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Original Article

Effect of *Urena procumbens* on CYP450 isoforms activity of rats by cocktail method

Lianguo Chen[1], Shihui Bao[2], Shujun Wang[1], Jianhua Xiong[1], Paul Lee[3], Bik Luen Leung[4], Chongliang Lin[5], Lufeng Hu[5], Xuezhi Yang[5], Guanyang Lin[5], Yingxia Tan[5]

[1]Department of Pharmacy, Wenzhou People's Hospital Wenzhou 325000, China; [2]The Second Affiliated Hospital and Yuying Children's Hospital, Wenzhou Medical University, Wenzhou 325000, China; [3]Department of Medicine, The Third Affiliated Queen Mary Hospital, University of Hong Kong, Pokfulam, Hong Kong, China; [4]Department of Geriatric Medicine, The Fourth Affiliated Queen Elizabeth Hospital, Kowloon, Hong Kong, China; [5]The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325035, Zhejiang, PR, China

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Abstract: Urena procumbens, a traditional Chinese medicine, is widely used in diuretic, antipyretics applications, and also for the treatment of cough and rheumatic disease. In order to investigate the effects of Urena procumbens on the metabolic capacity of cytochrome P450 (CYP) enzymes, in the present study, a cocktail method was employed to evaluate the activities of CYP2B6, CYP1A2, CYP2C9, CYP2D6, CYP3A4 and CYP2C19 after Urena procumbens treatment. Twenty-four Sprague-Dawley rats were randomly divided into three groups (Low-group, High-group and control group with 8 rats in each group). Two different Urena procumbens treated group (Low-group, High-group) were given Urena procumbens 5, 10 g/kg one time by intragastric administration at every morning, and lasted for 7 days, respectively. Control group were given saline using the same method. On the eighth day, six probe drugs (10 mg/kg of bupropion, phenacetin, metroprolol, testosterone, omeprazole, and 1 mg/kg of tolbutamide) were given to 24 rats through intragastric administration, and the plasma concentrations were determined by Ultra Performance Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (UPLC-MS/MS). Compared to the control group, the pharmacokinetic parameters of bupropion, phenacetin, tolbutamide and metroprolol from Urena procumbens treated group experienced substantial changes with decreased $AUC_{(0-t)}$ ($P<0.05$) and increased CL ($P<0.05$) after dosage increase. It showed that Urena procumbens could induce the activities of CYP2B6, CYP1A2, CYP2C9, and CYP2D6. No significant difference was observed for AUC of omeprazole ($P>0.05$). The Urena procumbens was not able to induce or inhibit the activity of CYP2C19 enzyme. Induction of drug metabolizing enzyme by Urena procumbens would reduce the efficacy of other drugs which metabolized by CYP2B6, CYP1A2, CYP2C9, and CYP2D6. Therefore, careful attention should be paid when Urena procumbens and other drugs are administrated together.

Keywords: CYP450, *Urena procumbens*, cocktail, UPLC-MS/MS, rat

Introduction

Urena procumbens (Fan-tian-hua) is native to China, but it is grown in many tropical countries, including South Africa, the Americas, Africa, Australia and the United States (Florida). In traditional Chinese medicine, *Urena procumbens* has been used for diuretic, antipyretics applications, but also for the treatment of cough and rheumatic disease. Therefore, exploring the effect of *Urena procumbens* on CYP enzyme would facilitate to understand its metabolic behavior and avoid undesirable drug-drug interactions.

Cytochrome P450 (CYP) enzymes are essential for most biotransformation steps of xenobiotics and endogenous molecules [1, 2]. Furthermore, the CYP enzymes play a critical role in drug metabolism and supplement-drug interactions [3-5]. So far, no study about the effects of *Urena procumbens* on the metabolic capacity of CYP enzyme was reported. To avoid adverse effects from drug-drug interactions, it is highly important to understand the effects of a new chemical entity on drug-metabolizing enzymes [4, 6]. Therefore, in this study, six probe drugs were employed to evaluate effect of *Urena procumbens* on the metabolic capacity of CYP2B6,

CYP1A2, CYP2C9, CYP2D6, CYP3A4 and CYP2C19.

Material and methods

Chemicals

Bupropion, phenacetin, tolbutamide, metroprolol, testosterone, omeprazole (all >98%) and the internal standard diazepam (IS) were obtained from Sigma-Aldrich Company (St. Louis, USA). Ultra-pure water was prepared by Millipore Milli-Q purification system (Bedford, USA). Methanol and acetonitrile (HPLC grade) were obtained from Merck Company (Darmstadt, Germany).

Animals

Sprague-Dawley rats (male, 220 ± 20 g) purchased from Shanghai SLAC Laboratory Animal Co., Ltd. Animals were housed under a natural light-dark cycle conditions with controlled temperature (22°C). All twenty-four rats were housed at Wenzhou Medical University Laboratory Animal Research Center. All experimental procedures were approved ethically by the Wenzhou Medical University Administration Committee of Experimental Animals.

UPLC-MS/MS conditions

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

Bupropion, phenacetin, tolbutamide, metroprolol, testosterone, omeprazole and diazepam (IS) were separated using a Waters BEH C18 column (2.1 mm×100 mm, 1.7 μm) at constant temperature 40°C. The initial mobile phase consisted of 0.1% formic acid and acetonitrile with gradient elution at a flow rate of 0.4 mL/min and an injection volume of 2 μL. Elution was in a linear gradient, with acetonitrile 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.

The mass spectrometric detection was performed in a positive mode. Nitrogen was used as the cone gas (50 L/h) and desolvation gas (1000 L/h). The mass conditions were set as follows: source temperature 150°C; capillary voltage 2.5 kV; desolvation temperature 500°C. The multiple reaction monitoring (MRM) mode with m/z 180.1→109.9 for phenacetin, m/z 268.1→115.8 for metroprolol, m/z 289.0→97.0 for testosterone, 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.

Pharmacokinetics of probe drugs

Twenty-four rats (220 ± 20 g) were randomly divided into three different dosages of *Urena procumbens Linn* groups (Low-group, High-group and control group with 8 rats in each group). Two different dosages of *Urena procumbens Linn* group (Low-group, High-group) were given at 5, 10 g/kg one time by intragastric administration at every morning, and lasted for 7 days, respectively. Control group were given saline using the same administration method. At 8 days morning, six probe drugs (10 mg/kg of bupropion, phenacetin, metroprolol, testosterone and omeprazole, 1 mg/kg of tolbutamide) were mixed in corn oil and given to the rats in two different dosages of *Urena procumbens Linn* treated groups and control group by intragastric administration at a single dosage.

Blood (0.3 mL) samples 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 intragastric administration of six probe drugs. 100 μL of plasma was obtained from blood after centrifugation at 4000 *g* for 10 min. In a 1.5 mL centrifuge tube, 200 μL of acetonitrile (containing 50 ng/mL IS) was added into 100 μL of collected plasma. After vortex-mixing for 1.0 min, the sample was centrifuged at 13000 *g* for 15 min. Then supernatant (2 μL) was injected into the UPLC-MS/MS system for analysis.

Concentration of plasma probe drugs versus time was analyzed by using Version 3.0 Data Analysis System (Wenzhou Medical University, China).

Table 1. Pharmacokinetic parameters of bupropion and phenacetin in control group and *Urena procumbens* treated group rats (mean ± SD, n=8)

Parameters		$AUC_{(0-t)}$	$AUC_{(0-\infty)}$	$t_{1/2z}$	$C_{Lz/F}$	$V_{z/F}$	C_{max}
		ng/mL*h	ng/mL*h	h	L/h/kg	L/kg	ng/mL
Bupropion (CYP2B6)	Control	403.8 ± 158.9	432.0 ± 165.4	1.2 ± 0.4	26.5 ± 10.6	47.4 ± 32.1	194.0 ± 102.9
	Low	232.3 ± 156.8*	267.2 ± 160.8*	1.7 ± 1.4	50.0 ± 28.6*	109.9 ± 75.4*	118.7 ± 71.1
	High	97.0 ± 69.8**	101.1 ± 73.4**	1.1 ± 0.4	182.0 ± 151.5*	248.5 ± 198.5*	52.2 ± 26.8**
Phenacetin (CYP1A2)	Control	8053.4 ± 2480.7	8056.1 ± 2481.1	0.5 ± 0.1	1.4 ± 0.5	0.9 ± 0.5	5398.5 ± 1002.0
	Low	2487.1 ± 1836.3**	2495.9 ± 1832.0**	0.6 ± 0.3	6.2 ± 4.2**	5.8 ± 4.3**	2217.4 ± 1513.2**
	High	1795.5 ± 1407.9**	1798.9 ± 1408.0**	0.7 ± 0.2**	11.7 ± 11.6*	13.0 ± 16.0*	1788.1 ± 1448.4**

Compared *Urena procumbens* treated group with the control group, *: P<0.05, **: P<0.01.

Table 2. Pharmacokinetic parameters of tolbutamide and metroprolol in control group and *Urena procumbens* group rats (mean ± SD, n=8)

Parameters		$AUC_{(0-t)}$	$AUC_{(0-\infty)}$	$t_{1/2z}$	$C_{Lz/F}$	$V_{z/F}$	C_{max}
		ng/mL*h	ng/mL*h	h	L/h/kg	L/kg	ng/mL
Tolbutamide (CYP2C9)	Control	101706.2 ± 12808.6	106342.7 ± 13587.9	5.2 ± 0.3	0.010 ± 0.001	0.071 ± 0.008	9457.4 ± 1136.5
	Low	38208.2 ± 8946.9**	41070.0 ±10862.1**	5.6 ± 1.7	0.026 ± 0.007**	0.200 ± 0.039**	3548.6 ± 911.3**
	High	29803.9 ± 3331.7**	30560.1 ± 3554.9**	4.2 ± 0.5**	0.033 ± 0.004**	0.198 ± 0.016**	3276.5 ± 867.5**
Metroprolol (CYP2D6)	Control	1048.5 ± 272.4	1051.5 ± 272.4	0.7 ± 0.1	10.0 ± 2.3	9.7 ± 2.4	458.1 ± 124.1
	Low	766.1 ± 452.2	969.2 ± 511.8	1.7 ± 1.0*	13.1 ± 6.5	30.1 ± 19.3*	340.5 ± 195.3
	High	361.9 ± 184.9**	419.8 ± 224.6**	1.3 ± 0.7*	31.3 ± 17.3**	55.2 ± 30.7**	187.4 ± 116.4**

Compared *Urena procumbens* treated group with the control group, *: P<0.05, **: P<0.01.

Table 3. Pharmacokinetic parameters of testosterone and omeprazole in control group and *Urena procumbens* group rats (mean ± SD, n=8)

Parameters		$AUC_{(0-t)}$	$AUC_{(0-\infty)}$	$t_{1/2z}$	$C_{Lz/F}$	$V_{z/F}$	C_{max}
		ng/mL*h	ng/mL*h	h	L/h/kg	L/kg	ng/mL
Testosterone (CYP3A4)	Control	199.3 ±89.1	221.5 ± 86.2	3.3 ± 1.9	51.3 ± 20.2	265.6 ± 203.8	194.4 ± 281.5
	Low	658.9 ±176.4**	687.0 ± 179.9**	2.6 ± 0.8	15.4 ± 3.9**	56.5 ± 17.8*	212.1 ± 66.5
	High	290.6 ± 82.3*	345.7 ± 100.2*	4.0 ± 5.5	31.1 ± 8.8*	144.2 ± 155.7	127.1 ± 110.9
Omeprazole (CYP2C19)	Control	496.2 ± 157.1	503.0 ± 157.7	0.9 ± 0.5	22.9 ± 11.8	30.7 ± 21.2	452.9 ± 182.4
	Low	733.4 ± 347.5	755.2 ± 340.9	1.1 ± 0.4	15.5 ± 5.8	25.4 ± 13.2	481.7 ± 351.4
	High	399.7 ± 241.1	432.8 ± 229.4	1.5 ± 1.3	30.8 ± 17.8	68.2 ± 69.7	367.2 ± 302.7

Compared *Urena procumbens* treated group with the control group, *: P<0.05, **: P<0.01.

Statistical analysis

The main pharmacokinetic parameters of the *Urena procumbens Linn* treated group and control group were analyzed by using SPSS l8.0 statistical software, statistical significance was assessed by an independent sample t-test (P<0.05 was considered as statistically significant).

Results and discussion

Method validation

The concentrations of bupropion, phenacetin, tolbutamide, metroprolol, testosterone and omeprazole in rat plasma were simultaneously determined by a sensitive and simple UPLC-MS/MS method [7]. The LLOQ for each probe drug in plasma was 2 ng/mL. The RSD of the six probe drugs were less than 12%. The calibration plot of the probe drugs is in the range of 2-2000 ng/mL (r>0.995). The intra-day and inter-day accuracy ranged from 90% to 112%. The matrix effects were more than 85% or less than 113%. The extraction recoveries were better than 84%.

Pharmacokinetics of probe drugs

The main pharmacokinetic parameters of bupropion, phenacetin, tolbutamide, metropro-

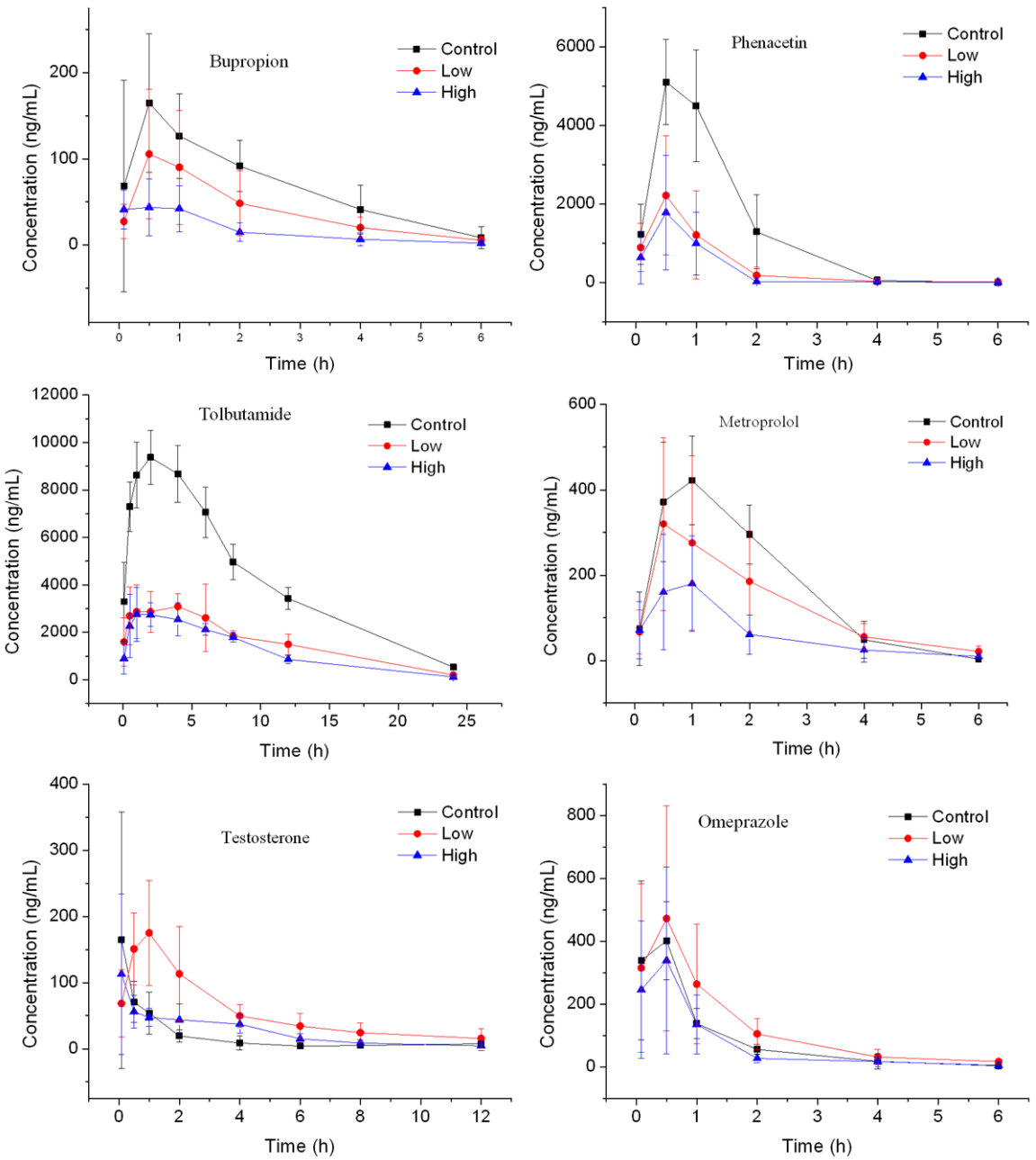

Figure 1. The pharmacokinetic profiles of bupropion (CYP2B6), phenacetin (CYP1A2), tolbutamide (CYP2C9), metroprolol (CYP2D6), testosterone (CYP3A4) and omeprazole (CYP2C19) in control group and *Urena procumbens* treated group (Low, High) rats (n=8).

lol, testosterone and omeprazole calculated from non-compartment model analysis were summarized in **Tables 1-3**. The representative profiles of concentration of drugs (phenacetin, metroprolol, testosterone, omeprazole, tolbutamide and bupropion) vs. time were presented in **Figure 1**.

From the **Table 1**, compared with the control group, the pharmacokinetic parameters of bupropion and phenacetin experienced obvious change with decreased $AUC_{(0-t)}$ ($P<0.05$) and increased CL ($P<0.05$) after the dosage increase of *Urena procumbens*. This result indicates that a 7 days-intragastric administration of *Urena procumbens* may induce the metabolism of bupropion (CYP2B6), phenacetin (CYP1A2) in rat. The similar results were also found for tolbutamide (CYP2C9) and metroprolol (CYP2D6), therefore, the 7 days-intragastric

administration of *Urena procumbens* may also induce the metabolism of tolbutamide (CYP2C9) and metroprolol (CYP2D6).

On the other hand, no significant difference for AUC, $t_{1/2}$ of omeprazole (CYP2C19) ($P>0.05$) between the *Urena procumbens* treated group and control group was observed. The pharmacokinetic parameters of testosterone experienced obvious change with increased $AUC_{(0-t)}$ ($P<0.05$) and decreased CL ($P<0.05$), which indicated that the 7 days-intragastric administration of *Urena procumbens* may inhibit the metabolism of testosterone (CYP3A4). However, the pharmacokinetic parameters of Low-group and High-group *Urena procumbens Linn* group were not non-linear changes, the effect of *Urena procumbens* on CYP3A4 in rats should be further study.

In general, changes in pharmacokinetic profile of drugs are attributed to drug-drug or drug-food interactions [8]. In pharmacokinetic characterization, drug metabolic enzymes are considered to be the most important interactive sites for drug metabolism. For example, 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 enzyme-catalyzed step [9, 10]. Similarly, supplement-drug interactions involving CYP enzyme-catalyzed metabolism are also associated with severe adverse effect events of drugs. Additionally, as *Urena procumbens* may be administrated in combination with other drugs, interactions between *Urena procumbens* and other drugs would increase the risk of either diminished efficacy or adverse effects. For these reasons, we investigated the effects of 7 days-intragastric administration of *Urena procumbens* on the activity of CYP enzymes in *vivo*. CYP isoforms such as CYP2B6, CYP1A2, CYP2C9, CYP2D6, CYP3A4 and CYP2C19 were investigated in this study since more than 90% of drugs are metabolized by those 6 CYP enzymes [11, 12]. We found a decreased $AUC_{(0-t)}$ ($P<0.05$) and an increased CL ($P<0.05$) of bupropion, phenacetin, tolbutamide and metroprolol after dosage increase of Urena procumbens. While no significant difference was observed for AUC of omeprazole ($P>0.05$).

Conclusion

In summary, we used a sensitive and simple UPLC-MS/MS method to simultaneously determine the concentrations of bupropion, phenacetin, tolbutamide, metroprolol, testosterone and omeprazole in rat plasma after *Urena procumbens* treatment. We found that 7 days-intragastric administration of *Urena procumbens* induce the metabolism of bupropion (CYP2B6), phenacetin (CYP1A2), tolbutamide (CYP2C9) and metroprolol (CYP2D6). This effect will result in decreased concentration of effective drugs, which would lead to unsatisfactory therapeutic effect. Therefore, careful attention should be paid to *Urena procumbens* when it was used in combination with other drugs which metabolized by CYP2B6, CYP1A2, CYP2C9, and CYP2D6.

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Disclosure of conflict of interest

None.

Address correspondence to: Drs. Yingxia Tan and Guanyang Lin, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325035, Zhejiang, PR, China. Tel: (+86) 577-86699156; E-mail: 522010936@qq.com (YXT); guanyanglinwzmc@gmail.com (GYL)

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