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Short communication

Quantitative determination of astilbin in rabbit plasma by liquid chromatography

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Abstract

A simple method for determining the concentration of astilbin, a flavanone, in rabbit plasma has been developed. After liquid–liquid extraction, the flavanone was detected by HPLC on a 4.6-μm octadecylsilica column (Nova-Pak C-18) at 291 nm. Linear calibration graphs for astilbin were constructed from 0.44 to 22.17 μM. The limit of quantitation was 0.44 μM in plasma. The method has been applied to pharmacokinetic studies after a single i.v. and an oral administration of the compound to rabbits.

Keyword: Astilbin

1. Introduction

Astilbin, 3,3′,4′,5,7-pentahydroxyflavanone 3-(6-deoxy-(L-mannopyranoside)) (Fig. 1), was isolated from the rhizome of *Smilax glabra*, a Liliaceae plant. The flavanone was also reported in many other plants such as *Dimrophandra mollis*, *Hypericum perforatum*, *Virola oleifera*, and *Engelhardtia chrysolepis* et al. [1–4]. In some wines such as Chardonnay, Pinot Noir vintages, and French varietal wines, the existence of astilbin was also detected [5,6].

This flavanone has been reported to show insecticidal [1], coenzyme A reductase-inhibiting [7], aldose reductase-inhibiting [4], hepatoprotective [8], anti-oedematogenic [9], and anti-oxidative activities [10,11]. In our previous works, we documented that this compound showed a significant inhibition on both ear contact dermatitis and liver injury induced by a delayed-type hypersensitivity reaction at the effector phase. Its mechanism was found to involve a selective induction of apoptosis for the liver-infiltrating T lymphocytes and the mitogen-activated Jurkat T cells without affecting hepatocytes, non-activated Jurkat cells and other cells [12,13]. Also the flavanone significantly suppressed collagen-induced arthritis and delayed-type hypersensitivity via causing the dysfunction of lymphocytes and inhibiting lymphocyte migration [14,15]. Such selective activity of astilbin is quite different from immunosuppressors so far [16] and will be useful as a novel immunosuppressive agent for the treatment of immunologically related diseases. Also, as a flavanone, the immunosuppression of astilbin is of significance for developing a new immunosuppressor from flavones since there is rarely reported for the immunosuppressive activity in other flavones except for several data on anti-inflammation activity [17]. For exploring how astilbin takes the effects, we need to know the pharmacokinetic characteristics of this interesting compound. Although several methods have been developed to determine the content of astilbin in plants recently [5,6,18], there is no analytical method for the quantitation of astilbin in plasma. Therefore, the present study aims at developing a HPLC method to evaluate the plasma profile of astilbin in rabbit after single i.v. or oral dosing.

2. Experimental

2.1. Chemicals and reagents

Analytical-grade ethyl acetate and acetic acid, and HPLC-grade methanol were used. They were obtained from

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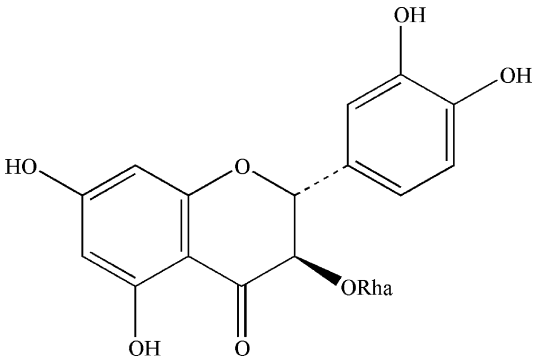

Fig. 1. Structure of astilbin.

Jiangsu Hanbon Science and Technology Co. Ltd. (Nanjing, Jiangsu, China). Water used in the experiment was double glass-distilled. Astilbin was isolated and purified in our laboratory as described in our previous paper. The structure of this compound has been elucidated by MS and NMR including NOE, DEPT, ^{1}H-^{1}H-COSY, ^{1}H-^{13}C-COSY, and COLOC experiments as reported by us previously [19]. The purity was determined to be above 98% by HPLC.

2.2. Sample preparation

Rabbit plasma (200 μl) collected was mixed with ethyl acetate (5 ml per sample) and vortexed thoroughly for 1 min. After centrifugation for 10 min at 2000 × *g*, 2.5 ml of the organic phase were carefully transferred to another tube and evaporated to dryness under a stream of nitrogen at 40 °C. The residue was dissolved in 100 μl of mobile phase and 20 μl was injected into the HPLC apparatus.

2.3. Equipment

The HPLC system (Shimadzu, Kyoto, Japan) consisted of a pump (LC-10AT), a UV detector (SPD-10A) and a chromatopac (C-R6A). A Nova-Pak C-18 column (4.6 μm, 150 mm × 3.9 mm) was obtained from Waters Corporation (Miliford, MA, USA).

2.4. Chromatographic analysis

The mobile phase was prepared from methanol–water–acetic acid (36:63.7:0.3, v/v/v) at a 0.8 ml/min flow-rate using isocratic elution. The detector was operated at 291 nm and 0.001 AUFS. The analysis was performed under room temperature. Under such conditions the retention time for astilbin was 9.0 min.

Standard calibration curves (peak areas versus concentration) were obtained from samples into which known amounts of astilbin were added. Briefly, astilbin was dissolved in methanol as the stock solution (2.217 mM). The solution was then diluted gradiently with methanol and 10 μl of them were added in 1 ml of plasma from naive rabbits to make different concentrations (0.44, 1.11, 2.22, 4.44, 11.1, 22.17 μM). Calibration graphs were constructed by plotting the concentration of plasma astilbin (*y*-axis) (μM) versus the peak area (*x*-axis) using linear regression.

2.5. Recovery

The absolute recovery of astilbin from rabbit plasma was determined in replicate samples ($n = 5$). Namely, different amounts of astilbin were added into the plasma from a naive rabbit (0.44, 2.22 and 22.17 μM). The compound was then extracted according to the above extraction procedures shown in Section 2.2 and quantitated from the peak area. The extraction recovery (ER) was calculated from the determined amounts against the added amounts of astilbin.

2.6. Animals

New Zealand white rabbits ($n = 5$; 2–2.5 kg) were purchased from the Experimental Animal Center of China Pharmaceutical University (Nanjing, China) and kept at 21±2 °C. Animal welfare and experimental procedures were strictly in accordance with the guide for the care and use of laboratory animals (National Research Council of USA, 1996) and the related ethical regulations of our university. All efforts were made to minimize suffering and to reduce the number of rabbits used. Before administration of compound, rabbits were fasted for 12 h. Astilbin was dissolved in distilled water and 10% ethanol for oral and i.v. administration, respectively. The blood samples (0.5 ml) were collected from ear vein of rabbits at 5, 10, 20, 40, 60, 90, 120, 240 min after oral and 5, 10, 15, 20, 30, 40, 60, 120 min after i.v. administration, respectively.

3. Results

3.1. Linearity and precision

The standard curve of astilbin in plasma was linear over the concentration range (0.44–22.17 μM) examined. The regression equation for astilbin was $y = 2e^{-05}x - 0.1076$ ($r = 0.9998$), where *y* was the plasma concentration of astilbin and *x* was the peak area. The intra- and inter-day precision was determined at three different concentrations (0.44, 2.22 and 22.17 μM). The intra-assay relative standard deviation (R.S.D.) for every studied concentration was less than 7.5%, indicating the precision of the method for routine purposes. The inter-assay precision was studied using spiked samples that were analyzed at least five times within a 5-day period. The results show that the concentration values are reproducible with an inter-assay R.S.D.

Table 1
Intra- and inter-assay accuracy and precision of the method for determining astilbin concentration in rabbit plasma

Concentrations (μM)	Mean (μM)		R.S.D. (%)	
	Intra-assay	Inter-assay	Intra-assay	Inter-assay
0.44	0.48	0.51	7.4	10.4
2.2	2.07	2.16	7.2	9.9
22.17	22.31	23.51	2.3	3.3

Each value indicates the mean of five experiments, and for other details, see the description in Section 2.5.

at the studied concentrations of less than 10.5%. Intra- and inter-day accuracy and precision data are shown in Table 1.

3.2. Recovery

The mean (±S.D.) recovery for astilbin from plasma samples was 70.7 ± 7.4, 74.9 ± 7.2 and 87.9 ± 2.3% at 0.44, 2.22 and 22.17 μM, respectively.

3.3. Limit of quantitation

Based on a signal-to-noise ratio (S/N) = 10 (R.S.D. < 20%), the limit of quantitation of astilbin was 0.44 μM in plasma.

The proposed HPLC method was applied to determine astilbin plasma concentrations in the rabbit following single i.v. or oral dosing of the compound during a preliminary pharmacokinetic study. Fig. 2 shows a good separation of astilbin to the plasma constituents in each case of direct astilbin spiking to plasma, oral and i.v. administration of astilbin (Fig. 2B–D). The kinetics of astilbin in the plasma of five treated rabbits were shown in Fig. 3. Data were processed by 3P87 (The Chinese Pharmacological Society), and the pharmacokinetic parameters of astilbin were shown in Table 2.

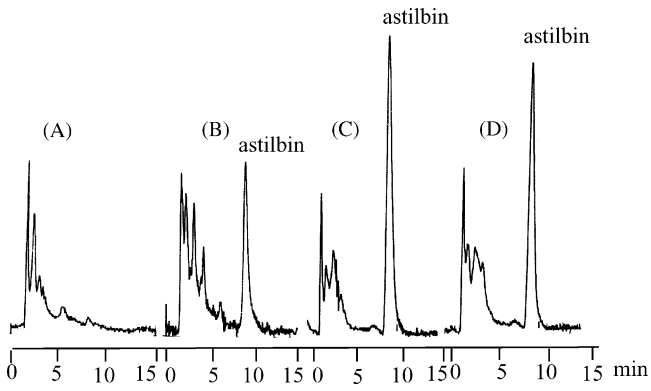

Fig. 2. Typical chromatograms of the extracts from rabbit plasma: (A) blank plasma; (B) plasma spiked with 0.88 μM astilbin; (C) plasma sample following an oral administration of astilbin (100 mg/kg) 40 min after the administration; (D) plasma sample following an i.v. administration of astilbin (10 mg/kg) 40 min after the injection. The mobile phase was methanol–water–acetic acid (36:63.7:0.3, v/v/v).

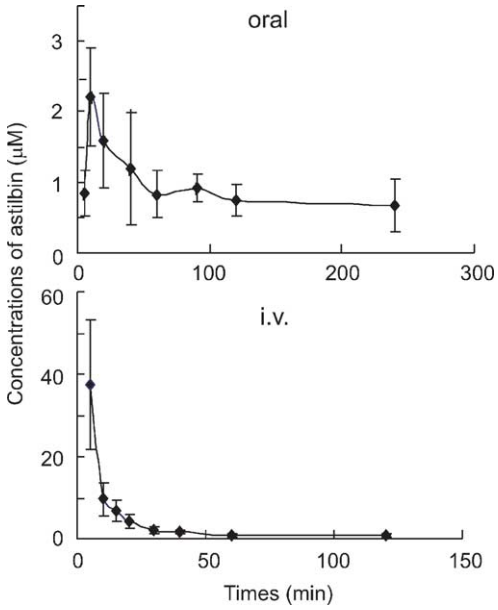

Fig. 3. Kinetics of astilbin in plasma of five rabbits after single oral administration (100 mg/kg) or i.v. (10 mg/kg) injection.

Table 2
Pharmacokinetic parameters of astilbin after single oral (100 mg/kg) and i.v. (10 mg/kg) administration

Parameter	Values (oral)	Values (i.v.)
C_{max} (μM)	2.38	
t_{max} (min)	2.22	
$t_{1/2\alpha}$ (min)	12.99	5.57
$t_{1/2\beta}$ (min)	636.97	382.83
$t_{1/2ka}$ (min)	0.40	
AUC (μM min)	839.39	916.23

4. Discussion

It is known that astilbin has been determined in plants with gradient HPLC methods by using acetonitrile as the mobile phase. Here we developed another HPLC method using methanol as the mobile phase, which successfully separated astilbin and other interfering substances existing in plasma by a suitable mobile phase (Fig. 2). Also in the mobile phase we added acetic acid in order to inhibit the ionization of astilbin. The accuracy of this method for determining astilbin in plasma by HPLC was demonstrated in the intra- and inter-assay by multi-injection of astilbin (Table 1).

By using this method, we found that astilbin showed a longer $t_{1/2}$ when administered orally than intravenously (Table 2). This phenomenon may be due to the difference in hepatoenteral circulation property between oral and intravenous administration of the flavanone. These pharmacokinetic characteristics might be helpful for exploring the effective mechanisms of astilbin and its related compounds in vivo as well as for the possible application in the clinical treatment of diseases. Also, astilbin was absorbed very

quickly after oral administration, where astilbin was detected in plasma at low concentration only after 5 min of the administration (Fig. 3).

Since astilbin was isolated from the ethyl acetate part of plant [19], ethyl acetate was selected as the extraction solvent.

5. Conclusions

This report describes a successful development of HPLC assay for astilbin, which has a novel immunosuppressive activity, and its application to pharmacokinetic studies in rabbits. The sensitivity of this method appeared satisfactory for monitoring the plasma concentration.

Acknowledgements

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