JPET Fast Forward. Published on November 6, 2007 as DOI: 10.1124/jpet.107.131615
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JPET Fast Forward. Published on November 6, 2007 as DOI: 10.1124/jpet.107.131615

JPET #131615

Title Page

The Guinea Pig as a Preclinical Model for Demonstrating the Efficacy and Safety of Statins

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Running Title Page

a) Running title: *Guinea pig as a model for statin-induced efficacy and safety*

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c) Document statistics:

Number of text pages: 20

Number of tables: 6

Number of figures: 6

Number of references: 39

Number of words in Abstract: 204

Number of words in Introduction: 658

Number of words in Discussion: 1496

d) List of non-standard abbreviations: Adult Treatment Panel (ATP); Bristol-Myers Squibb Co. (BMS); 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR); creatine kinase (CK); liquid chromatography tandem mass spectroscopy (LC/MS/MS); reversed-phase high-performance liquid chromatography (RP-HPLC); National Cholesterol Education Program (NCEP); upper limit of normal (ULN); low density lipoprotein cholesterol (LDLc)

e) Recommended section assignment: Cardiovascular

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Abstract

Statins, because of their excellent efficacy and manageable safety profile, represent a key component in the current armamentarium for the treatment of hypercholesterolemia. Nonetheless, myopathy remains a safety concern for this important drug class. Cerivastatin was withdrawn from the market for myotoxicity safety concerns. BMS-423526, similar to cerivastatin in potency and lipophilicity, was terminated in early clinical development due to an unacceptable myotoxicity profile. In this report, we describe the guinea pig as a model of statin-induced cholesterol lowering and myotoxicity, and show that this model can distinguish statins with unacceptable myotoxicity profiles from statins with acceptable safety profiles. In our guinea pig model, both cerivastatin and BMS-423526 induced mytoxicity at doses near the ED_{50} for total cholesterol (TC) lowering in plasma. In contrast, wide differences between myotoxic and TC-lowering doses were established for the currently marketed, more hydrophilic statins, pravastatin, rosuvastatin, and atorvastatin. This *in vivo* model compared favorably to an *in vitro* model which utilized statin inhibition of cholesterol synthesis in rat hepatocytes and L6 myoblasts as surrogates of potential efficacy and toxicity, respectively. Our conclusion is that the guinea pig is a useful preclincal *in vivo* model for demonstrating whether a statin is likely to have an acceptable therapeutic safety margin.

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Introduction

Three-hydroxy-3-methylglutaryl-CoA reductase (HMGR) inhibitors, known as statins, represent the cornerstone for treating hypercholesterolemia and mixed dyslipidemia. Several landmark clinical trials have firmly established the effectiveness of statins in lowering low density lipoprotein cholesterol (LDLc) and their benefits in reducing cardiovascular events and overall mortality (Kreisberg and Oberman, 2002). Accordingly, guidelines from the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III emphasize the need for aggressive lipid lowering in patients with other risk factors, such as previous heart attack, hypertension, or diabetes mellitus (Grundy et al., 2004). Achieving high treatment-to-goal rates may require increasing the dosage of a statin or adding with another LDLc-lowering agent (McKenney, 2005). The development of novel, more efficacious, LDLc lowering agents would also be helpful.

Myopathy potentially leading to life-threatening rhabdomyolysis is an important side effect of chronic statin therapy (Arora et al., 2006; Thompson et al., 2006). Myopathy is estimated to occur in approximately 0.1% of patients who receive statin monotherapy (Hamilton-Craig, 2001). Rhabdomyolysis, though rare, is more likely to occur when initial clinical signs of statin-induced myopathy are not recognized and statin therapy is continued (Omar et al., 2001). The most dangerous pathologic sequela of rhabdomyolysis is acute renal failure. Currently, plasma level of creatine kinase (CK) activity is the key biomarker for the clinical diagnosis of myopathy. Creatine kinase generates adenosine triphosphate via phosphorylation of adenosine diphosphate and is found primarily in skeletal muscle and the myocardium (Langer and Levy, 1968). Circulating levels of CK rise after muscle cell membrane damage and subsequent leakage into the systemic circulation. Rhabdomyolysis, as defined by the NCEP, is plasma CK activity >10 times the upper limit of normal (ULN) with renal compromise (Pasternak et al., 2002).

The incidence of drug-related myopathy requiring hospitalization is low for all currently marketed statins (Cziraky et al., 2006). However, prior to its voluntary withdrawal from the market in 2001, cerivastatin was clearly overrepresented among cases of statin-associated

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rhabdomyolysis demonstrating differences in safety among statins (Wooltorton, 2001). The preponderance of data indicate that the toxicological effects of statins on the myocyte are the direct result of HMGR inhibition and subsequent depletion of downstream products; however, the exact mechanism(s) of statin-induced myotoxicity remains elusive (Laaksonen, 2006).

To estimate safety margins preclinically, a facile model that discriminates between cerivastatin and currently marketed statins with respect to efficacy and also safety would be useful as an *in vivo* screen of novel statins, or potentially novel LDLc-lowering agents combined with a statin. Rats, especially juveniles, are susceptible to statin-induced myotoxicity (Reijneveld et al., 1996; Westwood et al., 2005). However, because the rat liver responds to statins by increasing hepatic cholesterol synthesis, use of plasma cholesterol as an indication of statin efficacy precludes the rat from being an optimal model for estimating therapeutic safety margins (Krause and Newton, 1995). In contrast to rat, the guinea pig, due to its similarities in lipid metabolism with humans, is an excellent model for studying LDLc lowering agents, including statins [reviewed by (Fernandez and Volek, 2006)]. However, to date, no studies have been described for guinea pig which characterizes the effects of statin treatment on skeletal muscle.

We conducted a series of studies in guinea pigs using cerivastatin, pravastatin, atorvastatin, rosuvastatin, and BMS-423526. Similar to cerivastatin, BMS-423526 is both highly potent and lipophilic, and demonstrated an unacceptable myotoxic profile in early studies in humans[1]. These 5 statins were given orally to guinea pigs for 10 days at doses that provided a range of efficacious exposures. On the final day of study we evaluated plasma TC level as a marker of efficacy concurrently with plasma CK activity and skeletal muscle histopathology as markers of myotoxicity. Results from these studies demonstrate that this guinea pig model discriminated between statins with an acceptable therapeutic safety margin from those without. This model may be of value as new statins, and possibly other cholesterol-lowering agents to be co-administered with statins, are being developed to meet current ATPIII guidelines for LDLc lowering.

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METHODS

Animal care. All procedures were approved by the Bristol-Myers Squibb Institutional Animal Care and Use Committee (IACUC). Experimental procedures utilized either male Hartley guinea pigs (250-300g) or Sprague-Dawley rats (150-200g) purchased from Charles River Laboratories, Inc. (Wilmington, MA). Upon receipt, animals were housed in a temperature-controlled environment (22-25^{o}C), and allowed free access to both water and food [for guinea pigs, Purina Guinea Pig Chow 5025 (Richmond, IN) and for rats, Teklad Rodent Chow 2018 (Madison, WI)]. Rats were maintained under reverse light-cycle conditions.

Statins. Atorvastatin {(3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-yl-pyrrol-1-yl]-3,5-dihydroxy-heptanoic acid} calcium salt was generously provided by Parke-Davis. Rosuvastatin {(3R,5R)-7-[4-(4-fluorophenyl)-2-(methyl-methylsulfonyl-amino)-6-propan-2-yl-pyrimidin-5-yl]-3,5-dihydroxy-heptanoic acid} calcium salt was purchased commercially from Astra-Zeneca. BMS-423526 {(3R,5S)-7-[4-(4-Fluorophenyl)-6,7-dihydro-2-(1-methylethyl)-5H-benzo[6,7]cyclohepta[1,2-b]pyridin-3-yl]-3,5-dihydroxy-heptenoic acid} sodium salt , cerivastatin {(3R,5S)-7-[4-(4-fluorophenyl)-5-(methoxymethyl)-2,6-dipropan-2-yl-pyridin-3-yl]-3,5-dihydroxy-heptanoic acid} sodium salt and pravastatin {(3R,5R)-7-[(1S,2R,6S,8S,8aR)-6-hydroxy-2-methyl-8-[(2S)-2-methylbutanoyl]oxy-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxy-heptanoic acid} sodium salt were synthesized by the Chemistry Department at BMS. The structures of all five statins tested are provided in Table 1. The structure and synthetic route for BMS-423526 is described in a past patent application as Example 2 (Robl, 2003).

Preparation of rat liver microsomes. Prior to liver harvesting, animals were maintained for 5 days on powdered rat chow supplemented with 5% cholestyramine. At the mid-dark point in the light /dark cycle the animals were anesthetized with CO_2, decapitated, and their livers harvested. Livers were homogenized in 25 ml per liver of ice-cold buffer A [0.04 M potassium phosphate (pH 7.2); 0.05 M KCl; 0.1 M sucrose; 0.03 M EDTA; 0.002 M dithiothreitol (pH 7.2); aprotinin (500 KI units/ml)]. The homogenate was centrifuged at 16,000 x g at 4°C for 15 min. The supernatant was collected and re-centrifuged under the same conditions. The supernatant was then centrifuged at 100,000 x g at 4°C for 70 min. Pelleted microsomes were re-suspended in a

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minimum volume of buffer A (3-5 ml per liver), and homogenized on ice using a glass/glass homogenizer. Microsomal preparations were aliquoted, snap-frozen on acetone/dry ice, and stored at –80°C.

HMGR enzymatic assay. HMGR activity was assayed by ion exchange separation of substrate and product essentially as described previously (Beg et al., 1977). The final reaction mixture in a total volume of 0.25 ml contained: 0.04 M potassium phosphate (pH 7.0), 0.05 M KCl, 0.1 M sucrose, 0.03 M EDTA, 0.01 M dithiothreitol, 3.5 mM NaCl, 1% dimethyl sulfoxide, 200 μg microsomal protein prepared from rat liver, 100 μM ^{14}C-HMG-CoA (0.05 μCi, 30-60 mCi/mmol) (Amersham Pharmacia Biotech, Piscataway, NJ) and 2.7 mM NADPH. Inhibitors (dissolved in DMSO) were pre-incubated with microsomal enzyme in the presence of NADPH at 37^{o}C for 15 min. The reaction was initiated with the addition of ^{14}C-HMG-CoA substrate and terminated after 20 min with the addition of 25 μl of 33% potassium hydroxide. Upon termination, ^{3}H-mevalonic acid (0.05 μCi, 10-40 Ci/mmol) (PerkinElmer, Waltham, MA) was added to the sample as an internal control for subsequent steps. The reaction mixture was incubated at room temperature for 30 min. Under the described conditions, in the absence of inhibitor, enzyme activity increased linearly up to 300 μg microsomal protein per reaction mixture, and was linear with respect to incubation time up to 30 min. The standard incubation time (20 min) resulted in 12-15% conversion of HMG-CoA substrate to the mevalonic acid product. Lactonization of the mevalonic acid was achieved by adding 5N HCl (50 μl) and incubating the mixture at room temperature for an additional 30 min. Ten μl of a 0.1% solution of bromophenol blue was added to monitor pH. The reaction mixture was layered onto an AG 1-X8 anion exchange resin prepacked in a 0.8 x 4 cm column (Bio Rad; Hercules, CA) and eluted with 2.0 ml H_2O. Ten ml of Opti-fluor scintillation fluid was added to the final 1.5 ml of eluate. The number of radioactive counts was determined using a 2500 TR Packard liquid scintillation analyzer. Results were calculated as nmoles mevalonic acid produced per 20 min, and were corrected to 100% recovery of ^{3}H. Drug effects on enzyme activity are expressed as IC_{50} values (concentration of drug producing 50% inhibition of product formation) derived from composite concentration response data using a four-parameter

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logistic fit model (Levenburg-Marquardt algorithm) and the Microsoft Excel software package (Microsoft, Redmond, WA).

Rat hepatocyte isolation. Primary rat hepatocytes were isolated as described previously (Berry and Friend, 1969). The freshly dissected rat liver was submerged in Kreb's Ringer Buffer (KRB: 0.12 M sodium chloride, 5.4 mM potassium chloride, 5.5 mM glucose, 25 mM sodium bicarbonate, 0.4 mM HEPES, pH 7.4) and gently agitated with surgical rakes to free hepatocytes. The released cells were sequentially filtered through 250μm and 62μm filters with KRB. The filtrate was spun down at 300 x g at 4°C for 5 min. The cell pellets were re-suspended in 50 ml of KRB. The suspension was mixed with an equal volume of 90% Percoll/10x HBSS [53.7 mM potassium chloride, 4.4 mM potassium phosphate monobasic, 1.37 M sodium chloride, 41.7 mM sodium bicarbonate, 3.4 mM sodium phosphate dibasic, 55.5 mM glucose (pH 7.4)] and centrifuged at 500 x g at 4°C for 5 min. The supernatant containing dead cells was aspirated and the pellet of viable cells were re-suspended in KRB up to 50 ml and spun down at 300 x g at 4°C for 5 min. Purified cells were re-suspended in KRB.

Inhibition of cholesterol synthesis in cells. Cellular synthesis of cholesterol was measured, in the presence or absence of drug, as incorporation of ^{14}C-acetate into cholesterol using an adaptation from a previously described method (Capuzzi and Margolis, 1971). Freshly isolated rat hepatocytes were plated in hepatocyte InVitroGRO medium (In Vitro Technology, Baltimore, MD) at 1X10^6 cells/well. Cells were pre-incubated with drug dissolved in DMSO for 30 min at 37^{o}C. (1-^{14}C)-sodium acetate (2 μCi/ml/well; 1-3 mCi/mmol) (Amersham Pharmacia Biotech, Piscataway, NJ) was then added to each well. Following a 4-hr incubation period, the cells were centrifuged at 500 x g for 10 min and lysed with the addition of 1 ml H_2O. Cellular lipids were extracted essentially as described (Bligh and Dyer, 1959). The organic phase containing lipids was collected and dried under N_2. The dried pellet was resuspended in 50 μl of $CHCl_3/CH_3OH$, 2:1 (v/v) and lipids were separated by thin layer chromatography using CH_2Cl_2/CH_3COCH_3, 60:1 (v/v) as a developing solvent. Plates were air-dried and the amount of radiolabeled cholesterol was quantitated with a Packard Instant Imager. Cholesterol synthesis in rat L6 skeletal muscle myoblasts was measured in a similar manner as performed for hepatocytes. L6 cells (rat skeletal

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muscle myoblasts) were obtained from American Type Culture Collection (Manassas, VA) and maintained in culture using the provider's suggested protocol. Monolayers of L6 cells were cultured in 6-well plates and treated with drug upon reaching 80-90% confluency. Following incubation with ^{14}C-acetate, cells were washed twice with PBS and lipids were extracted from the monolayer by incubation with 0.2 ml of isopropanol/heptane, 1:2 (v/v) by gentle agitation for 40 min at room temperature. For both hepatocytes and L6 cells, drug effects were expressed as IC_{50} values. The degree of cell-selectivity for a particular statin was defined as the IC_{50} for cholesterol synthesis inhibition in L6 myoblasts divided by the IC_{50} value for cholesterol synthesis inhibition in hepatocytes.

Experimental protocol for 10-day oral dose studies in guinea pigs. Following a 3-day acclimation period, animals were dosed orally once-daily for 10 days with drug dissolved in 0.5% carboxymethylcellulose (EM Science, Gibbstown, NJ). The total volume of each dosing solution was 0.5 ml. To allow the animals to become accustomed to human handling and dosing, during the acclimation period and prior to actual compound administration, guinea pigs were dosed orally with water. Animals were dosed using a 1 ml syringe (no gavage needle). The syringe is placed toward the back of the throat and ~1/3 volume of the dosing solution is slowly dispensed (~30 seconds). Without removing the syringe from the animal's mouth, the animal is allowed sufficient time (~10 seconds) to swallow the solution. The above step is repeated until the full dose is delivered to the animal. Complete intake of the dose is verified by visual inspection of the oral cavity. Animals were fasted overnight prior to administration of the final dose on Day 10. On Day 10, at 1 hr following administration of final dose, animals were sacrificed by CO_2 asphyxiation and blood was collected from the vena cava in 0.05% EDTA. Following perfusion with 0.9% saline via the portal vein using a Baxter Quik-Cath (2N-11-12-I, 18G X 5.1 cm) for 8 min, liver and skeletal muscle (diaphragm, quadriceps femoris, triceps brachii and, for some animals, gastrocnemius, and psoas minor) samples were collected in 10% buffered formalin and prepared for histologic analysis. Plasma samples were collected for determination of drug concentrations as well as analysis of levels of total cholesterol (TC) and creatine kinase (CK) activity using an autoanalyzer (Hitachi 912, Roche Diagnostics; Nutley, NJ). Additional liver and muscle tissues were collected for determination of drug levels by LC/MS/MS (see below). Total

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cholesterol values from individual animals and were utilized to derive the mean value (± standard deviation) for each treatment group. Percent total cholesterol lowering was determined by dividing the mean value of the drug-treatment group with the mean value of the vehicle-treatment group. CK values >2.5-fold the mean for the vehicle control group were considered moderately elevated, whereas CK values >10-fold the mean for the vehicle control group were considered severely elevated. For statistical evaluation, each group of actively treated animals was compared with the corresponding vehicle-treated control groups using Student's *t*-Test.

Histopathology. Samples from at least 2 of the following skeletal muscles were collected from each animal: diaphragm, gastrocnemius, psoas minor, quadriceps femoris, and triceps brachii. For some studies, muscle samples were collected from only 2 animals in the vehicle group, and from only 5 animals in the 2 highest dose groups and not from animals in the lower dose groups. At necropsy, skeletal muscles were immersed in 10% neutral buffered formalin for at least 24 hrs, routinely processed into paraffin, sectioned at approximately 6 microns, stained with hematoxylin and eosin, and examined by light microscopy for morphologic evidence of myopathy. We attempted to process and examine at least 1 transversely and 1 longitudinally orientated section of each sample.

Log $D_{7.0}$ determinations using reversed-phase high-performance liquid chromatography (RP-HPLC). All samples were prepared at a concentration of 0.25 mg/ml and were analyzed using a Shimadzu HPLC with a diode array detector. Mobile phase buffer: 55% methanol/ 45% potassium phosphate buffer (pH 7.0). HPLC system parameters were as follows: flow rate was 1.0 ml/min; injection volume was 10 µl; oven temp was 40°C; YMC C18 ODS-A 4.0 X 50 mm column (Waters Corp., Milford, MA) was utilized; wavelength set to 210 nm; with an approximate run time of 250 min. Calibration standards (p-methoxyphenol, p-cresol, 1-naphthol, thymol, diphenylether, and hexachlorobenzene) were prepared as a composite with each solution having a concentration of 0.25 mg/ml. Sodium nitrate was utilized to determine void volume.

Quantitation of drug levels by LC/MS/MS. Plasma samples were prepared for analysis by precipitating plasma proteins with two volumes of acetonitrile containing an internal standard. The samples were mixed by manual vortexing and centrifuged. The resulting supernatant was transferred to an auto-sampler vial and a volume of 10 µL was injected for analysis by

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LC/MS/MS. For the analysis of drug concentrations in muscle (quadriceps) and liver tissue, the samples were homogenized on ice with 9 ml of water per gram of tissue using an auto-homogenizer (Tomtec, Hamden, CT). Blank homogenate was used to prepare standards. Sample preparation and analysis were done as for plasma samples, described above. The HPLC system consisted of two Shimadzu LC10AD pumps (Columbia, MD), a Shimadzu SIL-HTC autosampler (Columbia, MD), and a Hewlett Packard Series 1100 column compartment (Palo Alto, CA). The column was an YMC Pro C18 (2.0 x 50 mm, 3 µm particles). The mobile phase system was 0.1 % formic acid and 10 mM ammonium formate in methanol/water. Gradient chromatography was used and the total analysis time was 4.5 minutes. The HPLC was interfaced to a Micromass Ultima tandem mass spectrometer (Waters, Milford, MA) equipped with an electrospray ionization source. Data acquisition utilized selected reaction monitoring.

The analytical ranges of the assays were from 1 to 10000 ng/mL. Standards were analyzed in duplicate and quality control samples (three concentrations within the range of the calibration curve) were analyzed in triplicate along with the study samples to comprise a complete analytical set.

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Results

Activity of statins in enzymatic and cell-based selectivity assays. The four reference statins and BMS-423526 were tested for HMGR inhibitory activity in a standard enzyme assay using rat liver microsomes (Table 1). The partition coefficient value (Log $D_{7.0}$) for each of the five statins (hydroxyacid forms) was also determined. BMS-423526, with a mean IC_{50} value of 1.3 nM and a Log $D_{7.0}$ value of 4.8, was determined to be the most potent and lipophilic of the five statins tested. Cerivastatin, with a mean IC_{50} value of 9.8 nM and Log $D_{7.0}$ value of 4.5, though less potent, was the most similar to BMS-423526 with respect to lipophilicity. Pravastatin was determined to be the least potent (mean IC_{50} = 31.6 nM) and least lipophilic (Log $D_{7.0}$ = 2.2) of the statins tested. To understand how potency and lipophilicity can affect activity in different cell types, each statin was tested for inhibition of cholesterol synthesis in both rat primary hepatocytes and rat L6 myoblasts grown in culture. The two most lipophilic statins, cerivastatin and BMS-423526, were determined to be slightly more potent in the L6 myoblast than in the hepatocyte. The L6 myoblast/hepatocyte IC_{50} ratio (cell-selectivity) for cholesterol synthesis inhibition was less than one for these two statins. In contrast, the three statins with relatively lower Log $D_{7.0}$ values (atorvastatin, pravastatin, and rosuvastatin) were considerably more potent in the hepatocyte than in the L6 myoblast. The degree of cell-selectivity for cholesterol synthesis inhibition for these three statins ranged from 31 to 114, with rosuvastatin being the most hepatocyte-selective of the three.

Cerivastatin-induced cholesterol lowering and myotoxicity in guinea pig. Because of its withdrawal from the market due to an unacceptable safety profile based on incidences of myopathy, cerivastatin was evaluated first to demonstrate that the guinea pig was susceptible to statin-induced myopathy. Cerivastatin lowered plasma levels of TC in a dose-dependent manner with approximately 50% TC lowering at the highest dose of 1.2 mg/kg (Table 2). Plasma CK values for individual animals within the vehicle-treated group were highly similar with a mean value of 306 ± 56 (U/L) (Fig. 1). At the 0.6 mg/kg dose there was a significant increase in the mean CK value and considerable intra-group variability among individual animals ranging from

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normal (i.e., similar to vehicle-treated animals) to >10,000 U/L. The dynamics of this range increased further in the 1.2 mg/kg group ranging from near normal to almost 100,000 U/L. Increases in plasma CK activity correlated on an individual animal basis with increasing amounts of drug. Histopathologic evidence of myopathy was observed at ≥ 0.3 mg/kg/day with an incidence of 0/5, 0/5, 1/5, 3/5, and 5/5 for the 0, 0.1, 0.3, 0.6, and 1.2 mg/kg groups respectively. Histologically, myopathy was characterized by coagulation, retraction, lysis and fragmentation of the sarcoplasm with varying degrees of macrophage infiltration and nuclear hypertrophy/hyperplasia of satellite cells, indicative of early regeneration. In many affected myofibers, macrophages had infiltrated through the sarcolemma and had partially phagocytized coagulated remnants of the sarcoplasm. These histopathologic changes were essentially identical to those observed in the muscles of guinea pigs given BMS-423526 (Fig. 2). The most severe and widespread myopathic lesions correlated with plasma CK increases of >2.5x vehicle mean. However, muscle lesions were observed in some animals with plasma CK activities within the range of the vehicle group. For example, muscle lesions were observed in two animals dosed with 1.2 mg/kg/day with plasma CK activity of only 303 and 326 U/L.

Increases in plasma levels of drug were roughly linear with dose increase, as were muscle and liver concentrations. Intra-group variability for these samples was quite high, in some cases the standard deviation was equivalent to the mean value. However, these values do not represent true exposures based upon area under the plasma concentration curve-time 0 to 24 hr ($AUC_{0\text{-}24hr}$), but rather, only a single time point at 1 hr post-dose. Thus, these values may not reflect overall drug exposure in the animal. Additionally, because samples were taken on the tenth day of study, this analysis may be confounded by possible cumulative effects of chronic dosing. Finally, what fraction of drug was intracellular as opposed to extracellular was not determinable. With these caveats in mind, we noted that the mean concentration of cerivastatin in muscle was roughly 3.5-fold higher for the 0.6 as for the 0.3 mg/kg/day group (46 nM vs. 13 nM). This narrow dose range, in which plasma TC lowering increased from 28.7% to 41.8%, appeared to represent the threshold for myotoxicity based on plasma CK elevation and histopathology. For animals with marked increases in plasma CK activity, a rough correlation

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with muscle drug levels was noted. The 3 animals with the highest plasma CK values (14,338, 61,930, and 93,640 U/L) also had the highest muscle concentrations of drug (119, 189, and 216 nM, respectively). However, at the lower plasma CK values (~300 to 1500 U/L) no clear correlation with muscle levels of drug were noted. Mean muscle levels of drug in the highest dose group (1.2 mg/kg/day) were approximately 3-fold and 14-fold less than plasma and liver levels, respectively. These differences indicate that cerivastatin preferentially distributes to the liver relative to muscle.

BMS-423526-induced cholesterol lowering and myotoxicity in guinea pig. To further validate the guinea pig as a model for discriminating among statins, we investigated a second compound, BMS-423526. BMS-423526 was given orally to guinea pigs at doses of 0.25, 0.5, 1.0, 1.5, and 2.0 mg/kg/day. The ED_{50} of BMS-423526 for plasma TC lowering was approximately 0.5 mg/kg (Table 3). Increases in mean CK activity were noted at ≥0.25 mg/kg/day and were dose-dependent (Fig. 3). At 1.0 mg/kg/day animals with plasma CK activity >2.5-fold vehicle group mean were noted and at ≥1.5 mg/kg/day several animals had plasma CK activity >100-fold the vehicle mean. This remarkably steep dose response effect for increases in plasma CK activity is highly similar to cerivastatin in this model. Samples of skeletal muscle (diaphragm, quadriceps femoris, and triceps brachii) from 5 animals in the 2 highest dose groups were examined for histopathologic evidence of myopathy. Skeletal muscle lesions were observed in animals with plasma CK activity >20,000 U/L but not in those with plasma CK activity <10,000 U/L. As shown in Fig. 2, histopathologic changes were identical to those observed in animals given cerivastatin.

Plasma levels of BMS-423526 increased in a non-linear fashion with escalating dose. However, liver to plasma ratios (ranging from 1.3 to 2.2) and muscle to plasma ratios (ranging from 0.04 to 0.09) of drug were essentially maintained across all doses. At the 0.5 mg/kg dose, which resulted in nearly 50% TC lowering, the mean liver concentration of BMS-423526 (2352 nM) was approximately 27-fold greater than the mean concentration of drug present in muscle

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(88 nM). Thus, BMS-423526 preferentially distributes to the liver relative to muscle. The mean concentration of BMS-423526 in muscle increased by approximately 2-fold from 88 nM to 178 nM for the 0.5 and 1.0 mg/kg treatment groups, respectively. Similar to observations made for cerivastatin, this small increase in dose, in which plasma TC decreased from 46.6% to 57.1%, appeared to represent the threshold for myotoxicity. An analysis of individual animals revealed that there was no absolute correlation between levels of drug in muscle and plasma levels of CK. For example, two animals within the 2.0 mg/kg treatment group had plasma CK values greater than 60,000 U/L. One of these animals had the lowest concentration of drug in muscle (190 nM) for that treatment group while the other animal had the highest concentration of drug in muscle (552 nM).

Rosuvastatin- and pravastatin-induced cholesterol lowering and myotoxicity in guinea pig. Relative to cerivastatin and BMS-423526, rosuvastatin has a reduced incidence of myotoxicity in the clinic and, as shown in Table 1, is less lipophilic and considerably more cell-selective. At doses of 0, 10, 25, 50, 75, and 100 mg/kg, rosuvastatin lowered plasma TC levels (relative to vehicle mean) by 31.0, 38.6, 49.6, 47.9, and 54.3 percent, respectively (Table 4). Thus, the ED_{50} for TC lowering was ~50 mg/kg. Plasma levels of rosuvastatin increased in a slightly greater than dose-proportional manner, most likely due to saturation of hepatic extraction at the higher doses. The dose-dependent decrease in the liver-to-plasma drug concentration ratios also suggests saturation of hepatic uptake with increasing dose. Drug concentrations in liver relative to plasma were only moderately greater, with a maximum liver-to-plasma ratio equal to 2.5 for the lowest dose (10 mg/kg). Mean muscle levels of drug at the top dose of 100 mg/kg were approximately 13-fold and 9-fold less than plasma and liver levels, respectively. These differences indicate that rosuvastatin preferentially distributes to the liver relative to muscle.

At the ED_{50} for TC lowering, the mean CK level (399 ± 93 U/L) was moderately, but significantly ($p < 0.05$) greater than the mean CK level of the vehicle-treated animals (311 ± 33 U/L) (Fig. 4). However, there were no animals within the 50 mg/kg group with CK values >2.5-fold the vehicle mean. Plasma CK activity >2.5-fold the mean CK value of the vehicle group was

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noted in only one high-dose (100 mg/kg) animal and no histopathologic evidence of myopathy was observed in any animal.

Pravastatin was dosed to guinea pigs (n = 10/vehicle group; n = 5/treatment groups) at 50, 100, 150, and 200 mg/kg. Percent TC lowering (relative to mean of vehicle group) was 31, 32, 31, and 25, respectively. Thus, a dose at which TC was lowered by 50% was not identified for pravastatin. Plasma levels of drug increased proportional to dose with mean values of 294 ± 197 nM (50 mg/kg), 435 ± 178 nM (100 mg/kg), 548 ± 212 nM (150 mg/kg), and 1273 ± 1430 nM (200 mg/kg). Concentrations of drug in liver and muscle were not determined for this study. The mean plasma CK value of the vehicle group was 310 ± 44 U/L. The 200 mg/kg treatment group had the highest mean plasma CK value (416 ± 36 U/L) which was significantly different ($p < 0.05$) from the vehicle mean. The highest individual plasma CK values (453 U/L) were observed for two animals within the 200 mg/kg group. No animals had CK values >2.5-fold the vehicle mean and no histopathologic evidence of myopathy was observed in any animal.

Atorvastatin-induced cholesterol lowering and myotoxicity in guinea pig. Atorvastatin was utilized in the guinea pig (n = 8/group) to explore the potential for myotoxicity at very high exposure levels of drug. A single dose of cerivastatin (1.2 mg/kg; n = 8) was also included within this study to assess reproducibility of previous results. At doses of 25, 50, 100, 150, 200, 250 mg/kg, atorvastatin reduced TC by 37.1, 48.4, 63.5, 63.2, 62.9, and 68.2 percent, respectively. Plasma TC was decreased by 42.6% for the cerivastatin-treated animals (Table 5). Two of eight guinea pigs within the 150 mg/kg group were declared moribund and euthanized prior to study termination on Day 7. Similarly, 6 guinea pigs within the 200 mg/kg group and 5 guinea pigs within the 250 mg/kg group were sacrificed prior to study termination (between Days 6 to 10) or were found dead in their cage. Blood and tissue samples were obtained from moribund animals prior to sacrifice. Although the exact cause of the adverse effects observed at the higher doses of atorvastatin (≥ 150 mg/kg) is unknown, all of the animals that died or were sacrificed early prior to Day 10 experienced reduced body weight gain and body weight loss, and displayed clinical

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signs such as reduced grooming and hunched posture. Similar toxicological findings at these doses of atorvastatin have been described for rat (Henck et al., 1998) and dog (Walsh et al., 1996), and are likely due, in part, to adverse effects on liver. Simvastatin was also demonstrated to cause dose-dependent hepatotoxicity in guinea pig (Horsmans et al., 1990). At the higher doses, in multiple guinea pigs, we noted significant increases in plasma levels of bilirubin which is consistent with adverse effects on liver (data not shown).

Analysis of plasma CK activities revealed that one animal within the vehicle group had a value of 769 U/L. What caused this abnormally high CK level is unknown; however, it is possible that fighting with cage mates and subsequent tissue trauma may have occurred. Although this value was included in all calculations, it is worth noting that exclusion of this single value reduces the overall CK mean for the vehicle group from 392 to 338 U/L resulting in an incidence (CK >2.5-fold vehicle mean) rate of 3/5 within the 100 mg/kg group. With all values included, the CK group means were significantly ($p < 0.05$) elevated relative to the vehicle mean beginning with the 100 mg/kg dose, but the first CK incidence (>2.5-fold) was first observed within the 150 mg/kg group in 4 of 8 animals (Fig. 5). Of note, the two animals within the 150 mg/kg group that were declared moribund and sacrificed early on Day 7 had normal or only moderately elevated plasma CK levels (289 and 459 U/L, respectively). Blood samples were obtained from only 3 animals in the 200 mg/kg group, including one animal that was sacrificed moribund on Day 6. Plasma CK activity was >2.5-fold but <10-fold vehicle mean in all 3 of these animals (ranging from 1216 to 1533 U/L). The maximal plasma CK activity for atorvastatin-treated animals was 2294 U/L (250 mg/kg group). No histopathologic evidence of myopathy was observed in any animal. Plasma CK activity for the 1.2 mg/kg cerivastatin group were in agreement with previous results.

Plasma levels of drug were equal to or greater than dose-proportional up through the 250 mg/kg dose. The increase in plasma exposures at the higher doses likely reflects saturation of hepatic uptake. Considerable variability in drug concentrations within a treatment group was noted at the higher doses. The plasma drug levels at 50 mg/kg, which generated ~50% TC lowering (~ED_{50}), and at 250 mg/kg, a dose 5-fold greater, were 0.72 μM and 38.6 μM,

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respectively. Thus, even with a 54-fold increase in drug exposure, increases in plasma CK levels were relatively moderate (<10-fold the vehicle mean value). The relative drug concentrations in liver were much greater than in muscle at the lower doses, but less so at the highest doses. For example, at the 50 mg/kg dose liver and muscle concentrations of drug were 11.1 and 0.05 μM, respectively. This represents a 222-fold differential. At the 250 mg/kg dose, liver and muscle concentrations of drug were 47.0 and 13.4 μM, respectively. This represents a 3.5-fold differential, considerably less than that observed for the 50 mg/kg dose. Atorvastatin is metabolized by CYP3A4 in humans and other species to active more polar forms in which one of the pendent phenyl rings is hydroxylated at either the *ortho* or *para* position (Kantola et al., 1998; Black et al., 1999). Thus, we determined plasma concentrations of atorvastatin (parent molecule) and its *ortho*- and *para*-hydroxy metabolites in guinea pig (Fig. 6). Both metabolites were readily detected in blood with the *ortho*-hydroxy being by far the most predominant form. The concentration of combined metabolites (*ortho* + *para*) as a percent of total drug concentration (parent + *ortho* + *para*) increased with increasing dose, ranging from 47% to 84% (25 and 250 mg/kg, respectively).

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Discussion

The mean IC_{50} values for inhibition of rat HMGR and relative rank-ordering based on lipophilicity for the four reference statins (pravastatin, atorvastatin, cerivastatin, and rosuvastatin) are in good agreement with previous reports which include IC_{50} values for the highly homologous (~97% amino acid sequence identity) rat and human enzymes (Ishigami et al., 2001; Istvan and Deisenhofer, 2001; McTaggart et al., 2001; Davidson, 2002). A comparison of IC_{50} values for cholesterol synthesis inhibition in freshly isolated primary rat hepatocytes are similar, although not in perfect agreement, to a previous report in which all four reference statins were compared head-to-head (McTaggart et al., 2001). A comparison of activities in the primary rat hepatocyte versus the L6 myoblasts shows a clear dichotomy amongst the tested statins. The two most lipophilic statins, cerivastatin and BMS-423526, were determined to be slightly more potent in the L6 myoblast while the other three more hydrophilic statins (pravastatin, atorvastatin, and rosuvastatin) were considerably more potent in the hepatocyte. Although the absolute differential is not as great, these data are in agreement with a previous report which compared the activities of the four reference statins in isolated rat primary hepatocytes and rat fibroblasts grown in culture (McTaggart et al., 2001). The exact mechanisms which dictate the degree of cell-selectivity for a statin are unknown. Previous studies indicate that carrier-mediated transport by the hepatocyte plays an important role in the uptake of hydrophilic statins (Nezasa et al., 2003), whereas passive diffusion across the hepatocyte cell membrane is thought to be the primary uptake mechanism for lipophilic statins (Schachter, 2005). The hepatocyte and L6 myoblast data generated in this study, in particular the lack of cell-selectivity observed for BMS-423526, a highly potent and lipophilic statin, strongly support these earlier studies demonstrating that the physicochemical properties of a statin can greatly influence its activity in different cell types.

An analysis of all guinea pig data for the five statins profiled in this report continue to support the existence of a dichotomy between cerivastatin and BMS-423526 and the other three statins with respect to the apparent size of the safety window for TC lowering and induction of myotoxicity (Table 6). With regard to efficacy, excluding pravastatin, the guinea pig model did not

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readily distinguish amongst the statins for ability to maximally lower TC. Of the five statins tested, pravastatin was the least efficacious in guinea pig and is also the least efficacious in the clinic. Maximal TC lowering for the other four statins ranged from approximately 50% to 65%, with atorvastatin being the most efficacious. Although a head-to-head comparison within a single study was not performed, atorvastatin and rosuvastatin appeared to be essentially equipotent in the guinea pig. In humans, rosuvastatin is approximately twice as potent and slightly more efficacious for lowering LDL cholesterol than atorvastatin (McKenney, 2005). The relatively small number of animals utilized per treatment group, the dynamic range in values within a treatment group, as well as differences in mean plasma TC values between comparator vehicle control groups (ranging from 43.1 to 56.4 mg/dL), are all contributing factors which preclude certain meaningful direct comparisons (e.g., rank-ordering based on maximal TC lowering). With that said, the model did predict the potency for cholesterol lowering of cerivastatin versus atorvastatin/rosuvastatin in humans. A comparison of potency and efficacy data to previous reports which studied statin effects on plasma TC in guinea pigs revealed both similarities (Suzuki et al., 1999), as well as discrepancies (Conde et al., 1999). For the later study, difference in routes of administration (diet admix versus once-daily oral dosing) and increased duration on atorvastatin (21 days versus 10 days) may have contributed to the observed differences in potency. A lack of data prevents a direct comparison based on pharmacokinetics.

It is well-established that statin-induced myopathy in humans is drug-related (Pasternak et al., 2002). Cerivastatin and BMS-423526, when dosed orally to guinea pigs, generated a dose-dependent increase in plasma, liver, and muscle levels of drug as well as a dose-dependent increase in myotoxicity. Both statins appeared to have a threshold effect for induction of myotoxicity. Likewise, dramatic increases in cerivastatin-induced myotoxicity, with minimal increases in dose (2-fold), led to the proposal of a threshold effect for statin-induced myotoxicity in humans (Jacobson, 2006). The concentration of cerivastatin in muscle was determined to be highest (ranging from 119 to 216 nM) in the 3 animals with highest plasma CK values (>10,000 U/L). For these three animals, the muscle concentrations of cerivastatin were between 70- and 127-fold greater than the IC_{50} value (1.7 nM) for cholesterol synthesis inhibition in rat L6

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myoblasts. In contrast, for BMS-423526, there was no obvious correlation between muscle concentrations of drug and myotoxicity. Of note, some animals without evidence of myotoxicity had greater concentrations of BMS-423526 in muscle than did individuals with overt myotoxicity. The fact that data was obtained from only a single time point may explain, at least in part, the absence of a strong correlation between drug concentration in muscle and evidence of myotoxicity, especially at the lower doses. Nevertheless, this apparent lack of correlation is not unique to this model. Although it is generally thought that intramuscular statin levels are inherent to the myotoxic process, a thorough review of statin-related myopathies by the Muscle Expert Panel could find no direct evidence relating intramuscular statin concentrations to myopathy in humans (Thompson et al., 2006). It is of interest that several animals dosed with cerivastatin had normal plasma CK values and yet histopathologic analysis revealed myofiber damage. Similar findings of histopathologic evidence of muscle tissue damage in the absence of elevated plasma CK have been reported for humans suffering from statin-induced myopathy (Phillips et al., 2002). Kinetic studies in dog showed that plasma CK is rapidly cleared with a half-life of about 2 hours (Aktas et al., 1993). Thus, the absence of elevated plasma CK may indicate that statin-induced muscle damage and subsequent CK release is to some degree episodic or transient in nature. For future studies, it may be of value to investigate whether additional sampling of plasma during the 10-day study period would reveal elevations in CK.

In contrast to cerivastatin and BMS-423526, pravastatin, rosuvastatin, and atorvastatin were markedly less myotoxic in the guinea pig. At comparable doses (up to 100 mg/kg), rosuvastatin and atorvastatin were very similar with respect to plasma TC lowering and lack of myotoxicity. Although the mean plasma CK values were elevated relative to their respective vehicle mean values, the maximum plasma CK value for an individual animal at the 100 mg/kg dose (2-times the ED_{50} for TC lowering) for both rosuvastatin and atorvastatin was only moderately increased (845 and 944 U/L, respectively). Muscle levels of rosuvastatin and atorvastatin at the 100 mg/kg dose were also quite similar, 276 and 170 nM, respectively. These concentrations of drug are approximately 4- and 2-fold greater than the derived IC_{50} values for rosuvastatin and atorvastatin for cholesterol synthesis inhibition in L6 myoblasts, respectively.

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These values are considerably less than the >70-fold values observed for cerivastatin (at the ED_{50}) and BMS-423526 (at 2-times the ED_{50}).

High doses (≥150 mg/kg) of atorvastatin resulted in adverse effects, most likely hepatotoxicity, leading to euthanasia of several animals. The lack of a robust increase in plasma CK strongly suggests that the observed adverse effects were not due to myotoxicity. A remarkable finding for this particular study is the significant concentrations of atorvastatin detected in muscle tissue and yet only mild myotoxicity was evident. One potential explanation as to why atorvastatin is not more myotoxic at high doses is the fact that the majority of atorvastatin found present in blood is in the form of polar active metabolites. Similar to guinea pig, the acid form of the *ortho*-hydroxy metabolite represents a major portion (2.6-times that of parent form at the 80 mg dose) of circulating levels of active drug in humans (Lins et al., 2003). These more polar, active metabolites of atorvastatin, which are less likely to penetrate the myocyte cell membrane, may be, at least in part, responsible for the relatively low incidence of myopathy observed in the clinic (Wierzbicki, 2001).

In summary, we demonstrated that the guinea pig, as a model for statin-induced cholesterol lowering and myotoxicity, is capable of distinguishing between statins with relatively poor myotoxicity profiles in the clinic (cerivastatin and BMS-423526) and those with an acceptable safety profile (pravastatin, rosuvastatin, and atorvastatin). The model was also qualitatively predictive of human potency and efficacy. By correlating lipophilicity and differential activities in hepatocytes and myoblasts and relating these results to cholesterol lowering and induction of myotoxicity in the animal, these data lend strong support to the hypothesis that a statin's physicochemical properties greatly influence its therapeutic potential. It will be of interest to test the utility of this model with other agents in development that may eventually be co-administered with a statin as a therapeutic modality and determine if potentiation of myotoxicity occurs. This model may also be of value for the testing of agents, unrelated to statins, but suspected to cause myotoxicity.

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Acknowledgments

The authors greatly appreciate the technical assistance of Christopher Hassan, Karen Phillips, Debra Wescott, and Roseann Riley. The authors would also like to thank Richard Reeves and William Schumacher for critical reading of the manuscript.

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Footnotes

[1]An ascending multiple-dose clinical study was conducted in healthy subjects to examine the safety, pharmacokinetics, and pharmacodynamics of BMS-423526 following once daily administration for 14 days. The mean percent decrease in plasma levels of LDLc relative to baseline was 18% (0.5 mg), 34% (1 mg), 38% (2.5 mg), 54% (5 mg), 52% (10 mg), 57% (20 mg), and 56% (40 mg). Elevated plasma CK (>10 times the ULN) was noted in 1 subject (out of 6) at the 20 mg dose and also in 4 of 6 subjects treated with 40 mg, 3 of whom manifested reversible rhabdomyolysis.

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Legends for Figures

Fig. 1. Individual plasma creatine kinase (CK) activity levels (circles) and group means (bars with adjacent values) from animals dosed with cerivastatin (n = 5 per group). Plasma samples were obtained on final day of study (Day 10) at 1 hr post-dose. CK values greater than 2.5-fold and 10-fold the vehicle (0 mg/kg) mean value are indicated by dotted lines and by brackets. *denotes significance from its respective vehicle-treated group ($p < 0.05$).

Fig. 2. Histopathology of skeletal muscle (quadriceps femoris) from guinea pigs given either (**A**) 2 mg/kg BMS-423526 or (**B**) Vehicle (control) once daily for 10 days. Myofiber necrosis is present in the skeletal muscle from the guinea pig given BMS-423526. Necrotic myofibers are characterized by coagulation, retraction, lysis and/or fragmentation of the sarcoplasm (open arrow). Macrophages have infiltrated the disrupted sarcolemma to phagocytosize sarcoplasmic remnants (closed arrow). Nuclear pyknosis and reactive hypertrophy/hyperplasia are also evident. Plasma creatine kinase activity in this guinea pig was 60,920 U/L (vehicle mean = 270 U/L). Bar = 50 µm.

Fig. 3. Individual plasma creatine kinase (CK) activity levels (circles) and group means (bars with adjacent values) from animals dosed with BMS-423526 (n = 8 per group). Plasma samples were obtained on final day of study (Day 10) at 1 hr post-dose. CK values greater than 2.5-fold and 10-fold the vehicle (0 mg/kg) mean value are indicated by dotted lines and by brackets. *denotes significance from its respective vehicle-treated group ($p < 0.05$).

Fig. 4. Individual plasma creatine kinase (CK) activity levels (circles) and group means (bars with adjacent values) from animals dosed with rosuvastatin (n = 8 per group). Plasma samples were obtained on final day of study (Day 10) at 1 hr post-dose. CK values greater than 2.5-fold and 10-fold the vehicle (0 mg/kg) mean value are indicated by dotted lines and by brackets. *denotes significance from its respective vehicle-treated group ($p < 0.05$).

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Fig. 5. Individual plasma creatine kinase (CK) activity levels (circles) and group means (bars with adjacent values) from animals dosed with atorvastatin or cerivastatin (n = 8 per group). Due to premature deaths, samples were obtained from only 3 and 7 animals dosed with atorvastatin at 200 and 250 mg/kg, respectively. Plasma samples were obtained on final day of study (Day 10) at 1 hr post-dose. Animals declared moribund were euthanized prior to Day 10 and the plasma samples collected (150 mg/kg: 2 animals on Day 6; 200 mg/kg: 1 animal on Day 5; 250 mg/kg: 1 animal on Day 6, 2 animals on Day 7, 2 animals on Day 8). CK values greater than 2.5-fold and 10-fold the vehicle (0 mg/kg) mean value are indicated by dotted lines and by brackets. *denotes significance from its respective vehicle-treated group ($p < 0.05$).

Fig. 6. Plasma levels of parent and metabolite forms (*ortho*-hydroxy and *para*-hydroxy) of atorvastatin in guinea pigs expressed as a percentage of total drug (parent + *ortho*-hydroxy + *para*-hydroxy) set to 100. Plasma samples were collected at 1 hr post-dose.

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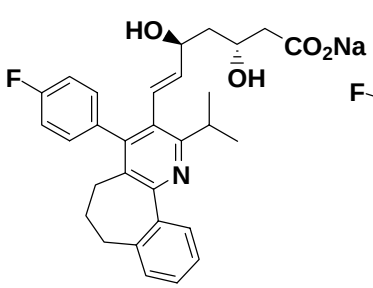
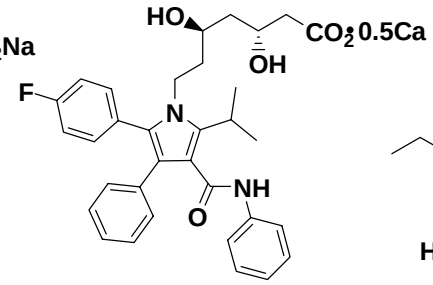
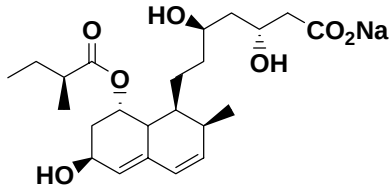
TABLE 1. Profiling of BMS-423526 and four reference statins in enzymatic and cell-based selectivity assays.



BMS-423526 sodium



atorvastatin calcium



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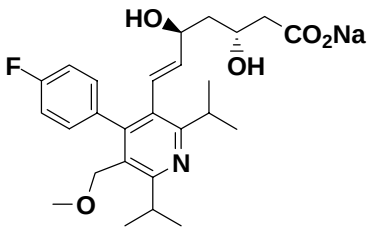
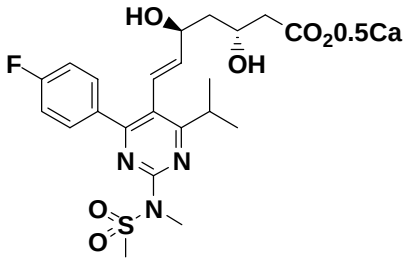


cerivastatin sodium



rosuvastatin calcium

Assay Data	BMS-423526	cerivastatin	atorvastatin	rosuvastatin	pravastatin
Rat HMGR[a] IC_{50} ± S.D. (nM)	1.3 ± 0.4	9.8 ± 2.8	6.2 ± 1.7	3.1 ± 0.4	31.6 ± 4.4
Rat 1° hep[a] IC_{50} ± S.D. (nM)	4.5 ± 0.3	2.3 ± 0.6	2.5 ± 1.1	0.6 ± 0.2	28.4 ± 4.9
Rat L6 myo[a] IC_{50} ± S.D. (nM)	2.3 ± 0.2	1.7 ± 0.9	77.1 ± 31.0	64.5 ± 30.1	1520 ± 514
IC_{50} Ratio (L6/Hep)	0.5	0.7	31.4	113.8	53.5
Lipophilicity[b] (Log $D_{7.0}$)	4.8	4.5	3.8	2.4	2.2

[a] mean IC_{50} values ± standard deviation (n ≥ 2 for each statin tested)

[b] distribution coefficient measurements (Log $D_{7.0}$) for hydroxyacid forms of reference statins

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TABLE 2. Cerivastatin-induced total cholesterol lowering and tissue levels of drug in guinea pig.

Cerivastatin Dose (mg/kg)	TC[a] ± S.D.	TC Percent Lowering[b]	Drug Levels (nM)		
			Plasma Mean ± S.D. (Ratio)[c]	Liver Mean ± S.D. (Ratio)	Muscle Mean ± S.D. (Ratio)
0	56.4 ± 12.3				
0.1	43.8 ± 10.3	22.3	24 ± 23 (1)	158 ± 108 (6.6)	≤LLQ[d]
0.3	40.2 ± 7.3	28.7	33 ± 33 (1)	425 ± 407 (12.9)	13 ± 20 (0.4)
0.6	32.8 ± 9.6	41.8	170 ± 124 (1)	1203 ± 805 (7.1)	46 ± 44 (0.3)
1.2	28.4 ± 9.1	49.6	292 ± 292 (1)	1416 ± 1041 (4.8)	101 ± 92 (0.3)

[a] n = 5 per treatment group; mean plasma total cholesterol (TC) values in mg/dL

[b] relative to vehicle mean

[c] relative to mean plasma drug level set to one

[d] lower limits of quantitation

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TABLE 3. BMS-423526-induced total cholesterol lowering and tissue levels of drug in guinea pig.

BMS-423526 Dose (mg/kg)	TC[a] ± S.D.	TC Percent Lowering[b]	Drug Levels (nM)		
			Plasma Mean ± S.D. (Ratio)[c]	Liver Mean ± S.D. (Ratio)	Muscle Mean ± S.D. (Ratio)
0	47.8 ± 3.6				
0.25	30.5 ± 9.5	36.1	618 ± 135	1346 ± 596	49 ± 13
			(1)	(2.2)	(0.08)
0.5	25.5 ± 2.1	46.6	1227 ± 289	2352 ± 629	88 ± 25
			(1)	(1.9)	(0.07)
1.0	20.5 ± 8.9	57.1	3363 ± 489	4347 ± 849	178 ± 38
			(1)	(1.3)	(0.05)
1.5	22.9 ± 5.1	52.1	4213 ± 2075	6261 ± 1518	182 ± 59
			(1)	(1.5)	(0.04)
2.0	24.0 ± 10.7	49.7	4643 ± 958	8771 ± 1895	416 ± 125
			(1)	(1.9)	(0.09)

[a] n = 8 per group; mean plasma total cholesterol (TC) values in mg/dL

[b] relative to vehicle mean

[c] relative to mean plasma drug level set to one

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TABLE 4. Rosuvastatin-induced total cholesterol lowering and tissue levels of drug in guinea pig.

Rosuvastatin Dose (mg/kg)	TC[a] ± S.D.	TC Percent Lowering[b]	Drug Levels (nM)		
			Plasma Mean ± S.D. (Ratio)[c]	Liver Mean ± S.D. (Ratio)	Muscle Mean ± S.D. (Ratio)
0	50.9 ± 9.4				
10	35.1 ± 3.9	31.0	179 ± 50	444 ± 169	25 ± 21
			(1)	(2.5)	(0.14)
25	31.3 ± 7.3	38.6	519 ± 129	1022 ± 406	108 ± 77
			(1)	(2.0)	(0.21)
50	25.6 ± 6.9	49.6	951 ± 206	1412 ± 353	83 ± 21
			(1)	(1.3)	(0.08)
75	26.5 ± 6.5	47.9	2140 ± 1470	3385 ± 1386	182 ± 72
			(1)	(1.5)	(0.09)
100	23.3 ± 6.9	54.3	3626 ± 2286	2536 ± 1477	276 ± 103
			(1)	(0.7)	(0.08)

[a] n = 8 per group; mean plasma total cholesterol (TC) values in mg/dL

[b] relative to vehicle mean

[c] relative to mean plasma drug level set to one

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TABLE 5. Atorvastatin-induced total cholesterol lowering and tissue levels of drug in guinea pig.

Atorvastatin Dose (mg/kg)	Number of animals analyzed per group of 8	TC[a] ± S.D.	TC Percent Lowering[b]	Drug Levels (μM)[d] Plasma Mean ± S.D. (Ratio)[c]	Liver Mean ± S.D. (Ratio)	Muscle Mean ± S.D. (Ratio)
0	8/8	43.1 ± 12.3				
25	8/8	27.1 ± 8.5	37.1	0.51 ± 0.27 (1)	7.5 ± 2.8 (14.7)	0.02 ± 0.01 (<0.1)
50	8/8	22.3 ± 4.3	48.4	0.72 ± 0.46 (1)	11.1 ± 7.9 (15.4)	0.05 ± 0.03 (<0.1)
100	8/8	15.8 ± 4.0	63.5	3.14 ± 1.67 (1)	20.4 ± 8.2 (6.5)	0.17 ± 0.09 (<0.1)
150	8/8[e]	15.9 ± 3.5	63.2	6.91 ± 5.40 (1)	22.3 ± 8.7 (3.2)	0.33 ± 0.28 (<0.1)
200	3/8[f]	16.0 ± 6.9	62.9	26.50 ± 19.92 (1)	54.3 ± 48.4 (2.0)	6.05 ± 7.07 (0.23)
250	7/8[g]	13.7 ± 7.1	68.2	38.57 ± 86.89 (1)	47.0 ± 52.9 (1.2)	13.41 ± 30.68 (0.35)
1.2 ceriva	8/8	24.8 ± 10.5	42.6	ND	ND	ND

[a] mean plasma total cholesterol (TC) values in mg/dL

[b] relative to vehicle mean

[c] relative to mean plasma drug level set to one

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[d] values represent sum total of parent, ortho- and para-hydroxy metabolites

[e] 2 animals were declared moribund on Day 6; samples were collected prior to euthanizing

[f] 5 animals died prematurely and no samples were collected; 1 animal was declared moribund on Day 6, sample was collected prior to euthanizing

[g] 1 animal died prematurely, no sample was collected; 1 animal on Day 6, 2 animals on Day 7, and 2 animals on Day 8 were declared moribund, samples were collected prior to euthanizing

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TABLE 6. Incidence of myotoxicity in relationship to dose multiples of the ED_{50} for plasma TC lowering.

Statin	Approximate ED_{50}[a] (% TC lowering at indicated dose)	Number of animals per treatment group	Number of animals with CK >2.5-fold at 1X ED_{50}	Number of animals with CK >10-fold at 1X ED_{50}	Number of animals with CK >2.5.-fold at 2X ED_{50}	Number of animals with CK >10-fold at 2X ED_{50}	Number of animals with CK >2.5-fold at 3X ED_{50}	Number of animals with CK >10-fold at 3X ED_{50}
Cerivastatin[b]	1.2 (45.7)	13	**6/13**	**6/13**	N/A[c]	N/A	N/A	N/A
BMS-423526	0.5 (46.6)	8	0/8	0/8	**3/8**	0/8	**6/8**	**5/8**
Rosuvastatin	50 (49.6)	8	0/8	0/8	**1/8**	0/8	N/A	N/A
Pravastatin	200[d] (24.5)	5	0/5[d]	0/5[d]	N/A	N/A	N/A	N/A
Atorvastatin	50 (48.4)	8	0/8	0/8	0/8	0/8	**4/8**	0/8

[a] dose at which plasma TC was lowered by ~50% relative to vehicle control group. Units for ED_{50} are mg/kg

[b] combined data from Tables 1 & 5 for the 1.2 mg/kg dose of cerivastatin

[c] not applicable (statin not dosed at this multiple)

[d] ED_{50} not reached; 200 mg/kg represents maximum dose tested

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Figure 1

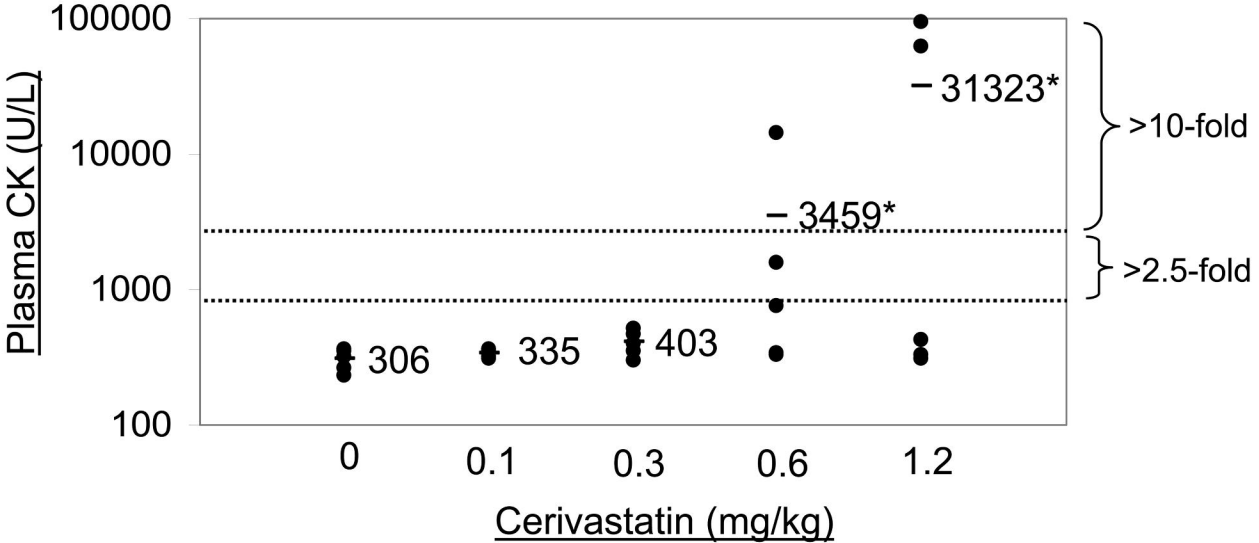

100000
10000
1000
100
Plasma CK (U/L)
31323*
3459*
306
335
403
>10-fold
>2.5-fold
0
0.1
0.3
0.6
1.2
Cerivastatin (mg/kg)

Figure 2

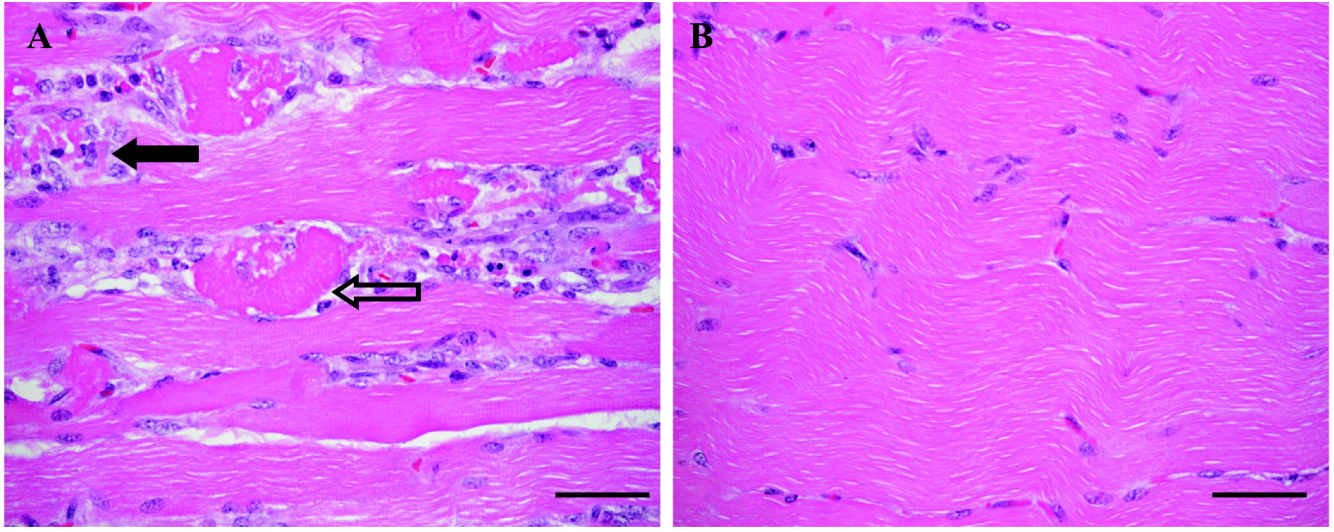


Figure 3

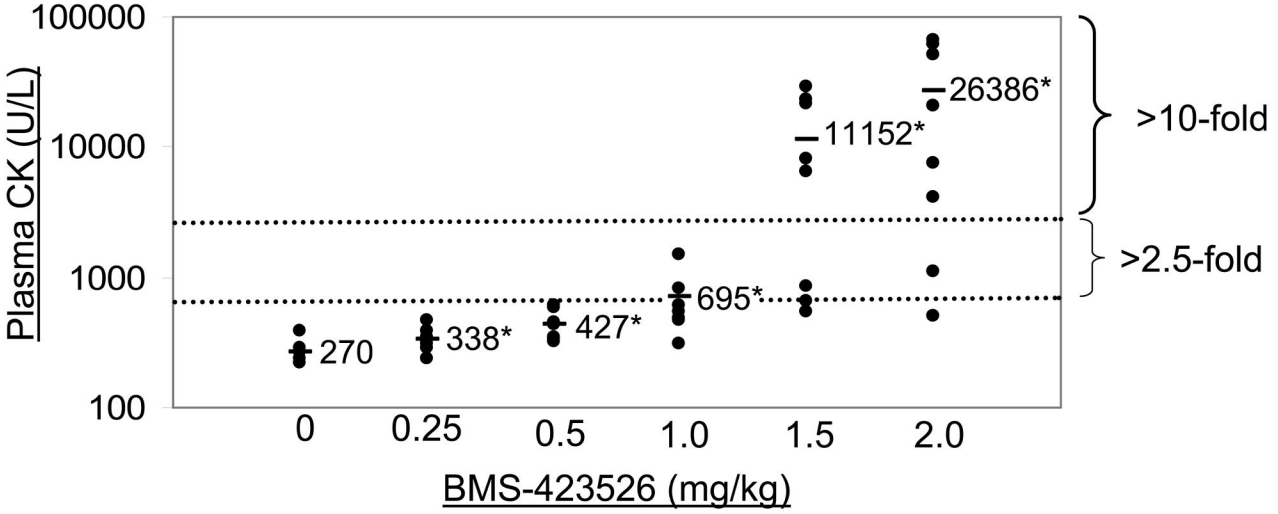

100000
10000
1000
100
Plasma CK (U/L)
270
338*
427*
695*
11152*
26386*
>10-fold
>2.5-fold
0
0.25
0.5
1.0
1.5
2.0
BMS-423526 (mg/kg)

Figure 4

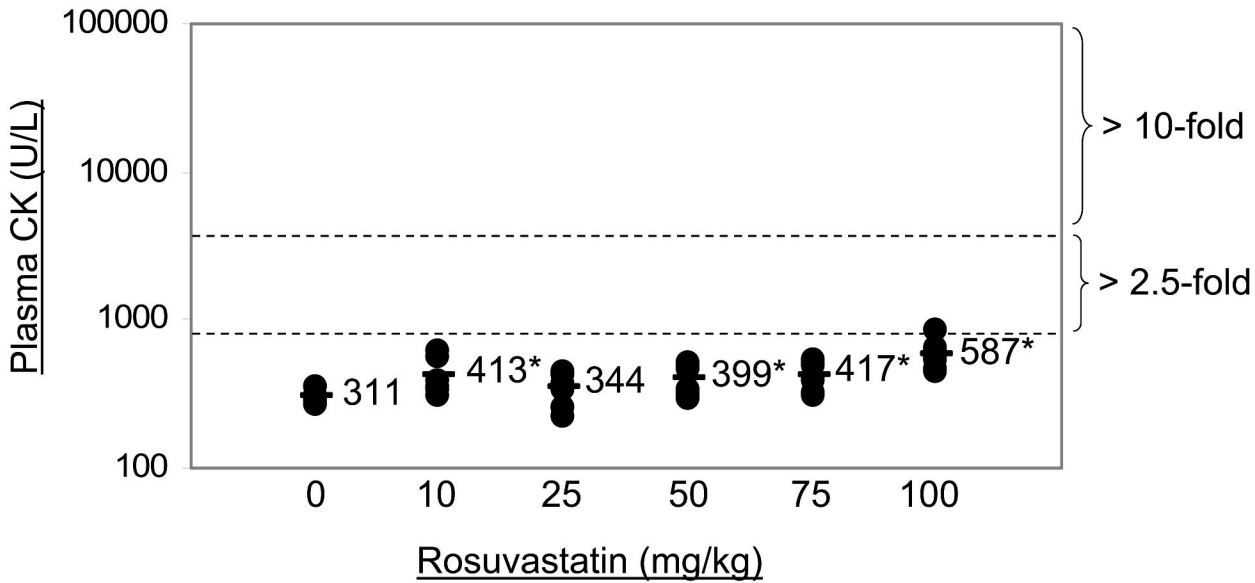

100000
10000
1000
100
Plasma CK (U/L)
> 10-fold
> 2.5-fold
311
413*
344
399*
417*
587*
0
10
25
50
75
100
Rosuvastatin (mg/kg)

Figure 5

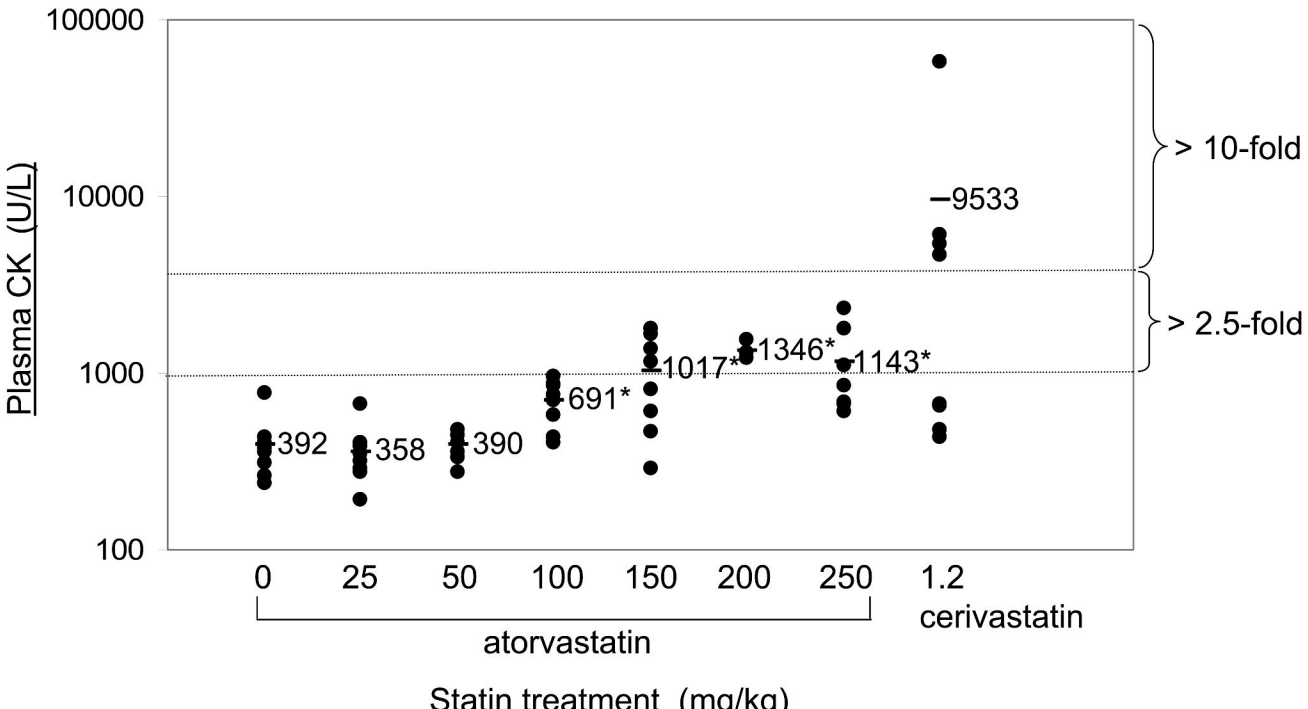
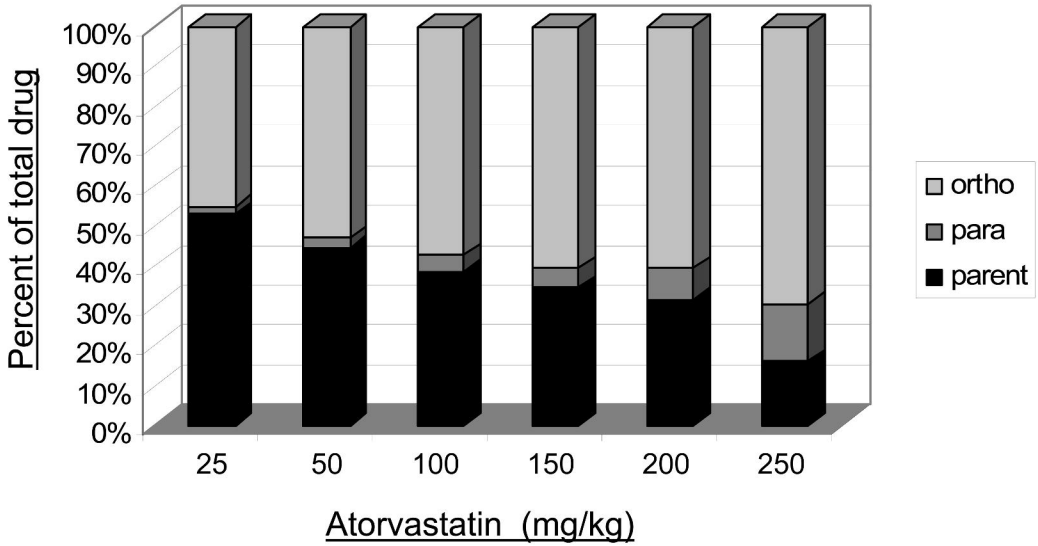

100000
10000
1000
100
Plasma CK (U/L)
> 10-fold
> 2.5-fold
9533
392
358
390
691*
1017*
1346*
1143*
0
25
50
100
150
200
250
1.2
atorvastatin
cerivastatin
Statin treatment (mg/kg)

Figure 6


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percent of total drug
25
50
100
150
200
250
Atorvastatin (mg/kg)
ortho
para
parent