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Quantification of desoxyrhapontigenin (4-methoxyresveratrol) in rat plasma by LC–MS/MS: Application to pre-clinical pharmacokinetic study

Yu Dai [a], Amanda Li Cheng Tan [a], Huan Chen [a], Pei Shi Ong [a], Xiaoqiang Xiang [b], Jinzhu Wu [c], Hai-Shu Lin [a,*]

[a] Department of Pharmacy, National University of Singapore, 10 Kent Ridge Crescent, 119260, Singapore
[b] Department of Clinical Pharmacy, School of Pharmacy, Fudan University, 826 Zhangheng Road, Shanghai, 201203, China
[c] Department of Chemistry, School of Science, Harbin Institute of Technology, Harbin, 150001, China

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ABSTRACT

Desoxyrhapontigenin (DRG, 4-methoxyresveratrol or *trans*-3,5-dihydroxy-4′-methoxystilbene) is a naturally occurring resveratrol (RES) derivative with a variety of biological activities. To facilitate its further medicinal exploration, a reliable LC–MS/MS method was developed and validated for the quantification of DRG in rat plasma using heavy isotope labelled RES as an internal standard. The ESI was operated in its negative ion mode while DRG and RES were determined by multiple reaction monitoring (MRM) using precursor-to-product ion transitions of m/z 241.1 → 180.8 and m/z 233.0 → 191.0, respectively. This LC–MS/MS method displayed excellent selectivity, sensitivity (LLOQ = 2.5 ng/ml), accuracy (both intra- and interday mean analytical recovery within 100 ± 15%) and precision (both intra- and interday CV < 15%). The mean matrix factors were all within 1.000 ± 0.150 with CV < 15%. The pharmacokinetic profiles of DRG were subsequently examined in Sprague-Dawley rats. Upon intravenous administration (4 or 10 mg/kg), DRG displayed very rapid clearance (Cl = 338 ± 66 or 275 ± 30 ml/min/kg) and short mean residence time (MRT = 12.9 ± 4.7 or 10.4 ± 0.5 min). After oral administration of DRG fully solubilized by 2-hydroxypropyl-β-cyclodextrin (HP-β-CD), the plasma profiles of DRG were highly erratic with a low absolute bioavailability (F < 9.83 ± 5.31%). When DRG was given at a higher dose (50 mg/kg) in suspension form, the F was increased to 24.1 ± 5.6%. The pharmacokinetic comparison among DRG, RES and some of its hydroxyl analogues stilbenes was performed. The information obtained from this study will facilitate further exploration on DRG as well as other RES derivatives.

1. Introduction

Resveratrol (RES, *trans*-3,5,4′-trihydroxystilbene), a phytoalexin that is present in grapes, red wine and some other edible plants, has attracted substantial interests in scientific community over the past decades [1]. Its beneficial biological activities including anti-ageing, anti-cancer, anti-diabetic, anti-inflammation, anti-obesity, anti-oxidation, cardio-, hepato- and neuro-protection have been observed in numerous pre-clinical studies [1]. Recently, the interests in RES has been extended to its dietary/herbal derivatives.

Desoxyrhapontigenin (DRG, 4-methoxyresveratrol or *trans*-3,5-dihydroxy-4′-methoxystilbene) is a monomethylether of RES that can be found in rhubarb (rhizome of *Rheum*), a remedy widely used in oriental herbal medicines [2–4]. Similar to RES and many of its derivatives, DRG possessed various health-promoting effects such as anti-androgenic [5], anti-diabetic [6], anti-inflammation [2,4], anti-oxidation [2,7] and anti-platelet [8]. Moreover, as it was found to be an inhibitor of cytochrome P450 1A1 and 1B1, which are responsible for the metabolic activation of various chemical carcinogens, DRG displayed cancer chemo-preventive potential as well [9]. Clearly, DRG has emerged as a promising candidate for further medicinal exploration.

Pharmacokinetic study plays a significant role in drug discovery and development. Obtaining the pharmacokinetic profile(s) of a lead candidate and/or its active metabolite(s) in plasma over time enables a better correlation of its dosing regimen to its exposure and responses. However, to the authors' knowledge, the pharmacokinetic profile of DGR has not been reported in literature although

* Corresponding author.
E-mail address: phalh@nus.edu.sg (H.-S. Lin).

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it was detected as a metabolite in rat urine after oral dosing of *Resina Draconis* extract [10]. In addition, there is a lack of a sensitive analytical assay for the quantification of DGR in biological samples, further hindering its pharmacokinetic profiling.

In the present study, a sensitive and reliable LC–MS/MS method was developed and validated for quantifying DGR in rat plasma. The pharmacokinetic profile of DRG was subsequently examined in Sprague-Dawley rats following oral and intravenous administration. To the authors' knowledge, this is the first report on the LC–MS/MS quantification and pharmacokinetics of DRG. The information obtained from this study will facilitate further exploration on DRG as well as other RES analogues.

2. Experimental

2.1. Special precautions

In order to prevent the photo-isomerisation of light-sensitive stilbenes, all laboratory handling of DRG and RES was carried out in a dimly-lit environment [11,12].

2.2. Chemicals and reagents

Desoxyrhapontigenin (4-methoxyresveratrol or *trans*-3,5-dihydroxy-4′-methoxystilbene, DRG) (purity: 99.7%) was obtained from Xi'an Leader Biochemical Engineering Co., Ltd (Xi'an, China). *Trans*-3,5,4′-trihydroxystilbene-$^{13}C_6$ (isotopically labelled resveratrol with a mass shift of M+6, an isotopic purity of 99% and a chemical purity of >97%) was purchased from Sigma-Aldrich (St Louis, MO, USA) and used as an internal standard (IS). 2-hydroxypropyl-β-cyclodextrin (HP-β-CD, degree of substitution: about 0.6) was kindly donated by Wacker (Burghausen, Germany). Sodium salt of carboxymethylcellulose (CMC), a suspending vehicle was purchased from Sigma-Aldrich. DMSO (analytical grade) and acetonitrile (HPLC grade) were obtained from MP Biomedical (Santa Ana, CA, USA) and Fisher Scientific (Singapore), respectively. Ultrapure water (18.2 MΩ cm at 25 °C) was dispensed from a Millipore Direct-Q® ultra-pure water system (Billerica, MA, USA) and applied to prepare mobile phase as well as dosing vehicles.

2.3. Preparation of calibration standards and QC samples

Stock solutions of DRG (1.00 mg/ml) were prepared in DMSO and they were stored at room temperature. The stock solutions were further diluted using pooled blank Sprague-Dawley rat plasma to prepare the calibration standards (2.5, 5, 10, 20, 50, 100 and 250 ng/ml) and QC samples (7.5, 75 and 200 ng/ml). The stock solution of IS was prepared in acetonitrile at a concentration of 10.0 μg/ml, 100 μl of this stock solution was aliquoted into several 1.5 ml polypropylene tubes and frozen at −80 °C. Prior to sample preparation, the IS stock solution was further diluted with acetonitrile to a working concentration of 10 ng/ml, then stored at 4 °C.

2.4. Sample preparation

A simple protein precipitation method was used to clean up the plasma samples prior to LC–MS/MS analysis [11,13]. One hundred and five μl IS working solution were added to 35 μl plasma sample. After vigorous vortex, centrifugation of samples was performed at 15,000*g* for 10 min at 4 °C. Subsequently, the clear supernatants were transferred into HPLC vials. During each LC–MS/MS analysis, 2 μl of supernatant was injected into the HPLC.

2.5. Instrumentation

The assay was performed with an Agilent 1290 Infinity Liquid Chromatography system (Agilent Technologies, CA, USA) using a reversed-phase C18 column (Agilent Poroshell 120 EC-C18: 75 × 3.0 mm, 2.7 μm), which was protected by a guard column (Agilent ZORBAX Eclipse Plus C18 12.5 × 4.6 mm, 5 μm). This chromatographic system was coupled to ABSciex QTRAP® 5500 (AB Sciex, MA, USA) mass spectrometer equipped with TurboIon Spray™ (electrospray ionisation (ESI)) probe. The LC–MS/MS system was controlled by the Analyst 1.5.2 software (AB Sciex) and chromatographic data analysis was performed using the same software. Chromatographic separation was obtained by a 6-min gradient delivery of acetonitrile and water at a flow rate of 0.7 ml/min at 40 °C. The gradient program was: (a) 0.0–1.0 min: 15% acetonitrile; (b) 1.0–1.5 min: 15% → 50% acetonitrile; (c) 1.5–2.5 min: 50% acetonitrile; (d) 2.5–4.0 min: 50% → 95% acetonitrile; (e) 4.0–4.5 min: 95% acetonitrile; (f) 4.5–5.0 min: 95% → 15% acetonitrile; (g) 5.0–6.0 min: 15% acetonitrile. Only the eluent from 2.0–2.9 min was directed into the mass spectrometer via electrospray ionization (ESI) operated in the negative ionization mode.

In mass spectrometer, nitrogen was used as nebulising, curtain and collision gases. Optimization of the operating parameters of the ESI ion source for the DRG and IS was carried out through tuning as reported in previous studies [11,13]. Both the negative and positive ion mode were attempted to detect the parent ions and the ion mode with highest detection sensitivity for the parent ion of DRG was used to further optimize the operating parameters of the MS. The source parameters, including curtain gas, gas 1, gas 2, temperature, and ion spray voltage were set at 20 psi, 40 psi, 30 psi, 600 °C and −4500 V, respectively. Sequential ramping of the operation potentials identified the optimal compound parameters, comprising of declustering potential (DP), entrance potential (EP), collision energy (CE) and collision exit cell potential (CXP), to be −108.2 V, −9.1 V, −27.8 V and −12.7 V for DRG and −74.6 V, −3.9 V, −26.9 V and −12.2 V for IS, respectively. Using the optimized parameters, the MS detector was operated in multiple reaction monitoring (MRM) mode using a dwell time of 100 ms and at unit mass resolution.

2.6. Method validation

The validation of this LC–MS/MS method was performed by examining its selectivity, sensitivity, linearity, accuracy, precision, absolute recovery, dilution integrity, matrix effect and stability under different conditions [11,13].

Selectivity was established by comparing the chromatograms of six individual blank rat plasma samples with same plasma samples spiked with DRG and IS. The selectivity was further confirmed by chromatographic comparison between pre-dosing and post-dosing plasma samples collected from pharmacokinetic study.

The sensitivity of this assay was denoted by the lower LOQ, which was defined as the minimal concentration that produces a signal-to-noise ratio not less than 5 with acceptable accuracy (mean analytical recovery: 80% − 120%) and precision (CV ≤ 20%).

The analytical response was expressed as the peak area ratio of the DRG and IS. Linear regression analysis was performed where x was the concentration of compound, y was the analytical response and $1/x^2$ was chosen as the weighting factor. The goodness-of-fit of linear regression is represented by the R^2. Linearity was assessed on 3 consecutive days using calibration standards (2.5, 5, 10, 20, 50, 100, 250 ng/ml). In day 1, five replicate samples were prepared while in day 2 and 3, duplicate samples were prepared.

Intra- and inter-day accuracy and precision were assessed using quality control samples at high, medium and low concen-