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ORIGINAL ARTICLE

Low-dose Simvastatin Increases Skeletal Muscle Sensitivity to Caffeine and Halothane

Xu-lei Cui¹, Ying-lin Wang², Gang Tan¹, Ai-lun Luo^{1*},
and Xiang-yang Guo^{3*}

¹Department of Anesthesiology, Peking Union Medical College Hospital,
Chinese Academy of Medical Sciences & Peking Union Medical
College, Beijing 100730, China

²Department of Anesthesiology, People's Hospital (Affiliated Haikou Hospital),
Xiangya School of Medicine, Central South University,
Changsha 410008, China

³Department of Anesthesiology, Peking University Third Hospital,
the Third School of Clinical Medicine of Peking University, Beijing 100191, China

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Objective To determine whether the myotoxic side effects of statin simvastatin affect skeletal muscle's sensitivity to caffeine and halothane.

Methods Primary cultured neonate rat skeletal myotubes were treated with 0.01-5.0 $\mu\text{mol/L}$ simvastatin for 48 hours. MTT was used to evaluate cellular viability. The gross morphology and microstructure of the myotubes were observed with a light and electron microscope, respectively. The intracellular calcium concentrations ($[\text{Ca}^{2+}]_i$) at rest and in response to caffeine and halothane were investigated by fluorescence calcium imaging. Data were analyzed by analysis of variance (ANOVA) test.

Results Simvastatin (0.01-5.0 $\mu\text{mol/L}$) decreased myotube viability, changed their morphological features and microstructure, and increased the resting $[\text{Ca}^{2+}]_i$ in a dose-dependent manner. Simvastatin did not change myotube's sensitivity to low doses of caffeine (0.625-2.5 mmol/L) or halothane (1.0-5.0 mmol/L). In response to high-dose caffeine (10.0 mmol/L, 20.0 mmol/L) and halothane (20.0 mmol/L, 40.0 mmol/L), myotubes treated with 0.01 $\mu\text{mol/L}$ simvastatin showed a significant increase in sensitivity, but those treated with 1.0 $\mu\text{mol/L}$ and 5.0 $\mu\text{mol/L}$ simvastatin showed a significant decrease. The sarcoplasmic reticulum Ca^{2+} storage peaked in the myotubes treated with 0.01 $\mu\text{mol/L}$ simvastatin, but it decreased when cells were treated with higher doses of simvastatin (0.1-5.0 $\mu\text{mol/L}$).

Conclusions The myotoxic side effect of simvastatin was found to change the sensitivity of myotubes in response to high-dose caffeine and halothane. When dose was low, sensitivity increased mainly because of increased Ca^{2+} content in the sarcoplasmic reticulum, which might explain why some individuals with statin-induced myotoxic symptoms may show positive caffeine-halothane contracture test results. However, when the dose was high and the damage to the myotubes was severer, sensitivity was lower. It is here supposed that the damage itself might put individuals with statin-induced myotoxic symptoms at greater risks of presenting with rhabdomyolysis during surgery or while under anesthesia.

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STATINS acting as 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors are widely used in patients with hypercholesterolemia to reduce cardiovascular and cerebrovascular events.¹

The drugs are well tolerated in most healthy adults and produce very few side effects, although reports of statin-induced myopathy (SIM) are frequent. Symptoms in affected patients include increased creatine kinase (CK) level, muscle soreness and fatigue or even life threatening rhabdomyolysis (RM).² Overall, 1%-10% of patients on a statin regimen display muscle related side effects among whom 0.1% will suffer from RM.^{1, 3}

The mechanism responsible for SIM is still largely unknown and is currently under active investigation. Hypotheses include direct impacts on lipid synthesis and resulting increases in cell membrane permeability,⁴ inhibition of mitochondria co-enzyme Q10 synthesis and impaired energy production,⁵ and dysregulation of calcium homeostasis within muscle cells.⁶ The latter is not only believed to mediate statins-induced side effects in muscles, but may also play a crucial role in other pathophysiological states of muscle such as malignant hyperthermia (MH). MH is an inherited pharmacogenetic disorder which triggered by halogenated inhaled anesthetics including halothane.⁷ Typical MH symptoms include skeletal muscle rigidity, a rapid and sustained rise in body temperature, severe metabolic acidosis and other symptoms of RM such as increased CK levels and myoglobinuria.⁸ If not properly treated, MH can rapidly lead to severe tissue damage and even death.

The caffeine-halothane contracture test (CHCT) is the gold standard of MH susceptible (MHS) diagnosis. In CHCT, MHS skeletal muscles have stronger contracture reactions to stimulation by caffeine and halothane than MH non-susceptible (MHN) skeletal muscles.⁹ Studies have shown the myotoxic side effects of statins could increase skeletal muscles sensitivity to caffeine and halothane stimulation. Krivosic-Horber *et al*¹⁰ demonstrated that patients suffering from SIM with increased serum CK levels tested positive in

the CHCT. Guis *et al*¹¹ also found that 7 of 9 patients with SIM tested positive in the CHCT. This means these patients may be at risk of developing MH when they were exposed to halogenated inhaled anesthetics. However, Evans *et al*¹² also reported that patients with atypical MH symptoms (increased CK or RM without high temperature) had taken statins irregularly before surgery and suggested that the myotoxic side effects might have increased sensitivity to inhaled anesthetics.

The increasing number of patients receiving statins during perioperative periods developing the rare, but very serious adverse effects of statins, illustrates the importance of understanding the mechanisms for such adverse effects, which until recently have been understudied. Simvastatin produces the highest incidence of SIM in humans and was therefore used at different doses to determine their effects on skeletal muscle sensitivity to caffeine and halothane.

MATERIALS AND METHODS

Experimental rats

Healthy Sprague-Dawley rats born within 24 hours (Beijing Vital River Laboratory Animal Technology Co., Ltd., China) were used for the experiments. There was no restriction on gender. The experiments were performed according to an animal use protocol that was reviewed and approved by animal use committee of Peking Union Medical College Hospital.

Chemicals and solutions

Type II collagenase, trypsin, D-Hank's, MEM medium, fetal bovine serum (FBS), and horse serum were purchased from cell culture center of Institute of Basic Medical Sciences (Beijing, China). Type I collagen, simvastatin, halothane, and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO, USA). Fura-2-acetoxymethyl ester (AM) and pluronic F-127 were from Biotium (Hayward, CA, USA). Caffeine was from Peking Union Medical College Hospital. Triton X-100 and dimethyl sulphoxide (DMSO)

were purchased from Amresco (Solon, OH, USA).

Simvastatin was dissolved in 100% ethanol and was diluted in differentiation medium at the final concentrations of 0-10 $\mu\text{mol/L}$ (0, 0.001, 0.01, 0.1, 1.0, 5.0, 10.0 $\mu\text{mol/L}$) for cell culture. The final concentration of ethanol was less than 0.3% in culture medium.¹³ The skeletal muscle extracellular solution was prepared as following: 140 mmol/L NaCl, 5.4 mmol/L KCl, 1.4 mmol/L MgCl_2 , 0.4 mmol/L NaH_2PO_4 , 1 mmol/L CaCl_2 , 10 mmol/L glucose, and 5 mmol/L Hepes, pH 7.2 in triple-distilled water. Potassium depolarization solution was made as: 85.4 mmol/L NaCl, 60 mmol/L KCl, 1.4 mmol/L MgCl_2 , 0.4 mmol/L NaH_2PO_4 , 1 mmol/L CaCl_2 , 10 mmol/L glucose, and 5 mmol/L Hepes, pH 7.2.

Using the research method described by Wehner *et al.*,¹⁴ halothane was dissolved in DMSO on the day of experiment to produce a 2 mmol/L stock solution which was immediately diluted in extracellular solution to produce simulation solutions with corresponding concentrations before every experiment. The 20 mmol/L caffeine stock solution was produced in extracellular solution that was further diluted to prepare stimulation solutions at designated concentrations.

Extraction and culture of skeletal myotubes

Recently published methods were modified for cell culture of primary myotubes.¹⁴ After removal of connective tissue and fat, the chest and thigh muscles of the neonatal rats were cut into small pieces. Myoblasts were isolated enzymatically in D-Hank's solution supplemented with 0.17% trypsin, and 0.085% type II collagenase was added for digestion at 37°C for 45-50 minutes. Satellite cells were released by repeated pipetting of the tissue. The tissue was filtered and the filtrate was plated in an uncoated culture dish for 1 hour to reduce the number of fibroblasts.¹⁴ The suspension cells were collected and resuspended with proliferation medium (MEM with 20% FBS) to a density of $1 \times 10^4/200 \mu\text{l}$ per well in a 96-well plate, $1 \times 10^5/1.5 \text{ ml}$ per well in a 24-well plate, $5 \times 10^5/5 \text{ ml}$ per flask in T25 flasks. All flasks and plates were coated with 0.01% type I collagen. After most of the cells adhered and reached 50%-60% confluence, the medium was changed to differentiation medium (MEM with 10% horse serum, 2% FBS) to continue the incubation for 18-24 hours. During this time the satellite cells fused to form myotubes, which gradually increased in number over time. After 48 hours of differentiation, simvastatin was added at different concentrations when the differentiation medium was changed and the culture was continued for 24-72 hours. Mock treated cells treated with 0.3% ethanol carrier served as control.

MTT assay

These assays were performed as described by Masters *et al.*¹⁵ The myotubes were treated with simvastatin at different concentrations for 24, 48, or 72 hours. Then culture medium was discarded by centrifugation followed by adding serum-free MEM containing 0.05% MTT. After another 4 hours of incubation, the resultant formazan crystals were dissolved in DMSO. A microplate reader was then used to measure absorbance at 570 nm. Optimal concentrations and incubation times were determined empirically and used in subsequent experiments.

Morphology and ultrastructure of myotubes

Assessments of cell morphology using light microscopy is widely used as a health status indicator of muscular fibers and myotubes.¹⁶ Based on the MTT results, a 48-hour time point was selected for microscopical analysis of myotubes treated with different concentrations of simvastatin. The number of myotubes and their morphology were examined and photographed by a 40 $\times$ lens on a light microscope.

The ultrastructure of myotubes was analyzed using a transmission electron microscope. Briefly, myotubes were trypsinized and collected. After centrifugation, myotubes were fixed with 2.5% glutaraldehyde for 2 hours at 4°C and then with 1% osmium tetroxide. Samples were dehydrated with alcohol, soaked in acetone, and embedded in epoxy before sectioning. Ultrathin sections of grid were analyzed for changes in subcellular structure after double staining with 2% uranyl acetate and lead citrate.

Measurement of intracellular calcium concentrations ($[\text{Ca}^{2+}]_i$)

A 48-hour time point was selected to assess the effect of simvastatin on intracellular calcium homeostasis. Glass slides with myotubes were washed 2-3 times with extracellular solution and then incubated in loading solution containing 5 $\mu\text{mol/L}$ Fura-2-AM for 45 minutes at 37°C. Calcium imaging was performed using a fluorescence imaging set for fast photometric detection (Till Photonics, Gräfelfing, Germany) and an inverted microscope (Olympus IX71, Japan). Extracellular solution was perfused constantly at 1 ml/second and at a controlled temperature of 37°C. The fluorescence intensity was recorded at excitation wavelengths of 340 nm and 380 nm every 5 seconds using Aqua Cosmos 2.5 software. The fluorescent intensity ratio (340 nm/380 nm) was obtained and has been previously shown to change in proportion to $[\text{Ca}^{2+}]_i$ concentrations.¹⁷

The $[\text{Ca}^{2+}]_i$ was here represented as the fluorescence

ratio (340 nm/380 nm). The amount of calcium released into the cytoplasm was presented as the difference between the stimulated peak fluorescence ratio and resting fluorescence ratio (ΔC -fluorescence ratio). The maximum amount of calcium released from sarcoplasmic reticulum (SR), represented in this study as ΔC_{max} -fluorescent ratio (340 nm/380 nm), can indicate calcium storage capacity.^{14, 18} After stimulation with a high dose of caffeine or halothane, the maximum amount of calcium is released from SR.¹⁸

Myotube selection criteria for studying intracellular calcium homeostasis

A field containing a relatively large number of myotubes (about 4 to 6 per experiment) was selected. Criteria for inclusion were typical multinuclear fiber-like morphology and a rapid (>1.6 fold) increase in $[Ca^{2+}]_i$, induced by chemical depolarization with 60 mmol/L KCl, followed by a fast decline in $[Ca^{2+}]_i$. Myotubes that did not meet this requirement were excluded.¹⁸

Caffeine and halothane were applied sequentially in increasing doses with a resting period of 5 minutes before the next dose. During this time, the recording chamber was rinsed with extracellular solution.

Statistical analysis

Quantitative data were presented as mean $\pm$ standard error of mean (SEM). The comparison among quantitative data sets was conducted by one-way analysis of variance (ANOVA). Least significant difference method was used to compare homogeneity of variances, and Dunnett's T3 method was used to compare heterogeneity of variances. SPSS 13.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis and $P < 0.05$ was considered to be statistically significant.

RESULTS

Simvastatin reduced cell viability and altered morphology

Simvastatin reduced the viability of neonate rat myotubes in a dose and time dependent fashion. As shown in Table 1, the half maximal inhibitory concentration (IC₅₀) of simvastatin on myotubes was 51.70, 41.7, and 28.45 $\mu\text{mol/L}$ for 24, 48, and 72 hours, respectively.

Under light microscopy, myotubes in the control group grew densely in a full, thick, and long shape with smooth surface outlines (Fig. 1A). As the concentration of simvastatin was increased, the number of myotubes was gradually decreased and the morphology gradually changed to be thin and short. When the concentration of simvastatin reached 1.0 $\mu\text{mol/L}$ or above, the number of myotubes was significantly decreased. In the groups where 5.0 $\mu\text{mol/L}$ or above were used, the surface of myotubes was no longer smooth and membrane distortion was observed. When simvastatin concentration was increased even higher to 10.0 $\mu\text{mol/L}$, some myotubes came off the culture plate.

Electron microscopy was employed to further observe the ultrastructure of myotubes. There was an abundance of mitochondria in the myotubes of the control group with clear mitochondrial crista, and cells were filled with filament bundles. When the concentration of simvastatin was 0.01 $\mu\text{mol/L}$, there were no obvious changes in the ultrastructure of myotubes. As the concentration of simvastatin was increased, the amounts of mitochondria and filaments was gradually decreased, glycogen accumulation gradually became distinct, mitochondria crista gradually disturbed, and mitochondria gradually expanded, vacuolated with a large amount of lipid droplets gradually emerged (Fig. 1B).

Table 1. Viability of primary cultured neonate rat myotubes treated with various concentrations of simvastatin for 24, 48, and 72 hours, respectively[§]

Simvastatin ($\mu\text{mol/L}$)	Percentage of viable cells (compare to control)		
	Acting 24 hours	Acting 48 hours	Acting 72 hours
Control	1	1	1
0.001	0.97 $\pm$ 0.05	0.95 $\pm$ 0.04	0.93 $\pm$ 0.03*
0.01	0.96 $\pm$ 0.02	0.93 $\pm$ 0.03	0.85 $\pm$ 0.05**
0.1	0.96 $\pm$ 0.02	0.92 $\pm$ 0.02	0.84 $\pm$ 0.01**
1.0	0.94 $\pm$ 0.03	0.89 $\pm$ 0.04*	0.80 $\pm$ 0.05**
5.0	0.91 $\pm$ 0.06*	0.84 $\pm$ 0.08*	0.74 $\pm$ 0.04*
10.0	0.89 $\pm$ 0.08*	0.80 $\pm$ 0.06*	0.66 $\pm$ 0.04**

§: Plus-minus values are means $\pm$ SEM.

* $P < 0.05$ compared with the controls, * $P < 0.05$ compared with the myotubes incubated with simvastatin for 24 hours, * $P < 0.05$ compared with the myotubes incubated with simvastatin for 48 hours.

Influence of simvastatin on myotube calcium homeostasis

The resting fluorescence ratio did not change significantly in myotubes incubated with 0.01 $\mu\text{mol/L}$ simvastatin (Fig. 2A). When simvastatin concentration was further increased to 0.1, 1.0, and 5.0 $\mu\text{mol/L}$, resting fluorescence ratio increased by 4.20%, 5.04%, and 9.00%, respectively, which was statistically significant (all $P < 0.01$).

After application of 60 mmol/L KCl, the peak flores-

cence ratio in the myotubes treated with 0.01 $\mu\text{mol/L}$ simvastatin increased to 11.61% more than the control ($P < 0.01$). As doses of simvastatin were increased from 0.1 $\mu\text{mol/L}$ to 5.0 $\mu\text{mol/L}$, the peak fluorescence ratio gradually decreased (Fig. 2B). In the myotubes treated with 0.01 $\mu\text{mol/L}$ simvastatin, the ΔC -fluorescence ratio peaked at 20.38% more than the controls ($P < 0.01$). The ΔC -fluorescence ratio decreased gradually as the doses of simvastatin were increased (Fig. 3B).

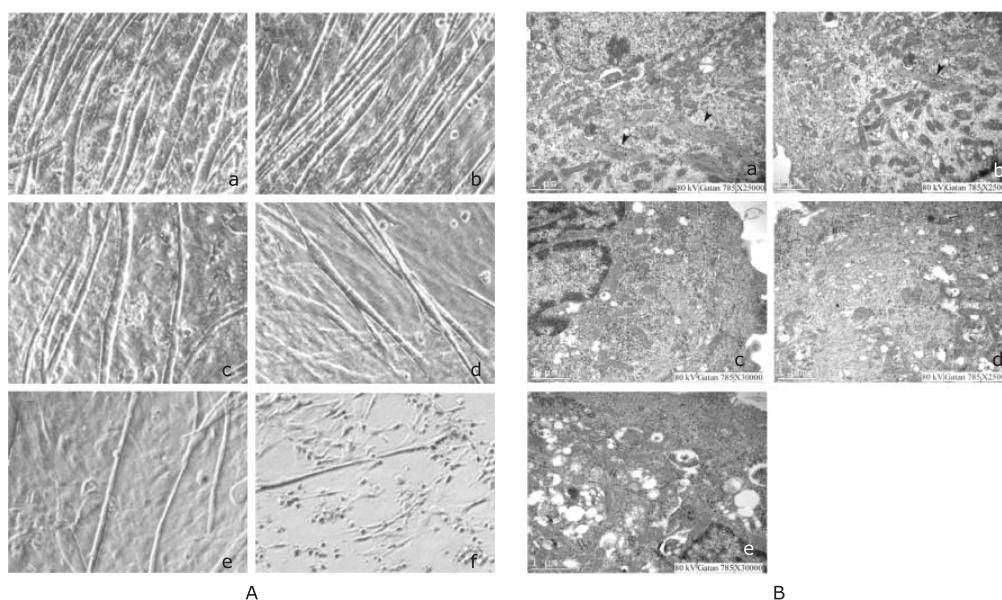


Figure 1. Simvastatin alters myotube morphology.

- A. Light micrograph of cultured primary neonatal rat myotubes treated for 48 hours with (A-a) 0, (A-b) 0.01, (A-c) 0.1, (A-d) 1.0, (A-e) 5.0 or (A-f) 10.0 $\mu\text{mol/L}$ simvastatin.
- B. Electron micrograph of primary cultured neonate rat myotubes treated with (B-a) 0, (B-b) 0.01, (B-c) 0.1, (B-d) 1.0, or (B-e) 5.0 $\mu\text{mol/L}$ simvastatin for 48 hours. Arrowheads indicate myosin.

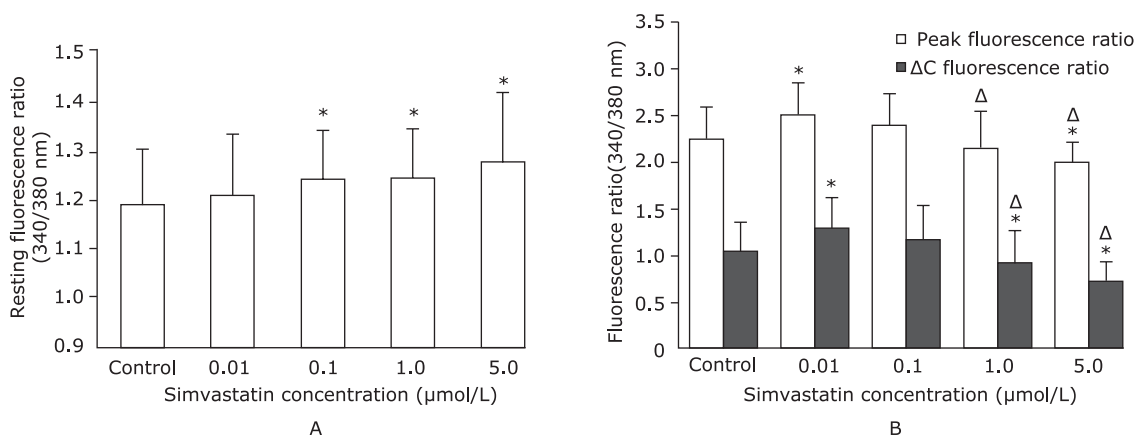


Figure 2. Simvastatin regulation of myotube intracellular calcium concentrations ($[\text{Ca}^{2+}]_i$).

- A. Resting $[\text{Ca}^{2+}]_i$ levels were measured in the cultured primary neonatal rat myotubes after 48 hours in differentiation medium containing different concentrations of simvastatin. * $P < 0.01$ compared with the control.
- B. $[\text{Ca}^{2+}]_i$ and calcium release (ΔC) in the myotubes depolarized by 60 mmol/L KCl and cultured in different doses of simvastatin. * $P < 0.01$ compared with the control. $\Delta P < 0.01$ compared with the myotubes treated with 0.01 $\mu\text{mol/L}$ simvastatin.

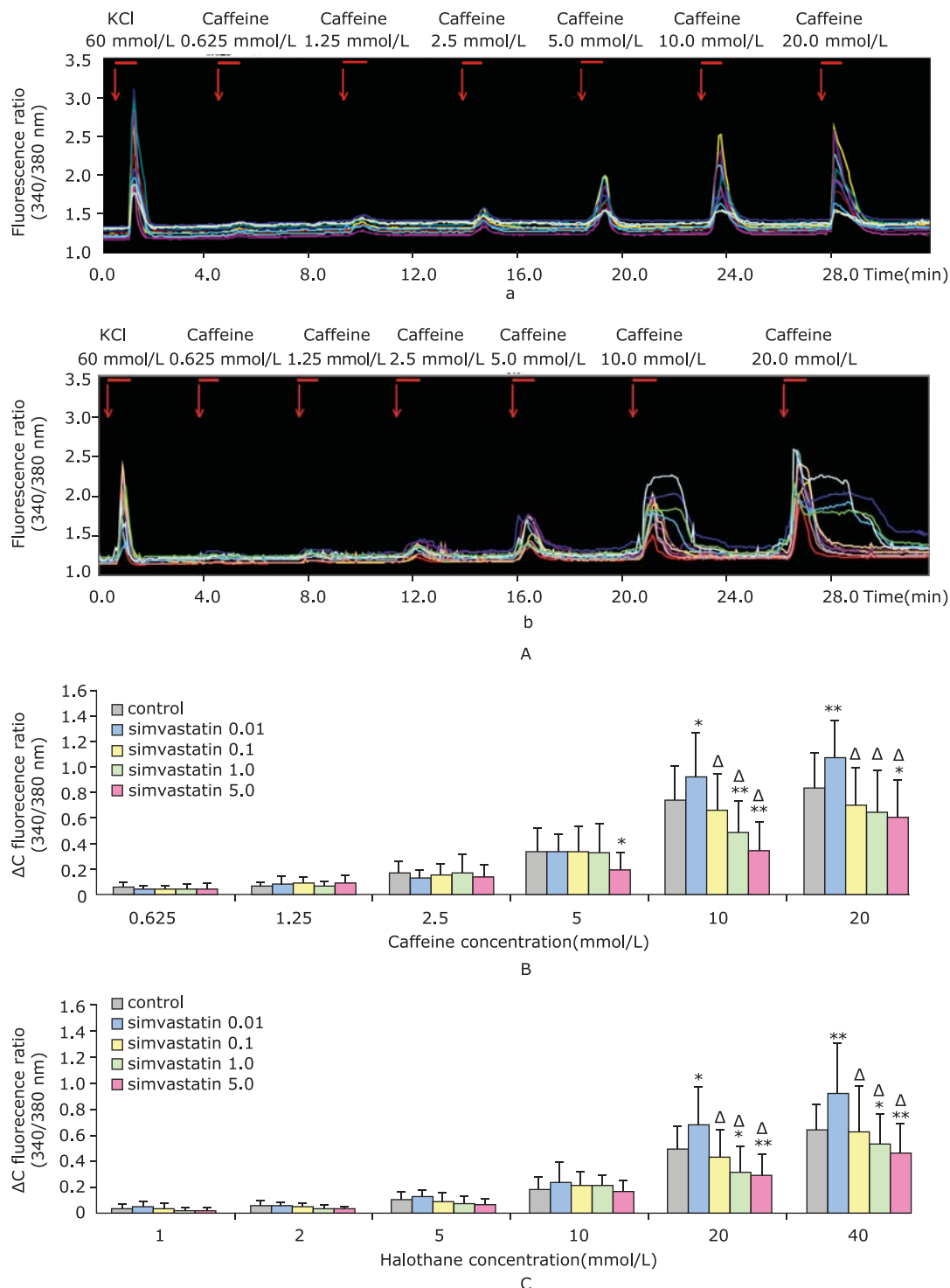


Figure 3. Influence of simvastatin on different doses of caffeine- and halothane-induced calcium release.

A. Typical course of calcium release in response to caffeine induction. The effect of sequentially applied caffeine on calcium release in the myotubes treated without simvastatin (A-a) or with 5.0 $\mu\text{mol/L}$ simvastatin (A-b) for 48 hours. Each line represents the averaged fluorescence ratio (340/380 nm) in one myotube. Red arrows and red bars indicate the application of caffeine.

B. Effect of simvastatin on calcium release induced by increasing doses of caffeine.

C. Effect of simvastatin on calcium release induced by increasing doses of halothane.

* $P < 0.05$, ** $P < 0.01$ compared with the control; $\Delta P < 0.01$ compared with the myotubes treated with 0.01 $\mu\text{mol/L}$ simvastatin.

Impact of simvastatin on the response of myotubes to caffeine or halothane induction

Fig. 3 A-a represents a typical course of fluorescent ratio (340 nm/380 nm) in response to the stimulation of grading caffeine doses. Maximum calcium release was induced with 10.0 mmol/L or 20.0 mmol/L caffeine or halothane at concentrations of 20.0 mmol/L or 40.0 mmol/L. Higher concentrations of caffeine did not increase the fluorescence ratio, but caused myotoxicity as evidenced by morphological changes in myotubes (image not shown). As shown in Fig. 3A-b, when caffeine was used at 10.0 mmol/L or 20.0 mmol/L, the myotubes received 5.0 $\mu\text{mol/L}$ simvastatin treatment showed delayed descending branches compared with that of the control group.

As shown in Fig. 3B and 3C, caffeine and halothane induced calcium release in a clear dose dependent fashion. No significant difference was observed when the stimulating

concentration of caffeine was less than 5.0 mmol/L and that of halothane was no more than 10.0 mmol/L. However, 0.01 $\mu\text{mol/L}$ simvastatin significantly increased the peak fluorescence ratio when cells were stimulated with 10.0 mmol/L caffeine or 20.0 mmol/L halothane ($P < 0.05$), although higher doses of either attenuated the induction of calcium release, a pattern similar to that observed in Fig. 2B.

Effect of simvastatin on calcium storage capacity in SR

As shown in Fig. 4, treatment with 0.01 $\mu\text{mol/L}$ simvastatin increased caffeine- and halothane-induced ΔCmax -fluorescent ratios by 28.57% and 44.60%, respectively ($P < 0.05$). Higher doses of simvastatin, however, reduced the ΔCmax -fluorescent ratio by 27.38% (caffeine, Fig. 4A) and 27.69% (halothane, Fig. 4B) ($P < 0.05$), which is again consistent with patterns described in other figures.

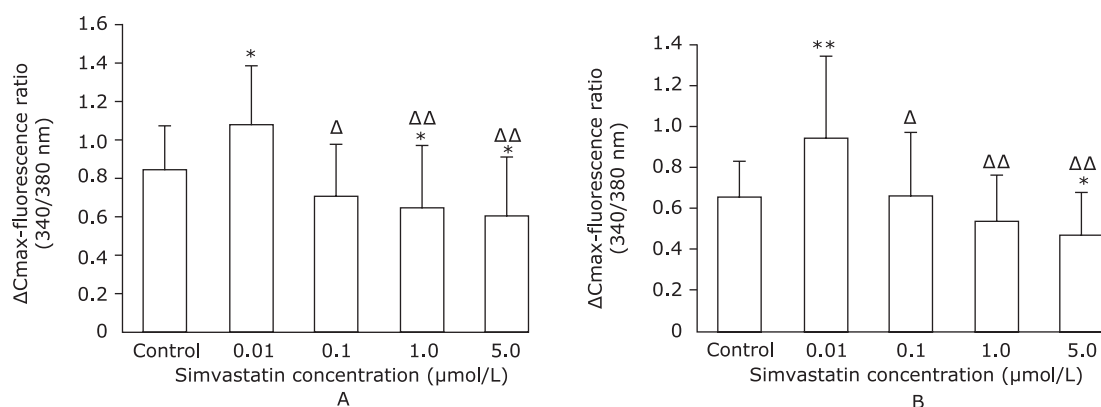


Figure 4. Effect of simvastatin on maximum increase of $[\text{Ca}^{2+}]_i$ (ΔCmax fluorescence) in response to caffeine (A) or halothane (B). * $P < 0.05$, ** $P < 0.01$ compared with the control; $\Delta P < 0.05$, $\Delta\Delta P < 0.01$ compared with the myotubes treated with 0.01 $\mu\text{mol/L}$ simvastatin.

DISCUSSION

After prolonged treatment with 0.01 $\mu\text{mol/L}$ simvastatin, the resting $[\text{Ca}^{2+}]_i$ did not change significantly, and the myotubes' SR calcium storage capacity significantly increased. Under these conditions, there were no significant changes in myotube viability, gross morphology, or microstructure. However, it is here speculated that the myotubes membrane's structure might have been changed. This caused leakage of extracellular Ca^{2+} into the cells along the concentration gradient. However, the sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) still functioned normally, and pumped excess intracellular Ca^{2+} to the SR to maintain a normal level of resting $[\text{Ca}^{2+}]_i$. Further experiments will be needed.

As the concentration of simvastatin was increased (0.1-5.0 $\mu\text{mol/L}$), gradual changes in myotube viability and gross morphology indicated a dose-dependent myotoxic effect. The microstructure also showed fewer mitochondria and filaments, suggesting functional damage, which could affect all the intracellular energy consuming Ca^{2+} pumps. In the following investigation of myotubes' reaction to caffeine and halothane, SR recycling instantly elevated Ca^{2+} , and this effect lasted longer in myotubes treated with 5.0 $\mu\text{mol/L}$ simvastatin, which suggested the dysfunction of energy consuming calcium recycling in the SERCA.^{19, 20} As a result, the myotubes resting $[\text{Ca}^{2+}]_i$ increased and calcium storage in SR gradually decreased as the myotoxic side effects of simvastatin increased.

Caffeine and halothane have also been used to differentiate *in vitro* cultured MHS and MHN skeletal muscle

cells because MHS skeletal muscle cells have stronger calcium release reactions induced by these two drugs than MHN skeletal muscle cells.¹⁴ In this study, caffeine and halothane were used to stimulate the myotubes. In myotubes treated with 0.01 $\mu\text{mol/L}$ simvastatin, the sensitivity of the release of calcium to high dose caffeine and halothane increased significantly. This may have been primarily caused by the increased SR calcium storage capacity in this group of myotubes. Lamb *et al*²¹ found the SR Ca^{2+} load in rat slow-twitch fiber to be increased with much stronger sensitivity SR calcium release reaction than in fast-twitch fiber. When both fibers had the same SR Ca^{2+} load, the sensitivity of calcium release tended to be similar.²¹ López *et al*²² reduced the SR Ca^{2+} load in human MHN and MHS type *ex vivo* muscle fibers, and found that the sensitivity of RyR1-induced calcium release decreased in both. On the cytological level, the results of this study might explain why some patients taking statins had positive CHCT results. However, in myotubes treated with high concentrations of simvastatin (1.0 $\mu\text{mol/L}$ and 5.0 $\mu\text{mol/L}$), the sensitivity of the release of calcium to high doses of caffeine and halothane was significant decreased, which may have been caused by their low SR calcium storage capacity.

Some studies indicate that the increase in resting $[\text{Ca}^{2+}]_i$ could lead to increased sensitivity of SR towards calcium release stimuli.^{22, 23} However, in this experiment, myotubes resting $[\text{Ca}^{2+}]_i$ increased with the increasing myotoxic side effects of simvastatin. The sensitivity of their release of calcium conversely decreased. Liantonio *et al*⁶ already observed that the phenomenon of reduced reactivity of skeletal muscles to SR calcium release stimulations, when resting $[\text{Ca}^{2+}]_i$ increased. The researchers fed mice with 5.0 mg/kg or 20.0 mg/kg of fluvastatin daily for two months. Results showed that this could increase muscle fiber resting $[\text{Ca}^{2+}]_i$ by 13% and 59%, respectively. However, the skeletal muscles of mice fed 20.0 mg/kg fluvastatin showed significantly less reactivity to high potassium depolarization. Liantonio *et al*^{6, 24} believed this to be related to T tube structural damages caused by the high concentration of fluvastatin and to a series of protein hydrolases induced by excessive resting $[\text{Ca}^{2+}]_i$, which damaged the intracellular calcium channel protein. This might explain the reduced sensitivity of myotubes towards calcium release stimulations caused by large doses of simvastatin and the reduction of SR calcium storage capacity. Further study is needed.

This study also showed that different concentrations of simvastatin did not change the sensitivity of myotubes to low doses of caffeine and halothane. Nevertheless, $[\text{Ca}^{2+}]_i$ increased in all myotubes that received stimulation. If the

increase of $[\text{Ca}^{2+}]_i$ exceeded a certain threshold, it would trigger a series of cell apoptotic processes.²⁵ It can be speculated that patients taking statins who have elevated skeletal muscle resting $[\text{Ca}^{2+}]_i$ could be at risk of further skeletal muscle damage when exposed to halogenated inhaled anesthetics that stimulate further increases in skeletal muscle $[\text{Ca}^{2+}]_i$.²⁶

The toxic effect of simvastatin could change skeletal muscle's calcium homeostasis. For patients with SIM, exposure to halogenated anesthetics increases the risk of worsening skeletal muscle damages, though the underlying mechanism may be different.

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