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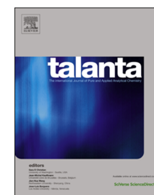

Study on pharmacokinetic and tissue distribution of lycorine in mice plasma and tissues by liquid chromatography–mass spectrometry

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ABSTRACT

A fast and simple liquid chromatography–mass spectrometry method for the determination of lycorine in mice plasma and tissues was developed and well used in the pharmacokinetic and tissue distribution study of lycorine after tail vein injection and intraperitoneal administration. Biological samples were processed with ethyl acetate by liquid–liquid extraction, and evodiamine was used as the internal standard. Chromatographic separation was performed on an Amethyst C18 column (4.6 × 150 mm) with a mobile phase consisting of methanol and water. Quantification was performed by selected ion monitoring with m/z 288 $[M+H]^+$ for lycorine and m/z 304 $[M+H]^+$ for the internal standard. Good linearity was observed over the concentration ranges. Limits of quantification were low up to 10.0 ng/mL in plasma samples, 9.0 ng/g for lung, 12.0 ng/g for heart, 18.0 ng/g for spleen and 6.5 ng/g for other tested tissues. The intraday accuracy and precision in plasma and tissues ranged from −7.4% to 9.1%. Recoveries in plasma and tissue were more than 80%. The method was rapid, accurate and fully validated. It was successfully applied to the investigation of the pharmacokinetics and tissue distribution of lycorine in mice.

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1. Introduction

Lycorine is a natural alkaloid extracted from Amaryllidaceae species including flowers and bulbs of daffodil (Narcissus), snowdrop (Galanthus) or spider lily (Lycoris) [1]. It exhibits stronger inhibition than aspirin in acetic-acid induced abdominal stretching, indicating powerful antinociceptive activity [2]. It also has antiviral and antimalarial properties as well as the ability to inhibit protein synthesis in eukaryotic cells [3–5]. In addition, lycorine could weakly inhibit acetylcholinesterase activity, which might restore the acetylcholine levels and cholinergic functions of the brains of patients with Alzheimer's disease [6]. In particular, during in vitro and in vivo study, lycorine was also found to have potent anticancer effects by suppressing growth and inducing apoptosis of tumor cells like HL-60 cells (human myeloid leukemia) [7], human APL cells [8], KM3 cells [9], etc. All these functions suggest that lycorine may be a good anticancer candidate for further new drug development. However, in the literatures there are no reports about the pharmacokinetic and biodistribution in vivo studies, which are very important for new drug discovery. In order to explore the potential of lycorine as an anticancer agent, it is necessary to further study the in vivo pharmacokinetic and distribution characteristics of lycorine.

Different analytical techniques have been described for the qualitative and quantitative determination of lycorine in various parts of different Amaryllidaceae plants including high-performance thin layer chromatography [10], capillary gas chromatography–mass spectrometry [11], high performance liquid chromatography (HPLC) with a diode array detector [12], and non-aqueous capillary electrophoresis with an electrospray ionization mass spectrometer (ESI-MS) [13]. However, the sensitivity of these methods is not sufficient for the pharmacological study which requires the lower limit of quantification (LLOQ). Therefore, it was critical to develop a sensitive method for the determination of lycorine in biological samples. LC–MS proves to be a feasible alternative due to good separation and detection performance. Moreover, LC–MS has been extensively applied in the bioanalysis and pharmacokinetic studies of numerous drugs [14–16].

In the present work we described a sensitive HPLC–ESI-MS method for the determination of lycorine in biological matrices after a single-step liquid–liquid extraction with ethyl acetate. To improve the accuracy and the precision of the method, evodiamine was used as an internal standard (IS). The method was sensitive, accurate, reproducible and suitable for application in the pharmacokinetic and tissue distribution study following with intraperitoneal (IP) and intravenous (IV) injection.

2. Experimental

2.1. Chemicals and reagents

Lycorine and evodiamine were supplied by Aladdin reagent (Shanghai, China). HPLC-grade methanol was obtained from Tedia

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Company Inc. (Fairfield, USA). Helium (purity, 99.999%) and liquid nitrogen were obtained from Wuhan Analytical Instrument Factory (Wuhan, China). Ultra-pure water used throughout the study was of Milli-Q quality (Millipore Corp., Bedford, MA, USA). All other reagents used in the experiment were commercially available and were of analytical grade.

2.2. HPLC–MS conditions

The HPLC separation was conducted on an Agilent HP1100 HPLC system (Agilent, California America) equipped with a Amethyst C18 column (4.6 × 150 mm, 5 μm). The mobile phase consisted of water and methanol (15:85, v/v) at a flow rate of 0.5 mL/min. The column temperature was set at 40 °C. The injection volume was 10 μL.

The Agilent 1100 HPLC system was coupled on-line to an ion-trap mass spectrometer (Agilent Corp, Waldbronn, Germany) equipped with an ESI source. The AutoMS operation parameters are described as follows: positive ion mode (ESI^+); nitrogen drying gas, 10 L/min; nebulizer, 50 psi; gas temperature, 350 °C; compound stability, 80%; and mass range, 100–1000 *m/z*. Detection of lycorine and IS was performed in selected ion monitoring (SIM) mode with ion *m/z* of 288 and 304, respectively.

2.3. Animals

All experimental protocols involving animals were reviewed and approved by the Institutional Animal Experimentation Committee of Wuhan University. KM mice, weighing 22–25 g, were supplied by the Experimental Animal Center of Wuhan University (Wuhan, China). All mice were maintained under standard conditions with normal access to food and water.

2.4. Standard and quality control samples preparation

Appropriate amount of lycorine and IS was respectively dissolved in methanol to prepare a stock solution of 1.0 mg/mL. Then stock solutions were diluted with methanol to the concentration of 100.0 μg/mL for lycorine and 10.0 μg/mL for IS as working standard solutions and kept at 4 °C before use.

Plasma calibration standards and quality controls were prepared by spiking blank plasma with the appropriate amount of working standard solutions and 20 μL of working IS solution. Calibration standards were prepared at seven concentrations ranging from 0.1 μg/mL to 10 μg/mL and plasma quality control (QC) samples were prepared at three concentrations of 0.1, 2.5 and 10 μg/mL.

Calibration standards for various tissues including heart, liver, spleen, lung, kidney, cerebrum, intestine and stomach were prepared by spiking blank tissues of certain weight (300 mg of liver, kidney, cerebrum, intestine and stomach or 150 mg of heart or 80 mg of spleen or 200 mg of lung) with working standard solutions (in the concentration ranges of 1–100 μg/mL) and 20 μL of working IS solution. QC samples at three levels were prepared in the same fashion.

Standard calibration samples and QCs were stored at −20 °C until analysis.

2.5. Samples pretreatment

As for plasma samples, 20 μL aliquot of working IS solution and 0.6 mL ethyl acetate was added to 200 μL of plasma sample. After vortex-mixed for 2 min, the samples were centrifuged at 10,000 g for 10 min. The supernatant was transferred to another tube and evaporated under a stream of nitrogen at 40 °C. The residue was reconstituted with 100 μL mobile phase and centrifuged again. 10 μL Of the supernatant was injected into the HPLC–MS systems for analysis.

For tissue samples, 20 μL aliquot of working IS solution and 3.0 mL ethyl acetate was added to small slices of tissues. The samples were then treated as the same fashion as the plasma sample. And also 10 μL of the supernatant was injected into the HPLC–MS systems for analysis.

2.6. Method validation

The method was validated for selectivity, linearity, precision and accuracy, matrix effects and extraction recovery according to the FDA guidelines for the bioanalytical method [17].

2.6.1. Specificity

Specificity was assessed by analyzing blank matrices, blank matrices spiked with lycorine and IS, and real plasma and liver sample from mice after IV administration of lycorine.

2.6.2. Linearity of calibration curves and lower limits of quantification

Calibration curves were generated by plotting the peak area ratios (analyte/IS) (y) against the theoretical concentration (x) using a $1/x^2$ weighting. The LLOQ was defined as the lowest drug concentration that could be detected with a relative error and precision (relative standard deviation, RSD) no more than 20%.

2.6.3. Accuracy and precision

Intraday assay accuracy and precision were established by analyzing six replicates of the QC samples at the three concentrations described. Interday assay accuracy and precision were established through the performance of 3 consecutive days. The accuracy was determined as the percentage of deviation (relative error, RE%) between the measured and nominal concentrations. The precision was evaluated from the relative standard deviation (RSD) of the concentration measurements. Intra- and interrun accuracies and precisions for QC concentrations of less than or equal to 15% were deemed to be acceptable.

2.6.4. Recovery and matrix effect

Extraction recovery in mice matrices for lycorine was determined at the three levels of QC and calculated as the ratio of analyte peak area from extracted QC samples to that from extracted blank matrices spiked with lycorine standard solution.

The matrix effect (plasma and tissue homogenates) was evaluated using extracted blank samples spiked with lycorine at three QC concentrations and determined as the ratio of analyte peak area from extracted blank matrices spiked with the neat solution to the mean peak area of the neat solution at the same concentration prepared in mobile phase.

2.7. Pharmacokinetic study

The mice received a 10 mg/kg dose of lycorine by IV and IP injection. Blood samples were serially withdrawn from each animal at 0, 5, 10, 20, 30, 45, 60, 90 and 120 min after administration to an eppendorf tube rinsed with heparin (0.25 IU/mL). Then each blood sample was immediately centrifuged at 4000 g for 10 min and a 200 μL aliquot of supernatant plasma was transferred into another tube and stored at −20 °C until treatment.

2.8. Tissue distribution study

Various tissue samples of certain weight including liver (300 mg), intestine (300 mg), kidney (300 mg), cerebrum (300 mg), heart

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