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Short communication

An HPLC method for the pharmacokinetic study of daidzein-loaded nanoparticle formulations after injection to rats

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ABSTRACT

This study was aimed at developing a simple HPLC method for the detection of daidzein in rat plasma. Daidzein was extracted from rat plasma with ethylparaben as internal standards (IS). Chromatographic separation of daidzein and IS was achieved by a Dikma Dimonsil C18 column (200 mm × 4.6 mm) with the mobile phase consisting of methanol–water (55:45, v/v) at a flow rate of 1.0 mL/min. The injection volume was 20 μL and the detecting wavelength was 249 nm. The calibration curve was linear over a concentration range from 0.05 to 5 μg/mL, and the accuracy was within a range of 93.4–126.2%. This HPLC method was applied successfully to the pharmacokinetic study of two kinds of daidzein-loaded poly(lactide-co-glycolide) (PLGA) nanoparticles (D-NPs) and daidzein suspension after intravenous injection in rats. Significant differences in main pharmacokinetic parameters of daidzein suspension and D-NPs were observed.

1. Introduction

Daidzein (Fig. 1) (4,7-dihydroxyl isoflavone), an important component of Chinese herb Kudzuvine root, is a water-insoluble isoflavone (with the water solubility of 3.85 μg/mL) [1]. It is applied in the clinical treatment of cardiovascular disease, cerebral thrombosis, hypertension and women menopausal syndrome [2–4]. It can also inhibit the growth of cancer cells dependent on cell death pathway and prevent diabetes onset [5–8].

To date, many methods of determining daidzein in plasma have been reported, such as liquid chromatography–mass spectrometry methods [9–11], coulometer detection method [12], and enzyme-linked immunosorbent method [13]. Recently, a simultaneous method was established to determine daidzein by a solid-phase HLB cartridge [14], and another chromatographic separation method was reported using a mobile phase containing 20 mM sodium acetate, 0.25 mM EDTA, pH 4.3, 4% methanol and 11% acetonitrile in water [15]. Li and Shen [16] detected daidzein by a simple HPLC method without internal standard.

Although these methods are precise, the sample handling and detection processes are time-costing and usually demand complex conditions. These complex conditions are not practical in laboratory analysis, while other simple quantification methods may not be accurate enough to measure the actual drug concentration in plasma.

In the present work, two kinds of stabilized daidzein-loaded PLGA nanoparticles, daidzein-loaded phospholipid complex PLGA nanoparticles (D-PNPs) and daidzein-loaded cyclodextrin inclusion compound PLGA nanoparticles (D-CNPs) were designed, and a simple and sensitive HPLC method for determining daidzein in rat plasma was developed. In this method, ethylparaben was selected as the internal standard, and chromatographic separation of daidzein and IS was completed within 10 min. Besides, highly efficient drug extraction from plasma was attained, and mobile phase composition was suitable to the pharmacokinetic study of D-PNPs and D-CNPs in rats.

2. Materials and methods

2.1. Reagents and chemicals

Daidzein (purity > 98%) was obtained from Qingze Co. Ltd. (Nanjing, China). CMC-Na, acetonitrile and methanol were purchased from Shanghai Chemical Reagent Co. Ltd. (Shanghai, China). Distilled water was produced by a Milli-Q purification system (Millipore, Billerica, MA, USA). All other chemicals and reagents were of analytical grade.

The daidzein-phospholipid PLGA nanoparticles (D-PNPs) and the daidzein-β-cyclodextrin inclusion compound PLGA nanoparticles (D-CNPs) were prepared by an emulsification–solvent evaporation method and a double emulsion method, respectively.

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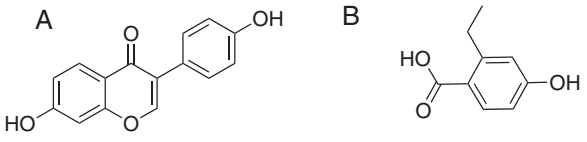

Fig. 1. Structure of (A) daidzein and (B) ethylparaben.

2.2. Animal handling

Healthy male Sprague–Dawley rats (220 ± 20 g) were obtained from the Laboratory Animal Center, Shanghai Jiao Tong University, China. The animal experimental protocols were performed according to the guideline of the Experimental Animal Ethics Committee of Shanghai Jiao Tong University. Rats were kept in a room under a controlled temperature of 21 ± 1 °C and a relative humidity of 50 ± 10%, and housed in groups of three in stainless-steel wire-mesh cages. After the collection of blood, animals were sacrificed by cervical dislocation during ether anesthesia.

2.3. HPLC assay

2.3.1. Chromatography system

The chromatography system used was composed of a Shimadzu LC-20A series chromatographic system (Shimadzu, Kyoto, Japan) with a LC-20 AT binary pump and a SPD-20A UV–vis detector. Data processing was performed with LC Solution software. Analysis was carried out on a Dikma Dimonsil C18 column (200 mm × 4.6 mm i.d.), from Dikma Technologies, China.

2.3.2. Preparation of samples

Stock solutions (1 mg/mL) of daidzein and ethylparaben in methanol were prepared and stored at −20 °C. The working solutions of daidzein at concentrations of 0.5, 1, 5, 10 and 50 μg/mL were prepared by serial dilution of daidzein stock solution. Ethylparaben working solution at a concentration of 5 μg/mL was also prepared by diluting ethylparaben stock solution.

Plasma sample was prepared by the following steps: 100 μL blank plasma was obtained by centrifugation of orbital blood; then 10 μL daidzein and 10 μL IS working solutions were, respectively, spiked into the blank plasma; and 900 μL acetonitrile was added to remove protein. After centrifuging, the supernatant acetonitrile was withdrawn and dried by nitrogen gas. Finally the dry matters were redissolved by 100 μL methanol.

2.3.3. Calibration curve

20 μL of mixture (composed of 10 μL daidzein and 10 μL IS working solutions) was mixed with 100 μL plasma to prepare HPLC samples as described above. The peak areas of daidzein (A_D) and ethylparaben (A_E) were recorded, and the values of A_D/A_E and the concentrations of daidzein were used to plot the calibration curve ($y = ax + b$).

2.3.4. Accuracy and precision

Each daidzein quality control (QC) sample was prepared with daidzein working solutions and at the final concentration of 0.05, 0.5 or 5 μg/mL. The preparation of IS working solution and blank plasma followed the steps in Section 2.3.3. The intra- and inter-assay accuracy and precision of the method were determined by assaying six replicates of each QC sample in different times on three random days.

2.3.5. Recovery

Daidzein and IS working solutions were added into the blank plasma at QC levels, and then the QC samples were dealed with using the method described in Section 2.3.2. The peak area ratio (A_D/A_E) was compared to that for daidzein and IS dissolved in methanol at the same concentrations.

2.4. Pharmacokinetic study of daidzein-loaded PLGA nanoparticles

An in vivo pharmacokinetic and bioavailability study was undertaken to determine the enhanced absorption of daidzein existed in D-PNPs and D-CNPs.

2.4.1. Animal administration and sampling

The healthy male SD rats were randomly divided into three groups, each animal was fasted overnight with free access to water before drug administration. Rats in control group were tail-intravenously injected with the daidzein (10 mg/kg) suspended in 1 mL 0.5% CMC-Na solution, the other groups were injected with 173 mg D-PNPs or 126 mg D-CNPs formulations which were prepared by suspending freeze-dried powders of nanoparticles in 2 mL saline solution at the same dose of the control group. After administration, 300 μL blood samples collected from orbit at the time points of 0.25, 0.75, 1.5, 2, 4, 8, 12, 24, 36 and 48 h were placed into heparinized micro-centrifuge tubes (100 IU/mL blood). The blood samples were centrifuged at 5000 rpm for 5 min to obtain 100 μL plasma. The preparation is described in Section 2.3.2.

2.4.2. Calculations and statistics

HPLC results of samples were analyzed with the LC Solution software (Shimadzu, Kyoto, Japan), pharmacokinetic analysis of plasma daidzein concentrations versus time data was performed via the software Kinetica 4.0, which followed the macro-intravenous injection model. A value of $p < 0.05$ was considered to indicate significant differences.

3. Results and discussion

3.1. HPLC method development and sample handling

The most convenient and stable detection method was chosen from many HPLC methods. Mobile phase must contain more than 50% methanol, or else daidzein cannot be dissolved. Three methanol–water mixtures which were in the proportions of 50:50, 55:45 and 60:40 were tested as mobile phases, and the results showed that when the proportion of methanol:water was 55:45 (v/v) and the flow rate was 1.0 mL/min, the elution of both daidzein and IS was finished within 10 min with complete separation from the impurity peaks. The UV detection wavelength was set at 249 nm, with reference of the full wavelength scan result of daidzein.

Several chemicals were tested as IS, for example, genistein, fluconazole, ethylparaben etc. Genistein interfered with daidzein in all of the mobile phases we tested, while the fluconazole had tailing peaks in these conditions. In our experiments, the separation factor (Rs) of genistein-to-daidzein was 1.119, and the tailing factor (T) of fluconazole was 1.259. Ethylparaben was selected owing to its stability in plasma, symmetrical peak shape ($T = 0.998$) and good separation from daidzein peak under the chosen HPLC method.

The method of plasma sample handling was extracting daidzein from plasma by adding 900 μL acetonitrile to 100 μL plasma. Compared with methanol and ethyl acetate, which were often used to remove impurities and extract drugs, this volume of acetonitrile can precipitate more plasma protein and extract more drugs.

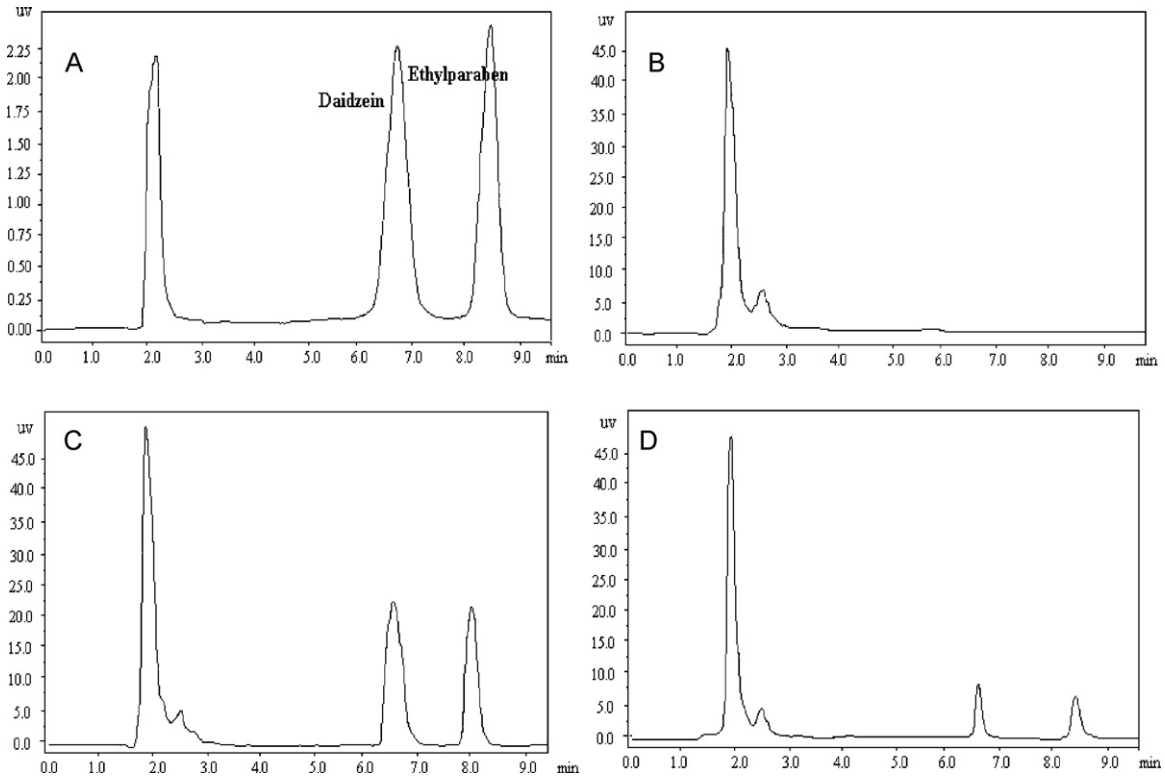

Fig. 2. Representative chromatograms of (A) daidzein and IS; (B) blank plasma; (C) plasma added with daidzein and IS; (D) plasma sample after a single intravenous injection of D-PNPs with IS.

3.2. HPLC method validation

3.2.1. Selectivity

Daidzein peaks were well shaped in both blending daidzein plasma samples and endogenous daidzein plasma samples, IS peaks can be separated well from daidzein peaks without any interferences. The average elution time of daidzein and ethylparaben was 6.78 min and 8.42 min, respectively (Fig. 2).

3.2.2. Linearity of calibration curve

The calibration curve was linear over the range of 0.05–5 μg/mL. The regression equations of the calibration curve are $A_D/A_E = 2.731C - 0.008$, $r^2 = 0.9997$ ($n = 3$). In addition to the plasma concentration of control group at 12 and 24 h, all data are in the calibration curve range.

3.2.3. Precision and accuracy

Table 1 shows the precision and accuracy of daidzein QC samples described above. In the measured concentration range, the precision (R.S.D., %) of intra-day and inter-day ranged from 1.5 to 4.9% and 9.8 to 10.5%, respectively; while the accuracy of intra-day and inter-day ranged from 93.4 to 96.9% and 93.7 to 126.2%.

Table 1
The validation of intra- and inter-day precision and accuracy with daidzein QC samples ($n = 6$).

Concentration (μg/mL)	Precision (%R.S.D.)		Accuracy (%)	
	Intra-day	Inter-day	Intra-day	Inter-day
0.05	4.9	9.8	96.9	126.2
0.5	3.4	10.5	93.4	108.4
5	1.5	9.8	93.9	93.7

3.2.4. Recovery

The mean recoveries of 6 repeated daidzein QC samples for each of the three concentrations, 0.05 μg/mL, 0.5 μg/mL and 5 μg/mL, are 89.5 ± 0.3%, 97.2 ± 2.7% and 93.9 ± 2.1%, respectively.

3.3. Pharmacokinetic study of D-NPs

Plasma concentration–time curves of daidzein are shown in Fig. 3. The relevant pharmacokinetic parameters, including AUC, MRT, C_{max} $T_{1/2\alpha}$ and $T_{1/2\beta}$, are listed in Table 2. Compared to the control group, both of the D-PNPs and D-CNPs treated rats groups have higher concentrations of daidzein in plasma during 0.75–24 h,

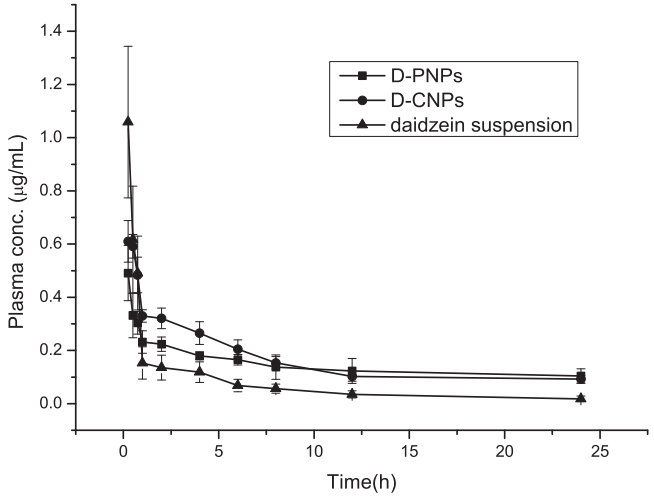

Fig. 3. Mean plasma concentration–time profiles of daidzein in SD rats ($n = 3$) after a single intravenous injection of D-PNPs, D-CNPs and daidzein suspension at the dose of 10 mg/kg, respectively.

Table 2
Pharmacokinetic parameters (mean ± S.D., $n = 3$) of daidzein after a single intravenous injection of D-PNPs, D-CNPs and daidzein suspension at the dose of 10 mg/kg.

Parameters	Daidzein suspension	D-PNPs	D-CNPs
AUC (h μg/mL)	2.28 ± 1.17	6.21 ± 1.63*	4.61 ± 0.65*
MRT (h)	7.66 ± 0.93	26.97 ± 7.72**	12.53 ± 3.95*
C_{max} (μg/mL)	1.86 ± 0.65	0.76 ± 0.25*	0.79 ± 0.28*
$T_{1/2\alpha}$ (h)	0.28 ± 0.09	0.25 ± 0.07	0.48 ± 0.11*
$T_{1/2\beta}$ (h)	7.80 ± 0.67	19.30 ± 4.02*	9.29 ± 2.03
Cl (mL/h)	461.44 ± 57	161.38 ± 23.08*	215.36 ± 17.92*

* $p < 0.05$ compared with daidzein suspension group.
** $p < 0.01$ compared with daidzein suspension group.

and the area under the plasma concentration–time curve (AUC) is significantly increased by 3.08 and 1.77 times, respectively. The mean residence time (MRT) and clearance (Cl) of D-NPs, the distribution half-life ($T_{1/2\alpha}$) of D-CNPs, and the elimination half-life ($T_{1/2\beta}$) of D-PNPs are also increased significantly, inversely the maximum plasma concentrations (C_{max}) of D-NPs were both lower than the daidzein control group.

3.4. Discussion

According to the results of the HPLC method validation and material selection, daidzein and IS peaks eluted in 10 min and were separated completely with high precision. It suggests that the quantification method of daidzein is quick and easy with high accuracy, especially for determination of daidzein at low concentrations. Besides, the high recovery rate indicated that the method for plasma handling is effective and accurate for extracting daidzein and removing plasma proteins and other impurities. With regards of mobile phase, methanol and water were used without other additives, avoiding the column or pipe damage caused by salt or other ions.

The results of pharmacokinetic study clearly showed that two kinds of nanoparticles and the prototype drugs had great difference in the bioavailability. Plasma concentration of daidzein suspension administrated group decreased rapidly while those of the D-PNPs and D-CNPs administrated group decreased slowly. This phenomenon could be explained by the different liver metabolic rates of D-NPs and daidzein suspension. To our knowledge, prototype daidzein has a strong metabolism in intestine and liver and can be quickly eliminated from blood [1,17]. According to the results and references, we conjecture that daidzein entrapped in PLGA nanoparticles may be transported through the blood vessel wall and re-distributed to other organs, or be protected by the PLGA shell to avoid elimination [18–21]. The differences in bioavailability between D-PNPs and D-CNPs may be related to their different particle sizes, while the specific reason will be for a future research.

4. Conclusion

In this study, an effective HPLC method was provided for the determination of daidzein with good accuracy and precision. This method was successfully applied to the pharmacokinetic analysis of two kinds of daidzein-loaded nanoparticles in rats. The results showed that both D-PNPs and D-CNPs can prolong the residence time of daidzein and enlarge the AUC. Compared with the prototype drugs, daidzein-loaded nanoparticles have a significantly higher bioavailability.

Acknowledgements

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