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Pharmacokinetic studies of active triterpenoid saponins and the total secondary saponin from *Anemone raddeana* Regel

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ABSTRACT

The rhizome of *Anemone raddeana* Regel, a Traditional Chinese Medicine (TCM) which has a robust history treating rheumatism and neuralgia. The total secondary saponin (TSS) from it has demonstrated antitumor activity. In this study, a rapid and validated LC–MS/MS method was developed to simultaneously determine the active compounds (Hederacolchiside A1 and Eleutheroside K). Analytes were separated on a reverse-phase C18 column with acetonitrile-water (5 mmol/L ammonium acetate) as the mobile phase. This assay showed acceptable linearity ($r > 0.99$) over the concentration range 5–1000 nmol/L for two analytes. The intra- and inter-day precision was within 8.06% and accuracy was ranged from −3.16% to 3.34% for two analytes. The mean extraction recoveries of analytes and IS from rat plasma were all more than 76.0%. Under the developed analytical conditions, the obtained values of main pharmacokinetic parameters (C_{max} and AUC_{0-t}) indicated that the pure compounds were more efficient than the TSS extract in Hederacolchiside A1 and Eleutheroside K absorption. In addition, pharmacokinetic studies of two individual compounds demonstrated their poor oral absorption in rat ($^{a}F\%$, 0.019–1.521). In the study of absorption and transportation of Hederacolchiside A1 and Eleutheroside K in Caco-2 cell monolayer model, the uptake permeability was in 10^{-6} cm/sec range suggesting poor absorption, which confirmed the previous pharmacokinetic profiles *in vivo*. Interestingly, the uptake ratio of them declined significantly when treated with phloridzin (SGLT1 inhibitor). It indicated that the absorption of Hederacolchiside A1 in intestine was mainly through positive transport and SGLT1 might participate in its active absorption.

1. Introduction

Anemone raddeana Regel belonging Ranunculaceae family is widely distributed in the northeast of China, Russia (Far East), Japan and Korea [1]. The rhizome of *Anemone raddeana* Regel, also called "Liangtoujian", is a Traditional Chinese Medicine (TCM) which has been officially listed in the Chinese Pharmacopeia for the treatment of rheumatism and neuralgia [2]. Extensive phytochemical and pharmacological studies on this plant proved that triterpenoid saponins were the main bioactive components of this plant [3–6]. The crude saponin from *Anemone raddeana* Regel was demonstrated to significant antitumor activity *in vitro* and *in vivo*. It had been reported a significant inhibition on KB, HCT-8, MCF-7WT and MCF-7/ADR cell lines measured with MTT *in vitro*, and the 50% inhibition concentration (IC_{50}) was 7.68, 18.52, 17.34 and 19.43 μg/mL respectively. Furthermore, obvious inhibitory effect on the growth of EAC, sarcoma S180, H22 and cervical carcinoma was observed after an oral administration with the crude saponin of "Liangtoujian" [7,8]. Our previous pharmacological research of the total secondary saponin (TSS) prepared by alkali hydrolyzation showed significant cytotoxicity on Hep-G2, MCF-7 and A549. The pharmacological activity was indicating that TSS possibly a potential anti-cancer medicine, however, little endeavors had been made to its pharmacokinetic studies.

In our preliminary study, Hederacolchiside A1 and Eleutheroside K were isolated and identified as the major active components in TSS. Their anti-tumor mechanism of membrane-damaging had been reported in literatures [9,10], which might be related to the anti-cancer effect of TSS. Hence, Hederacolchiside A1 and Eleutheroside K were chosen as the marker compounds for the pharmacokinetic studies of TSS. Furthermore, herbal ingredient–ingredient interactions have been reported in many medicine herbs [11–13]. Potential interactions among components in TCMs are getting more and more attention [14]. Therefore, in

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the present study, we not only investigated the pharmacokinetics of TSS in rats, but also compared the pharmacokinetic profiles of the active components in rats after consumptions of TSS versus pure compounds. Poor oral bioavailability have been considered as one of the general characteristics of triterpenoid saponins, which was proved by abundant of studies, such as ginsenosides, licorice saponins, astragalosides and saikosaponins [15–18]. The membrane permeability of triterpenoid saponins across Caco-2 cell monolayers revealed that the poor absorption mainly contributed to their low bioavailability [19]. However, there is still lacking of the information of intestinal absorption and transportation of Hederacolchiside A1 and Eleutheroside K.

Till now, limited analytical methods were developed for the detection of *Anemone raddeana* Regel [20,21]. Several HPLC-UV methods had ever been used in the quantification of the extract of this plant. But the low sensitivity and long detection time of the HPLC-UV analysis couldn't meet the requirement for pharmacokinetic research. LC–MS/MS is an effective and sensitive technique for compound analysis in biosamples. Liu et al. [22] and Luan et al. [23] utilized LC–MS/MS to investigate the pharmacokinetic studies of Raddeanin A, which was another representative triterpenoid saponin in *Anemone raddeana* Regel. An LC–MS/MS method was established for quantification of Hederacolchiside A1 and Eleutheroside K in rat plasma after administration of extract of *Pulsatilla chinensis* [24]. In the reported method, solid-phase extraction (SPE) was used for sample preparation. In the present research, a sensitive and convenient LC–MS/MS with protein precipitation was developed for the measurement of Hederacolchiside A1 and Eleutheroside K in rat plasma to investigate the pharmacokinetics of TSS, the potential interaction of TSS, and the absorption mechanism of the test compounds. This information will be beneficial for understanding pharmacological and even clinical effects of such plant materials.

2. Materials and methods

2.1. Chemicals and materials

The rhizome of *Anemone raddeana* Regel (Ranunculaceae) was purchased from AnguoTongling Medicinal Materials Co., Ltd (Place of origin: Jilin, Batch lot: 20100916), and authenticated by Prof. Jincai Lu, the School of Traditional Chinese Material Medica, Shenyang Pharmaceutical University. Two standard triterpenoid saponins (Hederacolchiside A1and Eleutheroside K) (purity > 95%) and the TSS extract were prepared in Pharmacognosy laboratory, the School of Traditional Chinese Material Medica, Shenyang Pharmaceutical University Shenyang, China. They were characterized by spectral methods, including ^{1}H- and ^{13}C NMR spectroscopy. The data were consistent with those reported in literature [25]. Triamcinolone acetonide (purity > 98%) used as the internal standard (IS), was purchased from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). The structures of the compounds are shown in Fig. 1. Acetonitrile (HPLC grade) was used for LC–MS/MS analysis and plasma sample preparation obtained from Dikma Technologies Inc (USA). De-ionized water was obtained from a Milli-Q water purification system (USA). All the other reagents were of analytical purity.

2.2. Animals and treatments

Male Sprague-Dawley rats (n = 24, 180–220 g) were provided by the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, PR China), animal license number: SCXK Liao 2014-0001. All animals were kept under the same laboratory conditions of temperature (25 ± 2 °C) and lighting (12:12 h, light:dark cycle) and were given free access to standard laboratory chow and tap water for seven days until 12 h prior to experiments. Animal experiments were performed in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China.

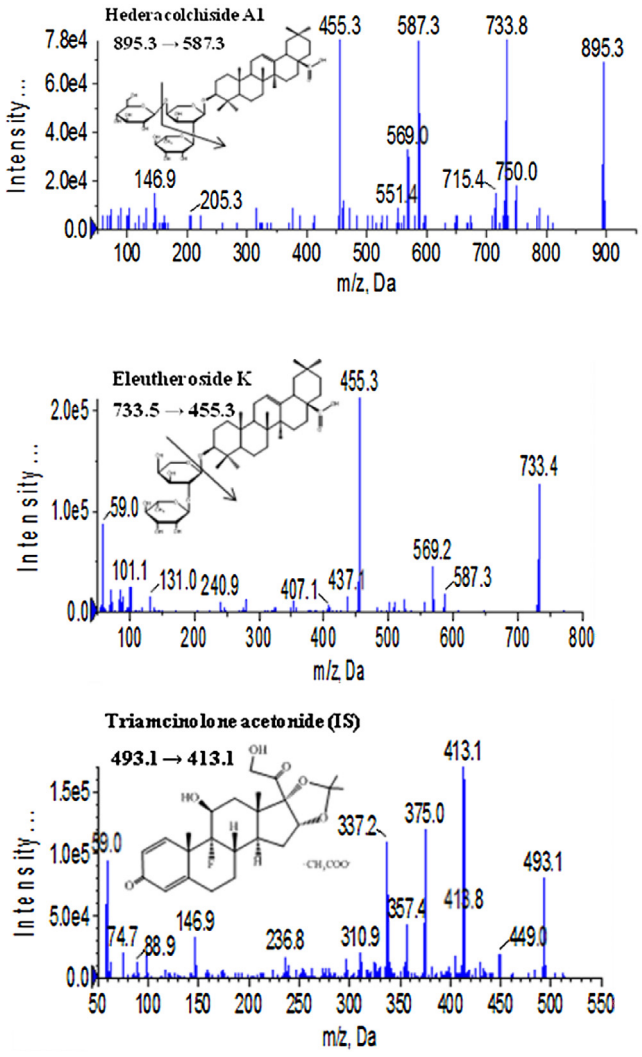

Fig. 1. Product ion spectra and monitored transitions of analytes.

2.3. Preparation of the TSS extract

The rhizome of *Anemone raddeana* Regel (Ranunculaceae) was soaked in 70% ethanol-alkali (PH = 13) solution overnight, then refluxed for 1.5 h at 90 °C three times. The extract was subsequently dried under reduced pressure conditions. The residue was chromatographed on a resin column, and eluted with water, 20% ethanol and 80% ethanol sequentially. The fractions eluted with 80% ethanol were combined, dried in vacuum, and a powder (TSS) was obtained.

2.4. Determination of the two saponins' contents in the TSS extract

2.4.1. HPLC-UV analytical conditions

To calculate the doses to be administered, the amounts of two maker compounds in the TSS extract were quantitatively determined using HPLC-UV. Analysis was performed on a Lab PC 3000 liquid chromatography system (Lab Alliance Co., USA) connected to an EZ Chrome software. Chromatographic separation was car-