Roles of Exercise and Pharmacokinetics in Cerivastatin-Induced Skeletal Muscle Toxicity

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Three-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors are associated with adverse skeletal muscle effects, but the underlying mechanisms remain unclear. To determine whether toxicity involves the level of drug exposure in muscle tissue and to test the effect of exercise on cerivastatin (CVA)-induced skeletal muscle damage, female rats were administered vehicle or CVA at 0.1, 0.5, and 1.0 mg/kg/day by gavage for two weeks and exercised or not on treadmills for 20 min/day. Clinical chemistry and plasma and tissue pharmacokinetics were evaluated; light and transmission electron microscopy (TEM) of Type I and Type II fiber-predominant skeletal muscles were performed. Serum levels of AST, ALT, CK, and plasma lactic acid were significantly elevated dose-dependently. CVA treatment decreased psoas and quadriceps weights. At 1 mg/kg all muscles except soleus demonstrated degeneration. Exercise-exacerbated severity of CVA-induced degeneration was evident in all muscles sampled except soleus and quadriceps. Early mitochondrial involvement in toxicity is suggested by the numerous membranous whorls and degenerate mitochondria observed in muscles at 0.5 mg/kg. No significant differences in CVA concentrations between either EDL and soleus or plasma and muscle were found. We found that CVA had no effect on cleaved caspase 3. In summary, we found that treadmill exercise exacerbated the incidence and severity of CVA-induced damage in Type II fiber-predominant muscles. Tissue exposure is likely not the key factor mediating CVA-induced skeletal muscle toxicity.

Key Words: pharmacokinetics; ultrastructure; muscle weight; degeneration; treadmill; statins.

Three-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) inhibitors (statins) are the largest class of drugs on the market, with an estimated 11 million patients currently using this therapy nationwide (Verispan, 2005). Statins attenuate cholesterol biosynthesis by inhibiting the rate-limiting enzyme HMG-CoA reductase (Alberts, 1988) and thereby effectively lower cholesterol and reduce cardiovascular-related mortality. Adverse effects of statins on muscle occur at a rate of 1–5% and range from mild pain and weakness to rhabdomyolysis, which includes muscle breakdown and myoglobinuria (Ucar et al., 2000). Rhabdomyolysis may lead to acute renal failure and death. The incidence of statin-induced rhabdomyolysis is less than 0.5% and variable among compounds (Baker and Tarnopolsky, 2001; Thompson et al., 2003). However, Bayer’s Baycol (cerivastatin [CVA]) was withdrawn from the market in 2001 in the wake of 31 reported fatal rhabdomyolysis cases in the U.S. linked to the drug (Baker and Tarnopolsky, 2001). Due to the recently discovered, cholesterol-independent pleiotropic effects of statins, research into their potential as therapies for disorders including cancer, osteoporosis, multiple sclerosis, rheumatoid arthritis, Type II diabetes, and Alzheimer’s disease (Carmena and Betteridge, 2004; Liao and Laufs, 2005; Sleijfer et al., 2005) is being performed. Therefore, the number of patients taking statins is expected to increase. It is crucial to understand the mechanism of statin-induced myopathy.

In statin patients muscular problems occur more frequently during and after exercise (Franc et al., 2003). Conflicting reports exist on whether exercise elevates creatine kinase (CK) levels in lovastatin-treated patients (Reust et al., 1991; Thompson et al., 1991, 1997). Recently, Sinzinger and O’Grady (2004) found that 78% of professional athletes with familial hypercholesterolemia could not tolerate therapy with any statin, due to muscle pain and cramps. In most of these cases, either minimal or no increase in serum CK levels was observed (Sinzinger and O’Grady, 2004). The extent to which exercise contributes to statin-induced muscle injury is unclear. Exercise may affect the absorption, distribution, metabolism, and excretion of a variety of pharmaceuticals, leading to altered pharmacokinetics (Lenz et al., 2004). For example, following exercise on a cycle ergometer, serum levels of digoxin were significantly decreased, while skeletal muscle digoxin concentrations were significantly increased (Joreteg and Jorestrand, 1984). Mechanisms of potential exercise-exacerbated statin-induced muscle toxicity have not been investigated. It is possible that exercise, by virtue of the increased blood flow to muscle increases exposure (Lenz et al., 2004), leading to...
increased drug levels in muscle tissue and possible subsequent toxicity. Statin kinetics in skeletal muscle tissue has not been previously investigated.

Statin promote apoptosis in different cell types including vascular smooth muscle cells, endothelial cells, melanoma cell lines, and leukocytes (Kaneta et al., 2003; Shellman et al., 2005; Wibaut-Berlamont et al., 2005). However, they may also inhibit cardiomyocyte apoptosis (Chen et al., 2004). Recently, CVA-induced apoptosis in rat and human myocyte cultures has been reported (Johnson et al., 2004; Sacher et al., 2005). Although unregulated apoptosis is a proposed mechanism of many myopathies and of decreased muscle function with aging, its contribution to the death of in vivo skeletal myofibers is not fully known.

In the present study CVA was selected as a model statin because it produces myopathy in rats (Schaefer et al., 2004; von Keutz and Schluter, 1998; Westwood et al., 2005) and had a reporting rate of fatal rhabdomyolysis 10 to 50 times higher than that of other statins in humans (Staffa et al., 2002). We used female rats because of their increased sensitivity to statin-induced myopathy (Schaefer et al., 2004). In order to confirm Type I-fiber resistance to statin effects (Schaefer et al., 2004; Waclawik et al., 1993; Westwood et al., 2005), we sampled Type I fiber- (soleus) and Type II fiber- (extensor digitorum longus [EDL] and quadriceps) predominant muscles, as well as gastrocnemius, psoas, and tongue.

Many hypotheses have been proposed to explain statin myotoxicity; however, the mechanisms are still not fully understood. Therefore, the overall goal of this study was to investigate the mechanisms of CVA-induced muscle toxicity, using an in vivo rat model. Specifically, we evaluated the pharmacokinetics of CVA in skeletal muscle. Additionally, we investigated the effects of treadmill exercise on CVA-induced muscle degeneration. Finally, by evaluating expression of the executioner caspase 3 in skeletal muscle of CVA-treated and vehicle-treated rats, we sought to establish whether this compound promotes apoptosis in in vivo skeletal myocytes.

MATERIALS AND METHODS

Animals. Female Sprague-Dawley rats, strain Crl:CD(SD)IGS BR, were obtained from Charles River Laboratory (Raleigh, NC). Animals were individually housed in stainless steel wire cages in temperature (21–26°C) and humidity (30–70%) controlled rooms with a 12-h light/dark cycle. Animals were fed powdered Lab Diet 5002 Certified Rodent Diet and water ad libitum and acclimated for six days prior to dosing. Animals were 10 weeks old at the start of dosing. All procedures were performed with the approval of the Institutional Animal Care and Use Committee.

Compound and administration. CVA, purity 99.2%, was obtained from Sequoia Research Products (Oxford, U.K.) and was formulated for dosing as a suspension in 0.5% methylcellulose. Rats were randomly allocated either to control groups that received vehicle or one of three treatment groups receiving 0.1, 0.5, or 1.0 mg/kg/day of CVA in vehicle for 14 days by oral gavage (10 ml/kg). Dosages were selected based on a previous study that defined a no-effect (0.1 mg/kg) and a myopathy-producing (1 mg/kg) dose (Schaefer et al., 2004). Half the animals were exercised on rodent treadmills and half were not exercised.

Treadmill exercise. Animals in Groups 5 through 8 were acclimated to Exer 3R rodent treadmills (Columbus Instruments, Columbus, OH) for six days, prior to study initiation. Acclimation consisted of 5 to 10 min/day at 10 m/min for the first three days, increased to 20–25 m/min at 10 m/min for the fourth day. On the final two days, animals were exercised for 5 min at 10 m/min followed by 10 to 15 min/day at 20 m/min. During study, animals exercised at 10 m/min for 5 min, as a warm-up, then ran at 20 m/min at a 15° incline, for 15 to 25 min, 5 days/week at approximately 0.5 h postdose, which represented low to moderate intensity exercise, similar to protocols previously described (Hildebrandt et al., 2003; Mitchell et al., 2004). Rats exhibiting difficulty in running were removed promptly from the treadmill. Nonexercised controls were handled daily. To control for effects of noise and other stresses associated with treadmill use, treadmills were operated in close proximity to cages of the sedentary animals.

Clinical signs and body weight. All animals were observed for clinical signs predose: at 0.5 and 1 h postdose daily; and prior to necropsy. Signs of fatigue (hopping gait) and exhaustion (reduced pace) were recorded 1 h postdose (during and immediately following treadmill exercise). Body weights were recorded pretest, prior to dosing on Days 1 and 8, and at necropsy.

Clinical chemistry. Blood was sampled from all animals at necropsy. Serum was analyzed for alanine aminotransferase (ALT), alkaline phosphatase (AP), aspartate aminotransferase (AST), total bilirubin, CK, glucose, total protein, cholesterol, urea nitrogen (BUN), and lactate dehydrogenase (LDH) using a Vitros 950 with reagents from Ortho-Clinical Diagnostics (Raritan, NJ). Plasma lactic acid was also analyzed with the Vitros 950. Analysis of serum myoglobin concentration was performed on the Immulite 2000, with reagents from Diagnostic Products Corporation (Los Angeles, CA).

Pharmacokinetics. On Day 14, blood was collected from the jugular vein into ethylene diamine tetra-acetic acid (EDTA) tubes at approximately 0.5, 1, 2, 4, 7, and 24 h postdose for pharmacokinetic analysis. At necropsy (Day 15), samples of liver, soleus, and EDL were collected, snap-frozen in liquid nitrogen and stored at −70°C pending analysis. Liver samples were homogenized in high performance liquid chromatography (HPLC) grade water using the Polytron PT 10–35 (Brinkmann). Soleus and EDL samples were weighed, 5 ml HPLC grade water was added/g muscle tissue, several 2.4 mm zirconia beads were added and samples were processed with a Mini beater apparatus. Muscle and liver homogenates were stored at −70°C until analysis. Plasma and tissue homogenate samples (0.1 ml) were extracted with 1 ml of methyl tertiary butyl ether/dichloromethane (70:30 v/v). Samples were vortexed for 5 min, centrifuged, and the organic layer was transferred and dried under nitrogen. The residue was reconstituted in acetonitrile/water (50:50) for LC/MS-MS analysis. Liquid chromatography was performed using a Shimadzu integrated HPLC system. Mass spectrometry was performed using a Scieix API 4000 in the TurboLyn Spray positive ion mode using multiple reaction monitoring. The lower limit of quantitation (LLOQ) was 0.05 ng/ml (plasma), 0.05 ng/g (muscle), and 0.10 ng/g (liver). Concentrations below the LLOQ were reported as zero and were used in evaluation of mean concentrations.

Necropsy and tissue collection. On Day 15, animals were anesthetized with 5% isoflurane/95% oxygen and euthanized by exsanguination. Tongue, gastrocnemius, psoas, soleus, EDL, and quadriceps were examined grossly and subjectively graded as minimal, mild, moderate, or severe based on relative intensity and/or area of degenerative changes in skeletal muscle.

Light microscopy. Tissues were harvested and fixed in buffered 10% neutral buffered formalin (Sigma Chemical Co., St. Louis, MO) for 24 h. Paraffin-embedded samples were sectioned (transverse and longitudinal), stained with haematoxylin and eosin (H & E) and examined by light microscopy. Severity was subjectively graded as minimal, mild, moderate, or severe based on relative intensity and/or area of degenerative changes in skeletal muscle.
**Transmission electron microscopy.** Samples of EDL and psoas collected for ultrastructural assessment were fixed in a mixture of 4% paraformaldehyde/2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2 (SC), washed in SC, postfixed in SC-buffered 1% osmium tetroxide and rinsed again. Samples were subsequently processed and embedded to resin blocks for longitudinal- and cross-sectioning. “Thick” sections (approximately 2 µm) of embedded tissue were collected and stained with toluidine blue for light microscopic examination. Subsequently, ultrathin (70–90-nm) resin sections were cut and stained with 4% aqueous uranyl acetate and 0.3% lead citrate. Ultrastructural morphology was examined on a Phillips CM-100 BioTWIN transmission electron microscope using 60 kV accelerating voltage. Images were acquired with a Kodak 4.21 digital camera and AMT software.

**Statistical analysis.** Treatment comparisons were performed on rank-transformed animal organ weight data, quantitative clinical laboratory data, and pharmacokinetic data using a dose-trend test sequentially applied at the two-tailed 5% level of significance within a two-factor analysis of variance (ANOVA). In addition, comparisons between exercised and non-exercised animals were made for each treatment group within the 2-factor ANOVA at the 5% level of significance. Analysis of the original (non-transformed) data was also performed. Treatment comparisons were performed on body weight data for each time point (Days 1, 8, and 15) using a dose-trend test sequentially applied at the 5% significance level, within a two-way ANOVA with repeated measures. In addition, comparisons between exercised and non-exercised animal body weights were made for each treatment group at each time point within the 2-way ANOVA at the 5% level of significance. Pharmacokinetic parameter values (Cmax [maximum concentration], tmax [maximum time], and AUC [area under the plasma concentration-time curve]) were calculated from the plasma concentration-time profiles using non-compartmental analysis.

**RESULTS**

**Clinical Observations during Treadmill Exercise**

In order to determine the effects of CVA on muscle symptoms in rats, we assessed the clinical signs associated with treadmill exercise. A hopping gait was demonstrated when animals lifted one limb or another and hopped while on the treadmill; a behavior that may indicate discomfort or weakness in the affected limb. Only mid- and high dose animals demonstrated a hopping gait (Table 1). Rats in the high dose group more frequently refused to exercise. Moreover, at 1 mg/kg, animals were less able to maintain pace on the treadmill, termed reduced pace, over the 14-day study, compared to controls. These signs may qualitatively indicate muscular effects such as fatigue, discomfort, or weakness associated with CVA dosing.

**Effects of CVA and Exercise on Clinical Chemistry Parameters**

Serum AST, ALT, LDH, and CK were elevated in a dose-dependent manner (Fig. 1A). At 0.5 and 1 mg/kg without exercise, significant increases compared to control (p < 0.05) were observed in CK (0.7, 11.8-fold), AST (0.62, 21.5-fold), and ALT (0.15, 5-fold), respectively. At 1 mg/kg without exercise glucose and total protein decreased 27.4 and 12.5%, respectively. At 1 mg/kg with exercise, significant increases compared to control (p < 0.05) were noted in CK (1.3-fold), ALT (2.5-fold), LDH (94%), AST (9.1-fold), and cholesterol (18%); urea nitrogen, glucose, and total protein significantly decreased (p < 0.05) 13, 24.2, and 15.6%, respectively. Of the total serum CK measured in the high dose groups, the majority consisted of the skeletal muscle-specific CK-MM isoenzyme (Fig. 1A). No clear shift in LDH isoenzymes was identified. Exercise increased (p < 0.05) the levels of AST (90.3%), ALT (57%), myoglobin (2.9-fold), and CK (3.5-fold), in the control group (Fig. 1A). At 0.5 mg/kg, exercise significantly increased (p < 0.05) AST by 42.6%. Plasma levels of lactic acid were elevated 95% in nonexercised, high dose rats, compared to nonexercised controls (Fig. 1B). In exercised, low, mid-, and high dose groups, lactic acid was significantly increased (p < 0.05) 36.3, 75, and 139%, respectively. No significant treatment-related effects were noted on total serum bilirubin, AP or myoglobin.

**Effect of CVA on Muscle Weights**

Weight of quadriceps from the high dose, exercised group was significantly decreased (p < 0.05) by 13%, from exercised controls (Fig. 2A). Weight of psoas from high dose, nonexercised rats was significantly decreased (p < 0.05) by 21.6% from nonexercised controls (Fig. 2B). Furthermore, exercise significantly decreased (p < 0.05) quadriceps weight 14.1% at 1 mg/kg. Total body weights and weights of soleus, gastrocnemius, and EDL were unaffected by CVA treatment.

**Plasma and Tissue CVA Concentrations**

Exercise had no effect on the mean Cmax and AUC values for CVA (Fig. 3). In exercise and nonexercise groups, the mean Cmax and AUC values for CVA increased (p < 0.05) with dose,

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<th>Cervinastatin dose (mg/kg)</th>
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<td>Refused to exercise</td>
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aAnimals that lifted one limb or another and hopped while on the treadmill.

bClinical signs were recorded 1 h postdose (during and immediately following treadmill exercise) daily for 14 days.

cAnimals unable to maintain pace on the treadmill.
and the increase in systemic exposure was greater than the dose increment over the range evaluated (Fig. 3). The mean $t_{\text{max}}$ values for CVA were between 2.5 and 5.2 h and did not differ between groups. There was no significant difference ($p < 0.05$) in systemic exposure to CVA between exercise and nonexercise groups at the corresponding dose levels. Likewise, exercise had no effect on mean plasma concentrations of CVA at 24-h postdose (Fig. 4C). However, within the exercise and
nonexercise groups, mean plasma concentrations were significantly different \((p < 0.05)\) between dose groups.

Mean muscle concentrations of CVA at 24-h postdose increased significantly \((p < 0.05)\) in a dose-related manner (Figs. 4A and 4B). At 0.1 mg/kg, muscle concentrations at 24-h postdose were below LLOQ in all animals in the nonexercised group and in five of six animals in the exercised group. Mean muscle levels of CVA were not different from those of plasma at 24-h postdose following repeated dose administration of CVA. Concentration in Type II fiber-rich EDL was similar to that in Type I fiber-rich soleus muscles. Exercise had no effect on muscle concentrations.

Mean liver concentrations of CVA at 24-h postdose increased significantly \((p < 0.05)\) in a dose-related manner and were substantially higher than those in plasma and muscles (Fig. 4D). For instance, at 0.5 mg/kg, mean concentrations were 1339\% higher than those in muscles and 2346\% higher than those in plasma; at 1 mg/kg they were 290\% higher than those in muscles and plasma. Exercise had no significant effect on liver CVA concentrations.

**Exercise Exacerbates Incidence and Severity of CVA-Induced Skeletal Muscle Degeneration**

Compared to control tissue (Fig. 5A) with evident cross-striations and peripherally localized nuclei, 1 mg/kg CVA induced lesions consisting of fragmented sarcoplasm and vacuoles, internalized nuclei and absent cross-striations (Fig. 5C, arrows vs. A). At 1 mg/kg, severity of degeneration ranged from minimal to mild without exercise and minimal to moderate with exercise (Table 2; Figs. 5C and 5D). CVA-induced degeneration occurred in all muscle groups sampled.
except soleus; incidence in tongue was sporadic and not related to drug or exercise (not shown). A representative micrograph of quadriceps muscle at 0.5 mg/kg demonstrates minimal, single fiber degeneration, also seen occasionally in controls (Fig. 5B). Exercise exacerbated the incidence and severity of CV A-induced degeneration in gastrocnemius, psoas, and EDL muscles (Table 2). We found that exercise aggravated the severity of damage induced by 1 mg/kg CV A in quadriceps (Figs. 5C and 5D) but had no effect on soleus (Figs. 5E and 5F). In addition to fragmented, vacuolated sarcoplasm, absence of cross-striations, and internalized nuclei, exercise plus CV A induced a greater infiltration of mixed inflammatory cells when compared with nonexercised counterparts (Fig. 5D). Moreover, more severe damage with exercise was also noted by the mineralization of fibers in degenerative areas (Fig. 5D). Exercise alone had no effect on muscle histology since muscles from the controls that exercised resembled those that did not exercise (not shown).

**CVA and Exercise Effects on Skeletal Muscle Ultrastructure**

Psoas and EDL muscles from mid- and high dose groups were selected for ultrastructural examination and compared to controls. At 0.5 mg/kg the only effects detected by TEM that were not seen in controls, were changes in the structure of mitochondria. Specifically, in nondegenerated muscles at 0.5 mg/kg, we observed mitochondria with vacuoles, cleared matrices, and cristolysis (Figs. 6C and 6D, arrows). Effects of CV A were seen on mitochondria located in subsarcolemmal regions, almost exclusively. All other organelles, as well as myofibril structure and banding, were unremarkable, and similar to controls (Fig. 6B). A common finding was that unaffected mitochondria were observed in close proximity to damaged mitochondria (Figs. 6C and 6D, arrows). Exercise had no observable effect on muscle ultrastructure of controls.

At 1 mg/kg, in nonexercised EDL we observed accumulated degenerate, heterogeneous organelles and vacuoles in subsarcolemmal regions (Figs. 6E and 6F). Some membranous whorls and vacuoles resembled degenerate mitochondria (Fig. 6E, arrow). With the addition of exercise, we detected more areas of degeneration plus mixed inflammatory cell infiltration (Fig. 6G). Moreover, myofiber structure was disorganized, Z bands of the sarcomere were absent in many fibers (left in Fig. 6G), myofibrils were fragmented, glycogen was accumulated and apparent degraded contractile material was evident (Fig. 6H, arrows). Thus, exercise exacerbated
CV A-induced effects in fast-twitch, glycolytic muscles were evident by both light microscopy and TEM.

**Effect of CV A on Expression of Activated Caspase 3**

In order to evaluate the potential of CV A to induce apoptosis in vivo, we assessed the activity of caspase 3 in muscle tissue from vehicle- and CV A-treated rats, using immunohistochemistry (IHC). Using antibodies against cleaved caspase 3 we observed no differences in staining in muscles from CV A-dosed groups compared to controls (data not shown).

**DISCUSSION**

To investigate the toxicity of CV A in muscle, we measured its concentration in Type I fiber-rich soleus, Type II fiber-rich soleus, and the EDL muscle.
FIG. 6. Cerivastatin and exercise affects skeletal muscle ultrastructure. Representative transmission electron micrographs of vehicle (A, B) and 0.5 mg/kg nonexercised (C), 0.5 mg/kg exercised (D), 1 mg/kg nonexercised (E, F), and 1 mg/kg exercised (G, H) EDL muscle samples. CVA dosing induces mitochondrial cristolysis and swelling (arrows in C, D). Note that affected mitochondria are in close proximity to mitochondria with normal morphology (C, D). CVA induces accumulation of degenerate, vacuolar structures; some resemble degenerate mitochondria (arrow in E). Also note the CVA-induced infiltration of inflammatory cells (arrow in G), the fragmented sarcomeres, and destructed myofibrillar components (arrow in H). Bars = 0.5 μm, except where indicated otherwise.
EDL muscles, and plasma, examined the effects of exercise on muscle histology from CVA-dosed rats, and tested whether CVA promotes apoptosis in rat skeletal muscle.

Immunosuppressants, Ca\(^{2+}\) channel blockers, niacin, and gemfibrozil are known to influence statin pharmacokinetics (Backman et al., 2000; Bolego et al., 2002; Luh and Karnath, 2003). Exercise can also affect pharmacokinetics (Lenz et al., 2004). Up to 46% of patients taking statins report muscular pain exacerbated by physical exercise (Franc et al., 2003). Thus, we aimed to determine whether treadmill exercise exacerbates toxicity by influencing CVA kinetics in plasma and muscle tissue. Given that the risk of myopathy increases with statin dose, factors that increase its concentration in muscle tissue may enhance its potential toxicity. Since statin concentration in muscle has not been previously assessed, as far as we are aware, we sought to determine CVA muscle concentration and establish whether its level in muscle differs from that in plasma. Here we provide evidence that CVA concentration in muscle does not differ from that in plasma at each corresponding dose following repeated dose administration, suggesting that CVA unlikely accumulates in muscle. In this study, 0.5 and 1 mg/kg yielded plasma C\(_{\text{max}}\) values of 4.85 (10.5 nM) and 24.8 ng/ml (53.9 nM), respectively, similar to the C\(_{\text{max}}\) of 13.8 ng/ml reported in hypercholesterolaemic patients (Stein et al., 1999).

Type I slow-twitch fibers are oxidative with a high content of mitochondria, whereas Type II fast-twitch fibers have a lower content of mitochondria, are predominantly glycolytic and store more glycogen (Goll et al., 1977). Similar to earlier reports (Schaefer et al., 2004; Waclawik et al., 1993; Westwood et al., 2005), we found no evidence of degeneration of Type I fiber-predominant soleus from CVA-dosed rats. Interestingly, we found similar concentrations of CVA in both degenerated Type II fiber-predominant EDL and in nondegenerated, Type I fiber-abundant soleus muscle. Evidently, this type of muscle fiber is insensitive to CVA-induced damage.

Conflicting data exist as to whether or not exercise exacerbates statin-induced skeletal muscle toxicity (Reust et al., 1991; Thompson et al., 1991, 1997). We found that although serum CK levels at 1 mg/kg were significantly greater than controls, they were not further elevated by exercise. However, CK should not be the only parameter to assess muscle degeneration; our data indicate that exercise did exacerbate the incidence and severity of CVA-induced myofiber damage, evident by light microscopy and TEM. Furthermore, muscle symptoms are seen in statin patients lacking elevated CK (Phillips et al., 2002). Exercise may have sensitized the muscle to injury, allowing it to occur earlier than in the nonexercised counterparts. In the present study, two weeks of treadmill exercise aggravated the minimal to mild damage produced at 1 mg/kg in all muscles except soleus, and increased the susceptibility of gastrocnemius, psoas, and EDL muscles to damage, but had no effect on muscle at 0.1 and 0.5 mg/kg. We conclude that treadmill exercise exacerbates CVA-induced muscle toxicity.

At 0.5 mg/kg the only subcellular alterations observed in the present study in nondegenerated fast-twitch EDL by TEM were distended mitochondria with disrupted cristae and cleared matrices. Our results are consistent with recent reports demonstrating statin effects on mitochondria as a potential early event in myotoxicity (Bergman et al., 2003; Gambelli et al., 2004; Sirvent et al., 2005; Westwood et al., 2005). Similar to findings of Westwood and others (2005), we observed changes to morphology of mitochondria in fibers with no concurrent changes to contractile elements, endoplasmic reticulum, or other subcellular compartments. Moreover, Sirvent et al. (2005) recently demonstrated that simvastatin treatment of human Type II fiber-rich vastus lateralis muscle triggers mitochondrial membrane depolarization and rapid Ca\(^{2+}\) efflux from the sarcoplasmic reticulum. In agreement with our results, statin effects on mitochondria morphology in patients, suggesting impaired oxidative metabolism, have also been shown (Gambelli et al., 2004). However, other groups have concluded that mitochondrial abnormalities are secondary effects of statin-induced muscle toxicity (Nakahara et al., 1998; Schaefer et al., 2004). In these studies, disrupted mitochondria were observed only in severely degenerated fibers. In contrast, our data show CVA-induced changes in mitochondria not limited to degenerated fibers. Our results contribute to the evidence for mitochondria effects as likely primary perturbations by CVA leading to subsequent myofiber degeneration. Direct or indirect effects of statins on mitochondria and potentially fatty acid oxidation likely play roles in the mechanism of toxicity.

Toxicity was achieved at 1 mg/kg, evident by significant increases in serum CK, ALT, and AST. Plasma lactic acid was significantly (\(p < 0.05\)) increased in the high dose, nonexercised and low, mid-, and high dose, exercised groups, compared to controls. These data are consistent with reports of statin patients with increased blood lactate/pyruvate ratios, elevated plasma lactate, and lactic acidosis (De Pinieux et al., 1996; Livingstone et al., 2004; Neale et al., 2004). Lactic acid may also accumulate within the muscle itself, which would contribute to the observed dose-dependent increase in fatigue during treadmill exercise.

In the present study weights of psoas and quadriceps, but not soleus, were significantly decreased in high dose groups compared to controls, consistent with a report in which simvastatin significantly decreased the mass of gastrocnemius but not soleus muscles, in tumor-bearing rats (Muscaritoli et al., 2003). To our knowledge this is the first report to show that CVA promotes Type II fiber-rich muscle weight loss in rats, at the dose that produces toxicity. Moreover, exercise decreased quadriceps weight at 1 mg/kg. Larger muscle groups, such as the quadriceps, may be more susceptible to myopathy (Franc et al., 2003), but the underlying mechanism of this observation is unknown.
In order to investigate mechanisms of toxicity, we tested whether CVA induces apoptosis in vivo. Although data have recently appeared for cell culture models (Johnson et al., 2004; Sacher et al., 2005), we are not aware of studies evaluating effects of statins on apoptotic processes in vivo skeletal muscle. A 24 h exposure of rat L6 myocytes to 50 and 100 nM CVA was shown to increase TdT-mediated dUTP nick end labeling (TUNEL) staining, a measure of apoptosis (Johnson et al., 2004). In this study, at 1 mg/kg and 24 h postdose, the plasma concentration of CVA without and with exercise was 53.9 ± 30.8 nM and 85.3 ± 30.8 nM, respectively. However, at this dose we found no evidence of active caspase 3 in EDL muscle or tongue. Our data imply that CVA may not promote apoptosis in vivo rat muscle. Caspase activation is an early marker of apoptosis so it is possible that its activity is enhanced within hours following CVA dosing in vivo, and that following 24 h, it is no longer activated.

In conclusion, we report that CVA-induced skeletal muscle toxicity does not involve increased concentrations of parent compound in muscle tissue itself, as we observed no difference between muscle and plasma CVA levels, or between damaged EDL and spared soleus muscles. In contrast to in vitro results in which CVA treatment enhanced TUNEL staining (Johnson et al., 2004), and caspase 3 and 9 activity (Sacher et al., 2005), a 14-day CVA treatment did not affect expression of active caspase 3 in skeletal muscle in the present in vivo study. It follows that CVA most likely does not promote apoptosis in vivo, as a mechanism of myodegeneration. In our model, treadmill exercise increases the incidence and severity of CVA-induced skeletal muscle damage, except in Type I-fiber-rich muscles. Thus, depleted energy or impaired fuel substrate use are likely mechanisms of toxicity. Finally, injury to mitochondria in Type II fiber-rich muscles appears to be an initiating event in CVA-induced skeletal muscle degeneration. Exercise training leads to fiber-type switching from Type II to Type I fibers (Simoneau et al., 1985). Why does exercise/training decrease tolerance of statin therapy (Sinzinger and O'Grady, 2004), when Type I-predominant fibers are apparently resistant to statin damage? Clearly, more investigations are necessary to fully comprehend the multifactorial mechanisms of CVA-induced skeletal muscle toxicity.

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