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ORIGINAL ARTICLE

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

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Key words: simvastatin; myopathy; calcium homeostasis; caffeine; halothane; calcium release

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 μmol/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 ($[Ca^{2+}]i$) 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 μmol/L) decreased myotube viability, changed their morphological features and microstructure, and increased the resting $[Ca^{2+}]i$ 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 μmol/L simvastatin showed a significant increase in sensitivity, but those treated with 1.0 μmol/L and 5.0 μmol/L simvastatin showed a significant decrease. The sarcoplasmic reticulum Ca^{2+} storage peaked in the myotubes treated with 0.01 μmol/L simvastatin, but it decreased when cells were treated with higher doses of simvastatin (0.1-5.0 μmol/L).

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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)