ORIGINAL ARTICLE

Determination and pharmacokinetic study of catechin in rat plasma by HPLC

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Abstract A high performance liquid chromatographic method was developed and validated for the quantitative determination of catechin in rat plasma and its pharmacokinetic study after intragastric administration of Catechu and Xiongdanjiangre Wan into SD rats. Plasma samples were prepared by protein precipitation using methanol–5% aqueous zinc sulfate (70:30, v/v) as precipitant. Chromatographic separation was achieved on Hypersil C18 column (250 mm × 4.6 mm, 10 μm) with acetonitrile–water–triethylamine (6:94:0.3, v/v/v, pH 4.0 ± 0.1, adjusted with phosphoric acid) as mobile phase, followed by a UV detection at 207 nm. Good linearity was obtained over the range of 0.143–7.15 mg/L of catechin, with correlation coefficient of 0.9992. The method was simple, sensitive, accurate and reproducible and has been successfully applied to the pharmacokinetic study of catechin in rat plasma.

1. Introduction

Xiongdanjiangre Wan was made of seven Chinese crude drugs, mainly including Catechu, fel ursi and Rhubarb. It has been widely used in detoxication and purgation. Catechu, one of the basic composition units of this compound preparation, has already been recorded in Chinese Pharmacopoeia (2010 Edition) and been used to treat eczema, bleeding wound, aphtha and so on. Catechin (Fig. 1) is one of the active components in Catechu and Xiongdanjiangre Wan. Previous studies also revealed that catechin showed strong activities of anti-atherosclerosis, antibiosis, anticancer and antioxidant [1–3].

A number of analytical techniques have been applied to the quantification and pharmacokinetic study of catechin in rabbit plasma and rat plasma [4,5]. Only a few studies on pharmacokinetic of catechin in different formulations have been reported [6]. But there is no literature report on the quantification and pharmacokinetic study of catechin in rat plasma after intragastric administration of Catechu and Xiongdanjiangre Wan. Since catechin possesses various biological effects, it is necessary to examine the difference on pharmacokinetic of catechin between Catechu and Xiongdanjiangre Wan after intragastric administration. In this study, a simple HPLC method with a simple
2. Experimental

2.1. Materials, chemicals and reagents

Xiongdanjiangre Wan and Catechu were provided by West China School of Pharmacy (Sichuan University, Chengdu, China). The reference standard of catechin was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC-grade acetonitrile was obtained from Fisher Scientific (Pittsburgh, PA, USA). Other reagents were all of analytical grade. Ultra-pure water was generated by using a water-purification system (PFC-10, Pin Cheng, Chengdu, China).

2.2. Animals

Healthy SD rats (180–200 g, grade: CV, production license No.: SCXK (Chuan) 2009-09, animal use license No.: SYXK (Chuan) 2009-045) were purchased from the Experimental Animal Center of Sichuan University. The rats were free to water and fed with a standard laboratory diet before the experiment. Animal experiments were conducted in accordance with the Guidelines provided by the Institutional Ethics Committee for Care and Use of Laboratory Animals of Sichuan Province and protocols approved by the Animal Care Committee of Sichuan University.

2.3. LC instruments and analytical conditions

Chromatography analysis was performed on an LC-10ATvp LC chromatograph (Shimadzu, Japan) with an SPD-10A vp UV–vis detector (Shimadzu, Japan) and a column oven. The detector was operated at 207 nm. The analytical column was a Hypersil C18 column (250 mm × 4.6 mm, 10 μm, Dalian Elite Analytical Instruments Co. Ltd., Dalian, China). The chromatographic data was processed by a Chromtek software (version 5.3, Alltech, USA). All analyses were conducted in isocratic mode, the mobile phase consisted of acetonitrile–water–triethylamine (6:94:0.3, v/v/v, pH 4.0 ± 0.1, adjusted with phosphoric acid) with a flow rate of 1.0 mL/min and column temperature of 35 °C.

2.4. Preparation of standard solution and working reference solution

Standard solution was prepared by weighing proper amounts of the catechin reference standard and dissolving it in methanol (24 mg/L). In order to obtain a true result reflecting the influence of coexisting compounds, the Xiongdanjiangre Wan stock extract, in which the content of catechin had been standardized by corresponding standard solution, was diluted to a certain concentration and used as the working reference solution instead of standard solution. An accurately weighed fine powder (1.0 g, through a 20-mesh sieve) of Xiongdanjiangre Wan was extracted with methanol (50 mL) for 20 min of ultrasonication, and then filtered. The filtrate was used as the stock extract. A diluted extract was prepared by diluting the stock extract to its 10 percent of concentration. An aliquot of 5 μL of the diluted extract and standard solution of catechin (24 mg/L) was injected into the HPLC system for analysis, and then the concentration of catechin in the diluted extract was obtained (28.6 mg/L) and that in the working reference stock solution was also calculated (286 mg/L). The working reference solution was prepared by diluting the working reference stock solution into the concentration of 0.715 mg/L and stored at 4 °C.

2.5. Preparation of calibration samples and quality control samples

Different volumes (10, 30, 50, 200, 300 and 500 μL) of the working reference solution were dried by nitrogen gas and then mixed with 50 μL blank rat plasma, respectively. The final concentrations of catechin in calibration samples were 0.143, 0.429, 0.715, 2.86, 4.29 and 7.15 mg/L. Quality control (QC) samples were generated with the same process to yield final three different concentrations levels (0.429, 2.86 and 7.15 mg/L). All the samples were stored at 4 °C and brought to room temperature before use.

2.6. Preparation of Catechu suspension and Xiongdanjiangre Wan suspension

Catechu suspension was prepared as follows: about 10.0 g Catechu crude herb was crushed to powder, and then was sifted through a 20-mesh (0.9 mm) sieve. 1.0 g powder was extracted with water (50 mL) in a heating–refluxing bath for 1 h after soaked for 24 h. And the extract was filtered. The filtrate was evaporated to dryness and the residue was dissolved in 50 mL water (0.02 g/mL Catechu powder in water).

Xiongdanjiangre Wan suspension was prepared as follows: 6.0 g of Xiongdanjiangre Wan powder (prepared in the same way of Catechu powder) was weighed, and prepared as the procedure of Catechu suspension, giving an equal content of Catechu to the catechu suspension.

2.7. Preparation of plasma samples

A mixture consisting of 50 μL of plasma sample, 10 mg of sodium fluoride and 100 μL of methanol–5% aqueous zinc sulfate (70:30, v/v) was vortex-mixed for 5 min and then centrifuged for 10 min at 12,000 rpm. An aliquot of 10 μL of
the supernatant was injected into the HPLC system for the
determination of catechin.

2.8. Pharmacokinetic study

The rats were randomly divided into two groups (I and II) in
half respectively male and female. An aliquot of 2.5 mL of
Catechu suspension was administered to group I, while the
same volume of Xiongdanjiangre Wan suspension to group II.
Serial blood samples (0.5 mL) were collected into heparinized
tubes from the vena caudalis at 0.25, 0.5, 0.75, 1, 1.5, 2.5, 3.5,
5, 7, 10, 12 and 24 h. Samples were immediately centrifuged at
12,000 rpm for 10 min and stored at −20°C until analysis.
The pharmacokinetic parameters of catechin were calculated
by DAS 2.1 software.

3. Results and discussion

3.1. Optimization of plasma sample pretreatment conditions

Catechin is a strongly polar compound, and can be dissolved
in methanol and acetonitrile easily. Therefore, a simple protein
precipitation procedure was available to prepare the plasma
samples. The calibration sample with a concentration of
2.86 mg/L of catechin was used as a typical one to do the
optimization. Methanol, acetonitrile and methanol–5% aqu-
eous zinc sulfate (70:30, v/v) were investigated as the pre-
cipitants. Methanol-5% aqueous zinc sulfate (70:30, v/v) was
chosen as the precipitant for the highest value of peak area.
Three governing parameters: precipitant volume (the different
ratios of selected precipitant and blank plasma: 2:1, 1:1 and
1:2), balance time for the conjugation between drug and
protein (15, 30 and 45 min), and the amount of enzyme
inhibitor (Sodium Fluoride; none, 5, 10 and 20 mg) were
optimized by univariate approach. When the volume of
methanol-5% aqueous zinc sulfate (70:30, v/v) was 2 times
of that of plasma, balance time was approximately 30 min and
sodium fluoride was 10 mg, the peak area of catechin reached
the highest value. Therefore, the best pretreatment conditions
of plasma samples were as follows: plasma samples (50 μL)
spiked with 10 mg sodium fluoride, 100 μL methanol-5%
aqueous zinc sulfate (70:30, v/v) and equilibrated for 30 min.

3.2. Method validation

3.2.1. Specificity

The specificity of the method to analyze catechin in the
presence of other endogenous substances in plasma was
investigated. Fig. 2 shows that the resolution factor measured
for the peaks from the nearest peak in plasma was at least
equal to 1.5 under optimized chromatographic conditions.

3.2.2. Linearity and LLOQ

The calibration curve was constructed by plotting the
peak areas versus the concentrations of catechin (range from
0.143–7.15 μg/mL) in standard plasma samples at six

Figure 2  Typical chromatograms of a blank rat plasma (A), blank rat plasma spiked with working reference solution (B), a rat plasma
sample 45 min after intragastric administration of Catechu suspension (C) and a rat plasma sample 45 min after intragastric
administration of Xiongdanjiangre Wan suspension (D). 1, catechin.
concentration levels. The lower limit of quantification (LLOQ) was set as the lowest concentration on the calibration curve. Good linearity was obtained with the equation of $Y = 9.561 \times 10^3X + 831.6$ with correlation coefficient of 0.9992. The LLOQ was 0.143 µg/mL, suggesting a good sensitivity of the method.

3.2.3. Precision
In this study, the precision was measured by means of intra-day and inter-day precision. Intra-day precision was evaluated by replicate determinations ($n=5$) of catechin in QC samples at different concentrations on the same day, whereas inter-day precision was estimated on three different days. The measured concentrations of catechin were calculated based on linearity plots. In all situations, the relative standard deviation (RSD) values were less than 5% (Table 1), which was considered to be acceptable.

3.2.4. Accuracy
The recovery of the method was determined by analyzing the percentage recovery of catechin in plasma samples. This experiment was carried out by analyzing replicates ($n=3$) at three QC levels. The deviation between the measured value and the true value was evaluated by the relative standard deviation (RSD). As a result, the average recovery of catechin was 95.4% with RSD of 1.66%, confirming that the method was accurate.

3.2.5. Stability
The stability of the working reference solution was evaluated at 4°C over 30 days. Short-term (at room temperature over 4 h), long-term (at −20°C for 30 days), and two freeze-thaw cycles stability had been evaluated by analyzing replicates ($n=3$) at three QC levels. The working reference solution was stable in 30 days and the RSD of peak areas was 1.87. The stability results of spiked plasma are shown in Table 2, which indicated that catechin had no significant degradation under the conditions previously described.

| Spiked concentration (mg/L) | Intra-day precision ($n=5$) Mean±SD (mg/L) | RSD (%) | Inter-day precision ($n=5$) Mean±SD (mg/L) | RSD (%) |
|-----------------------------|---------------------------------------------|---------|---------------------------------------------|---------|
| 0.429                       | 0.427±0.590 1.4                             | 2.1     | 0.427±0.900 2.1                             |         |
| 2.86                        | 2.87±3.90 1.4                                | 1.6     | 2.84±4.62 1.6                              |         |
| 7.15                        | 7.15±5.26 0.7                                | 1.1     | 7.13±7.95 1.1                              |         |

3.3. Pharmacokinetic application
The developed and validated LC method was applied to a pharmacokinetic study of catechin in rats after intragastric administration of Catechu and Xiongdanjianjane Wan containing equal amounts of Catechu. The plasma concentrations of catechin at different points were expressed as mean±SD, and the mean concentration-time curve is shown in Fig. 3. The calculated pharmacokinetic parameters are summarized in Table 3. The results showed that catechin behaved as an opened two-compartment model after intragastric administration of the suspension of Catechu and that of Xiongdanjianjane Wan. The data showed that the absorption and distribution of catechin were very fast, with $T_{max}$ of 45 min. The elimination halftime ($T_{1/2}$) of catechin in Catechu was longer than that in Xiongdanjianjane Wan and the relative bioavailability ($AUC_{0-\infty}$) of catechin in Catechu was higher than that in Xiongdanjianjane Wan. There were significant differences in CL and relative bioavailability ($AUC_{0-\infty}$) of catechin between Catechu and Xiongdanjianjane Wan, respectively. The elimination time was shorter and the $AUC_{0-\infty}$ was lower for catechin after intragastric administration of Xiongdanjianjane Wan than those of Catechu. These results demonstrated that Xiongdanjianjane Wan did not increase the bioavailabilities of catechin in vivo, compared with Catechu, and the mechanism of this difference needs further study.

4. Conclusion
An effective, rapid and reliable HPLC method was developed for the determination of catechin in rat plasma. The method was proved to be simple, special, precise and accurate, and has
been successfully applied to determine the pharmacokinetic parameters of catechin in rat plasma after intragastric administration of Catechu and Xiongdanjiangre Wan. The pharmacokinetic parameters obtained may be useful for the clinical application and further studies of Catechu and its Chinese medicinal preparations.

| Parameters               | Catechu      | Xiongdanjiangre Wan |
|--------------------------|--------------|---------------------|
| $T_{1/2a}$ (h)           | 1.832 ± 1.961| 0.499 ± 0.260       |
| $T_{1/2b}$ (h)           | 12.433 ± 4.036| 7.779 ± 2.568      |
| AUC$_{0-\infty}$ (mg/L h)| 16.704 ± 2.360| 7.444 ± 1.176     |
| $C_{max}$ (mg/L)         | 3.387 ± 0.638| 1.666 ± 0.184      |
| $T_{max}$ (h)            | 0.75 ± 0     | 0.75 ± 0            |
| $CL/F$ (L/h/kg)          | 3.720 ± 0.617| 7.993 ± 1.540     |

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