Journal of Pharmaceutical and Biomedical Analysis 134 (2017) 137–142

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Short communication

Comparative tissue distribution and excretion study of alkaloids from Herba *Ephedrae-Radix Aconiti Lateralis* extracts in rats

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ARTICLE INFO

Article history:
Received 15 July 2016
Received in revised form 9 November 2016
Accepted 11 November 2016
Available online 13 November 2016

Keywords:
Mahuang-Fuzi
Alkaloids
UPLC-MS-MS
Distribution
Excretion

ABSTRACT

Herba *Ephedrae-Radix Aconiti Lateralis*, composed of *Ephedrae* (*Mahuang* in Chinese) and *Radix Aconiti Lateralis* (*Fuzi* in Chinese), is a classical herbal combination proven to be effective in treating common cold, asthma, and rheumatoid arthritis. Alkaloids, bioactive components of the herbal extract, have been associated with many side effects. Nine alkaloids, including norephedrine, norpseudoephedrine, ephedrine, pseudoephedrine, methylephedrine, hypaconitine, benzoylaconine, benzoylmesaconine and benzoylhypaconine, were simultaneously quantified within 14.5 min, by a validated ultra-performance liquid chromatography-tandem mass spectrometry method in various rat tissues, urine, and feces after oral administration of *Mahuang-Fuzi* and single-herb extracts. The results indicated that the alkaloids were widely distributed in the heart, liver, spleen, lung, kidney, and brain. Lower bioavailability and higher clearance of some alkaloids were observed for the herbal combination, but hypaconitine showed a longer residence time and lower clearance. Elimination kinetics demonstrated that ephedra and aconitum alkaloids were mainly excreted in urine and feces, respectively. The tissue distribution and excretion of ephedra and aconitum alkaloids are comprehensively reported for the first time for the *Mahuang-Fuzi* combination. Compared with single-herb extracts, lower extraction efficiencies of alkaloids in vitro were observed which may result in their lower intake. However, the combination showed a prolonged residence time and delayed elimination of aconitum alkaloids, which increases the risk of drug accumulation. The study demonstrated potential risks of intoxication with aconitum alkaloids, associated with the use of *Fuzi* in combination with *Mahuang*. *Mahuang-Fuzi* is a classical combination used in clinics, further investigation is needed.

1. Introduction

Herba *Ephedrae-Radix Aconiti Lateralis* is a classical herbal combination derived from some traditional formulas, such as Mahuangfuzixixin Tang and Wutou Tang, used for the treatment of common cold, asthma, and rheumatoid arthritis [1,2].

Ephedrae-Radix Aconiti Lateralis is composed of *Ephedrae* (*Mahuang* in Chinese), dried herbaceous stems of *Ephedra sinica* Stapf., and *Radix Aconiti Lateralis* (*Fuzi* in Chinese), dried lateral roots of *Aconitum carmichaelii* Debx. *Ephedrae* is commonly used to treat common cold and asthma [3], and previous studies have shown that the active ingredients of *Mahuang* are mainly ephedra alkaloids [4,5]. *Radix Aconiti Lateralis* is used in the treatment of asthma and rheumatoid arthritis with cold symptoms [6,7]. Similarly, aconitum alkaloids are the main effective components, including diester alkaloids [aconitine (AC), hypaconitine (HA), and mesaconitine (MA)] and monoester alkaloids [benzoylaconine (BAC), benzoylhypaconine (BHA), and benzoylmesaconine (BMA)]. All these alkaloids appear to be the toxic as well as the effective compounds. The *Mahuang-Fuzi* combination has been widely used for thousands of years in China, but it is noteworthy that *Mahuang* and *Fuzi* have both been reported to cause adverse effects on the cardiovascular system [8–10].

It has been reported that aconitum alkaloids may contribute to toxicity through their analgesic activity [11]. Drug combination

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http://dx.doi.org/10.1016/j.jpba.2016.11.027
0731-7085/

analysis shows acute toxicity and an analgesic effect with overlaid dose distributions (dose <62.0 g/kg) for combined application of *Mahuang* and *Fuzi* (unpublished observation). Furthermore, our previous pharmacokinetics data also showed that, compared with the single-herb extracts, slower elimination of alkaloids, except ME, BAC, and BHA, was observed for the *Mahuang-Fuzi* combination, which increases the risk of drug accumulation [12].

Compared with plasma concentration, variations in drug distribution and execration altering the concentrations of drugs in target tissues and its elimination in vivo, have a closer correlation with its efficacy and toxicity [13]. In this study, a simple ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method was validated for the separation and quantification of 11 alkaloids in three biological matrices, including tissue homogenates, urine, and feces. In addition, tissue distribution and excretion kinetics of alkaloids were compared in rats after oral administration of *Mahuang*, *Fuzi*, and *Mahuang-Fuzi* aqueous extracts.

2. Experimental

2.1. Chemicals and reagents

Ephedrine (E) hydrochloride, methylephedrine (ME) hydrochloride, pseudoephedrine (PE) hydrochloride, benzoylaconine (BAC), benzoylhypaconine (BHA), benzoylmesaconine (BMA), aconitine (AC), hypaconitine (HA), mesaconitine (MA), diphenhydramine hydrochloride (used as internal standard-1, IS-1), and lappaconite hydrobromide (IS-2) were obtained from the National Institutes for Food and Drug Control (Beijing, China). Norephedrine (NE) hydrochloride and norpseudoephedrine (PNE) hydrochloride (purity ≥98%) were supplied by Chifeng Mengxin Pharmaceutical Co., Ltd. (Chifeng, China). Methanol, acetonitrile (ACN; HPLC grade, Merck, Darmstadt, Germany), and formic acid (HPLC grade, Kermel Chemical Reagent Co., Ltd.) were used for high-performance liquid chromatography (HPLC) analysis. Ultrapure-grade water was generated using a Milli-Q ultra-pure water system (Millipore, Bedford, MA, USA).

The herb medicines Herba *Ephedrae* (batch number: 20110901) and *Radix Aconiti Lateralis* (batch number: 20130801) purchased from Guangzhou Zhixin Chinese Herbal Medicine Co., Ltd. (Guangzhou, China) were authenticated by Professor Ji Ma (Department of Chinese Medicine Authentication, School of Traditional Chinese Medicine, Southern Medical University, Guangzhou, China).

2.2. Laboratory animals

Adult male Wistar rats (weighing 200–240 g) were supplied by the Experimental Animal Center of Southern Medical University (license No. 44002100002118), Guangzhou, China. The animals were housed at a temperature of 18–24 °C, with a relative humidity of 45–75% and a 12-h light/dark cycle, and allowed to acclimate for seven days before the experiment. All animals were fasted for 12 h before the assays and euthanized by cervical dislocation at the end of the experiments.

This study was approved by the Animal Ethics Committee of The First Affiliated Hospital of Southern Medical University, Guangzhou, China (approval No. NFYY-2011-08).

2.3. Preparation of herb extracts and standard solutions

Mahuang (120 g) was immersed in 8000 mL of distilled water for 30 min and then boiled for 30 min *Fuzi* (220 g) was subsequently added, and the mixture was simmered for 70 min. Single-herb extracts were prepared in the same way. The extraction method was described in the Treatise on Febrile Diseases, an ancient Chinese medical book. Water extracts collected by filtration were concentrated under reduced pressure to 0.6, 1.1, and 1.7 g/mL of the *Mahuang*, *Fuzi*, and *Mahuang-Fuzi* extract, respectively.

Stock solutions were prepared by accurately weighing and dissolving the 13 standard and reference compounds either in methanol (for the ephedrines, IS-1, and IS-2) or in ACN containing 0.1% hydrochloric acid (for the *Aconitum* alkaloids). A mixed stock solution was prepared by mixing the five stock solutions of the ephedrine compounds to yield the following final concentrations (mg/mL): NE, 20.2; NPE, 20.1; E, 202.4; PE, 101.2; and ME, 41.4. Mixed *Aconitum* alkaloids and internal standards were prepared by similar methods to yield the following final concentrations: AC, 40.4 mg/mL; MA, 40.2 mg/mL; HA, 40.6 mg/mL; BAC, 80.3 mg/mL; BMA, 80.0 mg/mL; BHA, 79.9 mg/mL; IS-1, 400.0 ng/mL; and IS-2, 100.0 ng/mL. Serial dilutions were prepared for quality control samples and calibration curves.

2.4. Quantification of alkaloids in Mahuang, Fuzi, and Mahuang-*Fuzi* extracts

The contents of the ephedra alkaloids (NE, NPE, E, PE, and ME) and aconitum alkaloids (AC, MA, HA, BAC, BMA, and BHA) in *Mahuang-Fuzi* and single-herb extracts were determined using validated HPLC methods established in our previous study [14] and those documented in the Chinese Pharmacopeia [15], respectively.

2.5. Instrumentation and analytical conditions

Separation and quantification of the analytes in tissue, urinary, and fecal samples were achieved under the same UPLC-MS/MS conditions as described in our previous work [15]. Briefly, an Agilent 1290 UPLC system (Agilent Technologies, Wilmington, DE, USA), coupled with a tandem 6410 B triple quadrupole mass spectrometer (Agilent Technologies), was used to separate and quantify the analytes in tissue, urinary, and fecal samples in 14.5 min.

Separation of the analytes was achieved on a ZorbaxSB-Aq column (100 mm × 2.1 mm, 3.5 μm; Agilent Technologies), with a mobile phase that consisted of ACN(A) and a 0.1% aqueous solution of formic acid (B), using a gradient program: 0%A at 0–2.0 min; 0%A–5%A at 2.1–5.0 min; 5%A–35%A at 8.0–9.5 min; 35%A at 9.5–12.5 min; 35%A–0%A at 12.5–13.0 min. The flow rate was 0.3 mL/min, the injection volume was 5 mL, and the column temperature was set at 25 °C. The analytes were detected using multiple reaction monitoring (MRM) with an electrospray source in a positive mode. An Agilent Mass Hunter workstation was used for data acquisition and analysis. The source parameters were set as follows: capillary voltage, 4000 V; MS heater temperature, 100 °C; drying gas flow, 10 L/min; drying gas temperature, 350 °C; nebulizer pressure, 40 psi.

2.6. Method validation

Validation assays for the previously developed UPLC-MS/MS method were conducted according to the guidelines for industry bioanalytical method validation [16], including specificity, linearity, lower limit of quantification (LLOQ), recovery, matrix effect, precision, accuracy, and stability.

2.7. Tissue distribution study

Three groups (n = 20 per group) of male Wistar rats were treated intragastrically with a single dose of 10 mL/kg of the *Mahuang*, *Fuzi*, and *Mahaung-Fuzi* extracts and subsequently sacrificed at 0.5, 1, 2, and 4 h (n = 5 each) after the administration. The heart, liver, spleen, lung, kidney, and brain tissues were timely removed, then rinsed