Pharmacokinetics of astragaloside iv in beagle dogs

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SUMMARY

In this study, the pharmacokinetics of Astragaloside iv (AGS-IV) in Beagle dogs was studied by high performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (MS). The concentrations of the drugs in plasma were determined after i.v. administration of 0.5, 1, 2 mg·kg⁻¹ AGS-IV and p.o. administration of 10 mg·kg⁻¹ AGS-IV. The areas under concentration-time curve (AUC) were linearly correlated to the doses administrated. The absolute bioavailability of AGS-IV after p.o. administration was found to be 7.4%. The plasma protein binding rate of AGS-IV was about 90% within a concentration range of 250–1000 ng·ml⁻¹. There was no significant species difference regarding the pharmacokinetics of AGS-IV between the rat and the Beagle dog.

INTRODUCTION

Radix astragali is one of the most commonly used traditional Chinese herbal medicines, which was prepared from the roots of Astragalus membranaceus and Astragalus membranaceus var. mongolicus (Leguminosae). Pharmacological tests have shown that it possesses hepatoprotective, antioxidative, antiviral, antihypertensive and immunostimulatory activity (1–3). Astragaloside iv (AGS-IV) (Fig. 1), a 9,19-cycloartaneype tri-terpene glycoside, has been regarded as being one of the typical and active constituents of Radix astragali. It has been reported in recent studies that AGS-IV has neuroprotective, cardioprotective, antiinflammatory and immunostimulatory properties (4,5). Analytical approaches for the determination of AGS-IV have included thinlayer chromatography (TLC), high performance liquid chromatography and evaporative light scattering detection (HPLC–ELSD), precolumn derivatization HPLC and liquid chromatography-mass spectrometry (LC–MS) with solid phase extraction (SPE) (6). Recently, HPLC/MS and HPLC/MS/MS have been established as the optimal techniques the for quantitative determination of AGS-IV in biological samples as they have high sensitivity and selectivity (6,7).

Further investigation regarding AGS-IV will require detailed pharmacokinetic studies in preclinical animal models. The pharmacokinetics of this compound in rats has been studied in our lab (8). Having considered the differences between genera, two or more animal's species should be studied before a reliable result can be provided for use in clinical medicine. To our knowledge, there have been no reports so far on the pharmacokinetic parameters of AGS-IV after i.v. and p.o. administration in Beagle dogs.

As is commonly known, most drugs combine reversibly with plasma protein in the blood system,
so there are two patterns of drug behavior in the body, i.e. an associative and a dissociative pattern. Only the associative drugs could have their distribution, absorption, metabolism, and excretion in the body, so the protein binding rate of drugs would influence their fate in the body. Clinically, two kinds of drugs with high protein binding drugs should not be used simultaneously. And the protein binding rate of AGS-IV in Beagle dogs was also a blank. Such pharmacokinetic results regarding AGS-IV may limit its application in clinical practice.

**MATERIALS AND METHODS**

**Animals**

Beagle dogs (3♀, 3♂) weighing 8-10 kg obtained from the animal center of the China Pharmaceutical University were used in this study. The experimental procedures adopted were in accordance with the guidelines of the National Institutes of Health and were approved by our Animal Care Committee.

**Chemicals**

AGS-IV was provided by the Institute of Pharmaceutical Research (Jiangsu, People’s Republic of China). Digoxin, which was used as internal standard (I.S.), was obtained from the National Institute of Pharmaceutical and Biological Products (Beijing, People’s Republic of China). Acetonitrile (chromatographically pure grade) was obtained from Fisher Scientific (USA). Heparin sodium (0.1%) was provided by Huixing Biochemical Reagent Company (People’s Republic of China). Distilled, deionized water was provided by a Milli-Q Reagent Water System (Millipore, MA, USA). Other reagents were of analytical grade.

**Instruments and chromatographic conditions**

An LC/MS/MS system was used for analysis was LC/MS/MS system (API2000, USA). The Separation was carried out on a Kromasil C18 (USA) analytical column (5 μm, 150*2.0 mm) at 25°C. The mobile phase consisted of acetonitrile:water containing 5 μM NaAc (60:40, v/v) and the flow rate was 0.2 ml/min. Positive ionization with selected ion monitoring (SIM) was used for the analysis. The quasi-molecular ions [M+Na]+ were detected at a wave length of m/z 807 for AGS-IV and m/z 804 for digoxin.

**Sample preparation**

Oasis HLB cartridge solid phase extraction columns were treated with 1 ml methanol, followed by 1ml distilled water. Samples plasma (500 μl) were mixed with 12.5 μl (0.04 mg.ml⁻¹) of I.S. solution and water (500 μl) and loaded onto SPE columns. The columns were then washed in 4 ml water and eluted with 1ml methanol. Methanol extracts were evaporated to dryness under nitrogen at 45°C. The residues were reconstituted as 200μl aliquots of HPLC mobile phase and centrifuged at 15000 rpm, at 4°C for 10 min. 10 μl of the supernatants were injected onto the LC/MS/MS for analysis.

**Assay validation**

AGS-IV plasma standards (5.0, 10.0, 50.0, 100, 500, 1000, 2500, and 5000 ng.ml⁻¹) were prepared by dilution and then treated in the same way as the samples. The calibration curve for AGS-IV was based on the peak area ratio of AGS-IV to the I.S. Recovery was based on the analysis of plasma samples spiked with AGS-IV at concentrations of 10, 500, and 2500 ng.ml⁻¹. Precision was determined at the same three concentrations. The detection limit was determined at a signal-to-noise ratio of 3.