Comparative Pharmacokinetic Behavior of Glycyrrhetic Acid after Oral Administration of Glycyrrhizic Acid and Gancao-Fuzi-Tang

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Comparative pharmacokinetic profiles of glycyrrhetic acid (GA), glycyrrhizic acid (GL) and Gancao-Fuzi-Tang (KF) after oral administration of GL and KF were studied. Plasma samples taken from rats were acidified with acetic acid and GA was extracted with isopropanol–ethanol (1 : 1). Separation of GA was performed on a C18 column with the detection wavelength set at 254 nm. The mobile phase was methanol–acetonitrile–water–acetic acid (58 : 18 : 24 : 1 v/v). The results showed that the mean residence time and area under the curve of GA in KF-administered rats were 27.6±1.5 h and 122.8±46.7 μg·h/ml respectively, which were significantly different from those in GL-administered rats (15.0±2.0 h and 40.9±9.6 μg·h/ml respectively). The results suggest the increased effect of GA after oral administration of KF in comparison with GL.

Key words Gancao-Fuzi-Tang; glycyrrhizic acid; glycyrrhetic acid; pharmacokinetics

Glycyrrhiza uralensis Fisch. is one of the most often used drugs in traditional Chinese medicine (TCM) prescriptions. Glycyrrhizic acid (GL), the glycoside of glycyrrhetic acid (GA), is one of the major ingredient in licorice.1 GL has been used in the treatment of hepatitis and chronic hepatitis.2,3 GL is also effective against allergic disorder4 and gastric ulcer.5 Both GL and GA possess the anti-inflammatory activity.5,7

Gancao-Fuzi-Tang (KF, Kanzo-Bushi-To in Japanese) is reported originally in Treatise on Febrile Disease written by Zhang Zhongjing. The prescription consists of Glycyrrhiza uralensis Fisch., Aconitum carmichaeli Debx., Atractyloides macrocephala Koiz. and Cinnamomum cassia Presl. It has been used in treating rheumatic and rheumatoid disease for a long time and has produced quite a favorable effect.8,9 When GL was orally administered, GA instead of GL was detected in serum.10 GL administered orally was converted to GA by intestinal bacteria in rats.11,12 It was also found that GL was subjected to presystemic metabolism and enterhepatic circulation.13,14 Many studies report the pharmacokinetics of GL, while only one literature concern the pharmacokinetics of KF, only cinnamic acid and 6E,12E-tetradecadiene-8,10-diyne-1,3-diol diacetate were detected in plasma.15 In the present study, the pharmacokinetic behavior of GA after oral administration of KF in rats was compared with that after oral administration of GL.

MATERIALS AND METHODS

Materials Gancao (Glycyrrhiza uralensis Fisch.), Fuzi (Aconitum carmichaeli Debx.), Baizhu (Atractyloides macrocephala Koiz.), Guizhi (Cinnamomum cassia Presl.) were purchased from Tianyitang (Shenyang, China), glycyrrhizic acid and glycyrrhetic acid were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Diphenyl and nandroline phenylpropionate were ordered from the National Institute for the control of Pharmaceutical and Biological Products (Beijing, China). Methanol and acetonitrile were of chromatographic grade. All the other reagents were of analytical grade.

Animals Male Wistar rats (250—300 g) were obtained from the Laboratory Animal Center of Shenyang Pharmaceutical University. All animal use procedures were in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of People’s Republic of China on November 14th, 1988. The rats were fasted for 12 h and with free access to water prior to the experiments.

Chromatographic Condition The HPLC system consisted of a LC-10AD pump, SPD-10A UV spectrophotometric detector (Shimadzu, Co., Ltd., Kyoto, Japan) and an LC workstation for data collection. Separation was performed on a Hypersil C18 column (5 μm particle size, 250×4.6 mm i.d.) from Yilite Co. (Dalian, China) at room temperature. The mobile phase was methanol–acetonitrile–water–acetic acid (24 : 29 : 47 : 1, 58 : 18 : 24 : 1 v/v) for the detection of GL and GA respectively. The detection wavelength was set at 254 nm and the flow rate was 0.8 ml/min.

Preparation of KF Gancao 5 g, Fuzi 5 g, Baizhu 5 g, Guizhi 10 g were extracted twice by refluxing with 250 ml 50% ethanol, each time for 2 h. After evaporating the solvent under reduced pressure, the residue was redissolved with water.

Drug Administration and Blood Sampling GL water solvent was administered orally at a dose of 100 mg/kg. The dose of KF was equally calculated as the amount of GL and GA wasn’t detected in KF after HPLC determination. The administered volume was 2 ml. Blood samples (about 800 μl) were collected in the heparinized tubes according to a specific schedule (at 0, 4, 8, 10, 12, 14, 16, 25, 28, 32, 36, 40, 50, 55 and 60 h after dosing). The blood samples were immediately centrifuged at 3000g for 10 min to get the plasma. Because large volume of blood samples were required, 10 rats were used in each group, in which 5 rats were used for blood collecting at the seven time points ahead and the other 5 rats were used at the remained eight time points to make up for the blood loss.

Pretreatment of Plasma Sample Aliquot portion (400 μl) plasma sample was spiked with 10 μl of acetic acid solution containing internal standard (0.104 mg/ml of diphenyl for GL and 0.152 mg/ml of nandroline phenylpropionate for GA), then the mixture was extracted with isopropanol–ethanol (1 : 1) 2 ml. The resulting solution was vortexed for 5 min and then centrifuged at 3000g for 5 min.
The supernatant was dried at 40 °C water bath under a stream of nitrogen. The residue was reconstituted in 100 μl methanol and stored at −20 °C until use. A 10 μl aliquot was injected into HPLC system for analysis.

**Validation of the Method** The calibration curve of GL and GA was linear in the range of 1.25—200 μg/ml and 0.506—81.0 μg/ml respectively. The detection limit of GL and GA was 0.5 μg/ml and 0.2 μg/ml respectively. The within day precision and between day precision of GL and GA at low, medium and high concentrations were found to be less than 10.7%. The accuracy of GL and GA at low, medium and high concentrations were 7.1%, 0.6%, −4.0% and 2.9%, 3.2%, −3.3% respectively. The recoveries of GL and GA were above 94.7% and 88.9% respectively. The precision, accuracy and the recovery were conformed with the principle of bio-sample analysis. The representative chromatograms are shown in Fig. 1.

**Pharmacokinetic Analysis** Experimental data and the pharmacokinetic parameters were expressed as mean±S.D. The concentration–time curve was plotted and all the data were processed with the computer program TOPFIT (V2.0, Godecke, Schering, Thomae). Due to the existence of entero-hepatic circulation, statistical moment theory instead of compartment model was used to calculate the pharmacokinetic parameters such as mean residence time (MRT) and area under the curve (AUC_0−∞). The maximum concentration (C_{max}) and the time to C_{max} (T_{max}) were obtained from the observed data. The terminal elimination rate constant (λ) was calculated by fitting individual data for three terminal points of the plasma concentration profile with a log-linear regres-

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![Fig. 1. Representative Chromatograms of Blank Sample (A), Blank Plasma Spiked with GL and Internal Standard (B), Plasma Sample of Rat Given KF (C) under the Chromatographic Condition for GL Determination and Representative Chromatograms of Blank Sample (D), Blank Plasma Spiked with GA and Internal Standard (E), Plasma Sample of Rat Given KF (F) under the Chromatographic Condition for GA Determination](image)

The pharmacokinetic parameters are shown in Table 1. The pharmacokinetic profile of GA in the absorption phase showed that the process which GL was metabolized to GA by β-glucuronidase wasn’t influenced by the other constituents in KF, and the formation of GA wasn’t significantly changed. The results also showed that the elimination of GA after oral administration of KF was very much longer than that after oral administration of GL. GA could not be detected after 28 h after administration of GL, whereas GA could still be detected until 60 h after oral administration of KF. MRT was prolonged to 27.6 h. Cantelli-Forti et al. reported that the bile flow in rats administered orally with licorice extract was significantly increased compared with that in rats administered with pure GL. Under the effect of enterohepatic circulation, GA was transported from blood to the intestinal tract via the biliary excretion. The increase of bile flow could increase the reuptake of GA, which meant the less excretion, followed that AUC was increased significantly. GA could also combine with the alkaloids in KF and released slowly, resulted in the delayed excretion and the prolonged MRT. We assume that the increased AUC may be due to the inhibition of metabolism of GA by the other constituents in KF. Akao et al. reported that GA was metabolized to the 22α-hydroxyglycyrrhetinic acid and 24-hydroxyglycyrrhetinic acid. Further experiments were needed to prove the inhibition effect on the hydroxylase by the other constituents in KF. In the treatment of rheumatic disease, it usually needs a long duration, the high bioavailability and long-term effect of KF is very meaningful for the treatment.

**REFERENCES**