Pharmacokinetics of a Chinese Traditional Medicine, Danshen (3,4-Dihydroxyphenylactic Acid), in Rabbits Using High-Performance Liquid Chromatography

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An injectable solution of Danshen was prepared and its in vivo disposition was examined in rabbits. The presence of Danshen, one of the active components of Danshen, in the obtained solution was confirmed by a simple high-performance liquid chromatographic (HPLC) method. The pharmacokinetics of Danshen in rabbits was evaluated by the HPLC method for plasma Danshen. The calibration curve for Danshen was linear (r = 0.998) over the concentration range of 0.25—40.0 μg/ml. The intra-assay coefficients of variation (CV) were 3.8, 3.1, and 3.1% at 1, 10, and 50 μg/ml, respectively, and the inter-assay CV were 5.3, 5.3, and 2.9% at 1, 10, and 50 μg/ml, respectively. The analytical recovery of Danshen in plasma averaged 95.2%. From the plasma concentration profile of Danshen after its intravenous administration, the t1/2, mean residence time (MRT), Vdss and Cltot were determined as 32 min, 48 min, 149 ml/kg, and 3.13 ml/min/kg, respectively.

Key words Danshen; Danshen HPLC; rabbit; pharmacokinetics

Danshen (Salvia miltiorrhiza) is listed in the Chinese Pharmacopoeia and has been used for the treatment of menstrual disorder, menostasis, menorrhagia, insomnia, blood circulation diseases, and angina pectoris.12 Danshen injection is an effective medicine used widely in China for the treatment of myocardial infarction, and Danshen 3,4-dihydroxyphenylactic acid (Fig. 1) is one of its active components. It has been found that Danshen dilates an isolated swine coronary artery and antagonizes the constricting response elicited by morphine and propranolol.21 Recent experiments indicated that Danshen can effectively scavenge superoxide anions generated from the xanthine–xanthine oxidase system and protect the myocardial mitochondrial membranes from lipid peroxidation;23 this is assumed to be the major reason that Danshen injection can cure heart disease. In this study, we prepared an injectable solution of Danshen, established a simple HPLC method for Danshen and applied it to a pharmacokinetic study of the Chinese traditional medicine in rabbits after intravenous (i.v.) administration.

MATERIALS AND METHODS

Materials Standard Danshensu was kindly provided by Shanghai Medical University. Radix Salvia miltiorrhiza was provided by Heilongjian Pharmaceutical Corp. (Harbin, China). Other reagents were commercially available and of analytical grade.

Extraction Procedure An aliquot (0.5 ml) of plasma was treated with 1 ml of 2.5% HClO4 and centrifuged at 4000 rpm for 10 min. A portion (20 μl) of the supernatant was used for HPLC analysis described below.

HPLC System The HPLC system consisted of a pump (LC-6A, Shimadzu, Kyoto, Japan), a Rheodyne 7125 syringe-loading sample injector valve (Cotati, CA, U.S.A.), and a UV detector (SPD-6A, Shimadzu). A reversed-phase WYG C18 column (150 x 4.6 mm i.d.; pore size 5 μm) (Dalian, China) was used. The mobile phase was water—methanol—glacial acetic acid (80: 19: 1.2). The mobile phase was delivered at a flow rate of 1.2 ml/min. Detection was performed at a wavelength of 280 nm under a constant temperature (25 °C). Chromatograms were recorded by an integrator (C-R3A, Shimadzu).

Preparation of Danshen Injection The preparation procedure of Danshen injectable solution is as follows:
(1) Radix Salvia miltiorrhiza was extracted with 65% ethanol 3 times and filtered with a glass filter (3—4 μm, Changchuen, China).
(2) The above filtrate was concentrated, added with distilled water and the solution was then filtered.
(3) pH of the filtrate was adjusted to 3.0 with HCl and filtered again.
(4) The filtrate was added with 0.3% of activated charcoal and filtered again.
(5) pH of the filtrate was adjusted to 8.0 and filtered again. Finally, the filtrate was sterilized to obtain a solution for injection.

Animal Experiment Five male white rabbits weighing 2.5—3 kg were used after being fasted for 24 h. A preparation of Salvia miltiorrhiza (Danshensu 1.25 mg/ml) was injected at a dose of 6.25 mg/kg via the right marginal ear vein for 3 min. Blood (2 ml) was collected in a heparinized tube via the left marginal ear vein at 0, 5, 15, 30, 60, 120, and 240 min using a disposable syringe. Plasma was separated and kept at −80 °C until analysis.

Pharmacokinetic Analysis The model-independent pharmacokinetic parameters of half-life (t1/2), mean

Fig. 1. Chemical Structure of Danshen
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residence time (MRT), area under the plasma concentration–time curve (AUC), total clearance (CLtot), and steady-state volume of distribution (Vss) were calculated by the following equations, based on a moment theory:  

$$AUC = \int_0^\infty C_p \, dt$$  

$$MRT = \int_0^\infty t \cdot C_p \, dt / \int_0^\infty C_p \, dt$$  

$$V_{ss} = CL_{tot} \cdot MRT$$  

$$CL_{tot} = D/AUC$$

where \( C_p \) represents the plasma concentration of Danshensu at time \( t \) after i.v. administration; and \( D \) denotes the dose administered.

RESULTS AND DISCUSSION

The HPLC chromatograms of blank rabbit plasma and the spiked plasma are shown in Fig. 2. The retention time of Danshensu was 2.7 min. Since Danshensu was eluted as a sharp single peak, we could not discriminate between the S- and R-enantiomers of the compound. There were no interfering peaks near that of Danshensu.

The calibration curve for Danshensu was linear \((r=0.998)\) over the concentration range of 0.25—40.0 \( \mu g/ml \) in rabbit plasma. The intra-assay coefficient of variations (CV) were 3.8, 3.1, and 3.1\% at 1, 10, and 50 \( \mu g/ml \), respectively, and the inter-assay CV were 5.3, 5.3, and 2.9\% at 1, 10, and 50 \( \mu g/ml \), respectively. The analytical recovery of Danshensu in plasma averaged 95.2\%. These lines of evidence indicate that the method is applicable to a pharmacokinetic study of Danshensu.

There is little information on the pharmacokinetic behavior of Chinese traditional medicines due to the difficulty of their chemical analysis in plasma. This study was undertaken to clarify the disposition profile of Danshensu after i.v. administration, because its i.v. formulation is known to be effective for the treatment of myocardial infarction,\(^3\) ischemic stroke,\(^5\) coronary heart disease,\(^6\) chronic active hepatitis,\(^7\) and so on. The plasma concentration versus time profile of Danshensu in rabbits after i.v. administration is presented in Fig. 3, and the pharmacokinetic parameters are listed in Table 1. The plasma level of Danshensu declined rapidly with a half-life of 32 min, and the concentration fell below the quantifiable limit (0.25 \( \mu g/ml \)) after 4 h. The \( V_{ss} \) was estimated to be 149 ml/kg. It is thus likely that Danshensu does not distribute inside the cells due to its low lipophilicity. This observation does not contradict the pharmacological effect (i.e., vasodilatation), considering that the site of action may be on the surface of endothelial cells of blood capillaries, but not within the cells.

The ratio of S- and R-enantiomers of Danshensu was not determined in this study, since our HPLC method was not stereospecific. There is yet no information on the pharmacological difference between the S- and R-enantiomers of Danshensu. Moreover, there are some other active components in Danshen injection such as Tanshinone, which have been proved to effectively scavenge the lipid free radicals generated from lipid peroxidation of myocardial mitochondrial membranes.\(^3\)

The pharmacokinetics of Tanshinone, however, remains to be clarified.

In conclusion, a simple HPLC method was developed for Danshensu in plasma and successfully applied to the pharmacokinetic study of this Chinese traditional medicine. The pharmacokinetics of Chinese traditional drugs should be investigated considering their potential interactions with other drugs.

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Fig. 2. Chromatograms of Danshensu
A, blank plasma sample; B, 1.68 mg/kg standard in plasma; C, plasma from rabbit study. The arrows indicate the elution peak of Danshensu.

Fig. 3. Plasma Concentration Profile of Danshensu after Its Intravenous Administration in Rabbits
Dose: Danshensu 6.25 mg/kg. Each point and bar represent the mean ± S.D. of 5 rabbits.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (±S.D.)</th>
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<tbody>
<tr>
<td>t1/2 (min)</td>
<td>31.7 ± 2.5</td>
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<tr>
<td>AUC (μg/ml·min)</td>
<td>1996 ± 356</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>47.5 ± 9.6</td>
</tr>
<tr>
<td>Vss (ml/kg)</td>
<td>149 ± 23</td>
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<tr>
<td>CLtot (ml/min/kg)</td>
<td>3.13 ± 0.97</td>
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Each value represents the mean ± S.D. (n = 5).

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REFERENCES