Steroids 106 (2016) 62–69

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

A new quantitation method of protodioscin by HPLC–ESI-MS/MS in rat plasma and its application to the pharmacokinetic study

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ARTICLE INFO

Article history:
Received 18 March 2015
Received in revised form 24 October 2015
Accepted 11 December 2015
Available online 17 December 2015

Keywords:
Protodioscin
Ginsenoside Rb_1
HPLC–MS/MS
Pharmacokinetics
Rat plasma

ABSTRACT

A specific high performance liquid chromatography tandem mass spectrometry (HPLC–MS/MS method) was established for determining the concentration of protodioscin (PG) in rat plasma after intragastric administration of its standard form. Ginsenoside Rb_1 was selected as the internal standard (IS). The plasma sample was prepared using one-step deproteinization procedure by adding three parts of acetonitrile to precipitate proteins. The chromatographic separation was accomplished on an Inersil ODS-3 C_{18} column (250 × 4.6 mm, 5 μm) with a mobile phase composed with acetonitrile and water containing 0.1% formic acid under a gradient elution mode at a flow rate of 1 mL min^{-1}. A 3:1 portion of the eluent after a microsplit was detected on a triple quadrupole tandem mass spectrometer coupled with electrospray ionization (ESI) in positive ion and multiple reaction monitoring (MRM) scanning modes. The mass transitions were selected as 888.1 → 1050.2 for PG and 948.2 → 1110.3 for IS, respectively. After careful validation, the plasma samples were always stable under different storage conditions. These analytical results rendered sensitive, selective, and reliable values by this established method which displayed high accuracy, adequate extracted recoveries, and almost negligible matrix effects. This method was applied to the pharmacokinetic studies on PG level in the rat plasma and its pharmacokinetic effect. The results of our studies suggest that the present method may be a useful tool for further clinical study of PG.

1. Introduction

Protodioscin (PG, Fig. 1) is known as a typical furostanol steroid saponin with the chemical name of 25(R)-26-O-β-D-glucopyranosyl-furost-$\triangle^{5(6)}$-en-3β, 22α, 26-triol-3-O-α-L-rhamnopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside [1]. Although this compound exists abundantly in many oriental vegetables as well as medicinal plants, it is mainly isolated from the roots of three *Dioscorea* plants including *Dioscorea nipponica* Makino, *Dioscorea panthaica* Prain et Burkill, and *Dioscorea zingiberensis* C.H. Wright. This hydrophilic steroid saponin has been successfully developed into famous Chinese Herb Medicines, such as Dioscornin Tablet, Di'ao Xinxuekang Capsule, and Dunye Guanxinning Tablets, for treating cardiovascular diseases (CVD) since 1970s [2–4]. Modern pharmacological investigations revealed that PG has a cytotoxic activity against many cell lines from leukemia and solid tumors like HL-60 according to the accumulating data *in vitro* [5]. What's more, methyl protodioscin, an ester derivative of PG bearing a methoxy group (–OCH_3) in place of a hydroxyl group (–OH) at the C-22 position, reveals anti-proliferative effect against HepG2 and K562 cells through causing G2/M arrest as well as inducing apoptosis [6,7]. This evidence demonstrates that PG and its derivative are potential anticancer agents. In addition, its beneficial effect against ICVD (Ischemic Cerebrovascular Disease) has been studied in our laboratory.

Since few chromophores in the structure of PG cause low UV absorption, HPLC-ELSD has been often used to determine the PG in crude extracts of raw herbs, medicine or food *in vitro* [8]. However, due to the low detection limit of ELSD, this method is not suitable for detecting the trace amount of PG present in biological matrices such as blood plasma, urine, feces, and tissues *in vivo* [9]. So it is urgent to develop a new bio-analytical method to solve this intractable problem and further explore the pharmacokinetic profile of a single active constituent, which is crucial for understanding its pharmacological function and indispensible in drug development. With the recent advances in analytical techniques, high performance liquid chromatography coupled with tandem mass spectrometry (HPLC–MS/MS method) has become a preferred tool in drug analysis due to its improved sensitivity, selectivity, and specificity [10,11]. This powerful technique has been considered as

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http://dx.doi.org/10.1016/j.steroids.2015.12.009
0039-128X/

a promising method and applied in a wide range of fields like environmental and pharmaceutical research, especially for quantitating drugs or their metabolites from biological samples [12,13]. However, to the best of our knowledge, no report about determining the concentration of PG in rat plasma and its pharmacokinetic effects after oral administration has been published until now. Although the pharmacokinetics of PG in rat plasma has been reported after intravenous administration [14], the effects may be different according to the administration methods. When PG was orally administrated, it should first pass through the digestive system, and then the lipid bilayer of cell membrane. And finally, its prototype could be found in the blood sample. On the contrary, its prototype could be determined in blood sample immediately after intravenous administration. Since the pharmacokinetic parameters must be different according to the above two administration methods, it was essential to carry out the present research.

Thus, the objective of the current study is to establish a reproducible, simple, efficient and fast HPLC–MS/MS method to measure the PG concentration in rat plasma through optimizing the extraction and separation conditions following its oral administration. After validation based on the USFDA guidelines, this new method was applied to pharmacokinetic studies on PG through calculating its parameters, and evaluating its pharmacokinetic effects in rats.

2. Materials and methods

2.1. Chemicals and reagents

PG was isolated from the rhizomes of *Dioscorea zingiberensis* C. H. Wright in our laboratory with the purity of over 98% determined by HPLC-ELSD, and its chemical structure was identified by MS and NMR. The ginsenoside Rb_1 (Fig. 1, purity >98%, NO. 0704-9508) was chosen as the internal standard (IS) and purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile (NO. MS1122-001) and methanol (NO. MS-1922) were chromatographic grade and offered by Sigma Aldrich (St. Louis, MO, USA) and Merck Company (Darmstadt, Germany), respectively. Formic acid (NO. XK13-2014316) was of MS grade and purchased from Aladdin Reagent Co., Ltd (Shanghai, China). Distilled water used throughout the experiment was prepared from a Milli-Q purification reagent water system (18.2 Ω, Bedford, MA, USA). All other chemicals were higher than analytical grade and obtained from commercial sources.

2.2. Animals

The healthy adult male Sprague–Dawley (SD) rats, weighting 250–280 g and 8–10 weeks old, were supplied by the Experimental Animal Center of the Fourth Military Medical University (Xi'an, China). They were raised single in polyethylene cage under a constant and friendly SPF environment (24 ± 1 °C, 55–65% relative humidity, and a 12 h light:12 h dark cycle). The animals were acclimated for this normal condition at least one week with free access to standard rodent chow and purified tap water. Twelve hours prior to this experiment, the food was withdrawn and the rats were fasted overnight but with water *ad libitum*. All experimental procedures and protocols were reviewed and approved by the Animal Ethics Committee of the Fourth Military Medical University (approved on September 10, 2014, No. FMMU 2014-09), which was in accordance with the guidance for the Care and Use of Laboratory Animals.

2.3. Instrument and HPLC–MS/MS

2.3.1. Chromatographic conditions

The chromatographic analysis was performed on a Waters 2695 HPLC system equipped with a quaternary pump, a vacuum degasser, a thermostatted column compartment, and an autosampler. The sample separation was accomplished on an Inersil ODS-3 column (250 × 4.6 mm, 5 μm). The mobile phase consisted of acetonitrile (A) and water containing 0.1% formic acid (B), and was eluted at a flow rate of 1 mL min^{-1} in a linear gradient mode. The optimal gradient elution program was as follows: 0–5 min, 20–30% A; 5–8 min, 30–40% A; 8–15 min, 40–60 A%. The injection volume was 10 μL. A subsequent re-equilibration was performed before next injection, and the needle was rinsed with 50% methanol. The column and autosampler temperature was optioned at 25 °C and 4 °C, respectively.

2.3.2. Mass spectrometry conditions

Mass spectrometric analysis was carried out on a Varian triple quadrupole mass spectrometer (320-MS) coupled with

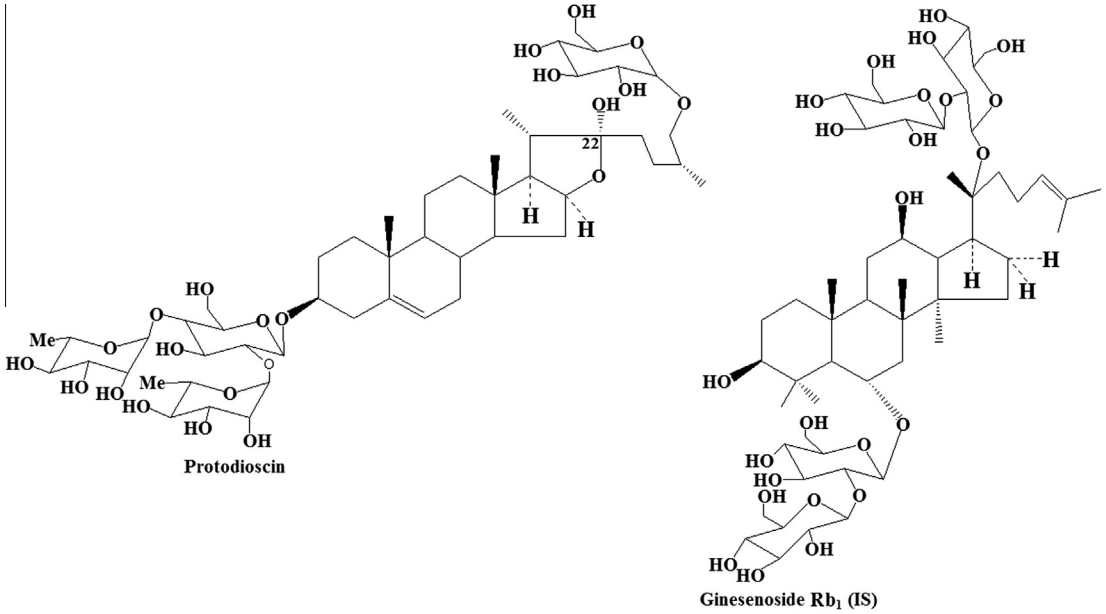

Fig. 1. The chemical structure of protodioscin and ginsenoside Rb_1 (IS).