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Original Article

Effect of Radix Sophorae Tonkinensis on the activity of cytochrome P450 isoforms in rats

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Abstract: *Radix Sophorae Tonkinensis* (S. tonkinensis) is the processed lateral root of *Sophora subprostrata* (Leguminosae) that widely distributed over the southwest China. *Radix Sophorae Tonkinensis* has been widely used as a Chinese medicinal herb for the treatment of disease such as jaundice, inflammation, and aches. Herein, in order to investigate the effects of *Radix Sophorae Tonkinensis* on the metabolic capacity of rat cytochrome P450 (CYP) enzymes, we employed a cocktail method to evaluate the activities of CYP1A2, CYP2D6, CYP3A4, CYP2C19, CYP2C9 and CYP2B6. The experimental rats were randomly divided into two groups (control group and *Radix Sophorae Tonkinensis* treated group). The *Radix Sophorae Tonkinensis* treated group rats were given 5 g/kg *Radix Sophorae Tonkinensis* by continuous intragastric administration for 14 days. The mixture of six probes (phenacetin, metroprolol, midazolam, omeprazole, tolbutamide and bupropion) was given to rats by intragastric administration. The concentrations of probe drugs in rat plasma were measured by UPLC-MS/MS. The results showed that continuous intragastric administration for 14 days may inhibit the activities of rat CYP450 isoforms CYP2D6, CYP2C19 and CYP2B6. This finding may provide guidance for rational clinical uses of *Radix Sophorae Tonkinensis*.

Keywords: CYP450, *Radix Sophorae Tonkinensis*, cocktail, rat

Introduction

The dried roots and rhizomes of *Sophora tonkinensis* Gapnep (Leguminosae) named as shandou-gen (*Radix Sophorae Tonkinensis*) have been widely used as a Chinese herb for the treatment of acute pharyngolaryngeal infections and sore throats [1, 2]. In *Radix Sophorae Tonkinensis*, the quinolizidine alkaloids are the most important ingredient of this poisonous Chinese herb. However, some of alkaloids exhibit potentially useful pharmacological effects such as analgesic, antipyretic, anti-inflammatory, anti-tumor and anti-arrhythmia activities because of their toxicity to humans and livestock [3].

Cytochrome P450 (CYP450) is one of the most important drug-metabolizing enzymes that present in the liver with largest number and highest abundance [4-6]. Previous studies show that CYP450 are essential for most biotransformation of xenobiotics and play a critical role in the drug metabolism in both humans and rats [7]. Furthermore, various probe drugs have been widely employed in clinical investigations of drug metabolism for the evaluation of CYP450 activities [8-11]. Probe drugs are one kind of compounds that catalyzed by CYP isoforms specifically, and the activities of CYP isoforms can be reflected by the pharmacokinetic changes of specific probe drug.

To our knowledge, few studies have been reported about the hepatic toxicity of *Radix Sophorae Tonkinensis* from literatures. In this study, the cocktail probe drugs approach was used to evaluate the induction or inhibition effects of *Radix Sophorae Tonkinensis* on the activities of rat CYP450 isoforms such as CYP1A2, CYP2D6, CYP3A4, CYP2C19, CYP2C9 and CYP2B6. The induction or inhibition effects

are expressed by the pharmacokinetic changes of six specific probe drugs.

Experimental

Chemicals and reagents

Phenacetin, metroprolol, midazolam, omeprazole, tolbutamide and bupropion (all > 98%) and the internal standard diazepam (IS) were purchased from Sigma-Aldrich Company (St. Louis, USA). HPLC grade acetonitrile and methanol were purchased from Merck Company (Darmstadt, Germany). All other chemicals were of analytical grade. Ultra-pure water (resistance > 18 mΩ) were prepared by Millipore Milli-Q purification system (Bedford, USA).

Animals

Male Sprague-Dawley rats (250 ± 20 g) were obtained from Shanghai Laboratory Animal Co., Ltd. The animal license number was SCXK (Shanghai) 2012-0005. All twenty rats were housed at Laboratory Animal Research Center of Wenzhou Medical University. Animals were housed under controlled conditions (22°C) with a natural light-dark cycle. All experimental procedures were conducted according to the Institutional Animal Care guidelines and approved ethically by the Administration Committee of Experimental Animals, Laboratory Animal Center of Wenzhou Medical University.

Instrumentation and conditions

The compounds were analyzed by using UPLC-MS/MS with ACQUITY I-Class UPLC and a XEVO TQD triple quadrupole mass spectrometer (Waters Corp., Milford, MA, USA) that equipped with an electrospray ionization (ESI) interface. The UPLC system included a Binary Solvent Manager (BSM) and a Sample Manager with Flow-Through Needle (SM-FTN). Data acquisition and instrument control were performed on the Masslynx 4.1 software (Waters Corp., Milford, MA, USA).

Phenacetin, metroprolol, midazolam, omeprazole, tolbutamide, bupropion and diazepam (IS) were separated with a UPLC® BEH C18 column (2.1 mm × 100 mm, 1.7 μm, Waters, USA) at constant temperature 40°C. The initial mobile phase consisted of acetonitrile and water (containing 0.1% formic acid) with gradient elution at a flow rate of 0.4 mL/min and an injection volume of 2 μL. Elution was performed in a linear gradient, with the acetonitrile content changing from 30 to 60% in 0.3-1.8 min and increasing up to 95% over 0.2 min. The acetonitrile content was maintained at 95% for 0.5 min and decreased to 30% within 0.1 min, and then maintained at 30% for 0.4 min. The total run time of the analysis was 3 min. After each injection, the sample manager was cleaned by a needle wash process with a strong wash (methanol-water, 50/50, V/V) and a weak wash (methanol-water, 10/90, V/V).

The mass spectrometric detection was performed on a triple-quadrupole mass spectrometer that equipped with an ESI interface in a positive mode. Nitrogen was used as the desolvation gas (1000 L/h) and cone gas (50 L/h). The selected ion monitoring conditions were defined as follows: capillary voltage 2.5 kV; source temperature 150°C; desolvation temperature 500°C. The multiple reaction monitoring (MRM) mode of m/z 180.1→109.9 for phenacetin, m/z 268.1→115.8 for metroprolol, m/z 326.0→291.0 for midazolam, m/z 346.1→197.8 for omeprazole, m/z 271.2→155.1 for tolbutamide, m/z 240.1→184.1 for bupropion and m/z 285.1→193.1 for IS was used for quantitative analysis.

Preparation of standard solutions

Stock solutions of phenacetin, metroprolol, midazolam, omeprazole, tolbutamide, bupropion and IS in 1.0 mg/mL were prepared in methanol, respectively. The working solutions of each analyte were prepared by serial dilution of the stock solution with methanol. All of the solutions were stored at 4°C before use.

The calibration standards were prepared by spiking blank rat plasma with appropriate amounts of phenacetin, metroprolol, midazolam, omeprazole, tolbutamide and bupropion. Calibration plots of each probe drug in plasma were constructed in the range of 10-2000 ng/mL (2, 10, 20, 50, 100, 200, 500, 1000 and 2000 ng/mL).

Pharmacokinetic study

Twenty male Sprague-Dawley rats (250 ± 20 g) were randomly divided to control group and *Radix Sophorae Tonkinensis* group (n = 10).

Table 1. Pharmacokinetic parameters of bupropion, omeprazole, phenacetin, tolbutamide, midazolam and metroprolol in control group and *Radix Sophorae Tonkinensis* treated group rats (mean ± SD, n = 10)

Compound	Group	$AUC_{(0-t)}$	$AUC_{(0-\infty)}$	t1/2z	T_{max}	CLz/F	Vz/F	C_{max}
		μg/L*h	μg/L*h	h	h	L/h/kg	L/kg	ug/L
Bupropion	Control	184.4 ± 211.7	194.5 ± 217.9	3.3 ± 1.2	0.4 ± 0.2	83.0 ± 38.4	394.8 ± 212.4	83.6 ± 69.2
	Radix Sophorae Tonkinensis	601.3 ± 367.2**	610.1 ± 370.4**	2.0 ± 0.4**	0.5	21.2 ± 10.7**	62.6 ± 42.7**	188.3 ± 87.7*
Omeprazole	Control	76.0 ± 47.4	78.2 ± 47.7	0.6 ± 0.2	0.2 ± 0.2	154.5 ± 56.3	135.3 ± 52.2	86.4 ± 43.3
	Radix Sophorae Tonkinensis	331.2 ± 114.6**	355.1 ± 141.8**	1.0 ± 0.5*	0.2 ± 0.2	32.3 ± 13.3**	43.1 ± 15.8**	351.7 ± 167.0**
Phenacetin	Control	584.6 ± 584.6	598.5 ± 581.5	1.9 ± 2.1	0.2 ± 0.2	24.7 ± 11.6	81.2 ± 96.9	657.6 ± 252.8
	Radix Sophorae Tonkinensis	1220.3 ± 767.2	1230.9 ± 783.7	0.5 ± 0.1	0.6 ± 0.6	15.2 ± 18.2	10.3 ± 13.4	1160.2 ± 531.3*
Tolbutamide	Control	63308.6± 16700.4	124720.9 ± 91266.1	41.1 ± 35.4	11.9 ± 8.7	0.013 ± 0.010	0.5 ± 0.2	1893.6 ± 560.8
	Radix Sophorae Tonkinensis	73704.6 ± 21138.8	77987.5 ± 19819.3	10.9 ± 5.8*	3.6 ± 3.2**	0.014 ± 0.004	0.2 ± 0.1**	2834.5 ± 586.0**
Midazolam	Control	242.0 ± 195.8	264.3 ± 218.0	0.9 ± 0.3	0.2 ± 0.2	51.8 ± 52.5	53.5 ± 44.3	156.3 ± 114.2
	Radix Sophorae Tonkinensis	565.6 ± 533.6	645.6 ± 645.3	1.2 ± 0.4*	0.6 ± 0.2**	13.2 ± 7.3	23.6 ± 17.2	278.3 ± 197.8
Metroprolol	Control	1577.9 ± 769.3	1603.7 ± 778.0	2.1 ± 0.6	0.5 ± 0.0	8.2 ± 5.9	24.5 ± 18.6	765.2 ± 383.8
	Radix Sophorae Tonkinensis	3567.6 ± 2148.5*	3593.6 ± 2143.5*	1.3 ± 1.1	0.6 ± 0.2	3.7 ± 2.5*	6.6 ± 5.5*	1372.2 ± 689.6*

(Compared Radix Sophorae Tonkinensis treated group with the control group, *: $P < 0.05$, **: $P < 0.01$).

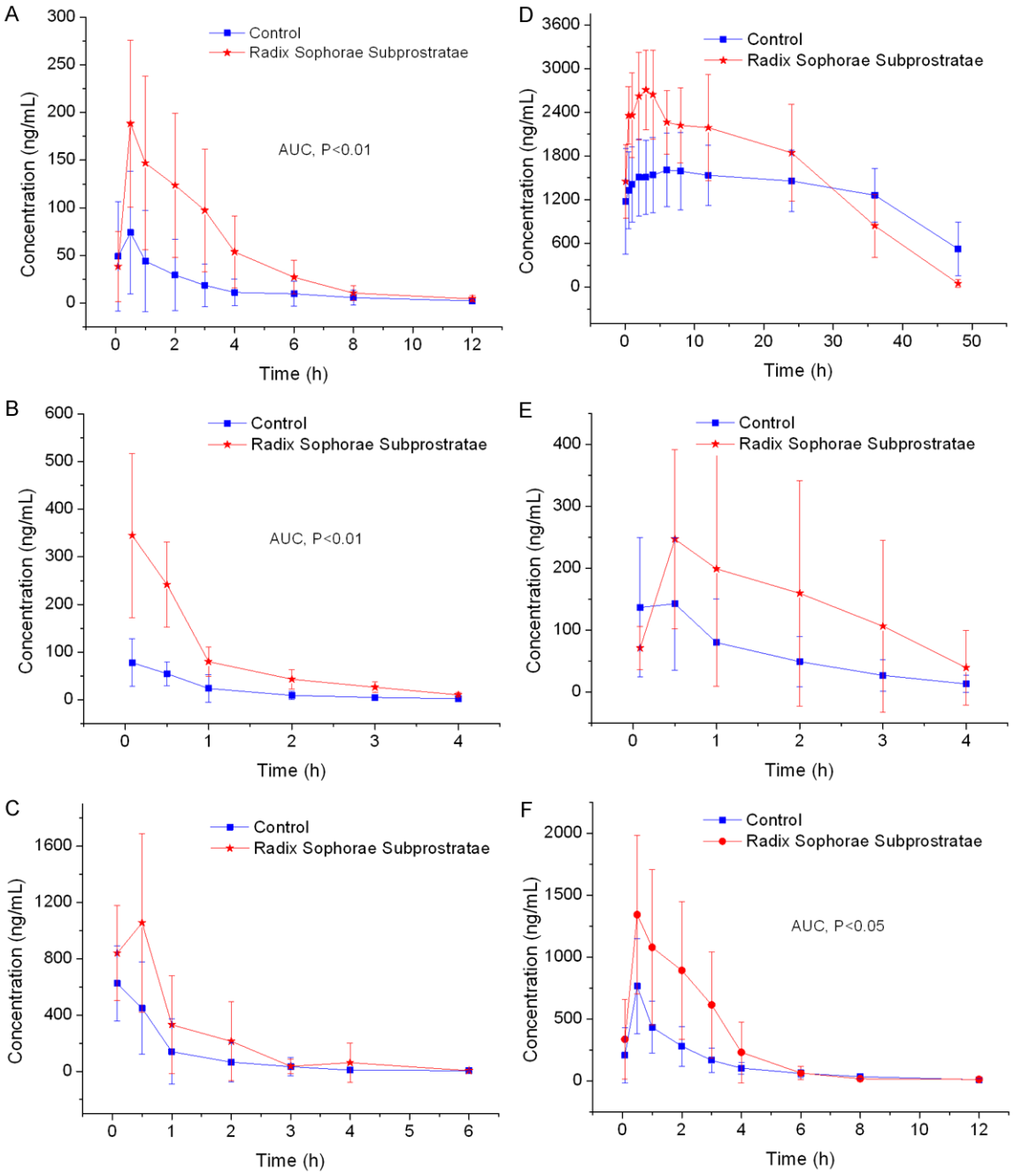

Figure 1. The pharmacokinetics profiles of bupropion (A), omeprazole (B), phenacetin (C), tolbutamide (D), midazolam (E) and metroprolol (F) in control group and Radix Sophorae Tonkinensis treated group rats (n = 10).

Saline was given to control group by continuous intragastric administration for 14 days; while *Radix Sophorae Tonkinensis* was given to *Radix Sophorae Tonkinensis* group (5 g/kg) by continuous intragastric administration for 14 days. After two days, the mixed six probe drugs (phenacetin, metroprolol, midazolam, omeprazole, tolbutamide and bupropion were 10 mg/kg, 10 mg/kg, 5 mg/kg, 10 mg/kg, 1 mg/kg and 10 mg/kg) were given to the *Radix Sophorae Tonkinensis* and control group by oral administration, respectively.

Blood samples (0.3 mL) were collected into heparinized 1.5 mL polythene tubes from the tail vein at 0.0833, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 h after oral administration of probe drugs. 100 μL of plasma was obtained from each

blood sample after centrifugation at 8000 r/min for 5 min.

The plasma samples were extracted and analyzed by UPLC-MS/MS. In a 1.5 mL centrifuge tube, an aliquot of 10 μL of the internal standard working solution (0.5 μg/mL) was added into 100 μL of collected plasma sample, and followed by the addition of 0.2 mL acetonitrile. After vortex-mixing for 1.0 min, the sample was centrifuged at 15000 rmp for 10 min. The supernatant (2 μL) was injected into the UPLC-MS/MS system for analysis.

Concentration of probe drugs in plasma versus time for each rat was analyzed by using DAS software (Version 3.0, Drug Clinical Research Center of Shanghai University of T.C.M and Shanghai BioGuider Medicinal Technology Co., Ltd., China). The pharmacokinetic parameters of the test group and control group were analyzed by SPSS 18.0 statistical software; statistical significance was assessed by t-test ($p < 0.05$ was considered as statistically significant).

Results

Method validation

The concentrations of phenacetin, metroprolol, midazolam, omeprazole, tolbutamide and bupropion in rat plasma were simultaneously determined by a sensitive and simple UPLC-MS/MS method. Calibration curves for six probe drugs were built from linear regression of peak area ratios against concentrations, respectively. The calibration plot of the probe drugs is in the range of 2-2000 ng/mL ($r > 0.995$). The LLOQ for each probe drug in plasma was 2 ng/mL. The relative standard deviation (RSD%) of the six probe drugs in low, medium and high concentrations were less than 14%. The intra-day and inter-day relative error (RE %) ranged from -8% to 12%. The results demonstrate that the values were within the acceptable range and the method was accurate. The extraction recoveries were ranged from 85% to 98%. The matrix effect and percent nominal concentration were more than 86% or less than 115%, respectively.

Pharmacokinetic study

The main pharmacokinetic parameters of phenacetin, metroprolol, midazolam, omeprazole, tolbutamide and bupropion from non-compartment model analysis were summarized in **Table 1**. The representative profiles of concentration of probe drugs (phenacetin, metroprolol, midazolam, omeprazole, tolbutamide and bupropion) vs. time in 20 rats were presented in **Figure 1**. From **Figure 1**, we observed that the AUC and C_{max} of metroprolol, omeprazole and bupropion in *Radix Sophorae Tonkinensis* treated group is higher than that of control group, which is consistent with the result in **Table 1**.

Comparing the results of *Radix Sophorae Tonkinensis* treated group with the control group in **Table 1**, we also observed that the pharmacokinetic parameters of metroprolol, omeprazole and bupropion experienced changes with increased $AUC_{(0-t)}$ ($P < 0.01$ or 0.05) and decreased CL ($P < 0.01$ or 0.05). The results indicate that continuous administration of *Radix Sophorae Tonkinensis* may inhibit the activities of rat CYP2D6, CYP2C19 and CYP2B6 enzyme. On the other hand, no significant difference for AUC of phenacetin, midazolam and tolbutamide ($P > 0.05$) was observed between *Radix Sophorae Tonkinensis* treated group with the control group. The results indicated that the *Radix Sophorae Tonkinensis* may not induce or inhibit the activities of rat CYP1A2, CYP3A4 and CYP2C9 enzyme.

Discussion

Extract of *Radix Sophorae Tonkinensis* exhibits pharmacological activity with therapeutic effect, but it also causes side effects such as liver toxicity [12]. The therapeutic effect versus the toxicity of oxymatrine and matrine has been extensively discussed in the literature for many years [1]. As *Radix Sophorae Tonkinensis* is always administered in combination with other drugs, interactions between *Radix Sophorae Tonkinensis* and other drugs may cause the risk of either adverse effects or diminished efficacy.

In general, changes in pharmacokinetics are thought to be caused by drug-drug or drug-food interactions [13]. On the other hand, a large number of drugs are metabolized by CYP enzymes in the liver, and more than 90% of drug-drug interactions occur at the CYP-catalyzed step [14, 15]. Additionally, supplement-drug interactions that involved CYP-catalyzed reaction are also found to cause

severe adverse effects. For these reasons, we evaluated the effects of 14 days-intragastric administration of *Radix Sophorae Tonkinensis* on the activities of rat CYP enzymes using a cocktail method. More than 90% of drugs are known to be metabolized by the 6 CYP enzymes (CYP1A2, CYP2D6, CYP3A4, CYP2C19, CYP2C9 and CYP2B6), therefore, these 6 CYP enzymes were investigated in this study [16, 17].

In this study, we found that continuous intragastric administration of *Radix Sophorae Tonkinensis* for 14 days may inhibit the activities of rat CYP450 isoforms CYP2D6, CYP2C19 and CYP2B6. These results would give us valuable information for the clinicians about the interactions of *Radix Sophorae Tonkinensis* with other drugs.

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Disclosure of conflict of interest

None.

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