Revista Brasileira de Farmacognosia xxx (2017) xxx–xxx

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Original Article

Quantitative determination of monotropein in rat plasma and tissue by LC–MS/MS and its application to pharmacokinetic and tissue distribution studies

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ARTICLE INFO

Article history:
Received 28 February 2018
Accepted 8 May 2018
Available online xxx

Keywords:
Monotropein
Pharmacokinetics
Tissue distribution
LC–MS/MS

ABSTRACT

A selective and sensitive liquid chromatography tandem with mass spectrometry was developed and validated for accurate determination of monotropein in rat plasma and tissues. All biological samples were prepared by simple protein precipitation method using catalpol as an internal standard. The analyte and internal standard were separated on a C_{18} analytical column with 2 min of run time, at flow rate of 0.5 ml/min. The detection was performed on a triple-quadrupole tandem mass spectrometer equipped with negative-ion electrospray ionization by selected-reaction monitoring of the transitions at m/z 389→147 for monotropein and m/z 361→169 for the internal standard. The calibration curves for plasma and tissue samples were linear over the concentration range of 4–2000 ng/ml, with a lower limit of quantification of 4 ng/ml. The method was successfully applied to a pharmacokinetic and tissue distribution study of monotropein in rats.

Introduction

Monotropein (**1**) is an iridoid glycoside isolated from the root of *Morinda officinalis* F.C.How, Rubiaceae (Yang et al., 2016; Zhao et al., 2017a). Monotropein exhibits an evident anti-inflammatory effect that is mainly related to the inhibition of the expressions of inflammatory mediators *via* NF-κB inactivation (Shin et al., 2013). Furthermore, administration of monotropein exerts good bone protective effects by increasing bone mineral content and volume fraction, and improving bonemicro structure and biochemical properties in an ovariectomy mice model of osteoporosis (Zhang et al., 2016). Therefore, it may be a promising candidate for the prevention and treatment of osteoporosis. In addition, monotropein displays anti-apoptosis, antioxidant, antinociceptive and anti-catabolic activities *in vitro* (Choi et al., 2005; Wang et al., 2014, 2017; Zhu et al., 2016; Yang et al., 2017). As such, this compound has been extensively investigated. Therefore, it is important to establish analytical methods for the determination of monotropein in biological fluids and to study pharmacokinetics and tissue distribution in animals.

Several methods have been reported to analyze monotropein in plants and herbal prescriptions, including high-performance liquid chromatography (HPLC) (Yang et al., 2008; Liang et al., 2008; Zhao et al., 2017b), gas chromatography (Inouye et al., 1976), and HPLC coupled with mass spectrometry (Li et al., 2016a). Recently, an LC tandem with mass spectrometry (LC–MS/MS) method was reported to quantify monotropein and another iridoids glycoside deacetylasperulosidic acid in rats after consumption of *Morinda officinalis* extract (Li et al., 2016b), but the pharmacokinetic properties after given with pure monotropein still remain unknown so far. Here a sensitive and robust LC-MS/MS method was established and validated for the determination of monotropein in rat plasma and various tissues. The method was successfully applied to pharmacokinetics and tissue distribution of monotropein in rats after intragastric administration.

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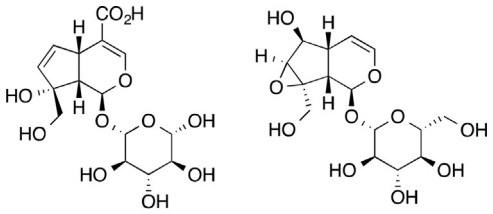
https://doi.org/10.1016/j.bjp.2018.05.005

Please cite this article in press as: Zhou, Q., et al. Quantitative determination of monotropein in rat plasma and tissue by LC–MS/MS and its application to pharmacokinetic and tissue distribution studies. Revista Brasileira de Farmacognosia (2017), https://doi.org/10.1016/j.bjp.2018.05.005

Materials and methods

Chemicals and reagents

Reference standards of monotropein (**1**) (purity 94.2%) and catalpol (**2**) (internal standard, IS; purity 98.1%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC-grade methanol and acetonitrile were purchased from Tedia Co. (Ohio OH, USA). Deionized water was prepared using a Milli-Q Purification System (Millipore, Bedford, MA, USA). All other reagents were of analytical reagent grade.



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Instruments and chromatographic condition

The LC–MS/MS system was a Dionex Ultimate 3000 RSLC system equipped with a triple-quadrupole TSQ Vantage mass spectrometer (Thermo Scientific, San Jose, CA, USA). The stationary phase was a Thermo Scientific Hypersil GOLD C_{18} column (4.6 mm × 50 mm, 3.0 μm) with 40 °C column temperature. The mixture of methanol and water (50:50, v/v) was used as the mobile phase with an isocratic elution pattern at a flow rate of 0.5 ml/min. Mass spectral ionization, fragmentation and acquisition parameters were optimized by directly injecting monotropein and catalpol standard solutions in the negative ESI mode. Nitrogen was employed as the sheath and auxiliary gases at the pressures of 45 psi and 15 psi, respectively. Argon was employed as the collision gas. The data were acquired under selected-reaction monitoring (SRM) mode with precursor to product qualifier transition m/z 389→147 for monotropein at collision energy of 22 eV and m/z 361→169 for IS at collision energy of 20 eV, respectively (Fig. 1).

Preparation of standard and quality control (QC) samples

A stock solution of monotropein (**1**) (0.4 mg/ml) was prepared by dissolving 4 mg in 10 ml of methanol-water (50:50, v/v). A stock solution of catalpol (**2**, IS) was prepared in methanol-water at a concentration of 0.5 mg/ml and further diluted with methanol given the working solution at the concentration of 50 ng/ml. All stock solutions were stored at −60 °C until use. Calibration curves were prepared by spiking 10 μl of the appropriate standard solution with 190 μl of blank rat plasma or rat tissue homogenate. The effective calibration concentrations in all biological matrices were 4.0, 10, 20, 50, 200, 500, 1000 and 2000 ng/ml for monotropein. Another stock solution of monotropein (0.4 mg/ml) in methanol-water (50:50, v/v) was used to prepare QC samples. The QC samples were prepared in the same way as calibration standard at concentrations of 8 (low), 100 (medium) and 1600 ng/ml (high). 50 μl of the calibration standard and QC samples were then pipetted into plastic tubes and treated as '*Extraction of plasma and tissue samples*' section.

Extraction of plasma and tissue samples

Plasma or tissue homogenate (50 μl), IS (50 μl, 50 ng/ml) and acetonitrile (300 μl) were drawn into a plastic tube, vortexed for 3 min then centrifuged at 13,680 × *g* for 10 min. The organic layer was separated and evaporated to dryness at 40 °C under a nitrogen stream. The residue was reconstituted in 200 μl of the mobile phase and 5 μl was injected for LC–MS/MS analysis. Similarly, tissue homogenates were processed and analyzed by LC–MS/MS.

Method validation

The LC–MS/MS method was validated for carryover, selectivity, linearity, sensitivity, precision, accuracy, matrix effect, stability and extraction recovery in accordance with the Bioanalytical Method Validation of FDA (US Food and Drug Administration, 2001). Blank matrix samples obtained from six rats were screened to evaluate selectivity. Calibration curves were constructed by plotting the peak area ratios (y-axis) of the analyte to IS against the spiked concentrations (x-axis) and analyzed by weighted ($1/x^2$) least squares linear regression. The lower limit of quantification (LLOQ) was defined as the lowest concentration that yield a signal-to-noise ratio (S/N) greater than or equal to 10. Intra- and inter-day accuracy and precision were evaluated with five replicates at three QC levels on a single assay and on three consecutive validation days. Acceptable criteria were within 15% of precision and accuracy, except the LLOQ, which was within 20%. Recovery was determined by comparing peak areas of analyte in extracted QC samples with those of the spiked at corresponding concentrations to post-extracted blank matrix. Matrix effect was studied by comparing peak areas of analyte spiked in post-extracted blank matrix with those of spiked in post-extracted water. Stability was investigated under four different conditions as follows: short-term (ambient temperature for 6 h), long-term (−60 °C for 30 days), freeze and thaw (three cycles at −60 °C and room temperature), and post-preparation stability (autosampler at 4 °C for 12 h).

Stability study of monotropein in simulated gastric and intestinal fluids

Monotropein (**1**, 2000 ng/ml) was incubated in simulated gastric fluid (pH 1.2), and simulated intestinal fluid (pH 6.8) for 2 h in a shaking water bath (37 °C and 100 rpm). Organic content in the reaction mixture was kept within 0.5%. 100 μl of samples were taken at 0, 0.25, 0.5, 1.0, 1.5, and 2.0 h and quenched with 300 μl of acetonitrile containing 50 ng/ml IS (**2**). Samples were vortexed and centrifuged at 13,680 × *g* for 10 min. The organic layer was separated and evaporated to dryness at 40 °C under a nitrogen stream. The residue was reconstituted in the mobile phase and analyzed using the developed LC–MS/MS method.

Pharmacokinetic and tissue distribution studies

The animal study was approved by the Institutional Animal Ethical Committee of the First Hospital of Jilin University (Approval Number: 2017-123). For pharmacokinetic study, eighteen rats (220 ± 20 g) were randomly divided into three groups (six rats per group). Monotropein freshly prepared by suspending the required amounts in 0.5% sodium carboxymethyl cellulose (CMC-Na) solution was given to rats *via* intragastric administration at doses of 10, 20 and 40 mg/kg, respectively. Rats were fasted 12 h prior to the monotropein dose. Approximately 250 μl blood samples were collected into heparinized tubes from the tail vein into heparinized 1.5 ml polythene tubes at 0 (predose), 0.083, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12 and 24 h after intragastric administration. The samples were immediately centrifuged (860 × *g*, 10 min), then the supernatant layer was collected and stored at −60 °C until analysis. The pharmacokinetic parameters, including maximum plasma concentration (C_{max}), time reaching C_{max} (T_{max}), elimination half-life ($t_{1/2}$), area under the plasma concentration–time curve (AUC), and mean

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