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Determination of sinomenine sustained-release capsules in healthy Chinese volunteers by liquid chromatography–tandem mass spectrometry

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ABSTRACT

A sensitive and selective liquid chromatographic tandem mass spectrometric method was developed and validated for the determination of sinomenine in human plasma. Plasma samples were precipitated using methanol with metronidazole as internal standard. Separation was carried out on an Inertsil ODS-3 column using a mixture of 0.2% ammonium acetate solution (A) and methanol (B) as the mobile phase with linear gradient elution as follows: 0 min (50%B) → 1.5 min (80%B) → 4.5 min (80%B) → 4.6 min (50%B) → 6.0 min (50%B). All mass data were obtained in the positive ion mode, and the fragmentation transitions for the selective multiple reaction monitoring were m/z 330 → 181 and 172 → 128 for sinomenine and metronidazole, respectively. The method was fully validated to be accurate and precise with a linear range of 0.5–500 ng/mL and applied to a single- and multiple-dose pharmacokinetics study of sustained-release capsules of sinomenine hydrochloride in 20 healthy Chinese volunteers. After oral administration of a single 60-mg dose, the T_{max}, C_{max}, AUC_{0-96} and $t_{1/2}$ were 7.9 ± 2.0 h, 123 ± 22 ng/mL, 3032 ± 682 ng h/mL and 13.4 ± 1.6 h, respectively. After oral administration of the 60 mg capsules twice-daily for 7 consecutive days, these parameters were 4.4 ± 3.6 h, 279 ± 69 ng/mL, 7333 ± 2096 ng h/mL and 15.1 ± 1.3 h, respectively. The AUC and C_{max} values after multiple-dose treatment were significantly higher than those after a single-dose treatment (P < 0.01), with an accumulation factor of 2.49 ± 0.77.

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1. Introduction

Sinomenine [(9alpha, 13alpha, 14alpha)-7,8-didehydro-4-hydroxy-3,7-dimethoxy-17-methylmorphinan-6-one; Fig. 1] is an alkaloid extracted from the stem of the Chinese medicinal plant, *Sinomenium acutum*, Rehder & E.H. Wilson (Family Menispermaceae). In vitro and in vivo studies have indicated that sinomenine has an array of biological activities, including immunosuppression [1], anti-inflammatory [2,3], anti-arthritic effect [4,5], inhibition of lymphocyte proliferation [6] and prevention of antagonize cartilage degradation and chondrocyte apoptosis [7]. It has shown significant beneficial effects as monotherapy in the treatment of rheumatoid arthritis [8–11]. Although the enteric-coated tablet of 20-mg sinomenine hydrochloride has been marketed in China for many years, the very detailed information about human pharmacokinetics was limited.

Similar to the non-steroidal anti-inflammatory drugs (NSAIDs), conventional formulations of sinomenine hydrochloride occasionally caused adverse effects in the digestive system [11]. Accordingly, it is necessary to develop sustained-release formulation to overcome some of the pharmacokinetic limitations and safety concerns associated with the conventional formulation [12,13]. The formulation in this study is a hard gelatin capsule containing pellets coated with ethylcellulose for film-controlled release of sinomenine hydrochloride. Multiparticulate formulations have the significant advantage of less intra- and inter-subject variability in terms of pharmacokinetics than their monolithic counterparts. The twice-daily formulation assessed in this study has been characterized in vitro as a stable and sustained-release drug, which is active for at least 12 h.

To characterize the pharmacokinetics of sinomenine, a highly sensitive and selective method is required. Currently, the primary focus of quantification of sinomenine hydrochloride in biological samples is based on the high-performance liquid chromatography with ultraviolet detector (LC–UV) [14–20], which required either a complicated extraction or long analysis time (>10 min). Furthermore, the lower limits of quantitation (LLOQ) of these methods in a range of 6–320 ng/mL were not suitable for sinomenine

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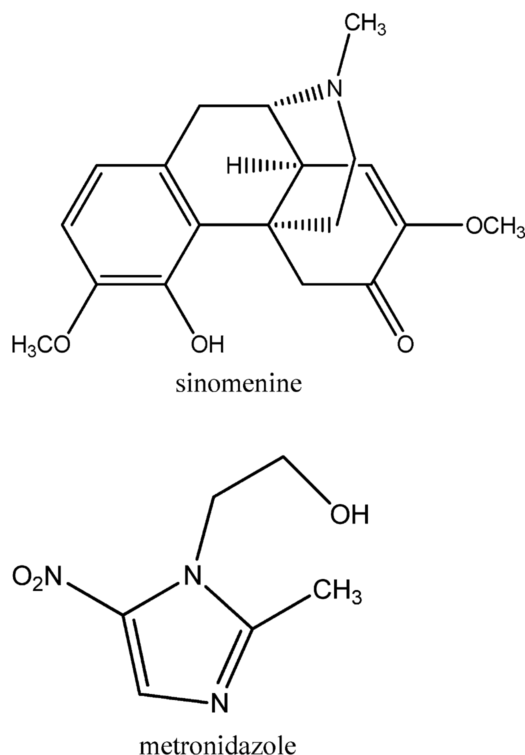

Fig. 1. Chemical structures of sinomenine and internal standard (metronidazole).

pharmacokinetic study. Some more sensitive methods using LC/MS have been reported to detect this drug in rat's brain [20] or skin [21]. Only one study using ion trap mass spectrometry has been published for the determination of sinomenine in human plasma with an LLOQ of 0.5 ng/mL [22], but the analyses were performed only with spiked plasma samples so pharmacokinetic parameters were not available.

The present study developed and fully validated a selective and sensitive high performance liquid chromatography–electrospray ionization-tandem mass spectrometric (LC/MS/MS) method for the determination of sinomenine in human plasma. The established LC/MS/MS method was applied to the pharmacokinetic study of the 60-mg sinomenine hydrochloride sustained-release capsules after a single and multiple oral doses in 20 healthy Chinese volunteers.

2. Materials and methods

2.1. Reagents and materials

Sinomenine hydrochloride standard substance (purity > 99% HPLC) and sinomenine hydrochloride sustained-release capsules were supplied by Jiangsu Chiatai Qingjiang Pharmaceutical Co., Ltd. (Jiangsu, Huai'an, PR China). Internal standard, metronidazole was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (lot 100191-200305, Beijing, PR China). Methanol of HPLC/Spectro grade was obtained from Tedia Company Inc. (Fairfield, OH, USA). Other chemicals were all of analytical grades, and purchased from Nanjing Chemical Reagent Factory (Nanjing, PR China). Deionized water was purified through a PL5242 Purelab Classic UV (PALL Co., Ltd, USA) before use. Blank plasma was supplied by the Red Cross Society of China, Nanjing Branch.

2.2. LC/MS/MS conditions

A Shimadzu LC-2010CHT system (Shimadzu, Kyoto, Japan) was hyphenated with a Waters Quattro-micro tandem mass spectrometer equipped with electrospray ionization (ESI) source (Micromass, Manchester, UK). Data acquisition was performed with Masslynx software (Version 4.0). An Inertsil ODS-3 column (5 μm, 250 mm × 4.6 mm i.d.) was used for the chromatographic separations. The mobile phase A was 0.2% ammonium acetate solution (pH 6.6), and the mobile phase B was methanol. The linear gradient elution program was performed as follows: 0 min (50%B) → 1.5 min (80%B) → 4.5 min (80%B) → 4.6 min (50%B) → 6.0 min (50%B) with a flow rate of 1.0 mL/min and 30% of the effluent was split into the MS inlet for the determination. To assure the reproducibility of the retention time, the column temperature was maintained at 30 °C. For optimal stability, the auto-sampler temperature was set at 4 °C.

All analyses were carried out in positive-ion ESI and monitored in multiple selective reactions monitoring (SRM) mode. The MS conditions were optimized as follows: the spray voltage was set at 2.5 kV with the source temperature at 120 °C, and the desolvation nitrogen gas temperature was set at 400 °C with a desolvation gas flow of 450 L/h and a cone gas flow of 30 L/h. The $[M+H]^+$ ion of sinomenine was monitored with a transition of m/z 330 → 181, cone voltage of 38 volts (V) and collision energy of 35 electrovolts (eV). The $[M+H]^+$ ion of metronidazole (internal standard) was monitored with a transition of m/z 172 → 128, cone energy of 38 V and collision energy of 15 eV. The extractor voltage was 2 V and RF lens voltage 0.1 V. Argon gas of 0.2 Pa was used for collision-induced dissociation.

2.3. Sample preparation

To an aliquot 200 μL plasma samples in 1.5 mL Eppendorf tube, 20 μL of methanol (when preparing calibration and quality control (QC) samples, standard solution was added instead of methanol), 20 μL of IS solution (400 ng/mL) and 600 μL methanol were added and vortex-mixed for 1 min for protein precipitation and centrifuged at 10,000 × g for 10 min. An aliquot of 20 μL of the supernatant was injected into the LC/MS/MS system.

2.4. Preparation of standard solutions, calibration and QC samples

Standard stock solution of sinomenine was prepared by dissolving an accurately weighed appropriate amount in a 25 mL volumetric flask with methanol to achieve a concentration of 500 μg/mL. Stock solution of the IS was also prepared in methanol and diluted to 400 ng/mL. The sinomenine standard solutions of 5–5000 ng/mL were prepared by serial dilution of the stock solution. All of the solutions were stored at 4 °C, and brought to room temperature before use. Plasma calibration standards of 0.5–500 ng/mL were prepared by spiking 20 μL each of the standard solutions with aliquots of 0.200 mL blank human plasma. QC samples were prepared in the same way with four levels of 2.5, 20, 150, and 400 ng/mL.

2.5. Method validation

2.5.1. Selectivity

Selectivity was demonstrated by the chromatograms of six different blank plasma samples obtained from six subjects with reference to those spiked with sinomenine and metronidazole.

2.5.2. Linearity and lower limit of quantification (LLOQ)

Calibration curves for sinomenine in plasma were prepared in the range from 0.5 to 500 ng/mL. The linearity of each

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