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Extensive Intestinal First-Pass Elimination and Predominant Hepatic Distribution of Berberine Explain Its Low Plasma Levels in Rats

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Running short title: First-pass elimination and tissue distribution of berberine

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Abbreviations: IG, intragastric; ID, intraduodenal; IPV, intraportal; IV, intravenous.

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ABSTRACT

Berberine, one of the most commonly used natural products, exhibits poor plasma concentration-effect relationship whose underlying mechanisms remain largely unclear. This study was designed to test the hypothesis that extensive first-pass elimination and abundant tissue distribution of berberine may be its specific pharmacokinetic properties. For that, four different dosing routes – intragastric (IG), intraduodenal (ID), intraportal (IPV) and intravenous (IV) – were utilized to investigate the gastric, intestinal, and hepatic first-pass elimination of berberine, respectively. After IG dosing, approximately half of berberine run intact through the gastrointestinal tract and another half was disposed of by the small intestine, leading to an extremely low extent of absolute oral bioavailability in rats (0.36%). Moreover, the major berberine metabolites were identified and quantified in rat enterocyte S9 fractions, portal vein plasma, and intestinal perfusates; plasma concentrations and tissue distribution of berberine and its major metabolites were determined as well. Data indicated that M1, M2-glucuronide, and M3 were the major metabolites generated from the small intestine, and that there was a 70-fold increase in the ratio of the AUC_{0-t} value of berberine (liver vs. plasma). We conclude that intestinal first-pass elimination of berberine is the major barrier of its oral bioavailability, and that its high extraction and distribution in the liver could be another important cause that leads to its low plasma levels in rats.

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Introduction

Accumulating evidence has well documented that some (if not all) herbal products are characterized by very low extent of absolute oral bioavailability and exposure, but that they can exert their pharmacological effects well (Hao et al., 2007), suggesting that the distribution of their active ingredients in certain tissues or organs, particularly inside the active sites, may be contributory. Berberine, one of the frequently used herbal medications in Eastern Asians, has been found to have various pharmacological activities for treating certain diseases, such as antibacterial (Amin et al., 1969), anti-tumor (Ho et al., 2009), anti-oxidation (Hwang et al., 2002), cholesterol-lowering (Kong et al., 2004), anti-hyperglycemia (Zhang et al.), and anti-inflammatory effects (Kuo et al., 2004). Similar to some other herbal products, berberine exhibits extremely low but variable plasma concentrations after oral administration in humans (Li et al., 2000; Hua et al., 2007), insufficiently achieving its effective concentrations required in the in vitro assays (Kong et al., 2004). For example, maximum concentration (C_{max}) of berberine in human plasma was measured at 0.4 ng/ml after a single oral dose of 400 mg (Hua et al., 2007). Furthermore, our most recent study also demonstrated that C_{max} of berberine was estimated at 4 ng/ml after oral administration of 100 mg/kg in rats (Liu et al., 2009). Such low exposure alone cannot explain its effective concentrations required for the in vitro assays. For example, the concentrations of berberine that can induce expression of LDLR (low density lipoprotein receptor) mRNA were ≥ 2.5 ug/ml in HepG2 cells (Kong et al., 2004). Clearly, low plasma berberine concentrations in the body cannot be used to better explain its clinical efficacy being measured in patient care.

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Efforts have been made to explore why berberine exhibits low bioavailability in the body after oral intake (Zuo et al., 2006; Qiu et al., 2008). For example, the metabolism of berberine was found to be catalyzed by several cytochrome P450s and UGTs (UDP-glucuronosyltransferase) in rat liver microsomes, whose major metabolic pathways were oxidative demethylenation and subsequent glucuronidation after iv administration (Liu et al., 2009). However, less is known about how the small intestine and liver make their relative contributions to extensive first-pass elimination of berberine in the body. To do that, this study was designed to dissect the entire first-pass elimination of berberine and tissue distribution of both berberine and its major metabolites (which is chemically synthesized in advance) in rats after dosing via four different routes – intragastric (IG), intraduodenal (ID), intraportal (IPV) and intravenous (IV) – and to further investigate the intestinal metabolism of berberine by identification and quantification of its major metabolites being generated in rat enterocyte S9 fractions, intestinal perfusates (using a single-pass intestinal perfusion model), and portal vein blood after ID dosing of berberine, respectively.

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Materials and Methods

Chemicals and Reagents

Berberine, tetrahydroberineper and jatrorrhizine (purity > 99% each) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Glucose-6-phosphate (G6P, 98-100%), glucose-6-phosphate dehydrogenase (G6PD, ≥150 units/mg protein), β-nicotinamide adenine dinucleotide phosphate (NADP, approximately 98%), uridine 5'-diphosphoglucuronic acid (UDPGA, 98-100%), D-saccharic acid 1, 4-lactone (D-saccharolactone, ≥ 98%), alamethicin (≥ 90%), and β-glucuronidase (1240 units/mg solid) were purchased from Sigma Chemical (St. Louis, Mo, USA). HPLC grade acetonitrile was obtained from Fisher Scientific (Toronto, Canada). Deionized water was purified using a Milli-Q system (Millipore, Milford, MA, USA). All other reagents were of the highest grades available from standard commercial sources.

Synthesis of Two Major Phase I Metabolites – berberrubine (M1) and demethyleneberberine (M2)

To prepare berberrubine (M1), berberine chloride (0.7 g) was heated at 190°C in a dry oven under vacuum (20-30 mmHg) for 20 min. The crude product was eluted with $CHCl_3$/MeOH (10/1, v/v) through a silica gel column to separate M1 (0.35g, yield: 52.2%; m.p. 255–259°C).

For the preparation of demethyleneberberine (M2), berberine chloride (1.0g, 2.7mmol) and phenol (0.76g, 8.1mmol) were mixed with 60% H_2SO_4 (40 ml) and stirred at 100°C in an oil bath for 8 h. The mixture was cooled, filtered and subject to anion exchange into chloride form in H_2O-acetone (1:1). The crude product was concentrated to prepare M2 (0.31g, yield:

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31.3%). After synthesis of M1 and M2, LC/MS and ^{1}H NMR were used to confirm their structural features. M1 was the demethylation product of berberine at the C-9 position.

Animals

Sprague-Dawley rats (180 – 250g, 6-7 weeks) were obtained from the Experimental Animal Center, China Pharmaceutical University, and housed with free access to food and water. The animals were maintained on a 12 h light/dark cycle (light on from 8:00 to 20:00) at ambient temperature (22 – 24°C) with 60% relative humidity. Rats were fasted for 12 h prior to all experimental studies. The study protocol was approved by the Animal Care and Use Committee, College of Pharmacy, China Pharmaceutical University.

Instruments

The LC-MS/MS analysis was performed using a Finnigan Surveyor™ HPLC and TSQ Quantum Discovery max system (Thermo Electron, San Jose, CA, USA). The separation and determination were performed using Shim-pack VP-ODS analytical column (150 mm × 2.0 mm id, Shimadzu, Kyoto, Japan). Samples were eluted through the column with a gradient of water-formic acid 100:0.05 (v/v) and acetonitrile (0 min, 85:15; 5min, 25:75; 6min, 85:15; 10min, 85:15) at a flow rate of 0.2 ml/min at 40°C in the column oven. Berberine, tetrahydroberineper, M1 (berberrubine), M2 (demethyleneberberine), and M3 jatrorrhizine standards were used to validate the LC-MS/MS methods.

Relative Contribution of the Small Intestine and Liver to the First-Pass Elimination of Berberine

To differentiate the hepatic and gastrointestinal first-pass elimination of berberine, four different dosing routes – IG, ID, IPV and IV – were adopted (Kim and Lee, 2002). Briefly, the

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carotid artery, jugular vein, and portal vein of each rat were cannulated under urethane anesthesia. Berberine was dosed after 24 hour of recovery. Rats were infused with berberine, intravenously or intraportally, for 15 min at a dose of 4mg/kg, whereas the dose was set at 100 mg/kg for the intragastric or intraduodenal administration. Blood (about 0.15 mL) was collected from the carotid artery into the heparinized tubes at predose and at 0.083, 0.25 (the time for infusion), 0.5, 1, 2, 4, 6, 8, 12, 24 and 36 h postdose. The rats were euthanized by cervical dislocation at the end of the experiment and total gastrointestinal tract and its contents were collected to determine berberine. The quantification of plasma berberine concentrations was conducted using a LC/MS/MS system with tetrahydroberineper used as an internal standard.

Preparation of Enterocyte Fractions S9

The small intestines from five male rats were rinsed with 10mmol phosphate buffer, pH 7.4, containing 0.5% NaCl (phosphate-buffered saline), and then cut longitudinally; the mucous membrane was scraped gently with a slide. The mucosal scrapings were then mixed with 5 volumes (w/v) of 10 mmol phosphate buffer containing 5 mmol EDTA, pH 7.4, and homogenized to prepare 20% homogenates. The homogenates were subjected to centrifugation to isolate cellular fractions S9 as described elsewhere (van de Kerkhof et al., 2007). The protein concentrations of the cellular fractions S9 were determined using a commercially available kit (BCA protein assay; Pierce Chemical Co., Rockford, IL, USA) and following the manufacturer's instructions. The cellular fractions S9 were stored at - 80°C before use.

In Vitro Incubation

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The phase I reactions were conducted in a medium containing 100 mmol potassium phosphate buffer (pH 7.4), 10 mmol $MgCl_2$, 0.5 mmol NADP, 10 mmol G6P, 1Unit/ml G6PD, 25 µmol berberine (based on the Km values determined in rat liver microsomes) (Liu et al., 2009) and 2 mg/mL protein of intestinal S9 in a total volume of 200 µL. After preincubation for 5 min, the reaction was initiated by addition of the NADPH generating system. After incubation at 37°C for 120 min (long incubation time was designed to generate sufficient metabolites), the reaction was terminated after adding two volumes of cold acetonitrile. Blank incubations were performed without NADP or with boiled microsomes.

In order to determine the phase II glucuronide-conjugated metabolites, the intestinal S9 preparations were pretreated with alamethicin (a helical pore-forming peptide) at 25 µg of alamethicin/mg of subcellular protein on ice for 15 min (Hao et al., 2007). The reaction mixture consisted of 25 µmol berberine, 2 mg of subcellular protein, 5 mmol UDPGA, 10 mmol $MgCl_2$, 1 mmol saccharic acid-1,4-lactone, NADPH generating system (containing 0.5 mmol NADP, 10 mmol G6P, 1Unit/ml G6PD, and 100 mmol potassium phosphate buffer, pH 7.4) in a final volume of 200 µl. After preincubation for 5 min at 37°C, the reaction was started by adding UDPGA. Blank incubations were carried out without UDPGA or NADPH-generating system or with boiled subcellular proteins. All reactions were incubated at 37 °C for 120 min and terminated by adding two volumes of cold acetonitrile.

Identification of the Major Metabolites in Portal Vein Plasma

Rats were anesthetized with urethane and a cannula was inserted into the portal vein to collect blood samples. An aliquot of blood was collected from the portal vein 24 hour after surgery. Then berberine (100 mg/kg) was instilled into the duodenum, and portal blood

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was collected at 5, 15 and 30min after dosing to identify the intestinal metabolites of berberine. The blood was centrifuged immediately at 14,*000 g* for 5 min at room temperature to obtain plasma. Plasma samples were extracted with five volumes of n-butanol.

Single-Pass Intestinal Perfusion (SPIP)

SPIP studies were performed using an established method as described elsewhere(Cook and Shenoy, 2003). Briefly, Sprague-Dawley rats (male, 180-220 g) were anesthetized using an intraperitoneal injection of ethyl urethane and placed on a heated pad to keep body temperature normal. The small intestine was surgically exposed and 20cm of duodenum and jejunum were ligated for perfusion and cannulated with plastic tubing (0.04 in. i.d., 0.085 in. o.d.). The cannulated segment was rinsed with saline (37°C) and attached to a syringe pump. Bilary duct was ligated to prevent enterohepatic circulation. Blank perfusion buffer was infused for 20min followed by the perfusion of berberine (20 ug/ml) at a flow rate of 0.2 ml/min for 120min. Phenol Red (20 ug/ml) was added as a non-absorbable marker for water flux. The perfusate was collected every 20min through microtubes. The length and perimeter of segment were measured following the last collection and finally the animal was sacrificed with a cardiac injection of saturated solution of KCl. Samples were frozen immediately and stored at -20°C until analysis. After thawing at 37°C,β-glucuronidase (100 units) was mixed with a 100 µL of the perfusate and incubated for 16 h at 37°C (Zuo et al., 2006). Samples pretreated with or without β-glucuronidase were extracted by two volumes of acetonitrile for later LC/MS/MS analysis.

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Pharmacokinetic Study of Berberine and its Major Metabolites after Oral and Intravenous Administrations

Rats were given with berberine of either 100 mg/kg via IG or 4 mg/kg via IV. Blood was collected pre-dose and at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 36, and 48 h post-dose from five rats and kept in the heparinized Eppendorf tubes. Plasma (100 µL) was separated by centrifugation at 5000 *g* for 10 min and stored at -20°C before analysis. β-Glucuronidase (50 units) was mixed with a half volume of thawed plasma (50 µL) and incubated for 16 h at 37°C (Zuo et al., 2006). Plasma samples pretreated with or without β-glucuronidase were extracted by five volumes of n-butanol.

Tissue Distribution of Berberine and Its Major Metabolites after Oral Administration

After rats were given with berberine of a single oral dose of 100 mg/kg, their heart, liver, spleen, lung, kidney and brain were collected at 0.5, 2, 8, and 24 h post dose, respectively. There were five rats for each time point. These tissue samples were homogenized to make 20 % homogenates and stored at -20°C before analysis. β-glucuronidase (100 units) was mixed with a 100µL of the homogenates and incubated for 16 h at 37°C (Zuo et al., 2006). Tissue samples pretreated with or without β-glucuronidase were extracted by five volumes of n-butanol.

Data Handling and Biostatistical Analysis

The area under the drug concentration-time curve (AUC_{0-t}) was estimated by trapezoidal rule (non-compartmental analysis).

Assuming that berberine is chemically stable in the gastrointestinal tracts and metabolized in the small intestine and liver in rats, and that PK profiling of berberine is linear

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in the range of berberine doses used, total absolute oral bioavailability (F_t), hepatic bioavailability (F_h), and gastrointestinal bioavailability (F_g) were calculated using the following equations:

$$F_t = F_a \times F_h \times F_g \quad \text{(eq. 1)}$$

$$F_t = AUC_{ig}/AUC_{iv} \times D_{iv}/D_{ig} \quad \text{(eq. 2)}$$

$$F_h = AUC_{ipv}/AUC_{iv} \times D_{iv}/D_{ipv} \quad \text{(eq. 3)}$$

$$F_g = 1/F_a \times F_t/F_h = 1/F_a \times AUC_{id}/AUC_{ipv} \times D_{ipv}/D_{id} \quad \text{(eq. 4)}$$

where D denotes the dose of berberine, and F_a is the fraction of absorption, which equals total dosage minus the sum of both fraction not being absorbed and fraction excreted by the efflux transporters into the intestinal lumen and bile after berberine was absorbed.

The effective permeability coefficient of berberine (P_{eff}) was calculated using the following equation (Zakeri-Milani et al., 2007) according to the parallel model:

$$P_{eff} = - Q \ln [C_{out} / C_{in}] / 2\pi rl \quad \text{(eq. 5)}$$

where C_{in} denotes the inlet concentration, and C_{out} is the outlet concentration of berberine, both of which are normalized by volume change in segment using phenol red concentration in inlet and outlet tubing. Q denotes the flow rate (0.2 ml/min), r is the intestinal radius of each rat, and l is the length of the segment. In addition, the following equation was used to make a correction.

$$Conc_{corrected} = Conc_{measured} \times [\text{phenol red}]_{in} / [\text{phenol red}]_{out} \quad \text{(eq. 6)}$$

To estimate the velocity of the major phase I metabolites of berberine being present in the perfusate, the equation used is as follows:

$$Velocity = C_{metabolite} \times Volume_{perfusate} / t / 2\pi rl \quad \text{(eq. 7)}$$

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where $C_{metabolite}$ denotes the concentration of phase I metabolite in the perfusate, t is the time of perfusion (20 min each point), r is the intestinal radius of each rat, and l is the length of the segment.

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Results

Hepatic First-Pass Elimination of Berberine

As shown in **Table 1**, after 15min infusion of berberine at 4 mg/kg intraportally or intravenously, intraportal administration (ruling out the role played by the small intestine in the overall first-pass elimination of berberine) led to a significant reduction in AUC_{0-t} of berberine in plasma compared to IV dosing (466.0 ± 109.9 versus 649.2 ± 99.5 ng·h/ml; n = 5, $P < 0.05$). According to the equation 3, the F_h was estimated at 71.8 % of the fraction of berberine that entered the portal vein. In other words, first-pass elimination of berberine in the liver was estimated at 28.2% of the fraction of berberine that entered the portal vein.

Gastrointestinal (GI) First-Pass Elimination of Berberine

The fraction of berberine not being absorbed during the period of 36 h after IG administration was at least 56% of the total dose given, in which 32% was recovered from rat stools and 24% was from the entire GI tract. Thus, the F_a value was estimated at 44%.

According to the equations 2 and 4, the F_t and F_g values were estimated at 0.37% and 1.16%, respectively. Moreover, the GI bioavailability (F_{GI}) after drug intake by IG or ID was estimated by the following equations, respectively.

$$F_{GI} = AUC_{ig} \times D_{ipv} / AUC_{ipv} \times D_{ig} \quad \text{(eq. 8)}$$

$$F_{GI} = AUC_{id} \times D_{ipv} / AUC_{ipv} \times D_{id} \quad \text{(eq. 9)}$$

According to the equations 8 and 9, the F_{GI} was 0.50% and 0.51%, respectively. If hepatic first-pass effect was ignored, the first-pass elimination in the GI was 99.5%, in which gastric first-pass effect was 0.01% and intestinal first-pass effect was 99.49%. When taking the fraction of berberine not being absorbed (56%) into account, gastric and intestinal

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first-pass effects were adjusted as 0.004% and 43.8%, respectively. Therefore, the fraction of berberine that entered the portal vein was estimated at 0.2% of the total oral dose, and hepatic first-pass effect was 0.056% after oral intake of berberine in rats.

As shown in Table 1, the AUC_{0-t} values of berberine after IG administration were not significantly different from that after ID dosing, indicating that the gastric first-pass elimination was almost negligible (if any) in rats. However, the AUC_{0-t} values of berberine after ID dosing were significantly less than that after IPV administration (that is, mean AUC_{id} was nearly 0.5% of that of the AUC_{ipv} when normalized by the dose given), suggesting that first-pass elimination of berberine occurs predominantly in rat small intestine but not in the liver and stomach.

Identification of Major Metabolites of Berberine *in vivo* and *in vitro*

The major metabolites of berberine were identified in several important matrices, including systemic plasma, portal plasma, enterocyte fractions S9, and intestinal perfusates. LC/MS/MS spectrum analysis together with the synthetic standards of berberine metabolites indicated that berberine phase I metabolites were M1 via demethylation, M2 via demthylenation and M3 (jatrorrhizine), whereas its phase II metabolites were their corresponding glucuronide conjugates of M1, M2 and M3, respectively (Figure 1). These results were consistent with previous findings as described elsewhere (Tsai and Tsai, 2004; Zuo et al., 2006). All six metabolites appeared in systemic plasma and portal vein plasma after IG and ID dosing. M1, M2, M3, M1-glucuronide and M2-glucuronide were identified in enterocyte fractions S9 and intestinal perfusates.

Single-Pass Intestinal Perfusion (SPIP)

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An effective permeability coefficient (P_{eff}) was estimated based on the berberine steady- state concentrations (occurring at about 60min post perfusion, as measured by desired phenol red concentrations at steady state) in collected perfusates. Consequently, P_{eff} of berberine was estimated at 1.78 ± 0.90 cm/s (10^{-5}), according to the equation 5.

For the major metabolites of berberine being identified in the perfusates, their formation velocity was calculated and summarized in Table 2. M1, M2-glucuronide, and M3 are the major metabolites generated from the small intestine.

The Metabolism of Berberine and the Formation of Its Major Metabolites Vary by Its Dosing Route

The AUC_{0-t} values of berberine and its phase I metabolites (M1, M2 and M3) after IG and IV dosing were summarized in Table 3. Oxidative demethylation (M1) and its subsequent glucuronidation were predominantly metabolic pathways after oral intake. In contrast with our more recent study(Liu et al., 2009), in which oxidative demethylenation (M2) of berberine and subsequent glucuronidation of M2 were major metabolic pathways after IV dosing in rats. After digestion with β-glucuronidase, the AUC_{0-t} ratio of M1 to berberine was 6.8-fold, while the AUC_{0-t} ratio of M2 to berberine was 2.7-fold after IG dosing. By contrast, the AUC_{0-t} ratio of M1 to berberine was 37 %, while the AUC_{0-t} ratio of M2 to berberine was 1.6-fold after IV dosing. These data suggest that intestinal first-pass elimination of berberine is the major barrier of its absolute oral bioavailability, and that its metabolic pathway is dependent on its dosing route.

Tissue Distribution of Berberine and Its Major Metabolites after Oral Intake

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The AUC_{0-t} values of berberine and its phase I metabolites (M1, M2, M3) in different tissues as well as their ratios (tissue/ plasma) after oral intake were summarized in Table 4. In addition, the concentrations of berberine and its phase I metabolites at different time points in the liver were also summarized in Figure 2. After IG dosing, berberine was widely distributed into various tissues, including liver, heart, kidney, spleen, lung, and even brain, with the liver being the most predominant organ, where the mean level of berberine was about 70-fold greater than that in plasma (Table 4). As for the major metabolites of berberine, the un-conjugated metabolites were the major forms present in tissues, including liver, heart, and kidney; however, glucuronides of phase I metabolites of berberine were the major forms in plasma after oral intake. The liver is key organ for the distribution of berberine and its un-conjugated metabolites (Figure 2), where there was a ranking order at their amount: M2 > berberine > M1 >> M3, consistent with our most recent studies on the metabolism of berberine in the liver(Liu et al., 2009).

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Discussions

In this study, we dissected the role the GI tract and liver could play in the overall first-pass elimination of berberine in rats, and found that the first-pass elimination of berberine occurs predominantly in the small intestine rather than in the liver and stomach, and that the liver dominates over the other organs in its tissues distribution, and that oxidative demethylation of berberine (generating M1) and subsequent glucuronidation are the major mechanisms responsible for the intestinal metabolism of berberine after oral intake. Using four different dosing routes (IG, ID, IPV, and IV) to estimate various bioavailability of berberine, we observed a very low F_t (0.36%) and F_{GI} value (0.5% each by IG or ID), confirming a very low exposure of berberine after oral intake in rats and further providing some underlying mechanisms to explain this observation.

In theory, an extremely low plasma concentrations of berberine is associated mainly with the following PK causes: 1) extensive metabolism in the gut and/or liver (Tsai and Tsai, 2004; Zuo et al., 2006; Hao et al., 2007; Qiu et al., 2008); 2) marked excretion to intestinal lumen, bile (Tsai and Tsai, 2004) and urine (Qiu et al., 2008) (as a substrate of certain efflux transporters) (Xie, 2010), as well as enterohepatic circulation (Xie, 2010 ; Zuo et al., 2006); 3) poor absorption (due to some unique physicochemical properties); and 4) predominant tissue distribution.

Previous studies in vivo (Tsai and Tsai, 2004; Zuo et al., 2006; Qiu et al., 2008), including our more recent study in vitro (Liu et al., 2009), have well demonstrated that berberine is extensively metabolized in the body to generate M1 and M1-glucuronide via oxidative demethylation and subsequent glucuronidation, and to form M2 and

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M2-glucuronide via oxidative demethylenation and subsequent glucuronidation. Therefore, UGTs have been identified as the major drug-metabolizing enzymes responsible for the formation of the phase II metabolites of berberine as shown by our more recent study in vitro (Liu et al., 2009) and this study.

Marked efflux transporter-mediated excretion of berberine to intestinal lumen, bile and urine may be a second PK cause leading to such a very low absorption and exposure of berberine after oral intake. Previous observations have shown that, in addition to being a substrate of certain influx transporters (OCT1 and OCT2) (Nies et al., 2008), berberine is a substrate of P-glycoprotein (P-gp) (Pan et al., 2002; Nies et al., 2008). As anticipated, when co-administered with a P-gp inhibitor (cyclosporine or verapamil), there was a marked increase in the absorption of berberine in Caco2 cells (Pan et al., 2002), indicating that intestinal efflux transporters may be involved in the excretion of berberine into intestinal lumen, leading to poor absorption and thus low bioavailability. Furthermore, concurrent intake with quinine (an inhibitor of P-gp and OCT) resulted in a significant reduction in biliary excretion of unbound berberine (Tsai and Tsai, 2004), suggesting that the efflux transporters expressed in the liver may play an important role in bilary excretion of berberine. However, intravenous co-administration with cyclosporine – an inhibitor of P-gp (Cummins et al., 2004), OATP (Shitara et al., 2009), BCRP (Xia et al., 2007) and CYP3A (Cummins et al., 2004) – led to a dramatic decrease in unbound concentrations of berberine in the liver and bile but not in blood (Tsai and Tsai, 2004). Therefore, the role drug transporters may play in the entire first-pass elimination of berberine would be more complex than we assumed, and a

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knock-out mouse model of each transporter (in particular P-gp) will be required to clarify this issue if no highly specific chemical inhibitor of each of drug transporters is available.

Tissue distribution of berberine, in particular in certain target organs and the active sites in the body, can also be used as evidence to explain why a very low exposure of an oral drug is associated with certain efficacy of berberine to achieve, because pharmacological effects are exerted by unbound fraction of the drug that is absorbed into blood and then distributed into target tissues to exert its efficacy. In this study, a relatively dominant tissue distribution of berberine was observed in the liver as shown in Table 4 and Figure 2, which can be used to well explain lipid-lowering effects of berberine by inducing expression of LDLR mRNA in the liver, which required higher berberine concentrations (≥ 2.5 ug/ml) (Kong et al., 2004).

In summary, we found in this study that extensive elimination in the small intestine and the high hepatic extraction are the major PK causes that result in a very low plasma concentrations of berberine in rats. In addition, this study would also be helpful for guiding the future research on how to explore the mechanism by which low plasma concentrations of other natural compounds occur.

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Footnote

Y.T. L., H.P. H, and H.G. X contributed equally to this work.

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Legend for Figures

Figure 1. Structures of berberine and its major metabolites.

Figure 2. The time course of the concentrations (ng/ml) of berberine and its phase I metabolites (M1, M2, M3) in the liver after oral intake (n = 5 each).

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Table 1. The AUC_{0-t} values (mean ± SD) of berberine after four different dosing routes (n = 5 each)

Route	Dose (mg/kg)	AUC_{0-t} (ng·h/ml)
IG	100	57.9 ± 20.6
ID	100	59.3 ± 17.0
IPV	4	466.0 ± 109.9*
IV	4	649.2 ± 99.5

* $p < 0.05$ (IPV vs. IV).

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Table 2. The estimated velocity (ng/min/cm^2, mean ± SD) of the major metabolites of berberine being formed in the purfusates (n= 5 each).

	M1	M2	M3
Enzyme-digested	0.181± 0.057	0.148 ± 0.09*	0.42± 0.11
undigested	0.163± 0.042	0.022 ± 0.012	0.44 ± 0.122

* $p < 0.05$ vs. undigested.

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Table 3. The AUC_{0-t} values (mean± SD, ng•h/ml) of berberine and phase I metabolites after oral or intravenous administration (n = 5 each)

	Berberine	M1	M2	M3
IG				
Enzyme-digested	65.1±3.2*	441.5±109.3**	178.6±53.9	13.9±2.4
undigested	73.5±7.3	83.3±29.7	0	0
IV				
Enzyme-digested	631.1±77.6	234.7±35.8	1040.2±145.2**	82.7±15.9**
undigested	656.5±32.2	198.1±53.0	149.0±45.2	21.8±1.8

* $p < 0.05$, ** $p < 0.01$ vs. undigested.

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Table 4. The AUC_{0-t} values (ng•h/ml) of several important tissues and ratios of tissue/plasma of berberine and its phase I metabolites after oral dosage (n = 5 each).

AUC_{0-t}		Plasma	Liver	L/P	Heart	H/P	Kidney	K/P	Lung	L/P	Spleen	S/P	Brain	B/P
M1	undigested	22.2	1108.5	50.0	44.5	2.0	91.9	4.1	-	-	-	-	-	-
	digested	631.1	1036.3	1.6	42.4	0.1	98.7	0.2	-	-	-	-	-	-
M2	undigested	60.4	2001.3	33.1	85.1	1.4	58.5	1.0	-	-	-	-	-	-
	digested	683.1	2479.1	3.6	100.1	0.1	68.6	0.1	-	-	-	-	-	-
M3	undigested	0.6	106.7	171.4	49.5	79.5	17.2	27.7	-	-	-	-	-	-
	digested	80.6	296.4	3.7	81.0	1.0	38.1	0.5	-	-	-	-	-	-
Berberine	undigested	21.6	1645.9	76.3	120.0	5.6	49.6	2.3	31.2	1.4	50.2	2.3	9.9	0.5
	digested	25.1	1687.5	67.1	147.0	5.8	57.8	2.3	35.1	1.4	69.4	2.8	12.5	0.5

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Figure 1

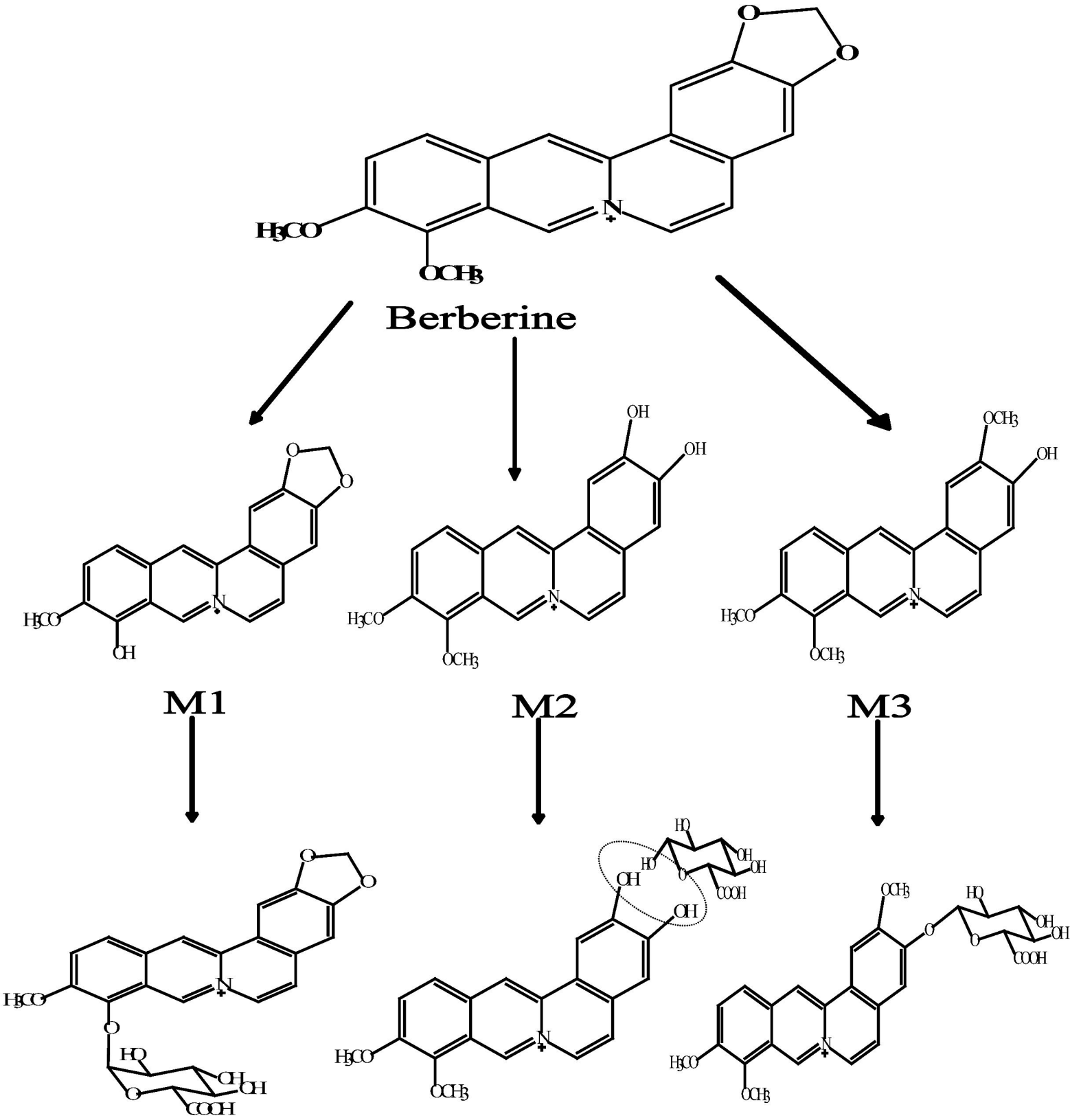
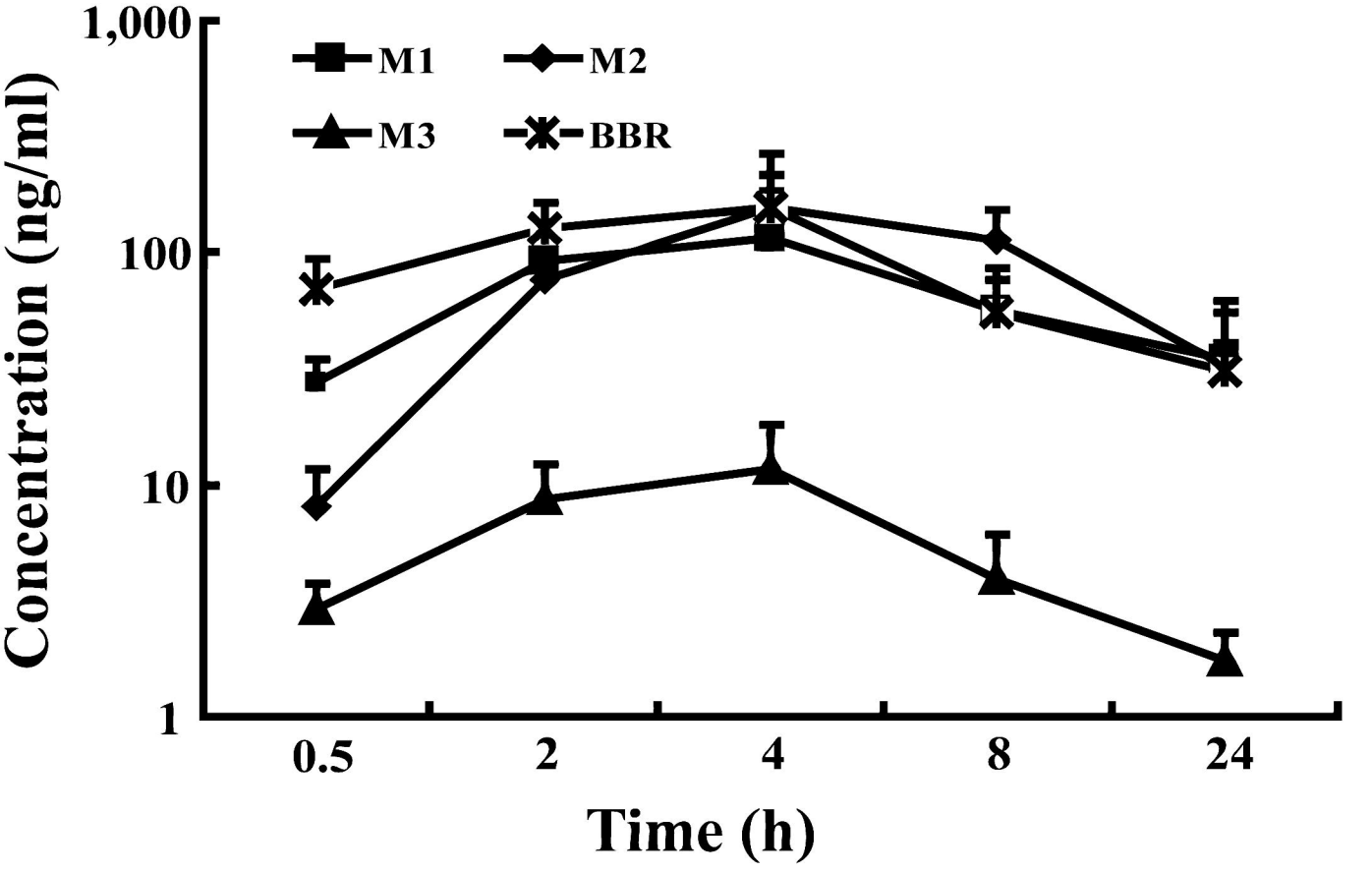
Figure 2