Acta Pharmaceutica Sinica B 2016;6(3):243–252

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

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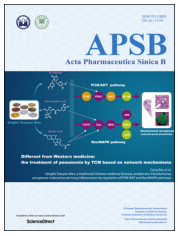

ORIGINAL ARTICLE

Pharmacokinetics, bioavailability, metabolism and excretion of δ-viniferin in rats

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Received 22 February 2016; received in revised form 17 March 2016; accepted 22 March 2016

KEY WORDS

Viniferin;
Pharmacokinetics;
Excretion;
Metabolism;
Bioavailability;
LC–MS/MS

Abstract A highly rapid and sensitive liquid chromatographic–electrospray ionization tandem mass spectrometric (LC–ESI-MS/MS) method was developed and validated for the determination of *trans*-δ-viniferin (Rs-1) in rat plasma, urine and feces. All biological samples were prepared by liquid–liquid extraction and hesperetin was included as an internal standard (IS). Chromatographic separation was achieved on a shim-pack XR-ODS column using a gradient mobile phase. MS/MS detection was performed by negative ion electrospray ionization. The method was sensitive with a lower limit of quantification of 1.42 ng/mL and linear over the range of 1.42–2172 ng/mL in all matrices. The method was applied to study the pharmacokinetics, bioavailability, metabolism, and excretion of Rs-1 in rats following a single oral or intravenous dose. Two metabolites, Rs-1 glucuronide and Rs-1 sulfate, were detected in plasma and in urine after administration of Rs-1. The absolute oral bioavailability of Rs-1 was 2.3%, and the total absorption rose to 31.5% with addition of its glucuronide and sulfate metabolites. Only 0.09% of the gavaged dose, including Rs-1 and metabolites, was excreted in the urine, while 60.3% was found in the feces in unchanged form. The results indicate that both poor absorption and extensive metabolism were the important factors that led to the poor bioavailability of Rs-1, which can provide a basis for further studies on structural modification and dosage form design.

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Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

http://dx.doi.org/10.1016/j.apsb.2016.03.008

1. Introduction

Resveratrol (*trans*-3,5,4′-trihydroxystilbene) and its oligomers, including their glucosides, are widely distributed in families, such as Dipterocarpaceae, Vitaceae, Cyperaceae, Leguminosae and Gnetaceae. Monomeric resveratrol displays a broad range of pharmacological activities including cardioprotection, antioxidation, protection against brain damage and prevention against cancers[1–4]. Resveratrol can be polymerized into resveratrol oligomers (so-called viniferins) in fungi or plants[5–9] and those resveratrol oligomers have more extensive pharmacological activities, such as anti-HIV and antitumor effects[10–13]. However, as with resveratrol and other stilbenes, the pharmacokinetic properties of those compounds are not favorable due to their poor bioavailability, extensive metabolism and rapid elimination[14,15].

Trans-δ-viniferin (Rs-1, Fig. 1), a resveratrol-*trans*-dehydrodimer, was first found in grapevine (*Vitis vinifera* L.) in 1977[16], and many studies have demonstrated that Rs-1 has numerous pharmacological properties, such as anti-inflammation[6,17], antimicrobial action[18,19], antioxidation[7], cardioprotection[8] and antidiabetes[20].

It has been shown that resveratrol has high absorption but very low bioavailability after oral administration in humans. At least 70% of oral dose was absorbed based on the urinary excretion data, and the relatively poor bioavailability was mainly owing to rapid metabolism[14]. A previous study has shown that the bioavailability of Rs-1 in rats was low[21], but whether poor absorption or rapid metabolism or both were responsible for the poor bioavailability was not determined. The bioavailability, metabolism and excretion of Rs-1 were investigated in the present study. A highly rapid and sensitive LC–MS/MS method was developed and validated for the determination of Rs-1 in rat plasma, urine and feces. This method yielded shorter sample turnover rate per sample and higher sensitivity compared with a previous study[21]. The method was applied to the assessment of the pharmacokinetics, bioavailability, metabolism and excretion of Rs-1 in healthy rats, and LC–MS/MS was used to identify Rs-1 metabolites.

2. Materials and methods

2.1. Materials and reagents

Rs-1 (purity >98%) was provided by the Institute of Materia Medica, Chinese Academy of Medical Sciences (Beijing, China). Hesperetin (Fig. 1), the internal standard (IS), was purchased from Sigma (St. Louis, MO, USA). Acetonitrile and methanol were purchased from ANPEL (Shanghai, China) and acetic acid was from OE Scientific Inc. (Newark, NJ, USA). All solvents were HPLC grade. Other chemicals used were analytical grade and obtained from Sigma–Aldrich (St. Louis, MO, USA). Water used in this study was prepared in a Milli-Q water purification system (Billerica, MA, USA).

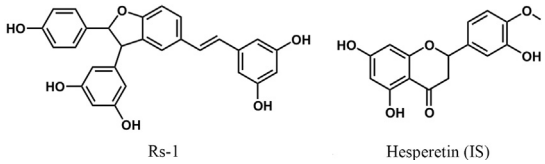

Figure 1 Chemical structures of Rs-1 and hesperetin (IS).

2.2. Liquid chromatography and tandem mass spectrometry conditions

Chromatographic separations were performed on a Shimadzu UFLC-20AD XR system (Shimadzu, Tokyo, Japan) with a shim-pack XR-ODS column (75 mm × 3 mm, 2.2 μm, Shimadzu, Tokyo, Japan) maintained at a temperature of 40 °C and a flow rate of 0.3 mL/min. The mobile phase was composed of A (0.1% formic acid–water, *v*/*v*) and B (0.1% formic acid–acetonitrile, *v*/*v*). A gradient elution program was as follow: 0–0.3 min, 45%–75% B; 0.3–1.8 min, 75% B; 1.8–2.1 min, 75%–45% B; 2.1–4 min, 45% B. The injection volume was 10 μL.

The Shimadzu UFLC-20AD XR system was coupled on-line to an Applied Biosystems Sciex Qtrap 5500 (MDS-Sciex, Concord, Canada) with an electrospray ionization (ESI) interface. The mass spectrometry was carried out using multiple reaction monitoring (MRM) and operated by electrospray ionization in negative mode for Rs-1 and IS. Transitions of precursor→product ion were monitored at *m*/*z* 453.2→411.2 for Rs-1 with a declustering potential (DP) of –178 V, collision energy (CE) of –35 V, and *m*/*z* 301.1→163.9 for IS with a DP of –100 V, CE of –32 V. Their collision exit potential (CXP) and entrance potential (EP) were both –10 V. The source parameters were optimized as follows: ion spray voltage, –4500 V; temperature, 550 °C; curtain gas (nitrogen), 28 psi; nebulizer and turbo gases (nitrogen), 50 and 50 psi, respectively. MRM transition was monitored with a dwell time of 200 ms. Quantification was determined from the peak area ratio of Rs-1 to IS. Analyst 1.6 software was used for control of equipment, data acquisition and analysis. The full scan product mass spectra of Rs-1 and IS are shown in Fig. 2.

2.3. Preparation of stock and working solutions

Stock solutions of Rs-1 and hesperetin (IS) at 0.2 mg/mL were prepared by dissolving the proper amount of the accurately weighed reference material in methanol. A series of working solutions for calibration standards (CS) in a concentration range of 14.2–21,720 ng/mL and quality control (QC) at 14.2, 35.6, 3480 and 17,380 ng/mL were prepared by subsequently diluting Rs-1 stock solution with methanol. The IS working solution at 200 ng/mL was prepared by diluting IS stock solution with methanol. All of the solutions were stored at –20 °C.

2.4. Preparation of standard and quality control samples

CS and QC samples of Rs-1 were prepared freshly by spiking the appropriate amount of the working solution into 100 μL of blank plasma, urine, feces suspension (1 g feces was homogenized with 20 mL of saline for 2 min). CS samples were prepared at 9 concentrations of 1.42–2172 ng/mL and QC samples were at 4 levels of 1.42 (lower limit of quantification QC), 3.56 (low QC), 348 (middle QC) and 1738 (high QC) ng/mL.

2.5. Sample preparation

A liquid–liquid extraction (LLE) method was used to extract the analyte from biological samples (plasma, urine and feces). For Rs-1 analysis, an aliquot of 100 μL of each plasma, urine or feces suspension sample was mixed with 20 μL of IS working solution and the mixture was vortex-mixed for 30 s. For Rs-1 in plasma and urine, 600 μL ethyl acetate was added, and in feces, 600 μL diethyl