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Short Communication

Determination of glycyrrhizin in rabbit plasma by high-performance liquid chromatography with photodiode-array ultraviolet detection and its pharmacokinetics application

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ABSTRACT

A simple and sensitive high-performance liquid chromatographic method for the determination of glycyrrhizin in rabbit plasma has been developed. Up to 0.1 ml of plasma containing glycyrrhizin was deproteinized by acetonitrile, which contained an internal standard (indomethacin). The supernatant was injected onto a LiChrospher RP-18 column using a methanol–water–ammonia solution (80:20:0.1, v/v, pH 3.0–3.2, adjusted with perchloric acid) as the mobile phase and ultraviolet detection at 254 nm, followed by ultraviolet spectrum identification (between 200 and 380 nm) with a photodiode-array detector. The method is rapid, easily reproduced, selective and sensitive. It was applied to pharmacokinetic studies of glycyrrhizin in rabbit, after a 2 mg/kg intravenous administration. A biphasic phenomenon with a rapid distribution followed by a slower elimination phase was observed from the plasma concentration–time curve. Compartmental analysis yielded a two-compartment model.

INTRODUCTION

Liquorice (*Glycyrrhizae radix;* Chinese name: Gancao) is one of the most commonly used herbal drugs in traditional Chinese prescriptions, mainly as a demulcent and sweetener [1]. It has been reported that 58.5 mg of glycyrrhizin, 5.9 μg of 18α-glycyrrhetinic acid and 95.3 μg of 18β-glycyrrhetinic acid are contained in an aqueous extract of 1 g of liquorice [2]. In our previous study [3], a simple high-performance liquid chromatographic (HPLC) method was developed for the separation and determination of 18α-glycyrrhetinic acid and 18β-glycyrrhetinic acid, and this method was applied to study their pharmacokinetics. Recently, a number of methods involving HPLC and UV detection [4,5] have been described. In the present paper, we report an HPLC method using photodiode-array detection and UV spectrum identification of glycyrrhizin in

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rabbit plasma and apply it to the study of pharmacokinetics [3,6,7].

EXPERIMENTAL

Chemicals and reagents

Glycyrrhizin was purchased from Sigma (St. Louis, MO, USA). Methanol and acetonitrile (HPLC grade), indomethacin, perchloric acid (70%) and ammonium solution (32%) were obtained from Merck (Darmstadt, Germany). Three times deionized water (Millipore, Bedford, MA, USA) was used for all preparations. The stock standard solution of glycyrrhizin was prepared by dissolving 10 mg of glycyrrhizin in 100 ml of water, and indomethacin was dissolved in acetonitrile at a concentration of 5 μg/ml and stored at 4°C. These solutions were stable for at least one month.

Chromatography

The HPLC system consisted of a Waters U6K injector, a Waters M 990 photodiode-array detector and a Waters 510 chromatographic pump (Milford, MA, USA). Separation was achieved on a reversed-phase LiChrospher RP-18 column mm, 125 mm × 4 mm I.D., 5 μm particle size, fitted with a guard column, LiChrospher, 4 mm × 4 mm I.D., 5 μm particle size (Merck). The mobile phase was a methanol–water–ammonium solution (80:20:0.1, v/v, pH 3.0–3.2 adjusted with perchloric acid) at a flow-rate of 1.0 ml/min.

Animals

Male New Zealand albino rabbits (2.5–3.0 kg) were obtained from the Laboratory Animal Center at the National Taiwan University. These animals were kept in our own environmentally controlled quarters, with the temperature mantained at 24 ± 1°C and a 12-h light–dark cycle (light 07:00–19:00 h) for at least one week before use. Water and standard laboratory chow were given *ad libitum* until 18 h before the experiments, at which time only food was withdrawn.

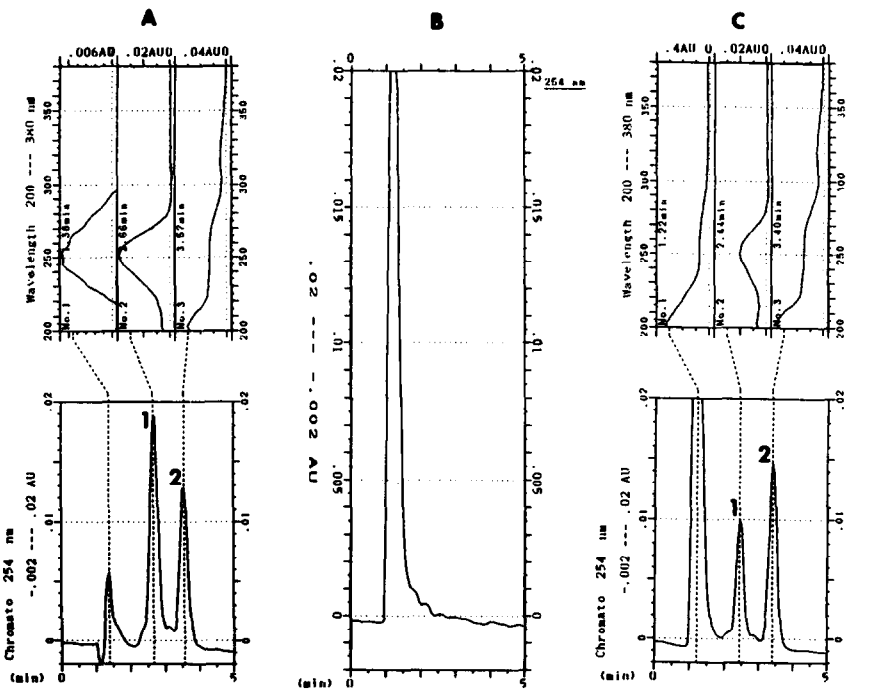

Fig. 1. (A) Peak and UV spectra of authentic glycyrrhizin and indomethacin, respectively. (B) Chromatogram of blank plasma. (C) Peak of glycyrrhizin and its UV spectrum of extracted plasma sample 10 min after a 2 mg/kg intravenous administration of glycyrrhizin (1.83 μg/ml). Peaks: 1 = glycyrrhizin; 2 = indomethacin.

Sample preparation

A 0.5-ml blood sample was directly withdrawn from the ear vein of conscious rabbits, which were minimally restrained in a rabbit holder. Blood samples were collected at time intervals of 5, 10, 15, 20, 30, 45, 60 and 90 min and 2, 3 and 4 h after intravenous administration of glycyrrhizin (2 mg/kg). Six animals were used for the test. The blood sample was then transferred to a heparinized microfuge tube and centrifuged at 8000 *g* for 5 min (Eppendorf 5402). The resulting plasma (0.1 ml) was mixed with a 0.2-ml portion of acetonitrile, which contained 5 μg/ml indomethacin as internal standard. The denatured protein precipitate was separated again by centrifuging at 8000 *g* for 5 min. The supernatant (20 μl) was direclty injected onto the HPLC system for analysis.

RESULTS AND DISCUSSION

Under the conditions described above, the retention times of glycyrrhizin and indomethacin were 2.66 and 3.57 min, respectively (Fig. 1). Fig. 1A shows the chromatograms and the UV spectra of the authentic compound. Fig. 1B shows the blank rabbit plasma and no background interference from endogenous constituents. Fig. 1C shows the chromatogram and the UV spectra of a plasma sample which was obtained from rabbit 10 min after intravenous administration with glycyrrhizin (2 mg/kg), while the concentration of glycyrrhizin was 1.83 μg/ml. The main characteristic spectral data obtained in the mobile phase were absorption maxima at 254 nm for glycyrrhizin, and at 206, 263 and 320 nm for indomethacin. The equation of the calibration curve was $y = 0.0612x - 0.0083$ for rabbit plasma, where x is amount of compound analysed and y is response in peak-area ratio. The correlation coefficient was 0.999.

For recovery studies, 1-ml portions of blank plasma spiked with 0.1, 1 or 10 μg of glycyrrhizin ($n = 4$) were extracted as described above. The recoveries were between 92 and 94% [coefficient of variation (C.V.) = 3.5–5.7%] for plasma.

The reproducibility and accuracy of the method were indicated by the intra-day C.V.s of 5.36, 5.01 and 2.33% and accuracies of 101.21, 98.41 and 98.37%, and by inter-day C.V.s of 6.53, 5.23

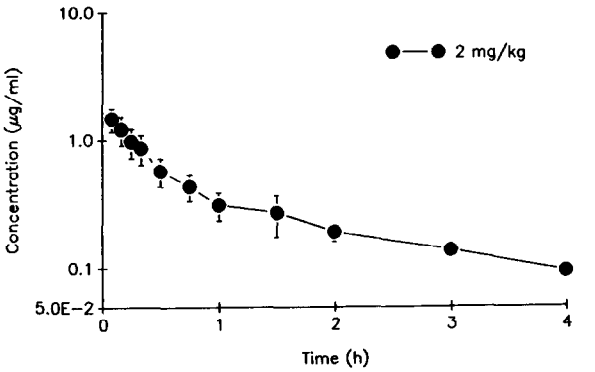

Fig. 2. Plasma concentration–time curve for glycyrrhizin following intravenous administration of 2 mg/kg to rabbits showing a two-compartment model ($n = 6$).

TABLE I

ESTIMATES OF PHARMACOKINETIC PARAMETERS ACCORDING TO A TWO-COMPARTMENT OPEN MODEL WITH ELIMINATION FROM THE CENTRAL COMPARTMENT WHEN A DOSE OF 2 mg/kg GLYCYRRHIZIN WAS INTRAVENOUSLY ADMINISTERED TO MALE RABBITS

Data are expressed as mean ± standard error of the mean ($n = 6$).

Parameter[a]	Estimate
A (μg/ml)	1.82 ± 0.08
B (μg/ml)	0.60 ± 0.03
α (1/h)	3.25 ± 0.34
β (1/h)	0.39 ± 0.02
k_{10} (1/h)	1.18 ± 0.12
k_{12} (1/h)	1.34 ± 0.19
k_{21} (1/h)	1.23 ± 0.27
$T_{1/2,\beta}$ (h)	1.80 ± 0.12
C_{max} (μg/ml)	2.42 ± 0.12
AUC (μg h/ml)	2.10 ± 0.29
Vol (ml/kg)	729.97 ± 40.86

[a] A and B = concentration intercepts for fast and slow disposition; α and β = disposition rate constants for fast- and slow-disposition phases; k_{10} = elimination rate constants from central compartment; k_{12} and k_{21} = transfer rate constants from central to peripheral and from peripheral to central compartment, respectively; $T_{1/2,\beta}$ = elimination half-life; C_{max} = concentration of drug in plasma at $t = 0$; AUC = area under the concentration–time curve; Vol = volume of distribution.

and 3.94% and accuracies of 97.43, 95.87 and 97.94% over a period of six days for the three concentrations (0.1, 1 and 10 μg/ml) tested. The detection limit obtained for plasma glycyrrhizin was 100 ng/ml (signal-to-noise ratio = 3).

Kinetics analyses through a JANA and PCNONLIN program (purchased from SCI Software, Lexington, KY, USA) suggested a two-compartmental model. The equation of the disposition curve was $c = 1.82e^{-3.25t} + 0.60e^{-0.39t}$, aftger glycyrrhizin (2 mg/kg, intravenously) administration (Fig. 2). The estimates of these pharmacokinetic parameters are listed in Table I.

In conclusion, the UV spectrum identification, extraction and chromatographic procedures described in this study allow the quantitation of glycyrrhizin from rabbit plasma. The pharmacokinetic study of glycyrrhizin (2 mg/kg, intravenously) was characterized by the two-compartmental model.

REFERENCES

1 Juangsu New Medical College, *Zhong Yao Da Ci Dian (Dictionary of Chinese Materia Medica)*, Shanghai Scientific and Technological Publishers, Shanghai, 1985, pp. 567–575.
2 T. H. Tsai and C. F. Chen, *J. Chromatogr.*, 542 (1991) 521.
3 T. H. Tsai and C. F. Chen, *J. Chromatogr.*, 567 (1991) 405.
4 X. Y. Zhang, R. J. Wu, J. Chen and D. K. An, *J. Chromatogr.*, 495 (1989) 343.
5 Y. Yamamura, J. Kawakami, T. Santa, H. Kotaki, K. Uchino, Y. Sawada and T. Iga, *J. Chromatogr.*, 567 (1991) 151.
6 T. Ichikawa, S. Ishida, Y. Sakiya and Y. Akada, *Chem. Pharm. Bull.*, 32 (1984) 3734.
7 T. Ichikawa, S. Ishida, Y. Sakiya, Y. Sawada and M. Hanano, *J. Pharm. Sci.*, 75 (1986) 672.