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Research Paper

Pharmacokinetics of aconitine-type alkaloids after oral administration of Fuzi (*Aconiti Lateralis Radix Praeparata*) in rats with chronic heart failure by microdialysis and ultra-high performance liquid chromatography–tandem mass spectrometry

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ABSTRACT

Ethnopharmacological relevance: Fuzi [the lateral root of *Aconitum carmichaeli* Debx (Ranunculaceae)] is a well-known traditional medicinal herb used to treat chronic heart failure (CHF). Aconitine-type alkaloids are major alkaloids that are responsible for the pharmacological activity and toxicity of this herb. To investigate therapeutic effects and pharmacokinetic profiles of aconitine-type alkaloids in CHF rats.
Materials and methods: The plasma pharmacokinetic profiles of aconitine, mesaconitine, and hypaconitine were investigated after once treatment of Fuzi extract (containing aconitine 0.086 mg/g, mesaconitine 0.84 mg/g, and hypaconitine 1.97 mg/g) using a rapid and sensitive combinative method of ultra-high performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) and microdialysis (MD). The cardiac function and antioxidant enzyme activities were also evaluated.
Results: Recoveries of MD sampling ranged from 35.06% to 45.74% with RSD below 6.05%. Fuzi extract improved the myocardial function and antioxidant enzymatic activities of rats with CHF. Aconitine, mesaconitine, and hypaconitine exhibited slower absorption into the bloodstream, and yielded 11-fold less values of area under concentration–time curve (AUC) in the CHF rats than those in normal rats. The plasma AUC showed that the maximum blood concentration (C_{max}) was 5.561 ng/mL for aconitine, 17.30 ng/mL for mesaconitine, and 17.78 ng/mL for hypaconitine in normal rats, while these were 0.6059 ng/mL, 2.430, and 0.7461 ng/mL in CHF rats, respectively.
Conclusion: Aconitine-type alkaloids associated with Fuzi's efficacy have lower intake and slower elimination in the CHF rats, indicating a non-interdependent relationship between its efficacy and toxicity. It may contribute to the depth understanding of the toxicological and pharmacological profiles of Fuzi and further benefit the herbal drug development with safety and efficacy for CHF treatment.

1. Introduction

Chronic heart failure (CHF) is a common, costly, disabling, and potentially deadly condition (Irving et al., 2013; Dilokthornsakul et al., 2012). Approximately 1–2% of adult populations in developed countries suffer from CHF, in which prevalence has increased to ≥10% among persons aged ≥70 years (Juillière et al., 2013). The pathogenesis of CHF is complicated and influenced by several factors. Although cardiac rehabilitation exercise training and CHF self-care counseling have been shown to improve clinical status and clinical outcomes in CHF (Goodman et al., 2013), a very limited and relatively expensive preventive technique is required to solve such a pandemic, if this pandemic occurs (MacIver et al., 2013).

Fuzi (also known as aconite or monkshood) is the fire-processed product of the lateral root of *Aconitum carmichaelii* Debx. (Ranunculaceae); Fuzi has been used widely in Asia as an essential herbal drug for 2000 years. In traditional folk medicine, the preparations of Fuzi and various Fuzi-containing herbal formulations, which are usually combined with other commonly used herbal medicines, such as *Radix ginseng*, *Rhizoma zingiberis*, and *Radix glycyrrhizae*, have been frequently prescribed by Chinese physicians to promote blood circulation; these formulations have also been recommended to strengthen the immune system and treat heart failure, neuralgia, rheumatism, gout, and several endocrinal disorders, such as irregular menstruation (Singhuber et al., 2009). Studies have further shown that Fuzi exhibits various

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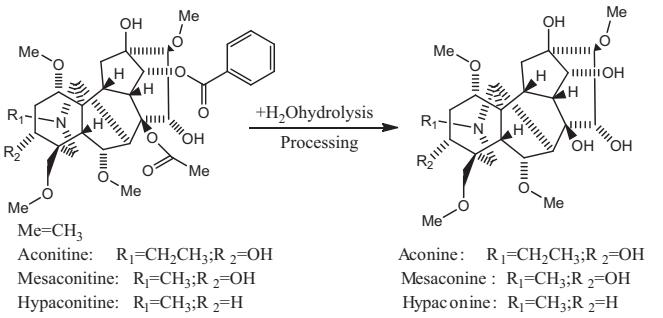

Fig. 1. Chemical structures of major aconitine-type alkaloids and the reaction from toxic aconitines to nontoxic aconines.

pharmacological properties, such as analgesic, antitumor, anti-inflammatory, anti-asthmatic, and anti-epileptic (Liou et al., 2005; Voss et al., 2008; Li et al., 2010; Zhao et al., 2012; Du et al., 2013; Wang et al., 2013). Evidence has also revealed that Fuzi and its active components elicit cardiac electrophysiological effects by blocking sodium channels (Luo et al., 2008) and improved inotropic effect and left ventricular diastolic function on myocardial ischemia-reperfusion injury in rats (Liu et al., 2012). Cardioprotective effect of Fuzi may be partially related to the scavenging of hydroxyl radicals or inhibition of lipid peroxidation (Zheng et al., 2004; Zhao et al., 2012). In a clinical investigation, aconite tuber possibly increases NO production in humans, thereby improving peripheral blood circulation and preventing myocardial infarct (Yamada et al., 2005).

Aconitine-type alkaloids (aconitine, mesaconitine, and hypaconitine), sharing a common C19-norditerpenoid skeleton as shown in Fig. 1, are the main bioactive components in Fuzi. However, they are also toxic substances found in this herbal medicine because of their hyperpolarization and activation effect on the voltage-dependent sodium channel function in cells (Chen et al., 2008; Friese et al., 1997). The improper use of aconitum in Asian countries causes a high risk of severe intoxications (Gao et al., 2012; Qin et al., 2012; Tong et al., 2013). The traditional preparation of soaking, saturating, and boiling has been designed in Fuzi processing, in which aconitine-type alkaloids can be hydrolyzed to form non-toxic derivatives (non-ester diterpenoid alkaloids) by losing their acetyl and benzoyl ester groups at C8 and C14, respectively (Fig. 1); as a result, toxicity is reduced (Singhuber et al., 2009; Chen et al., 2008). Considering that toxic alkaloids remain present at hazardous levels, researchers should investigate the pharmacokinetics of aconitine-type alkaloids *in vivo via* oral Fuzi administration to provide a safe and effective clinical application of this herbal medicine in CHF treatment. Therefore, the present study aimed to investigate pharmacokinetic profiles of aconitine-type alkaloids (aconitine, mesaconitine, and hypaconitine) in normal rats and CHF rats by applying ultra-high performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) combined with MD after Fuzi extract (FZE) was orally administered. Pharmacokinetic data could provide additional information on pharmacokinetics and potential cardioprotective effects of FZE *in vivo*.

2. Materials and methods

2.1. Chemicals

The crude herb Fuzi was obtained from Chinese Medicine Herbal Factory (Zhejiang, China), and identified by Prof. Shui-Li Zhang, college of Pharmacy, Zhejiang Chinese Medical University. A voucher specimen (No. 110824) has been deposited at the college of Pharmacy, Zhejiang Chinese Medical University, Zhejiang, China. Fuzi (5 kg) was soaked in distilled water for 30 min and then boiled in 50 L of water (v/w) for 1 h and extracted twice in the same way. The suspension was then centrifuged (2000*g*, 20 min) and the supernatant was decompressed and concentrated to a final density of 1.08 g/ml determined by a hydrometer (Hangzhou Hongda Instrument CO., LTD., China). By HPLC analysis (Csupor et al., 2009), the contents of aconitine, mesaconitine, and hypaconitine in FZE were detected to be 0.086, 0.84 and 1.97 mg/g, respectively.

Aconitine-type alkaloids (aconitine, mesaconitine, and hypaconitine) were all purchased from Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Deionized water was purified on a Milli-Q system (Millipore, Bedford, USA). Methanol, acetonitrile and formic acid (HPLC grade) was purchased from Tjshield Fine Chemicals Co. Ltd., China. Other reagents were of analytical grade and obtained from Sinopharm Chemical Reagent Co. Ltd., Shanghai, China.

2.2. Apparatus and chromatographic conditions

2.2.1. Ultra-performance liquid chromatography (UPLC)

Chromatography was performed on an ACQUITY™ UPLC system (Waters Corp., Milford, MA, USA). Separation was carried out in an ACQUITY™ BEH C_{18} column (100 mm × 2.1 mm i.d., 1.7 μm) from Waters Corp., USA. The column temperature was maintained at 25 °C. Analysis was achieved with gradient elution using (mobile phase A) acetonitrile and (mobile phase B) water (containing 0.1% formic acid). The gradient elution program started with 30% (v/v) of phase A for 1 min, then increased linearly to 70% (v/v) of phase A for 3 min and finally washed with 30% (v/v) of phase A linearly for 4 min. Subsequently, the column was washed with phase A for 2 min and then equilibrated with the initial 30% phase A for 10 min. The total analysis time including the washing and equilibrium steps was 20 min. The flow rate was maintained at 0.2 mL/min. The injection volumes of the samples or standard solutions were 10 μL.

2.2.2. Mass spectrometer

Waters ACQUITY™ TQD triple-quadrupole tandem mass spectrometer (Waters Corp., Manchester, UK) was connected to the UPLC system *via* an electrospray ionization source (ESI) interface. The ESI source was operated in a positive ionization mode at a capillary voltage of 3.2 kV. The cone voltage was 20 V for aconitine-type alkaloids. Nitrogen was used as the desolvation gas (600 L/h) and cone gas (50 L/h). The temperatures of the source and desolvation were set at 150 and 400 °C, respectively. Argon was used as the collision gas, and the collision energy was 14 eV. Testosterone was used as an internal standard (IS). Quantification was performed by multiple-reaction monitoring (MRM) using electrospray ionization (ESI) operated in a positive ion mode. The following transitions were obtained: m/z 646.3 → m/z 586.3 for aconitine; m/z 632.3 → m/z 572.3 for mesaconitine; m/z 616.3 → m/z 556.3 for hypaconitine; and m/z 289.4 → m/z 108.9 for IS. All of the data were collected in a centroid mode by using Masslynx™ NT4.1 software (Waters Corp., Milford, MA, USA). Post-acquisition quantitative analyses were performed using a Quanlynx™ program (Waters Corp., Milford, MA, USA).

2.3. Animals and preparation of CHF model

Male special pathogen free Sprague–Dawley rats weighing 180–220 g were purchased from Animal Experimental Center, Zhejiang Academy of Medical Sciences, China. Animals were housed in groups of five per standard cage, on 12 h light/dark