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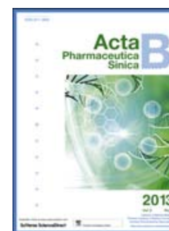

ORIGINAL ARTICLE

Pharmacokinetic study and metabolite identification of the bidesmosidic triterpenoid saponin BTS-1 in rat plasma

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Abstract Assays based on high-performance liquid chromatography (HPLC) and liquid chromatography tandem mass spectrometry (LC–MS^n) have been developed and validated for the determination and metabolite identification of the bidesmosidic triterpenoid saponin, BTS-1 (3-*O*-*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosyl gypsogenin 28-*O*-*α*-L-arabinopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranoside), in rat plasma. The assay was successfully applied to a pharmacokinetic study in rats given a single oral dose of BTS-1 (400 mg/kg). The results indicated that the compound was rapidly absorbed ($T_{max}=1.28\pm0.29$ h, $C_{max}=37.4\pm5.6$ μg/mL) and slowly eliminated ($t_{1/2}=13.2\pm6.6$ h). In addition, secondary glycosides and aglycones of BTS-1 were detected and identified. Since these metabolites are known to be active *α*-glucosidase inhibitors, they probably play an important role in mediating the pharmacological effects of the saponin.

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1. Introduction

Triterpenoid saponins, a large category of secondary plant metabolites, are believed to be the main constituents of many traditional Chinese medicines (TCM) and are considered responsible for numerous pharmacological effects[1]. One such compound known as BTS-1 with the chemical name 3-*O*-*β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucuronopyranosylgypsogenin 28-*O*-*α*-L-arabinopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranoside (Fig. 1) is isolated from the roots of *Gypsophila oldhamiana* (family Caryophyllaceae), a plant widely distributed in the Northern regions of China. The roots have been used as an alternative to the roots of *Stellria dichotoma* var. *Lanceolata Bge* (Yin-Chai-Hu), the most common TCM for the treatment of fever, consumptive disease and diabetes[2].

The triterpenoid saponins of the roots of *G. oldhamiana*, which contain BTS-1 as a major compound, are known to be oleanane-type, some of which are 3-*O*-monoglucosides, 28-*O*-monoglucosides and 3,28-*O*-bidesmosides[3–5]. These saponins have been reported to exhibit anticancer and antidiabetic effects[3,6,7]. In our previous research, the 28-*O*-monoglucosidic saponins and their alycones were shown to be the main constituents responsible for *α*-glucosidase inhibitory activity, while the 3,28-*O*-bidesmosidic saponins were found to be without this type of activity[3]. Furthermore, we found that the content of the monoglucosidic saponins was much lower than that of the 3,28-*O*-bidesmosidic saponins.

In order to understand the pharmacological effects of the roots of *G. oldhamiana*, it is important to determine whether orally administered 3,28-*O*-bidesmosidic saponins are absorbed from the intestine and metabolized to their monoglucosidic saponins or aglycones. To this end, we carried out a pharmacokinetic study of BTS-1 in rat using a fully validated liquid chromatography mass spectrometric assay and in addition identified its metabolites.

2. Experimental

2.1. Chemicals and reagents

The reference standards were isolated from *G. oldhamiana* in our laboratory and their structures were fully characterized using chemical and spectroscopic methods (UV, IR, NMR and MS). The purity of the standards was >98.0% as determined by HPLC. The internal standard (IS), glycyrrhizic acid (purity>98.0%, Fig. 1) was obtained from the TCM Institute of Chinese Materia Medica (Nanjing, China). Water was purified using a Milli-Q50 SP water purification system (Millipore, MA, USA). HPLC-grade acetonitrile was purchased from TEDIA Company (Tedia Fairfield, OH, USA). The other reagents were of analytical purity and used as received.

2.2. Animals

The animal pharmacokinetic study was approved by the Animal Ethics Committee of China Pharmaceutical University, Nanjing, China. Male Sprague–Dawley rats (weight 180–220 g) were bought from the Shanghai SIPPR/BK Experimental Animal Co., Ltd. The rats were maintained in an air-conditioned animal house at 22±2 °C and a relative humidity of 50±10%. Water and food (laboratory rodent chow, Nanjing, China) were allowed *ad libitum*. The animals were acclimatized to the facilities for a week and then fasted with free access to water for 12 h prior to an experiment.

2.3. Sample collection

Rats were administrated an oral dose of 400 mg/kg BTS-1 suspended in an aqueous solution of 0.5% carboxymethylcellulose sodium. Blood samples (200 μL) were collected from the oculi chorioideae vein into heparinized Eppendorf tubes before dosing and at 0.084, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 32, 40 and 48 h following the dose. Plasma samples were obtained by centrifuging at 4000 rpm for 10 min and frozen at −20 °C pending analysis.

2.4. Sample preparation

Plasma samples (50 μL) were spiked with 10 μL IS and extracted for 6 min with 1 mL *n*-butanol. After centrifugation (4000 rpm, 5 min), the supernatant was transferred to another vial and evaporated to dryness under a slow stream of nitrogen at room temperature. The residue was then reconstituted in 100 μL mobile phase (acetonitrile: 0.05% aqueous formic acid 30:70 *v*/*v*) and 20 μL injected into the HPLC system. Plasma samples prepared from blood samples taken 1 h after oral administration of BST-1 were used for to investigate metabolites.

2.5. Instrumentation and assay conditions

Chromatography was performed on an LC-2010 instrument (Shimadzu, Japan) equipped with a vacuum degasser, a quaternary pump, an autosampler and a UV detector set at 210 nm. Chromatographic separation was carried out on a C18 column (250 mm × 4.6 mm i.d., 5 μm; Welch Materials Inc., USA) maintained at 30 °C and protected by a C18 guard column (7.5 mm × 4.6 mm i.d.). The mobile phase consisted of (A) acetonitrile and (B) 0.05% (*v*/*v*) aqueous formic acid delivered at 1 mL/min according to the following linear gradient: 0–8 min 30–35% A; 8–16 min 35–36% A; 16–46 min 36–53% A; 46–56 min 30% A for re-equilibration. The injection volume was 20 μL.

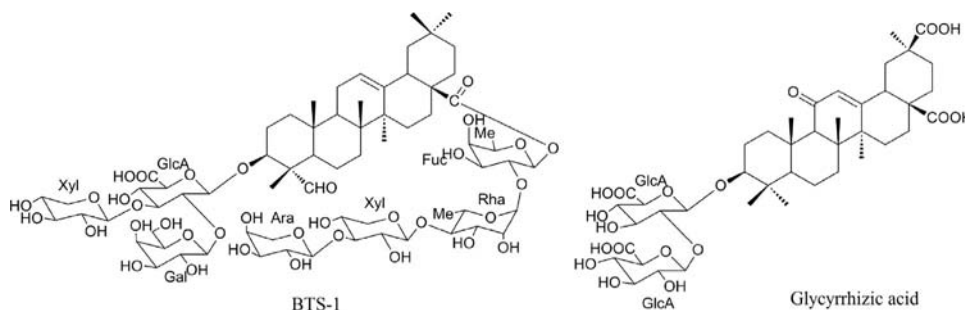

Figure 1 Chemical structures of BTS-1 and glycyrrhizic acid (IS).

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