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Determination and pharmacokinetic study of the novel anti-tumor candidate drug DG-7 in rat plasma by liquid chromatography–tandem mass spectrometry

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ABSTRACT

DG-7 (11,14-dihydroxy-7,20-epoxy-20-*O*-derivative of *ent*-kaurene diterpenoid) is a novel anti-tumor candidate drug. A sensitive and specific liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was developed and validated for the quantification of DG-7 in rat plasma. An aliquot of 50 μL plasma sample was prepared by liquid–liquid extraction with ethyl acetate. Chromatographic separation was accomplished on a Waters XTerra C_{18} column (2.1 mm × 150 mm, 5 μm) with an isocratic elution system consisting of methanol and water. Detection was performed by multiple reaction monitoring (MRM) mode using electrospray ionization in the positive ion mode. The optimized fragmentation transitions for MRM were m/z 590.1 → m/z 260.0 for DG-7 and m/z 180.3 → m/z 110.1 for phenacetin (internal standard). The method was linear over the concentration range of 5–2500 ng/mL. The intra- and inter-day precisions were less than 7.9% and the accuracy was within ±9.0%. The mean recovery of DG-7 ranged from 76.8% to 79.2%. The validated method has been successfully applied to a pharmacokinetic study in rats after intravenous administration of DG-7.

1. Introduction

Ent-kaurene diterpenoids are the major components of *Rabdosia rubescens* (Donglingcao in Chinese) [1–3]. Chemical investigations and pharmacological studies have demonstrated that *ent*-kaurene diterpenoids have a series of biological and physiological activities including anti-tumor [4–6], anti-bacterial [7,8], anti-inflammatory [9], induction of apoptosis [10,11], antioxidant [12] and cytotoxicity [13–16]. Oridonin is a natural *ent*-kaurane diterpenoid isolated from *R. rubescens* with its unique biological activity [17,18]. Cell culture experiments showed that oridonin had an inhibitory effect on a variety of human cancer cell lines, such as leukemia [19]; esophageal cancer [20]; gastric cancer [20]; liver cancer [21]; lung cancers [22] and uterine cancer [23]. However, there are still some issues associated with oridonin including a short half-life, poor water solubility and instability in plasma [24–26]. These inherent problems prompted us to synthesize novel anti-tumor *ent*-kaurene diterpenoid derivatives with better pharmacokinetic profiles.

As a part of our ongoing investigation to develop more potent anti-tumor agents, we have designed and synthesized many novel *ent*-kaurene diterpenoid derivatives. Out of all the derivatives, DG-7 (11,14-dihydroxy-7,20-epoxy-20-*O*-derivative of *ent*-kaurene diterpenoid (Fig. 1)) has shown increased anti-tumor activity. The IC_{50} values of DG-7 treatment for 48 h were estimated to be 7.81 μM in MCF-7 cells and 4.81 μM in SMMC7721 cells, respectively, which exhibited excellent *in vitro* anti-tumor activity relative to oridonin [27,28]. Based on the superior biological activity, DG-7 was selected for further development as a potential anti-tumor lead. During the development of a new drug candidate, it is essential to obtain early information regarding its pharmacokinetic parameters as early as possible [29].

To further understand and explore the pharmacokinetic profile and therapeutic effect of the DG-7, a sensitive and high throughput method is required for routine analysis in biological samples. In this study, we developed a rapid and sensitive HPLC–MS/MS method for determination of DG-7 in rat plasma. The method was fully validated in terms of selectivity, linearity, precision, accuracy, matrix effects, extraction recovery and stability, and then applied

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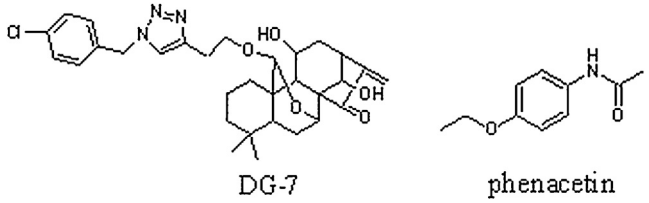

Fig. 1. Chemical structures of DG-7 and phenacetin (IS).

to investigate the pharmacokinetic study of DG-7 in rats after intravenous administration of DG-7.

2. Materials and methods

2.1. Reagents and chemicals

DG-7 (Fig. 1, purity > 99%) was provided by the New Drug Research & Development Center of Zhengzhou University. Phenacetin (Fig. 1, purity > 99%) was used as an internal standard (IS) and was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol and acetonitrile (HPLC grade) were purchased from J.T. Baker (USA). Ethyl acetate and formic acid were from Sigma–Aldrich Co. Ltd. (Poole, UK). All of the aqueous solutions were prepared with water purified using a Milli-Q water-purification system (Millipore, Bedford, MA, USA).

2.2. Apparatus and operating conditions

2.2.1. Liquid chromatography

Chromatography was performed on a Waters HPLC system (Waters Corp., Milford, MA, USA) including a 2695 separation module. HPLC separations were carried out using a Waters XTerra C18 column (2.1 mm × 150 mm, 5 μm) (Waters Corp., Milford, MA, USA) at ambient temperature. The isocratic mobile phase consisted of methanol and water (75:25, *v*/*v*), delivered at a flow rate of 0.2 mL/min. The total run time for sample analysis was 6 min. Data were acquired by MassLynx V4.1 software (Waters Corp., Milford, MA, USA) and processed for quantification with QuanLynx V4.1 (Waters Corp., Milford, MA, USA).

2.2.2. Mass spectrometry

Mass spectrometry was performed using a Micromass Quattro Micro™ API triple-quadrupole system (Waters, Manchester, UK) equipped with an electrospray ionization (ESI) source. The ESI instrumental settings were optimized for the analysis, the appropriate MRM transitions and MS/MS parameters were determined for each compound by direct infusion into the mass spectrometer. The following were the optimum operating parameters of the ESI interface in positive mode: capillary voltage of 3.0 kV, source temperature at 120 °C and desolvation temperature at 300 °C. Cone voltage was 30 V, and collision energies of 20 eV for DG-7 and 18 eV for IS. Quantification was determined using MRM mode for the transitions *m*/*z* 590.1 → *m*/*z* 260.0 for DG-7, and *m*/*z* 180.3 → *m*/*z* 110.1 for IS (Fig. 2).

2.3. Preparation of stock solutions, calibration samples, and quality controls

Stock solutions of DG-7 and phenacetin (IS) were prepared separately in methanol at a target concentration of 100 μg/mL. A series of working standard solutions were obtained by further diluting the stock solutions with 50% methanol. The IS working solution (500 ng/mL) was obtained by diluting the stock solution with 50% methanol. Calibration standards were prepared by spiking 10 μL working solutions into 50 μL blank plasma to yield final concentrations of 5, 10, 20, 50, 100, 200, 500, 1000 and 2500 ng/mL.

The quality control (QC) samples were used to assess the accuracy and precision of the assay method. The quality control (QC) samples were prepared by the same procedure as above at concentrations of 10, 100 and 2000 ng/mL for the low, medium and high concentration QC samples, respectively. All solutions were kept refrigerated (4 °C) and brought to room temperature before use.

2.4. Sample preparation

Plasma samples (50 μL) were spiked with 10 μL of IS solution (phenacetin, 500 ng/mL). The mixture was extracted with 1 mL ethyl acetate by vortex-mixing for 2 min. After centrifugation at 12,000 rpm for 10 min, the supernatant was transferred into a clean glass tube and evaporated to dryness under a gentle stream of nitrogen gas at 37 °C. The residue was reconstituted in 500 μL 50% methanol by ultrasonic method for 60 s and centrifuged at 12,000 rpm for 10 min. Finally, 5 μL supernatant was injected into the LC–MS/MS system for analysis.

2.5. Method validation

The method was validated according to the Food and Drug Administration (FDA) guidelines for selectivity, linearity, precision, accuracy, matrix effects, recovery and stability [30].

2.5.1. Selectivity

The selectivity of the LC–MS/MS method was evaluated by comparing the chromatograms of six different batches of the blank plasma with the corresponding standard plasma samples spiked with DG-7 and the internal standard, as well as the rat plasma samples after intravenous administration of DG-7.

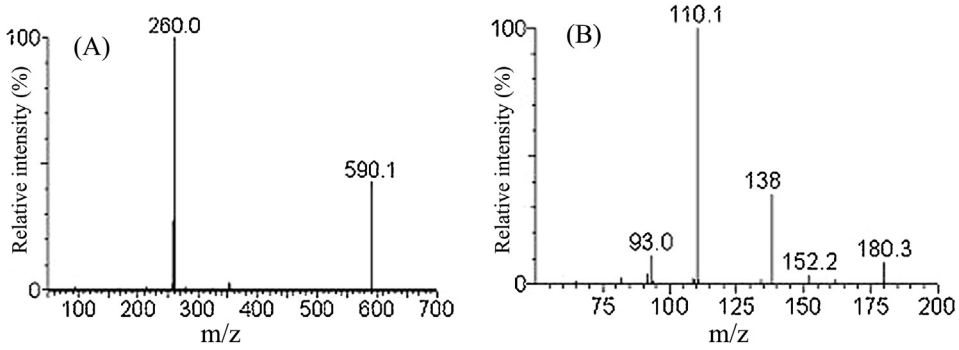

Fig. 2. Product ion spectra of (A) DG-7 and (B) IS.