High-Performance Liquid Chromatographic Method for the Determination and Pharmacokinetic Study of Mangiferin in Plasma of Rats Having Taken the Traditional Chinese Medicinal Preparation Zi-Shen Pill

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Abstract

A high-performance liquid chromatographic method for the determination and pharmacokinetic study of mangiferin in the plasma of rats that have been orally administered the traditional Chinese medicinal preparation Zi-Shen pill is established. Plasma samples taken from rats are pretreated by protein precipitation with acetonitrile. Separation of the main effective constituent mangiferin is accomplished on a C_{18} stationary phase and a mobile phase of methanol–water (25:75, v/v) with 0.6% glacial acetic acid. The UV detection wavelength is set at 320 nm, and the detection limit for mangiferin in plasma is 0.163 μg/mL. After validation, the method is used to take a limited view of pharmacokinetic profiles of the traditional Chinese medicinal preparation Zi-Shen pill.

Introduction

Traditional Chinese medicine (TCM) theories and practices have evolved over the past 4,000 years, and one unique characteristic of it is the use of a combination of herbs prepared in a formula for treatment. The principle of the combination is to enhance therapeutic action, decrease toxicity, improve pharmacokinetic and pharmacodynamic features, and increase absorption or modification of the metabolism of the active component of key herbs. Today, the application of these drugs is becoming popular.

The Zi-Shen pill was originally reported in *Secret Record of the Chamber of Orchids* (by Li Gao) in the years of Yuan Dynasty in China (1279–1368 A.D.) and was listed in the first edition of the *Drug Standard of Ministry of Public Health of the People's Republic of China* (1). The pill is prepared by mixing *Rhizoma anemarrhenae, Cortex phellodendri*, and *Cortex cinnamomi* with honey. In the clinical practice of TCM science, the pill has been used to treat prostatitis, ptostatomegaly, and infection of the

urinary system diseases over a very long period of time, and it has produced a quite good effect (2).

Rhizoma anemarrhenae is the main medicine of the Zi-Shen pill, and mangiferin (Figure 1) is the major active constituent of *Rhizoma anemarrhenae*. Thus, mangiferin is used as one of the marker compounds to tentatively characterize the pill. There are some determination methods for mangiferin (3–5). Because of the complexity of chemical constituents in TCM formulas, there are barely any reports of their pharmacokinetic studies. This paper, however, reports a pharmacokinetic study accomplished on the constituent mangiferin of the Zi-Shen pill to provide a limited view of its pharmacokinetic profiles.

Experimental

Materials and reagents

Zhimu (*Anemarrhena asphodeloides Bge*), Huangbo (*Phellodendron chinense Schneid* or *Phellodendron amurense Rupr*), and Rougui (*Cinnamomum cassia Presl*) all were purchased at Tianyitang TCM shop (Shenyang, China). The Zi-Shen pill was prepared according to the conventional method: with mangiferin and *p*-nitrophenol, both ordered from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Ethanol and glacial acetic acid were of analytical grade, and methanol and acetonitrile were of chromatographic grade.
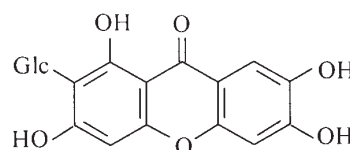

Figure 1. Structure of mangiferin.

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Chromatographic system

The system consisted of a Shimadzu LC-2010A composed of a quaternary gradient pump, high-speed autosampler, column oven, and UV detector; Class-VP Ver.6.0 chromatography data system; and a 200- × 4.6-mm-i.d. column with a stationary phase of hypersil ODS C_{18} (5-µm particle size) and a mobile phase of methanol–water (25:75, v/v) with 0.6% glacial acetic acid. The analyses were carried out at a flow rate of 0.9 mL/min with wavelength of 320 nm and operated at 23°C.

Standard solutions

Stock solutions of standard mangiferin and the internal standard p-nitrophenol were prepared with methanol. These solutions were spiked into drug-free plasma samples of rats to determine the recovery, precision, accuracy, and detection limit of the high-performance liquid chromatographic (HPLC) method. All standards were stored at 4°C before use.

Sample extraction

An appropriate amount of the Zi-Shen pill was extracted with 50% aqueous ethanol by refluxing on a water bath at 100°C and then filtered. The extraction was repeated twice. The extraction solvent was combined and ethanol was removed with reduced-pressure evaporation.

Plasma sample preparation

The Zi-Shen pill was orally administered to rats. After a designated time period, the cervical artery was cut under ether anesthesia, and the blood was collected. Plasma samples (0.5 mL) from rats were precipitated with 1.3 mL of acetonitrile containing p-nitrophenol (47.5 µg). After vortex mixing for 1 min and centrifuging at 2500 rpm for 10 min, the supernatant was evaporated to dryness under nitrogen at 60°C. The residue was reconstituted with 0.1 mL of the mobile phase, and an aliquot (20 µL) was injected into the HPLC system.

Calibration procedure

We used blank plasma from rats spiked with stock solutions of standard mangiferin for the construction of the calibration curve. Evaluation of the assay was performed with a six-point calibration curve in the concentration range of 0.163–16.32 µg/mL. The slope and the intercept of the calibration graphs were calculated through weighted least-squares linear regression (6) of each drug to internal standard peak-area ratios versus drug concentration. Experimental peak-area ratios were interpolated on the relative calibration curve, and the concentrations were calculated.

Recovery, precision, and accuracy

The recovery was determined by a standard addition method at three concentrations (0.163, 4.896, and 16.32 µg/mL), and the precision (within-day and day-to-day) of the method was calculated at the same three concentrations. The variability of the peak-area ratio at each concentration was determined as a measure of the precision of the assay. The accuracy was determined by comparing the measured concentration with its true value.

Results and Discussion

Typical chromatograms of the blank and spiked plasma are given in Figure 2A and 2B, in which the retention time was 14.5 min for mangiferin and 18.6 min for p-nitrophenol, respectively. There are no coeluting disturbing peaks in the vicinity of the two peaks on the chromatogram of the blank plasma. A chromatogram of plasma sample of rat taken 5 h after oral administration of the Zi-Shen pill is given in Figure 2C.

The calibration curve for the determination of mangiferin in rat plasma is linear over the range of 0.163–16.32 µg/mL with a coefficient of determination (r^2) of 0.998 ($n = 6$). The linearity range will permit the use of this method in future pharmacokinetic studies of this drug. The quantitation limit was 0.163 µg/mL. The within-day precision [percent relative standard deviation (%RSD)] was approximately 2.0–8.0% ($n = 18$), and the day-to-day precision (%RSD) was approximately 5.7–7.0% ($n = 18$). The accuracy was approximately –9.3% to 9.5% ($n = 18$). The recovery of mangiferin was obtained through the comparison of concentrations of its methanol extracts with those of the corresponding spiked plasma. The mean recovery was 77.0% ($n = 6$). In all instances, the accuracy, precision, and recovery showed satisfactory levels (7,8).
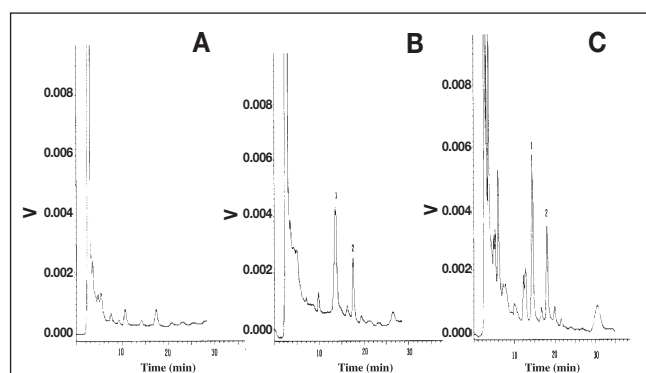
Figure 2. Typical chromatograms for determination of mangiferin in plasma samples. (A) Blank plasma sample, (B) plasma sample spiked with mangiferin and internal standard, and (C) plasma sample of rat taken 5 h after oral administration of the Zi-Shen pill. (1) Mangiferin and (2) internal standard.
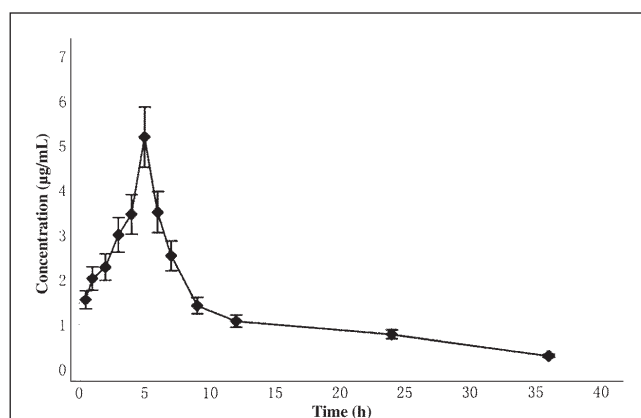
Figure 3. Plot of the mean concentration of mangiferin in plasma of rats against time after oral administration of the Zi-Shen pill.

The assay has been applied to the pharmacokinetic study of mangiferin in a TCM preparation of Zi-Shen pill. Plasma samples from rats were taken at 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 9.0, 12.0, 24.0, and 36.0 h after oral administration of the pill (an oral dosage of 19 g/kg, unconcentrated). Figure 3 is the mean plasma concentration–time plot of mangiferin, in which its pharmacokinetic parameters are obtained as follows: $t_{max} \approx 5$ h, $C_{max} = 5.187 \pm 2.8$ µg/mL, and $t_{1/2} = 12.2$ h.

Several pharmacologic studies of the Zi-Shen pill on antipyretic, anti-inflammatory, and immunocompetence have been attempted, and there were some indications that mangiferin satisfied the conditions necessary to be considered a main active component of the Zi-Shen pill. Traditionally, the Zi-Shen pill has been taken orally two times a day since the ancient times of record. The value of $t_{1/2}$, which was 12.2 h as shown in this study, was consistent with the traditional use of this pill. The feasible pharmacokinetic parameters C_{max} and $t_{1/2}$ of mangiferin suggest that it may be used as a marker compound to characterize some profiles of the Zi-Shen pill.

Because of the complexity of chemical constituents in TCM formulas, pharmacokinetic study of active constituents in TCM will play an important role in illustrating their action mechanism and investigating the impact of combination. This kind of study will be very useful for the further development of TCM.

Conclusion

This paper describes a sensitive, specific, and rapid HPLC method with UV detection for the determination of mangiferin in rat plasma. This method has been proven to be suitable for the use in pharmacokinetic studies of mangiferin in the TCM Zi-Shen pill.

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