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Short communication

Matrine determination and pharmacokinetics in human plasma using LC/MS/MS

Xiao-Lin Zhang [a], Hong-Rong Xu [a], Wei-Li Chen [a], Nan-Nan Chu [a], Xue-Ning Li [a,*], Gang-Yi Liu [b], Chen Yu [b]

[a] Department of Clinical Pharmacology, Zhong Shan Hospital, Fudan University, 180 Feng Lin Road, Shanghai 200032, China
[b] Shanghai Xu Hui District Central Hospital, 966 Huaihai Middle Road, Shanghai 200031, China

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ABSTRACT

A liquid chromatography/tandem mass spectrometry (LC/MS/MS) method was developed for the determination of matrine in human plasma extracted by isopropanol:ethyl acetate (v/v, 5:95). Rapid chromatographic separation was achieved in the mobile phase composition of 5-mM aqueous ammonium acetate and acetonitrile (v/v, 70:30) with a flow rate of 0.20 ml/min. Detection was carried out using positive-ion electrospray tandem mass spectrometry on a Sciex API3000. The method was accurate, specific and sensitive for the analysis of matrine in human plasma in the concentration range of 5–2000 ng/ml, when huperzine A was used as internal standard. The method facilitated a clinical pharmacokinetic study after oral administration of a single dose of matrine soft gelatin capsules (100, 200 and 400 mg) in a three-period crossover design. Dose-related linear trends were observed for the AUC_{0-t} and the C_{max} of matrine. The $t_{1/2}$ and the T_{max} of matrine were independent of the administered doses.

1. Introduction

Matrine (Fig. 1), a quinolizidine alkaloid isolated from *Sophora alopecuroides*, *Sophora flavescens* or *Sophora subprostrata* in traditional Chinese medicine, has been extensively used in China for the treatment of viral hepatitis, cancer, cardiac and skin diseases [1–4]. However, there is a lack of information on the pharmacokinetics of pure matrine in humans, especially after oral administration. In the only clinical pharmacokinetic study of pure matrine, serum matrine concentrations ranged from 1 to 6 μg/ml after a large-dose intravenous infusion (6 mg/kg) [5].

Several assay methods have been developed for determining matrine in biofluids. Nevertheless, with a limit of quantitation not better than 0.25 μg/ml, methods including high-performance liquid chromatography with ultraviolet detection [5–9], thin-layer chromatography [10] and high-performance capillary electrophoresis [11] are not practical or sensitive enough to detect matrine in the plasma of humans receiving a single oral dose of matrine soft gelatin capsule 100 mg, whose concentrations are expectedly lower than 1 μg/ml due to the much smaller given dose than the aforementioned study [5] and the loss before entering systemic circulation [9]. Although fluorescence quenching [10] and gas chromatography/mass spectrometry [12] detected plasma matrine at low ng/mg concentrations, both of them required complicated sample pretreatment. In comparison, liquid chromatography/mass spectrometry (LC/MS) [13–20] is frequently employed by the recent studies for the analysis of matrine in biomatrices because of its excellent sensitivity, speed and simplicity combined. Most of these studies quantified matrine in plasma samples extracted by 3–4 ml of chloroform at a sensitivity of 5–15 ng/ml and at a run time of 5–12 min.

To support a clinical pharmacokinetic investigation after oral administration of matrine soft gelatin capsules 100, 200 and 400 mg, a more rapid LC/MS/MS method with a lower limit of quantitation of 5 ng/ml was established in this paper to determine matrine in human plasma samples handled by 0.8 ml of less toxic isopropanol–ethyl acetate.

2. Experimental

2.1. Reagents and chemicals

Matrine (99.99% pure, batch number 20031010) was supplied by Shanghai Jiagu Pharmaceuticals (Shanghai, China). Huperzine A (internal standard, 99.0% pure, batch number 20020608) (Fig. 1) was obtained from the Shanghai Institute of Materia Medica, Chinese Academy of Sciences (Shanghai, China). Ammonium acetate was bought from Tedia (Fairfield, OH, USA). Isopropanol was purchased from Caledon (Georgetown, Ontario, Canada). Ethyl acetate was the provision of Dikma (Richmond Hill, ON, USA). Methanol and acetonitrile, both HPLC grade, were imported from Sigma–Aldrich Chemicals (St. Louis, MO, USA). Deionized/distilled (DI) water was prepared from tap water in our own department.

* Corresponding author. Tel.: +86 21 64437963/041990x2702; fax: +86 21 64437963.
E-mail address: li.xuening@zs-hospital.sh.cn (X.-N. Li).

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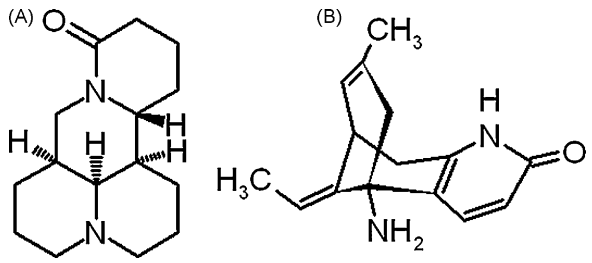

Fig. 1. Chemical structures of: (A) matrine and (B) huperzine A (internal standard, IS).

2.2. LC/MS/MS analysis

The liquid chromatography separation system comprised LC-10AD VP (pump), DGU-14AM (degasser) and SIL-HTc (autosampler) (Shimadzu, Kyoto, Japan). The separation column was a CAPCELL PAK C18 column (50 mm × 2.0 mm, 5 μm) (Shiseido, Tokyo, Japan). An XW-80 vortex was obtained from Shanghai Medical University Apparatus (Shanghai, China). The Biofuge 28RS centrifuge was introduced from Heraeus Sepatech (Osterode, Germany). The isocratic mobile phase consisted of 70% 5-mM aqueous ammonium acetate and 30% acetonitrile. The flow rate was set to 0.20 ml/min. The injection volume was 5 μl.

A Sciex API 3000 LC/MS/MS system (Foster City, CA, USA) operated under Analyst 1.3.1 software. The electrospray ion source was run in a positive-ionization mode. The typical ion source parameters were: declustering potential, 36 V; collision energy, 53 eV for matrine and 39 eV for huperzine A; focusing potential, 190 V; collision cell exit potential, 11 V; ionspray voltage, 1500 V; ion temperature, 400 °C. Nebulizer gas, curtain gas and collision gas using nitrogen was set to 10, 8 and 12 l/min, respectively. Samples were analyzed via multiple-reaction monitoring (MRM) with monitoring ion pairs at m/z 249.4 → 148.1 for matrine and m/z 243.4 → 210.3 for huperzine A. The scan dwell time was set at 0.2 s for every channel.

2.3. Matrine standard, quality control (QC) and internal standard (IS) preparation

Primary stock solutions of matrine (100 μg/ml) and huperzine A (100 μg/ml) were prepared in DI water. Working standard solutions of matrine and working solutions of huperzine A for internal standard (4 μg/ml) were prepared by diluting aliquots of corresponding primary stock solution with DI water. All solutions were stored at 4 °C in darkness when not in use. Calibration standards of matrine (5, 10, 50, 100, 500, 1000, 2000 ng/ml) were prepared by spiking the working standard solutions into a pool of blank human plasma.

QC stock solution of matrine (5 mg/ml) was prepared from a separate weighing and was also dissolved in DI water. The QC samples were prepared at high (1600 ng/ml), medium (800 ng/ml) and low (5 ng/ml) concentrations in the same way as the calibration standards. QCs were stored at −20 °C.

2.4. Plasma sample preparation

100 μl of plasma sample in 2.0-ml labeled microcentrifuge tubes was mixed with 10 μl of the working internal standard solution and vortexed for 5 s. Then 800 μl of isopropanol:ethyl acetate (v/v, 5:95) was added and vortexed for 1 min. The tubes were subsequently centrifuged at 18,000 × g for 2 min. The organic layer was transferred to another tube and evaporated to dryness at ambient temperature under a gentle stream of nitrogen. The residue was reconstituted in 400 μl of DI water and centrifuged at 26,000 × g and 4 °C for 20 min. The supernatant was injected into LC/MS/MS system.

2.5. Pharmacokinetic study protocol and parameters

The present investigation was an open-label, randomized and 3 × 3 crossover study with a washout of 7 days. The protocol was approved by the Ethic Committee of Zhongshan Hospital and conformed to the principles of the Declaration of Helsinki. Volunteers signed informed consent before any screening item was performed. Nine of them were recruited and given a single dose of matrine soft gelatin capsule (100, 200 or 400 mg) during each period. Blood samples were collected into heparinized vacutainers at pre-dose and serially at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 36 and 48 h post-dose, and immediately centrifuged at 1000 × g and 4 °C for 10 min. The plasma was pipetted into screw-cap polypropylene tubes and stored at the study center at −20 °C until shipment to the laboratory where the analyses were performed.

Pharmacokinetic parameters were estimated by non-compartmental model using 3p97 PK software programmed by Chinese Pharmacological Association. The elimination half-life ($t_{1/2}$) was 0.693/k_e, where k_e, the elimination rate constant, was calculated by linear regression from the terminal linear portion of plasma concentration–time curve. The maximum drug plasma concentrations (C_{max}) and time to C_{max} (T_{max}) were read directly from the observed data. The area under the plasma concentration–time curve from zero to the time of the final measurable sample (AUC_{0-t}) was calculated by use of the linear-trapezoidal rule. The area under the plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$) was determined by summing AUC_{0-t} and the extrapolated area. The extrapolated area was determined by dividing the last measurable concentration by k_e.

3. Results and discussion

3.1. LC/MS/MS optimization

The mobile phase was optimized for sensitivity, speed and peak shapes. At first, various proportions of methanol in water were used but gave very poor chromatography. When acetonitrile and water (v/v, 50:50) was employed, much better but asymmetrical peak shapes as well as over fast elution was observed. Therefore, the content of acetonitrile was reduced to 30%. Then acetic acid was added. It failed to increased peak symmetry while decreased response. The addition of ammonium acetate improved tailing factor without sensitivity loss and was included in the mobile phase. Under the final chromatographic conditions, huperzine A, one of the candidate compounds, showed similar retention and ionization to that of matrine and was adopted as internal standard.

In the process of sample pretreatment, protein precipitation with methanol or acetonitrile was tried with priority due to its simplicity and the successful examples reported [13,14]. However, consistent with other studies [15,16], it resulted in poor matrix effect. Then we used ethyl ether, methyl *tert*-butyl ether or ethyl acetate to extract samples. Ethyl acetate was chosen since it provided clean extracts and higher recoveries for the analyte and the IS. Instead of two extraction cycles, the addition of isopropanol to ethyl acetate (v/v, 5:95) further increased the recoveries without reducing the cleanliness of extracts.

In the present assay, a low ionspray voltage and MRM were found to improve signal-to-noise ratios better than high ionspray voltages and selected ion monitoring. Protonated matrine and huperzine A were the major ions when a m/z range of 50–300 amu was scanned during Q1 scan. They were selected as precursor ions to obtain their product ions, respectively. Under the optimized collision energy, matrine gave a major product ion at 148.1 and huperzine A gave a major product ion at 210.3 (Fig. 2).

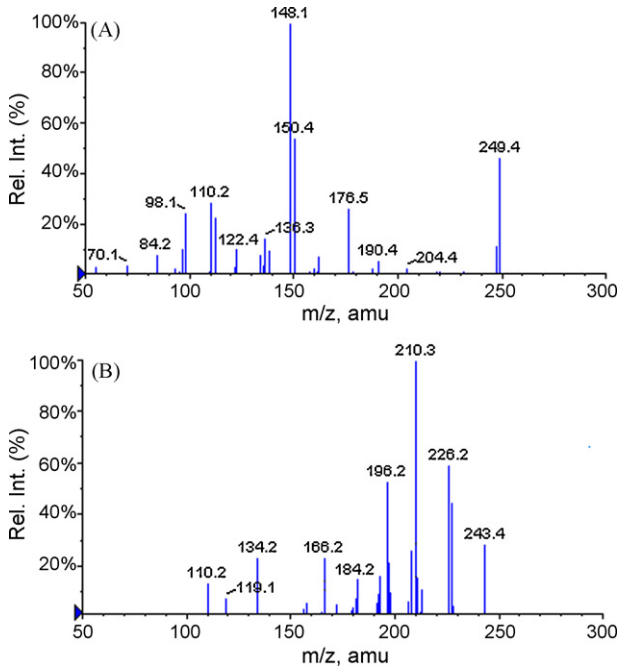

Fig. 2. Product ion spectra of protonated matrine (A) and hupeizine A (internal standard, IS) (B).

3.2. Method validation

3.2.1. Calibration curve and dilution protocol

Calibration curves were constructed using duplicate of seven calibration standards with concentrations in the range of 5–2000 ng/ml. Peak area ratios of matrine:IS were used for a weighted ($1/x$) least-squares linear regression analysis. One of the regression equations and its correlation coefficient (r) were: $y = 0.0074x + 0.016$ ($r = 0.9994$), in which y is the peak area ratios and x is the concentration of matrine. The equation showed good liner relationships between the peak areas and the concentrations. Matrine concentration (5 ng/ml) producing a signal that was ten times higher than the noise peaks was regarded as the limit of quantitation (LOQ).

The concentrations of matrine in unknown samples were determined by interpolation from the newly constructed calibration curves. However, some of them reached about 3600 ng/ml. Therefore, a dilution test was performed as follows: 50-μl human plasma spiked with matrine at a concentration of 4000 ng/ml was mixed with 50-μl blank human plasma to prepare 100-μl plasma sample. Six replicates of such plasma samples were pretreated as the descriptions in Section 2.4. The percentage of (detected concentration × 2)/4000 ranged from 92.6% to 101.0%, demonstrating samples with matrine concentrations exceeding 2000 ng/ml can be accurately measured by diluting these samples with the same volume of blank human plasma.

3.2.2. Specificity and matrix effects

Typical MRM chromatograms of human plasma with and without standards were showed in Fig. 3. Matrine and huperzine A gave retention times of 1.6 and 1.8 min, respectively. The overall chromatographic run time was within 2.5 min. No significant interfering peaks were present at the retention times of the analyte or the IS. The matrix effects of the analyte and the IS were assessed by comparing the peak areas of the analyte and the IS in post-extraction blank samples from six different individuals with those in neat solution at the corresponding concentrations. The matrix effects at concentrations of 5, 800 and 1600 ng/ml for the analyte were 87.4 ± 6.5%, 88.4 ± 3.8% and 88.2 ± 5.7%, respectively, and at concentration of 400 ng/ml for the IS was 86.2 ± 3.7%. Such low ionization suppression from plasma matrices did not compromise the performance of the assay.

3.2.3. Accuracy, precision and recovery

Accuracy was defined as (detected concentration − nominal concentration)/(nominal concentration) × 100% (denoted as RE). Precision was determined by calculating the coefficient of variation (denoted as %CV). As can be seen from Table 1, the matrine assay in plasma was accurate and precise on intra- and inter-batch for each QC level. The largest %CV appeared in the intra-batch precision of matrine, which was 12.15% for LOQ. All QC levels for matrine had intra- and inter-batch RE within ±6%.

The extraction recoveries of the analyte and the IS were evaluated by comparing the peak areas of the analyte and the IS in pre- and post-extraction plasma samples from six different individuals at the corresponding concentrations. The extraction recoveries

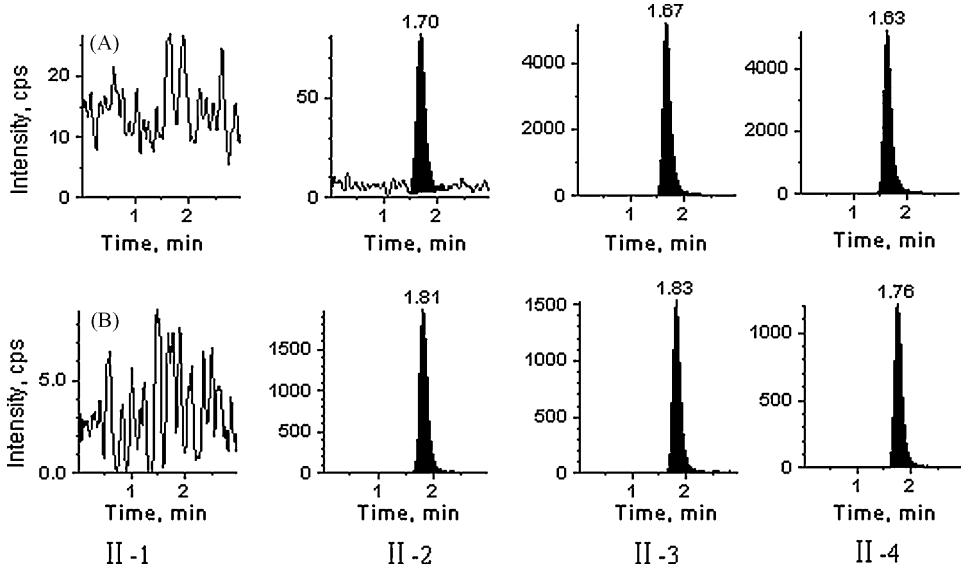

Fig. 3. Chromatography of (A) matrine and (B) huperzine A (internal standard, IS) in human plasma. II-1: blank plasma, II-2: 5 ng/ml matrine added into blank plasma (LOQ), II-3: 800 ng/ml matrine and IS added into blank plasma, and II-4: a human plasma collected 1.0 h after an oral single dose of matrine soft gelatin capsule 100 mg.

Table 1
Intra- and inter-batch accuracy and precision of matrine in human plasma.

	LOQ 5 ng/ml	Middle 800 ng/ml	High 1600 ng/ml
Intra-batch accuracy and precision			
Mean	5.29	774.00	1577.80
SD	0.64	17.32	63.11
%CV	12.15	2.24	4.00
RE	5.80	−3.25	−1.39
n	6	6	6
Inter-batch accuracy and precision			
Mean	5.10	830.30	1640.60
SD	0.43	40.03	95.76
%CV	8.40	4.82	5.84
RE	2.00	3.79	2.54
n	5	5	5

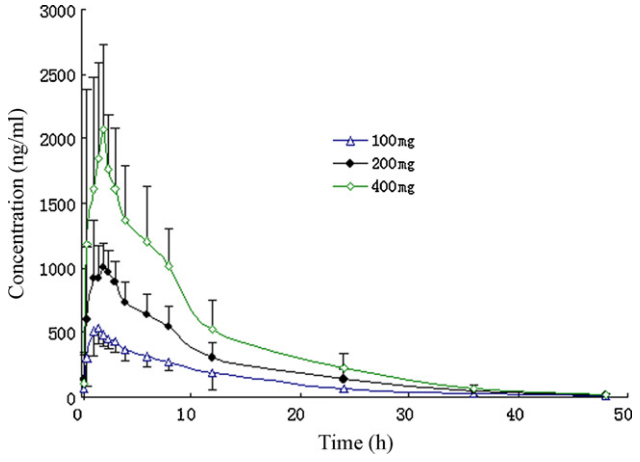

Fig. 4. Mean (±SD) plasma concentration–time curve of matrine for 9 healthy volunteers after an oral single dose of matrine soft gelatin capsules 100, 200 and 400 mg.

at concentrations of 5, 800 and 1600 ng/ml for the analyte were 76.9 ± 5.6%, 70.7 ± 2.9% and 74.6 ± 4.2%, respectively, and at a concentration of 400 ng/ml for the IS was 86.3 ± 3.1%. Compared with exclusively used chloroform [15–19], the present extract solvent was less toxic and consumed a much smaller volume for each sample (0.8 ml vs. 3–4 ml). Furthermore, the clean extracts and stable extraction achieved without pre-deprotein or basification [19] can make it a good choice for liquid–liquid extraction in the determination of plasma matrine concentration using LC/MS system.

3.2.4. Stability

The stability of matrine in human plasma was investigated by analyzing triplicates of three QC levels. The tested conditions included three freeze–thaw cycles, at room temperature for 3 h, at 4 °C for 24 h in the autosampler and at −20 °C for 30 days. Matrine was found to be stable under these conditions, since changes in the concentrations of matrine at any QC level were in the range of −7.15% to 13.81%.

3.3. Clinical application

The current assay facilitated the concentration–time profiles of matrine in human plasma after oral administration of matrine soft gelatin capsules (100, 200 and 400 mg) (Fig. 4). The C_{max} of matrine in nine volunteers ranged from 411.8 to 3608.5 ng/ml. The mean C_{max} at doses of 100, 200 and 400 mg were 603.3 ± 131.8, 1207.3 ± 332.0 and 2384.4 ± 720.7 ng/ml occurring at 1.3 ± 0.6, 1.6 ± 0.8 and 1.7 ± 0.7 h, respectively. The mean AUC_{0-t} were 6203 ± 2206, 12816 ± 4666 and 20078 ± 6842 ng h/ml, respectively, and the mean $AUC_{0-\infty}$ were 6375 ± 2253, 13047 ± 4781 and 20316 ± 6939 ng h/ml, respectively. The $t_{1/2}$ were 8.7 ± 1.3, 8.3 ± 1.0 and 7.8 ± 0.8 h, respectively, for the doses.

The pharmacokinetic results of matrine indicated that the mean C_{max} and the mean AUC_{0-t} increased in proportion to dose increase, the T_{max} and the $t_{1/2}$ had no apparent change as the dose ascended. Therefore, a conclusion can be drawn that matrine had linear pharmacokinetic trends in healthy Chinese volunteers. Overall, the $t_{1/2}$ in the present study were close to the previous study which recorded that matrine as the active metabolite of oxymatrine had a $t_{1/2}$ of 9.4 h in humans [13].

4. Conclusion

This paper describes a rapid, sensitive, accurate, and precise LC/MS/MS method using a simple, low-toxicity and economical liquid–liquid extraction for the determination of matrine in human plasma. The method allowed a high throughout of samples from a clinical pharmacokinetic study. The observed linear pharmacokinetic trends of matrine in healthy volunteers after oral administration of matrine soft gelatin capsules 100, 200 and 400 mg would be used as a suitable reference in clinical practice.

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