Research Article

Pharmacokinetics of 10-Hydroxy Mesaconitine in Rat Plasma by Ultra-Performance Liquid Chromatography-Tandem Quadrupole Mass Spectrometry

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Mesaconitine is the predominant active ingredient in Aconitum carmichaelii Debx. The compound 10-hydroxy mesaconitine is one known metabolite of mesaconitine and is toxic. In order to better understand its pharmacokinetics, UPLC-MS/MS was used in this paper to measure the concentration of 10-hydroxy mesaconitine in rat plasma after oral (5 mg/kg) and intravenous (0.1 mg/kg) administration of 10-hydroxy mesaconitine. The concentrations of 10-hydroxy mesaconitine in rat plasma measured in the standard curve covered the range of 0.3–60 ng/mL. The intraday and interday precisions of the samples of 10-hydroxy mesaconitine in rat plasma were lower than 15%. In addition, the accuracies ranged between 96.0% and 109.3%, the matrix effects ranged between 88.9% and 98.1%, and the recoveries were all higher than 79.1%. The AUC(0–t) values were 23.6 ± 5.9 and 207.6 ± 72.9 ng/mL · h for intravenous and oral administration, respectively, and the bioavailability of 10-hydroxy mesaconitine was 17.6%. Lastly, t1/2 was 1.3 ± 0.6 h and 3.1 ± 0.4 h for intravenous and oral administration, respectively.

1. Introduction

Aconitum, a genus of flowering plant species, is widely used in the clinic owing to its significant therapeutic effect in the treatment of rheumatic arthralgia [1–3]. Aconitum contains alkaloids such as mesaconitine and aconitine that exhibit anti-inflammatory and analgesic activity but at the same time suffer from strong toxicity and narrow therapeutic window [4–7]. In Aconitum, the amount of mesaconitine is higher than that of aconitine [4, 5]. Aconitum poisoning has no specific clinical symptoms because it rapidly decomposes in the body, making it difficult to detect [6, 7]. Metabolites of mesaconitine were detected in rat blood using liquid chromatography with tandem mass spectrometry (LC-MS/MS) [5], e.g., 10-hydroxyl-mesaconitine, hycaponitine, dehydrated mesaconitine 16-O-demethylmesaconitine, 16-O-demethylhycaponitine, and 16-O-demethyl-dehydrated hycaponitine. The metabolites of mesaconitine in rat urine were analyzed using LC-MS/MS [8], such as hypo-mesaconitine glucuronic acid conjugate, 10-hydroxy-mesaconitine, 1-O-demethyl mesaconitine, deoxymesaconitine, and hypo-mesaconitine. The metabolite 10-hydroxyl-mesaconitine is toxic and can be detected in both rat blood and urine; therefore, it was selected for quantification in this study.

LC-MS/MS is widely used in the detection and analysis of drugs [9–11], pharmaceutical impurities [12, 13], degradation products [14, 15], and metabolites [16, 17] because it has the advantages of high sensitivity, low detection limit, strong anti-interference ability, and accurate qualitative and quantitative measurability. Specifically, ultrahigh-performance liquid chromatography (UPLC) and a reliable internal standard can be used to reduce the effect of matrix effect on quantitative results. In addition, UPLC can
achieve rapid and efficient qualitative and quantitative analysis of each component, improve the detection sensitivity and accuracy, and reduce the measurement time.

Several studies have been reported on the pharmacokinetic analysis of mesaconitine in vivo using HPLC [18], LC-MS/MS [19], and UPLC-MS/MS [20]. However, the pharmacokinetic analysis of 10-hydroxy mesaconitine in vivo has not been reported. In this study, UPLC-MS/MS was used to analyze the metabolite 10-hydroxy mesaconitine in rat plasma after oral and intravenous administration. This study is useful for elucidating the pharmacological effects ofaconitine alkaloids.

2. Materials and Methods

2.1. Chemical. 10-hydroxy mesaconitine (purity >98%) and diazepam (purity >98%, internal standard) were purchased from Chengdu Mansite Biotechnology Co., Ltd. (Chengdu, China). HPLC-grade formic acid, methanol, and acetonitrile were purchased from Tedia Company, Inc. (Ohio, USA). Ultrapure water was prepared using a Millipore Milli-Q water purification system (Bedford, MA, USA).

2.2. Instrument and Condition. An ACQUITY H-Class UPLC coupled with a XEVO TQS-micro triple quadrupole mass spectrometer was used in this study for the chromatographic analysis (Waters Corporation, Milford, MA, USA). A UPLC BEH C18 (2.1 mm × 50 mm, 1.7 μm) column was used for chromatographic separation, and the mobile phase consisted of methanol and water (with 0.1% formic acid) with a flow rate of 0.5 mL/min. From 0 to 0.2 min, methanol was isocratic at 10%; from 0.2 min to 1.4 min, methanol was increased from 10% to 80%; from 1.4 min to 2.0 min, methanol was isocratic at 80%; from 2.0 min to 2.1 min, methanol was decreased from 80% to 10%; and from 2.1 min to 3.0 min, methanol was isocratic at 10%.

The capillary voltage was set to 2.4 kV, the desolvation temperature was 400°C, and the ion-source temperature was 150°C. Nitrogen was used as the desolvation (800 L/h) and nebulizing gas. Positive-ion mode ESI with multiple reaction monitoring (MRM) was used to monitor the molecular ion transitions of m/z 648 → 105 for 10-hydroxy mesaconitine and m/z 285 → 193 for the internal standard (IS) (Figure 1).

2.3. Preparation of Stock and Working Solutions. Stock solutions of 10-hydroxy mesaconitine (0.6 mg/mL) and diazepam (0.2 mg/mL) were prepared in methanol. Working stock solutions of 10-hydroxy mesaconitine at different concentrations (3, 10, 50, 150, 300, and 600 ng/mL) were prepared by diluting the stock solutions with methanol.

2.4. Preparation of the Standard Curve. Blank rat plasma was spiked with the working solutions of 10-hydroxy mesaconitine prepared previously to yield standard solutions of 10-hydroxy mesaconitine with concentrations of 0.3, 1, 5, 15, 30, and 60 ng/mL in plasma. Quality control samples (0.8, 12, and 50 ng/mL) were also prepared in the same manner.

2.5. Plasma Preparation. In a 1.5 mL centrifuge tube, acetonitrile (200 μL, containing 50 ng/mL of the IS) was added to plasma (50 μL). The solution was vortexed for 1 min and centrifuged at 13,000 rpm for 10 min. The resulting supernatant (150 μL) was transferred to an LC-MS vial, and 2 μL of this solution was injected into the UPLC-MS/MS for analysis.

2.6. Pharmacokinetics. Sprague Dawley (SD) rats (male, weight: 200–220 g) were obtained from the Animal Experimental Center of Wenzhou Medical University. Six rats were administered 5 mg/kg of 10-hydroxy mesaconitine orally (po), and six rats were administered 0.1 mg/kg of 10-hydroxy mesaconitine intravenously (iv). Food was prohibited for a period of 12 h before the start of the experiment, but water was freely available. Blood (150 μL) was withdrawn from the caudal vein 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h after administration of 10-hydroxy mesaconitine and was anticoagulated by heparin sodium. Plasma (50 μL) was collected after centrifugation at 3,000 rpm for 15 min and stored at −20°C.

3. Results

3.1. Method Validation. The selectivity of the method was evaluated by analyzing six lots of blank rat plasma, blank plasma spiked with 10-hydroxy mesaconitine and IS, and a rat plasma sample. Figure 2 illustrates the UPLC-MS/MS chromatograms of blank plasma samples spiked with 10-hydroxy mesaconitine and IS. No endogenous substances were found to intervene in the detection of 10-hydroxy mesaconitine.

To assess the linearity, calibration curves were obtained by analyzing the spiked calibration samples (0.3–60 ng/mL in rat plasma) and plotting the peak area ratios of 10-hydroxy mesaconitine to IS against their concentrations. The concentrations of the standard 10-hydroxy mesaconitine solutions in rat plasma that were measured in the standard curve covered the range of 0.3–60 ng/mL. The equation generated from the standard curve was $y = 0.0154x + 0.0124$ ($r = 0.9985$), where $y$ is the peak area ratio of 10-hydroxy mesaconitine to IS and $x$ represents the 10-hydroxy mesaconitine concentrations in the rat plasma. The LLOQ of 10-hydroxy mesaconitine in rat plasma was determined to be 0.3 ng/mL.

The accuracy and precision were evaluated by the determination of the LLOQ and QC samples (0.8, 12, and 50 ng/mL) in six replicates over three consecutive days. Intraday and interday precisions corresponding to the measurement of 10-hydroxy mesaconitine in rat plasma were lower than 15%, and the accuracies ranged between 96.0% and 109.3%.

To evaluate the matrix effect, blank rat plasma was extracted and spiked with the 10-hydroxy mesaconitine to obtain final concentrations of 0.8, 12, and 50 ng/mL of the alkaloid. The matrix effect was determined by comparing the corresponding peak areas of the three quality control samples to those of standard solutions prepared in 1:1
methanol and water (0.1% formic acid) at equivalent concentrations. The recovery of 10-hydroxy mesaconitine was evaluated by comparing the peak areas of the extracted QC samples to those of the reference QC solutions. From the analysis, the matrix effect ranged between 88.9% and 98.1%, and the recoveries were higher than 79.1% (Table 1).

The stability of 10-hydroxy mesaconitine in rat plasma was evaluated by analyzing the peak areas of the three QC samples (0.8, 12, and 50 ng/mL) in rat plasma after being exposed to three different storage conditions: room temperature for 2 h; three freeze-thaw cycles, followed by room temperature for 12 h (after processing); and −20°C for 30 days. These results were compared to the results of the freshly prepared standard samples. The variation in the stabilities of 10-hydroxy mesaconitine in rat plasma for the three different conditions was within ±14%, and the RSD of these measurements was within ±14% (Table 2).

3.2. Pharmacokinetics. The plasma concentration-time curves of 10-hydroxy mesaconitine are shown in Figure 3. The main pharmacokinetic parameters determined by the non-compartment model using the DAS 2.0 software (China Pharmaceutical University) are listed in Table 3.

4. Discussion

The choice of operating in either positive or negative mode for ESI is often evaluated in mass spectrometry methodologies [21, 22]. It was determined that ESI positive-ion mode was more suitable for the mass spectrometry analysis of 10-hydroxy mesaconitine compared to negative-ion mode. We optimized the ionization of 10-hydroxy mesaconitine and found that the fragment with an m/z ratio of 105 had the highest abundance. Therefore, the transition of m/z 648 → 105 for 10-hydroxy mesaconitine (cone voltage: 35 V; fragmentor voltage: 26 V) was monitored for quantitative analysis.

The mobile phase and stationary phase (column) play a decisive role in the chromatographic behavior [23–25]. We compared methanol-water (w/0.1% formic acid) and acetonitrile-water (w/0.1% formic acid) using BEH C18 columns. A suitable retention time and analyte peak shapes could be obtained by using methanol-water (w/0.1% formic acid) as the mobile phase. Compared to acetonitrile, methanol was more appropriate as the organic phase for a suitable retention of 10-hydroxy mesaconitine on the column, and it produced better peak shapes and sensitivity. UPLC can obtain excellent results by enabling the efficient separation of analytes with a wide linear velocity, flow rate, and back pressure. UPLC manifests improved chromatographic resolutions compared to HPLC by offering advantages that range from smaller (1.7 μm) particles to an increased column efficiency. In particular, the column efficiency provided by the 1.7 μm particles is three times higher compared to the efficiency of columns containing 5 μm particles. Using methanol-water (w/0.1% formic acid) as the mobile phase, the retention times of 10-hydroxy mesaconitine and the IS were 1.28 and 1.54 min, respectively.

Eliminating potential interferences is especially important for sample preparation [26–28]. The use of methanol-acetonitrile (1:1, v/v), acetonitrile, and methanol for precipitation protein was investigated. Acetonitrile seemed to be the best choice because it afforded better extraction recoveries and a lower background interference. The extraction recoveries with ethyl acetate and ethyl ether were about 60%, while the extraction recoveries with methanol and methanol-acetonitrile (1:1, v/v) were about 80%. However, the recovery with acetonitrile was about 90%.

In this experiment, a proprietary UPLC-MS/MS method was established to determine the concentration of 10-hydroxy mesaconitine in rat plasma. This method was sensitive, accurate, specific, and reliable. The analysis time for each sample was 3.0 min, the processing method of the plasma sample was trivial, the required sample volume was only 50 μL, and more than 300 plasma samples could be processed and tested every day. The developed UPLC-MS/MS method was applied in the pharmacokinetic analysis of 10-hydroxy mesaconitine in rats after oral (5 mg/kg) and intravenous (0.1 mg/kg) administration. The AUC(0–∞) values were 23.6 ± 5.9 and 207.6 ± 72.9 ng/mL·h for intravenous and oral administration, respectively, and the bioavailability was 17.6%. t1/2 was 1.3 ± 0.6 h and 3.1 ± 0.4 h for intravenous and oral administration, respectively.
Figure 2: UPLC-MS/MS chromatograms of 10-hydroxy mesaconitine and IS in rat plasma. (a) Blank rat plasma. (b) Blank rat plasma spiked with 10-hydroxy mesaconitine and IS. (c) Rat plasma after oral administration of 10-hydroxy mesaconitine.

Table 1: Accuracy, precision, matrix effect, and recovery of 10-hydroxy mesaconitine in rat plasma.

| Concentration (ng/mL) | Accuracy (%) | Precision (%RSD) | Matrix effect (%) | Recovery (%) |
|-----------------------|--------------|------------------|------------------|--------------|
|                       | Intraday     | Interday         | Intraday         | Interday     | Intraday | Interday | Intraday | Interday |
| 0.3                   | 107.9        | 109.3            | 11.8             | 15.0         | 88.9     | 7.8       | 93.0     | 6.7       |
| 0.8                   | 103.1        | 103.2            | 9.2              | 8.3          | 94.2     | 6.5       | 79.1     | 5.3       |
| 12                    | 98.1         | 94.3             | 6.8              | 11.3         | 96.5     | 4.5       | 87.7     | 3.2       |
| 50                    | 97.2         | 102.1            | 4.5              | 6.1          | 98.1     | 5.6       | 92.2     | 3.4       |

Table 2: Summary of the stability of 10-hydroxy mesaconitine under various storage conditions (n = 3).

| Concentration (ng/mL) | Autosampler (4°C, 12 h) | Ambient (2 h) | −20°C (30 d) | Freeze-thaw |
|-----------------------|--------------------------|---------------|-------------|------------|
|                       | Accuracy (%) | RSD (%) | Accuracy (%) | RSD (%) | Accuracy (%) | RSD (%) | Accuracy (%) | RSD (%) |
| 0.8                   | 97.2         | 1.8     | 102.9        | 8.0      | 112.7       | 9.0     | 89.9        | 12.4    |
| 12                    | 97.5         | 6.7     | 109.1        | 6.0      | 95.6        | 9.7     | 101.4       | 8.9     |
| 50                    | 103.0        | 0.5     | 94.5         | 2.1      | 97.0        | 10.4    | 86.7        | 10.6    |
5. Conclusion

*Aconitum* alkaloids get metabolized rapidly *in vivo*, which makes them difficult to detect. In this study, a rapid and selective UPLC-MS/MS method for the measurement of 10-hydroxy mesaconitine in rat plasma was developed. This method was applied in the pharmacokinetic analysis of 10-hydroxy mesaconitine in rats. The bioavailability was 17.6%.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors’ Contributions

Jinzhao Yang and Guowu Zeng contributed equally to this study.

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