Increased Systemic Exposure to Rhizoma Coptidis Alkaloids in Lipopolysaccharide-Pretreated Rats Attributable to Enhanced Intestinal Absorption

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ABSTRACT:

Rhizoma coptidis is a rhizome commonly used in traditional Chinese medicine. After oral administration of rhizoma coptidis extract, the plasma concentrations of its effective alkaloid constituents are so low that their systemic therapeutic actions cannot be explained. This study aimed to investigate the influence of lipopolysaccharide (LPS) on the pharmacokinetics of the rhizoma coptidis alkaloids. Pharmacokinetic experiments were performed with rats; both in vitro absorption and efflux experiments were carried out with everted rat gut sacs, whereas in vitro metabolism experiments were conducted with rat liver microsomes and intestinal S9 fractions. Mucosal changes were evaluated with light microscopy and transmission electron microscopy. The results showed that, in rat plasma, LPS pretreatment increased systemic alkaloid exposure. LPS pretreatment increased the in vitro absorption of the alkaloids and decreased their efflux. The efflux of vinblastine and rhodamine 123, P-glycoprotein substrates, also was decreased. The absorption of fluorescein isothiocyanate-labeled dextran (average molecular mass, 4 kDa), a gut paracellular permeability probe, was not influenced. Obvious damage was observed in the mucosa, but the tight junctions between epithelial cells remained intact. Intestinal, rather than hepatic, alkaloid metabolism was decreased. These findings indicated that LPS pretreatment increased systemic exposure to the alkaloids through enhancement of their absorption, which was related to decreased intestinal efflux and metabolism. The results add to the understanding of why rhizoma coptidis is active despite the low plasma concentrations of the rhizoma coptidis alkaloids measured in normal subjects and experimental animals.

Introduction

Rhizoma coptidis (Huang Lian) is the dried rhizome of several medicinal plants of the family Ranunculaceae, such as Coptis chinensis Franch. It is commonly used in traditional Chinese medicine because of its various pharmacological effects, including antibacterial, antifungal, antiviral, antidiabetic, anticancer, and anti-inflammatory activities, as well as activity against cardiovascular diseases (Yu et al., 2006). It yields alkaloids such as berberine, coptisine, palmatine, and jatrorrhizine (Chen et al., 2008) (Fig. 1). These alkaloids are the effective constituents in rhizoma coptidis, but berberine is the primary compound.

The plasma concentrations of the alkaloids are very low after oral administration of rhizoma coptidis extract (Yu et al., 2007). Likewise, berberine exhibits extremely low plasma concentrations after oral administration (Zuo et al., 2006; Liu et al., 2009). Researchers have proposed the following mechanisms for the low plasma concentrations of these alkaloids: 1) the alkaloids are metabolized in the intestine (Liu et al., 2010); 2) absorbed alkaloids are pumped out by intestinal (Zhang et al., 2011) or hepatic (Tsai and Tsai, 2004) P-glycoprotein (P-gp); 3) absorbed alkaloids are metabolized quickly and extensively after absorption (Tsai and Tsai, 2004; Zuo et al., 2006; Yang et al., 2010); and 4) absorbed alkaloids are distributed widely in tissues (Liu et al., 2010; Ma et al., 2010). The low plasma concentrations of the alkaloids are insufficient indicators of their in vivo bioactivities. The alkaloids may produce their systemic therapeutic actions by modulating effectors in the gut (Lu et al., 2009), through generated metabolites that are both abundant (Zuo et al., 2006) and active (Li et al., 2011), or on the basis of the relatively dominant tissue distribution of the alkaloids themselves (Liu et al., 2010; Ma et al., 2010). However, all of the aforementioned pharma-

ABBREVIATIONS: HPLC, high-performance liquid chromatography; LC, liquid chromatography; LPS, lipopolysaccharide; MS, mass spectrometry; MS/MS, tandem mass spectrometry; P-gp, P-glycoprotein; UDPGA, uridine-5’-diphosphoglucuronic acid; ESI, electrospray ionization; BCA, bicinchoninic acid; FD-4, fluorescein isothiocyanate-labeled dextran; Rh-123, rhodamine 123; AUC(0–24), area under the concentration-time curve from 0 to 24 h.
cokinetic studies were performed with normal subjects and experimental animals, and less is known about systemic exposure to rhizoma coptidis alkaloids in pathological cases, except for diabetic rats (Yu et al., 2010).

Orally administered rhizoma coptidis extract and its alkaloid constituents are broadly applied in clinics for the treatment of infectious, gastrointestinal, and cardiovascular diseases and diabetes mellitus (Xu et al., 2004; Vuddanda et al., 2010). Both the extract and its alkaloid constituents also show promise as novel antineoplastic agents (Tang et al., 2009). On one hand, inflammation is observed in all of the aforementioned diseases (Fearon and Fearon, 2008; Pandolfi et al., 2009; Goldfine et al., 2011; Grivennikov and Karin, 2011). On the other hand, both drug-metabolizing enzymes and transporters, which determine the fate of drugs in vivo, are regulated in these diseases (Morgan et al., 2008). Given that the rhizoma coptidis alkaloids are substrates of P-gp (Zhang et al., 2011) and are eliminated mainly through metabolism (Yang et al., 2010), we assume that the pharmacokinetic characteristics of the alkaloids change during inflammation, compared with normal conditions. We hypothesize that this change may be critical to elucidating why rhizoma coptidis cures the aforementioned inflammation-related diseases despite the low plasma concentrations of the alkaloids measured for normal subjects and experimental animals.

Materials and Methods

Materials. Rhizoma coptidis (Coptis chinensis Franch), which was produced according to The Preparing Standardization of the Crude Traditional Chinese Medicine (Shanghai Food and Drug Administration, 2008), was purchased from Shanghai Kang Qiao Herbal Pieces Co. (Shanghai, China), which is a good manufacturing practices-certificated manufacturer. The authentication of the herb was performed by Prof. Zhi-Li Zhao (Department of Botany, Shanghai University of Traditional Chinese Medicine) through comparison with appropriate voucher specimens and analysis of both physical and chemical properties according to The Pharmacopoeia of People’s Republic of China (National Pharmacopoeia Committee, 2010). All rhizoma coptidis alkaloid standards except coptisine were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The coptisine standard was purchased from Wako Pure Chemical Industries (Osaka, Japan). Vinblastine and vincristine were products of the United States Pharmacopoeia (Rockville, MD). A bicinchoninic acid (BCA) protein assay kit was obtained from the Shanghai Usen Biological Technology Co. (Shanghai, China), LPS, rhodamine 123 (Rh-123), fluorescein isothiocyanate-labeled dextran (FD-4) (average molecular mass of 4 kDa), phenacetin standard, NADPH, UDP-glucuronic acid (UDPGA), saccharic acid-1,4-lactone, and alamethicin were purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). The pure water used in the current study was prepared by using a Milli-Q system (Millipore Corporation, Billerica, MA). All other materials were of analytical grade or higher.

Standardized Preparation and Quality Control of Rhizoma Coptidis Extract. The rhizoma coptidis extract was prepared by using standardized procedures, according to a previously reported method (Ma et al., 2010). The yield was 37.1%. The active compounds were determined with HPLC, for quality control of the extract. The mass percentages of berberine, coptisine, palmatine, and jatrorrhizine in the extract were 15.5, 4.2, 1.9, and 3.2%, respectively.

Synthesis of Berberrubine and Demethyleneberberine. To obtain berberrubine, berberine chloride (0.5 g) was heated at 200°C for 20 min under vacuum (20–30 mm Hg). The solid produced was recrystallized three times with anhydrous ethanol. After that, the product was treated with ethanol/concentrated hydrochloric acid (95:5) to yield purified berberrubine (yield, 60%). To obtain demethyleneberberine, berberine chloride (0.5 g) and phloroglucin (0.5 g) were mixed with 60% $\text{H}_2\text{SO}_4$ (10 ml). The mixture was heated at 100°C for 8 h and then cooled in brine to yield precipitate. The precipitate was then purified with preparative HPLC to yield demethyleneberberine (yield, 26%). After the synthesis of berberrubine and demethyleneberberine, liquid chromatography (LC)-mass spectrometry (MS) and $^1$H NMR were used to confirm their structural features.

Animals. Sprague-Dawley rats (Grade II) weighing 240 to 260 g were purchased from the Shanghai Slac Laboratory Animal Co. (Shanghai, China). The rats were housed in an air-conditioned room at 22–24°C, with a 12-h dark/light cycle, and were given food and water ad libitum. All animal experiments were performed in accordance with the guidelines of the National Research Council.

Liver Microsome Preparation. Liver microsomes were prepared as described previously (Hill, 2003), except that the microsomes were resuspended and homogenized in ice-cold sucrose solution (0.25 M). Protein concentrations were determined with the BCA assay. The protein concentration was adjusted to 10 mg/ml with sucrose solution, and then 0.5-ml aliquots were dispensed into labeled tubes and stored at −80°C.

Intestinal S9 Fraction Preparation. Intestinal cellular S9 fractions were prepared as described previously (Masaki et al., 2007), with modifications. In Lipopolysaccharide (LPS), the outer membrane molecule of Gram-negative bacteria, has often been used to establish both in vitro and in vivo inflammatory experimental models (Morgan et al., 2008). It reportedly influences the pharmacokinetic characteristics of numerous drugs (Yang and Lee, 2008). In the present study, therefore, we aimed to assess the effect of LPS pretreatment on the pharmacokinetic characteristics of the rhizoma coptidis alkaloids and to study the underlying mechanisms.
brief, the small intestines were excised and rinsed with chilled 1.15% KCl. They were then cut into longitudinal strips. The mucosa was scraped and homogenized with 4 volumes of 50 mM ice-cold HEPES buffer (pH 7.4) containing 1.15% KCl. The homogenates were then centrifuged at 9000g for 20 min at 4°C. Protein concentrations were determined with the BCA assay. The protein concentration was adjusted to 10 mg/ml, and then 0.5-ml aliquots were dispensed into labeled tubes and stored at −80°C.

**Identification of Metabolites.** The experiments were performed with an LCQ ion-trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA) with an electrospray ionization (ESI) ion source, in positive-ion mode. Samples were eluted through a reverse-phase column (Thermo Hyperil-Keystone C18, 5 μm, 2.1 × 150 mm; Thermo Fisher Scientific) at 25°C with a linear gradient of solvent A (0.1% formic acid and 2 mM ammonium formate) and solvent B (methanol) (0 min, 80:20 solvent A/solvent B; 3 min, 80:20; 18 min, 52:48; 27 min, 5:95; 28 min, 80:20; 35 min, 80:20), at a flow rate of 0.3 ml/min. During the analysis, the ESI parameters were set as follows: sheath gas flow rate, 30 units; auxiliary sweep gas flow rate, 10 units; source spray voltage, 5 kV; capillary temperature, 300°C; capillary voltage, 5 V; tube lens offset, 10 V. The MS* product-ion spectra were produced through collision-induced dissociation of the molecular ions of all analytes at their respective HPLC retention times. Data acquisition was performed in full-scan LC/MS and MS/MS modes.

**HPLC-UV Analysis of Metabolites.** A validated biochemical analysis method based on HPLC was used to detect the metabolites of berberine; the HPLC was equipped with a 2695 separation module, a 2487 dual-wavelength absorbance detector, and an Empower2 Chemstation (Waters, Milford, MA). Separation and determination were performed by using an X-Terra RP 18 analytical column (5 μm, 3.9 × 150 mm; Waters) at 35°C with a detection wavelength of 296 nm. The samples were eluted through the column with a gradient of water/formic acid/methanol (100:0.1:0.2, v/v/v) at a flow rate of 0.5 ml/min. The standards had good linearity within their own quantitative range (0.078–2.5 μM for both metabolites, i.e., berberubine and demethylberberine). The quality control samples were prepared at three different concentrations (0.156, 0.625, and 2.25 μM).

**In Vitro Metabolism Incubations.** Both Phase I and Phase II liver microsomal or intestinal S9 incubations were performed as described previously (Liu et al., 2009), with modifications. In brief, reaction conditions, including the protein concentration and the incubation time, were optimized to ensure the linearity of the reactions. For the Phase I microsomal or intestinal S9 incubations, the reactions were conducted in medium containing 100 mM potassium phosphate buffer (pH 7.4) with 1 mM NADPH, 0.5 mg of microsomal protein or 2 mg of intestinal S9 fraction, and 10 or 50 μM berberine (for liver microsomes or intestinal S9 fractions, respectively). After preincubation for 5 min, the reaction was initiated with addition of the NADPH solution. After incubation at 37°C for 15 min for liver microsomes or 60 min for intestinal S9 fractions, the reactions were terminated with addition of an equal volume of ice-cold methanol with the internal standard. For the Phase II liver microsomal or intestinal S9 incubations, the reactions were conducted in medium containing 0.5 mg of microsomal protein or intestinal S9 fraction, 10 μM UDPGA, 10 mM MgCl2, 5 mM saccharic acid 1,4-lactone, 50 mM potassium phosphate buffer (pH 7.4), and 10 or 15 μM demethylberberine (for liver microsomes or intestinal S9 fractions, respectively). The microsomes were pretreated for 15 min on ice with 25 μg/ml alamethicin. After preincubation for an additional 5 min at 37°C, the reactions were initiated with addition of the UDPGA solution. After 2 min, the reactions were terminated with addition of an equal volume of ice-cold methanol with the internal standard. After centrifugation at 12,000 rpm for 15 min at 4°C, the samples were analyzed by using the validated HPLC method. The concentrations of the Phase II glucuronide metabolite were calculated semiquantitatively according to the accompanying calibration curve for demethylberberine. Control incubations with inactivated microsomes or intestinal S9 fractions (prepared through incubation at 90°C for 5 min) or without NADPH/UDPGA were performed in parallel. All reactions were performed in triplicate.

**Influence of LPS on Metabolism of Berberine in Rat Liver Microsomes and Intestinal S9 Fractions.** Twelve rats were used to study the influence of LPS pretreatment on the metabolism of berberine in rat liver microsomes, whereas 20 rats were used in experiments performed with rat intestinal S9 fractions. In each experiment, the rats were randomly divided into two groups (male/female ratio of 1:1). One group of rats received intraperitoneal injections of 1 mg/kg LPS, and the other received the same volume of saline solution. After pretreatment for 16 h, livers or intestines were excised, and liver microsomes or intestinal S9 fractions were prepared immediately and stored at −80°C for in vitro metabolism experiments.

**LC/MS/MS Analysis of Vinblastine.** A Shimadzu Prominance UFLCXR series HPLC system (Shimadzu, Kyoto, Japan) and a Thermo Fisher Scientific TSQ Quantum Ultra mass spectrometer (Thermo Fisher Scientific) equipped with an ESI source were used. After addition of 40 μM trichloroacetic acid and 10 μl of vincristine (500 ng/ml; internal standard) to 150-μl samples, the mixture was extracted with 2 ml of ethyl acetate. The supernatant was evaporated to dryness under a stream of nitrogen. The residue was redissolved in 150 μl of mobile phase (50% water phase/50% organic phase). After centrifugation at 16,000 rpm for 5 min at 4°C, 10-μl samples were injected into the LC/MS/MS system. The samples were eluted through a Hypersil Gold C18 analytical column (3 μm, 100 × 2.1 mm; Thermo Fisher Scientific) with a gradient of the aqueous phase (0.2% formic acid, v/v) and the methanol phase (0.2% formic acid, v/v; 0 min, 80:20; 3 min, 5:95; 4 min, 5:95; 6 min, 80:20; 9 min, 80:20), at a flow rate of 0.3 ml/min. The ESI source was set to positive-ion mode. Data acquisition was performed in the multiple-reaction monitoring mode for the selective mass transition for each compound. The transitions of the protonated precursor ions to the selected product ions were m/z 413.252 → m/z 362.300 for vincristine and m/z 406.203 → m/z 271.700 for vinblastine. The quantitative range for measuring vinblastine was 0.78 to 100 nM. The quality control samples were prepared at three concentrations (2, 15, and 90 nM). The accuracy, precision, recovery, and stability tests all met the requirements for quantitative determination in biological samples.

**LC/MS/MS Analysis of Rhizoma Coptidis Alkaloids.** A validated bioanalytical method based on LC/MS/MS was used. An Agilent 1200 series HPLC system and a triple-quadrupole mass spectrometer (AB4000; Applied Biosystems, Foster City, CA) equipped with an ESI source were used. The samples were precipitated with 3 volumes of acetonitrile, with phacentic as the internal standard. The supernatant was then mixed with an equal volume of pure water, and 10-μl samples were injected into the LC/MS/MS system. The samples were eluted through the X-Terra RP 18 analytical column at 40°C with the aqueous phase (0.08% formic acid, v/v, in 4 mM ammonium acetate) and the acetonitrile phase at 48:52, at a flow rate of 0.6 ml/min. The ESI source was set to positive-ion mode. Data acquisition was performed in the multiple-reaction monitoring mode for the selective mass transition for each compound. The transitions of the protonated precursor ions to the selected product ions were m/z 338.10 → m/z 322.90 for jatrorrhizine, m/z 320.20 → m/z 292.00 for coptisine, m/z 336.10 → m/z 292.00 for berberine, m/z 352.00 → m/z 335.90 for palmatine, and m/z 180.00 → m/z 110.00 for phacentic. The quantitative ranges were 0.625 to 500 ng/ml for all measured rhizoma coptidis alkaloids (berberine, coptisine, palmatine, and jatrorrhizine) in the tested biological samples. The quality control samples were prepared at three concentrations (1.5, 25, and 450 ng/ml). The accuracy, precision, recovery, and stability tests all met the requirements for quantitative determination in biological samples.

**Studies with Everted Gut Sacs.** The transport of Rh-123, vinblastine, FD-4, and the rhizoma coptidis alkaloids across rat everted sacs was evaluated as described (Veau et al., 2001), with modifications. In brief, 12 rats were used in each everted gut sac experiment. In each experiment, the rats were randomly divided into two groups (male/female ratio of 1:1). One group of rats received intraperitoneal injections of 1 mg/kg LPS, and the other received the same volume of saline solution. After 16 h, the rats were sacrificed and the ileum was removed 5 cm above the cecum, after laparotomy. The ileum was washed with chilled saline solution and everted. A 12-cm segment was cut and ligated at one end. As for the study of the absorption of the rhizoma coptidis alkaloids or FD-4, the everted gut sac was filled on the serosal side (inside) with 1 ml of blank Krebs-Ringer buffer (containing 118 mM NaCl, 25 mM NaHCO3, 1.2 mM MgSO4, 2.5 mM CaCl2, 11 mM glucose, 1.2 mM KH2PO4, and 4.7 mM KCl; pH 6.8). The other end was tightly ligated to create a gut sac. The sac was immediately incubated in a Magnus bath containing 19 ml of oxygenated Krebs-Ringer buffer at 37°C. After 5 min, 1 ml of rhizoma coptidis extract (1 mg/ml in Krebs-Ringer buffer) or FD-4 solution (2 mg/ml in Krebs-Ringer buffer) was added to the bath. Aliquots (200 μl) were taken from the serosal side every 15 to 60 min. The same volume of blank Krebs-Ringer buffer was
resupplied each time. For study of the efflux of Rh-123, vinblastine, or berberine across the everted sac, the sac was filled on the serosal side with 1 ml of Rh-123 (250\(\mu\)M in Krebs-Ringer buffer), vinblastine (10\(\mu\)M, dissolved in dimethyl sulfoxide and then diluted with Krebs-Ringer buffer; the final concentration of dimethyl sulfoxide was less than 1%), or berberine (1, 3, and 10\(\mu\)g/ml, dissolved in methanol and then diluted with Krebs-Ringer buffer; the final concentration of methanol was less than 1%) solution and was tightly ligated to create a gut sac. The gut sac was immediately incubated in a Magnus bath containing 20 ml of oxygenated Krebs-Ringer buffer at 37°C. Aliquots (200\(\mu\)l) were taken from the mucosal side (outside) every 15 to 60 min. The same volume of blank Krebs-Ringer buffer was resupplied each time. After termination of the incubation, the lengths of the sacs were measured. The concentrations of Rh-123 and FD-4 were determined by using a fluorescence microplate reader (BioTek Instruments, Winoski, VT), with excitation at 485 nm and emission at 528 nm. The concentrations of vinblastine and the rhizoma coptidis alkaloids were determined by using LC/MS/MS.

Time-Dependent Influence of LPS Pretreatment on Pharmacokinetics of Berberine in Rats That Received Rhizoma Coptidis Extract. Twelve male rats were randomly divided into four groups. Three groups of rats received intraperitoneal injections of 1 mg/kg LPS, and one group of rats received the same volume of saline solution, injected intraperitoneally, as a control. After 16, 24, or 36 h of LPS pretreatment, the rats were given 0.72 g/kg doses of the orally administered extract of rhizoma coptidis (the dose of berberine was 111.6 mg/kg). The rats in the control group were also given the orally administered extract. Venous blood samples (\(\sim\)0.3 ml) were collected in heparinized 1.5-ml tubes at 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h after rhizoma coptidis ingestion. Blood samples were centrifuged at 12,000 rpm for 10 min immediately after collection, and the plasma was
coptidis are shown in Fig. 3. The results showed that LPS pretreatment significantly decreased both the Phase I metabolism of berberine (0.72 ± 0.24 and 0.70 ± 0.18 μM · min⁻¹ · mg protein⁻¹ for M1 and M2 formation, respectively, with control microsomes) or the Phase II metabolism of demethyleneberberine to M3 (39.2 ± 9.5 μM · min⁻¹ · mg protein⁻¹ for M3 formation with control microsomes).

Influence of LPS on Metabolism of Berberine in Intestinal S9 Fractions. Similar to findings observed for the incubation with liver microsomes, both M1 and M2 were formed during the incubation of berberine with the intestinal S9 fraction in the presence of NADPH. However, the M1 generated was scarce. The incubation of demethylberberine in the presence of UDPGA led to the formation of the glucuronide of demethyleneberberine (Fig. 1), with fragment ions at m/z 500, according to the LC/MS/MS identification. As shown in Fig. 4, LPS pretreatment did not influence the Phase I metabolism of berberine (0.72 ± 0.24 and 0.70 ± 0.18 μM · min⁻¹ · mg protein⁻¹ for M1 and M2 formation, respectively, with control microsomes) or the Phase II metabolism of demethyleneberberine (39.2 ± 9.5 μM · min⁻¹ · mg protein⁻¹ for M3 formation with control microsomes).

Influence of LPS on Absorption of Rhizoma Coptidis Alkaloids in Rat Everted Gut Sacs. The mean absorption amount-time curves were formed during the incubation of berberine with rat liver microsomes in the presence of NADPH. The incubation of demethyleneberberine with rat liver microsomes in the presence of UDPGA led to the formation of the glucuronide of demethyleneberberine (Fig. 1), with fragment ions at m/z 500, according to the LC/MS/MS identification. As shown in Fig. 4, LPS pretreatment did not influence the Phase I metabolism of berberine (0.72 ± 0.24 and 0.70 ± 0.18 μM · min⁻¹ · mg protein⁻¹ for M1 and M2 formation, respectively, with control microsomes) or the Phase II metabolism of demethyleneberberine (39.2 ± 9.5 μM · min⁻¹ · mg protein⁻¹ for M3 formation with control microsomes).

Results

Time-Dependent Influence of LPS Pretreatment on Pharmacokinetics of Berberine in Rats That Received Rhizoma Coptidis Extract. The systemic exposures [area under the concentration-time curve from 0 to 24 h (AUCₚₒ₋₂₄)] to berberine in rhizoma coptidis are shown in Fig. 2. The results showed that LPS pretreatment for 16 h significantly increased the AUC₀₋₂₄ for berberine (P = 0.024).

Influence of LPS on Pharmacokinetics of Rhizoma Coptidis Alkaloids in Rats. Both the mean plasma concentration-time curves and the systemic exposures (AUC₀₋₂₄) to the alkaloids in rhizoma coptidis are shown in Fig. 3. The results showed that LPS pretreatment significantly increased the AUC₀₋₂₄ values for berberine (P = 0.025), coptisine (P = 0.03), and jatrorrhizine (P = 0.006).

Influence of LPS on Metabolism of Berberine in Rat Liver Microsomes. Two major Phase I metabolites (Fig. 1), M1 (berberrubine) and M2 (demethyleneberberine), with fragment ions at m/z 322 and m/z 324, respectively, according to the LC/MS/MS identification,
for the rhizoma coptidis alkaloids are shown in Fig. 6. The result showed that LPS pretreatment significantly increased the absorption of the rhizoma coptidis alkaloids \( (P = 0.036, \ P = 0.032, \ P = 0.005, \) and \( P = 0.003 \) for berberine, copistine, jatrorrhizine, and palmitine, respectively).

**Influence of LPS on Efflux of Berberine, Vinblastine, and Rh-123 in Rat Everted Gut Sacs.** The results showed that there was no significant difference between the efflux ratios for berberine at different concentrations. The mean efflux ratio-time curves for berberine are shown in Fig. 7A. The results showed that LPS pretreatment significantly decreased the efflux of berberine \( (P = 0.014) \). The efflux ratios for vinblastine \( (P = 0.003) \) and Rh-123 \( (P = 0.021) \) were also significantly decreased (Fig. 7, B and C).

**Influence of LPS on Absorption of FD-4 in Rat Everted Gut Sacs.** As shown in Fig. 8, LPS pretreatment had no significant influence on the absorption of FD-4.

**Influence of LPS on Mucosal Structure.** Compared with normal mucosa (Fig. 9A), LPS pretreatment caused significant damage to mucosal structures (Fig. 9B). The villi became shorter and blunt. The adjacent villi fused into one piece. Some epithelial cells were shed from the top of the villi. Obvious ultrastructural alterations (Fig. 9D), including sparse and ruptured microvilli, swollen mitochondria and endoplasmic reticulum, and apoptotic bodies in the nuclei, were also found, in comparison with normal control findings (Fig. 9C). However, the tight junctions between epithelial cells remained intact.

**Discussion and Conclusions**

Systemic exposure to LPS leads to systemic inflammation or endotoxemia. Generally speaking, LPS in the bloodstream might be derived from two sources: one is the contamination of tissues, fluids, or foreign bodies, and the other is bacterial and endotoxin translocation from the intestinal lumen (Hauser et al., 2011). Although the gastrointestinal tract contains a very high intraluminal concentration of living bacteria, the gut barrier restricts micromolecular permeation and almost completely restricts macromolecular permeation in normal subjects and experimental animals (Balzan et al., 2007). Therefore, translocation of intestinal bacteria and endotoxins does not normally occur in healthy adult subjects or experimental animals. Although it is regarded as a very severe form of infection, comparable to bacterial septicemia, intraperitoneal injection of LPS is still usually used to induce systemic inflammation or endotoxemia in experimental animals (Morgan et al., 2008). Several diseases for which rhizoma coptidis is useful therapy are associated with endotoxemia, including ulcerative colitis (Amati et al., 2003), acute gastrointestinal syndrome (Zhang et al., 2010), cardiac diseases (Mou et al., 2002), diabetes (Pusininen et al., 2011), and hepatic fibrogenesis (Lin et al., 2011). Therefore, the LPS model was used in this study.

The results showed increased systemic exposure to the four rhizoma coptidis alkaloids in rats that received orally administered rhizoma coptidis extract after LPS pretreatment, which indicated that the bioavailability of the rhizoma coptidis alkaloids improved in LPS-pretreated rats. These results add to the understanding of why rhizoma coptidis is curative in inflammation-related pathological conditions despite the low plasma concentrations of the alkaloids measured in normal subjects and experimental animals. Furthermore, we reported that LPS pretreatment increased the acute toxicity of the rhizoma coptidis extract in mice (Ma et al., 2011). Certain classic works of traditional Chinese medicine, such as *Ben Cao Yan Yi* (Augmented Materia Medica; Kou, 1997), have stated that, although rhizoma coptidis extract is an excellent therapeutic agent for dysentery, the dosage should be adjusted on the basis of the severity of the disease; otherwise, the health of the patient might be endangered. Berberine, the main toxic constituent of the rhizoma coptidis extract (Ma et al., 2010), has caused respiratory failure, extrapyramidal system reactions, severe arrhythmia, liver function injury, and even death in clinics in China (Li et al., 2008). These reports strongly suggested that rhizoma coptidis extract and its alkaloids not only are curative but also are potentially toxic and should be used carefully in clinics, especially in LPS-related inflammatory diseases.

We conclude that LPS increased the systemic exposure of the rhizoma coptidis alkaloids through the enhancement of their absorption, on the basis of the following results: 1) the in vitro absorption of the rhizoma coptidis alkaloids increased and 2) both the in vitro Phase I metabolism of berberine and the Phase II metabolism (glucuronidation) of demethyleneberberine were not significantly influenced in liver microsomes. Given that the rhizoma coptidis alkaloids are substrates of P-gp (Zhang et al., 2011), the reduced intestinal efflux of berberine indicated that the function of intestinal P-gp was decreased with LPS pretreatment. This assumption was confirmed by the reduced intestinal efflux of vinblastine and Rh-123, substrates of P-gp (Ogihara et al., 2006; Tomita et al., 2010). Because P-gp is expressed mainly on the brush border membrane of epithelial cells (Panwala et al., 1998), damage to mucosal structures, such as rupture of the microvilli and apoptosis of the epithelial cells, may be involved in the decreased function of P-gp caused by LPS pretreatment. Conversely, the intact tight junctions and unaffected absorption of FD-4 exclude the possibility that the increased absorption of the rhizoma coptidis alkaloids is attributable to paracellular transportation. Both the in vitro Phase I metabolism of berberine and the Phase II metabolism (glucu-
ronidation) of demethyleneberberine were significantly decreased in intestinal S9 fractions. Given that intestinal metabolism is involved in the low plasma levels of the rhizoma coptidis alkaloids (Liu et al., 2010), the results suggested that the increased intestinal absorption of the rhizoma coptidis alkaloids was attributable not only to decreased intestinal efflux but also to decreased intestinal metabolism.

The metabolites formed during the incubation of berberine and demethyleneberberine were consistent with previous findings (Liu et al., 2009). In the in vitro Phase II metabolism experiments, only the incubation of the M2 standard was tested. Formation of both M1 and M2 was reported to be catalyzed mainly by UDP-glucuronosyltransferase 1A1 (Liu et al., 2009), which suggests that the incubation of the M2 standard reflects the metabolic capability of UDP-glucuronosyltransferase 1A1. The results of the in vitro metabolism studies showed the different influences of LPS on hepatic and intestinal drug-metabolizing enzymes. LPS pretreatment was reported to show different influences on the expression of cytochromes P450 in the liver and intestine (Chung et al., 2008), which might be related to the tissue-specific concentrations of the effectors and the severity of pathological changes. The absence of LPS modulation of hepatic metabolism in the current work might have been caused by the different experimental settings, especially the time of initiation of the experiment after LPS administration. LPS pretreatment was reported to have time-dependent (Yang and Lee, 2008) and biphasic effects on some hepatic cytochrome P450 catalytic activities (Sewer and Morgan, 1998). For example, the hydroxylation of testosterone at the 2α, 16α, and 6β positions was decreased 6 h after LPS pretreatment and then returned to normal levels by 12 h before decreasing again at 24 h (Sewer and Morgan, 1998). Most of the changes in the enzyme activities in rat liver were observed more than 24 h after LPS pretreatment (Yang and Lee, 2008). According to the preliminary study, LPS pretreatment for 16 h showed the strongest influence on the systemic exposure of berberine, the major rhizoma coptidis alkaloid. Therefore, we studied only the influence of LPS pretreatment for 16 h in both the in vivo and in situ experiments, and the absence of LPS modulation of hepatic metabolism in this study was not a surprising outcome.

In conclusion, our results showed that LPS pretreatment increased systemic exposure to the rhizoma coptidis alkaloids in rats treated with orally administered rhizoma coptidis extract. This increase was attributable to enhancement of the intestinal absorption of the alkaloids, which was related to both decreased intestinal efflux and intestinal metabolism.

Authorship Contributions

Participated in research design: B.-L. Ma, Y.-M. Ma, C.-H. Wang, and X.-H. Wang.
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References


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