Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 44 (2007) 1113–1117
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Pharmacokinetics and bioavailability of gentiopicroside from decoctions of *Gentianae* and *Longdan Xiegan Tang* after oral administration in rats—Comparison with gentiopicroside alone

Chang-hong Wang [a,b,c], Xue-mei Cheng [a,c], S.W. Annie Bligh [d], Kenneth N. White [d], Christopher J. Branford-White [d], Zheng-tao Wang [a,b,c,*]

[a] *Key Laboratory of Standardization of Chinese Medicines, Ministry of Education, 1200 Cailun Road, Shanghai, China*
[b] *The Institute of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Shanghai, China*
[c] *Shanghai R&D Centre for Standardization of Chinese Medicines, 1200 Cailun Road, Shanghai, China*
[d] *Institute for Health Research and Policy, London Metropolitan University, 166-220 Holloway Road, London N7 8DB, United Kingdom*

Received 3 February 2007; received in revised form 26 March 2007; accepted 25 April 2007
Available online 5 May 2007

Abstract

The pharmacokinetics and bioavailability of gentiopicroside (GPS), an active component of the Gentian plant species, from orally administered decoctions of *Gentianae* (DG), or in combination with other plants in the prescription of *Longdan Xiegan Tang* (LXT), was compared in rats with oral administration of GPS alone, using doses adjusted to deliver equivalent amounts of GPS (150 mg/kg). Changes in plasma levels of GPS following oral administration of GPS or DG could be fitted to a one compartment open model with elimination half times ($T_{(1/2)K_e}$) of 3.35 ± 0.76 h and 6.21 ± 3.07 h, respectively. Kinetics of plasma GPS following oral administration of LXT could be fitted to a two compartments open model with an elimination half time ($T_{(1/2)\beta}$) of 3.83 ± 1.54 h. The bioavailability of GPS from DG was markedly better, and that from LXT markedly worse, compared with GPS alone, as judged by the area under concentration-time curve (AUC) values of 70.0 ± 13.9 μg h/ml (DG), 32.7 ± 12.9 μg h/ml (GPS) and 19.1 ± 5.9 μg h/ml (LXT). The study demonstrates the marked variability in pharmacokinetics and bioavailability of an active component from different herbal preparations.

Keywords: Gentiopicroside; *Gentianae*; *Longdan Xiegan Tang*; Pharmacokinetics; Bioavailability

1. Introduction

Gentiopicroside (GPS, Fig. 1), an iridoid glycoside, one of the main bitter principals of gentians, is the bioactive components of the gentian species of plants [1–3]. It has been used to treat inflammatory conditions such as rheumatoid arthritis, liver illness (hepatitis), fever, digestive and intestinal disorders [4–7]. Despite the widespread use of *Gentiana* extracts there have been few studies on the uptake of the active components of the herb. Since GPS has been demonstrated to exhibit these pharmacological effects, the pharmacokinetics of GPS is useful for designing an ideal dosing regimen in pharmacological studies. Furthermore, the pharmacokinetic profile contributes to the safety and efficacy of GPS in clinical applications.

Recent studies showed that GPS has a low bioavailability (*%F*) [8]. The low *%F* value, about 39.6% [8], may be attributed to the first-pass metabolism in the gut wall or liver, metabolism or decomposition in the intestine by bacterial microflora, and/or poor absorption from gastrointestinal tract [9,10]. To improve the clinical efficacy, therefore, it is important to efficiently increase the *%F* value of GPS and enhance its concentration in blood.

In clinical application, most traditional Chinese medicines (TCMs) have being prescribed by more than two herbal crude drugs to obtain the additive effects or to diminish the possible adverse responses. Therefore, to evaluate the effect of Chinese medicinal prescriptions on the pharmacokinetics of GPS is an important topic for further studies. The traditional Chi-

* Corresponding author at: Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Shanghai 201203, China. Tel.: +86 021 51322507; fax: +86 021 51322519.
E-mail address: wangzht@hotmail.com (Z.-t. Wang).

0731-7085/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2007.04.036

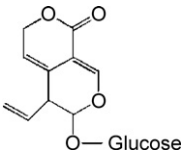

Fig. 1. Structure of gentiopicroside.

nese prescription *Longdan Xiegan Tang* (LXT, Chinese name), composed of ten important plants, *Radix Gentianae, Radix Scutellariae, Fructus Gardeniae, Rhizoma Alismatis, Caulis Hocquartiae, Semen Plantaginis, Radix Angelicae Sinensis, Radix Rehmanniae, Radix Bupleuri and Radix Glycyrrhizae*, is one of the famous Chinese prescriptions and is widely used in Chinese medication for anti-inflammatory, anti-infection, anti-bacteria, anti-allergy, liver protection, cholagogue, and immunostimulant [11,12]. The pharmacological effects of GPS are closely similar to those of LXT suggesting that GPS is one of the major active components of LXT [13]. The pharmacokinetics of GPS absorption and elimination, and its bioavailability from LXT has not been reported. To provide a firm basis for the design of dosing regimens in clinical applications and pharmacological experiments, the present study was designed to describe the plasma profiles and compare the pharmacokinetics of GPS in rats after oral administration of GPS alone, LXT or a signal herb decoction of *Radix Gentianae* (DG), 1 of the 10 plants used to prepare LXT.

2. Experimental

2.1. Materials

GPS with purity higher than 98.5% determined by HPLC, was supplied by Shanghai R&D Center for Standardization of TCM. Reference standard of theophylline was supplied by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). *Radix Gentianae* was purchased from Changchun Medicinal Materials Company. *Caulis Hocquartiae was* purchased from Changsha Medicinal Materials Company. *Radix Scutellariae, Fructus Gardeniae, Rhizoma Alismatis, Semen Plantaginis, Radix Angelicae Sinensis, Radix Rehmanniae, Radix Bupleuri and Radix Glycyrrhizae* were purchased from Longhua Hospital of Shanghai University of TCM. All of these plant materials were authenticated by Dr. Lihong Wu and voucher specimens were deposited in Shanghai R&D Center for Standardization of TCM, Shanghai, China. Methanol was of chromatographic grade. All the other reagents were of analytical grade.

2.2. Animals

Male and female Wistar rats weighing 240–260 g were purchased from the Shanghai Experiment Animal Center. 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 animals were housed in different cages (five animals per cage) and acclimated in the laboratory for at least 1 week prior to testing. Before experiments the animals were fasted for 12 h with free access to water, and maintained at 21 °C with 60% relative humidity and a 12 h light/12 h dark cycle. After surgery, rats were housed individually in metabolite cages for 1 week recovery and underwent pharmacokinetic treatment according to a jugular-catheterized rat model [14].

2.3. Preparation of DG and LXT

To prepare DG decoction, *Radix Gentianae* (12 g) was boiled with 50 ml water for 1 h and filtered. This was repeated three times and the filtrates were combined and concentrated to 30 ml by rotary evaporator at 80 °C.

To prepare LXT decoction, a mixture of *Radix Gentianae* (12 g), *Radix Scutellariae* (6 g), *Fructus Gardeniae* (6 g), *Radix Bupleuri* (12 g), *Rhizoma Alismatis* (12 g), *Caulis Hocquartiae* (6 g), *Semen Plantaginis* (6 g), *Radix Angelicae Sinensis* (6 g), *Radix Rehmanniae* (12 g), and *Radix Glycyrrhizae* (6 g) were coboiled with 350 ml water for 1 h and filtered. This was repeated three times and the filtrates were combined and concentrated to 200 ml by rotary evaporator at 80 °C.

In order to adjust the dosage protocol in experiment groups, the concentrations of GPS in each decoction were measured by HPLC before oral administration.

2.4. Animal surgery

Silicone medical grade tubing 100 mm long (Silastic Cat. No. 602-155; Dow Corning, Midland, MI, USA) with two stoppers made of medical adhesive silicone (Silastic Cat. No. 801; Dow Corning) was used. Rats were anesthetized by intraperitoneal administration of a solution of pentobarbital sodium (10 mg/ml), at a dosage of 40 mg/kg of body weight, and were anesthetized by inhalation of diethyl ether for maintenance. A longitudinal skin incision was made over the area where the right external jugular vein passed dorsal to the pectoralis major muscle. The catheter, filled with 20 units/ml heparinized physiologic saline, was put into the tight jugular vein and then advanced into the sinus venosus. The catheter was inserted up to the first silicone stopper and anchored in place by suturing the stopper to muscle. The free end of the catheter was passed under the skin of the dorsum of the neck just caudal to the ears and attached to the skin, together with a metal spring, which was covered with PVC tubing for protection of the outer part of the catheter. Finally, the catheter was filled with 500 units/ml heparinized saline, and a plug was inserted in the free end of the catheter [14,15].

2.5. Pharmacokinetic experiments in the jugular-catheterized rat model

Pharmacokinetic experiments were performed on 18 jugular-catheterized rats that were randomly divided into three groups for three protocols: oral administration of GPS, oral administration of DG, and oral administration of LXT. An aqueous solution of GPS was administered orally to rats by *gavage* at a dose containing 150 mg/kg GPS (1.5 ml per 100 g body weight). Doses

of DG and LXT were adjusted to deliver 150 mg/kg GPS, following analysis of GPS content by HPLC. After administration, jugular vein blood samples were collected (0.25 ml) from the rats into heparinized 1.5 ml microcentrifuge tubes at the following time intervals: 0, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 480, 600 and 720 min. The blood samples were centrifuged at 3000 × *g* for 10 min to obtain the plasma and stored at −20 °C until analysis. Data from these samples were used to construct pharmacokinetic profiles by plotting drug concentrations versus times.

2.6. Pre-treatment of plasma sample

GPS and the internal standard were purified from plasma by solid phase extraction (SPE) as described previously [8] with some modifications. In brief, an SPE cartridge (1 ml Supelclean LC-18 SPE column, Supelco Co.) was conditioned and washed with 2 ml of methanol and then 2 ml of deionized water. 100 μl of the plasma sample was spiked with 200 μl of internal standard solution (5.18 μg/ml of theophylline), and then 100 μl of 25% perchloric acid solution added. The mixture was vortexed for 1 min and then 500 μl of a mixture of 1 M monopotassium phosphate and 1 M potassium hydroxide was added. The resulting mixture was centrifuged at 4000 × *g* for 10 min at ambient temperature. The supernatant was applied to the SPE well under vacuum. The SPE wells were washed with 1 ml of water, and the analytes eluted with 1 ml of methanol. The eluate was evaporated to dryness in a gentle stream of nitrogen at 50 °C and the residue was reconstituted in 200 μl of a mixture of water and methanol (70:30). Finally a 30 μl aliquot was injected to HPLC for analysis.

2.7. HPLC determination for GPS and the validation of the assay

HPLC analyses were conducted by using a Waters 996 series HPLC system with a Diamonsil™ analytical guard column (5 μm particle size, 12.5mm × 4.6 mm, i.d.) used in tandem with a Diamonsil™ C_{18} column (5 μm particle size, 250mm × 4.6 mm, i.d.; Diamond Co.) at 40 °C. The detection was performed at 274 nm. For a better separation of GPS and internal standard from bioanalysis specimen of rat after oral administration of DG and LXT, the analytical method described previously [8] was made some modification with a gradient elution. The mobile phase consisted of methanol (A) and 0.4% phosphoric acid aqueous solution (B). The gradient elution was as follows: 0–10 min: 20% A at flow rate from 0.6 to 1.5 ml/min; 10–25 min: 20% A to 35% A at flow rate of 1.5 ml/min; 25–40 min: 35% A at flow rate of 1.5 ml/min; 40–45 min: 35% A to 85% A at flow rate of 1.5 ml/min; 45–50 min: 85% A at flow rate from 1.5 to 0.6 ml/min; 50–55 min: 85% A to 20% A at flow rate of 0.6 ml/min; 55–65 min: 20% A at flow rate of 0.6 ml/min.

2.7.1. Calibration curve

A calibration curve was constructed based on the HPLC analysis of blank rat plasma spiked with various concentrations (1.00, 2.01, 4.02, 8.03, 16.06, 32.13 and 64.26 μg/ml) of GPS, together with a fixed amount of theophylline (5.18 μg/ml). The various concentrations of GPS in the plasmas were calculated from the values of peak areas by using the equation for linear regression obtained from the calibration curve.

2.7.2. Recovery

Blank rat plasma samples were spiked with three different concentrations (2.01, 8.03 and 32.13 μg/ml) of GPS. After preparation of the plasma samples, fixed amounts of internal standard (theophylline) were added to the plasma for normalization. The resulting peak areas were compared with those standards carried in distilled water so as to calculate the recovery values.

2.7.3. Precision assay

The precision of the test was determined by five quintupled analyses of plasma samples ($n = 5$) spiked with three different concentrations (2.01, 8.03 and 32.13 μg/ml) of GPS. To determine intra-day variances, the assays were carried out on the same samples at five time intervals during one day. Inter-day variances were also determined by assaying the spiked samples over five consecutive days. Relative standard deviations (R.S.D.) were calculated from these sampled values of GPS.

2.8. Pharmacokinetic analysis

All data were subsequently processed by the pharmacokinetic software 3P97 edited by the Mathematics Pharmacological Committee, Chinese Pharmacological Society. The following pharmacokinetic parameters were calculated: absorption rate constant (K_a), distribution rate constant (α), elimination rate constant (K_e for GPS and DG, and β for LXT), peak concentration (C_{max}), time of maximum plasma concentration (T_{peak}), half-life ($T_{(1/2)K_e}$ for GPS and DG, and $T_{(1/2)\beta}$ for LXT), and other parameters. The area under the plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$) was calculated by means of the trapezoidal rule with extrapolation to infinity with terminal elimination rate constant K_e for GPS and DG, or β for LXT, respectively. The significance of differences was assessed by Student's *t*-test. Experimental data and the pharmacokinetic parameters were expressed as mean ± S.D.

3. Results

3.1. HPLC analyses of GPS in rat plasma

The representative HPLC chromatograms of blank plasma, plasma obtained at 60 min after oral administration of DG and LXT are shown in Fig. 2. The resolution of GPS and internal standard, theophylline, was greater than 3 (data not shown) and the retention times of GPS and internal standard were found to be around 22.5 and 15.0 min, respectively. No interfering peaks were observed within the time frame in which GPS and internal standard were detected.

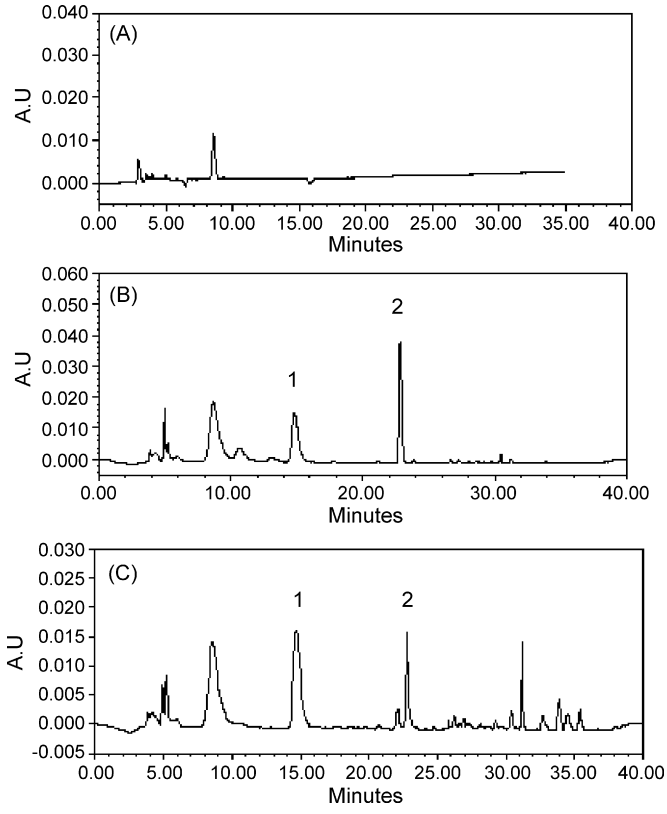

Fig. 2. The representative HPLC chromatograms of GPS in rat plasma. (A) Blank sample before oral administration; (B) Plasma sample obtained from rat at 60 min after oral administration of DG; (C) plasma sample obtained from rat at 60 min after oral administration of LXT. (1) Internal standard, (2) GPS.

3.1.1. Calibration curve

The calibration curves of GPS were linear in the measured range between 1.0 and 64.0 μg/ml with detection limit of 0.2 μg/ml. The calibration curve for area ratio of GPS to theophylline was linear ($R^2 = 0.9997$) over the range of concentrations of 1–64 μg/ml. With the least-squares method, a regression equation of $Y = 18.399X + 0.05146$ (Y was the concentration of GPS in plasma and X was the area ratio of GPS to theophylline) was obtained.

3.1.2. Recovery test and reproducibility

The data for validating the HPLC assay were shown in Table 1. The recovery was higher than 97.8%, the intra-day variation was lower than 8.7% and the inter-day variation was lower than 6.2%.

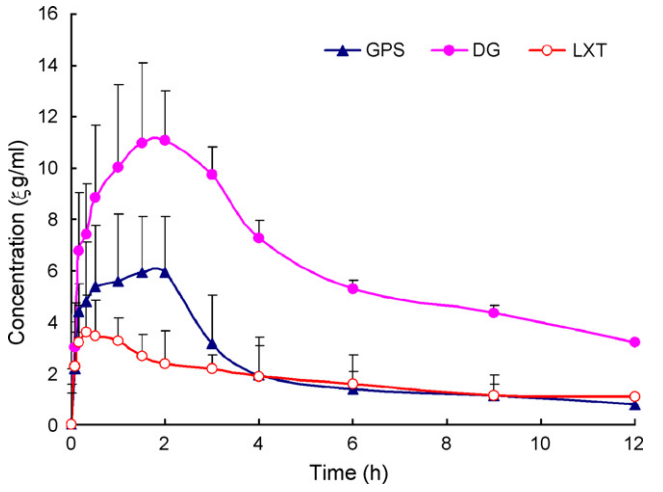

Fig. 3. Mean plasma concentration-time profile of GPS following oral administration of 150 mg/Kg GPS in rats. (▲) Oral administration of GPS alone; (●) oral administration of GPS in DG (150 mg/kg of GPS); and (o) oral administration of GPS in LXT (150 mg/kg of GPS).

3.2. Pharmacokinetics results of GPS

Representative time courses of plasma GPS after oral administration of GPS alone, DG or LXT, each at 150 mg/kg of GPS of body weight, were shown in Fig. 3. In each case there was rapid absorption, with detectable levels of GPS in the plasma 5 min after oral administration. After rapidly achieving maximal levels plasma concentrations of GPS declined sharply, followed by a slower phase of decrease until the levels fell below detection limits after 12 h in the cases of GPS administered alone or in LXT. However, levels of GPS remained significant high after 12 h administered from DG (Fig. 3). The highest levels of GPS were found after administration of DG and levels of GPS from LXT were notably lower than when GPS was administered alone.

The pharmacokinetic parameters calculated from plasma concentrations of GPS following oral administration of GPS alone or after oral administration of DG and LXT were summarized in Table 2. In comparison with GPS given alone, many parameters of GPS pharmacokinetics, including T_{peak}, C_{max}, AUC, and $T_{(1/2)K_e}$ or $T_{(1/2)\beta}$, differed significantly from DG and LXT. The mean maximum concentrations of GPS at 0.75∼1.6 h after the administration of GPS, DG or LXT were 5.78, 10.53, and 3.12 μg/ml, respectively. The plasma concentration-time curve of GPS after oral administration of LXT was corresponded to the two-compartmental open pharmacokinetic model (Table 2) with a half-life $T_{(1/2)\beta}$ of 3.83 ± 1.54 h. In contrast the

Table 1
Recovery, precision of the intra-day and the inter-day of the GPS assay

Test No.	Spiked concentration (μg/ml)	Measured value (μg/ml)[a]	Recovery (%)	Precision of the intra-day (R.S.D%)	Precision of the inter-day (R.E.D%)
1	2.008	2.1885 ± 0.039	108.99 ± 1.94	1.78	5.27
2	8.032	7.8535 ± 0.681	97.78 ± 8.48	8.68	4.55
3	32.128	31.928 ± 1.968	99.38 ± 6.13	6.16	6.19

[a] Data are expressed as rnean ± S.D. ($n = 5$).

Table 2
The main pharmacokinetic parameters of GPS after oral administration of GPS (150 mg/kg) alone, or co-administration of GPS (150 mg/kg) in DG and LXT to rats ($n = 6$), in which a one-compartmental open pharmacokinetic model was consistent to administration of GPS alone and DG, and a two-compartmental open pharmacokinetic model was consistent to administration of LXT

Parameters	GPS[a]	DG[a]	LXT[b]
K_a (1/h)	3.55 ± 2.01	4.01 ± 6.47	1.69 ± 1.S5
K_e (1/h)	0.22 ± 0.05	0.15 ± 0.12*	
α (l/h)			0.61 ± 0.26
β (l/h)			0.21 ± 0.15
$T_{(1/2)K_e}$(h)	0.21 ± 0.23	0.53 ± 0.45	0.55 ± 0.32
$T_{(1/2)K_a}$(h)	3.35 ± 0.76	6.21 ± 3.07*	
$T_{(1/2)\underline{\alpha}}$ (h)			1.24 ± 0.64
$T_{(1/2)\beta}$ (h)			3.83 ± 1.50
T_{peak} (h)	0.75 ± 0.62	1.61 ± 0.76*	1.31 ± 0.53*
C_{max} (μg/ml)	5.78 ± 2.24	10.53 ± 3.20*	3.12 ± 1.04*
AUC (μg h/ml)	32.7 ± 12.9	70.7 ± 13.9*	19.15 ± 5.91*
F_{rel} (%)		214	59

[a] One-compartmental open pharmacokinetic model.
[b] Two-compartmental open pharmacokinetic model.
* Significant difference from GPS group, $p < 0.05$.

plasma concentration-time curves of GPS after oral administration of GPS and DG were corresponded to the one-compartment pharmacokinetic model (Fig. 3 and Table 2) with half-life $T_{(1/2)K_e}$ of 3.35 ± 0.76 and 6.21 ± 3.07 h for administration of GPS alone and DG respectively. The relative bioavailability ($\%F_{rel}$) of GPS after oral administration of DG and LXT, in comparison to oral administration of GPS alone, was 214% and 59%, respectively. The data showed that GPS absorption from DG was enhanced two-fold contrasting with inhibition of GPS absorption from the decoction of LXT.

4. Discussion

Traditional Chinese Medicines (TCMs) were widely used in China, Southeast Asia and increasingly in the West and there was a need to relate clinical efficacy of TCMs to laboratory and clinical trial data. In addition to pharmacological studies, pharmacokinetic data were important. Because the interactions between herbs may increase or decrease the pharmacological or toxicological effects of each other, the herb–herb interactions should be documented. Pharmacokinetic study was one of the most important investigations for elucidating the clinical efficacy or/and explaining and predicting a variety of events related to the efficacy and toxicity of Chinese herbs [16,17].

Due to the complexity of herbal formulations, it was necessary to use purified components of herbs to provide a reference for interpreting the kinetics of different formulations. In this study we used purified GPS to compare availability of this active component from two decoctions, after oral administration in rats. In comparison with a previous study from our lab in mice [8], plasma levels of GPS after administration of pure GPS were much lower, at equivalent doses, in rats. In mice the maximal plasma levels of 55 μg/ml were found 1 h after administration, with a plasma half-life of 2.81 h [8], compared with a peak of 5.8 μg/ml at 45 min, with a half-life of 3.35 h in rats (Table 2). The evidence indicated that the species differences of pharmacokinetics of GPS resided in different animals. Interestingly the effect of other herbal components was marked, with a two-fold enhancement of bioavailability of GPS from the DG decoction, as shown by higher peak values of plasma GPS of 10.5 μg/ml, and a longer plasma half-life of 6.2 h (Table 2). A 50% reduction of GPS availability was found from the more complex LXT decoction, resulting in lower peak levels of 3.1 μg/ml (Table 2). GPS was known to have potent activity as a smooth muscle relaxant with an IC_{50} of 2.8 μg/ml [7] and levels of GPS from DG were maintained at useful concentrations of 4 μg/ml for up to 12 h, whereas GPS from LXT never reaches that level and levels from pure GPS decline below 4 μg/ml within 3 h (Fig. 3). These data illustrate the complexity of herbal pharmacokinetics and demonstrate the need to assess absorption characteristics for each type of herbal preparation. It was not safe to infer pharmacokinetic behaviour of a component of herbal mixtures from that of the sum of its individual herbs. In the present study the same amounts of *Gentianae* were used in the preparation of the DG and LXT decoctions, but clearly components of the other herbs used in LXT had a significant inhibitory affect on GPS absorption.

Acknowledgement

This study was supported by grants from the Shanghai Science & Technology Development Foundation (04DZ19851).

References

[1] R.X. Tan, L.D. Kong, Phytochemistry 46 (1997) 1035–1038.
[2] Q.S. Song, K.B. Gao, K.Z. Fu, Zhong Yao Tong Bao 12 (1987) 36–37.
[3] Y.H. Liu, X.C. Li, Y.Q. Liu, et al., Acta Bot. Yunnanica 16 (1994) 85–89.
[4] Y. Zhang, J.X. Jiang, W.M. Li, Yunnan Med. 12 (1991) 304–306.
[5] Y. Kondo, F. Takano, H. Hojo, Planta Med. 60 (1994) 414–416.
[6] Y.Q. Li, D.H. Zhao, B.R. Pan, J. Fourth Mil. Med. Univ. 22 (2001) 1645–1649.
[7] A. Rojas, M. Bah, J.I. Rojas, et al., Planta Med. 66 (2000) 765–767.
[8] C.H. Wang, Z.T. Wang, S.W. Bligh, et al., Eur. J. Drug Metab. Pharmacokinet. 29 (2004) 199–203.
[9] A.I. el-Sedawy, M. Hattori, K. Kobashi, et al., Chem. Pharm. Bull. 37 (1989) 2435–2437.
[10] X.F. Yang, C.Q. Song, Zhongguo Zhong Yao Za Zhi 25 (2000) 673–676.
[11] N.H. Zhang, J. Shandong Univ. Trad. Chin. Med. 21 (1997) 395–396.
[12] J. Zhang, J.J. Li, Clin J. AnHui Trad. Chin. Med. 15 (2003) 57–58.
[13] C.C. Chou, S.L. Pan, C.M. Teng, et al., Eur. J. Pharm. Sci. 19 (2003) 403–412.
[14] K.V. Thrivikraman, R.L. Huot, P.M. Plotsky, Brain Res. Protoc. 10 (2002) 84–94.
[15] Z.Q. Liu, H. Zhou, L. Liu, et al., J. Ethnopharmacol. 99 (2005) 61–67.
[16] P.A. De Smet, J.R. Brouwers, Clin. Pharmacokinet. 32 (1997) 427–436.
[17] P.A. De Smet, L. Rivier, J. Ethnopharmacol. 25 (1989) 127–138.