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Study on intestinal absorption and pharmacokinetic characterization of diester diterpenoid alkaloids in precipitation derived from Fuzi–Gancao herb-pair decoction for its potential interaction mechanism investigation

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ABSTRACT

Ethnopharmacological relevance: *Aconitum carmichaelii* Debx. (Fuzi in Chinese) has been widely clinically used to treat heart failure and rheumatism. Whereas its serious toxicity, *Radix et Rhizoma Glycyrrhizae* (Gancao in Chinese) was combined with it as traditional Chinese medicine (TCM) herb-pair for toxicity reduction and pharmacological effect improvement. Though some previous viewpoints about that has been reported, the underlying interaction mechanism of two herbs remain unknown and definitely worthy of investigating.

Aim of study: In present study, we focus on Fuzi–Gancao herb-pair precipitation (FGP), considering it related to the compatibility mechanism of Fuzi–Gancao herb-pair. The intestinal absorption and pharmacokinetic characters of 3 diester diterpenoid alkaloids in the precipitation were investigated.

Materials and methods: Both everted gut sac model and *in situ* single-pass intestinal perfusion model were used to investigate rat small intestinal permeability and transport mechanism of aconitine, hypaconitine and mesaconitine. Moreover, by means of determination of the plasma concentration, the pharmacokinetic characters of 3 alkaloid compounds in rats have been developed.

Results: In everted gut sac permeability experiment, the permeability of hypaconitine appeared best in ileum. Furthermore, their uptakes were increased in the presence of *P*-glycoprotein (*P*-gp) inhibitors. *In situ* single-pass intestinal perfusion uptake experiment, results revealed that the transport mechanism may fit the active transport mechanism. And 3 alkaloids in FPG could be absorbed well in rats, fitting 2-compartment model with 1^{st} order absorption and lag time.

Conclusions: Our results in present study indicated that 3 diester diterpenoid alkaloids in FGP could be dissolved out in gastrointestinal tract firstly and then absorbed in blood after oral administration, which could result in prolonging their mean residence time and adding their absorbed doses, avoiding dose dumping. The current study has significant enlightenments for further investigation on the interaction mechanisms of other acid-base herb-pairs as well as Fuzi–Gancao herb-pair.

1. Introduction

Herb-pair of TCM, the special types of drugs interaction, is a relatively fixed composition of two herbs in TCM clinical medication and also is a basic form of compatibility of TCM (Ung et al., 2007; Wang et al., 2012a). The application compatibility to herb-pairs of TCM always has the effect to reduce the toxicity and increase the efficacy of drugs (Pei et al., 2009). The study of herb-pairs which belongs to herb–drug interaction has gained much attention since the mid 1990s (Mohamed and Frye, 2011). There were an increasing number of researches which found that compatibility mechanisms of herb-pairs would ascribe to not only the synergic or antagonistic pharmacological effects by components in herb-pairs but also the result of regularity of active components *in vivo* (Ung et al., 2007). Thus, focusing on the intestinal absorption of active compounds would become very important for the oral drugs (Lin et al., 2010).

Aconitum carmichaelii Debx. (Fuzi in Chinese) is the daughter root of plant *Radix Aconiti Lateralis Preparata*, which is frequently used as an important both traditional Chinese medicine (TCM) and medicinal plants in Japan, Korea, and other Asian countries for rheumatism (Deng, 2008), heart failure (Wang et al., 2009b) and tumor (Gao et al., 2010). Yet, according to the records in TCM Classics and pharmacological experiments. it was also highly

Abbreviations: FGP, Precipitation of Fuzi–Gancao decoction; TCM, Traditional Chinese medicine.

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toxic and even could induce severely fatal cardio-toxicity and neurotoxicity (Lu et al., 2010; Wang et al., 2012b). In many TCM prescription (Gao et al., 2004), *Radix et Rhizoma Glycyrrhizae* (Gancao in Chinese), which is also one of the most frequently used Chinese herbs, is used by compatibility with *Aconitum carmichaelii* Debx. as classic herb-pairs.

There have been several reports about the compatibility effect of the herb-pairs. 3 diester diterpenoid alkaloids including aconitine, hypaconitine and mesaconitine have been approved as the main toxic as well pharmacological components (Bao et al., 2011; Liu et al., 2010; Wang et al., 2009a; Welch et al., 2011; Ye et al., 2011; Zhang et al., 2011). Chen et al. (2009) reported that the LD_{50} of mice after oral administration of *Aconitum carmichaelii* could be elevated by the compatibility of *Radix Glycyrrhizae*. Shen et al. (2011) reported that Gancao could enhance the tolerance of animals for side-effect and toxicity. Furthermore, more and more researchers began to focus on the compounds interactions in the herb-pairs. Due to the toxicity of diesterditerpene alkaloids, there has been a common opinion that the acid–alkaline compounds interaction would be the compatibility reason to reduce toxicity. Actually, it was still unable to explain the clinical cardio-tonic action of this two herbs compatibility. Thus, the underlying mechanism of the compatible effect between *Aconitum carmichaelii* and *Radix Glycyrrhizae* is also unknown thoroughly.

In our previous research, we have observed that absorption rate of hypaconitine could be delayed in Fuzi–Gancao decoction (Supplementary Fig. 1). And lots of precipitation had been derived from the Fuzi–Gancao decoction when *Aconitum carmichaelii* boil with *Radix Glycyrrhizae* together. We have discovered that there was a large amount of aconitine, hypaconitine and mesaconitine by LC-MS analysis (Supplementary Fig. 2) in precipitation of Fuzi–Gancao decoction (FGP). We analyzed its chemical constituents by LC-TOF-MSn, finding there are 11 kinds of compound from *Radix Glycyrrhizae* and 25 kinds of compound from *Aconitum carmichaelii* (Supplementary Table 1). Additionally, 45.76, 32.15, 64.42 μg content of aconitine, mesaconitine, hypaconitine, respectively, per 1 g FGP (in another publish paper approved) were uncovered. Besides, these alkaloids could be dissolved out slowly in gastrointestinal juice. Therefore, researching on the intestinal absorption characters of diester diterpenoid alkaloids in FGP would be helpful to investigate the compatibility mechanism between *Aconitum carmichaelii* and *Radix Glycyrrhizae*.

In present research, the intestinal transport and permeability mechanism of 3 diester diterpenoid alkaloids will be evaluated by using *in vitro* everted gut sac model and *in situ* single-pass intestinal perfusion model. Moreover, pharmacokinetic experiments in rats were used to proof the *in vivo* characters of 3 diester diterpenoid alkaloids in FGP through intestinal transport.

2. Materials and methods

2.1. Chemicals and reagents

Aconitine (No. 110798-200408), Hypaconitine (No. 110798-200405), Mesaconitine (No. 110798-200411) (purity ≥98%) and Berberine (No. 11073-200911) (purity ≥98%) were purchased from the National Institute for Food and Drug Control (China). Verapamil hydrochloride, digoxin, cyclosporine A, MK 571 sodium salt, probenecid, methotrexate, itraconazole and bis (*p*-nitrophenyl) phosphate sodium salt (BNPP) (purity ≥98%) were purchased from China Pharmaceutical Biological Products Analysis Institute (China).

HPLC grade Acetonitrile for liquid chromatography was purchased from Fisher Scientific (USA). Deionized water was prepared by Millipore Milli-Q Plus system (Milllipore Bedford, MA, USA). Krebs–Ringer (K–R) solution (pH7.4) was prepared based on report (Saito et al., 2011). Reagents which were not mentioned here were from a standard source and belonged to analytical pure grade.

2.2. Plant material

Aconitum carmichaelii Debx. (Fuzi) was collected from its native habitat (latitude 31°78′33″ north, and longitude 104°76′21″east), Jiangyou city, China in July of 2011. A voucher specimen was deposited at the Herbarium of Chengdu University of TCM (CDCM201107-3) and identified by Prof. Xianming Lu (Director of Chinese Materia Medica Herbarium of Chengdu University of TCM, Sichuan, China).

Radix et Rhizoma Glycyrrhizae (*Gaocao*) was cultivated in its native habitat (Inner Mongolia, latitude 40°20′25″ north, and longitude 106°59′27″ east) and harvested and processed to decoction slices by Kelun Pharmaceutical Co., Ltd. (China) in July of 2011.

2.3. Animals

Adult male Sprague-Dawley rats (weight, 180–220 g) were obtained from Institute of Laboratory Animals Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, China (production certificate NO.: SCXK2008-24). These animals lived in suitable conditions: temperature-controlled room (22±2 °C) with a 12 h light–dark cycle and with free access to standard rat food and water. All animal treatments followed the recommendations of the Regulations of the Administration of Affairs Concerning Experimental Animal and were approved by the Ethical Committee of Affiliated Hospital of Chengdu University of TCM.

2.4. Preparation of Fuzi–Gancao precipitation (FGP) experimental samples

The FGP (Supplementary Fig. 3) was dissolved by a little of artificial gastric juice (prepared according to the appendix of Chinese Pharmacopoeia, 2010 version) and filtered subsequently. The FGP solution was diluted with K–R solution (prepared as report (Chula et al., 2012)) as the gut sac sample with the terminal concentration of aconitine, hypaconitine and mesaconitine (46.6 μg mL^{-1}, 56.53 μg mL^{-1} and 39.64 μg mL^{-1}, respectively). And then, The FGP solution will be diluted with K–R solution to the low, middle and high concentration of aconitine, hypaconitine and mesaconitine, respectively, as the FGP perfusion samples for single-pass intestinal perfusion model.

2.5. Analytical condition

The LC system used was a high performance liquid chromatography system (1200 series, Agilent, USA). The separation was performed on an Agilent Eclipse Plus C_{18} column (3.5 μm particle size, 100 × 4.6 mm i.d.) from Agilent Co. (USA) at 30 °C. The mobile phase consisted of acetonitrile (contained 0.1% formic acid) and 0.03 M ammonium acetate (contained 0.1% glacial acetic acid) (50:50, v/v). The flow rate was 0.4 mL min^{-1}.

MS analysis was performed on a micrOQ-TOF mass spectrometer equipped with an ESI interface (Bruker Daltonic, Germany) in positive ion mode. The ionization parameters were set as follows: capillary voltage, 4.5 KV; desolvation gas, 240 L h^{-1}; desolvation temperature, 180 °C; atomization gas pressure, 2 bar. The data acquisition rate was set to 0.4 s, with 0.1 s inter scan delay. All data were acquired using the lock spray to ensure accuracy and reproducibility, in which sodium formate was used as the lock mass to calibrate the mass range of 150–1000 *m*/*z*. The collision energy of dissociation was set at 40 eV for alkaloid components in the MS/MS. The ions *m*/*z* 646.3/586.6, 616.1/556.5, 632.7/572.5 and 336.1/278.1 were used to detect aconitine, hypaconitine, mesaconitine and berberine in positiveion mode, respectively. Data were analyzed using Analyst QS date software (Bruker Daltonic, Germany).