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# Characterization of metabolites and cytochrome P450 isoforms involved in the microsomal metabolism of aconitine

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**Abstract**

Aconitine, a major *Aconitum* alkaloid, is well known for its high toxicity that induces severe arrhythmias leading to death. The current study investigated the metabolism of aconitine and the effects of selective cytochrome P450 (CYP) inhibitors on the metabolism of aconitine in rat liver microsomes. The metabolites were separated and assayed by liquid chromatography-ion trap mass spectrometry (LC/MS$^n$) and further identified by comparison of their mass spectra and chromatographic behaviors with reference substances. Various selective inhibitors of CYP were used to identify the isoforms of CYP, that involved in the metabolism of aconitine. A total of at least six metabolites were found and characterized in rat liver microsomal incubations. Result showed that the inhibitor of CYP 3A had an inhibitory effect on aconitine metabolism in a concentration-dependant manner, the inhibitor of CYP1A1/2 had a modest inhibitory effect, whereas inhibitors of CYP2B1/2, 2D and 2E1 had no obvious inhibitory effects on aconitine metabolism. Aconitine might be metabolized by CYP 3A and CYP1A1/2 isoforms in rat liver microsome.


*Keywords:* Aconitine; Cytochrome P450; Metabolites; Metabolism; Microsome

## 1. Introduction

Aconitine is a diesterditerpene alkaloid derived from some Chinese medicinal herbs of genus *Aconitum* in the family of Ranunculaceae, such as *Aconitum* carmichaeli Debx., *Aconitum* Kusnezoffii Reichb. and *Aconitum* carmichaeli Debx. These medicinal herbs are widely used in clinics in China and other East Asia countries because of their excellent effects against rheumatosis, rheumatoid arthritis and some other inflammations. In Europe, *Aconitum* alkaloids have also been used in homeopathic medicine. Aconitine, a major toxic *Aconitum* alkaloid, induces several arrhythmias with final outcome leading to death. The lethal dose 50% ($LD_{50}$) of aconitine for mice is 1.8 mg/kg (single dose, orally) [1] and in humans, the reported minimum lethal dose of orally ingested aconitine ranges from 2 to 6 mg. Aconitine poisoning frequently happened most likely because of its high toxicity. However, limited data are available on aconitine metabolism in human and other animal species. In mice, aconitine was distributed mainly in kidney and liver. The concentration of aconitine in organ and blood gradually decreased according to the repeated administration, whereas benzoylaconine and aconine increased [2]. Aconitine could be detected in blood samples at concentrations of 10.0 and 12.1 ng/g in two fatal cases with suspected aconite intoxication. In one case, aconitine could also be detected in the stomach content (3 ng/g) and in the urine (180 ng/mL) [3]. However, the role of cytochrome P450 (CYP) in aconitine metabolism remained unknown. CYP enzymes are the most important phase-I drug metabolizing enzymes that play an important role in xenobiotic metabolism and detoxification [4]. Nine isoenzymes have been identified in rats, and each isoenzyme plays a different role in xenobiotic metabolism. It is thus necessary to identify which CYP isoenzymes are involved in the major pathway of aconitine metabolism. Therefore, in the present study, we first detected the CYP isoenzymes principally responsible for the metabolism of aconitine in rat liver microsomes by chemical inhibition methods.

There are various *in vivo* studies that reported the metabolites of aconitine [5,6]. It is difficult to detect aconitine and its

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metabolites because of their lower doses in clinics; moreover, the amount of metabolites in some organs was near the detection limit, even though some metabolites were not detected. So it is necessary to understand the structure of metabolites of aconitine firstly for making further research in pharmacology and forensic medicine. For these reasons, an *in vitro* study was investigated to identify the metabolites of aconitine in rat liver microsomes by liquid chromatography-ion trap mass spectrometry (LC/$MS^n$). It may identify more metabolites at a higher concentration than *in vivo* studies. These results may contribute valuable information for aconitine study in toxicology, pharmacology and forensic medicine.

## 2. Conditions

### 2.1. Experimental

#### 2.1.1. Materials and methods

Aconitine (∼95%, HPLC grade), α-naphthoflavone (ANF) (99%), diphenhydramine (DPH) (≥98%), quinine (QUIN) (≥98%), diethylthiocarbamate (DDTC) (99%), and troleandomycin (TAO) (99%) were purchased from Sigma Chemical Co. (St. Louis, USA). β-Nicotinamine adenine dinucleotide phosphate (NADPH) in reduced form (≥98%) was purchased from Boehringer (Mannheim, Germany). Tris(hydroxymethyl)aminomethane (Tris, ultra pure grade) was purchased from ANGUS (Illinois, USA). Methanol (HPLC grade) was purchased from Fisher Chemical Co. (New Jersey, USA). All other reagents were of the highest quality commercially available.

#### 2.1.2. Animals and treatment

Male Sprague–Dawley rats were purchased from Vital River Laboratory Animal Co. Ltd. (Beijing, China). Animals were fed in a normal condition and acclimatized at 12 h light/dark cycle for 5 days before experimentation. Rats had been fasted for 12 h before being used for preparation of liver microsomes.

#### 2.1.3. Preparations of microsomes

In order to minimize degradation of CYP, all apparatus and solutions were cooled and stored at 4 °C prior to the experiment, and samples were maintained at 4 °C at all points in the microsomal preparation. Hepatic microsomes were prepared from each rat independently. In brief, rats were weighted and killed by cervical dislocation, livers were perfused with ice-cold saline (0.9%), finely minced and homogenized with four volumes of 0.05 mol/L Tris–HCl buffer (pH 7.4, containing 0.15 mol/L KCl, 1 mmol/L EDTA, 0.25 mol/L sucrose). The homogenate was centrifuged at 10,000 × *g* for 20 min at 4 °C and the resulting supernatant was ultracentrifuged at 100,000 × *g* for 60 min at 4 °C in order to obtain the microsomal pellet. The washed pellet was resuspended in 50 mmol/L Tris–HCl buffer (pH 7.4, containing 20% glycerol, 1 mmol/L EDTA, 0.25 mol/L sucrose) and stored in liquid nitrogen until use. The protein concentration was determined using the method of Lowry et al. [7] with bovine serum albumin as the protein standard. CYP and $b_5$ contents were determined using the method of Omura and Sato [8].

#### 2.1.4. In vitro microsomal incubation conditions

In order to identify the metabolites of aconitine, a typical reaction mixture contained 150 mmol/L Tris–HCl buffer (pH 7.4), 5 mmol/L $MgCl_2$, 1 g/L microsomal protein and 40 μmol/L aconitine in the final volume of 0.5 mL. The incubation mixture was preincubated at 37 °C for 3 min and reactions were initiated by adding 50 μL NADPH (20 mmol/L), then incubated at 37 °C in a waterbath shaker for 60 min. Reactions were linear with respect to protein concentration (0.1–1.0 g/L), time (5–60 min), and substrate concentration (linear part 2.5–40 μmol/L and saturation part 40–80 μmol/L). The reactions were terminated by adding 4 mL ice-cold dichloromethane, then vortexed and centrifuged at 4500 × *g* for 10 min to remove precipitated protein. The organic phase (3.5 mL) was drawn into another tube and dried with a gentle stream of nitrogen gas.

#### 2.1.5. Chemical inhibition study

The effect of specific CYP inhibitors such as ANF (CYP1A1/2) [9], DPH (CYP2B1/2) [10], QUIN (CYP2D) [11], DDTC (CYP2E1) [12] and TAO (CYP3A) [13] on aconitine metabolism was investigated in rat liver microsomes. Each inhibitor was tested in three randomly selected rat liver samples. Incubations contained 0.5 mg microsomal protein, 5 mmol/L $MgCl_2$, 40 μmol/L aconitine, 2 mmol/L NADPH, and various concentrations of the different inhibitors in 0.15 mol/L Tris–HCl buffer (pH 7.4). The concentration range of inhibitors was 0–10 μmol/L for ANF, 0–50 μmol/L for QUIN, 0–100 μmol/L for DPH, DDTC and TAO. The final incubation volume was 0.5 mL. With the exception of DPH and DDTC, which were dissolved in buffer, the ANF, QUIN and TAO were dissolved in methanol. In order to avoid possible effects of the solvent on metabolism, the organic solvent did not exceed 1% (v/v) when added to incubation. Incubation with no inhibitor added were regarded as controls. The mechanism-based inhibitor, TAO, was preincubated with microsomes and NADPH for 10 min to allow for TAO metabolism and the generation of CYP3A inhibitory metabolites before the reaction was started with substrate. All other reactions were started with NADPH. The incubation condition and extract methods were the same as described above. The metabolism of aconitine was analyzed by HPLC and expressed as aconitine disappearance rate [14].

#### 2.1.6. LC/$MS^n$ assays of identification of aconitine metabolites

Samples from the *in vitro* incubation mixture were extracted with dichloromethane; the organic phase was evaporated under a gentle stream of nitrogen gas. Residue was redissolved in 250 μL of HPLC mobile phase (see below), and an aliquot of 40 μL was injected and analyzed by Finnigan LCQ liquid chromatography-ion trap mass spectrometer (San Jose, USA) using the heated nebulizer and positive-ion detection. The spray voltage was set at 4.25 kV. The capillary voltage was fixed at 30 V, and the temperature was maintained at 180 °C. The HPLC fluid was nebulized using $N_2$, which was also the sheath gas and the auxiliary gas at a flow rate of 0.75 L/min and 0.15 L/min, respectively. The MS/MS spectra were produced by collision-induced dissociation (CID) of the selected precursor ions with He present in the

mass analyzer, and the relative collision energy was set at 30–40%. The samples were separated on a ZORBAX SB-$C_{18}$ column (5 μm, 4.6 mm × 150 mm), the mobile phase consisted of 55% methanol and 45% water (containing 0.04% triethylamine, 0.5% glacial acetic acid). Data were collected and analyzed by Navigator software (version 1.2, Finnigan, San Jose, USA).

#### 2.1.7. HPLC assay of inhibition study

Samples were prepared as described above. Residue was redissolved in 250 μL of HPLC mobile phase and an aliquot of 40 μL was injected and analyzed by Agilent 1100 HPLC system (HP, USA). The samples were separated on a ZORBAX SB-$C_{18}$ column (4.6 mm × 150 mm, 5 μm); the mobile phase used for aconitine consisted of 55% methanol and 45% water (containing 0.04% triethylamine, 0.5% glacial acetic acid), which was delivered at a constant flow rate of 1.0 mL/min; detection was at 230 nm.

#### 2.1.8. Data analysis

Data are expressed as mean ± S.E.M., and the cytochrome P450-mediated metabolism of aconitine in the presence of inhibitors is expressed as a percentage of the corresponding control values.

## 3. Results and discussion

### 3.1. Characteristics of rat liver microsomes

The P450 and $b_5$ contents of prepared liver microsomes were determined from six male Sprague–Dawley rats. The P450 content was 1.23 ± 0.26 nmol/mg protein, and $b_5$ content was 0.46 ± 0.14 nmol/mg protein. The P450 and $b_5$ contents of those rat liver microsomes are in the normal range, and the difference is minimal between individuals. These data suggested that the six microsomal samples could be used in metabolic experiments.

### 3.2. Metabolism profile of aconitine in rat liver microsomes by LC/$MS^n$ analysis

Compared with the controls, incubating aconitine with rat liver microsomes in the presence of NADPH resulted in metabolism with a decrease in aconitine (Fig. 1D), whereas the concentration of aconitine remained unchanged in the incubation without NADPH (Fig. 1B and C). The formation of these metabolites was dependent on the presence of NADPH.

### 3.3. Metabolite identification

The HPLC chromatograms and selective ion chromatograms for the positive ion electrospray LC/$MS^n$ analysis of an incubation of 40 μmol/L aconitine with rat liver microsome are shown in Figs. 2 and 3. Compared with the controls, six metabolites were detected in addition to unchanged aconitine (Fig. 2B). Each was generally determined on the basis of chromatographic behaviors and characteristic mass spectrometric fragmentation features, which were generated by electrospray ionization MS, MS/MS ($MS^2$), or $MS^3$ spectra of these compounds. The retention time and the associated information used in the identification are summarized in Table 1. Proposed metabolic pathways of aconitine in rat liver microsomes are shown in Fig. 4.

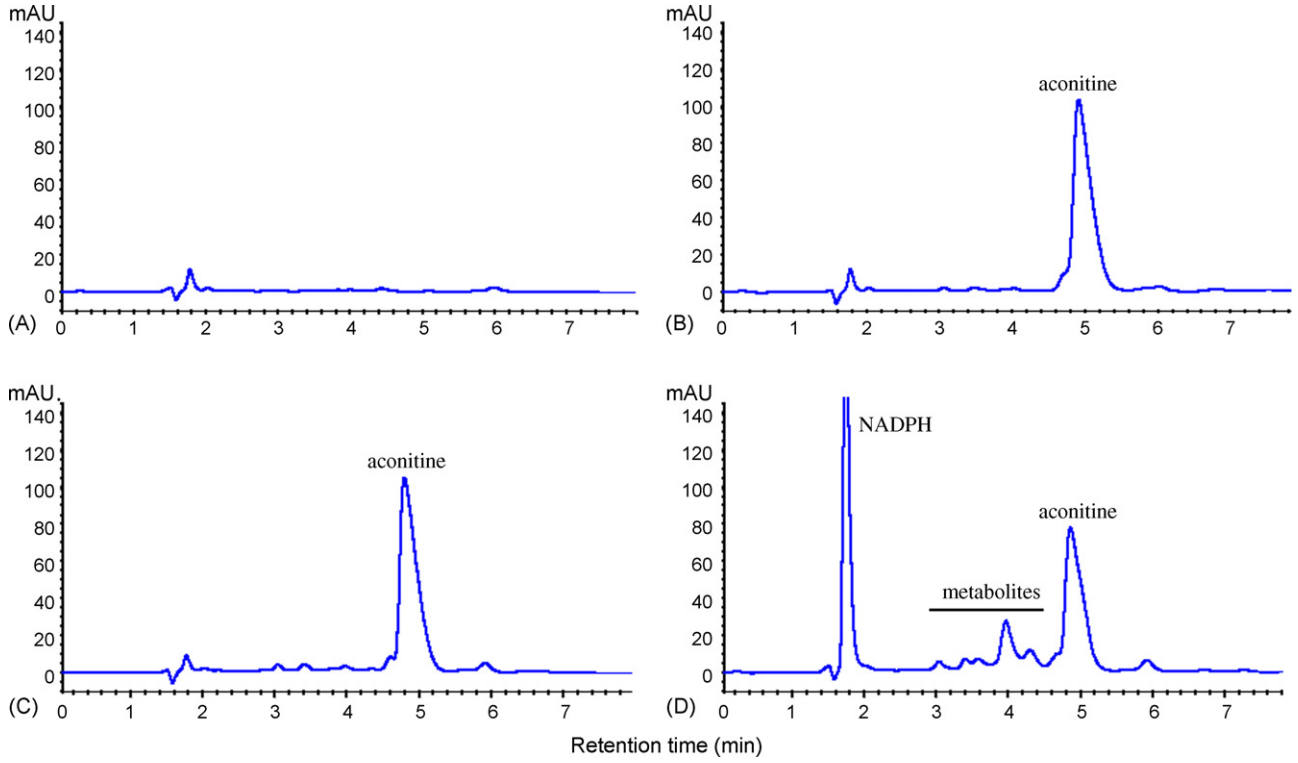


Fig. 1. HPLC chromatograms of aconitine in rat liver microsomal incubation: (A) blank microsome; (B) aconitine standard; (C) aconitine incubated at 37 °C for 60 min with microsome (no NADPH added); (D) aconitine incubated at 37 °C for 60 min with microsome (NADPH added).

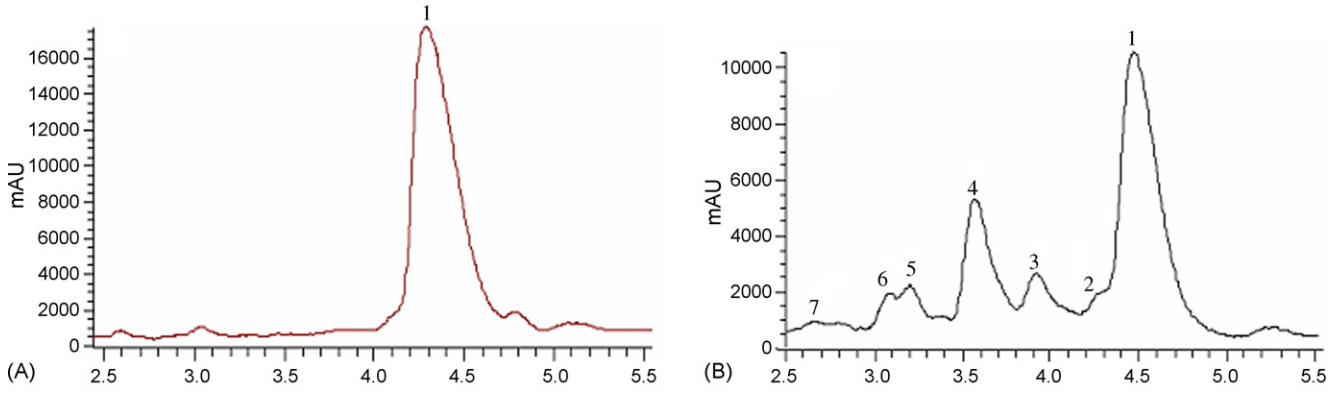


Fig. 2. HPLC chromatograms of aconitine and its metabolites formed by rat liver microsomes monitored by LC/MS: (A) aconitine incubated at 37 °C for 60 min with microsome (no NADPH added); (B) aconitine incubated at 37 °C for 60 min with microsome (NADPH added). Peak 1: aconitine; peak 2: M2; peak 3: M4; peak 4: M6; peak 5: M1; peak 6: M3; peak 7: M5.

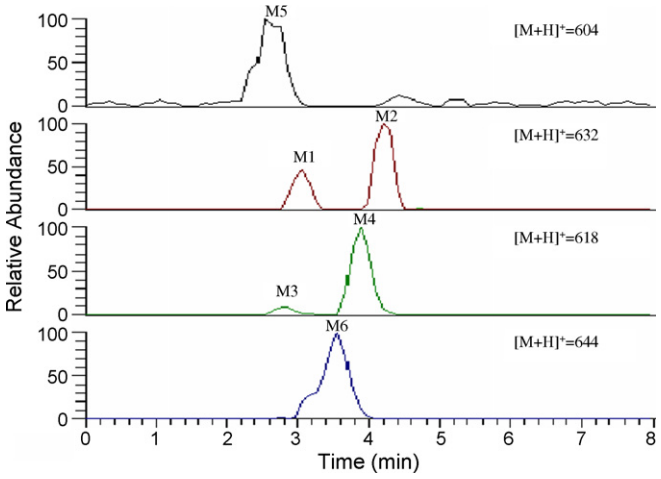


Fig. 3. Representative selective ion current chromatograms of aconitine metabolites in rat liver microsomes incubated with 40 μM aconitine for 60 min.

### 3.3.1. Aconitine

The compound eluting at 4.4 min possessed the same pseudo-molecule ion, full scan MS/MS spectrum and chromatographic behavior with authentic aconitine; therefore, it was identified as unchanged aconitine. Aconitine showed pseudo-molecule ion $[M+H]^+$ at *m/z* 646 in full scan mass spectrum, and $MS^2$, $MS^3$ spectrum of $[M+H]^+$ provided a number of characteristic fragment ions at *m/z* 586, 554, and 526 (Fig. 5), which are useful fragmental information in metabolite identification.

### 3.3.2. Metabolites M1 and M2

Metabolites M1 and M2 eluted at the retention times of 3.1 and 4.2 min, respectively. Both showed a protonated molecule ion at *m/z* 632 (Fig. 6A and C), a loss of 14 Da (*m/z* 646 → 632) from aconitine, suggesting that they were isomers of *O*-demethylated metabolites of aconitine. The difference between M1 and M2 lied in the position of *O*-demethylation in aconitine. The MS/MS spectra of *m/z* 632 from both metabolites contained major fragmentation ions at *m/z* 572, 512 (Fig. 6B and D), which were 14 Da lower than fragmentation ions *m/z* at 586, 526 (Fig. 5) of aconitines, respectively. The metabolites had the same fragmentation mode with aconitine, indicating that the diterpenoid sketch was intact. The relative abundance ratios of M1 and M2 were different and M2's relative abundance was larger than M1's (Fig. 3), suggesting that *O*-demethylation in M1 and M2 occurred at different positions on methoxyl group. 16-*O*-demethylation is relatively easier under the considerations of electronic effect, steric hindrance, and reference of the metabolic regulation of lappaconitine [15]. Therefore, M2 that is more abundant with retention time of 4.2 min was identified as 16-*O*-demethyl-aconitine. Except for the 16-*O*-methoxyl, positions 1, 6 and 18 (Fig. 5) all existed in methoxyl group, suggesting *O*-demethylation could be occur in any position of them. So M1 was determined to be *O*-demethyl-aconitine; the exact position of demethylation was not characterized.

### 3.3.3. Metabolite M3 and M4

Metabolites M3 and M4 had HPLC retention times of 2.9 and 3.9 min (Fig. 3), respectively. Both metabolites showed

Table 1
$LC/MS^n$ data of acontine and its metabolites after incubation with rat liver microsomes and NADPH

| Metabolite | Retention time (min) | $[M+H]^+$ (*m/z*) | Major fragment ions | Identification |
|---|---|---|---|---|
| Acontine | 4.4 | 646 | 586, 554, 526, 368 | Aconitine |
| M1 | 3.1 | 632 | 572, 512 | *O*-Demethyl-aconitine |
| M2 | 4.2 | 632 | 572, 512 | 16-*O*-Demethyl-aconitine |
| M3 | 2.9 | 618 | 558, 498 | *O*-Didemethyl-aconitine |
| M4 | 3.9 | 618 | 558, 526, 476 | *N*-Deethyl-aconitine |
| M5 | 2.5 | 604 | 554 | 8-*O*-Deacetyl-aconitine |
| M6 | 3.5 | 644 | 612, 584, 552 | Dehydrogen-aconitine |

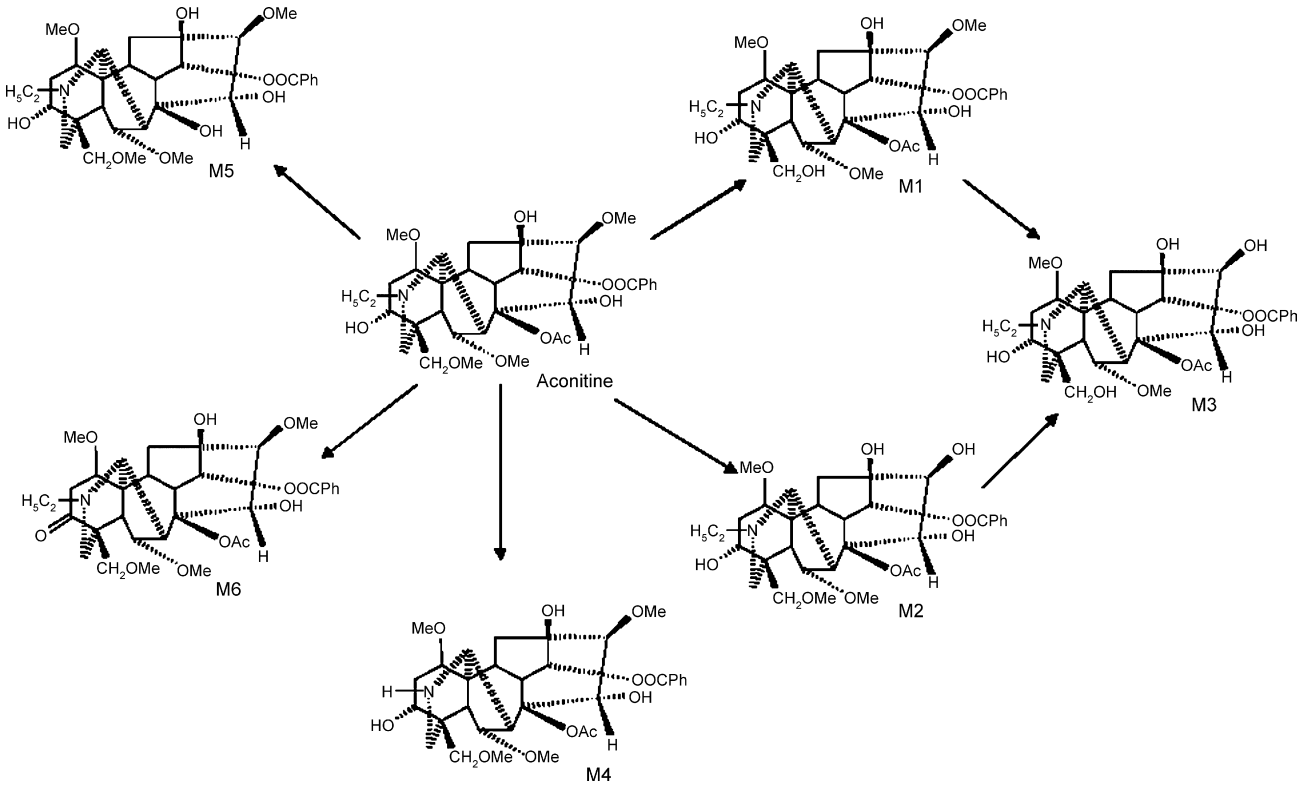


Fig. 4. Proposed metabolic pathways of aconitine in rat liver microsomes.

a pseudo-molecule $[M+H]^+$ ion at *m/z* 618 (Fig. 6E and G), indicating that they were isomers. Their pseudo-molecule ions were both 28 Da (*m/z* 646 → 618) lower than that of aconitine, suggesting that the loss of two methyl groups or a ethyl group from aconitine. The most informative fragment ion of $MS^2$ product ion of M3 was detected at *m/z* 498 (Fig. 6F) and corresponded to a loss of methoxyl ($-OCH_3$) and ethyl ($-C_2H_5$) from the precursor ion (*m/z* 558), which indicated that *N*-ethyl moiety in M3 was intact. The fragment ion at *m/z* 558 and 498 were 14 Da lower than those in the MS/MS spectra of M1 and

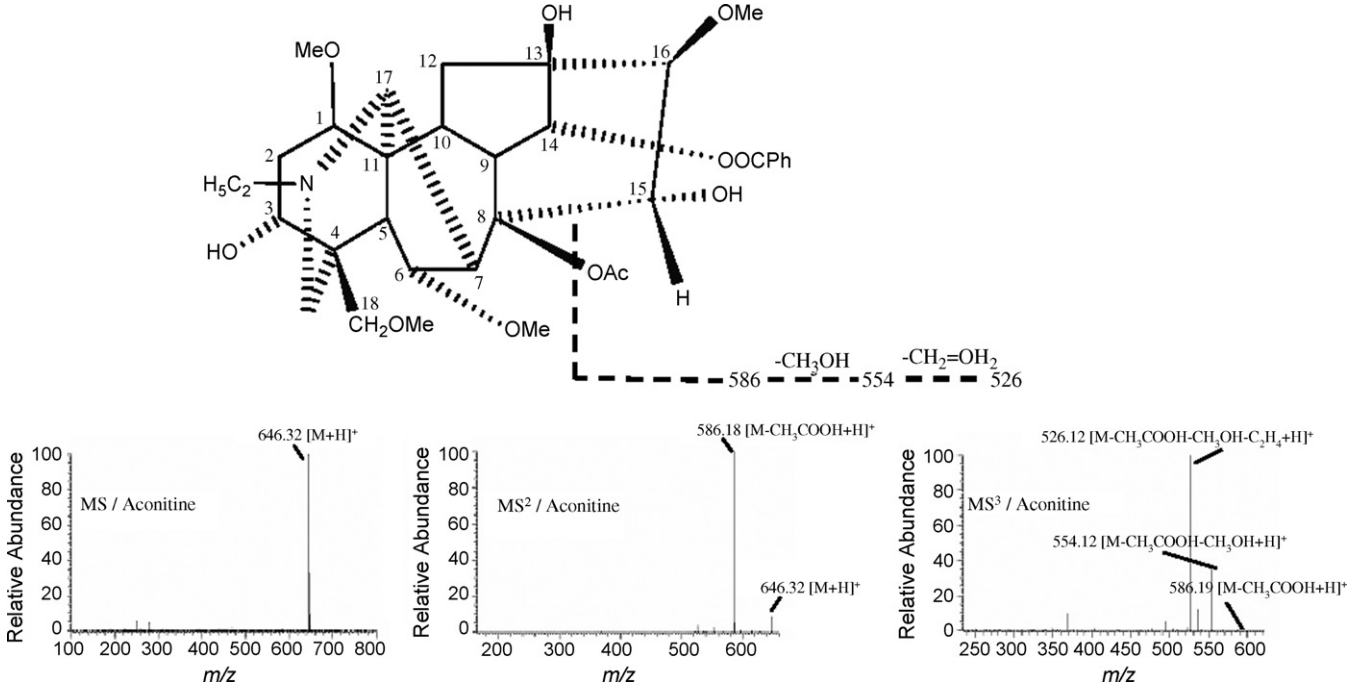


Fig. 5. Full scan, $MS^2$, and $MS^3$ spectra of $[M+H]^+$ (*m/z* 646) of aconitine.

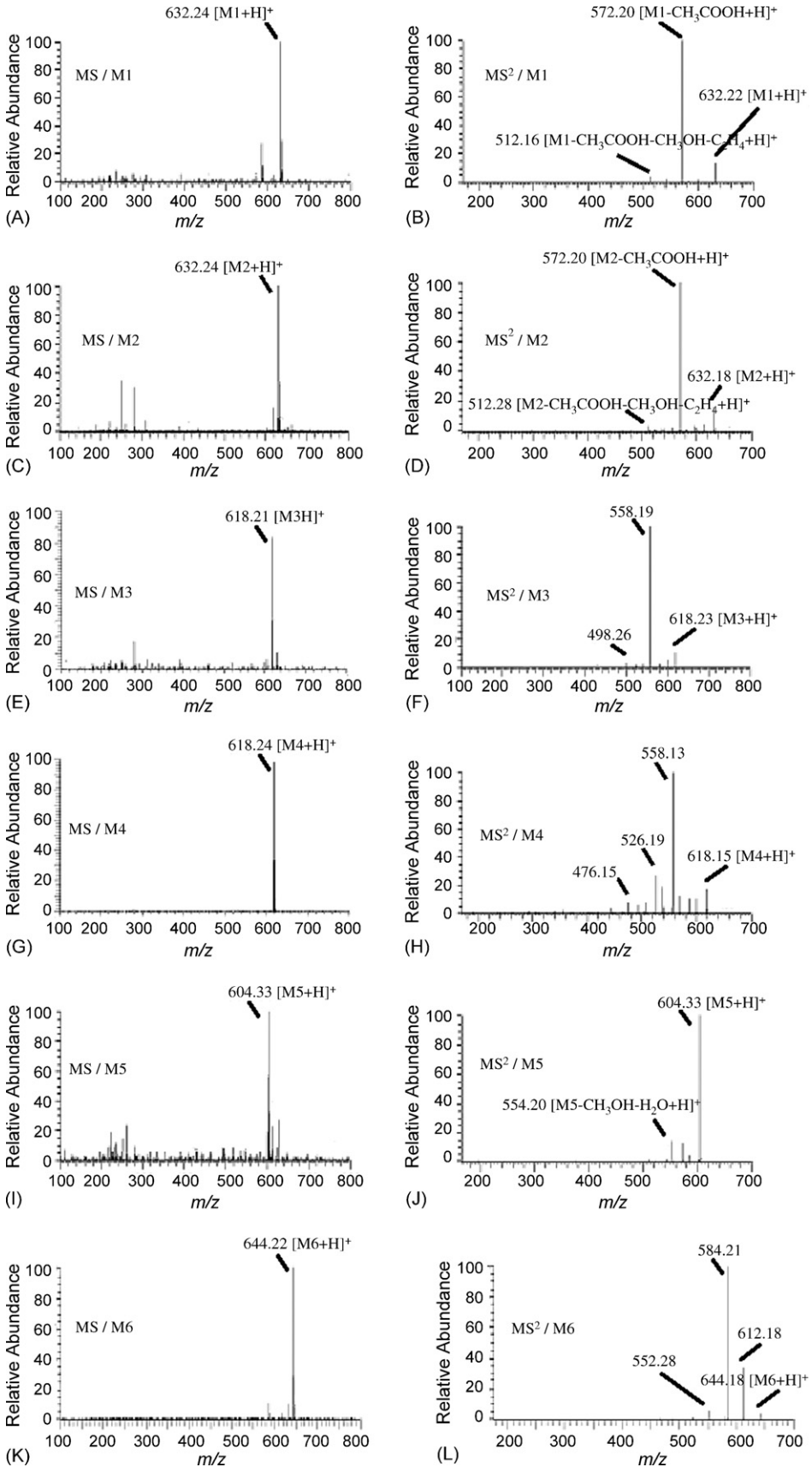


Fig. 6. LC/MS$^n$ spectra of acontine and its metabolites after incubation with rat liver microsomes and NADPH.

M2 (*m/z* 572, 512), suggesting that M3 was further demethylated derivate of M1 or M2. M3 was, therefore, identified as the *O*-didemethylated metabolite of aconitine. The MS/MS spectra of M4 showed fragment ions at *m/z* 558, 526 and 476 (Fig. 6H); the diagnostic ions at *m/z* 558, 526 were 28 Da lower than fragment ions in the MS/MS specta of aconitine, which indicated that the deethylation was carried out on the nitrogen atom, and therefore, M4 was identified as the *N*-deethylated metabolite of aconitine.

### 3.3.4. Metabolite M5

The retention time of M5 was 2.5 min (Fig. 3). It exhibited pseudo-molecule ion $[M+H]^+$ at *m/z* 604 (Fig. 6I), 42 Da (*m/z* 646 → 604) lower than that of aconitine, suggesting that M5 was deacetylation ($-COCH_3$) from aconitine. A CID product-ion spectrum of *m/z* 604 displayed major fragment ion at *m/z* 554 (Fig. 6J). The loss of 50 Da from the precursor ion (*m/z* 604 → 554) was proposed to arise via the loss of neutral molecules of $CH_3OH$ and $H_2O$. This fragmentation mode was different from aconitine, M1, M2, M3 and M4, which had a loss of the neutral molecular $CH_3COOH$ (neutral loss of 60 Da) and no loss of $H_2O$; so we postulated during the MS/MS scan of M5 thats the loss of $H_2O$ came from the hydroxyl formed by deacetylation of the acetate group [15]. M5, therefore, was confirmed as 8-*O*-deacetyl-aconitine, named benzoylaconitine.

### 3.3.5. Metabolite M6

Metabolite M6 had HPLC retention time of 3.5 min (Fig. 3). It had an $MH^+$ ion at *m/z* 644 (Fig. 6K). The pseudo molecule was 2 Da (*m/z* 646 → 644) lower than aconitine, the metabolic transformation might occur by direct dehydrogenation or by oxidation followed by a loss of water [16]. MS/MS scan of *m/z* 644 generated fragment ions at *m/z* 612, 584, and 552 (Fig. 6L); these fragment ions were all 2 mass unit lower than the corresponding fragments produced by the parent molecule, suggesting that no modification occurred on the parent molecule. M6, therefore, was identified as dehydrogenated metabolite of aconitine.

## 3.4. Influence of P450 inhibitors on aconitine metabolism

To determine which isoform of CYP could be involved in the aconitine metabolism, the effect of specific CYP inhibitors on aconitine metabolism at 40 μmol/L was investigated. TAO is the inhibitor of CYP3A, the increased concentration of TAO resulted in significant decrease in aconitine (Fig. 7E). The maximum inhibitory effect produced by CYP 3A inhibitor TAO was 81.3% (76–87%). As for CYP1A1/2 inhibitor ANF (Fig. 7A), the maximum inhibitory effect on the aconitine disappearance rate was 59.7% (28–63%). CYP2B1/2 inhibitor DPH, CYP2D inhibitor QUIN, and CYP2E1 inhibitor DDTC (Fig. 7B–D) did not produce obvious inhibitory effects on the aconitine disappearance rate.

## 3.5. Discussion

Aconitine is a neurotoxin that binds with receptor site II (associated with sodium channels). It causes arrhythmia in ventricular and Purkinje fiber preparations from the hearts and repetitive after-potentials and oscillations following stimulation of the nerve. These effects are caused by prolonged depolarization following action potentials that prevent complete repolarization of the excitable membrane [17]. It is well known that aconitine has high toxicity and can cause acute intoxication, but the detailed metabolism of aconitine and the cytochrome P450 isoforms involved had not been clarified. CYP isoenzymes collectively catalyze the oxidation of a multitude of xenobiotic and endobiotic substrates [18]. It is important to understand the metabolism of aconitine to identify the roles of CYP isoenzymes and metabolites of aconitine. In this experiment, study was focussed on characterization of the CYP isoenzymes participating in the metabolism of aconitine and identification of the metabolites of aconitine. We evaluated the metabolism of aconitine with an *in vitro* incubation of liver microsomes and chemical inhibition method.

Incubation with rat liver microsomes and NADPH indicated that the initial metabolic steps were catalyzed by CYP (Fig. 1). Therefore, the effects of CYP isoenzymes on aconitine metabolism was investigated using rat liver microsomes and specific inhibitors.

Medicinal plants are a major component of traditional medical system in Asia. Preparation of *Aconitum* root is employed in Chinese and Japanese medicine for analgesic, antirheumatic, and neurological indications. *Aconitum* root contains the highly toxic C19 diterpenoid alkaloid of aconitine. After ingestion, patients may present with typical signs and symptoms of aconitine poisoning. Death may occur from ventricular arrhythmias, which are most likely to develop within the first 24 h [19]. In the present study, the results from inhibition experiments given in Fig. 7 indicated that aconitine metabolism was mainly catalyzed by CYP3A with minor involvement of CYP1A1/2; obviously, CYP2E1, 2D and 2B1/2 did not participate in the metabolism of aconitine. These results suggested that metabolic change of aconitine may occur when drugs containing aconitine are used in combination with other drugs, that are inhibitors or inducers of CYP3A and/or CYP1A1/2, and may further affect the expression and activity of CYP3A and/or CYP1A1/2 and elicit drug interactions. Previous study reported that CYP1A2 activity can be induced by smoking, caffeinated drinks, and omeprazole, and inhibited by cimetidine, quinolones, furafylline, moclobemide, etc. [20]. CYP3A, the liver microsomal enzyme responsible for the oxidative metabolism of many drugs, is known to be induced by a variety of drugs, including dexamethasone, macrolide antibiotics [21] and ritonavir [22] and inhibited by itraconazole, ketoconazole, clarithromycin, erythromycin, etc. [23]. From a medical perspective, our results indicated that aconitine is likely to interact with the many drugs that are inducers or inhibitors of CYP3A and/or CYP1A. Indeed, there are already reports in the literature that many drugs change the metabolism of combined oral drugs; in some instances, these drug interactions can be life-threatening. A life-threatening ventricular arrhythmia associated with QT prolongation can occur when CYP3A inhibitors are coadministered with terfenadine, astemizole, cisapride or pimozide [23]. Studies on the molecular mechanism of the toxic effects of aconitine showed that it could

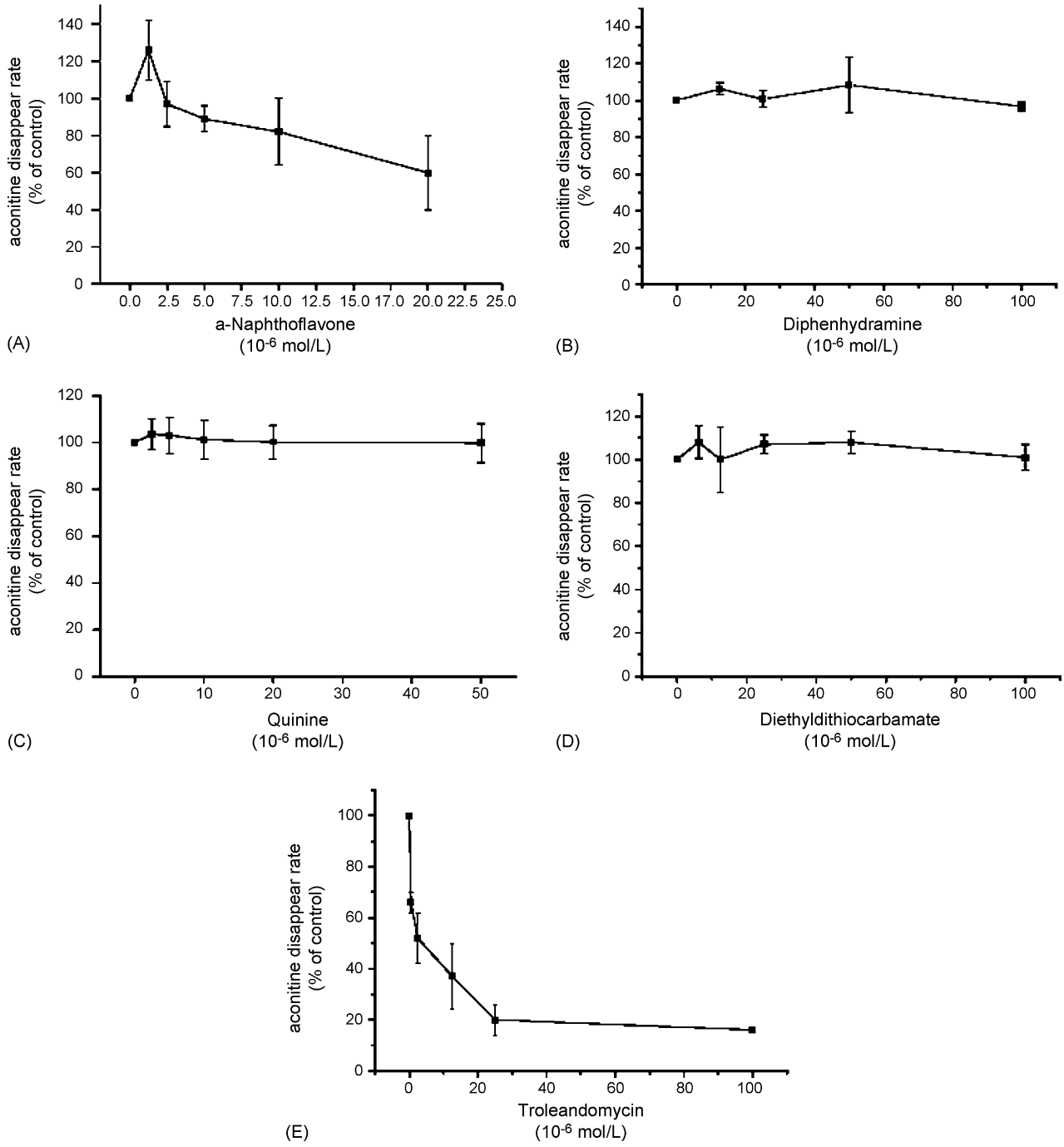


Fig. 7. Effects of various chemical inhibitors on cytochrome P450-mediated aconitine (40 μM) metabolism in rat liver microsomes. Data are expressed as mean ± S.E.M. ($n = 3$). The control value of aconitine disappearance rate was 72.0 ± 10.7 pmol/min/mg protein.

presumably induce arrhythmias by increasing ectopic impulse formation, making representative triggered activities, because of early as well as delayed after-depolarization [24]. Although the *Acontium* roots containing aconitine are highly toxic, they are frequently used as an important ingredient of many prescriptions used in traditional Chinese medicine to treat joint pain, arthritic and rheumatic diseases for over 2000 years by Chinese doctors. So our findings indicated that aconitine or *Aconitum* root containing aconitine should be used with caution by patients taking medications, especially those inhibitors or inducers of CYP3A and/or CYP1A1/2.

Previous studies [5,6] had identified some aconitine metabolites in human and rabbit urine *in vivo*. There appears to be some differences in the metabolites identified compared with the current *in vitro* study using rat liver microsomes; for example, metabolites of M1, M4 and M6 identified in rat liver microsomes appears to be novel. Firstly, we speculated it may be associated with an interspecies difference in aconitine metabolism. Sec-

ondly, the high toxicity of aconitine limits the higher oral dosage for *in vivo* study, whereas the current *in vitro* study using rat liver microsomes had no such a limited dosage and may contribute to identify more metabolites than an *in vitro* study.

Aconitine is metabolized to at least six major metabolites (M1–M6) in rat liver microsomes. Compared with aconitine, the metabolites only changed on side chains, suggesting that the metabolites may be also active. The high toxicity of *Aconitum* alkaloids are attributed to the acetyl group at C8, the hydroxyl group at C13, four methoxyl groups at C1, C6, C16, and C18, and the benzoylester group at C14 [25] and derivative by hydrolysis at the C8 acetyl ester (M5) is much less toxic than the parent drug (aconitine) [26]. M1, M2, and M3 also removed some functional groups responsible for toxicity from the parent molecule. So the metabolites of aconitine in rat liver microsomes may result in active compounds with less toxicity. When either the C8 or C14 groups were hydrolyzed and formed monoester structure, the derivatives were less toxic but had strong pharmacological effects of antinociceptive, antiarrhythmic, and antiepileptiform properties [25]. So the M5 with monoester group, to some extent, had less toxicity and more pharmacological effect than aconitine. These results may be useful for research on *Aconitum* alkaloid poisoning and metabolic detoxification.

## 4. Conclusion

Aconitine was biotransformed into at least six metabolites in rat liver microsomal incubates, and *O*-demethylation and *N*-deethylation were the main metabolic pathways. Aconitine metabolism in rat liver microsomes is mediated primarily by CYP 3A1/2, with a probable secondary contribution of CYP 1A1/2. Because CYP3A1/2 and 1A1/2 are involved in the metabolism of numerous important drugs and chemicals, further studies are required to ascertain whether aconitine has a clinically relevant metabolic interaction with CYP3A and/or CYP1A1/2 substrates and/or inhibitors, and to explain serious side-effects caused by drug–drug, drug–herb or herb–herb interactions based on CYP. The structure of aconitine is so complex and contains so many similar moieties that it would be extremely difficult to unambiguously identify metabolites solely using mass spectrometry; NMR data would be ideal for such compounds, but it may be difficult to generate sufficient amounts of metabolites. For these reasons, the metabolite characterization of the present study is rather preliminary and speculative, but it may be helpful to understand the metabolic profile of aconitine. Owing to the specie differences of P450, further studies using human liver microsomes will show whether the CYP-dependent metabolism of aconitine in rat and human is comparable.

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