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Absorption and enterohepatic circulation of baicalin in rats ☆

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Abstract

Pharmacokinetics of baicalin, in form of its parent drug (BG) and conjugated metabolites (BGM), were studied following intravenous and oral administration of baicalin to intact rats. The enterohepatic circulation of BG and BGM was also assessed in a linked-rat model. Multiple plasma and urine samples were collected, and concentrations of BG and BGM were determined using a liquid chromatography/tandem mass spectrometry method. The concentration of BGM was assayed in the form of baicalein after treatment with β-glucuronidase/sulfatase. After i.v. administration, plasma concentration of BG rapidly declined with the elimination half-life ($T_{1/2}$) of 0.1 till 4 h post dose, followed by slight increase from 4–8 h in plasma concentrations after drug administration. These plasma concentrations resulted in a significant prolongation of the terminal elimination half-life of BG ($T_{1/2\ TER}$, 9.7 h). BG also displayed slight increase in plasma concentrations (12–24 h) after oral administration, with $T_{1/2\ TER}$ of 12.1 h. Based on the AUC of BG and BGM, the absolute bioavailability of baicalin was 2.2±0.2% and 27.8±5.6%, respectively. The exposure of baicalin to the systemic circulation was approximately 118-fold lower than that of BGM after oral administration (AUC_{0-t}, 4.43 versus 523.97 nmol·h/mL). The high extent of glucuronidation suggested the possible presence of enterohepatic circulation, which was confirmed in the linked-rat model since plasma concentrations of BG and BGM were observed in bile-recipient rats at 4 to 36 h. The extent of enterohepatic circulation after intravenous administration of baicalin was 4.8% and 13.3% for BG and BGM, respectively. It was determined that 18.7% and 19.3% of the administered baicalin were subjected to enterohepatic circulation for BG and BGM, respectively, after oral administration. These results confirm that BG undergoes extensive first-pass glucuronidation and that enterohepatic circulation contributes significantly to the exposure of BG and BGM in rats.

Keywords: Baicalin; Absorption; First pass glucuronidation; Enterohepatic circulation

Introduction

Hepatic metabolism and enterohepatic circulation (EC) are reported to be some of the factors that affect the pharmacokinetics of certain drugs by influencing terminal half-life, the area under the plasma concentration–time curve and bioavailability (Tsai et al., 2000). Since many drugs (Marier et al., 2002; Horton and Pollack, 1991) are subject to enterohepatic circulation, measurement of EC would provide an additional characterization of drug pharmacokinetics. A linked-rat model was often used in the study of enterohepatic circulation (Tsai et al., 2000). A certain drug is administered intravenously or orally to bile-donor rats, and let their bile flow directly into the duodenum of bile-recipient rats via surgically implanted catheters so that the contribution of enterohepatic circulation to the overall disposition would be determined.

Baicalin (baicalein 7-*O*-β-glucopyranuronoside, BG) and its aglycone baicalein are bioactive flavonoids isolated from the root of *Scutellaria baicalensis* George, a medicinal herb that has been used since ancient times for the treatment of inflammation, fever and allergic diseases (Shen et al., 2003). Besides various pharmacological activities, baicalin might interact with coadministered drugs, such as cyclosporin (Lai et al., 2004). Furthermore, baicalin may induce the activity of cytochrome P450 (Hou et al., 2000). Glucuronidation is the main metabolic pathway for baicalin (Abe et al., 1990). Five biliary metabolites have been identified after oral administration of baicalin to rats. The main metabolites are baicalein 6-*O*-glucuronide-7-*O*-sulfate and baicalein 6,7-di-*O*-glucuronide. Baicalein 6-*O*-sulfate also has been identified after the

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administration of "Sho-Saiko-To" in humans (Muto et al., 1998). The conjugated metabolites are readily hydrolyzed by enzyme β-glucuronidase/sulfatase to free aglycone, and baicalin has been found to be absorbed from the gastrointestinal tract as its aglycone, which is restored to the baicalin parent drug by glucuronidation in the intestine and the liver in vivo (Akao et al., 2000). It is likely that part of baicalin could be reabsorbed and recirculated in the process known as enterohepatic circulation.

The pharmacokinetics of baicalin in rats has been reported (Lai et al., 2003). Baicalin was found absorbed more slowly and to a lesser extent than its aglycone, baicalein. Baicalin was primarily excreted to the bile in the form of glucuronides and the total cumulative amounts of its glucuronides after oral administration of baicalin was approximately 54% of the dose (Abe et al., 1990). The high biliary excretion suggests that baicalin might undergo enterohepatic circulation.

To further study the absorption and metabolism of baicalin, and to assess the contribution of enterohepatic circulation to the overall disposition of baicalin in rats, the systemic pharmacokinetics and the excretion of baicalin were examined in intact and bile duct-cannulated rats.

Materials and methods

Chemicals

Baicalin was purchased from Shifang Co. (purity > 98.3%, Sichuan, China). Daidzein was purchased from Xieli Co. (purity > 99.3%, Sichuan, China). Baicalein (purity > 99.5%) and 6-*O*-methyl-baicalein was obtained in our lab (a gift from Department of Medicinal Chemistry, Shenyang Pharmaceutical University, China). Structures of all the standards were confirmed by MS and NMR spectroscopy. β-Glucuronidase (with arylsulfatase activity, partial purified, G-4259) was purchased from Sigma Chemical Co. (St Louis, MO, USA). Methanol and acetonitrile were HPLC grade and other chemicals used were of analytical reagent grade.

Instrumentation

The HPLC system (Shimadzu, Kyoto, Japan) was equipped with a Thermo Finnigan TSQ Quantum mass spectrometer (San Jose, CA, USA) via an electrospray ionization interface. A Diamonsil C_{18} column (particle size 5 μm, 20 cm × 4.6 mm ID, Dikma, China) was used for sample separation. Mobile phase consisting of methanol–water–formic acid (60 : 40 : 0.5, v/v) was used at a flow rate of 0.4 mL/min for baicalin assay and acetonitrile–water–formic acid (50 : 50 : 0.5, v/v) at a flow rate of 0.5 mL/min for baicalein assay. Detection was performed on a triple quadrupole tandem mass spectrometer by selected reaction monitoring (SRM) mode recording the transitions from the respective $[M+H]^+$ ions to the following product ions: baicalin *m/z* 447 → *m/z* 271; baicalein *m/z* 271 → *m/z* 225, 253 and daidzein (internal standard) *m/z* 255 → *m/z* 199. The ionization was realized by applying spray voltage of 4.0 kV and capillary temperature of 320 °C. Nitrogen was used as both the sheath gas at a pressure of 35 Arb and auxiliary gas at a pressure of 3 Arb. MS spectra were obtained in the positive ion mode, using argon as collision gas at a pressure of 1.0 mTorr. Data were analyzed by Xcalibur software (version 1.4, Finnigan).

Drug administration and sample collection

Male Wistar rats (230–250 g) were supplied by Lab Animal Center of Shenyang Pharmaceutical University (Grade II, Certificate No. SYXK 2003-0012). The experimental protocol was approved by the University Ethics Committee for the use of experimental animals and conformed to the Guide for Care and Use of Laboratory Animals. Rats were maintained at 22 ± 2 °C and 55 ± 5% relative humidity on a 12-h light–dark cycle. They were fasted for 12 h before drug administration and for 3 h after dosing. Water was freely available. Surgical procedures pertaining to the linked-rat model were performed according to the method of Marier et al. (2002). Briefly, one catheter (PE-10) was inserted directly into the proximal portion of the bile duct toward the liver, and a second catheter (PE-50) was inserted directly into the duodenum. Both catheters were secured in place by ligation with surgical sutures and exteriorized subcutaneously at the back of the animal prior to closure of the abdomen. Finally, the catheters were connected via a dual swivel device to allow free flow of the bile in the animal. During the recovery period (3 days), bile duct and duodenum catheters remained connected together such that normal bile circulation within the animal was not interrupted. Approximately 2 h before dosing, rats were paired so that the bile cannula of one rat was connected to the duodenal cannula of a second rat.

The administered dose of baicalin to rats was chosen according to Lai et al. (2003). The intravenous bolus injection was given to intact rats ($n = 6$) via the tail vein at a dose of 37 μmol/kg. Baicalin was dissolved in phosphate solution (0.05 M, pH 7.4) for injection, and suspended in 0.5% carboxymethyl cellulose (CMC)–Na solution for oral administration ($n = 6$, 224 μmol/kg). Baicalin was also administered to bile-donor rats (i.v. 37 μmol/kg and p.o. 224 μmol/kg; $n = 3$) paired to bile-recipient rats ($n = 3$) in a linked-rat model. Blood samples of intact rats or bile-donor rats were withdrawn from the jugular vein before dosing and at 0.017, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 12.0 and 24.0 h after i.v. dosing, and at 0.5, 1.0, 3.0, 5.0, 8.0, 12.0, 24.0, 36.0 and 48.0 h after p.o. dosing. Blood samples at 1.0, 2.0, 4.0, 8.0, 12.0, 24.0, 36.0, 48.0 and 60.0 h were withdrawn from the jugular vein of bile-recipient rats. All heparinized blood samples were centrifuged at 9860 *g* for 15 min at 4 °C and the plasma was obtained. After i.v. or p.o. administration of baicalin to bile-donor rats, urine samples were taken at 8, 12, 24, 36, 48 and 60 h, each in 0.3 M hydrochloric acid (the final pH 4.0) with cooling, from bile-donor and bile-recipient rats. Bile samples were taken at 2, 4, 8, 12, 24, 36, 48 and 60 h (the bile of bile-donor rats without being paired was collected separately). All the biological samples were stored at −80 °C for later analysis.