Statin-induced apoptosis and skeletal myopathy

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Dirks, Amie J., and Kimberly M. Jones. Statin-induced apoptosis and skeletal myopathy. Am J Physiol Cell Physiol 291: C1208–C1212, 2006. First published August 2, 2006; doi:10.1152/ajpcell.00226.2006.—Over 100 million prescriptions were filled for statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) in 2004. Statins were originally developed to lower plasma cholesterol in patients with hypercholesterolemia and are the most effective drugs on the market in doing so. Because of the discovered pleiotropic effects of statins, the use has expanded to the treatment of many other conditions, including ventricular arrhythmias, idiopathic dilated cardiomyopathy, cancer, osteoporosis, and diabetes. The elderly population is growing. Therefore, it is estimated that the number of statin users will also increase. Fortunately, the use of statins is relatively safe with few side effects. Myopathy is the most common side effect with symptoms ranging from fatigue, weakness, and pain to symptoms associated with rhabdomyolysis which is a life-threatening condition. The development of statin-induced rhabdomyolysis is rare occurring in ~0.1% of patients; however, the occurrence of less severe symptoms is underreported and may be 1–5% or more. Physical exercise appears to increase the likelihood for the development of myopathy in patients taking statins. It is thought that as many as 25% of statin users who exercise may experience muscle fatigue, weakness, aches, and cramping due to statin therapy and potentially dismissed by the patient and physician (46). The development of myopathy can impact daily living and quality of life by affecting the patient’s ability to accomplish even simple tasks such as opening jars or may prevent participation in once enjoyable physical activities such as golf, tennis, or other forms of exercise. Currently, the only treatment for statin-induced myopathy is the discontinuation of statin use in affected patients, in which symptoms are most often reversible.

Identification of the mechanisms causing skeletal myopathy may lead to the development of effective preventative measures or treatments for patients who would benefit from statin therapy. The mechanisms that cause statin-induced myopathy have not been elucidated; however, research efforts suggest that apoptosis of myofibers may contribute. The mitochondrion is considered a regulatory center of apoptosis, and therefore its role in the induction of apoptosis will be discussed as well as the mechanism of statin-induced apoptosis and myopathy.

**CELLULAR PROCESSES OF APOPTOSIS**

Apoptosis is a cell suicide program that is highly regulated and executed via activation of specific signaling pathways. Hence, particular morphological, biochemical, and molecular events occur, such as DNA fragmentation, nuclear condensation, and formation of apoptotic bodies which are then engulfed by macrophages or neighboring cells without initiating an inflammatory response (20, 35, 36). Apoptosis allows for the death of a single cell without death or disruption to the surrounding tissue (20, 21). Apoptotic pathways can activate cysteine-dependent, aspartate-specific proteases (caspases), which are endopeptidases, and are integral to the final execution of cell death (20, 21). There are 14 known mammalian caspases (i.e., caspase-1 through caspase-14), which participate in the apoptotic process depending on the stimulus and respective signaling pathway activated and/or cell type undergoing apoptosis. Caspases normally exist in an inactivated state, called procaspases, in the cytoplasm but can be activated by proteolytic cleavage (39). Initiation of apoptosis leads to the activation of a “caspase cascade,” in which activation of “initiator” caspases (i.e., caspase-8, -9, and -12) cleave and activate “effector” caspases (i.e., caspase-3 and -7), which carry out the actual proteolytic events that result in cellular breakdown (39).
The mitochondrion plays a central role in regulating apoptosis (see Fig. 2). It can release cytochrome c into the cytosol, which then forms an “apoptosome” with Apaf-1, ATP, and procaspase-9. Once the apoptosome is formed, procaspase-9 can cleave and activate itself into caspase-9. Caspase-9 can then cleave and activate procaspase-3, leading to apoptosis. This process is highly regulated. The Bcl-2 family of proteins was the first described to affect the release of cytochrome c. This family consists of several proteins, which are antiapoptotic and proapoptotic. For example, Bcl-2 and Bcl-XL protect against cytochrome c release from the mitochondria. Often the Bcl-2/Bax ratio is used as an indicator of apoptotic potential where a high ratio protects against cytochrome c release, while Bax, Bak, Bad, and Bid favor cytochrome c release and are therefore proapoptotic. The ratio and interaction of the Bcl-2 family antiapoptotic and proapoptotic proteins determine the fate of cytochrome c release from the mitochondria. Often the Bcl-2/Bax ratio is used as an indicator of apoptotic potential where a high ratio protects against apoptosis and a low ratio favors apoptosis (2, 9, 34). Another regulatory protein that can regulate the release of cytochrome c from the mitochondria is apoptosis repressor with card (ARC) (11). Upon stimulation, ARC can translocate from the cytosol to the mitochondrial membrane to inhibit the release of cytochrome c. Recent data show that ARC may prevent apoptosis by binding to Bax and interfering with its activation, which would ultimately protect against cytochrome c release (18). Another family of proteins that regulate the apoptotic process is the inhibitor of apoptosis protein (IAP) family. The IAPs (i.e., XIAP, cIAP-1, and cIAP-2) can bind to cleaved and activated caspase-9 and -3 to inhibit their enzyme activity and to prevent apoptosis (29). Finally, the mitochondrion can release additional proteins, along with cytochrome c, to relieve the inhibition exerted by the IAPs so indeed apoptosis can occur. The mitochondrion can also play a role in inducing apoptosis in a caspase-independent manner. Upon stimulation, the mitochondrion can release proapoptotic proteins, such as apoptosis-inducing factor and endonuclease G, which translocate to the nucleus to induce DNA fragmentation (6, 7, 23, 50).

Other apoptotic signaling pathways require an alternate initiator caspase to initiate the caspase cascade (i.e., caspase-8 and caspase-12). Receptor-mediated pathways can be activated by various ligands binding to its receptor and inducing apoptosis in an effector cell by the activation of procaspase-8, which can cleave and activate procaspase-3 to initiate the caspase cascade (43). Once apoptosis is initiated via caspase-8, the release of cytochrome c from the mitochondrion and activation of the mitochondrion-mediated signaling may occur, but is downstream from caspase-8 activation. Active caspase-8 can cleave Bid, which then stimulates Bax and Bak activity resulting in cytochrome c release (26, 50). Some cell types require activation of the mitochondrion-mediated signaling via Bid to execute apoptosis and others do not. Endoplasmic reticulum stress and calcium dyshomeostasis could also contribute to apoptosis via the activation of calpains and/or procaspase-12, which then leads to activation of procaspase-3 (10, 24, 38, 42).
Evidence of apoptosis in statin-induced myopathy. Similar to the apoptosis-inducing effects of statins on many cell types, statins have also been shown to induce apoptosis in skeletal myocytes in vitro. It has recently been shown that various statins can induce apoptosis in skeletal myoblasts, myotubes, and in differentiated primary human skeletal muscle cells, most often in a concentration-dependent manner (19, 30, 32, 40). The data support that statin-induced apoptosis in skeletal muscle cells may be mitochondrial-mediated as shown by an increase in active caspase-9 and caspase-3 (19, 40). Sacher et al. (40) show that Bax translocates to the mitochondria in response to statin treatment, which may lead to the release of cytochrome c and activation of the mitochondrial-mediated apoptotic signaling pathway. Addition of mevalonate prevents statin-induced apoptosis and caspase-3 activation (19, 40). These results suggest that the depletion of downstream products of mevalonate synthesis induces apoptosis of skeletal muscle cells in a mitochondrial-mediated manner in vitro. These results are consistent with in vitro data generated in non-skeletal muscle cell lines, which also support a mitochondrial-mediated mechanism of statin-induced apoptosis. It has been shown in vascular smooth muscle cells that apoptosis induced by statins is associated with suppressed levels of Bcl-2, whereas Bax expression was not altered, and increased activation of caspase-9. Coincubation of cells with a caspase inhibitor significantly inhibited apoptosis (3). Statin-induced apoptosis of anaplastic thyroid cancer cells was associated with cytochrome c release from the mitochondria and an increase in activity of caspases-2, -3, and -9. Activities of caspases-1 and -8 were not affected (49). Therefore, statin-induced apoptosis is likely executed via a mitochondrial-mediated mechanism involving a decrease in the Bcl-2/Bax ratio leading to cytochrome c release and activation of caspase-9, followed by activation of caspase-3.

Although it is clear the statins induce apoptosis in vitro, it is not as clear whether statins induce skeletal muscle apoptosis in vivo. Only one study to date has been published which report the effects of statins on apoptotic markers in skeletal muscle in vivo (41). Seachrist et al. (41) report cervastatin-induced muscle damage after 14 days of administration to female rats. Protein levels of cleaved caspase-3 were only measured 24-h post dose and did not differ from controls. DNA fragmentation or other characteristic markers of apoptosis were not assessed. The data, although limited, suggest that skeletal muscle apoptosis may not occur in vivo. However, further in vivo studies will delineate if apoptosis may occur at later time points beyond 24 h and coincide with the appearance of muscle degeneration.

Mechanism of apoptosis in statin-induced myopathy. Despite ongoing research efforts, the underlying pathology of statin induced myopathy remains largely speculative. Although the primary clinical use of statins is to lower cholesterol levels by inhibiting de novo cholesterol synthesis, evidence supports that a decreased cholesterol level likely is not a significant factor contributing to myopathy. Experiments show that the inhibition of squalene synthase, an enzyme downstream of HMG-CoA reductase in the cholesterol biosynthetic pathway, does not cause myotoxicity (19). This suggests that myotoxicity due to HMG-CoA reductase inhibition by statins is not due to decreased synthesis of cholesterol but rather to suppressed synthesis of alternative downstream products of mevalonate metabolism, such as the isoprenoids.

Indeed, it has been shown in several cell types, including skeletal muscle cells, that depletion of isoprenoids are responsible for the induction of apoptosis (3, 8, 13, 14, 19, 27, 30, 33, 49). Isoprenoid intermediates serve as important lipid moieties for the posttranslational modifications of membrane-associated proteins such as Ras, Rho, and Rac (25, 28). Isoprenylation, farnesylation and geranylgeranylation, of these proteins regulate their translocation to the plasma membrane and/or activity (25, 28). For example, farnesylation of Ras is required for its translocation from the cytoplasm to the plasma membrane where it becomes activated and geranylgeranylation of Rho is required for its translocation to the plasma membrane (27). Isoprenoids are also required for the synthesis of ubiquinone, a component of the electron transport chain.

Research efforts have been focused on whether apoptosis induced by isoprenoid depletion is a result of impaired ubiquinone synthesis, farnesylation, and/or geranylgeranylation of signaling proteins. There is not sufficient evidence to support that statin induced apoptosis is due to ubiquinone depletion (19, 22, 45). Johnson et al. (19) found no correlation between statin-induced apoptosis of rat and human myotubes and ubiquinone levels. Moreover, coadministration of mevalonate with cervastatin prevented apoptosis but did not have an effect on concentration of ubiquinone. Several in vitro studies have shown that coadministration of geranylgeranyl pyrophosphate with statins prevented or decreased the occurrence of apoptosis in various cell types, while farnesyl pyrophosphate had little or no effect (8, 13, 19, 33, 49). It has been further shown that inhibition of geranylgeranylation and the RhoA pathway, using
a geranylgeranyltransferase inhibitor, in human endothelial cells induced apoptosis while inhibition of farnesylation did not (27). Similar results have been shown in anaplastic thyroid cancer cells treated with statin. Statin treatment of these cells is associated with a dose-dependent decrease in the translocation of RhoA and Rac1, but not Ras, from the cytosol to the membrane (49). Thus, in some cell types, statin-induced apoptosis may be stimulated by inhibition of geranylgeranylation while the inhibition of farnesylation is less important. However, statin-induced apoptosis in L6 rat myoblasts, an undifferentiated cell type, has been shown to be associated with depletion of membrane-farnesylated Ras depletion, rather than geranylgeranylated Rho (30). Also, statin-induced apoptosis of vascular smooth muscle cells is rescued by geranylgeranyl pyrophosphate or farnesyl pyrophosphate, suggesting that both cellular processes are important for cell survival (17). Taken together, these results suggest that statin-induced apoptosis is likely not due to depletion of ubiquinone but rather due to the depletion of geranylgeranylated proteins or farnesylated proteins, which may be dependent on cell type or the state of differentiation.

Statin-induced apoptosis in skeletal myoblasts and myotubes has also been associated with elevated levels of cytosolic calcium (32, 40). It has been shown that statins induce the phosphorylation of tyrosine, which leads to an increase in cytosolic calcium, resulting in apoptosis (32). It is speculated that the alteration in isoprenoid synthesis with statin therapy is responsible for the tyrosine phosphorylation and stimulation of pathways causing a rise in cytosolic calcium. Sacher et al. (40) have shown that the elevated calcium levels in response to statins activate calpain, which in turn leads to apoptosis via the translocation of Bax to the mitochondria and activation of caspase-9 and caspase-3. Inhibition of the mitochondrial transition pore, in which opening is required for cytochrome c release and thought to be regulated by Bax, prevented apoptosis and activation of caspase-9 and caspase-3. Further experimentation showed that chelation of calcium completely prevented calpain, caspase-9, and caspase-3 activation (40). Also, coadministration of mevalonate with the statin attenuated the rise in cytosolic calcium levels, although did not completely prevent it (40). Taken together, statin-induced apoptosis is largely in part due to the depletion of isoprenoids, which in turn can decrease protein geranylgeranylation and/or farnesylation, which, in turn, may lead to elevated levels of cytosolic calcium and activation of mitochondrial-mediated apoptotic signaling.

REFERENCES


