J Pharmacol Sci **123**, 289 – 294 (2013)

Journal of Pharmacological Sciences

Current Perspective

Mechanism of Statin-Induced Rhabdomyolysis

Kazuho Sakamoto[1,*] and Junko Kimura[1]

[1]*Department of Pharmacology, Fukushima Medical University School of Medicine, 1 Hikarigaoka, Fukushima, Fukushima 960-1295, Japan*

Received May 22, 2013; Accepted September 26, 2013

Abstract. Statins, a group of drugs used for the treatment of hypercholesterolemia, have adverse effects on skeletal muscle. The symptoms of these effects range from slight myalgia to severe rhabdomyolysis. The number of patients currently taking statins is estimated to be several millions worldwide. However, the mechanism of statins' myotoxic effects is unclear. Statins inhibit biosynthesis of mevalonate, a rate-limiting step of cholesterol synthesis, by inhibiting HMG-CoA reductase. Mevalonate is also an essential precursor for producing isoprenoids such as farnesylpyrophosphate and geranylgeranylpyrophosphate. These isoprenoids are especially important for anchoring small GTPases to the membrane before they function; e.g., Ras GTPases modulate proliferation and apoptosis, Rho GTPases control cytoskeleton formation, and Rab GTPases are essential for intracellular vesicle trafficking. Inactivation of these small GTPases alters cellular functions. Recently, we successfully reproduced statin-induced myotoxicity in culture dishes using in vitro skeletal muscle systems (e.g., skeletal myotubes and myofibers). This review summarizes our findings that statins induce depletion of isoprenoids and inactivation of small GTPases, especially Rab, which are critical for statin-induced myotoxicity. Although further study is required, our findings may contribute to the prevention and treatment of statins' adverse effects on skeletal muscle and development of safer anti-hypercholesterolemia drugs.

Keywords: prenylation, rhabdomyolysis, skeletal muscle, small GTPase, statin

1. Introduction

Increase of serum cholesterol and/or low-density lipoprotein (LDL cholesterol) accelerates atherosclerosis and promotes the risk of coronary heart disease (1). Cholesterol is one of the end products of the mevalonate pathway, in which the rate-limiting step is the conversion of HMG-CoA to mevalonate mediated by HMG-CoA reductase, which is localized in the endoplasmic reticulum (ER) (2). Statins are structural analogs of HMG-CoA, developed to inhibit HMG-CoA reductase. Orally administered statins are mainly distributed in the liver and inhibit the biosynthesis of mevalonate. As a consequence, expression of the LDL receptor increases in the liver, enhancing its receptor-mediated endocytosis, and reducing LDL cholesterol levels in the circulation (1). A meta-analysis based on five clinical trials showed that the administration of three different statins (lovastatin, simvastatin, and pravastatin) reduced major coronary diseases and mortality by 31% and 21%, respectively (3).

Although statins have been proved to be useful drugs, there are some patients who are intolerant to the adverse effects of statins on skeletal muscles. The risk of statins for myotoxicity depends on the dose (4). The symptoms of statins' adverse effects range from myalgia or myopathy (pain and contractile dysfunctions) to rhabdomyolysis. Especially, severe rhabdomyolysis can be lethal. Statins induce necrosis of skeletal muscle, and the muscular contents including myoglobin are released into the blood flow to develop acute renal failure. Serum creatine phosphokinase (CPK) also rises due to muscular damage induced by statins. If CPK levels exceed 5 times the upper limit of the normal range, statin administration should be stopped. If the excess becomes 100 times the upper limit of the normal CPK range, a diagnosis of rhabdomyolysis can be made and adequate hydration is required (4). Statins induce vacuolation in the cytoplasm

*Corresponding author. kazuho@fmu.ac.jp
Published online in J-STAGE on November 19, 2013
doi: 10.1254/jphs.13R06CP

Invited article

of myofibers due to dilation of the sarcoplasmic reticulum and mitochondria, which is a sign of muscle damage (5). There are two different types of myofibers; one is aerobic red (Type I) and the other is glycolytic white (Type IIA/IIB) myofibers. Statins' myotoxicity mainly appears in white myofibers (5).

The first clinical trial for statin was conducted in 1980, which revealed adverse effects of myopathy and CPK elevation (6). The seriousness of this side effect became apparent with the cerivastatin incident, in which 52 patients were killed by rhabdomyolysis, leading to the removal of cerivastatin from the market in 2001 (7). While statin monotherapy rarely causes myotoxicity, combinations of some other drugs with statins elevate statin blood levels and increase the adverse event rate. Orally administered statins are mainly absorbed in the small intestine, and are taken up by hepatocytes via organic anion transporting polypeptides (OATP in humans, Oatp in rodents). They are then metabolized by cytochrome P450. In the case of cerivastatin, its metabolizer CYP2C9 was inhibited by co-administered gemfibrozil to elevate the serum concentration of cerivastatin, which facilitated the development of rhabdomyolysis (7). Drug–drug interactions take place not only at P450 but also at OATP because inter drug competition at the liver OATP suppresses the transition of drugs from the blood to the liver (8). Furthermore, people with OATP1B1 single nucleotide polymorphisms (SNPs) that reduce the transporter activity were more susceptible to statin-induced myotoxicity than those without SNPs (8). However, the mechanism of statins to injure the skeletal muscle was unknown, because skeletal muscle cells were difficult to culture, and therefore sufficient in vitro models were not available to analyze the mechanism of the drug actions.

2. In vitro skeletal muscle models for statins myotoxicity

Skeletal muscle is mainly composed of special cells termed myofibers. Skeletal myofibers are long (up to tens of cm) and multinuclear, which makes them difficult to culture to test drug actions. Alternatively, primary cultured or immortalized myoblasts from skeletal muscles (9) have been used as a model for in vitro skeletal muscles. These cells are mononuclear, proliferative, migrative, and adhesive, while myofibers in vivo are not. Therefore, it is unclear whether these cells are adequate models. Some studies adopted these systems for the analysis of statin-induced myotoxicity, but the results did not represent the phenotypes observed in animal experiments and humans. One of the discrepancies was the style of cell death. As described in a previous report, skeletal muscles damaged by statins demonstrated necrotic features including the release of muscle contents and inflammatory markers, while the myoblasts showed apoptotic features (10). Another discrepancy was the sensitivity to statins. For example, a therapeutic serum concentration of fluvastatin in human was up to 1 μM, while the myoblasts required concentrations higher than 10 μM fluvastatin to induce cell death (11) (see Table 1). This discrepancy was more prominent in the case of hydrophilic statins. Although 5-μM serum concentration of pravastatin damaged the skeletal muscle in an animal (rat) experiment, this concentration and a concentration even as high as 1 mM did not cause cell death in cultured rat skeletal myofibers (11, 12). Therefore, it seemed inappropriate to analyze the adverse effects of statins using cultured myoblasts.

If myoblasts are cultured under a low level of growth factor, the cells differentiate into long and multinuclear myotubes (9). They are more sensitive to statins than myoblasts. The toxic effects of hydrophilic pravastatin on the myotubes were observed at a μM range (13). Therefore, these cells were closer to the practical concentration (Table 1). It was however difficult to observe statin-induced morphological changes in myotubes because the statin treatment made myotubes shrink, unlike skeletal muscle fibers (13). In addition, it was difficult to examine the contractility of the myotubes, so they were not ideal for the analysis of statin myotoxicity.

3. Primary cultured skeletal myofibers reproduce statin myotoxicity

Establishment of an ideal skeletal muscle model was required for the precise analysis of statin myotoxicity in vitro. We adopted an enzymatic technique that enabled the isolation of physiological skeletal myofibers from

Table 1. Comparison of practical and in vitro toxic concentrations of statins

Compounds	C_{max} (μM)	C_{max} fold increase	Myoblast (μM)	Myofiber (μM)
Fluvastatin	0.5 – 1.1	1.3 – 6.0	10-	0.1-
Pravastatin	0.1 – 0.2	22.8	> 1000	10-

C_{max}: maximum serum concentration in normal administration (40 mg/day each) (ref. 8), C_{max} fold increase: fold increase of C_{max} in patients due to drug–drug interaction (ref. 8), Myoblast: toxic concentration for rat L6 myoblasts (ref. 11), Myofiber: toxic concentration for rat FDB myofibers (ref. 11).

rodent flexor digitorum brevis (FDB) muscle, which consists of the shortest myofibers in the body (14). We could establish a primary culture system for the myofibers from rat FDB muscles (11, 15 – 17).

In the cultured rat FDB myofibers, application of 1 μM fluvastatin or 10 μM pravastatin for 3 – 4 days induced vacuolation, which was similar to those reported in in vivo animal experiments (15, 16). Longer (> 5 days) treatment of the statins caused death of myofibers with a release of cell contents (15, 16) (Fig. 1). The statin treatment reduced the contractility of the cultured myofibers and made them vulnerable to caffeine-induced contraction, resulting in cell damage (15). These results were similar to those of previous reports of statin-induced muscle weakness (4) and myotoxicity exacerbated by exercise (18). Our study also revealed that statins reduced intracellular ATP levels and Ca^{2+} storage in sarcoplasmic reticulum, and thereby muscle contraction was suppressed (16). Furthermore, our cultured myofibers, unlike myoblasts, were sensitive to both lipophilic and hydrophilic statins, and the toxic concentrations of the statins were close to the clinically reported range (Table 1). Furthermore, we found that myofibers expressed two isoforms of Oatp, Oatp1a4 and Oatp2b1 (rat), and these drug transporters contribute to the uptake of statins into myofibers (11). Recently, another group confirmed our results and reported that OATP2B1 facilitated intramuscular statin accumulation to enhance myotoxicity in human myotubes (19). Thus, primary cultured myofibers successfully reproduced the adverse effects of statins on skeletal muscles morphologically and in terms of sensitivity to statins. We have adopted this as an in vitro model for the further analysis of statin-induced myotoxicity.

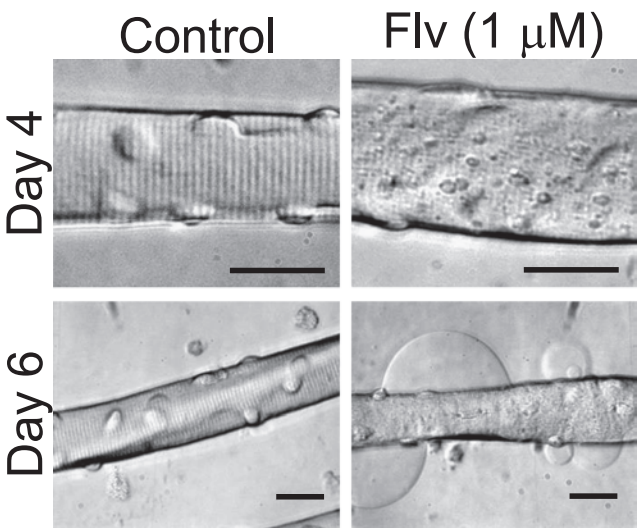

Fig. 1. Statin-induced morphological changes in FDB myofibers. Many vacuoles appeared in the myofibers cultured with 1 μM fluvastatin (Flv) for 4 days (upper right). Muscular contents were released from the myofibers cultured with 1 μM Flv for 6 days (lower-right). Bars: 20 μm. Modified from ref. 17.

4. Depletion of geranylgeranylpyrophosphate triggers statin myotoxicity

Among the biochemical products from the mevalonate pathway, farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP) are physiologically significant because these isoprenoids modify the functions of some small GTPases (Fig. 2), which work as a switch for cell functions. For example: Ras GTPases suppress apoptosis and enhance proliferation; Rho GTPases modulate cytoskeleton formation and adhesion; and Rab GTPases activate intracellular vesicle traffic. Modification of their C-terminus with FPP and/or GGPP is essential for the binding of these proteins to target membranes (20). Geranylgeranylation of Rho is mediated by geranylgeranyltransferase I (GGTase I); that of Rab is mediated by GGTase II; and farnesylation of Ras is mediated by farnesyltransferase (FTase) (20) (Fig. 2). Thus, statin-induced depletion of these isoprenoids invites inactivation of small GTPases. Durham et al. revealed that lovastatin reduced prenylation of small

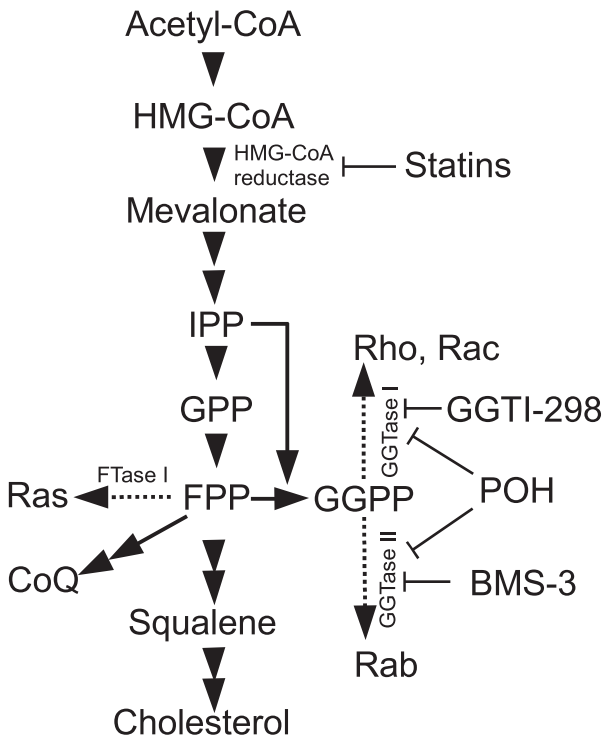

Fig. 2. Mevalonate pathway. CoQ: coenzyme Q, FPP: farnesylpyrophosphate, FTase: farnesyltransferase, GGPP: geranylgeranylpyrophosphate, GGTase: geranylgeranyltransferase, GPP: geranylpyrophosphate, IPP: isopentenylpyrophosphate.

GTPases and that supplementation of the geranylgeranyl group (geranylgeraniol: 1 – 30 μM) or farnesyl group (farnesol: 1 – 100 μM) canceled the lovastatin-induced loss of ATP in rat primary cultured myotubes (21). However, it is unclear in their study which isoprenoid depletion is more important for statin-induced myotoxicity. This is an important point for future drug development because FPP is a material for cholesterol production, while GGPP is not. Since GGPP is biosynthesized from FPP and isopentenylpyrophosphate, FPP alone does not recover GGPP level in statin-treated cells (Fig. 2). One group using rat immortalized L6 myoblasts reported that the depletion of FPP is critical for myotoxicity (22), but another laboratory using the same cell line suggested that GGPP was the critical isoprenoid (12). In our hands, it was not FPP (3 μM), but GGPP (3 μM) alone, because the addition of GGPP, not FPP, in the presence of fluvastatin completely canceled the toxic effects of statins in skeletal myofibers (15, 17). FPP also failed to rescue myotoxicity in a study using myotubes differentiated from C2C12 myoblasts (23). Therefore, we concluded that GGPP depletion must be critical for muscle death by the toxic effects of statins.

5. Inactivation of Rab GTPases reproduced statin myotoxicity in myofibers and myotubes

GGPP depletion should affect many geranylgeranylated protein activities. We further investigated which protein inactivation was critical for statin myotoxicity. It had been reported that inhibition of Rho GTPases and Rho-dependent kinase (ROCK) caused apoptotic cell death among proliferative adherent cells (24). L6 skeletal myoblasts were also damaged by statins due to the inactivation of the Rho/ROCK system (12). However, the inhibitors of either Rho or ROCK did not damage myofibers, unlike (in contrast to their effects on) myoblasts (15, 17). Furthermore, we tested the effect of GGTase inhibitors to determine whether geranylgeranylated small GTPases were involved in statin's adverse actions on myofibers. GGTI-298 (*N*-[4-[2(*R*)-amino-3-mercaptopropyl]amino-2-(1-naphthalenyl) benzoyl]-L-leucine methyl ester trifluoroacetate salt), a specific GGTase I inhibitor, did not reproduce the adverse effects of statins. On the other hand, perillyl alcohol, a selective GGTase II inhibitor, simulated vacuolation and cell death (15, 17). Why were differentiated muscle cells insensitive to the inactivation of the Rho/ROCK system? This is due to the system being closely involved in cell adhesion and proliferation, although neither was relevant to differentiated myocytes. Thus, myoblast adhesion and proliferation were inadequate features for examining the model of statin-induced myotoxicity.

There are more than 60 isoforms of Rab GTPases, and each isoform corresponds to different vesicle traffic pathways (25). We focused on Rab1, which was responsible for the traffic from the ER to the Golgi apparatus because this is the origin and the bottle-neck pathway for all the vesicle traffic systems. Application of 1 μM fluvastatin reduced the membrane-bound form of Rab1, and GGPP supplementation canceled the reduction of membrane-bound Rab1 as well as vacuolation and cell death (17). This directly indicates that fluvastatin treatment inactivated Rab1. Furthermore, the ER-Golgi traffic inhibitor brefeldin A also reproduced the effects of statins on myofibers (17). Therefore, statin-induced Rab1 inactivation must be an important step leading to the myotoxic effects of statins. Interestingly, the sensitivity of statin-induced GGPP depletion of small GTPases is non-uniform in neuroblastoma (26). Especially, Rab1 is one of the small GTPases most sensitive to GGPP depletion (26). If this is also true in the skeletal muscle, our theory would be the ultimate cause of muscle toxicity of statins.

6. How does Rab inactivation induce muscle cell death?

The final question in this review is how does the inactivation of Rab kill muscle cells? The retardation of ER-Golgi traffic induced ER stress to activate caspases in human hepatocytic carcinoma (27). However, neither fluvastatin nor brefeldin A increased ER stress in myofibers (17). The ER stress sensors in skeletal myofiber might be different from those in carcinoma.

Recently, one study reported that statins increased the expression of atrogin-1, an ubiquitin ligase that enhances muscular atrophy, in biopsied human skeletal muscle tissues (28), and this study also reported that it was due to the reduction of geranylgeranylated Rab (23). Thus, this might be the reason why statin-induced Rab inactivation injures skeletal muscles. However, it is hard to accept this Rab/atrogin-1 theory as the mechanism of rhabdomyolysis induced by statins because atrogin-1 induces atrophy, but not necrosis. On the other hand, this theory might be suitable for explaining why statins cause muscle weakness.

We found that both fluvastatin and brefeldin A reduced mitochondrial membrane potential and intracellular ATP levels in myofibers (16). These are observed not only in apoptosis but also in necrosis (29). Statin-induced ATP reduction was also reported using myotubes (19, 30). Wagner et al. (30) showed that BMS-3 [(*R*)-2,3,4,5-Tetrahydro-1-(1H-imidazol-4-ylmethyl)-3-(phenylmethyl)-4-(2-thienylsulfonyl)-1H-1,4-benzodiazepine-7-carboni-

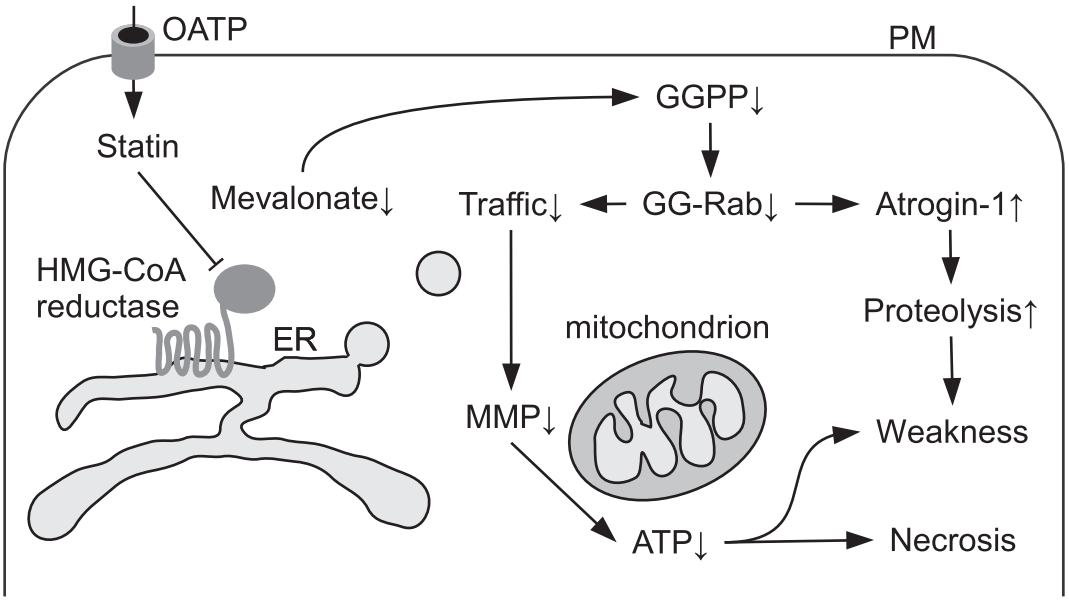

Fig. 3. Scheme for statin-induced rhabdomyolysis. ER: endoplasmic reticulum, GGPP: geranylgeranylpyrophosphate, GG-Rab: geranylgeranylated Rab GTPase, MMP: mitochondrial membrane potential, OATP: organic anion transporting polypeptide, PM: plasma membrane.

trile], a specific GGTase II inhibitor, also induced ATP reduction, while a specific GGTase I inhibitor did not (30). These reports suggest that the inhibition of vesicle traffic impairs mitochondrial respiration in differentiated myocytes. Further study is required to elucidate how "traffic jams" invite mitochondrial damage.

7. Conclusion

Statins are useful drugs for the prevention of cardiovascular events, but some patients are intolerant to their adverse effects on skeletal muscles. If this problem is resolved, a safer drug treatment will become available for hypercholesterolemia. A series of recent studies using differentiated muscle cells revealed that statins induce muscle cell damage by depleting GGPP, which reduces prenylated Rab, and intracellular vesicle traffic is consequently suppressed. This invites mitochondrial dysfunction and ATP depletion (Fig. 3). Based on these new findings, we hope for the development of a new drug for hypercholesterolemia, which will not induce myopathy during treatment.

Acknowledgments

We thank Ms. Midori Sasaki-Yatabe for editing the English of this manuscript. This work was supported by JSPS KAKENHI Grant Numbers 20790210, 22790257, and 25460338 to KS.

Conflicts of Interest

The authors declare no conflicts of interest.

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