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Determination of Chlorogenic Acid in Rat Plasma by High Performance Chromatography after Peritoneal Administration of Compound Daqingye Injection

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A simple and sensitive high performance liquid chromatographic method has been developed for the determination of chlorogenic acid (3-*O*-caffeoyl-D-quinic acid) in rat plasma and applied to its pharmacokinetic study in rats after peritoneal administration of compound Daqingye injection. Plasma samples are extracted with perchloric acid. HPLC analysis of the chlorogenic acid is performed on a C_{18} reversed-phase column using methanol-water (80: 20, v/v, pH 2.8) as mobile phase with UV detector set at 327 nm. The standard curves are linear in the range of 0.200—10.0 μg/ml ($r=0.9982$). The inter- and intra-day precision (relative standard deviation) was less than 9% and the accuracy (relative error) was less than 10%. The limit of quantitation was 0.200 μg/ml. The plasma concentration of chlorogenic acid shows a C_{max} of 7.53 ± 0.52 μg/ml at 13.33 ± 4.00 min with a $t_{1/2}$ of 59.10 ± 5.42 min.

Key words——Daqingye; chlorogenic acid; HPLC

INTRODUCTION

Daqingye injection is a famous traditional Chinese medicine (TCM) preparation and it has been widely used for the treatment of a number of inflammatory and viral diseases. The compound Daqingye injection is made of five herbal medicines Daqingye (Folium Isatidis), Jinyinhua (Flos Lonicerae), Qianghuo (Rhizoma Radix Notopterygii), Dahuang (Radix Rhizoma Rhei) and Quanshen (Rhizoma Bistortae). All the herbs are officially listed in Chinese Pharmacopoeia.[1] The main active constituent of the Daqingye injection is chlorogenic acid (Fig. 1), which has been reported to have the antiviral activity,[2] antioxidant activity,[3] anti-inflammatory activity[4] and inhibiting the hepatic glucose-6-phosphatase.[5] Thus chlorogenic acid can be considered as one of the marker constituents to characterize the quality of the compound Daqingye injection.

Pharmacokinetic studies of active constituent in Chinese herbs are considered to have great impaction on promoting the development of TCM. It is essential to clarify the pharmacokinetics of the active component in human body. However, owing to the complexity of chemical constituents in TCM formulas, there is scarcely any report of their pharmacokinetic studies. Several previous papers have described reversed-phase HPLC methods for the determination of chlorogenic acid with various detectors.[6—8] These papers mainly focus on the analysis of chlorogenic acid in plasma or urine after administration of single Chinese herb such as hawthorn,[6] Flos Lonicerate,[7] and vegetables and fruits.[8] Up to date, there is no validated method for the determination of pharmacokinetics of chlorogenic acid in compound Daqingye injection due to the more complex constituents contained in the compound Daqingye injection.

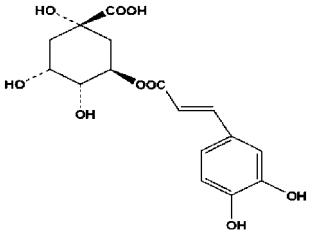

Fig. 1. Chemical Structure of Chlorogenic Acid

In this study, a simple and accurate method for the determination of chlorogenic acid in rat plasma after peritoneal administration of compound Daqingye injection was developed and the pharmacokinetics of the compound in rats was studied.

EXPERIMENTALS

1. Materials and Reagents Chlorogenic acid is provided by the China National Institute of Pharmaceutical and Biological Products (Bejing, China).

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Daqingye injection is provided by the Shandong Lunan Pharmaceutical Co., (Shandong, China). The concentration of chlorogenic acid in the injection was 616 mg/l, which was determined according the previous method.[9] To study the pharmacokinetics of chlorogenic acid in Daqingye injection, the injection was concentrated 50 times under reduced pressure. Methanol is of HPLC grade and all the other reagents are of analytical grade.

2. Sample Preparation Each rats ($n=6$) was fasted for 12 h with free access to water during the experiment and peritoneal administration of concentrated compound Daqingye injection at a dose of 2.5 ml/kg, equivalent to 74 mg/kg of chlorogenic acid. A blood sample (0.4 ml) was collected at 3, 5, 15, 25, 40, 60, 90, 120, 180, 240 and 300 min immediately transferred into heparinized tubes and centrifuged for 10 min at 12000 rpm. To 200 μl of plasma, 120 μl of perchloric acid (10%) was added. The tubes were vortexed for 30 s and centrifuged for 10 min at 12000 rpm. A 20 μl aliquot was injected onto the chromatographic column.

3. Standard Solutions and Quality Control Samples Stock solutions of standard chlorogenic acid were prepared in methanol and stored at -20°C. The calibration standards of concentration (0.200, 0.500, 1.00, 2.00, 5.00 and 10.0 μg/ml) were prepared by spiking 200 μl of blank plasma with 50 μl of appropriate standard stock solution. Quality control (QC) samples were prepared at low (0.200 μg/ml), medium (1.00 μg/ml), and high (8.00 μg/ml) concentrations in the same manner.

4. Precision and Accuracy The accuracy and precision were assessed by determining QC samples at three concentration levels on 3 different validation days. The accuracy was expressed by relative error (RE) and the precision by relative standard deviation (RSD).

5. Sample Pretreatment Recovery The sample pretreatment recoveries of chlorogenic acid at three QC levels were determined by assaying the samples as described above and comparing the peak areas of chlorogenic acid with those obtained from direct injection of the corresponding standards dissolved in the supernatant of the processed blank plasma.

6. Chromatographic System The essential parts of the HPLC system consisted of a Shimadzu LC-10AD pump (Shimadzu, Japan), a SPD-10AVP UV detector (Shimadzu, Japan) set at 327 nm, a 20 μl of injection loop and Ckchrom star workstation for data collection. The samples were determined at room temperature on a Diamonsil C_{18} analytical column (200×4.6 mm ID, 5 μm particle size). The mobile phase consisted of a binary mixture of methanol-water (80: 20, v/v) adjusted to pH 2.8 with acetic acid. The flow rate was 1 ml/min and operated at room temperature.

RESULTS AND DISCUSSION

1. Method Development The purpose of this study was to develop a simple and rapid HPLC method for the determination chlorogenic acid in rat plasma. Because the compound Daqingye injection is composed of five Chinese herbs, it contains very complex constitutes. Thus, it is very difficult to separate them with chlorogenic acid completely. The chlorogenic acid exhibits acidity due to the carboxyl group and two phenolic hydroxyls existed in its molecule (Fig. 1). Thus the decreased of the pH value of the mobile phase will enhance its retention in the reversed column and facilitate to decrease the interference of other components in the plasma or in the compound Daqingye injection. The optimal mobile phase consisted of a mixture of methanol-water (80: 20, v/v) with a pH of 2.8. The degree of interference by endogenous plasma constituents was assessed by inspection of chromatograms derived from processed blank plasma and spiked plasma samples.

2. Specificity Typical chromatograms of blank plasma, spiked plasma and rat plasma after administration of Daqingye injection were presented in Fig. 2. The chlorogenic acid was eluted at 18.4 min and there were no interfering peaks in the vicinity of the chlorogenic acid peak in the chromatogram of bank plasma.

3. Sample Preparation In previous paper,[6] the chlorogenic acid was extracted with EtOAc-MeOH (2: 1, v/v) from plasma samples pre-acidified with ascorbic acid. The usage of this sample preparation method, the chlorogenic acid was extracted from the plasma together with some other constituents and interfere the detection of chlorogenic acid. It was found that extraction of chlorogenic acid from plasma with other organic solvents also showed similar results. In this study, acetonitrile, methanol and perchloric acid was investigated to use as the protein precipitant. The usage of acetonitrile and methanol produced unsymmetrical peak of chlorogenic acid but

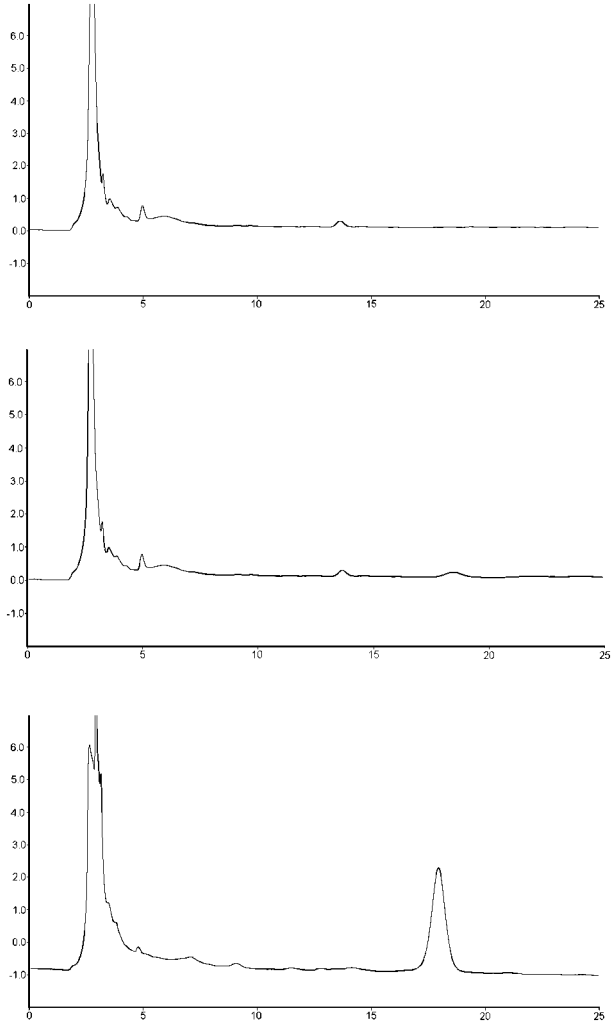

Fig. 2. Typical Chromatograms of Chlorogenic Acid
(A) blank plasma, (B) blank plasma spiked with chlorogenic acid (0.5 μg/ml), (C) plasma sample 15 min after peritoneal administration of concentrated compound Daqingye injection.

the perchloric acid produced better form of peak than acetonitrile and methanol. The protein precipitation method is simple and rapid over the extraction method. The average of sample pretreatment recovery of chlorogenic acid was 103.2%.

4. Calibration and Validation Three sets of calibration standards were prepared and analyzed on three separate days. The regression equation of mean value of three standard curve was $Y=1.40\times10^4X-2.78\times10^2$. The calibration curve for the determination chlorogenic acid was linear over the range of 0.200—10.0 μg/ml with a mean correlation coefficient of 0.9982. The linear range has showed adequate to the use of this method in the current pharmacokinetic studies of the chlorogenic acid. The LOQ of this HPLC method was 0.200 μg/ml. The accuracy and

Table 1. Precision and Accuracy of the HPLC Method to Determine the Chlorogenic Acid in Rat Plasma Samples

Concentration (μg/ml)		RSD (%)		Relative error (%)
Added	Found	Within day	Between day	
0.200	0.181	5.5	8.9	−9.6
1.00	1.04	4.1	7.2	4.0
8.00	7.71	3.5	5.0	−3.6

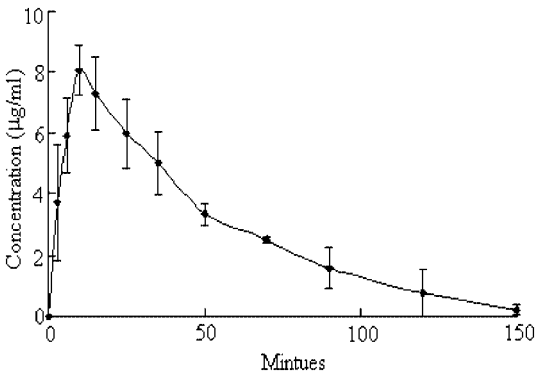

Fig. 3. Plot of the Mean Concentration of Chlorogenic Acid in Plasma of Rats versus Time after Peritoneal Administration of Concentrated Compound Daqingye Injection

precision of the method was evaluated with QC samples at concentrations of 0.200, 1.00 and 8.00 μg/ml. The results were shown in Table 1. The intra-day precision (RSD%) determined by assaying six replicates at each concentration on the same day was 3% to 6% ($n=18$) and inter-day precision (RSD%) determined by the three successive days was 5% to 9% ($n=18$). The accuracy (RE) ranged from −3.6% to −9.6% ($n=18$).

5. Pharmacokinetic Applicability The validated HPLC method was applied to study the pharmacokinetics of chlorogenic acid in compound Daqingye injection in rats. Plasma samples were taken at scheduled intervals after peritoneal administration of compound Daqingye injection. The mean plasma concentration-time profile is presented in Fig 3. The pharmacokinetic parameters were listed in Table 2. Chlorogenic acid was absorbed rapidly reaching C_{max} of 7.53 μg/ml at 13.33 min and metabolited quickly with $t_{1/2}$ of 59.1 min. The result is similar with the reported parameters.[10]

CONCLUSION

An HPLC method with UV detection was devel-

Table 2. Pharmacokinetic Parameters of Chlorogenic Acid after Peritoneal Administration of Concentrated Compound Daqingye Injection

Parameters	Non-compartment
K_e (1/min)	0.12±0.001
$t_{1/2}$ (min)	59.1±5.4
T_{max} (min)	13.33±4.00
C_{max} (μg/ml)	7.53±0.52
CL/F (L/min)	0.12±0.03
Vc/F (L)	1.3±0.5
AUC_{0-t} (μg·min/ml)	730.6±8.0

oped for the determination of chlorogenic acid in rat plasma after peritoneal administration of Daqingye injection. This method is sensitive, precise, and accurate and is successfully used in the study of pharmacokinetics of chlorogenic acid in rats.

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