Bioavailability of Salvianolic Acid B and Effect on Blood Viscosities after Oral Administration of Salvianolic Acids in Beagle Dogs

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(Received January 10, 2009/Revised April 10, 2009/Accepted April 16, 2009)

Salvianolic acid B (SalB) is an active component isolated from Chinese herbal medicine Salvia miltiorrhiza. The aim of this study was to investigate the extent of absolute oral bioavailability (F) of SalB in beagle dogs and the effect on blood viscosity after intravenous and oral administration of Salvianolic acids (SAs). A gradient elution HPLC method was developed and validated to determine the concentration of SalB and its three possible metabolites in plasma. After SAs (180 mg/kg, p.o.; 9 mg/kg, i.v.) were given, the AUCs of SalB were 1680 ± 670 and 7840 ± 1140 ng/mL·h, respectively. The F of SalB in dogs was calculated to be only 1.07 ± 0.43%. The blood viscosity was remarkably decreased after a single intravenous injection of SAs (9 mg/kg). However, no significant change of blood viscosity was observed after a single oral administration of SAs (180 mg/kg). The results suggested that the F of SalB was extremely low and single oral administered SAs had no effect on ameliorating blood viscosity in beagle dogs.

Key words: Salvianolic acids, Salvianolic acid B, Bioavailability, Blood viscosity, Beagle dogs

INTRODUCTION

Danshen, the dried root and rhizome of Salvia miltiorrhiza Bunge (National Pharmacopoeia Committee, 2005), is a kind of widely and extensively used traditional Chinese medicine for treating coronary heart disease (Ji et al., 2003), cerebrovascular disease (Sze et al., 2005), hepatitis (Chor et al., 2005) and hepatocirrhosis (Liu et al., 2000). Salvianolic acids (SAs) are water soluble active constituents isolated from Danshen and Salvianolic acid B (SalB) is the most abundant one (Lu and Foo, 2002). SalB has been reported to decrease atherosclerosis (Wu et al., 1998), improve regional cerebral blood flow (Tang et al., 2002), prohibit platelet aggregation (Gao et al., 2004) and ameliorate hemorheology parameters (Sun et al., 2003). As a result, there is a great interest in the therapeutic potential of SalB, and therefore it is worth to investigate the extent of absolute oral bioavailability (F) and pharmacokinetics of SalB thoroughly.

Determination and pharmacokinetics related to SalB have been studied in beagle dogs (Li et al., 2004; Li et al., 2005a) and human subjects (Gao et al., 2004; Li et al., 2005b) following intravenous administration. However, Danshen is mainly used as orally administrated decoction in traditional Chinese medicinal prescription, the extent of absolute oral bioavailability and pharmacokinetics of SalB should be studied in experimental models. Current reports described the pharmacokinetics of SalB in anesthetic (Zhang et al., 2005b; Kim et al., 2005), restrained (Zhang et al., 2004a; Zhang et al., 2004b) and conscious and freely moving (Wu et al., 2006) rats after oral administration and elucidated the low F of SalB in rats. Although it is very important to the rational design of dose regimen and dosage form of SAs and SalB, there has been no report yet on the pharmacokinetic and the extent of absolute oral bioavailability study of SalB in beagle dogs after oral administration.

The aim of the study was to elucidate the extent of absolute bioavailability of SalB in beagle dogs after
oral administration of SAs by plasma concentration and pharmacological response simultaneously. In this study, an HPLC method with ultraviolet detection and liquid-liquid sample extraction was established for the determination of SalB and its potential metabolites in the plasma of beagle dogs. Whole blood viscosity, which is an important hemorheology parameter, was assayed to evaluate the effect of SAs after single administration in beagle dogs. The results might provide useful information for clinical use of SAs and promote the development of new dosage forms of it.

MATERIALS AND METHODS

Chemicals and reagents
SAs was purchased from Dianhong Pharmaceutical Company (Kunming, China) and the content of SalB was determined to be 65.6% (w/w) by HPLC. SAs was dissolved in sodium chloride injection (0.9%) and filtered with 0.22 µm filter for intravenous injection administration and put into capsules with pregelatinized starch and aerosol for oral administration respectively in animal study.

SalB, protocatechuic aldehyde (Pal), lithospermic acid (LA) and naringin (the internal standard, IS) were purchased from National Institute for the Control of Biological and Pharmaceutical Drugs (Beijing, China). 3,4-dihydroxyphenyllactic acid (Danshensu, DS) was provided by the Department of Phytochemistry, School of Pharmacy, Fudan University (Shanghai, China). The purity of these compounds was over 98%, determined by HPLC. HPLC grade acetonitrile and methanol were obtained from TEDIA company, Inc. (Fairfield, USA.). Phosphoric acid, hydrochloric acid and ethyl acetate were of analytical grade from Sino-pharm Chemical Reagent Co., Ltd. (Shanghai, China). The purity of these compounds was over 98%, determined by HPLC. HPLC grade acetonitrile and methanol were obtained from TEDA company, Inc. (Fairfield, USA.).

Animals
Six male beagle dogs (8-12 kg) were obtained from Animal Center of Fudan University (Shanghai, China). Animals were kept in environmentally controlled breeding room for one week before starting the experiments. Standard laboratory food and water was given ad libitum. The animals were fasted overnight before drug administration maintained with physiological saline. All animal experimental procedures were based on the guideline recommended by the Animal Experimentation Ethics Committee of Fudan University.

HPLC analysis
HPLC-UV analysis was performed with a Shimadzu 20AB liquid chromatography system (Tokyo, Japan) equipped with a SPD-20A UV detector. A 5 µm Hypersil RP C18 column (4.6×250 mm) was used and maintained at 25°C with a flow rate of 1.0 mL/min. The detection wave length was 286 nm. The mobile phase consisted of acetonitrile (A) and phosphoric acid solution (2.6 mM, pH 2.5, B). A linear gradient elution of A and B was used starting with 2% A and 98%B to reach 21% A and 79% B at 18 min. The ratio was kept for 27 min. Then the system recovered to the initial conditions in 7 min.

200 µL plasma sample was vortex mixed with 15 µL of internal standard solution (3.96 µg/mL of naringin in water), 30 µL of 2 M HCL and 20 µL of Vitamin C (4 mg/mL) for 30 s and then extracted with 2 mL of ethyl acetate twice. The precipitation was separated by centrifugation at 3000×g for 10 min. The supernatant was transferred into a clean test tube and evaporated to dryness under a flow of nitrogen gas at 30°C. The residue was dissolved with 100 µL of methanol and centrifuged at 10000×g for 10 min. An aliquot of 20µL of the supernatant was injected into the HPLC-UV system for analysis. Calibration curves were constructed based on HPLC analysis of a standard mixture prior to each experiment.

Pharmacokinetic and pharmacodynamic study
The study was conducted according to a randomized three-way crossover design. SAs were administered (9 mg/kg i.v. and 180 mg/kg p.o., 1 mL/kg weight) to beagle dogs in experiment groups and physiological saline was given in control group. The solution of SAs was made shortly before administration. A 3 mL blood sample was withdrawn into a heparinized tube according to the programmed schedule from the other femoral vein at 0 h (predose), 0.17 h, 0.33 h, 0.5 h, 0.75 h, 1 h, 1.5 h, 2 h, 4 h, 6 h and 8 h after oral administration and at 0 h (predose), 0.033 h, 0.083 h, 0.17 h, 0.25 h, 0.5 h, 0.75 h, 1 h, 1.5 h, 2 h, 4 h and 6 h after intravenous injection of SAs and normal saline. 1 mL blood was centrifuged at 3000×g for 10 min. The resulting plasma sample was immediately frozen and kept at -30°C until analysis. 2 mL heparinized blood was taken to measure whole blood viscosity at predose and 0.5h, 1 h, 2 h and 6 h time post-dose for all three groups with LIANG-200 Type Capillaryviscosity meter (Shanghai Medical Instrument Factory, China). In order to reflect various blood flow rate, the shear rates ranged from 10 1/s to 110 1/s. A minimum one week was allowed for washout between each treatment.