Talanta 119 (2014) 401–406

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

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

Liping Ren, Haiyan Zhao, Zilin Chen *

Key Laboratory of Combinatorial Biosynthesis and Drug Discovery (Wuhan University), Ministry of Education, and Wuhan University School of Pharmaceutical Sciences, Wuhan 430071, China

ARTICLE INFO

Article history:
Received 29 July 2013
Received in revised form
24 October 2013
Accepted 1 November 2013
Available online 15 November 2013

Keywords:
Lycorine
HPLC–ESI-MS
Pharmacokinetics
Tissue distribution

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.

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

* Corresponding author. Tel.: +86 27 68759893; fax: +86 27 68759850.
E-mail address: chenzl@whu.edu.cn (Z. Chen).

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http://dx.doi.org/10.1016/j.talanta.2013.11.014

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

(150 mg), spleen (80 mg), and lung (200 mg) were collected from mice at 15, 30, 45 and 60 min after intravenous and intraperitoneal administration. Then tissues were rinsed with normal saline solution to remove the blood or content, blotted with paper towel and stored at −20 °C until treatment.

2.9. Data analysis

Non-compartmental analysis for lycorine was performed by the PK Solver 1.0 software supplied by China Pharmaceutical University (Nanjing, China) to obtain the pharmacokinetic parameters. Area under the curve ($AUC_{0-\infty}$) and area under the first moment curve ($AUMC_{0-\infty}$) were obtained by integrating concentration–time (C–t) data in plasma from time zero to infinity. Systemic clearance was defined as $CLs = \text{dose}/AUC_{0-\infty}$. For the parameters mean residence time (MRT) and volume of distribution at steady state (V_{ss}), estimates were obtained using the statistical moment theory, where $MRT = AUMC_{0-\infty}/AUC_{0-\infty}$, and $V_{ss} = CLs \times MRT$. C_{max} and T_{max} represent maximal concentration and time to reach maximal concentration respectively. The half-life ($t_{1/2}$) was calculated as $t_{1/2} = 0.693MRT$. The tissue distribution study of lycorine was evaluated by measuring the concentrations of lycorine at 15, 30, 45 and 60 min after administration. All data were expressed as means ± standard deviation.

3. Results and discussion

3.1. Conditions for MS and HPLC

The positive ion mode and ESI source were adopted for the assay of lycorine and IS, which were attributed to the amino groups in their structures. Fig. 1 shows the structures and typical mass spectra of lycorine and IS in positive ion mode. The base peak is $[M+H]^+$. Therefore, the quantitative analysis of lycorine and IS was performed at $[M+H]^+$ ions of m/z 288 and 304, respectively.

An ideal internal standard should meet several requirements such as similar chemical structure, similar retention time and well-resolved from the analyte. Evodiamine was chosen as the IS for the assay as the structure, retention and ionization behavior are very similar to lycorine.

3.2. Method validation

3.2.1. Specificity

Typical chromatograms of blank plasma and tissue homogenates, blank matrices spiked with lycorine and IS, and real sample after IV injection of lycorine are represented in Fig. 2. The retention time was about 7.1 min for lycorine and 9.8 min for IS. Due to the high selectivity of SIM mode, no significant endogenous components could interfere with the analyte and IS.

3.2.2. Linearity and lower limit of quantification

Linearity of the calibration curve was evaluated by a linear regression analysis using a 1/concentration^2 weighting in the given concentration ranges of lycorine in plasma and tissue samples. The calibration curves, determined coefficients and linear ranges of lycorine in plasma and each tissue are listed in Table 1. The calibration curves for all matrices showed good linearity ($R^2 > 0.99$) over the concentration ranges. The LLOQs were measured to be 10.0 ng/mL for 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.

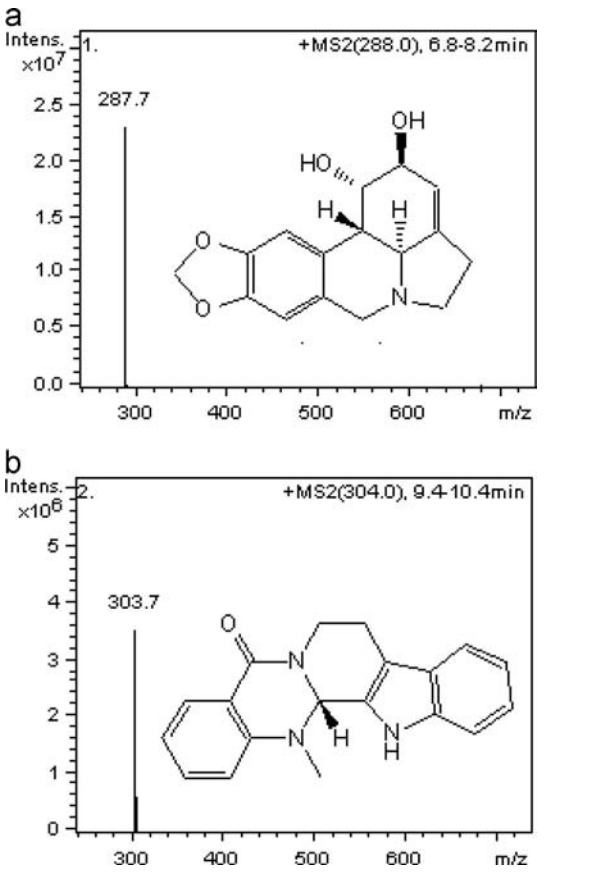

Fig. 1. Chemical structures and typical mass spectra of lycorine (a) and IS (b) in the positive mode.

3.2.3. Accuracy and precision

Intraday precision, interday precision, and accuracy for lycorine are exhibited in Table 2. All results for the samples tested ranged from −8.1% to 9.1% within the acceptable criteria of ±15% which suggested that the method was accurate and reproducible for the determination of lycorine in biological matrices.

3.2.4. Matrix effect, extraction recovery

All the variations of the matrix effect were in the range of 85–115%, which indicated that no significant ion suppression or enhancement existed in the HPLC–MS/MS method. The extraction efficiencies ranged from 85.0% to 95.3% for lycorine, which demonstrated that recoveries were consistent, precise and reproducible at different concentrations.

3.3. Pharmacokinetics study

The concentration of lycorine in plasma sample was determined by the present method. The mean plasma concentration versus time curve is presented in Fig. 3 and the major pharmacokinetic parameters calculated by non-compartmental model are listed in Table 3. It can be seen that there is no significant difference in the plots of two administration route. After administration, lycorine quickly achieved the peak concentration which was 4.73 ± 0.52 μg/mL at 10 min and 5.47 ± 0.68 μg/mL at 5 min after IP injection and IV injection, respectively. At 2 h post-dose, the concentration in plasma was undetectable, which indicated that lycorine cleared quickly from the body. The area under the plasma concentration–time curve was 145.09 ± 54.34 (μg/mL) min

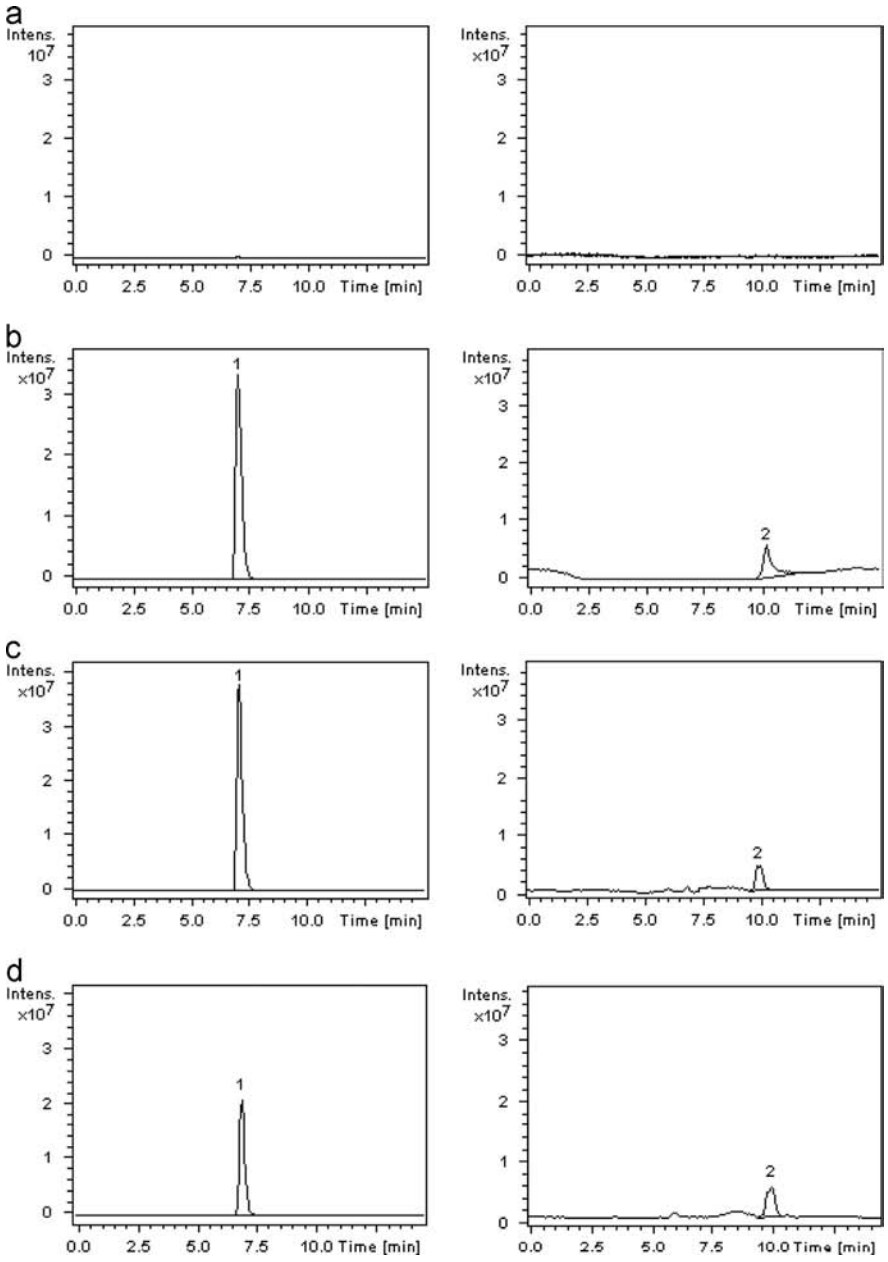

Fig. 2. Chromatograms of analytes under SIM mode from mice plasma and tissues: (a) blank plasma, (b) blank plasma spiked with lycorine and IS, (c) plasma sample obtained at 10 min after intravenous administration of lycorine, and (d) kidney sample obtained at 15 min after intravenous administration of lycorine. 1 – lycorine and 2 – IS.

Table 1
Standard curves, linear ranges, correlation coefficients and lower limit of quantification of lycorine in biological samples.

Sample	Calibration curve	R^2	Linear range (ng/g)	LLOQ (ng/g)
Plasma[b]	$Y=0.6356\times10^{-3}X-0.2337$	0.9923	500–10,000[a]	10.0[b]
Liver	$Y=3.2933\times10^{-3}X+0.4843$	0.9982	33–32,895	6.5
Intestinum	$Y=0.6797\times10^{-3}X+0.2813$	0.9969	33–32,895	6.5
Stomach	$Y=0.4469\times10^{-3}X+0.9875$	0.9960	33–32,895	6.5
Cerebrum	$Y=0.6124\times10^{-3}X+0.7107$	0.9981	33–32895	6.5
Kidney	$Y=0.7178\times10^{-3}X+0.2164$	0.9960	33–32,895	6.5
Lung	$Y=0.5385\times10^{-3}X+0.4440$	0.9980	46–46,053	9.0
Heart	$Y=0.8682\times10^{-3}X+0.2454$	0.9954	60–65,790	12.0
Spleen	$Y=0.6372\times10^{-3}X+0.2420$	0.9985	90–98,685	18.0

[a] Unit is ng/mL.
[b] Unit is ng/mL.

and 190.2 ± 40.12 (μg/mL) min for IP injection and IV injection, respectively.

3.4. Tissue distribution study

Lycorine is widely distributed in all tissues examined after IP and IV injection. As shown in Fig. 4, it extensively distributes into the extra-vascular system of animal body. Lycorine levels are significantly reduced to be undetectable in 2 h after both types of administration. The highest concentration is observed in stomach and kidney at 15 min after IP administration, followed by spleen, intestine, lung, heart, liver and cerebrum. Meanwhile, 15 min post-IV dose, the concentration descends in the order of kidney, spleen, intestine, lung, heart, liver, cerebrum and stomach. The high levels in kidney indicate that large quantity of lycorine is prone to be eliminated through kidney. The concentration in liver

Table 2
Accuracy and precision of lycorine in mice plasma samples and tissue homogenates ($n=5$).

Samples	Concentration spiked (ng/g)	Intraday		Interday	
		Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)
Plasma[a]	100[a]	3.9	3.5	−3.8	6.4
	2500[a]	0.5	3.9	−4.1	7.5
	10,000[a]	0.4	5.7	−6.9	4.9
Liver	66	7.5	4.8	−7.3	5.4
	3290	1.8	3.9	−6.4	8.1
	32,895	2.5	5.1	−8.1	7.9
Kidney	66	6.5	3.9	9.1	6.3
	3290	4.1	4.5	6.3	6.9
	32,895	2.3	6.1	−5.8	7.3
Cerebrum	66	8.3	5.3	−7.4	8.1
	3290	3.7	6.9	−6.5	6.9
	32,895	4.1	5.7	−6.3	4.2
Intestine	66	6.2	6.3	−3.6	7.5
	3290	0.1	5.1	−6.5	4.9
	32,895	3.8	6.8	−4.9	8.3
Stomach	66	7.9	7.5	−7.4	8.9
	3290	5.3	5.9	−6.3	7.2
	32,895	4.1	4.9	−8.1	4.9
Lung	100	5.8	6.3	3.5	5.9
	4936	5.3	5.1	−5.9	6.9
	46,053	1.4	4.9	−4.1	5.8
heart	140	6.3	4.2	−7.4	8.3
	6580	1.4	5.7	−5.9	7.9
	65,790	7.9	4.9	−4.8	6.5
Spleen	220	4.3	5.2	−6.1	5.1
	23,030	2.4	6.7	−6.9	7.4
	98,685	1.6	5.9	−3.5	7.1

[a] Unit is ng/mL.

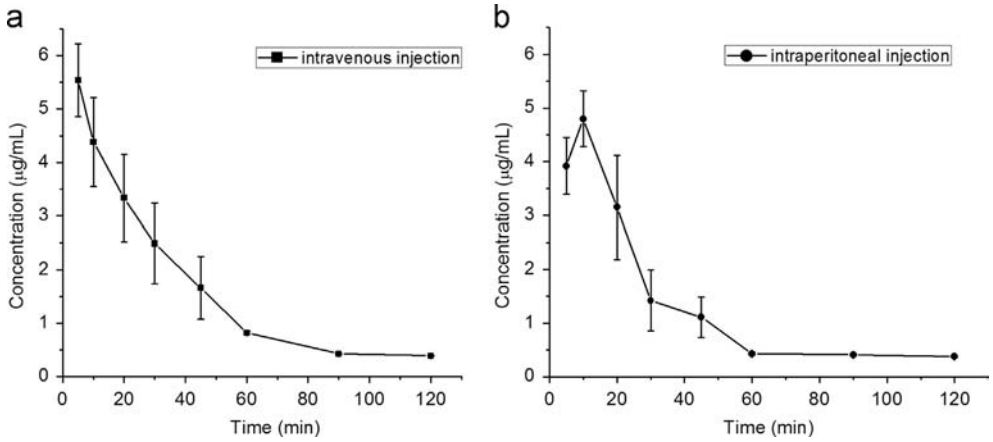

Fig. 3. Mean plasma concentration–time curves of lycorine in mice plasma after intravenous (a) and intraperitoneal (b) administration at a dose of 10 mg/kg ($n=4$).

Table 3
The pharmacokinetic parameters of lycorine in mice following intraperitoneal and intravenous injection at dose of 10 mg/kg ($n=4$).

Parameters	Intraperitoneal injection (mean ± SD)	Tail vain injection (mean ± SD)
C_{max} (μg/mL)	4.73 ± 0.52	5.47 ± 0.68
T_{max} (min)	10.00	5.00
$t_{1/2}$ (min)	335.05 ± 35.90	205.80 ± 46.88
MRT (min)	347.14 ± 86.36	175.17 ± 66.84
$AUMC_{0-\infty}$ (μg/mL min^2)	107,051.26 ± 18,713.54	51376.55 ± 17000.76
$AUC_{0-\infty}$ (μg/mL min)	145.09 ± 54.34	190.2 ± 40.12
CL (mL/min/mg)	0.03 ± 0.005	0.03 ± 0.002
V (mL/g)	15.73 ± 3.44	10.09 ± 2.82

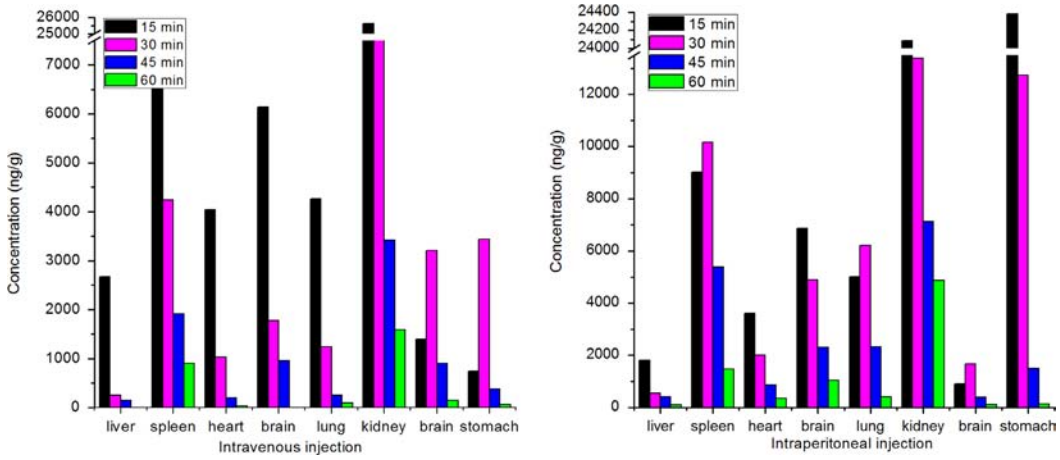

Fig. 4. Tissue distribution profile of lycorine in various tissues after intravenous and intraperitoneal administration at a dose of 10 mg/kg in mice.

is low, which suggests that lycorine may be apt to metabolize in liver. It agrees with the literature that phases I and II metabolites of lycorine would easily be found when in vitro incubated with liver microsomes [18]. Meanwhile, lycorine found in brain implies that it could cross the blood–brain barrier.

4. Conclusions

A simple and rapid LC–MS method for determination of lycorine presented in plasma and tissues has been developed and validated. The method was successfully applied to the pharmacokinetics and tissue distribution study of lycorine in mice. It is shown that there is no significant difference in the plasma concentration–time curves of lycorine after both types of administration. For the tissue distribution study, two types of administration route share the common sequence order of concentration in tissues except in stomach. The low levels in liver and high levels in kidney suggest that lycorine is easy to metabolize in liver and to be eliminated through kidney. Lycorine found in brain implies that it could cross the blood–brain barrier. The characteristics of pharmacokinetics and tissue distribution we got in this study would be instructive for clinical applications of lycorine.

Acknowledgments

This work was supported by the National Scientific Foundation of China (Grant nos.: 21375101, 90817103 and 30973672), Doctoral Fund of Ministry of Education of China (No. 20110141110024), Innovation Seed Fund and Translational Medical Research Fund of Wuhan University School of Medicine, and the Fundamental research Funds for the Central Universities.

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