Journal of Ethnopharmacology 155 (2014) 642–648

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Research Paper

Pharmacokinetic comparisons of five ephedrine alkaloids following oral administration of four different Mahuang–Guizhi herb-pair aqueous extracts ratios in rats

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ARTICLE INFO

Article history:
Received 21 January 2014
Received in revised form
23 May 2014
Accepted 31 May 2014
Available online 12 June 2014

Keywords:
Pharmacokinetic
Ephedrine alkaloids
Prescription compatibility
Mahuang–Guizhi herb-pair
UPLC–MS/MS

Chemical compounds studied in this article:
Ephedrine hydrochloride (PubChem CID:65326)
Pseudoephedrine hydrochloride (PubChem CID:9581)
Methylephedrine hydrochloride (PubChem CID:198190)
Norephedrine hydrochloride (PubChem CID:62943)
Norpseudoephedrine hydrochloride (PubChem CID:92920)
Amantadine hydrochloride (PubChem CID:64150)
Acetonitrile (PubChem CID:6342)
Formic acid (PubChem CID:284)
Diethyl ether (PubChem CID:3283)
Methylene chloride (PubChem CID:6344)
Sodium carbonate (PubChem CID:10340)

ABSTRACT

Ethnopharmacological relevance: *Herba Ephedra* (Mahuang in Chinese), is derived from dried *Ephedra sinica* Stapf stems and has been widely used to treat the common cold, coughs, asthma, and edema for thousands of years. The Mahuang–Guizhi herb-pair is a famous formula composed of Mahuang and *Ramulus Cinnamomi* (Guizhi in Chinese, the dried twig of *Cinnamomum cassia* Presl.), used to improve pharmacological effects and reduce toxicity. In order to investigate the influence of Mahuang–Guizhi herb-pair ratios on bioavailability, the plasma pharmacokinetics profiles of five ephedrine alkaloids were compared following oral administration of four different ratios to rats.

Materials and methods: Sprague-Dawley rats were randomly assigned to four groups and orally administered Mahuang–Guizhi (ratios 3:0; 3:1; 3:2; 3:4, w/w). Assays for five ephedrine alkaloids (ephedrine, pseudoephedrine, methylephedrine, norephedrine, and norpseudoephedrine) were developed and validated using ultra-high-performance liquid chromatography tandem mass spectrometry coupled with liquid-liquid extraction.

Results: Key pharmacokinetic parameters of the five ephedrine alkaloids (maximal plasma concentration, mean residence time, and half-life) were significantly different ($p < 0.05$) after oral administration of Mahuang–Guizhi herb-pair ratios, as compared to those of Mahuang.

Conclusion: Ephedrine alkaloid pharmacokinetic differences in rat plasma could help explain previous findings of pharmacological and toxicity differences between Mahuang and Mahuang–Guizhi herb-pair preparations. These results could facilitate future studies to increase the efficacy and decrease the toxicity of Mahuang and Guizhi.

1. Introduction

Herb pairs, as the basic composition units of Chinese herbal formulations, have special clinical significance in traditional Chinese medicine (TCM) (Ung et al., 2007; Deng et al., 2008), and are much simpler than other complex preparations, because their basic therapeutic features are not altered (Sun, 2006; Zhou et al., 2006). The Mahuang–Guizhi herb-pair (MHGZHP) is the main herb-pair present at different ratios in many TCM prescriptions, such as Mahuang-Tang and Xiaoqinglong-Tang. MHGZHP is a combination of *Herba Ephedra* (Mahuang [MH] in Chinese, the dried herbaceous stems of *Ephedra sinica* Stapf.) and *Ramulus Cinnamomi* (Guizhi [GZ] in Chinese, the dried twig of *Cinnamomum cassia* Presl.). In TCM, MHGZHP is used to induce diaphoresis, to dispel wind (as a pathogenic factor) and disperse cold (the

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http://dx.doi.org/10.1016/j.jep.2014.05.065
0378-8741/

pathogenic cold factor) (Chuai, 2009; Wang and Zhu, 2011), and it has been used clinically to treat the common cold, rheumatic diseases, cough and asthma (Wang and Zhu, 2011; Zhang and Liu, 2012).

Although MH is commonly used in TCM, it has been reported to induce neurotoxicity and cardio-toxicity (Haller et al., 2005; Sun et al., 2006; Chen et al., 2010). Combination with GZ, was reported to enhance the pharmacological effects of MH (Shen et al., 1986; Huang et al., 2004; Xu et al., 2013) and reduce toxicity, with acute toxicity experiments showing that the LD_{50} of different MHGZHP ratios were 1.5 to 2.2 fold that of MH (Xu, 2012). Ephedrine alkaloids are the main bioactive ingredients of MH (Kimura and Hikino, 1957; Krizevski et al., 2010), and are also used to treat bronchial asthma and common cold in modern Western medicine (Lee et al., 2000; Tseng et al., 2003). It has been reported that the ephedrine alkaloids present in MH were also responsible for its toxic effects (Wang, 1985; Madhusudan et al., 2004).

However, few comparative pharmacokinetics studies of ephedrine alkaloids after oral administration of different ratios of MHGZHP have been reported. The present study therefore investigated the pharmacokinetic profiles of ephedrine, pseudoephedrine, methylephedrine, norephedrine, and norpseudoephedrine in rat plasma after oral administration of four different MHGZHP extract ratios. To achieve this, ultra performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS) assays were developed and validated for simultaneous determination of these five ephedrine alkaloids in rat plasma. The overall aim of this study was to reveal pharmacokinetic variation following oral administration of different MHGZHP ratios, which would improve understanding of potential in vivo herb–herb interactions and inform rational administration in the clinic.

2. Material and methods

2.1. Material

The standards (purity > 99.8%) of ephedrine hydrochloride (E), pseudoephedrine hydrochloride (PE), methylephedrine hydrochloride (ME), and amantadine hydrochloride (internal standard, IS) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Norephedrine hydrochloride (NME) was purchased from Sigma-Aldrich (Schnelldorf, Germany). Norpseudoephedrine hydrochloride (NMP) was obtained from Lipomed AG (Arlesheim, Switzerland). Acetonitrile (Merck, Darmstadt, Germany) and formic acid (Tedia, Fairfield, USA) were of high-performance liquid chromatography (HPLC) grade. Water was prepared using a Milli-Q plotwater purification system (Millipore, Bedford, MA, USA). Other chemicals were of analytical grade. MH (*Ephedra Sinica* Stapf) and GZ (*Cinnamomum cassia* Presl.) were supplied by Guangzhou Zhixin Pharmaceutical Co., Ltd. (Guangzhou, China) and authenticated by Professor Ji Ma (Southern Medical University, Guangzhou, China).

2.2. Preparation of herbal aqueous extracts

MH (90 g) was soaked with 2.4 L of water for 30 min, and boiled for 20 min, GZ (30 g) was then added and the mixture was boiled for 30 min. An aqueous solution was obtained by filtration and concentrated under reduced pressure at 55 °C to contain the equivalent of approximately 1.0 g of MH raw material per mL. This preparation was the $MHGZHP_1$ aqueous extract. The same procedure was followed for the preparation of the other MHGZHP extracts: using the starting materials of 90 g MH and 0 g GZ to produce MH aqueous extract; 90 g MH and 60 g GZ to produce $MHGZHP_2$ aqueous extract; and 90 g MH and 120 g GZ to produce $MHGZHP_3$ aqueous extract. To calculate the dosage employed, the levels of E, PE, ME, NME and NMP in the aqueous extracts were quantitatively determined by UPLC–MS/MS as described in Section 2.4.

2.3. Animal treatment

Sprague-Dawley rats ($n=24$) weighing 230–250 g were obtained from Southern Medical University Experimental Animal Center (Guangzhu, China). All animals were housed under controlled conditions (25 ± 2 °C, relative humidity 50 ± 5%) with a natural light-dark cycle for at least one week before the experiments. The rats were given a commercial rat chow and water ad libitum. They were fasted for 12 h with free access to water before administration. The studies were carried out according to the Guide for Care National Institutes of Health Ethical approvals for the animal studies obtained from the ethical committees of Southern Medical University.

The rats were divided into four groups at random (six rats per group), i.e., MH group, $MHGZHP_1$ group, $MHGZHP_2$ group, and $MHGZHP_3$ group. The same dose of MH (the equivalent of 8.1 g crude MH/kg) was given to each rat in these four groups. Blood samples were collected from the orbital plexus of the eyes at 0.08, 0.25, 0.5, 0.75, 1.5, 3, 4, 6, 8, 12, 24, and 48 h post administration. The samples were immediately transferred to heparinized tubes and centrifuged at 4000 rpm for 10 min, the plasma supernatant was transferred to clean tubes and stored at −20 °C until analysis.

2.4. Blood sample preparation

The thawed plasma sample (100 μL) was transferred to another EP tube, before adding 30 μL amantadine hydrochloride (IS, 452.8 ng/mL) and vortex-mixing for 30 s. The mixture was alkalinized with 40 μL saturated sodium carbonate, and then extracted with 1.2 mL diethyl ether-methylene chloride (6:4, v/v). After vortex-mixing for 2 min, the resulting mixture was centrifuged at 14000 rpm for 10 min. The supernatant was transferred into another EP tube and evaporated to dryness under a stream of nitrogen. The residue was re-dissolved in 100 μL mobile phase (acetonitrile: 0.1% formic acid, 3:97, v/v). An aliquot of 1 μL was injected into the UPLC–MS/MS system for analysis.

2.5. Instrumentation and analytical conditions

Analyses were performed on an Agilent 1290 UPLC system and a 6410 triple quadrupole mass spectrometer equipped with an electrospray ionization source (Agilent Technologies, Inc., USA). Separation of the analytes was performed on an Agilent Zorbax SB-C_{18} column (3.5 μm and 2.1 × 100 mm). The mobile phase consisted of acetonitrile (A) and 0.1% formic acid (B) with a gradient elution program: 97% (v/v) B at 0–3.0 min; 97–96% B at 3.0–5.0 min; 96% B at 5.0–7.0 min; 96–82% B at 7.0–7.5 min; 82% B at 7.5–9.0 min; 82–97% B at 9.0–9.1 min, flowing at 0.4 mL/min. The column temperature was maintained at 35 °C and the sample injection volume was 1 μL.

The analytes were determined by MS–MS in positive ionization mode by monitoring the precursor-to-product combination using multiple-reaction monitoring (MRM). High purity nitrogen was used in all cases. The MS drying gas temperature was 350 °C, with a capillary voltage of 4000 V, a drying gas flow of 8.0 L/min and a nebulizer pressure of 30 psi. The precursor-to-product ion-pairs, fragmentor, collision energy, and chemical structures for each analyte and IS are shown in Fig. 1. All data acquisition and peak integration were performed using Qualitative Analysis B.04.00.