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Pharmacokinetic study of calenduloside E and its active metabolite oleanolic acid in beagle dog using liquid chromatography–tandem mass spectrometry

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ABSTRACT

Aralia mandshrica is a well-known traditional Chinese medicine from Northeast China commonly used to treat digestive, circulatory and immune system disorders. Calenduloside E is one of its bioactive components currently under evaluation as a pure drug. In this study, a highly sensitive and rapid method based on liquid chromatography–tandem mass spectrometry (LC–MS/MS) for the simultaneous quantitation of calenduloside E and its active metabolite oleanolic acid in beagle dog plasma has been developed and validated. Samples containing the ammonium salt of simvastatin acid as internal standard (IS) were purified by solid phase extraction and separated on a SUPELCO Ascentis-C_{18} column (50 mm × 4.6 mm i.d., 5 μm) using gradient elution with 0.35% formic acid and acetonitrile. Analytes and IS were detected in a cycle time of 5 min after ionization in the negative ion mode by multiple reaction monitoring of the precursor-to-product ion transitions at m/z 631.4 → 455.4 and m/z 435.4 → 319.0 for calenduloside E and IS respectively and by single ion monitoring of the ion at m/z 455.4 for oleanolic acid. The method was linear over the concentration range 0.4–100 ng/mL for both analytes using 0.5 mL plasma. Inter- and intra-day precisions were both <6.96% with accuracies <6.40%. In the pharmacokinetic (PK) study, beagle dogs were given oral doses of calenduloside E (1.05, 2.10 and 4.20 mg/kg) and an intravenous injection of 2.10 mg/kg. The absolute bioavailability of calenduloside E was only 0.58%. Area under the plasma concentration time curve ($AUC_{(0-t)}$) for the oral doses of calenduloside E was approximately dose proportional while other PK parameters ($t_{1/2}$, T_{max} and MRT) showed no significant differences among the three doses ($P > 0.05$). The PK data provide a useful platform on which to base future clinical studies of calenduloside E.

1. Introduction

Calenduloside E (3-O-[β-D-glucuronopyranosyl]oleanolic acid, Fig. 1A), the glucuronic acid conjugate of oleanolic acid (Fig. 1B), is a triterpenoid saponin present in a wide variety of *Aralia* plants, in which the content of calenduloside E is about 0.02% [1]. A number of studies of the biological activity of calenduloside E has been reported since its first isolation by Kasprzyk and Wojciechowski [2]. These studies indicate that calenduloside E has hypoglycemic activity and is potentially useful in the treatment of diabetes and morbid obesity [3,4]. It may also be useful as a spermicidal contraceptive [5] and as an antiarrhythmic agent [1,6,7].

Because of its wide-ranging biological activity and low toxicity [8], the potential clinical use of calenduloside E has attracted much attention in recent years. Furthermore, its active metabolite, oleanolic acid, has been shown to possess analgesic, anti-inflammatory, hepatoprotective, antitumor and hypolipidemic effects as well as having hypoglycemic activity [9,10]. In fact it may be responsible for all the pharmacological activity ascribed to calenduloside E. As a result, it is necessary to delineate the pharmacokinetics of oleanolic acid the two compounds after calenduloside E administration to fully understand its therapeutic potential.

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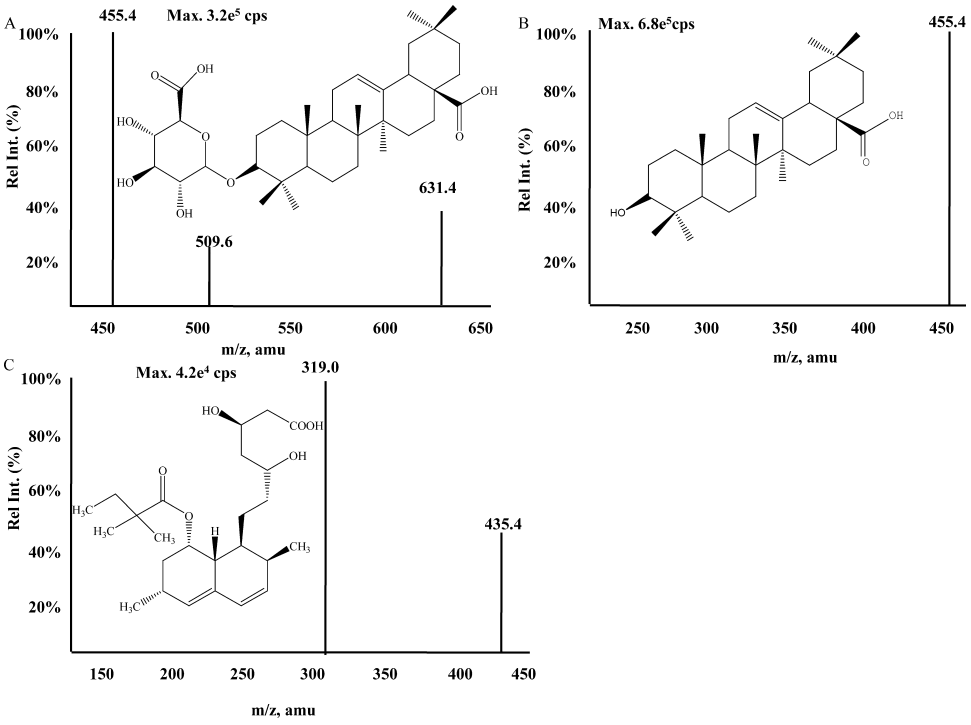

Fig. 1. Full-scan product ion mass spectra of $[M-H]^-$ ions of (A) calenduloside E, (B) oleanolic acid and (C) simvastatin acid (IS).

Although several bioanalytical methods have been reported for oleanolic acid based on HPLC [11,12], capillary electrophoresis [13–16] and liquid chromatography–tandem mass spectrometry (LC–MS/MS) [9], there are no reports of assays for calenduloside E nor for the simultaneous determination of the two compounds. This paper reports the first LC–MS/MS method for the simultaneous determination of calenduloside E and oleanolic acid in biological fluids. The method combines excellent sensitivity, specificity and recovery with a short run time (5 min/sample) and low matrix effects. The assay was successfully applied to a pharmacokinetic (PK) study involving oral and intravenous (i.v.) administration of calenduloside E to beagle dogs. The main aims of the PK study were to determine the oral bioavailability of calenduloside E and to evaluate the linearity of oral dosing in order to better inform its future clinical use.

2. Experimental

2.1. Chemicals and reagents

Calenduloside E (purity >98.0%) was obtained from the Traditional Chinese Medicine Academy of Sciences of Jilin Province (Changchun, PR China). Oleanolic acid (purity >98.0%) and the ammonium salt of simvastatin acid (purity >99.0%) for use as internal standard (IS) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). Formic acid and Discovery-pH solid phase extraction (SPE) columns (100 mg/1 mL) were purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC grade acetonitrile was obtained from Fisher (Fair Lawn, NJ, USA). All other chemicals were analytical grade and used as received. Milli Q water (Milli Q Water Systems, Millipore, Bedford, USA) was used throughout the study.

2.2. Animals

Six healthy beagle dogs (3 male, 3 female, weight 10.0 ± 1.5 kg, Certificate no. SCXK 2008-24) were purchased from the Institute of Chengdu Dashuo Biological Technology Co. Ltd. (Chengdu, China). The dogs were healthy and free of disease and parasites based on physical examination before and after completion of the study. Animals were housed under standard conditions of temperature, humidity and light with food and water provided ad libitum. Before drug administration, dogs were fasted overnight with free access of water. All animal experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of Jilin University.

2.3. Drug administration and sample collection

Calenduloside E was administered as single oral and intravenous doses to dogs in a randomized, crossover design with a 7-day washout period. The oral dose was formulated in a capsule with β-cyclodextrin as excipient and administered at doses of 1.05, 2.10 and 4.20 mg/kg. The doses of 1.05, 2.10 and 4.20 mg/kg were chosen in order to investigate dose linearity over the range of the effective dose of calenduloside E as an antiarrythmic agent established in a rat model of arrhythmia [1,6,7]. The intravenous dose was formulated at a concentration of 5 mg/mL in a vehicle of polyethylene glycol 400:saline (2:1, v/v) and administered at a dose of 2.10 mg/kg. Blood samples (2 mL) were collected from the fore vein into heparinized tubes before dosing and at 0.083, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h after dosing in the oral study and at 0.083, 0.167, 0.333, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12, 24 and 48 h after dosing in the intravenous study. Blood samples were centrifuged at 15,000 rpm for 5 min at 4 °C and 1 mL plasma samples removed and stored at −80 °C until analysis.

2.4. LC–MS/MS conditions

Chromatography was carried out using a Shimadzu UFLC SIL-20A XR system equipped with a SUPELCO Ascentis-C_{18} column (50 mm × 4.6 mm i.d., 5 μm, Sigma, USA) maintained at 40 °C and protected by a SecurityGuard C_{18} guard column (4 mm × 3.0 mm i.d., Phenomenex Inc., USA). The mobile phase consisted of solvent A [0.35% formic acid (pH 2.5)] and solvent B (acetonitrile) delivered