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# Co-administration with simvastatin or lovastatin alters the pharmacokinetic profile of sinomenine in rats through cytochrome P450-mediated pathways

Yuan Wang[1], Yi Jin[1], Xinming Yun, Meijing Wang, Yue Dai*, Yufeng Xia*

*School of Traditional Chinese Pharmacy, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, China*

ARTICLE INFO

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Sinomenine (PubChem CID: 5459308)
Simvastatin (PubChem CID: 54454)
Lovastatin (PubChem CID: 53232)
Dapsone (PubChem CID: 2955)
Dextromethorphan (PubChem CID: 5360696)
Propranolol hydrochloride (PubChem CID: 62882)
Verapamil (PubChem CID: 2520)
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Atorvastatin (PubChem CID: 60823)
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*Keywords:*
Sinomenine
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ABSTRACT

*Aims:* Sinomenine, an anti-rheumatoid arthritis drug used in China for decades, is usually co-administered with cardiovascular (CV) drugs to reduce arthritis-related risk of cardiovascular diseases. This study was to investigate whether and how CV drugs affect the pharmacokinetic profile of sinomenine.

*Main methods:* In rat liver microsomes (RLMs), the key metabolic enzymes of sinomenine were identified by using specific inhibitors. The influences of CV drugs, including propranolol, verapamil, warfarin, atorvastatin, simvastatin, and lovastatin, on the metabolism of sinomenine were examined. Cocktail probe, RT-qPCR, and western blotting were performed to unveil the underlying mechanism of the drug-drug interaction.

*Key findings:* The key metabolic enzymes of sinomenine were identified to be CYP3A1/2 and CYP2D1 in RLMs. Among the CV drugs screened, simvastatin and lovastatin were shown to inhibit the liver metabolism of sinomenine with *Ki* values of 13.00 and 25.83 μM, respectively. Single administration of simvastatin or lovastatin in rats increased the *AUC* value of sinomenine to 1.40- or 1.50-fold, and decreased the *CLz/F* value to 68.19% or 65.44%, respectively. In contrast, multiple administrations of simvastatin, but not lovastatin, increased the *CLz/F* value of sinomenine to 1.38-fold and decreased the *AUC* value to 71.59%. Further studies showed that the long-term administration of simvastatin could up-regulate the expression of CYP3A1/2 to account for the effect.

*Significance:* This study demonstrated the potential effect of simvastatin and lovastatin on the metabolism of sinomenine for the first time. The findings provide guidelines for the co-administration of sinomenine with simvastatin or lovastatin in clinic.

## 1. Introduction

Sinomenine, an isoquinoline alkaloid (Fig. 1) isolated from the Sinomenii Caulis, the dried plant stems of *Sinomenium acutum* (Thunb) Rehd. et Wils. or *S. acutum* (Thunb.) Rehd. et Wils var. *cinereum* Rehd. et Wils., has a variety of pharmacological activities including anti-arthritic, immunosuppressive and anti-cancer effects [1–4]. It has been approved in China as an anti-rheumatoid arthritis (RA) drug with a trade name of ZhengQing FengTongNing and marketed for decades [5,6]. The therapeutic efficacy of sinomenine in patients has been well confirmed with few side effects documented.

RA, a systemic autoimmune disease with a prevalence of approximate 0.42% in China [7], requires long-term administration of therapeutic drugs and is incurable. Epidemiologic studies show that RA causes dyslipidemia and atherosclerosis along with the disease course, increasing the risk of coronary heart diseases in RA patients. The incidence cardiovascular (CV) diseases in RA patients is 2–5 times higher than in normal humans, and the mortality increases by about 40% [8,9]. With coronary heart diseases being the primary cause of RA-related death, CV drugs, such as propranolol, verapamil, warfarin and statins, are frequently co-administered with sinomenine to reduce the risk of CV diseases. However, the potential interactions between the CV drugs and sinomenine remains poorly understood.

The pharmacokinetics of sinomenine has been investigated in different species, including rats, dogs and human beings. The results have established oral uptake as the optimal administration route. After oral administration, sinomenine was quickly absorbed and widely distributed, and might be metabolized in the liver and kidney [10–12]. Although the precise pharmacokinetic profile of sinomenine and its effect on cytochrome P450 (CYP) enzymes are still not fully elucidated,

*Abbreviations:* RA, rheumatoid arthritis; CV, cardiovascular; CYP enzyme, cytochrome P450 enzyme; DDI, drug-drug interaction; RLMs, rat liver microsomes; IS, internal standard

* Corresponding authors.
*E-mail addresses:* yuedaicpu@126.com (Y. Dai), yfxiacpu@126.com (Y. Xia).
[1] These authors contributed equally to this work.

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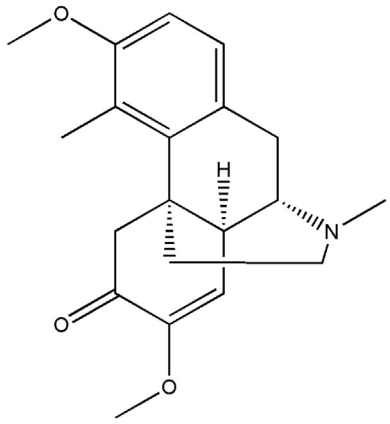

**Fig. 1.** The chemical structure of sinomenine.

one report indicated that sinomenine could inhibit the activity of CYP2C19 in the human liver microsomes *in vitro* [13]. On the other hand, most CV drugs are metabolized by CYP enzymes [14–16], and have potentials to alter the activities of CYP enzymes by either inhibition or induction [17]. These findings suggest that CV drugs may affect the pharmacokinetic profile, and therefore interfere with the therapeutic efficacy of sinomenine when they are co-administered.

The present study aimed to investigate the metabolic characteristics of sinomenine using rat liver microsomes (RLM) and identify the key metabolic enzymes. Furthermore, cocktail probe, RT-qPCR, and western blotting were performed to study the underlying mechanisms by which the statin drugs affect the activities of CYP enzymes and therefore the metabolism of sinomenine. The findings will provide a guideline for the safe and effective use of sinomenine.

## 2. Materials and methods

### 2.1. Materials and chemicals

Sinomenine, strychnine (internal standard, IS), α-naphthoflavone, cimetidine, quinine, 4-methylpyrazole, ketoconazole, simvastatin, warfarin sodium, propranolol hydrochloride, verapamil, atorvastatin, lovastatin, dapsone, dextromethorphan, berberine (IS, purity > 99%) were provided by National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). BCA protein assay kit was purchased from Beyotime Biotechnology (Jiangsu, China). HiScript ™ Q RT Super Mix and AceQ ™ qPCR SYBR® Green Master Mix were obtained from Vazyme Biotech Co., Ltd. (Nanjing, China). Human recombinant P450s were purchased from Cypex Ltd. (Shanghai, China). β-Nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate (NADPH, purity > 93%), CYP3A1 polyclonal antibody was purchased from Sunbio Technology Co., Ltd. (Nanjing, China), and CYP3A2 polyclonal antibody was purchased from Abcam (Cambridge, UK). β-actin antibody was the product of Aksomics (Shanghai, China). HPLC-grade acetonitrile was from Merck Millipore (Darmstadt, Germany). Other analytical grade chemicals and agents were obtained commercially. Ultrapure water was prepared using a Milli-Q reagent water system (Millipore, Bedford, MA, USA).

### 2.2. Animals

Male Sprague-Dawley (SD) rats (weighing 200–220 g) were supplied by the Qinglongshan Experimental Animal Center (Nanjing, China). The rats were housed under controlled conditions of temperature (22 ± 2 °C) and relative humidity (50 ± 10%) with a 12 h light/dark cycle with water and food freely available. The rats were acclimated to the housing environment for 7 days, and then fasted overnight before use with free access to water. All the studies were conducted in accordance with the guidelines of current ethical regulations for institutional animal care and use at China Pharmaceutical University.

### 2.3. Enzymatic kinetics of sinomenine

RLMs were prepared by a differential centrifugation following a previously published protocol with a slight modification [18]. Briefly, rats were sacrificed by a decapitation, and the livers were removed and minced carefully using a pair of scissors. The small pieces of liver were homogenized with ice-cold Tris-HCl buffer (50 mM, pH 7.4) and centrifuged at 9000 ×*g* for 20 min. The supernatant was collected and centrifuged again at 100000 ×*g* for 60 min to collect the pellet. All the procedures were performed at 0–4 °C. The pooled microsome pellets were resuspended in 30% glycerol-Tris-HCl buffer. The preparations were stored at −80 °C until use. The microsomal protein concentration was determined using a BCA protein assay kit.

The incubation mixture prepared in Tris-HCl buffer (50 mM, pH 7.4) consisted of RLMs (0.4 mg protein/mL), different concentrations (1, 2, 5, 10, 20, 50, 100, 200, 300, 400, 500 μM) of sinomenine, $MgCl_2$ (5 mM) and NADPH (1 mM) with a total volume of 200 μL. The organic solvent used in the incubation mixture was < 1‰ to minimize its influence on the enzyme activity. The mixture was preincubated at 37 °C for 5 min and initiated by adding NADPH. After incubation at 37 °C for 40 min in a shaking water bath, the reaction was terminated by adding 1 mL ice-cold ethyl acetate. Then, 10 μL IS (strychnine, 74.63 μM) and 100 μL NaOH (1 M) were added to the final mixtures. After vortex-shaking for 3 min and centrifugation at 12000 rpm for 10 min, the supernatant (organic phase, about 800 μL) was evaporated to dryness under vacuum at 37 °C. The residue was reconstituted in 100 μL phosphoric acid (0.05%) and centrifugated at 12000 rpm for 10 min. The supernatant was transferred into an autosampler vial for HPLC analysis. All the experiments were conducted in triplicates.

An Agilent 1260 HPLC system (Agilent Technologies, Palo Alto, CA, USA) was used to inject 20 μL aliquots of the processed samples on a Shim-pack CLC-ODS $C_{18}$ column (5 μm, 6 mm × 150 mm, Shimadzu Corporation, Kyoto, Japan), which was kept at ambient temperature. The mobile phase was acetonitrile and 0.05% phosphoric acid (15:85, v/v) at a flow rate of 1.0 mL/min. The detection wavelength was 263 nm, and the column temperature was maintained at 40 °C.

### 2.4. Identification of the CYP enzymes responsible for the metabolism of sinomenine

CYP enzyme subtypes responsible for the metabolism of sinomenine in RLMs were identified by using specific chemical inhibitors. The incubation mixture consisted of sinomenine (100 μM) and various chemical inhibitors, including ketoconazole (1, 2, 5 μM; inhibitor of CYP3A1/2), α-naphthoflavone (1, 2, 5 μM; inhibitor of CYP1A2), quinine (1, 5, 10 μM; inhibitor of CYP2D1), 4-methylpyrazole (1, 5, 10 μM; inhibitor of CYP2E1), or cimetidine (1, 5, 10 μM; inhibitor of CYP2C6/11). Control incubation was performed in the absence of chemical inhibitors. Other conditions and methods of the incubation system were the same as those of enzymatic kinetics assay.

### 2.5. Metabolism of sinomenine by CYP3A4 or CYP2D6 using recombinant human CYP enzymes

Studies were performed to investigate the metabolism of sinomenine by CYP3A4 or CYP2D6 using recombinant human CYP enzymes. The incubation mixture prepared in appropriate buffers consisted of recombinant human CYP enzymes (CYP3A4 or CYP2D6, 500 pmol/mL), sinomenine (0.5 μM), and NADPH (2 mM) with a total volume of 80 μL.