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Pharmacokinetics of aconitine as the targeted marker of *Fuzi* (*Aconitum carmichaeli*) following single and multiple oral administrations of *Fuzi* extracts in rat by UPLC/MS/MS

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ABSTRACT

Ethnopharmacological relevance: Fuzi, which is the processed lateral roots of *Aconitum Carmichaeli*. Debx and is widely distributed over the southwest provinces of China, is recognised for its anti-inflammatory and analgesic effects.

Aim of the study: The pharmacokinetic properties of *Fuzi* are inadequately understood. Aconitine, the primary highly toxic ingredient of *Fuzi*, is well known as the target marker of *Fuzi*. The purpose of the present study is to investigate the pharmacokinetic behaviours of aconitine *in vivo* following single and multiple administrations of processed *Fuzi* extracts and to compare the pharmacokinetic characteristics of aconitine after administrations of pure aconitine or *Fuzi* extracts as well as compare the difference at single dose and multiple doses. The *in vitro* aconitine protein binding in plasma through equilibrium dialysis was also examined.

Methods: A high performance liquid chromatography (HPLC) method was developed for the determination of aconitine in *Fuzi* crude extracts and a fast ultra performance liquid chromatography-tandem mass spectrometry (UPLC/MS/MS) was developed to investigate the pharmacokinetic behaviour of aconitine as the targeted marker of *Fuzi*.

Results: The absolute bioavailability (F%) after the administration of 0.5 mg/kg aconitine and *Fuzi* extract (0.118 mg/kg aconitine) in rat was 8.24 ± 2.52% and 4.72 ± 2.66%, respectively. Aconitine absorption was very fast at the t_{max} 30.08 ± 9.73 min for pure aconitine and 58.00 ± 21.68 min for *Fuzi* extract administration. Aconitine was also eliminated rapidly with a short half-life (i.v., 80.98 ± 6.40 min) and a low rate of protein bounding (23.9–31.9%). No significance was observed on all the pharmacokinetics parameters following the single and multiple doses of pure aconitine (ANOVA, $p > 0.05$). However, the absorption of aconitine after multiple administrations of *Fuzi* extract was much faster than that of a single dose (t_{max}: 58.00 ± 21.68 vs. 20.00 ± 8.66 min, $p < 0.05$), and the area under the plasma concentration–time curve (AUC) was higher than that of a single dose.

Conclusions: The pharmacokinetic behaviour of processed *Fuzi* was determined in this paper. The aconitine has low bioavailability. No variation in the pharmacokinetic behaviours of pure aconitine was observed after single and multiple administrations. In contrast, multiple administrations of processed *Fuzi* extract could result in variations in its pharmacokinetic behaviour in AUC and t_{max} indicating that multiple dose might increase the bioavailability of aconitine, which may result in its toxicity. In addition, aconitine has a low protein bounding (23.9–31.9%), resulting in its rapid elimination.

1. Introduction

Fuzi, which is the processed lateral roots of *Aconitum carmichaeli*. Debx or other near-relative species of the same genus and is widely distributed over the southwest provinces of China, has been used as a famous Chinese medicinal herb for the treatment of colds, polyarthralgia, diarrhea, heart failure, beriberi, and edema for thousands of years (Murayama et al., 1991). Raw *Fuzi* are used only after processing due to their toxicity, which results in side effects and adverse clinical reactions such as severe arrhythmia (Zhou et al.,

Abbreviations: ACN, acetonitrile; ANOVA, analysis of variance; AUC, area under curve; CL, clearance; HPLC, high performance liquid chromatography; IS, internal standard; MRT, mean residence time; MRM, multiple reaction monitoring; QC, quality control; SE, standard error; UPLC/MS/MS, ultra performance liquid chromatography–tandem mass spectrometry.

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1984; Chan et al., 1994). However, poisoning may still occur even after the consumption of processed *Fuzi* (Tai et al., 1992) for the herb contains high toxic alkaloids. Thus, the determination of the safe application of processed *Fuzi* is urgent and essential. The first stage requires an in-depth understanding of its pharmacokinetic properties.

The available information on the characterisation of the pharmacokinetics of *Fuzi* is poorly understood. Researchers have recently reported that aconitine alkaloids, the main ingredients of *Fuzi*, are known for their high toxicity and pharmacological activity as well as being the target markers of the crude methanol extracts of *Fuzi* (Chen et al., 2008). Aconitine is the foremost highly toxic diester-diterpene in *Aconitum* alkaloids (Chen and Liang, 1982; Hikino et al., 1983; Chen et al., 2008). This alkaloid has a narrow therapeutic index. Our recent study evaluated the analgesic effect of aconitine on mice by twisting reaction induced by 0.7% acetic acid; a 95% effective dose (ED_{95}) of 0.2508 mg/kg was obtained. However, the 5% lethal dose (LD_5) and half-lethal dose (LD_{50}) of aconitine were only 0.2602 mg/kg and 2.01 mg/ml in mice, respectively. The results indicate the very narrow safety window of aconitine. The therapeutic versus toxic potential of *Aconitum* alkaloids, including aconitine, has been a extensively discussed in the literature for many years (Ameri, 1998). Therefore, the main purpose of the present study is to characterise the pharmacokinetic behaviours of aconitine as the targeted marker of *Fuzi*. The prolonged administration of *Fuzi* for the treatment of chronic diseases, such as polyarthralgia, increases the danger of toxic reaction over time. Thus, more importantly, elucidating the pharmacokinetic behaviour of *Fuzi* following multiple administrations is very essential.

Although some basic information about the bioavailability and bioefficacy of aconitine is readily accessible, no data on its plasma protein binding are presently available. The pharmacological activity of a compound depends on its pharmacokinetic as well as its pharmacodynamic properties, which may be affected by plasma protein binding (Schmidt et al., 2010). Hence, the determination of aconitine protein binding should be revealed to gain further insight into the bioactivity of *Fuzi* extract.

Researchers have reported low aconitine plasma level ($C_{max} = 5.83 \pm 0.97$ ng/ml) after oral aconitine administration (0.2 mg/kg) in rats with short t_{max} (0.70 ± 0.18 h) and absolute bioavailability of 22.80%. According to Tazawa et al. (2003), the aconitine absolute bioavailability is only 1.3% using different analytical methods. A study by Zhang et al. (2008) involving a liquid chromatography–tandem mass spectrometry (LC/MS/MS) method for the pharmacokinetic evaluation of "SHEN-FU" injectable powder containing aconitine reported an undetectable aconitine plasma concentration. Researchers also published a simultaneous quantification of aconitine, mesaconitine, and hypaconitine in rat plasma after oral administration of Sini decoction (He et al., 2009).

Although a few studies have conducted on the PK of pure aconitine and *Fuzi*, little researches have investigated the pharmacokinetics after administration of multiple doses of pure aconitine or *Fuzi* extracts. Comparison of pharmacokinetic characteristics of pure aconitine and *Fuzi* extracts or the comparison between single dose and multiple doses also have not been investigated. Here, we developed a high performance liquid chromatography (HPLC) method for the determination of aconitine in *Fuzi* crude extracts considering the presence of the complex matrix of Chinese herbs. We further developed a fast ultra performance liquid chromatography–tandem mass spectrometry (UPLC/MS/MS) to investigate the pharmacokinetic behaviours of aconitine following single and multiple administrations of pure aconitine and processed *Fuzi* extracts in order to compare the pharmacokinetic characteristics of aconitine between pure aconitine and *Fuzi* extracts as well as compare the difference at single dose and multiple doses. Gaining information on the toxic and pharmacological effects of *Fuzi* is of utmost importance.

2. Materials and methods

2.1. Materials

Aconitine reference standards were purchased from the Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China, purity >98%, confirmed by LC/MS/MS). Processed *Fuzi* was provided by the He-Hua-Chi Chinese Pharmaceutical Company (Chengdu, China) in May 2009, and was identified as the lateral roots of *Aconitum carmichaeli* Debx. (Ranunculaceae) by Sichuan Academy of Chinese Medicine Sciences, Sichuan, China. The authentic sample (AC200905) is deposited in School of Pharmaceutical Sciences, Southern Medical University. The internal standard (IS) oxpentifylline was purchased from LC laboratories (Woburn, MA). Potassium phosphate dibasic (K_2HPO_4) and potassium dihydrogen phosphate (KH_2PO_4) were purchased from BD Biosciences (Woburn, MA). All other materials (typically of analytical grade or better) were used as received.

2.2. Animals

Male Sprague-Dawley rats (250–300 g, 8–10 wk old) were obtained from the South Medical University (Guangzhou, China) and kept in an environmentally controlled room (temperature: 25 ± 2 °C, humidity: 50 ± 5%, 12 h dark–light cycle) for at least 3 d before the experiments. The animal experiments were performed in accordance with the Guide for the Care National Institutes of Health. Ethical approvals for the animal studies were obtained from the Ethical committees of Southern Medical University. The animal protocols used in the present study were approved by the Institutional Animal Care and Uses Committee of the South Medical University.

2.3. Determination of aconitine concentration in Fuzi extracts

2.3.1. Preparation of aconitine samples

Each ground crude *Fuzi* sample powder (50 g) was soaked in 75% methanol (200 ml) for 0.5 h and then sonicated twice in 75% methanol (200 ml) at 25 °C for 30 min. The extracts were collected and concentrated to a final volume of 50 ml at 35 °C. The mixture was then centrifuged at 13,000 rpm for 15 min. The supernate was collected. An aliquot (500 μl) of the sample solution was mixed with an equal volume (200 μl) of the IS solution prior to analysis. The sample (200 μl) was injected into the HPLC system for aconitine quantitative analysis.

2.3.2. HPLC chromatographic conditions

The LC analyses were conducted using Agilent HP1200 equipped with two solvent pumps, a VWD 1100 UV detector (Agilent Technologies, Waldbronn, Germany), and an Agilent Eclipse plus C18 (4.6 mm × 150 mm, 5 μm). Flow rate was 0.1 ml/min. Phase A consisted of water and 0.05% trifluoroacetic acid (v/v), whereas phase B comprised 100% acetonitrile (ACN). Column separation was performed at room temperature using a gradient elution program, 0–3 min, 20% phase B; 3–6 min, 25% phase B; 6–13 min, 28% B; 13–15 min, 95% phase B; 15–22 min, 20% phase B min. Wavelength was at 231 nm. Calibration standard samples for aconitine were prepared in 50% methanol at concentrations of from 1.5625 to 100 μg/ml. The quality control (QC) samples were prepared at low (1.5625 μg/ml), medium (12.5 μg/ml), and high (100 μg/ml) concentrations in 50% ACN for calibration.