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Mdr1a plays a crucial role in regulating the analgesic effect and toxicity of aconitine by altering its pharmacokinetic characteristics

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ABSTRACT

Aconitine (AC) is the primary bioactive/toxic alkaloid in plants of the *Aconitum* species. Our previous study demonstrated that Mdr1 was involved in efflux of AC. However, the mechanism by which Mdr1 regulates the efficacy/toxicity of AC in vivo remains unclear. The present study aimed to determine the effects of Mdr1a on the efficacy/toxicity and pharmacokinetics of AC in wild-type and *Mdr1a*$^{-/-}$ FVB mice. After oral administration of AC, significantly higher analgesic effect was observed in *Mdr1a*$^{-/-}$ mice (49% to 105%) compared to wild-type mice ($P < 0.05$). The levels of s100-β protein and creatine kinase, which indicate cerebral and myocardial damage, respectively, were also significantly increased ($P < 0.05$) in *Mdr1a*$^{-/-}$ mice. Histopathological examination revealed that the *Mdr1a*$^{-/-}$ mice suffered from evident cerebral and myocardial damages, but the wild-type mice did not. These findings suggested that Mdr1a deficiency significantly promoted the analgesic effect of AC and exacerbated its toxicity. Pharmacokinetic experiments showed that $T_{1/2}$ of AC in the *Mdr1a*$^{-/-}$ mice was significantly higher (from 87% to 300%) than that in wild-type mice ($P < 0.05$). The distribution of AC in the brain of *Mdr1a*$^{-/-}$ mice was 2- to 32-fold higher than that in the brains of wild-type mice ($P < 0.05$). Toxic reactions were more severe in *Mdr1a*$^{-/-}$ mice compared to wild-type mice. In conclusion, Mdr1a deficiency significantly enhanced the analgesic effect of AC and exacerbated its toxicity by upregulating its distribution to the brain and decreasing its plasma elimination rate. Thus, Mdr1a dysfunction may cause severe AC poisoning.

1. Introduction

Extracts from plants of the *Aconitum* species are widely used in clinical practice to treat rheumatism, cardiac dysfunction, and pain (Singhuber et al., 2009). Aconitine (AC) is a bioactive alkaloid accounting for >60% of the total diester-diterpenoid alkaloids in *Aconitum* species (Liu et al., 2013). AC stimulates various biological activities, including anti-inflammatory, antiarrhythmic, and analgesic activities (Zhu et al., 2013). AC is also effective against various tumor cell lines, such as HepG2, metastatic Lewis lung carcinoma cells, and the melanoma cell line B16 (Du et al., 2013; Gao et al., 2012; Solyanik et al., 2004). However, AC has a narrow therapeutic index. For instance, the 5% lethal dose (LD_5) and half-lethal dose (LD_{50}) of orally administered AC in mice are 0.26 mg/kg and 2.0 mg/kg, respectively (Tang et al., 2012). Indeed, AC is the main contributor of frequent fatal poisoning related to use of *Aconitum* species in clinical practice, folk treatment, and ingestion (Niitsu et al., 2013). Direct effects on the central nervous system and cardiovascular system by AC underlies its various toxic effects, including nausea, vomiting, diarrhea, palpitation, convulsion, hypotension, arrhythmia, and death (Singhuber et al., 2009). However, safe use of *Aconitum* species in clinical practice has been impeded by conflicting findings about the relationship between its dosage and efficacy/toxicity. *Fuzi* extract obtained from the lateral root of *Aconitum carmichaelii* improves the myocardial function and antioxidant enzymatic activities of rats with chronic heart failure (CHF) (Yu et al., 2015). However, diester-diterpenoid *Aconitum* alkaloids, such as aconitine, mesaconitine, and hypaconitine, result in 11-fold lower values of area under concentration–time curve (AUC) in CHF rats than in normal rats (Yu et al., 2015). Thus, the relationship between efficacy or toxicity and disposition of AC with its mechanism should be investigated to promote reasonable and safe use of the *Aconitum* species in clinical practice.

AC is absorbed through a pH-dependent carrier-mediated active transport process (Yang et al., 2013a). However, AC exhibits poor bioavailability. The absolute bioavailabilities of orally administered AC and *Fuzi* water extract are 8.24% and 4.72%, respectively, probably because AC undergoes extensive hydrolysis, metabolism, and efflux in vivo (Tang et al., 2012). By losing an acetyl group, AC is easily

Abbreviations: AC, aconitine; AUC, area under concentration-time curve; cTnI, cardia troppnin I; CL, clearance; CK, creatine kinase; LD_{50}, 5% half-lethal dose; LD_{50}, half-lethal dose; NSE, neurone specific enolase; P-gp, P-glycoprotein; S100-β, neuron-enriched S100 beta; $T_{1/2}$, half life.

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hydrolyzed to produce monoester-diterpenoid alkaloids with 1/5th to 1/500th of the toxicity of diester-diterpenoid alkaloids (Huang et al., 2007; Wang et al., 2003). Metabolism of AC is mainly mediated by CYP450 enzymes. CYP3A4/5 and CYP2D6 are the main enzymes responsible for the demethylation, *N*-deethylation, dehydrogenation, and hydroxylation of AC in vitro (Tang et al., 2011). In vitro studies have also demonstrated that the extensive efflux of AC is induced by P-glycoprotein (P-gp, MDR1) (Chen et al., 2009; Ye et al., 2013). P-gp is an important efflux transporter with many substrates, including antineoplastic agents and neutral or positively charged hydrophobic compounds (Kobori et al., 2014; Wu et al., 2016). However, the ability of P-gp on the efficacy/toxicity of AC in vivo remains unknown.

P-gp is an important ATP-dependent efflux transporter widely expressed on the apical membrane of various epithelial cells, such as those in the intestine, liver, and blood-brain barrier (BBB) (Ye et al., 2013). The efflux pump effect of P-gp on external chemicals plays a crucial protective role in the human body. P-gp is also the primary cause of multidrug resistance to anti-tumor drugs and poor bioavailability of candidate treatment agents (Yang and Liu, 2016). However, P-gp expression and activity are susceptible to various agents, which potentially affect the pharmacokinetics, bioavailability, therapeutic index, and toxicity of therapeutic drugs. Thus, P-gp is considered an important cause of drug-drug interaction (DDI) (Rigalli et al., 2011; Verschraagen et al., 1999; Zhou, 2008). AC is also widely known for its central nervous system and cardiovascular system toxicity, which results in nerve paralysis, arrhythmia, and death (Zhou et al., 2013). P-gp is an important transporter in the BBB, which limits the uptake of xenobiotics into the brain and thereby protects it from damage (Nikandish et al., 2016; Ravikumar Reddy et al., 2016). Thus, the effect of P-gp variability on the efficacy/toxicity and disposition of AC should be investigated to facilitate reasonable and safe use of AC in clinical practice.

In this study, the effects of P-gp on the efficacy, toxicity, and disposition of AC in vivo were elucidated by using male wild-type and *Mdr1a*$^{-/-}$ FVB mice. The analgesic and anti-inflammatory effects of AC were evaluated at oral doses of 0.05 mg/kg and 0.1 mg/kg. Plasma biochemical analyses and tissue histological analyses were performed to confirm the toxic effects of AC on mice. The pharmacokinetics and tissue distribution of AC at oral doses of 0.1 mg/kg and 0.2 mg/kg were also evaluated.

2. Material and methods

2.1. Chemicals and reagents

2.1.1. Chemicals. Aconitine was purchased from Chengdu Mansite Pharmaceutical Co., Ltd. (Chengdu, China), Diclofenac sodium was obtained from Dalian Meilune Biological Technology Co., Ltd. (Dalian, China). Testosterone and carrageenan were purchased from Sigma-Aldrich Co., Ltd. (St. Louis, MO, USA). High-performance liquid chromatography grade acetonitrile was purchased from Thermo Fisher Scientific (St. Louis, MO, USA). All other chemicals and solvents were of analytical grade or better.

2.1.2. Animals. Male wild-type FVB mice were supplied by Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Male *Mdr1a*$^{-/-}$ FVB mice were obtained from Shanghai Biomodel Organism Science & Technology Development Co., Ltd. (Shanghai, China). *Mdr1a*$^{-/-}$ mice were all of >99% FVB genetic background. The *Mdr1a*$^{-/-}$ FVB mice were established by the CRISPR/Cas9 technique and identified by the Sanger sequencing method (Chain Termination Method). All mice were 8 to 12 weeks old and were maintained under temperature-controlled conditions with 12 h light/dark cycle and unlimited access to food and water. The animal protocols used in this study were approved by Institutional Animal Care and Uses Committee of Guangzhou University of Chinese Medicine.

2.2. Methods

2.2.1. Analgesic effect of AC in wild-type and Mdr1a$^{-/-}$ *FVB mice.* Wild-type mice (or *Mdr1a*$^{-/-}$ mice) were randomly divided into 4 groups with 6 mice per group. Mice in the vehicle control group and positive control group were gavaged with saline (10 mL/kg) and 50 mg/kg diclofenac sodium, respectively. Saline was used to dissolve AC and diclofenac sodium. Mice in the other two tested groups were gavaged with 0.05 mg/kg or 0.1 mg/kg AC, respectively. After 30 min of treatment with saline, diclofenac sodium and AC, mice were injected intraperitoneally with 1% acetic acid. The number of abdominal constrictions (writhes) were counted after 5 min of acetic acid injection for a period of 15 min.

2.2.2. Anti-inflammatory effect of AC in wild-type and Mdr1a$^{-/-}$ *FVB mice.* Wild-type mice (or *Mdr1a*$^{-/-}$ mice) were randomly divided into 4 groups with 6 mice per group. Mice in the vehicle control and positive control groups were gavaged with saline (10 mL/kg) and 50 mg/kg diclofenac sodium, respectively. Saline was used to dissolve AC and diclofenac sodium. Mice in the other two tested groups were gavaged with 0.05 mg/kg or 0.1 mg/kg AC, respectively. After 30 min of treatment, carrageenan (1%, 0.05 mL) was injected subcutaneously in the sub plantar tissue of the right hind paw of each mouse. Paw thickness was measured immediately before, and at 1 h, 2 h, 3 h, and 4 h after carrageenan treatment. The average foot swelling readings from the drug and standard treated animals was compared with that of vehicle control group.

2.2.3. Biochemical and histological analyses. Wild-type mice (or *Mdr1a*$^{-/-}$ mice) were randomly divided into 2 groups with 6 mice per group. Mice in the vehicle control group were gavaged with saline (10 mL/kg). Mice in the other groups were gavaged with 0.1 mg/kg AC. After 6 h of treatment, mice were anesthetized with diethyl ether and blood was collected from the suborbital vein. Then, mice were sacrificed by cervical dislocation the brains and hearts were rapidly removed. Tissues were fixed in 4% paraformaldehyde for 24 h. Blood samples were centrifuged at 4800 $\times g$ for 6 min at 4 °C, and the plasma fraction was collected and stored at −20 °C. The plasma level of creatine kinase (CK) was detected using a CK biochemical kit (Nanjing Jiancheng Bioengineering Instititek, China), while neuron specific enolase (NSE), neuron-enriched s100 beta (s100-β) and cardiac troponin I (cTnI) were detected using ELISA kits (Nanjing Jiancheng Bioengineering Instititek, China) following the manufacturer's protocol.

After fixation in 4% paraformaldehyde for 24 h, brain and heart samples were embedded in paraffin. Serial sections were cut in the coronal plane with a thickness of 6 μm. After deparaffinization with xylene and graded alcohols, the mounted tissue sections were stained with hematoxylin and eosin (H&E). The microscope was equipped with a digital video camera controlled by the Leica Application Suite software program for image acquisition. Tissue sections were visualized using 10× and 40× objective lenses.

2.2.4. Pharmacokinetics of AC in wild-type and Mdr1a$^{-/-}$ *FVB mice.* AC has poor and variable bioavailability. Using a low dose of AC, we were unable to obtain a reliable pharmacokinetic profile of AC in our preliminary study. Therefore, we conducted pharmacokinetics experiments using 0.1 mg/kg and 0.2 mg/kg of AC. AC was dissolved in saline. Mice were fasted for 10 h before 0.1 mg/kg or 0.2 mg/kg of AC were gavaged. Multiple blood samples (about 25 μL) were collected from the tail vein at 0 min, 5 min, 10 min, 20 min, 30 min, 50 min, 80 min, 120 min, 240 min, 360 min, 480 min, and 720 min using heparinized tubes and stored at −20 °C until analysis.

2.2.5. Tissue distribution of AC in wild-type and Mdr1a$^{-/-}$ *FVB mice.* Wild-type mice (or *Mdr1a*$^{-/-}$ mice) were gavaged with 0.1 mg/kg or 0.2 mg/kg of AC. AC was dissolved in saline. At 10 min,