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Simultaneous determination of gypenoside LVI, gypenoside XLVI, 2α-OH-protopanaxadiol and their two metabolites in rat plasma by LC–MS/MS and its application to pharmacokinetic studies

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ABSTRACT

Gypenoside LVI and gypenoside XLVI are the major bioactive dammarane saponins from *Gynostemma pentaphyllum*. Gypenoside LVI, gypenoside XLVI, and their metabolite 2α-OH-protopanaxadiol (2α-OH-PPD) possess potent non-small cell lung carcinoma A549 cell inhibitory activity. A sensitive liquid chromatography tandem mass spectrometry method was developed and validated to study the pharmacokinetics of gypenoside LVI and XLVI, 2α-OH-PPD, metabolite 1 (M1), and metabolite 2 (M2) after administration of gypenosides or 2α-OH-PPD. Plasma samples from rats were protein precipitated with methanol. Analytes were detected by triple quadrupole MS/MS with an electrospray ionization source in the positive multiple reaction monitoring mode. The transition m/z 441.4 → 109.2 was selected to quantify gypenoside LVI and XLVI, and 2α-OH-PPD, because of the extensive conversion of the gypenosides to aglycone in the ionization source. M1 and M2 are isomers that shared the transition m/z 493.4 → 143.1. To avoid interference, the baseline separation of each analyte was performed on a SunFire C18 column with a gradient of acetonitrile (0.1% formic acid, v/v) and water (0.1% formic acid, v/v). The chromatographic run time was 10 min. The linearity was validated over a plasma concentration range from 2.00 to 2000 ng/mL for M1 and M2, and from 10.0 to 2000 for gypenosides LVI and XLVI, and 2α-OH-protopanaxadiol. The lower limits of quantification were 10.0, 10.0, 10.0, 2.00, and 2.00 ng/mL for gypenoside LVI, gypenoside XLVI, 2α-OH-PPD, M1, and M2, respectively, with acceptable intra-/inter-day precision and accuracy. The extraction recovery rates were >86.9% for each compound. No apparent matrix effect or instability was observed during each step of the bioanalysis. After full validation, this method was proved to be simple, fast, and efficient in analyzing large batches of plasma samples for the analytes.

1. Introduction

Gynostemma pentaphyllum (*G. pentaphyllum*) is widely used as a herbal medicine in Asian countries to lower cholesterol, strengthen the immune system, and inhibit tumors [1,2]. Gypenosides, a group of dammarane triterpene saponins, are the principal bioactive constituents in *G. pentaphyllum* responsible for these beneficial properties [3,4]. Gypenosides possess many biological and pharmacological effects, including anti-cancer [5–7], anti-oxidation [8], anti-inflammatory [9], anti-hyperlipidemic [10], and ameliorating chronic stress-induced anxiety [11]. A recent in vitro study demonstrated the potent cytotoxic activity of gypenosides LVI (Fig. 1) and XLVI, and the corresponding aglycone 2α-OH-protopanaxadiol (2α-OH-PPD) against non-small cell lung carcinoma A549 cells [12]. We performed a metabolic study, which showed that after oral administration of 2α-OH-PPD the major metabolic pathway of 2α-OH-PPD was epoxidation and cyclization of the double bond to yield the isomeric metabolites 1 and 2 (M1 and M2). Similar metabolic pathways were observed for ginsenosides [13], and the metabolites showed similar pharmacological activities to their parent drugs. According to the structure-activity relationship, M1 and M2 may exhibit similar antitumor activity to 2α-OH-PPD [13]. Consequently, more research has focused on the pharmacological activities of gypenoside LVI and XLVI, 2α-OH-PPD, metabolite 1 (M1), and metabolite 2 (M2). To predict the therapeutic efficacy based on the pharmacokinetics of gypenosides, an analytical

Abbreviations: 2α-OH-PPD, 2α-OH-protopanaxadiol; *G. pentaphyllum*, *Gynostemma pentaphyllum*; GI, gastrointestinal; IS, internal standard; LC–MS/MS, liquid chromatography–tandem mass spectrometry; LLE, liquid–liquid extraction; LLOQ, lower limit of quantification; MRM, multiple reaction monitoring; QC, quality control; RE, relative error; RSD, relative standard deviation.

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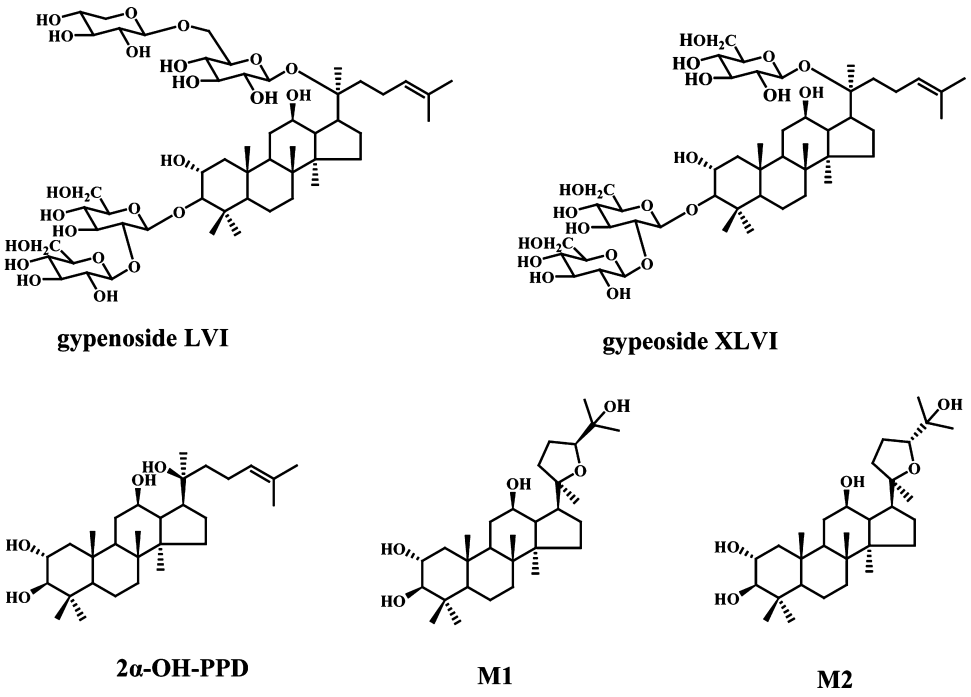

Fig. 1. Structures of analytes.

method is required to determine the concentrations of these analytes.

As far as we know, there are few reports describing the bioassay or the pharmacokinetic study of gypenoside LVI and XLVI, 2α-OH-PPD, M1, and M2. In this study, we established a rapid, sensitive liquid chromatography tandem mass spectrometry (LC–MS/MS) method for the simultaneous determination of gypenoside LVI and XLVI, 2α-OH-PPD, M1, and M2 in rat plasma. We used our method to study the pharmacokinetic behaviors of gypenoside LVI and XLVI, 2α-OH-PPD, M1, and M2 in rats after a single oral administration of gypenosides or 2α-OH-PPD.

2. Experimental

2.1. Chemicals and reagents

Gypenosides (total saponins of sweet *G. pentaphyllum*) were purchased from Orient Plant Health Care Science and Technology Co., Ltd. (no. 20070617, Huizhou, Guangdong Province, People's Republic of China). Gypenoside LVI and XLVI account for 57% and 24%, respectively, of the total saponins of sweet *G. pentaphyllum*. Gypenoside LVI (molecular mass 1094 g/mol), gypenoside XLVI (molecular mass 962 g/mol), 2α-OH-PPD (molecular mass 476 g/mol), M1 (molecular mass 492 g/mol), and M2 (molecular mass 492 g/mol) standards were kindly provided by Prof. Lihong Hu (Shanghai Institute of Materia Medica, Shanghai, China). The purity of the compounds was >98%. High-performance liquid chromatography (HPLC)-grade acetonitrile and methanol were purchased from Merck Chemical Co. (Darmstadt, Germany). Formic acid for HPLC was purchased from CNW Technologies (Dusseldorf, Germany). Water for HPLC analysis was purified with a PW Ultrapure Water System (Heal Force Co., Shanghai, China).

2.2. Experimental animals

Male Sprague-Dawley rats (specific pathogen free, body weight 200 ± 20 g) were provided by the Shanghai Laboratory Animal Corporation (Shanghai, China), and housed in the Experimental Animal Center of Shanghai Institute of Materia Medica under a 12 h light/dark cycle. The rats were kept in air-conditioned rooms, and were allowed free access to food and water.

2.3. Instruments

HPLC analysis was performed on an LC-20A system (Shimadzu, Kyoto, Japan), comprising an LC-20AD binary pump, a DGU-20AIII degasser, a SIL-20AC auto-sampler, and a CTO-20AC thermostatic column compartment. Detection was performed by triple quadrupole MS/MS (model 8030, Shimadzu).

2.4. HPLC–MS/MS conditions

Chromatographic separation was performed on a SunFire C18 column (50 × 2.1 mm, i.d., 3.5 μm; Waters, Milford, MA, USA). The mobile phases were (A) acetonitrile (0.1% formic acid, v/v) and (B) water (0.1% formic acid, v/v). The gradient elution was as follows: 12% A for 0.5 min; 12–40% A over 0.5 min; 40% A for 1.5 min; 40–80% A over 0.5 min; 80% A for 4 min; 80–12% A over 0.2 min; and 12% A for 2.8 min. The flow rate was kept at 0.2 mL/min and the column temperature was maintained at 40 °C. A needle wash was performed before and after injection with 100 μL methanol: water (50/50, v/v). The total run time was <10 min.

The MS was performed in positive electrospray ionization mode. The DL and heat block temperatures were set at 250 and 400 °C, respectively. The nebulizing gas (nitrogen) and drying gas (nitrogen) flow rates were set at 3 and 12 L/min, respectively. The collision gas (argon) pressure was 230 kPa. The same multiple reaction mode (MRM) transition (m/z 441.4 → 109.2) was used to quantify gypenoside LVI and XLVI, and 2α-OH-PPD. The transition for M1 and M2 was m/z 493.4 → 143.1. The transition of m/z 271.5 → 154.7 was recorded for the internal standard (IS), tolbutamide.

2.5. Preparation of standard and quality control samples

Standard stock solutions were prepared by weighing gypenoside LVI, gypenoside XLVI, 2α-OH-PPD, M1, and M2 standards,