Influence of 3-hydroxy-3-methylglutaryl Coenzyme A Reductase Inhibitors on Ubiquinone Levels in Rat Skeletal Muscle and Heart: Relationship to Cytotoxicity and Inhibitory Activity for Cholesterol Synthesis in Human Skeletal Muscle Cells

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Although statins are prescribed as relatively safe and effective drugs for hypercholesterolemic patients, it has been reported that a significant side effect, myopathy, occurs infrequently during medication. Moreover, because statins decrease cardiac ubiquinone levels, the risk of cardiac dysfunction has been suggested. This study sought to evaluate and compare the cytotoxicity of statins (cerivastatin, pitavastatin, fluvastatin, simvastatin, atorvastatin and pravastatin) in cultured human skeletal muscle cells (HSkMCs) and the effects on ubiquinone levels in statin-treated rat skeletal muscle and heart. Cerivastatin, the most potent inhibitor of HMG-CoA reductase, showed the strongest cytotoxicity (over 10-fold) among the statins examined, while the effects of the others were in a similar range. In rat experiments, neither pitavastatin nor cerivastatin decreased ubiquinone levels in skeletal muscle, but both dose-dependently lowered ubiquinone levels in the heart. As the rates of reduction by pitavastatin (9.6% at 30 mg/kg) and cerivastatin (9.7% at 0.3 mg/kg) were almost equal, it was estimated that cerivastatin reduced ubiquinone levels in the rat heart approximately 100-fold more strongly than pitavastatin, based on the effective doses. We found that cerivastatin showed the most potent cytotoxicity in HSkMCs and strongly lowered ubiquinone levels in the rat heart.


Key words; Statin, Ubiquinone, Cytotoxicity, Myopathy

Introduction

Hydroxymethylglutaryl-coenzym A (HMG-CoA) reductase inhibitors, statins, are the most frequently used drugs to reduce plasma cholesterol levels and decrease the frequency of cardiovascular events¹-². Although statins are generally well-tolerated in patients, some adverse reactions have been observed infrequent-
at the bifurcation for other biosynthetic pathways\(^\text{10, 11}\), statins inhibit not only the biosynthesis of cholesterol but also the biosynthesis of dolichol, hem A, and isopentenyladenosine, the prenylation of proteins and the biosynthesis of the lipophilic side chain of ubiquinone\(^\text{10, 12}\). Therefore, several possible mechanisms of statin-associated myopathy have been proposed recently: (I) depletion of secondary metabolic intermediates including ubiquinones; (II) induction of apoptosis by the suppression of protein prenylation; and (III) alterations of chloride channel conductance within myocytes\(^\text{13}\).

The ubiquinone concentration and mitochondrial function in rat skeletal muscle treated with cerivastatin have been evaluated in detail\(^\text{14-16}\). The results showed that although the ubiquinone levels in type II skeletal muscle were slightly lowered by cerivastatin treatment, the mitochondrial function in skeletal muscle was not affected by changes in ubiquinone levels. Also, neither mitochondrial dysfunction nor a decrease in ubiquinone levels was correlated with histological changes in cerivastatin-treated rat muscle. On the other hand, just recently, it has been reported that high-dose treatment with simvastatin (80 mg/d) decreased ubiquinone levels in human skeletal muscle and decreased respiratory chain enzyme activity in a minority of patients with a substantial reduction in muscle ubiquinone level\(^\text{17}\). In addition, because ubiquinones play an important role in the mitochondrial respiratory chain, the possibility of the influence of statins on the myocardial energy generating system and the risk of cardiac dysfunction associated with a decreased cardiac ubiquinone level have been pointed out\(^\text{14, 16}\). Especially in the ischemic heart, lipophilic statins increased myocardial contractile dysfunction during reperfusion after ischemia (stunning), with a reduction of tissue adenosine triphosphate (ATP) in dogs\(^\text{17}\).

Pitavastatin is a new chemically synthesized statin. It has strong HMG-CoA reductase inhibiting activity\(^\text{18}\) and relatively strong plasma total cholesterol (TC) -lowering activity as compared with other statins\(^\text{19, 20}\). In clinical trials involving patients with hyperlipidemia, it was confirmed that pitavastatin has an excellent serum cholesterol-lowering effect\(^\text{21, 22}\). Pitavastatin is classified as a lipophilic statin. Its log P value is 1.49, which suggests that its lipophilicity is moderate compared to that of other lipophilic statins, such as simvastatin (1.88 in acid form and 4.40 in lactone form), atorvastatin (1.53) and cerivastatin (2.32)\(^\text{23, 24}\). The moderate lipophilicity of pitavastatin is thought to be one of the important chemical properties of this compound and accounts in part for its influence on living bodies.

The present study was designed to compare the \textit{in vitro} potencies of cytotoxicity induced by various kinds of statins in cultured human skeletal muscle cells (HSkMCs) and to compare the effects of statins on ubiquinone levels in statin-treated rat skeletal muscle and heart. In HSkMCs, cerivastatin, which was the most potent inhibitor of cholesterol biosynthesis among the statins tested here\(^\text{25}\), potently lowered cellular cholesterol, ubiquinone and protein levels, and showed the most potent cytotoxic activity in this study. In addition, we evaluated the levels of ubiquinones in rat skeletal muscle and heart treated with pitavastatin or cerivastatin. We found that cerivastatin seemed to reduce ubiquinone levels more potently than pitavastatin in rat hearts.

**Materials and Methods**

**Reagents**

Pitavastatin was synthesized by Nissan Chemical Industries (Tokyo, Japan) while atorvastatin, simvastatin, cerivastatin, fluvastatin and pravastatin were synthesized and purified in our laboratory. Coenzyme Q9 (CoQ9) was purchased from Sigma Chemicals (St. Louis, MO, USA). Ubiquinone-10 (CoQ10) and ubiquinone-11 (CoQ11) were purchased from Wako Pure Chemical Industries (Osaka, Japan). All other reagents were purchased commercially and were of HPLC grade or extra pure grade.

**Cells and Cell Culture**

Human skeletal muscle cells (HSkMCs, CELL APPLICATIONS, Inc.) were purchased from TOYO-BO (Osaka, Japan). These HSkMCs, which were derived from normal human fetal skeletal muscle, were positive for sarcomeric myosin and were fused into multinucleated myotubes. The cells were cultured at 37°C in a 5% CO\(_2\) atmosphere and grown in skeletal muscle growth medium (CELL APPLICATIONS, Inc.). The cells were plated in 24-well culture plates or 6-well culture plates and grown to semi-confluence in growth medium. The cells were then induced to differentiate by changing the growth medium to skeletal muscle cell differentiation medium (CELL APPLICATIONS, Inc.). The experimental treatments began 3 days later, at which point myotubes were evident.

**Estimation of Cytotoxicity**

Cell viability was estimated by measuring active mitochondrial dehydrogenase activity (MTT assay)\(^\text{26}\). Briefly, HSkMCs were cultured in 24-well culture plates and grown to semi-confluence. The cells were then induced to differentiate by changing the growth
medium to differentiation medium. Three days later, various concentrations of drugs were added and incubated for 24 hours. The cells were examined by light microscopy and were supplemented with one-tenth the culture volume of 5 mg/mL MTT and incubated for 4 hours. The precipitates were dissolved in 0.04 mol/L HCl solution in 2-propanol and the absorbance at a wavelength of 540 nm with background subtraction at 620 nm was measured. All assays were carried out with quadruplicate sets of wells.

**Animals and Dosing**

Male Wistar (Slc/Wistar) rats (Japan SLC, Shizuoka, Japan) were approximately 7 weeks old and weighed approximately 150-190 g at study initiation. All rats were housed in a room maintained at 23 ± 3°C, with relative humidity at 55 ± 15%, under a constant light/dark cycle (light from 7:00 a.m. to 7:00 p.m.), and allowed ad libitum access to water and standard rodent chow (CE-2; CLEA Japan, Inc., Tokyo, Japan). All the experimental procedures were approved by the Animal Ethics Committee of Kowa Company according to internationally valid guidelines. Pitavastatin and cerivastatin were suspended in a 0.5% sodium carboxymethylcellulose solution and stored at 4°C until use. Pitavastatin (3, 10, 30 mg/kg), cerivastatin (0.3, 1, 3 mg/kg) or the vehicle alone (control) was administered daily at 4:00 p.m. for 2 weeks by oral gavage (2 mL/kg).

**Necropsy and Tissue Collection**

Eighteen hours after the final administration, venous blood samples were taken from the abdominal vein under pentobarbital anesthesia. The plasma was separated and used to measure TC, triglyceride (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and creatine phosphokinase (CPK) levels. The assay kits, Cholesterol E-test Wako, Triglyceride E-test Wako, Transaminase CII-test Wako, LDH CII-test Wako, and CPKII-test Wako were purchased from Wako Pure Chemical Industries. Heart and lateral great muscle were collected, weighed, quickly frozen in liquid nitrogen, and stored at −80°C for subsequent analysis of ubiquinone concentrations.

**Sample Preparation and HPLC Analysis**

CoQ9 and CoQ10 stock standard solutions were prepared by dissolving appropriate amounts of the compound in 2-propanol to a final concentration of 100-200 µg/mL and stored at −80°C. Working solutions for the preparation of calibration and quality control standards were prepared by diluting the stock solutions in 2-propanol. CoQ11 stock internal standard solution was prepared by dissolving the appropriate amounts of the compound in 2-propanol to a final concentration of 100 µg/mL and stored at −80°C. HPLC analysis was performed according to the rapid determination method with some modifications. Briefly, the tissue samples were removed from a −80°C freezer, kept on ice, and minced with scissors. Into each sample tube was added 1 mL of Milli-Q water (Millipore, Milford, MA) and the mixture was homogenized for approximately 2 min using a Physcotron Handy Micro Homogenizer (NS-310E, NITI-ON, Chiba, Japan). The samples were supplemented with 2.5 mL of ethanol, 50 µL of appropriate CoQ11 internal standard stock solution, and 300 µL of 2% FeCl₃ solution in ethanol to convert ubiquinones to the oxidized form, briefly vortex-mixed, and allowed to sit for 5 min at room temperature. The ubiquinones were extracted as follows. Five milliliters of n-hexane was added to the sample tube, vigorously shaken for 5 min at 250 min⁻¹, and centrifuged for 5 min at 3,000 rpm. After centrifugation, an appropriate aliquot of the supernatant was collected and evaporated to dryness under a nitrogen flow. The residue was dissolved in 200 µL of 2-propanol and a 10-µL aliquot was subjected to HPLC. Chromatographic separation was performed using a Shimadzu CLASS-LC10A system. The sample was injected onto a Waters spherisorb 5 m reversed phase column (4.6 x 150 mm, maintained at 40°C). The solvent was methanol/ethanol (1:1). The flow rate was 1.2 mL/min and the program time 10 min. The eluate was monitored at 275 nm. All the peaks were well resolved and quantitation was based on the peak height, using internal standards to compensate for sample losses.

**Determination of Tissue Lipid Contents**

For the determination of tissue TC and TG, an approximately 300-mg portion of the tissue was extracted with 10 mL of chloroform/methanol (2:1) in a Polytron homogenizer (PT-3100, KINEMATICA, Switzerland). An appropriate aliquot of the lower phase was taken, evaporated to dryness and dissolved in 50 µL of 2-propanol. The lipid concentration was determined enzymatically as described above.

**Determination of Cellular Cholesterol, Ubiquinone and Protein Contents**

For determination of the cellular cholesterol levels, lipids were extracted from cells cultured in a 6-well plate with n-hexane/2-propanol (3:2). The extracts were evaporated to dryness and dissolved in 250 µL of 2-propanol containing 10% Triton X-100. The cho-
lesterol content was determined enzymatically as described above. Intracellular CoQ10 levels were determined by HPLC analysis as described above. For determination of the cellular protein contents, the cells were digested with 1 mol/L NaOH, and protein contents in the diluted cell digests were measured with Micro BCA Protein Assay Reagent (Pierce, Rockford, IL, USA).

**Statistical Analysis**

Statistical analysis was performed by ANOVA for multiple comparisons, followed by Dunnett’s or Tukey’s test using EXSAS Ver.5.00 software (Scientist Co., Tokyo, Japan) and SAS System Release 6.12 (SAS Institute, Tokyo, Japan). Differences were considered significant at $p < 0.05$.

**Results**

**Effects of Statins on the Viability of Differentiated Human Skeletal Muscle Cells**

As it has been reported that some patients treated with statins develop rhabdomyolysis, we studied the effects of statins on the viability of differentiated, myotube-forming human skeletal muscle cells. Light microscopic evaluation revealed that cell cultures treated with concentrations of cerivastatin starting at 0.1 μmol/L showed many spherical or floating cells (Fig. 1). In contrast, pitavastatin-, fluvastatin-, simvastatin-, and atorvastatin-treated cells looked normal, at least at that drug concentration. No apparent changes were observed even in cells treated with 10 μmol/L pravastatin (not shown). The effects of statins on cell viability were tested by the MTT assay (Fig. 2). Cerivastatin concentration-dependently lowered cell viability. Significant reductions of viability compared to control cells were observed at 1 and 10 μmol/L. Pitavastatin and atorvastatin significantly lowered cell viability at 10 μmol/L. Fluvastatin, simvastatin and pravastatin had no significant effects even at the concentration of 10 μmol/L. Cerivastatin showed the most potent cytotoxic activity among the tested drugs. To investigate whether statin-induced cytotoxicity was dependent on mevalonate depletion, the cells were incubated with cerivastatin (10 μmol/L) or pitavastatin (10 μmol/L) in the presence or absence of mevalonolactone (1 mmol/L). The reduced cell viability induced by cerivastatin was abolished by the addition of mevalonolactone (Fig. 3).

**Fig. 1.** Cerivastatin-induced cell damage in cultured human skeletal muscle-like cells.

Cells were cultured in 24-well culture plates and grown to semi-confluence. They were then induced to differentiate by changing the growth medium to differentiation medium. Three days later, various concentrations of drugs were added and the cultures were incubated for 24 hours. Cells were photographed under a light microscope.
Effects of Statins on Cellular Cholesterol, Ubiquinone and Protein Levels

To evaluate the relationship to the cytotoxicity induced by statins, cellular cholesterol, ubiquinone and protein levels in cerivastatin- and pitavastatin-treated cells were measured. Both statins concentration-dependently lowered cellular cholesterol, CoQ10 and protein levels (Fig. 4). Cerivastatin lowered cellular cholesterol levels significantly compared to the control at 0.1 μmol/L or more, and pitavastatin lowered cholesterol levels significantly at 1 μmol/L or more in this experiment. Cerivastatin seemed to reduce cellular...
cholesterol levels approximately 10-fold more potently than pitavastatin, as indicated by the effective doses. Similarly, cerivastatin seemed to reduce cellular protein levels approximately 10-fold more potently than pitavastatin, as compared with the reduction rates of protein levels. The reduction of cellular CoQ10 levels induced by cerivastatin was almost abolished by the addition of mevalonolactone (Fig. 5).

**Effects of Statins on Tissue Ubiquinone Levels in Rats**

Ubiquinone levels (CoQ9 and CoQ10) in skeletal muscle (lateral great muscle) and heart of rats were measured by HPLC analysis. CoQ9 was the major form of ubiquinone in both tissues in rats. The concentration of CoQ10 in both tissues was much lower than that of CoQ9. Ubiquinone levels in the skeletal muscle and heart were different, but the ratios of CoQ9 and CoQ10 were very similar. CoQ9 and CoQ10 levels in the skeletal muscle are shown in Fig. 6. Neither pitavastatin nor cerivastatin showed a significant effect on the CoQ9 or CoQ10 concentration in skeletal muscle. Fig. 7 shows the effects of pitavastatin and cerivastatin on CoQ9 and CoQ10 concentrations in the heart. Pitavastatin lowered ubiquinone levels significantly compared to the control at 30 mg/kg, while cerivastatin lowered ubiquinone levels significantly at 0.3 mg/kg or more. The
rates of reduction by pitavastatin (9.6% at 30 mg/kg) and cerivastatin (9.7% at 0.3 mg/kg) were almost equal in this experiment. Thus, cerivastatin seemed to reduce ubiquinone levels in the rat heart approximately 100-fold more potently than pitavastatin.

**Effects of Statins on Plasma Lipid Levels in Rats**

The plasma TC and TG levels after 2 weeks of treatment with pitavastatin and cerivastatin are shown in Table 1. Neither pitavastatin nor cerivastatin showed any effect on the plasma TC level. Pitavastatin (10 mg/kg) and cerivastatin (1 and 3 mg/kg) lowered the plasma TG level significantly compared to in control rats.

**Fig. 6.** Effects of pitavastatin and cerivastatin on ubiquinone levels in rat skeletal muscle.

Pitavastatin (3, 10, 30 mg/kg, n=8), cerivastatin (0.3, 1, 3 mg/kg, n=6) or the vehicle alone (control, n=8) was administered daily for 2 weeks by oral gavage. Eighteen hours after the final administration, the lateral great muscles were collected under pentobarbital anesthesia. Ubiquinone levels were determined by HPLC analysis as described in Materials and Methods. A, Coenzyme Q9 content; B, Coenzyme Q10 content. All data represent the mean ± SD of 6 or 8 animals.

**Fig. 7.** Effects of pitavastatin and cerivastatin on ubiquinone levels in rat heart.

Experimental procedures were the same as in Fig. 4. Ubiquinone levels in rat hearts were determined by HPLC analysis. A, Coenzyme Q9 content; B, Coenzyme Q10 content. All data represent the mean ± SD of 6 or 8 animals. *p<0.05, **p<0.01, ***p<0.001 compared with the control.

**Effects of Statins on Plasma AST, ALT, LDH and CPK Activities in Rats**

The plasma AST, ALT, LDH and CPK activities in the rats are shown in Table 1. Neither pitavastatin nor cerivastatin showed any effect on plasma CPK and LDH activities. In rats treated with cerivastatin, the plasma LDH activity tended to increase slightly, but not significantly. Plasma AST and ALT activities in rats treated with cerivastatin at 1 mg/kg increased significantly, but we could not demonstrate the dose-dependency of the effect.

**Effects of Statins on Tissue Lipid Levels in Rats**

The lipid contents in tissues after 2 weeks of treatment with pitavastatin and cerivastatin are shown
Table 1. Determination of various biochemical blood indices for the evaluation of the effect of pitavastatin and cerivastatin treatment in rats

<table>
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<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>TC (mg/dL)</th>
<th>TG (mg/dL)</th>
<th>AST (unit)</th>
<th>ALT (unit)</th>
<th>LDH (unit)</th>
<th>CPK (IU/L)</th>
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<td>119.7</td>
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<td>10</td>
<td>8</td>
<td>Mean</td>
<td>56.5</td>
<td>64.0**</td>
<td>65.4</td>
<td>23.1</td>
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<td>Mean</td>
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<td>57.0**</td>
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<td>140.2</td>
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Pitavastatin, cerivastatin or the vehicle alone (control) was administered daily for 2 weeks by oral gavage. Venous blood samples were taken from the abdominal vein under pentobarbital anesthesia. The plasma was separated and measured for TC, TG, AST, ALT, LDH, and CPK levels as described in Materials and Methods. *p < 0.05, **p < 0.01 compared with the control.

in Table 2. Pitavastatin did not have any effect on the lipid content in either skeletal muscle or the heart. Cerivastatin (3 mg/kg) slightly but significantly decreased the TC content in skeletal muscle. Cerivastatin did not have any effect on the TG content in either tissue.

Discussion

Although statins are known to decrease the mortality and occurrence of cardiovascular events, and have been used successfully clinically, some adverse effects have been observed at a relatively low frequency (<0.5%)<sup>3–5</sup>). The most serious adverse effect is skeletal muscle problems, i.e., myopathy, including clinically significant myositis and rhabdomyolysis<sup>5</sup>). There have been many reports of studies of statin-induced myopathy in animals and humans<sup>3–7</sup>, but the precise mechanism has not been elucidated clearly. Statins inhibit cholesterol biosynthesis by inhibiting HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis<sup>8, 9</sup>). Inhibition of HMG-CoA reductase by statins decreases the level of mevalonate and farnesyl pyrophosphate, one of the metabolites produced from mevalonate in the pathway. As farnesyl pyrophosphate is the key substance at the bifurcation of other biosynthetic pathways<sup>10, 11</sup>, statins inhibit not only the biosynthesis of cholesterol but also the biosynthesis of dolichol, hem A, and isopentenyladenosine, the prenylation of proteins, and the biosynthesis of the lipophilic side chain of ubiquinone<sup>10, 12</sup>). Therefore, several possible mechanisms for statin-associated myopathy have been proposed recently: (I) depletion of secondary metabolic intermediates; (II) induction of apoptosis; and (III) alterations of chloride channel conductance within myocytes<sup>5</sup>.

It has been thought that statin-induced myopathy <i>in vivo</i> might result from damage to skeletal muscle cells resulting from the inhibition of HMG-CoA reductase activity. Therefore, we compared the <i>in vitro</i> potency of cytotoxicity induced by various kinds of statins in differentiated, myotube-forming human skel-
etal muscle cells (HSkMCs). In the MTT assay, cerivastatin concentration-dependently lowered cell viability. Significant reductions of viability compared to control cells were observed at 1 and 10 μmol/L cerivastatin, while pitavastatin and atorvastatin lowered cell viability significantly at 10 μmol/L. The other statins tested, fluvastatin, simvastatin and pravastatin, had no effect even at the concentration of 10 μmol/L. The cytotoxic potency of cerivastatin was approximately 10-fold or more higher than pitavastatin, atorvastatin, fluvastatin, simvastatin and pravastatin. The reduction of cell viability induced by cerivastatin was prevented by the addition of mevalonolactone. These results suggest that the cytotoxicity induced by statins in cells is related to the inhibition of HMG-CoA reductase. In this study, both cerivastatin and pitavastatin concentration-dependently reduced cellular cholesterol, ubiquinone and protein levels. As compared with the lowering potency, cerivastatin seemed to reduce cellular cholesterol, CoQ10 and protein levels approximately 10-fold more potently than pitavastatin. The reduction of cellular CoQ10 levels induced by cerivastatin was abolished by the addition of mevalonate similarly to that of cell viability. So, it seemed that the inhibition of cellular mevalonate biosynthesis by statins followed by the reduction of downstream metabolites in the pathway was one of the possible causes of statin-induced cytotoxicity in cells. The inhibitory effects of pitavastatin and other statins on sterol synthesis were previously demonstrated in HSkMCs and hepatocyte cells\(^{25}\). Each statin inhibited sterol synthesis concentration-dependently in both cell culture systems. Among the statins tested, cerivastatin showed the most potent inhibitory effect, with 50% inhibitory concentrations (IC\(_{50}\)) of 0.3 nmol/L in HSkMCs and 3.8 nmol/L in hepatocytes. In HSkMCs, the potency of cerivastatin was approximately 10-fold stronger than pitavastatin, fluvastatin, simvastatin and atorvastatin, which had IC\(_{50}\) values of 3.4, 4.1, 4.8, 4.7 nmol/L, respectively. Pravastatin, with an IC\(_{50}\) value of 164.4 nmol/L, was the weakest inhibitor among these statins. In hepatocytes, the IC\(_{50}\) values of pitavastatin, fluvastatin, simv-
astatin and atorvastatin were 24.5, 23.9, 106.0 and 209.0 nmol/L, respectively, while the IC$_{50}$ value of pravastatin was over 1000 nmol/L. Thus, it seemed that pitavastatin and fluvastatin were several times more effective than simvastatin or atorvastatin for the inhibition of hepatocytic cholesterol biosynthesis. Also, there might be differences in the inhibitory potencies of statins depending on the cell type$^{[29]}$. The results of this study revealed that cerivastatin, which was the most potent inhibitor of cholesterol biosynthesis in HSkMCs, lowered cellular cholesterol, ubiquinone and protein levels strongly, and showed the most potent cytotoxic activity in cells. It has been suggested that statin-induced apoptosis in human myotube cultures similar to our cell culture system is related to the inhibition of HMG-CoA reductase and in part to the inhibition of protein geranylgeranylation$^{[38]}$. At present, it seemed that the potency of cytotoxicity caused by statin treatment of HSkMCs was mainly correlated with the inhibitory activities of statins toward cholesterol biosynthesis, namely inhibitory activities toward mevalonate biosynthesis followed by the reduction of downstream metabolites in the pathway.

Ubiquinone biosynthesis has been extensively studied in the past$^{[29]}$. Circulating ubiquinone is in a reduced form (ubiquinol). It has been demonstrated that after hepatic biosynthesis, ubiquinol is transported into lipoproteins$^{[30]}$ where it acts as a highly reactive antioxidant$^{[31]}$. Ubiquinone is present in variable amounts in all tissues. Ubiquinone has a very important endogenous role in mitochondrial electron transfer and energy metabolism$^{[32]}$. It has been suggested that tissue ubiquinone levels are essentially dependent on de novo synthesis through the multiple-step pathway of cholesterol biosynthesis$^{[33]}$, and that tissue ubiquinones are not redistributed via the circulation$^{[34]}$. In skeletal muscle, as the decrease of ubiquinone levels is thought to be one of the causative phenomena of muscle disorders, evaluation of the effect of statins on ubiquinone levels in skeletal muscle is considered useful to predict the possibility that statins will cause the development of myopathy. In humans, simvastatin at low doses (20 mg/d) did not decrease skeletal muscle ubiquinone$^{[35, 36]}$, but high-dose treatment with simvastatin (80 mg/d) decreased ubiquinone levels in human skeletal muscle and decreased respiratory chain enzyme activity in a minority of patients with a substantial reduction in the muscle ubiquinone level$^{[33]}$. In that study, simvastatin and atorvastatin similarly reduced cholesterol and ubiquinone levels in serum, but only simvastatin treatment reduced skeletal muscle ubiquinone levels. Thus, the reduction in plasma ubiquinone levels may not be used as a marker for skeletal muscle ubiquinone levels$^{[13]}$.

Clinical studies on long-term treatment with statins have been reported$^{[37-39]}$, and they have all shown that statins slowed and reduced the development of atherosclerosis in the coronary arteries. Large randomized double-blind studies with simvastatin and pravastatin have shown that these statin treatments are associated with a reduced risk of coronary heart disease$^{[2]}$. Moreover, in a study with simvastatin, long-term prevention reduced the occurrence of heart failure in a cohort of patients with coronary heart disease$^{[40]}$. Lovastatin is also used for the treatment of hypercholesterolemia and successfully lowers cholesterol levels: it has been reported that lovastatin lowered the blood level of CoQ10 and increased cardiac dysfunction$^{[41]}$. Lovastatin also decreased the level of CoQ9 in rat blood, heart and liver$^{[42]}$. It has been reported that the administration of pravastatin and simvastatin for 3 weeks to dogs did not change mitochondrial respiratory indices in the non-ischemic heart, while simvastatin treatment worsened some mitochondrial respiratory indices in the ischemic heart$^{[43]}$. Similar results have been obtained in rat experiments$^{[44]}$. An HMG-CoA reductase inhibitor, particularly a hydrophobic one, may cause cardiac dysfunction by worsening the mitochondrial respiratory function during ischemia$^{[43, 44]}$.

In our study of male rats, pitavastatin and cerivastatin did not show obvious effects on ubiquinone and cholesterol contents in skeletal muscle. So, it remains unclear how these potent statins affect them in rat skeletal muscle. On the other hand, in the rat heart, both pitavastatin and cerivastatin dose-dependently lowered ubiquinone levels, but no effects were observed on the cholesterol contents. It has been suggested that tissue ubiquinone levels are essentially dependent on de novo synthesis$^{[33]}$, and that tissue ubiquinones are not redistributed via the circulation$^{[34]}$. On the other hand, it is well-known that tissue cholesterol homeostasis is regulated by the lipoprotein receptor system$^{[45]}$. So, tissue ubiquinone levels might be more sensitive to the inhibitory effects of statins than tissue cholesterol levels. In this study, there was a difference in the effect of statin treatment on ubiquinone levels between the rat skeletal muscle and heart. There might also be differences in sensitivity to the inhibitory effects of statins depending on the cell type as described above$^{[29]}$. Ubiquinone levels in the heart were decreased 9.6% from the control levels by pitavastatin treatment at 30 mg/kg/day for 2 weeks. Cerivastatin lowered the ubiquinone levels significantly at doses of 0.3 mg/kg or higher: ubiquinone levels were decreased 9.7, 11.7 and 12.5% by cerivastatin dosing at 0.3, 1 and 3 mg/kg, respectively. The rates of reduction caused by pitavastatin (30 mg/kg) and cerivastatin (0.3 mg/kg) were almost
equal in this experiment. Thus, cerivastatin seemed to reduce ubiquinone levels in the rat heart approximately 100-fold more potently than pitavastatin, as indicated by the effective doses.

Selectivity of tissue distribution among statins has been reported previously\(^\text{46-49}\). Pravastatin is the most hydrophilic statin among the statins now in use and inhibits cholesterol biosynthesis selectively in hepatocytes\(^\text{49}\), while simvastatin, a hydrophobic statin, inhibits HMG-CoA reductase in all organs\(^\text{46-49}\). It has been reported that the relatively selective inhibition of hepatic cholesterol synthesis by hydrophilic pravastatin in vivo might be due to the existence of a specific uptake mechanism mediated by the Na\(+\)-independent multispecific anion transporter system in the liver\(^\text{50, 51}\).

In the present study, we found that cerivastatin lowered ubiquinone levels in the rat heart more potently than pitavastatin. As the biodistribution of cerivastatin in the rat is not known, it remains unexplained why cerivastatin potently decreased ubiquinone levels in the rat heart. It is known that the hydrophobicity of cerivastatin (log P\(_{\text{aqua}}\) = 2.32) is greater than that of simvastatin (log P\(_{\text{aqua}}\) = 1.88 in acid form)\(^\text{24}\), and cerivastatin may also be distributed to all organs, similarly to simvastatin. Although pitavastatin is also a hydrophobic statin, the hydrophobicity of pitavastatin (log P\(_{\text{aqua}}\) = 1.49) is lower than that of cerivastatin or simvastatin\(^\text{23}\). There might be a difference in the distribution to rat tissues between cerivastatin and pitavastatin.

In summary, cerivastatin, which was the most potent inhibitor of cholesterol synthesis in cultured human skeletal muscle cells, potently lowered cellular cholesterol and ubiquinone levels, and showed the most potent cytotoxic activity in this study. It seemed that the cytotoxic activities of statins mainly correlated with their inhibitory potencies toward cholesterol synthesis in this culture system. Neither pitavastatin nor cerivastatin decreased ubiquinone levels in rat skeletal muscle. On the other hand, both pitavastatin and cerivastatin dose-dependently lowered ubiquinone levels in the heart. Cerivastatin seemed to reduce ubiquinone levels in rat hearts approximately 100-fold more potently than pitavastatin. It has not been clarified whether these ubiquinone-lowering effects of statins in the rat heart in fact led to some dysfunction in the heart. Cerivastatin, a hydrophobic and the most potent inhibitor of cholesterol biosynthesis, showed potent reduction of tissue ubiquinone levels. Further investigations should be performed not only on the favorable pharmacological actions of statins but also on their adverse effects to enable their safe use.

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