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Short communication

The experimental study of *Astragalus membranaceus* on meridian tropsim: The distribution study of astragaloside IV in rat tissues

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ABSTRACT

According to Traditional Chinese Medicine (TCM) theories, TCM with different meridian tropism have different therapeutic effects. In view of the meridian tropism of *Astragalus membranaceus* (Huangqi), astragaloside IV, one of the effective phytochemicals of Huangqi, was appointed and observed its distribution in rat tissues following a single intravenous (i.v.) dose. A simple and accurate LC-ESI-MS/MS method was developed and validated for astragaloside IV quantification in heart, liver, spleen, lung and kidney using warfarin as an internal standard (IS). Chromatographic separation was performed on a Eclipse plus C18 (4.6 mm × 100 mm, 1.8 μm) when the flow rate was set at 0.300 mL min^{-1} and ammonium acetate aqueous solution - acetonitrile was used as mobile phase. The intra- and inter-day precisions of the quality control samples were within 15% and accuracies were within 90.0–110%. The recoveries were more than 90.0% at high, medium and low concentrations, respectively. This method was successfully applied for distribution of astragaloside IV after intravenous (i.v.) dose of 4 mg kg^{-1} astragaloside IV in rats. Astragaloside IV concentration was highest in liver and kidney and remained much higher than that in other tissues over the experiment course. Lung, heart and spleen were also detected to contain astragaloside IV. The results clearly demonstrated that astragaloside IV was one of the material bases of the meridian tropism of Huangqi.

1. Introduction

Epidemiological studies have pointed out that regular consumption of Traditional Chinese Medicine (TCM), similar to plant food imparted health benefits [1,2]. These health benefits seem to be related to the content of active phytochemicals in TCM for medicinal purposes. Many studies have been reported that the composition and content of these phytochemicals were affected by several factors such as plant varieties, post-harvest practices, meteorological conditions, process method, soil and place of growing [3–12]. Tissue distribution was vital to investigate the major target sites and interpret the in vivo disposition of phytochemicals. Considering the growing significance of a potential beneficial role of phytochemicals in human health, detailed in vivo disposition studies of phytochemicals in TCM for medicinal or food purposes were required.

Meridian tropism theory was a core principle of TCM theories and played an essential role in clinical selection of TCM according to syndromes [13]. Based on meridian tropism theory, TCM had some special affinities for certain organs and meridians of the body and had primary or special treatments for diseases of these parts. Meridian tropism was the concept with both qualitative and positioning analysis of TCM and it was one of the basic contents of medicinal theory for instruction of medication [14]. The foundation of meridian tropism might be the selective effect of active phytochemicals in tissues and organs. Therefore, it was necessary to investigate the relationship between active phytochemicals distribution and meridian tropism.

Huangqi was the dry root of *Astragalus membranaceus* (Fisch.)Bge.var. mongholicus (Bge.)Hsiao or *A. membranaceus* (Fisch.)Bge. Its medical history was over 2000 years. About meridians of Huangqi, it distributed to triple energizers, spleen and kidney in “Tang Ye Ben Cao”; to triple energizers and lung in “Ben Cao Meng Quan”; to stomach and lung in “Ben Cao Jing Shu” and to lung, spleen and heart in “Ben Cao Xin Bian”. It also distributed to the lung and spleen in the Pharmacopeia of the People’s Republic of China (2010).

Astragaloside IV, one of the major active phytochemicals presents in Huangqi and its preparations have been reported to

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have various pharmacological properties [15]. It has been reported to protect against ischemia reperfusion [16], inhibit mitochondrial dysfunction and ROS production [17], improve barrier dysfunction [18], and protect dopaminergic neurons [19]. Although the pharmacokinetics and tissue distribution of astragaloside IV in rat and dogs were examined by using LC–MS assay [14,20] and astragaloside IV in rat plasma was determined by LC–MS/MS [21], the relation of meridian tropism and distribution study of astragaloside IV have not been reported.

In order to investigate the relationship between TCM active phytochemicals distribution and meridian tropism, a fast, sensitive and specific assay method needs to be developed to obtain a better estimate of the distribution of this ingredient. The matrix effects of astragaloside IV in heart, liver, spleen, lung and kidney were also investigated in this project since no report on this aspect has previously been reported in any published methods. Here we described a LC–MS/MS using MRM assay measurement of astragaloside IV in rat different tissues. This established method was expected more specific than single-ion monitoring (SIM) LC–MS assay.

2. Experimental

2.1. Chemicals and reagents

Acetonitrile (Dikma technologies Inc, USA) and methanol (Tianjin concord Science Co. Ltd., Tianjin, China) were of HPLC grade. Standard reference astragaloside IV and warfarin (IS) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Their purities were more than 98%. Ethyl acetate and ammonium acetate were of analytical grade. Deionized water was purified with a Milli-Q Academic ultra-pure water system (Billerica, MA, USA) prior to the use as HPLC mobile phase.

2.2. Instrument, analytical conditions and mass spectrometric conditions

The LC–MS/MS equipment included an Agilent 1200 system (Agilent Corporation, USA) consisting of a vacuum degasser, a binary pump, an autosampler, a column compartment and an AB MDS Sciex (Concord, Ontario, Canada) API 3200 triple quadrupole mass spectrometer equipped with a TurboIonSpray ionization (ESI) source. Data acquisition was carried out with Analyst 1.4.2 software (AB MDS Sciex).

The chromatographic separations were achieved using a Eclipse plus C18 (4.6 mm × 100 mm, 1.8 μm) column with a security guard (2.1 mm × 12.5 mm, 5 μm) C18 column. The mobile phases (delivered at 0.3 mL min^{-1}) consisted of acetonitrile for A and ammonium acetate aqueous solution (1 mM) for B. Mobile phase A and B ratios changed as follows: 0–5 min, 10–10%A; 5–10 min, 10–75%A; 10–17 min, 75–79%A and the re-equilibration time were 5 min.

The mass spectrometer was operated in the positive ion mode. The source parameters were as follows: curtain gas, collision gas, ionSpray voltage, temperature, ion source gas 1 and ion source gas 2 were set at 20 psi, 2 psi, 5500 V, 350 °C, 30 psi and 60 psi, respectively. Nitrogen was the only gas used in the experiment. Declustering potential (DP) was set at 42 and 54 V, collision energy (CE) at 30 and 21 V, entrance potential (EP) at 5 and 3 V, the collision cell exit potential (CXP) at 2 and 4 V for astragaloside IV and IS, respectively. Quantitation was acquired using MRM of the transitions of m/z 785.3 → 143.3 for astragaloside IV and 309.1 → 163.1 for IS. The mass spectrometer was operated at a unit resolution for both Q1 and Q3 with a dwell time of 600 ms in each transition.

2.3. Preparation of stock solutions

The standard stock solutions of astragaloside IV (1 mg mL^{-1}) were prepared by dissolving 5 mg in methanol in a 5 mL amber volumetric flask. Appropriate amounts of working solution were diluted with drug-free tissue homogenate to span a calibration standard range. All the solutions were stored at 4 °C and were brought to room temperature before use.

2.4. Preparation of samples and quality control samples

A precipitating protein directly with methanol procedure was used in this study for extraction of astragaloside IV from the different rat tissues. To a 100 μL aliquot of the tissue homogenate, 20 μL IS and 180 μL methanol was added. Then, the samples were vortexed for 0.5 min. After centrifugation for 10 min at 14,000 rpm at 4 °C, a 20 μL aliquot of the supernatant solution was injected into the LC-MS/MS system for analysis.

Quality control (QC) samples (10, 100, and 1000 ng mL^{-1}) for heart, spleen, lung and kidney and QC samples (50, 100, and 1000 ng mL^{-1}) for liver by spiking blank rat tissue homogenate with appropriate standard solutions to the required tissue homogenate concentrations, followed by the same samples preparation and operation method above.

2.5. Method validation

The established method was validated for specificity, sensitivity, linearity, accuracy and precision, recovery, matrix effect and stability following the USFDA guidelines [22].

The specificity was investigated by comparing the chromatograms of six different batches of blank rat tissue homogenate samples with the corresponding spiked tissue homogenate. The linearity was assessed by assaying calibration curves in tissue homogenate on three consecutive batches. The curves were fitted by a weighted ($1/x^2$) least-squares linear regression method through the measurement of the peak area ratio of the analyte to IS. The recoveries were obtained comparing peak areas of analytes in extracted samples with those in post-extracted spiked samples. The matrix effects were evaluated by comparing the peak area of the analytes in post-extracted spiked samples with those of the analytes in unextracted samples. The precisions were calculated by using the relative standard deviations (RSDs). Accuracy was determined by comparing the calculated concentration using calibration curve with the known concentration. The post-preparative stability was tested by re-analyzing QC samples at room temperature condition over the anticipated run time of 24 h. The stability was expressed as mean of percentage remains. All stability studies were conducted at three QC levels with five determinations for each.

2.6. Application

Eighteen healthy male Sprague-Dawley rats (220–240 g) were housed to a cage with unlimited access to food and water except at 12 h before the experiment. The animals were maintained on a 12 h light–dark cycle (light on from 8:00 to 20:00 h) at ambient temperature (22–24 °C) and 60% relative humidity. Astragaloside IV was injected into rats at an intravenous bolus dose of 4 mg kg^{-1} following overnight fasting for 12 h. Following i.v. injection, the rats were sacrificed by bleeding at the femoral artery. Selected tissues including the hearts, livers, spleens, lungs and kidneys were collected at 10, 30 and 60 min after intravenous injection. Approximately 0.5 g of heart, lung, spleen, liver, kidney, was quickly removed, rinsed with cold 0.9% NaCL injectable solution, minced and homogenized with 2 volumes of 0.9% NaCI injectable solution in a tissue homogenizer, and then centrifuged at 14,000 rpm at 4 °C, immediately.