, development, and fibre selectivity, *Toxicol. Pathol.* 2005, 33, 246-57
- [36] Baytan S.H., Alkanat M., Okuyan M., Ekinci M., Gedikli E., Ozeren M., Simvastatin impairs spatial memory in rats at a specific dose level, *Tohoku J. Exp. Med.*, 2008, 214, 341-9
- [37] Oms P., Assie N., Bruniquel F., Degryse A.D., van Haverbeke G., Delhon A., Biochemical changes and morphological alterations of liver and kidney in hamsters after administration of the HMG-coenzyme A reductase inhibitor, simvastatin: prevention and reversibility by mevalonate, *Pharmacol. Toxicol.*, 1995, 77, 391-6
- [38] Cenedella R.J., Neely A.R., Sexton P., Concentration and distribution of ubiquinone (coenzyme Q), the

- endogenous lipid antioxidant, in the rat lens: effect of treatment with simvastatin, *Mol. Vis.*, 2005, 11, 594-602
- [39] Sacher J., Weigl L., Werner M., Szegedi C., Hohenegger M., Delineation of myotoxicity induced by 3-hydroxy-3-methylglutaryl CoA reductase inhibitors in human skeletal muscle cells, *J. Pharmac. Exp. Ther.*, 2005, 314, 1032-41
- [40] Demyanets S., Kaun C., Pfaffenberger S., Hohensinner P.J., Rega G., Pammer J., et al., Hydroxymethylglutaryl-coenzyme A reductase inhibitors induce apoptosis in human cardiac myocytes in vitro, *Biochem. Pharmacol.*, 2006, 71, 1324-30
- [41] Cafforio P., Dammacco F., Gernone A., Silvestris F., Statins activate the mitochondrial pathway of apoptosis in human lymphoblasts and myeloma cells, *Carcinogenesis*, 2005, 26, 883-91
- [42] Boucher K., Siegel C.S., Sharma P., Hauschka P.V., Solomon K.R., HMG-CoA reductase inhibitors induce apoptosis in pericytes, *Microvasc. Res.*, 2006, 71, 91-102
- [43] Yokota K., Miyoshi F., Miyazaki T., Sato K., Yoshida Y., Asanuma Y., et al., High concentration simvastatin induces apoptosis in fibroblast-like synoviocytes from patients with rheumatoid arthritis, *J. Rheumatol.*, 2008, 35, 193-200
- [44] Hoque A., Chen H., Xu X.C., Statin induces apoptosis and cell growth arrest in prostate cancer cells, *Cancer Epidemiol. Biomarkers Prev.*, 2008, 17, 88-94
- [45] Oliveira K.A., Zecchin K.G., Alberici L.C., Castilho R.F., Vercesi A.E., Simvastatin inducing PC3 prostate cancer cell necrosis mediated by calcineurin and mitochondrial dysfunction, *J. Bioenerg. Biomembr.*, 2008, 40, 307-14
- [46] Tavintharan S., Ong C.N., Jeyaseelan K., Sivakumar M., Lim S.C., Sum C.F., Reduced mitochondrial coenzyme Q10 levels in HepG2 cells treated with high-dose simvastatin: a possible role in statin-induced hepatotoxicity? *Toxicol. Appl. Pharmacol.*, 2007, 223, 173-9
- [47] Kenis I., Tartakover-Matalon S., Cherepnin N., Drucker L., Fishman A., Pomeranz M., et al., Simvastatin has deleterious effects on human first trimester placental explants, *Hum. Reprod.*, 2005, 20, 2866-72
- [48] Van Vliet A.K., Nègre-Aminou P., van Thiel G.C., Bolhuis P.A., Cohen L.H., Action of lovastatin, simvastatin, and pravastatin on sterol synthesis and their antiproliferative effect in cultured myoblasts from human striated muscle, *Biochem. Pharmacol.*, 1996, 52, 1387-92
- [49] Tomiyama N., Matzno S., Kitada C., Nishiguchi E., Okamura N., Matsuyama K., The possibility of simvastatin as a chemotherapeutic agent for all-trans retinoic acid-resistant promyelocytic leukemia, *Biol. Pharm. Bull.*, 2008, 31, 369-74
- [50] Blanco-Colio L.M., Justo P., Daehn I., Lorz C., Ortiz A., Egido J., Bcl-xL overexpression protects from apoptosis induced by HMG-CoA reductase inhibitors in murine tubular cells, *Kidney Int.*, 2003, 64, 181-91
- [51] Kaneta S., Satoh K., Kano S., Kanda M., Ichihara K., All hydrophobic HMG-CoA reductase inhibitors induce apoptotic death in rat pulmonary vein endothelial cells, *Atherosclerosis*, 2003, 170, 237-43
- [52] Kaufmann P., Török M., Zahno A., Waldhauser K.M., Brecht K., Krähenbühl S., Toxicity of statins on rat skeletal muscle mitochondria, *Cell Mol. Life Sci.*, 2006, 63, 2415-25
- [53] März P., Otten U., Miserez A.R., Statins induce differentiation and cell death in neurons and astroglia, *Glia*, 2007, 55, 1-12
- [54] Xu G.Q., Huang W.F., Liu H., Yang Y.C., Liu W., Simvastatin-induced apoptosis of K562 cells is mediated by endoplasmic reticulum stress, *Yao Xue Bao*, 2008, 43, 371-7
- [55] Park D.S., So H.S., Lee J.H., Park H.Y., Lee Y.J., Cho J.H., et al., Simvastatin treatment induces morphology alterations and apoptosis in murine cochlear neuronal cells, *Acta Otolaryngol.*, 2009, 129, 166-74
- [56] Martinet W., Schrijvers D.M., Timmermans J.P., Bult H., Interactions between cell death induced by statins and 7-ketocholesterol in rabbit aorta smooth muscle cells, *Br. J. Pharmacol.*, 2008, 154, 1236-46
- [57] Ogunwobi O.O., Beales I.L., Statins inhibit proliferation and induce apoptosis in Barrett's esophageal adenocarcinoma cells, *Am. J. Gastroenterol.*, 2008, 103, 825-37
- [58] Kettawan A., Takahashi T., Kongkachuichai R., Charoenkiatkul S., Kishi T., Okamoto T., Protective effects of coenzyme Q10 on decreased oxidative stress resistance induced by simvastatin, *J. Clin. Biochem. Nutr.*, 2007, 40, 194-202
- [59] Glynn S.A., O'Sullivan D., Eustace A.J., Clynes M., O'Donovan N., The 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, simvastatin, lovastatin and mevastatin inhibit proliferation and invasion of melanoma cells, *BMC Cancer*, 2008, 8, 9
- [60] Demierre M.F., Higgins P.D., Gruber S.B., Hawk E., Lippman S.M., Statins and cancer prevention, *Nat.*

- Rev. Cancer, 2005, 5, 930-42
- [61] Kaji H., Naito J., Inoue Y., Sowa H., Sugimoto T., Chihara K., Statin suppresses apoptosis in osteoblastic cells: role of transforming growth factor-beta-Smad3 pathway, *Horm. Metab. Res.*, 2008, 40, 746-51
- [62] Schick B.A., Laaksonen R., Frohlich J.J., Päivä H., Lehtimäki T., Humphries K.H., et al., Decreased skeletal muscle mitochondrial DNA in patients treated with high-dose simvastatin, *Clin. Pharmacol. Ther.*, 2007, 81, 650-3
- [63] DePinieux G., Chariot P., AmmiSaid M., Louarn F., Lejonc J.L., Astier A., et al., Lipid-lowering drugs and mitochondrial function: Effects of HMG-CoA reductase inhibitors on serum ubiquinone and blood lactate/pyruvate ratio, *Brit. J. Clin. Pharmacol.*, 1996, 42, 333-7
- [64] Satoh K., Yamato A., Nakai T., Hoshi K., Ichihara K., Effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors on mitochondrial respiration in ischaemic dog hearts, *Br. J. Pharmacol.*, 1995, 116, 1894-8
- [65] Young J.M., Florkowski C.M., Molyneux S.L., McEwan R.G., Frampton C.M., George P.M., et al., Effect of coenzyme Q(10) supplementation on simvastatin-induced myalgia, *Am. J. Cardiol.*, 2007, 100, 1400-3
- [66] Marcoff L., Thompson P.D., The role of coenzyme Q10 in statin-associated myopathy: a systematic review, *J. Am. Coll. Cardiol.*, 2007, 49, 2231-7
- [67] Sirvent P., Bordenave S., Vermaelen M., Roels B., Vassort G., Mercier J., et al., Simvastatin induces impairment in skeletal muscle while heart is protected, *Biochem. Biophys. Res. Commun.*, 2005, 338, 1426-34
- [68] Nadanaciva S., Dykens J.A., Bernal A., Capaldi R.A., Will Y., Mitochondrial impairment by PPAR agonists and statins identified via immunocaptured OXPHOS complex activities and respiration, *Toxicol. Appl. Pharmacol.*, 2007, 223, 277-87
- [69] Velho J.A., Okanobo H., Degaspero G.R., Matsumoto M.Y., Alberici L.C., Cosso R.G., et al., Statins induce calcium-dependent mitochondrial permeability transition, *Toxicology*, 2006, 219, 124-32
- [70] Alnemri E.S., Livingston D.J., Nicholson D.W., Salvesen G., Thornberry N.A., Wong W.W., et al., Human ICE/CED-3 protease nomenclature, *Cell*, 1996, 87, 171
- [71] Jänicke R.U., Sprengart M.L., Wati M.R., Porter A.G., Caspase-3 is required for DNA fragmentation and morphological changes associated with apoptosis, *J. Biol. Chem.*, 1998, 273, 9357-60
- [72] Garland J.M., Rudin C., Cytochrome c induces caspase-dependent apoptosis in intact hematopoietic cells and overrides apoptosis suppression mediated by bcl-2, growth factor signaling, MAP-kinase-kinase, and malignant change, *Blood*, 1998, 92, 1235-46
- [73] Kluck R.M., Bossy-Wetzell E., Green D.R., Newmeyer D.D., The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis, *Science*, 1997, 275, 1132-6
- [74] Liu X., Kim C.N., Yang J., Jemmerson R., Wang X., Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c, *Cell*, 1996, 86, 147-57
- [75] Budihardjo I., Oliver H., Lutter M., Luo X., Wang X., Biochemical pathways of caspase activation during apoptosis, *Annu. Rev. Cell Dev. Biol.*, 1999, 15, 269-90
- [76] Li P., Nijhawan D., Budihardjo I., Srinivasula S.M., Ahmad M., Alnemri E.S., Wang X., Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade, *Cell*, 1997, 91, 479-89
- [77] Pan G., O'Rourke K., Dixit V.M., Caspase-9, Bcl-XL, and Apaf-1 form a ternary complex, *J. Biol. Chem.*, 1998, 273, 5841-5