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Bama Miniature Pigs (*Sus scrofa domestica*) as a Model for Drug Evaluation for Humans: Comparison of In Vitro Metabolism and In Vivo Pharmacokinetics of Lovastatin

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The objective of this study was to demonstrate that Bama miniature pigs are a suitable experimental animal model for the evaluation of drugs for man. To this end, in vitro lovastatin metabolism at the minipig liver microsomal level and in vivo pharmacokinetics were studied. Results were compared with those obtained from humans. Our data indicate that the main metabolites and enzyme kinetic parameters of lovastatin metabolism are similar in pigs and humans. Triacetyloleandomycin, a specific inhibitor of human CYP3A4, inhibited the metabolism of lovastatin in pig and human liver microsomes. In addition, the pharmacokinetic parameters and absolute bioavailability suggested that the absorption and elimination of lovastatin in Bama miniature pigs were similar to those in humans. Lovastatin was distributed across many organs in pigs, but the highest levels were found in the stomach, intestines, and liver. Within 96 h, 7% and 82% of the given dose was excreted in the urine and feces, respectively. In addition, no significant species differences in the plasma protein binding ratio of lovastatin and the rates of lovastatin hydrolysis to β-hydroxyacid lovastatin were apparent. From these results, we conclude that Bama miniature pigs are suitable for use in drug evaluation studies.

Abbreviations: CL_{int}, intrinsic clearance; CYP, cytochrome P450; HA, β-hydroxyacid lovastatin; TAO, triacetyloleandomycin.

Chinese Bama miniature pigs are genetically stable, highly inbred, small, inexpensive and share anatomical and physiological similarities with humans.[3,31,32] This makes the breed an excellent model for use in cardiovascular, gastrointestinal, renal system and skin pharmacological and xenotransplantation research.[2,34] Several strains of minipig such as the Göttingen, Yucatan, Sinclair, and Hanford, have been adopted as toxicological and pharmacological models in many countries. Minipigs also have become very popular for pharmaceutical studies in place of dogs and primates, especially in Europe.[28] Some sectors of the pharmaceutical industry have selected minipigs as animal models when developing dermally applied drugs and inhalations due to their specific responses to these kinds of drugs.[15,21] In addition, the State Food and Drug Administration of China recently announced that minipigs can be used for preclinical pharmacokinetic studies of new chemicals. [26] Our previous studies found several interesting similarities in drug metabolism by liver microsomes between Chinese Bama miniature pigs and humans. In particular, human cytochrome P450 (CYP) 3A4 may be well modeled by liver microsomes from Bama miniature pigs because of the similarity in their nifedipine oxidation activity and testosterone 6β-hydroxylation reaction.[16] However, despite the advantages of using minipigs as a drug evaluation model, researchers in the fields of pharmacology and toxicology generally still prefer to use dogs, monkeys, and, more often, rodents. This preference likely reflects a lack of basic research and the availability of kinetic data in these areas. Therefore, to promote the use of Bama miniature pigs in preclinical drug evaluation, additional comparative pharmacology and toxicology studies between these animals and humans are necessary.

Lovastatin, a prescription drug frequently used to treat cardiovascular disease and a specific substrate for CYP3A4 in the liver,[7,8,18] was selected as a model drug in the present study. Lovastatin is hydrolyzed to its active form, β-hydroxyacid lovastatin (HA), in vivo and then inhibits 3-hydroxy-3-methylglutaryl coenzyme A reductase and endogenetic cholesterol synthesis. Lovastatin has a short elimination half-life in humans and a high selectivity for the liver, its primary site of action, where it has a high first-pass effect.[13,29,30] Lovastatin reportedly is metabolized by CYP3A4 in human liver microsomes to yield 3 major metabolites: 6β-hydroxy lovastatin, HA, and 6′-exomethylene lovastatin.[13,29]

In the present experiment, we studied the similarities and differences in subcellular in vitro lovastatin metabolism in miniature pigs and humans. We also evaluated the pharmacokinetic parameters, absolute bioavailability, tissue distribution, excretion, and plasma protein binding of lovastatin in vivo. These data were compared with previously published results obtained from humans and other species.

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Materials and Methods

Chemicals. D-glucose-6-phosphate, glucose-6-phosphate dehydrogenase, $NADP^+$, and triacetyloleandomycin (TAO) were purchased from Sigma Chemicals (St Louis, MO). Lovastatin was obtained from Chongqing Daxin Pharmaceutical (Chongqing, China) whereas lovastatin standard was purchased from the National Institute for the Control of Pharmaceutical and Biological Products in China. Acetonitrile was obtained from Tedia (Fairfield, Ohio). All other chemicals were the highest grades available from commercial suppliers.

Standard solution. A stock solution of lovastatin was prepared in acetonitrile (1 mg/ml). The method to obtain the acid form of lovastatin (that is, HA) was obtained from the literature.[10] In all animal studies, lovastatin was suspended in 0.5% methyl cellulose for oral dosage or dissolved in 50% dimethyl sulfoxide in normal saline for intravenous use. The chromatographic retention times of lovastatin and HA were verified by comparison with standards.

Experimental animals and treatments. Bama miniature pigs *(Sus scrofa domestica*) were obtained from our laboratory. The animals weighed 10 to 12 kg (male; age, 6 mo) and were fed and treated as described.[16] Procedures involving animals were approved by our institutional animal care and use committee and complied with the Laboratory Animal Management Principles of China.[20] All animals were euthanized by intravenous injection of pentobarbital sodium (150 mg/kg body weight).

For the in vitro study, samples were taken from the left medial lobe of the liver of minipigs within 5 min of death. Samples of human liver from 3 Chinese autopsy samples (male; ages 33, 25, and 46 y) and plasma from 3 volunteers were obtained from Chongqing Southwest Hospital, China. These materials had been donated for research use, and this study was approved by the Ethics Committee of Third Military Medical University.

Thirty healthy Bama miniature pigs were used for the in vivo study. The animals were allowed to acclimate for 2 wk before the experiment was initiated; during the experimental period, minipigs were maintained on commercial concentrate piglet feed offered twice daily. Water was provided ad libitum. Animals were divided into 4 groups. Group I consisted of 8 animals which were used to study the pharmacokinetic parameters and absolute bioavailability of lovastatin after a single oral dose of 6 mg/kg body weight (4 animals) or intravenous dose of 1.2 mg/kg body weight (4 animals). Group II comprised 16 animals used to study tissue distribution: 4 animals were used as a negative control, and 4 each received a single daily oral dose of 45 mg/kg body weight at 1-, 4-, and 24-h intervals for 15 consecutive days. Group Ⅲcomprised 6 animals used to determine the excretion of lovastatin in urine and feces collected for 96 h after a single oral dose of 2.4 mg/kg body weight.

In vitro metabolism and analysis. Minipig liver microsomes were prepared from the livers of untreated male animals using procedures described in the literature.[5] Microsomal proteins were measured by the method of Lowry with bovine serum albumin as a standard.[17] Microsomal enzyme activity was measured by using a published approach.[30] The reaction tubes were centrifuged for 10 min at 14,300 × *g* and 4 °C, and the supernatants were analyzed by HPLC. To isolate metabolites for structure identification, semipreparative-scale mixtures in a final volume of 10 ml were incubated for 60 min.[29] The incubation mixtures were extracted with 20 ml ethyl acetate. After centrifugation, the organic layers were transferred into a glass tube and dried under a vacuum. The samples were redissolved in 100 µl acetonitrile.

Due to the lack of standards for 6′-β-hydroxy-lovastatin and 6′-exomethylene lovastatin, kinetic parameters (K_m and V_{max}) were evaluated based on loss of substrate rather that formation of metabolites. Concentrations of lovastatin ranging from 0 to 200 µM were used to determine kinetic parameters. TAO, an inhibitor of human CYP3A4,[22] was used in the inhibition studies at concentrations ranging from 0 to 500 µM. Reactions were initiated by adding lovastatin after the liver microsomes and TAO were preincubated for 20 min at 37 °C. Lovastatin consumption was expressed as a percentage of that of the control reaction without inhibitor.

The samples were analyzed by HPLC (1100 series, Agilent, Santa Clara, CA) with a XDB-C18 analytical column (4.6 × 250 mm, 5 µm). The column was eluted with a linear gradient of acetonitrile (30% to 90%) in 5 mM formic acid at a flow rate of 1.0 ml/min and a gradient change of 2%/min. Eluates were monitored continuously at 238 and 273 nm and the column temperature was set at 25 °C. The total run time for sample analysis was 30 min. Mass spectra of metabolite fractions were obtained by tandem mass spectrometry (API 2000, Applied Biosystems, Foster City, CA) at 70 V electron energy and a source temperature of 260 °C. Lovastatin metabolites were identified by their characteristic UV absorption and HPLC–mass spectrometry spectra.[29]

In vivo pharmacokinetic analysis. The collection and treatment of blood samples, urine, and feces was performed as described previously.[27] At 24 h before drug administration, the pigs underwent jugular vein cannulation with medical-grade silicon tubing by surgical intervention. Cannulas were introduced into the jugular veins of anesthetized minipigs; each cannula was directed toward the heart, and the free end was tunneled subcutaneously to the dorsal base of the neck. After surgery and before drug administration, the animals were fasted overnight (12 h), but water was made freely available.

Blood samples (4 ml) were collected from the cannulated jugular vein into heparinized glass test tubes at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, and 12 h after administration of drugs. Samples of urine and feces were collected at 12, 24, 48, 72, and 96 h after administration of lovastatin. Feces were dried, weighed precisely, homogenized with 100 mM potassium phosphate (pH 7.4) at a ratio of 1:15 (w/v), and stored at –20 °C. To study tissue distribution, animals were euthanized and representative 1-g samples were collected from the following tissues: liver, heart, spleen, lung, kidney, adrenal gland, pancreas, body and cardia of the stomach, testis, wall of the small and large intestines, prostate, brain, bladder, and skeletal muscle. Tissues were weighed, cut into small pieces, homogenized with 100 mM potassium phosphate (pH 7.4), diluted to 250 mg tissue/ml homogenates, and stored at –20°. Tissue and fecal homogenates as well as samples of plasma and urine (1 ml each) were extracted with 4 ml of acetic ether. After centrifugation at 1100 × *g* for 15 min, the organic layers were removed, and the aqueous layers were pooled and dried under vacuum, after which the samples were redissolved in 100 µl acetonitrile and centrifuged at 11,000 × g for 10 min. The supernatants underwent HPLC analysis using a Hypersil ODS column (4.0 × 250 mm, 5 µm; Agilent). The mobile phase consisted of 25 mM sodium dihydrogen phosphate solution (pH 4.5) and acetonitrile (40:60, v/v). The column was maintained at 50 °C with a flow rate of 1 ml/

min, and the UV detector was set to monitor at 238 nm. The total run time for sample analysis was 15 min.

Protein combination rate and hydrolysis of lovastatin in plasma. Binding of lovastatin by Bama miniature pig and human plasma protein was determined by equilibrium dialysis.[27] The rate of hydrolysis of 5 μg/ml lovastatin to HA in plasma was measured as described previously.[9]

Data analysis. The apparent K_m and V_{max} values were calculated from Lineweaver–Burk plots, and CL_{int} values were estimated. The pharmacokinetic parameters were evaluated with the 3p97 program (Committee of Mathematic Pharmacology, Chinese Society of Pharmacology), [33] and absolute bioavailability was assessed. The Student *t* test was used to analyze data, and a *P* value of less than 0.05 was considered statistically significant.

Results

Comparison of metabolites produced in vitro. The HPLC chromatogram revealed no discernible differences between the metabolites of lovastatin produced by minipig and human liver microsomes (Figure 1). Three main metabolites–HA, 6′-β-hydroxy-lovastatin, and 6′-exomethylene lovastatin–were produced. Peaks corresponding to HA (eluted at 19.7 min; peak 2) and lovastatin (24.7 min) were identified by using authentic standards. Two further metabolites were eluted at 11.5 min (peak 1) and 23.2 min (peak 3). UV spectrometry analysis of these peaks confirmed the identity of the metabolites in both pigs and human liver microsomes. Peak 1 had a λ_{max} at 238 nm and the characteristic structural pattern (triplet) of partially modified lovastatin (Figure 2 A). Peak 3 exhibited a large bathochromic shift with λ_{max} at 274 nm. Peak 1 showed a molecular ion peak at *m/z* 443 $[M+Na]^+$ whereas peak 3 showed a molecular ion peak at *m/z* 403 [M+H]. These results are similar to those described previously[29] and indicate that peaks 1 and 3 were 6′-β-hydroxy-lovastatin and 6′-exomethylene lovastatin, respectively. On the basis of results from human studies,[11,13] the metabolism of lovastatin is similar in the liver microsomes of Bama miniature pigs and humans.

Liver microsomes from pigs and humans catalyzed the biotransformation of lovastatin at rates of 3.76 and 3.92 nmol/mg/min, respectively. We compared the kinetic parameters of lovastatin metabolism in minipig liver microsomes (Table 1 and Figure 3) with those of humans. Although the K_m and V_{max} values were lower in pigs than humans, the CL_{int} values estimated by the K_m and V_{max} values were similar (Table 1).

Lovastatin was metabolized by CYP3A4 in human liver microsomes. The presence of enzymes in Bama miniature pigs similar to human CYP3A4[16] has been reported. Therefore, we studied the inhibitory effect of TAO, which is specific to CYP3A4 in humans, on the metabolism of lovastatin in minipigs. TAO clearly inhibited the conversion of lovastatin in the pig microsomes, similar to its effects in humans, and the same inhibition curve trend was present (Figure 4). However, TAO had a weaker inhibitory effect on pig microsomes than on those from humans: TAO at 100 μM inhibited the metabolism of lovastatin in human liver microsomes by 75% and in pigs by only 35%.

Pharmacokinetics of lovastatin in Bama miniature pigs. The HPLC chromatogram indicated the peak of lovastatin was eluted from pig plasma at 6.9 min (Figure 5). The concentration–time profiles of lovastatin are illustrated in Figure 6. The mean plasma concentration of lovastatin after a single oral administration (6 mg/kg) at 3 h was 32.99 ± 3.64 ng/ml, which declined to 1.34 ± 0.23 ng/ml by 24 h. After a single intravenous administration (1.2 mg/kg), the mean plasma concentration at 0.5 h was 421.05 ± 70.62 ng/ml, which declined to 2.04 ± 0.97 ng/ml by 24 h. The drug concentration–time data for both oral and intravenous administration routes fit a 1-compartment open model. The values of various pharmacokinetic parameters of oral and intravenous administration are presented in Tables 2 and 3, respectively. The absolute bioavailability of lovastatin was 4.97± 0.68%.

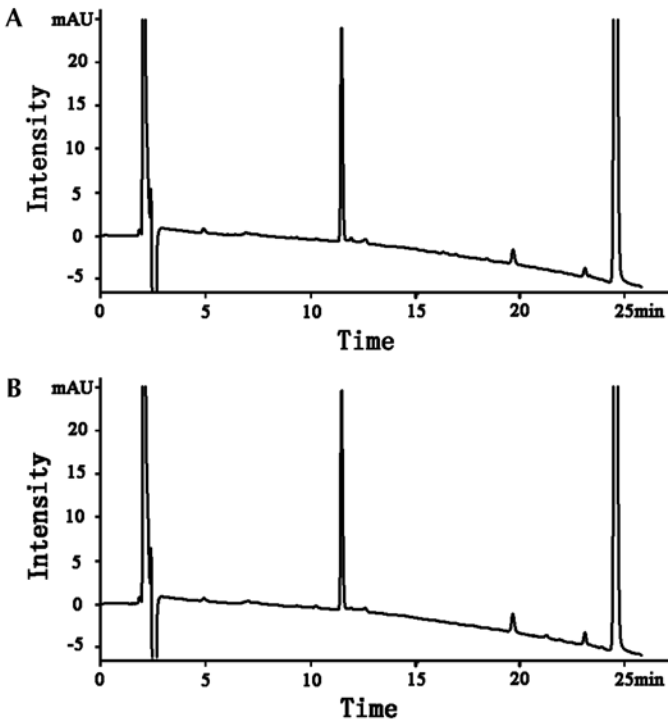

Figure 1. Spectra of lovastatin metabolites in liver microsomes from Bama miniature pigs and humans. (A) Bama miniature pigs. (B) Humans.

The concentrations of lovastatin in stomach cardia, stomach body, small intestine, and liver at 1 h after a single oral administration (45 mg/kg) were higher ($P < 0.05$) than in other tissues (Table 3). However, the tissue distribution of lovastatin after administration for 15 consecutive days and that at 4 h after a single oral administration did not differ. The concentration of lovastatin at 1 h was higher than at 4 h in the cardia of the stomach, body of the stomach and liver ($P < 0.05$). However, in the spleen, kidney, pancreas, testis, large intestine, brain, and plasma, the concentration was lower ($P < 0.05$) at 1 h than at 4 h. In other tissues, the distribution of lovastatin did not differ between the 1- and 4-h timepoints.

The time course of lovastatin excretion is shown in Figure 7 . Most of the total dose administered was eliminated within 96 h; around 7% of that excreted was excreted in the urine and 82% in the feces. The greatest amount of lovastatin eliminated was around 76% and occurred within 48 to 72 h of administration.

Comparison of PPBR and rates of lovastatin hydrolysis in Bama miniature pigs and humans. We compared protein binding of lovastatin in pig and human plasma at concentrations of 5, 50, and 500 μg/ml (Table 4). Overall means (pigs, 95.79% ± 1.42%; humans, 97.04% ± 1.72%) did not differ between species. Rates of

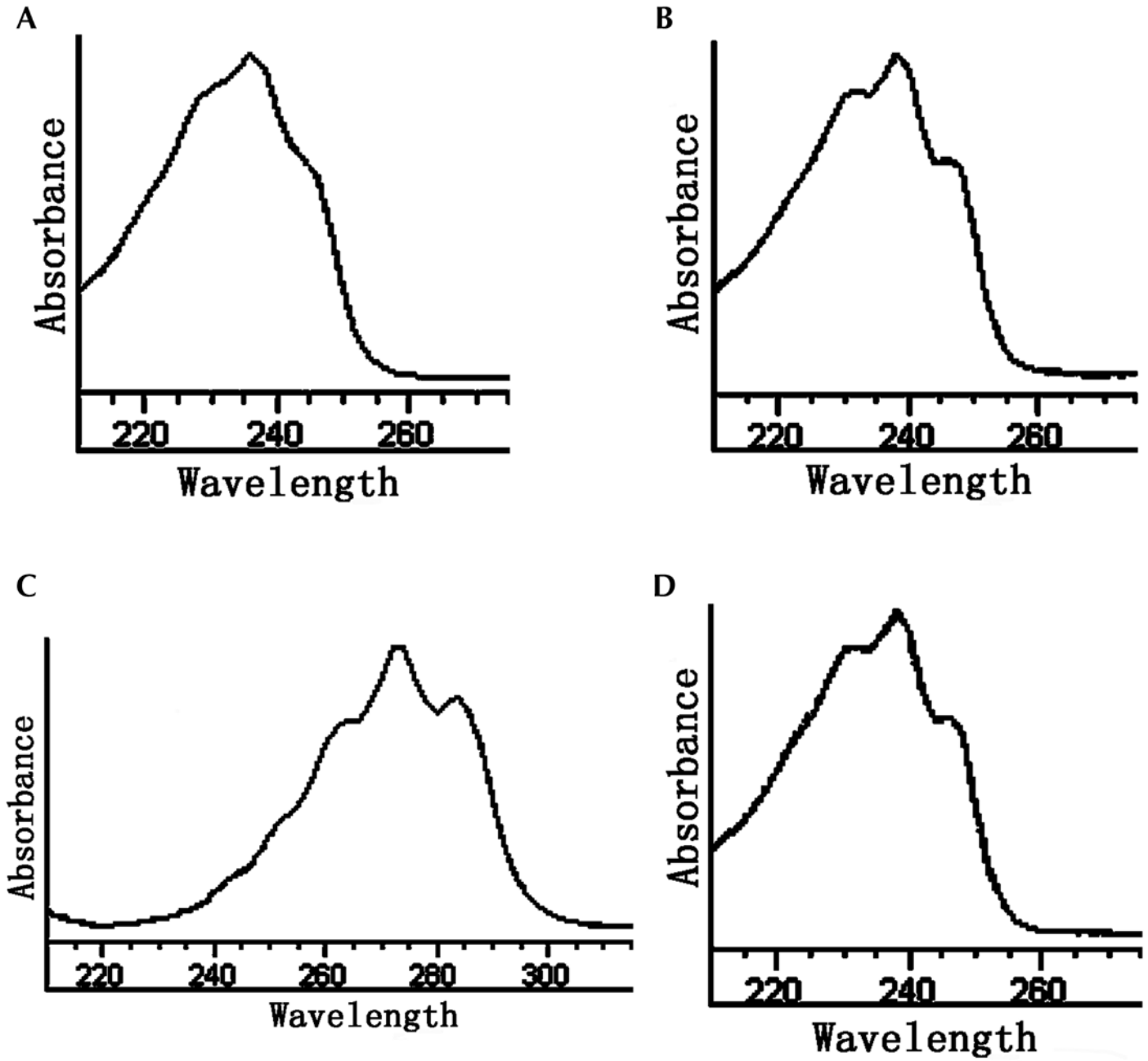

Figure 2. UV absorption spectra of metabolites in liver microsomes from Bama miniature pigs in the wavelength range of 200 to 400 nm. (A) 6′-β-Hydroxy-lovastatin. (B) HA. (C) 6′-Exomethylene lovastatin. (D) Lovastatin.

lovastatin hydrolysis to HA at 5 μg/ml were 0.74%/min for Bama miniature pigs and 0.66%/min for humans.

Discussion

Selection of the most suitable experimental animal for modeling drug evaluations in man requires an interspecies comparison of metabolic processes. Interspecific variation in drug metabolism mainly results from different liver CYP enzyme contents and activity.[24] Therefore, to identify animal models that are closely representative of humans, numerous studies have focused on comparing the kinetic parameters, activities, and inhibition profiles of enzymes from experimental animal liver microsomes with those obtained for humans.[1,12]

CYP450 3A from mice, male rats, minipigs, and monkeys is similar to that in humans in terms of testosterone 6β-hydroxylase activity and the inhibition profiles of ketoconazole.[1] Our study also showed that the metabolism of lovastatin by liver microsomes was similar between Bama miniature pigs and humans. First, the main metabolites detected in both pigs and humans were 6′-β-hydroxy-lovastatin, HA, and 6′-exomethylene lovastatin.[13,14] Second, significant differences in enzyme kinetic parameters were not found between pigs and humans. Although the metabolic

Table 1. Kinetic constants of lovastatin consumption in Bama miniature pigs and human liver microsomes

Microsome source	K_m (μM)	V_{max} (nmol/min/mg)	CL_{int} (ml/min/mg)
Bama minipigs	66.332 ± 2.646	6.465 ± 0.131	0.098 ± 0.006
Humans	87.642 ± 2.682	8.821 ± 1.564	0.101 ± 0.019

K_m and V_{max} values were determined by incubation of lovastatin (approximately 200μM), and the values were calculated from Lineweaver–Burk plots.

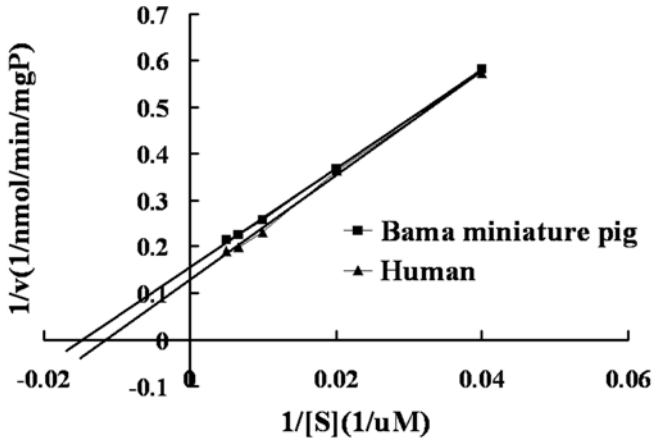

Figure 3. Lineweaver–Burk plots of lovastatin consumption in liver microsomes from Bama miniature pigs and humans. Kinetic parameters (K_m and V_{max}) were evaluated from substrate consumption. Lovastatin concentrations ranging from 0 to 200 μM were used to determined the kinetic parameters.

enzymes from pigs had greater affinity (lower K_m) for lovastatin than those from humans, the metabolic rate of lovastatin in pigs was highly similar to that in human microsomes. Third, TAO, a specific probe for human CYP3A4, had a pronounced inhibitory effect on the metabolism of lovastatin in Bama miniature pigs. In a previous study, ketoconazole, another highly potent selective inhibitor probe of human CYP3A4, also significantly inhibited nifedipine oxidation and testosterone 6β-hydroxylation activities in the same breed of pigs.[16] The similar selectivities of inhibitors of these activities suggest that liver microsomes from Bama miniature pigs exhibited activity characteristic of human CYP3A4. The CYP3A29 enzyme of minipigs, which showed 60% identity with human CYP3A4, seems to be an important form of CYP3A.[25,36] However, the CYP3A enzymes from Bama miniature pigs need further characterization to study interspecies differences and variations with humans.

The in vivo pharmacokinetics of lovastatin were also similar in Bama miniature pigs and humans. The maximum concentration achieved in pigs occurred at 2.12 ± 0.35 h, which is similar to that in humans (2 to 4 h).[6] In addition, significant interspecies differences in the absorption and elimination of lovastatin were not noted (pig: K_a = 0.97 ± 0.14 1/h, K_e = 0.19 ± 0.02 1/h; human: K_a = 0.82 ± 0.55 1/h, K_e =0.21 ± 0.05 1/h).[35] Because of poor absorption, minipigs had low bioavailability (F = 4.97± 0.68%), as also reported for humans (F < 5%).[4] Compared with those in mice, rats, and dogs, the percentage of plasma protein binding and the lovastatin

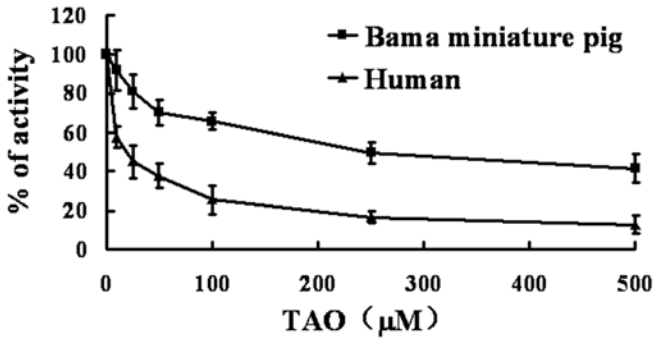

Figure 4. Inhibitory effect of TAO on the metabolism of lovastatin in liver microsomes from Bama miniature pigs and humans. The inhibitor was studied at concentrations ranging from 0 to 500 μM. The liver microsomes and TAO were preincubated for 20 min at 37 °C, after which lovastatin was added and the reaction was performed for 10 min. Each point represents the mean of triplicate experiments.

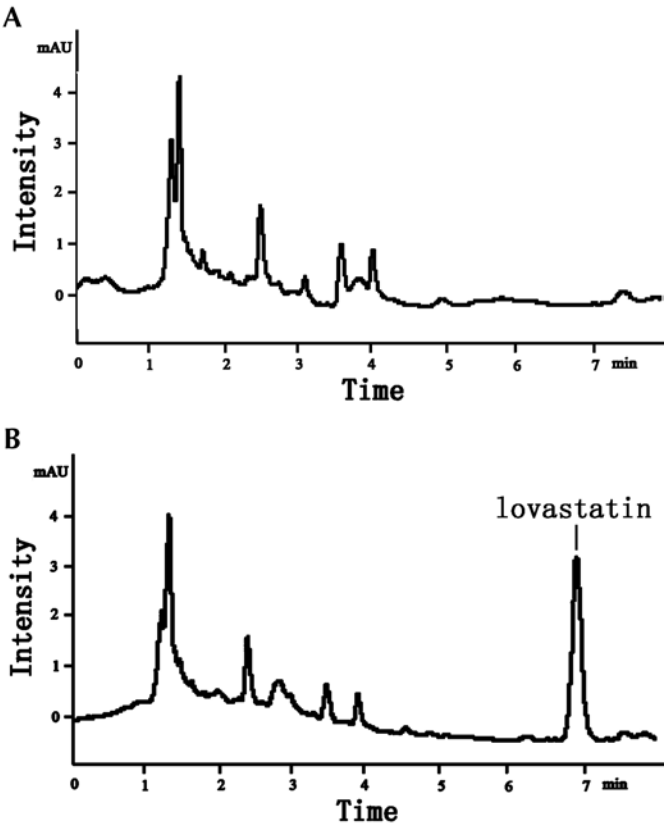

Figure 5. Representative chromatograms of lovastatin in Bama miniature pig plasma. (A) Plasma blank (no-lovastatin control). (B) Plasma sample after administration of lovastatin.

hydrolysis rate in Bama miniature pigs were more representative of those in humans.[9,19]

In the tissue residue study, lovastatin was distributed rapidly to different tissues. No noticeable differences were detected in tissue distribution after a single dose of lovastatin or doses administered over 15 d. This findings indicate that no accumulation occurred at the whole-body level as described previously.[9] High concentrations of lovastatin were detected in the stomach and intestines of

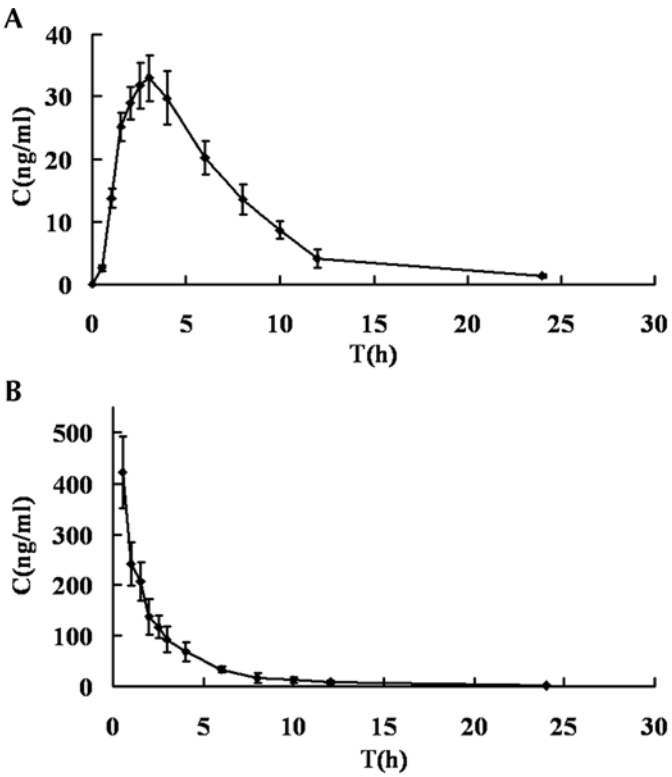

Figure 6. Profiles of average lovastatin concentration–time curves in Bama miniature pig plasma after (A) a single oral dose of 6 mg/kg and (B) an intravenous dose of 1.2 mg/kg (n = 4).

Table 2. Pharmacokinetic parameters of lovastatin in Bama miniature pigs (n = 4) after a single oral dose of 6 mg/kg or an intravenous dose of 1.2 mg/kg

Parameter	Oral	Intravenous
Kα (1/h)	0.97 ± 0.14	not applicable
K_{el} (1/h)	0.19 ± 0.02	0.31 ± 0.07
Lag time (h)	0.44 ± 0.01	not applicable
$t_{1/2}K_a$ (h)	0.72 ± 0.28	not applicable
$t_{1/2}K_e$ (h)	3.71 ± 0.20	2.24 ± 0.85
T_{peak} (h)	2.12 ± 0.35	not applicable
C_{max} (ng/ml)	27.83 ± 8.59	not applicable
AUC (ng/ml) × h	220.26 ± 56.60	886.48 ± 315.26

AUC, total area under the plasma concentration-versus-time curve ; C_{max}, maximal plasma concentration; Kα, absorption rate constant; K_{el}, elimination rate constant; $t_{1/2}K_a$, absorption half-life; $t_{1/2}K_e$, elimination half-life; T_{peak}, time to reach maximal plasma concentration
Pharmacokinetic parameters were evaluated with the 3p97 pharmacokinetics program.

Bama minipigs at1 h after oral administration, suggesting that the majority of lovastatin was metabolized or excreted by the gastrointestinal tract. Lovastatin exhibited high selectivity for the liver and achieved substantially higher concentrations in hepatic than nontarget tissues. This finding demonstrates that the liver is the primary site of action in pigs as well as humans, in which lovastatin underwent extensive first-pass extraction in hepatic tissue.[19] Therefore, the availability of lovastatin to the general circulation appears to be low, although its concentration in some tissues rose

Table 3. Lovastatin concentration in different tissues of Bama miniature pigs (n = 4) after oral administration

Tissue	Concentration of lovastatin in tissues (ng/g)		
	At 1 h after single dose	At 4 h after single dose	After administration for 15 d
Liver	4.10 ± 1.34	2.96 ± 0.91	3.06 ± 1.25
Heart	1.86 ± 0.61	1.23 ± 0.27	1.26 ± 0.35
Spleen	0.05 ± 0.01	0.25 ± 0.04	0.26 ± 0.02
Lung	1.45 ± 0.29	1.50 ± 0.17	1.70 ± 0.27
Kidney	0.68 ± 0.14	1.25 ± 0.18	1.34 ± 0.26
Adrenal gland	0.49 ± 0.09	0.48 ± 0.13	0.50 ± 0.05
Pancreas	0.41 ± 0.14	2.78 ± 0.78	2.47 ± 0.58
Stomach body	10.03 ± 1.78	4.39 ± 0.98	3.67 ± 0.75
Stomach cardia	10.63 ± 1.42	6.38 ± 1.25	6.55 ± 1.02
Testis	0.18 ± 0.01	0.70 ± 0.09	1.20 ± 0.39
Small intestine	5.79 ± 2.26	4.26 ± 1.46	5.35 ± 1.28
Large intestine	1.10 ± 0.35	2.90 ± 0.54	2.93 ± 0.75
Prostate	1.95 ± 0.45	2.45 ± 1.25	3.01 ± 1.10
Brain	0.14 ± 0.03	0.28 ± 0.09	0.54 ± 0.17
Bladder	0.40 ± 0.29	0.30 ± 0.35	0.35 ± 0.22
Muscle	0.75 ± 0.04	0.92 ± 0.16	1.01 ± 0.35
Plasma	0.16 ± 0.01	0.41 ± 0.09	0.39 ± 0.17

Samples of the 17 tissues were collected at 1 and 4 h after a single oral dose and at 4 h after multiple oral doses of 45 mg/kg.

gradually after an increase in plasma concentration. The presence of lovastatin in the brain suggested that the drug could cross the blood–brain barrier and enter the central nervous system due to its prodrug liposolubility characteristics.[23]

A high percentage of the total dose of lovastatin was recovered in the feces, indicating that, as in rats, dogs, monkeys, and humans,[22,35] fecal excretion is the main route of elimination in Bama minipigs. The major form of the parent drug was excreted in the bile, but less than 10% of lovastatin was excreted in the urine of minipigs.[9,19]

In summary, the combined results of in vitro metabolic and in vivo pharmacokinetic studies of lovastatin suggest that the liver microsomes of Bama miniature pigs exhibit a similar substrate specificity and activity to CYP3A4 in humans. This profile indicates that similar proteins to CYP3A4 may exist in Bama miniature pig liver microsomes. Thus, at the microsomal level, this breed of pigs seems to be a good model of drug metabolism when human CYP3A4 is involved. The overall pharmacokinetics of lovastatin in minipigs showed that these animals have the potential to be an ideal preclinical pharmacokinetic model of such cardiovascular drug classes. However, to promote the use of Bama miniature pigs for this purpose, more data comparing toxicologic pathologies is necessary. However, the characterization and comparison of the main metabolic enzymes in Bama miniature pigs is an important step in valid extrapolation of drug evaluation results to humans.

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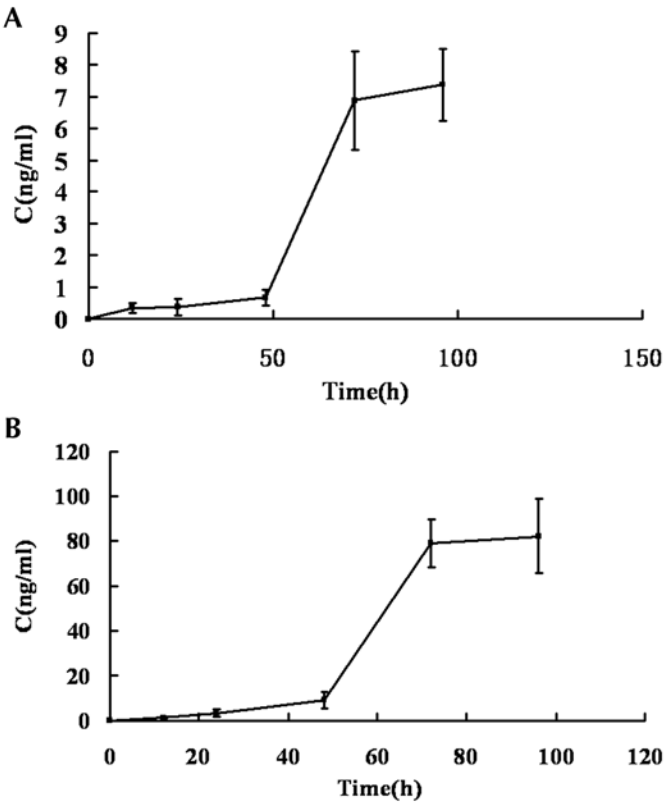

Figure 7. Profiles of average lovastatin concentration–time curves in the (A) urine and (B) feces of Bama miniature pigs after a single oral administration of 2.4 mg/kg (n = 6).

Table 4. Plasma protein binding rate of lovastatin in Bama miniature pigs and humans

	Percentage of protein binding (%)	
Concentration (μg/ml)	Bama miniature pigs	Humans
0.5	96.87 ± 1.25	97.88 ± 1.76
5	95.47 ± 1.10	97.01 ± 1.34
50	95.03 ± 1.57	96.27 ± 2.22
Overall mean	95.79 ± 1.42	97.04 ± 1.72

Plasma protein binding of lovastatin in pigs and humans were determined by equilibrium dialysis.[19] Plasma lovastatin concentrations of 0.5, 5, and 50 μg/ml were dialyzed for 24 h at 37 °C with phosphate buffer (0.02 M; pH 7.4; n = 3).

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References

1. **Bogaards JJP, Bertrand M, Jackson P, Oudshoorn MJ, Weaver RJ, van Bladeren PJ, Walther B.** 2000. Determining the best animal model for human cytochrome P450 activities: a comparison of mouse, rat, rabbit, dog, micropig, monkey, and man. Xenobiotica **30:**1131–1152.
2. **Cai M, Yin W, Li Q, Liao D, Tsutsumi K, Hou H, Liu Y, Zhang C, Li J, Wang Z, Xiao J.** 2006. Effects of NO-1886 on inflammation-associated cytokines in high-fat–high-sucrose–high-cholesterol-diet–fed miniature pigs. Eur J Pharmacol **540:**139–146.
3. **Chen BB, Wang CG, Zhou JH, Wei H, Wang AD, Gan SX.** 2003. [DNA fingerprinting analysis of Guangxi Bama and Guizhou miniature pigs.] Acta Acad Med Mili Tertiae **25:**620–622.
4. **Christians U, Jacobsen W, Floren LC.** 1998. Metabolism and drug interactions of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors in transplant patients—are the statins mechanistically similar? Pharmacol Ther **80:**1–34.
5. **Clement B, Demesmaeker M, Linne S.** 1996. Microsomal catalyzed N-hydroxylation of guanabenz and reduction of the N-hydroxylated metabolite: characterization of the two reactions and genotoxic potential of guanoxabenz. Chem Res Toxicol **9:**682–688.
6. **Corsini A, Bellosta S, Baetta R, Fumagalli R, Paoletti R, Bernini F.** 1999. New insights into the pharmacodynamic and pharmacokinetic properties of statins. Pharmacol Ther **84:**413–428.
7. **Davidson MH, Palmisano J, Wilson H, Liss C, Dicklin MR.** 2003. A multicenter, randomized, double-blind clinical trail comparing the low-density lipoprotein cholesterol-lowering ability of lovastatin 10, 20, and 40 mg/d with fluvastatin 20 and 40 mg/d. Clin Ther **25:**2738–2753.
8. **Downs JR, Clearfield M, Weis S, Whitney E, Shapiro DR, Beere PA, Langendorfer A, Stein EA, Kruyer W, Gotto AM Jr.** 1998. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. J Am Med Assoc **279:**1615–1622.
9. **Duggan DE, Chen IW, Bayne WF, Halpin RA, Duncan CA, Schwartz MS, Stubbs RJ, Vickers S.** 1989. The physiological disposition of lovastatin. Drug Metab Dispos **17:**166–173.
10. **Friedrich J, Zuzek M, Bencina M, Cimerman A, Strancar A, Radez I.** 1995. High-performance liquid chromatographic analysis of mevinolin as mevinolinic acid in fermentation broths. J Chromatogr A **704:**363–367.
11. **Greenspan MD, Yudkovitz JB, Alberts AW, Argenbright LS, Arison BH, Smith JL.** 1988. Metabolism of lovastatin by rat and human liver microsomes in vitro. Drug Metab Dispos **16:**678–682.
12. **Guengerich FP.** 1997. Comparisons of catalytic selectivity of cytochrome P450 subfamily enzymes from diferent species. Chem Biol Interact **106:**161–182.
13. **Halpin RA, Ulm EH, Till AE, Kari PH, Vyas KP, Hunninghake DB, Duggan DE.** 1993. Biotransformation of lovastatin. V. Species differences in in vivo metabolite profiles of mouse, rat, dog, and human. Drug Metab Dispos **21:**1003–1011.
14. **Jacobsen W, Kirchner G, Hallensleben K, Mancinelli L, Deters M, Hackbarth I, Benet LZ, Sewing KF, Christians U.** 1999. Comparison of cytochrome P450-dependent metabolism and drug interactions of the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors lovastatin and pravastatin in the liver. Drug Metab Dispos **27:**173–179.
15. **Koch W, Windt H, Walles M, Borlak J, Clausing P.** 2001. Inhalation studies with the Göttingen minipig. Inhal Toxicol **13:**249–259.
16. **Li J, Liu Y, Zhang JW, Wei H, Yang L.** 2006. Characterization of hepatic drug-metabolizing activities of Bama miniature pigs (*Sus scrofa domestica*): comparison with human enzyme analogs. Comp Med **56:**286–290.
17. **Lowry OH, Rosebrough NJ, Farr AL, Randall RJ.** 1951. Protein measurement with the folin phenol reagent. J Biol Chem **193:**265–275.
18. **Melin JM, Struble WE, Tipping RW, Reynolds JM, Vassil TC, Levy SJ, Petrohoy TM, Midgette P, Hemwall EL, Levine JG, Irvin JD.** 2004. A consumer use study of over-the-counter lovastatin (CUSTOM). Am J Cardiol **94:**1243–1248.
19. **Merck.** [Internet]. Mevacor (lovastatin) tablets: 2007 update [Issued December 2007]. Available at http://www.merck.com/product/usa/pi_circulars/m /mevacor/mevacor_pi.pdf.
20. **Ministry of Science and Technology of China.** [Internet]. The laboratory animal management principles of China. 2005 update [Issued 31 October 1988]. Available at http://www.most.gov.cn/bszn/new/dwjk/wjxz/200512/t20051225_55325.htm
21. **Mortensen JT, Brinck P, Lichtenberg J.** 1998. The minipig in dermal toxicology. A literature review. Scan J Lab Anim Sci **25 suppl. 1:**77–83.

22. **Reilly PEB, Mason SR, Read MA.** 1999. Human, rat, and crocodile liver microsomal monooxygenase activities measured using diazepam and nifedipine: effects of CYP3A inhibitors and relationship to immunochemically detected CYP3A apoprotein. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol **122:**197–204.
23. **Shitara Y, Sugiyama Y.** 2006. Pharmacokinetic and pharmacodynamic alterations of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors: drug–drug interactions and interindividual differences in transporter and metabolic enzyme functions. Pharmacol Ther **112:**71–105.
24. **Smith DA.** 1991. Species differences in metabolism and pharmacokinetics: are we close to an understanding. Drug Metab Rev **23:**355–373.
25. **Soucek P, Zuber R, Anzenbacherová E, Anzenbacher** P**, Guengerich FP.** [Internet]. Minipig cytochrome P450 3A, 2A and 2C enzymes have similar properties to human analogs. 2001 update. [cited 5 December 2001]. Available at http://www.biomedcentral.com/1471-2210/1/11
26. **State Food and Drug Administration**. 2006. Guidance for drug research. p 103–132. Bei Jing: Medicine Technology Press of China.
27. **Sultana M, Srivastava AK.** 2001. Pharmacokinetics, urinary excretion, and plasma protein binding of 2-PAM in calves. Indian J Pharmacol **33:**363–368.
28. **Svendsen O.** 2006. The minipig in toxicology. Exp Toxicol Pathol **57:**335–339.
29. **Vyas KP, Kari PH, Pitzenberger SM, Haplin RA, Ramjit HG, Arison B, Murphy JS, Hoffman WF, Schwartz MS, Ulm EH, Duggan DE.** 1990. Biotransformation of lovastatin. I. Structure elucidation of in vitro and in vivo metabolites in the rat and mouse. Drug Metab Dispos **18:**203–211.
30. **Wang RW, Kari PH, Lu AYH, Thomas PE, Guengerich FP, Vyas KP.** 1991. Biotransformation of lovastatin. IV. identification of cytochrome P450 3A proteins as the major enzymes responsible for the oxidative metabolism of lovastatin in rat and human liver microsomes. Arch Biochem Biophys **290:**355–361.
31. **Wu FC, Wei H, Wang AD.** 2001. [Analysis of genetic diversity in Bama and Guizhou miniature pigs by RAPD.] Acta Biol Exp Sinica **34:**115–119.
32. **Wu FC, Wei H, Wang AD.** 2002. [Study on genetic diversity of Bama miniature pigs by AFLP technique.] Chinese J Lab Anim Sci **12:**207–209.
33. **Xie HT, Huang XH, Sun ZY.** 2001. Introduction of domestic and foreign common pharmacokinetics software. Chin J Clin Pharmacol Ther **6:**289–292.
34. **Yu J, Wang Z, Wang B, Li HQ, Yi B, Yin WD.** 2007. [Study of lectin-like oxidized low-density lipoprotein receptor-1 expression in atherosclerotic of Guangxi bama minture swine.] Chin J Cardiovasc Rev **10:**764–766.
35. **Zhao F, Yuan Y, Shen XG.** 1997. [Determination of lovastatin in human plasma by RP-HPLC and its pharmacokinetics study.] J China Pharm Univ **28:**288–290.
36. **Zuber R, Anzenbacherova E, Anzenbacher P.** 2002. Cytochromes P450 and experimental models of drug metabolism. J Cell Mol Med **6:**189–198.