Original Article Evaluation of the impact of Flos Daturae on rat hepatic cytochrome P450 enzymes by cocktail probe drugs

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Abstract: Flos Daturae, known as "baimantuoluo" or "yangjinhua" in China, has been used for centuries in Traditional Chinese Medicine for the treatment of asthma, convulsions, pain, and rheumatism. To investigate the influences of *Flos Daturae* on the activities of rat CYP450 enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2B6, CYP2D6 and CYP3A4) using cocktail probe drugs in vivo. A cocktail solution at a dose of 5 mL/kg, which contained phenacetin (10 mg/kg), tolbutamide (1 mg/kg), omeprazole (10 mg/kg), bupropion (10 mg/kg), metoprolol (10 mg/kg) and testosterone (10 mg/kg), was intragastric administered to rats treated with a single low or high dose of *Flos Daturae* decotion for 7 days. Blood samples collected at a series of time-points in plasma were determined by UPLC-MS/ MS. The corresponding pharmacokinetic parameters were calculated by the software of DAS 3.0. The results from the present in vivo study showed that *Flos Daturae* induce the activity of CYP2D6 enzyme with the decreased C_{max}, AUC_(0-∞) (P < 0.05) and the increased CL (P < 0.05). However, there were no significant differences of other probe drugs in plasma concentration and pharmacokinetic parameters. There were no significant effects on rat CYP1A2, CYP3A4, CYP2B6, CYP2C9 and CYP2C19 by *Flos Daturae*. Therefore, the resulting data suggested that caution was needed when *Flos Daturae* was co-administered with CYP2D6 substrates, which may result in treatment failure and herb-drug interactions.

Keywords: Flos Daturae, UPLC-MS/MS, CYP450, cocktail, rat

Introduction

Flos Daturae, known as "baimantuoluo" or "yangjinhua" in China, is the dry flower of Daturae metel L. (Solanaceae), which is widely distributed in Jiangsu, Guangdong, and Fujian province of China [1]. Flos Daturae has been found to be rich in tropane alkaloids such as scopolamine anisodamine and atropine, and has been used for centuries in Traditional Chinese Medicine for the treatment of asthma, convulsions, pain, and rheumatism [2, 3]. Recently Flos Daturae also has been used clinically for the treatment of psoriasis in China [4]. Despite the widespread use of Flos Daturae, the effects of Flos Daturae on the cytochrome P450 (CYP450) involved drug metabolism remain ambiguous which may result in herb-drug interactions. The aim of this study is to examine the effects of Flos Daturae on CYP activities by using rats.

Cytochrome P450s superfamily expressed widely in organisms are known to play an important role in the biotransformation of many endogenous and exogenous substances, including more than 90% of all medications [5, 6]. Specific probe drugs have been widely used for assessing various individual CYP450 enzymes activities [7]. A probe drug is a kind of compound specially catalyzed by CYP isoforms, and the activities of CYP isoforms can be reflected by the metabolic rate of the probe drug [8]. In addition to individual assessments of CYP isoform activity, a "cocktail" approach can detect the activities of multiple cytochrome P450 (CYP) isoforms following the administration of multiple CYP-specific probe substrates in a single experiment [9, 10]. Thus, the cocktail approach is a powerful tool for the simultaneous and comprehensive evaluation of herb-drug interactions involving multiple CYP isoforms.



In this study, we used bupropion, phenacetin, tolbutamide, metroprolol, testosterone and omeprazole as probe drugs (**Figure 1**) respectively to investigate the effects of *Flos Daturae* on cytochrome P450 activities including CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. To our knowledge, the effect of *Flos Daturae* on the CYPs activities is still not well known. It is necessary to investigate the interactions between *Flos Daturae* and CYPs.

Materials and methods

Chemicals and reagents

Bupropion, phenacetin, tolbutamide, metroprolol, testosterone, omeprazole (all > 98%) and the internal standard diazepam 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) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. Animals were housed under a natural light-dark cycle conditions with controlled temperature (22°C). All 21 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.

Instrumentation and conditions

UPLC-MS/MS with ACQUITY I-Class UPLC and a XEVO TQD triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) interface (Waters Corp., Milford, MA, USA) were used to analyze the compounds. The UPLC system was comprised of a Sample Manager with Flow-Through Needle (SM-FTN) and a Binary Solvent Manager (BSM). The Masslynx 4.1 software was used for data acquisition and instrument control (Waters Corp., Milford, MA, USA).

Chromatographic separation was achieved on a UPLC® BEH C18 column (2.1 mm × 100 mm, 1.7 µm) maintained at 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 the acetonitrile changing from 30 to 60% between 0.3 and 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 maintained at 30% for 0.4 min. The total run time of the analytes needed 3 min. After each injection, the sample manager underwent a needle wash process, including 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 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 of m/z 180.1 \rightarrow 109.9 for phenacetin, m/z 240.1 \rightarrow 184.1 for bupropion, m/z 268.1 \rightarrow 115.8 for metroprolol, m/z 289.1 \rightarrow 97.0 for testosterone, m/z 346.1 \rightarrow 197.9 for omeprazole, m/z 271.2 \rightarrow 155.1 for tolbutamide, m/z 240.1 \rightarrow 184.1 for bupropion and m/z 285.1 \rightarrow 193.1 for IS was used as quantitative analysis.

Preparation of standard solutions

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

The calibration standards were prepared by spiking blank rat plasma with appropriate amounts of bupropion, phenacetin, tolbutamide, metroprolol, testosterone and omeprazole. Calibration plots of each probe drug were constructed in the range 2-2000 ng/mL for plasma (2, 5, 10, 20, 50, 100, 200, 500, 1000 and 2000 ng/mL). Quality-control (QC) samples were prepared by the same way as the calibration standards, three different plasma concentrations (4,800, and 1600 ng/mL). The analytical standards and QC samples were stored at -20°C.

Sample preparation

An aliquot of 10 μ L of the IS working solution (0.5 μ g/mL) was added to 100 μ L plasma sample followed by the addition of 200 μ L acetonitrile in a 1.5 mL centrifuge tube. The tubes were vortex mixed for 0.5 min. After centrifugation at 14900 g for 15 min, the supernatant (2 μ L) was injected into the UPLC-MS/MS system for analysis.

Preparation of Flos Daturae decoction and pharmacokinetics

The dry crude *Flos Daturae* was ground into powder and then boiled in distilled water (100 g/1 L). The rest filtrate was replenished with distilled water to make the final concentration 0.1 g/mL. Low dose (0.3 g/kg) and high dose (0.6 g/kg) of *Flos Daturae* decoction were conducted, and we studied the effect of *Flos Daturae* on rat CYP enzymes after rats were intragastric administered by a single dose of

Impact of Flos Daturae on rat hepatic cytochrome P450 enzymes

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Figure 2. The MRM chromatograms of bupropion, omeprazole, metopolol, tolbutamide, phenacetin, testosterone and diazepam (IS). A blank plasma sample (A), a blank plasma sample spiked with cocktail drugs and IS (B) and a plasma sample 0.5 h after dosing cocktail probes with intragastric administration (C).

Table 1. Regression equation, correlation coefficient for six probe drugs (*y* = peak area ratio of probe drugs *versus* IS; *x* = concentration of probe drugs)

Probe Drugs	Liner Range (ng/mL)	Regression Equation	Correlation Coefficient				
Bupropion	2-2000	y = 0.0233918x+0.226087	0.9986				
Metoprolol	2-2000	y = 0.00565082x+0.0623493	0.9950				
Testosterone	2-2000	y = 0.0039126x+0.0257208	0.9999				
Phenacetin	2-2000	y = 0.00663589x+0.0400272	0.9993				
Omeprazole	2-2000	y = 0.0143302x+0.201622	0.9948				
Tolbutamide	2-2000	y = 0.00112399x+0.00507379	0.9991				

Statistical analysis

Plasma probe drugs concentration versus time was analyzed by Version 3.0 Data Analysis System (Wenzhou Medical University, China) and statistical analyses were tested by t-test using SPSS I8.0 statistical software. A value of P < 0.05 was considered to be statistically significant.

Results

Determination method for probe drugs

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 (**Figure 2**). The precision levels for six probe drugs were both < 15%, while the measurement. The intra-day and inter-day accuracy was between 85.1 and 109.5%. Total

Flos Daturae decoction. Rats were randomly divided into three groups: control group (CG), low dose group (LG), high dose group (HG). The CG received 0.9% sodium chloride solution. The next day, all the rats were administered an intragastric cocktail solution containing phenacetin (10 mg/kg), tolbutamide (1 mg/kg), omeprazole (10 mg/kg), bupropion (10 mg/kg), metoprolol (10 mg/kg) and testosterone (10 mg/kg).

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Paramotors		MRT _(0-∞)	$AUC_{(0-\infty)}$	t _{1/2}	CL	C _{max}
Falameters		h	ng/mL·h	h	L/h/kg	ng/mL
Bupropion	Con	2.4±0.9	160.8±43.3	1.6±0.8	65.8±16.4	74±30.3
	Low	2.6±0.5	104.8±41.9	1.7±0.5	112.1±55.5	37±14.9
	High	2.6±0.8	141.1±33.1	1.7±0.7	73.7±15.6	52.8±8.7
Phenacetin	Con	9.1±8.2	2871±1171.8	9.9±8.1	3.9±1.4	956.6±404.9
	Low	5.8±6.4	2289.7±1609.8	4.2±2.8	5.9±2.9	924±530.1
	High	3.7±1.6	3761.4±1664.6	6.1±3.0	3.3±1.9	1646.9±849.7
Tolbutamide	Con	15.8±16.8	134368.7±96120.5	10±11.4	0.009±0.003	7790.8±753.9
	Low	9.8±1.4	93860.1±10930.3	5.9±1.4	0.011±0.001	6060.5±696.5**
	High	10.5±4.7	94622.6±21264.4	6.6±3.5	0.011±0.002	7300.3±2033.4
Omeprazole	Con	7.8±2.7	1015.3±382.3	6.2±2.1	10.8±3.1	215.3±50.3
	Low	5.7±2.4	740.2±247.3	5.5±2.8	14.8±4.9	211.8±51.5
	High	11.7±11.6	1524.5±1175.9	7.0±7.1	8.9±4.1	284.8±67.1
Metoprolol	Con	3.5±1.3	769.9±100.3	2.6±1.2	13.2±1.6	211.9±49.3
	Low	3.5±1.8	838±383.7	1.6±0.4	14.3±6.5	267.7±114.7
	High	2.7±0.4	480.6±133.2**	3.1±1.3	22.2±6.0*	134.9±35.2*
Testosterone	Con	10.3±3.3	338.8±92.7	8.9±2.9	31.5±9.1	61.7±9.3
	Low	10.1±5.1	324.2±118.9	8.8±4.6	34.4±11.9	55.4±24.6
	High	5.3±0.9**	365.1±52.5	4.9±2.1*	27.9±4.4	106.1±31.7*

Table 2. Effects of Flos Daturae on pharmacokinetic parameters of the six probe drugs

*P < 0.05; **P < 0.01 vs. control.

recovery of bupropion, phenacetin, tolbutamide, metroprolol, testosterone and omeprazole was between 93.2 and 110.4%. The matrix effects were between 93.6 and 108.5%. Linear regressions of the peak area ratios versus concentrations were fitted over the concentration range of 2-2000 ng/ml in rat plasma. Typical equation of the calibration curves were listed in Table 2, where y represents the ratio of the analyte peak area to that of the IS, and x represents the plasma concentration. The lowest limits of quantification (LLOQ) for probe drugs in plasma were 2 ng/mL. The precision at LLOQ were measured to be between 2.5% and 14.1%, and accuracy was between 85.1% and 109.5%, respectively. The stability study showed that the analytes were all stable under all testing conditions, including short-term storage (6 h at room temperature), long-term storage (2 weeks at -20°C), freeze-thaw cycling, and post-preparative storage. The Res calculated from the QCs under all testing conditions ranged from -11.5% to 12.9%. It took only 3 min to finish analyzing a plasma sample, which demonstrates that much time might be saved in experimental studies with hundreds of samples.

Pharmacokinetic interaction

The pharmacokinetic parameters of these probe drugs are listed in **Table 1** and mean plasma concentration-time curves in different groups are presented in **Figure 3**. Pretreatment with high dose of *Flos Daturae* decreased C_{max} (134.9±35.2 ng/ml vs. 211.9±49.3 ng/ml) and AUC_(0-∞)(480.6±133.2 ng/mL·h vs. 769.9±100.3 ng/mL·h) of metoprolol, and increased the CL (22.2±6.0 L/h/kg vs. 13.2±1.6 L/h/kg) of metoprolol, indicating that *Flos Daturae* could induce the activity of CYP2D6 enzyme.

Flos Daturae decreasing in t1/2 (4.9±2.1 h vs. 8.9±2.9 h) and MRT_(0-∞)(5.3±0.9 h vs. 10.3 ±3.3 h) of testosterone, and the corresponding increase in the C_{max}(106.1±31.7 ng/mL vs. 61.7±9.3 ng/mL) but without any significant changes in AUC_(0-∞) and CL suggested *Flos Daturae* had the potential to alter the rat hepatic CYP3A4 activity in a certain extent. The other probe drugs in the test rats hardly showed statistically significant difference from the control rats. It demonstrated that *Flos Daturae* did not influence on CYP1A2, CYP2C9, CYP2C19 and CYP2B6.

Discussion

The concomitant administration of herbal supplements and synthetic drugs has become increasingly popular. It is estimated by the World Health Organization (WHO) that approximately



Figure 3. Mean plasma concentration-time curves of bupropion (A), omeprazole (B), metopolol (C), tolbutamide (D), phenacetin (E) and testosterone (F) in untreated and *Flos Daturae* decotion treated rats. CG (control group) received 0.9% sodium chloride solution (control); LG (low dose group) received 3 mL 0.1 g/mL *Flos Daturae* decotion twice a day for one day; HG (high dose group) received 3 mL 0.1 g/mL *Flos Daturae* decotion twice a day for one day. Error bars represent S.D.

80% of the global population relies on traditional herbal medicines as part of standard health care [11]. Most consumers believe that herbal medicines are natural and therefore safe, which is a dangerous oversimplification. Moreover, herbal medicines are multi-component therapeutics, and these substances are effective through multiple targets and multiple pathways. Chinese herbal medicines contain a variety of biologically active ingredients. As a result, herb-drug interactions have become a common clinical problem. According to recent studies, the mechanisms underlying the interaction between herbal medicines and conventional drugs mainly involve induction or inhibition of the activities of metabolic enzymes and drug efflux proteins [12-14]. Therefore, potential herb-drug interactions involving Chinese herbal medicines are worthy of study based on the CYP system.

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Flos Daturae has been used in Traditional Chinese Medicine for the treatment of asthma, convulsions, pain, and rheumatism for centuries. The patients who suffer these diseases are commonly administered some other drugs that may be metabolized by CYP450 enzymes. There is no doubt that this has the potential to increase the risk for herb-drug interactions in patients.

Probe drugs have been extensively used for phenotyping many different kinds of CYP450 activities, and this approach has been widely applied in field of drug metabolism and pharmacokinetics [15-18]. In the past, a number of phenotyping cocktails have been proposed and evaluated. The phenotyping cocktail for simultaneous administration of multiple in vivo probe drugs including bupropion, phenacetin, tolbutamide, metroprolol, testosterone and omeprazole has previously been established in our laboratory. The cocktail demonstrated that the ability of the 6 probe drugs to determine the activities of their respective enzymes is not affected by those probe drugs.

Hence, in the present study, a developed and validated UPLC-MS/MS method was used investigated the effect of *Flos Daturae* on the activity of the major CYP isozymes in rats. Our results showed that pretreatment with single high dose of *Flos Daturae* could induce the enzyme activity of CYP2D6, which account for 2% of all hepatic CYPs but metabolize 25% of current drugs [19]. Typical substrates for CYP2D6 are largely lipophilic bases and include some antidepressants, antipsychotics, antiarrhythmics, antiemetics, b-adrenoceptor antagonists (b-blockers) and opioids [20, 21].

In addition, there was hardly any significant effect on the activities of all the CYP450 enzymes in LG of *Flos Daturae*, indicating that the dose dependency and kinetics of *Flos Daturae* on the activity of CYP450 remain to be further investigated. However, in consideration of the strong toxicity of *Flos Daturae*, it is not advised to use *Flos Daturae* at such a high dose [22, 23].

Conclusion

Our study showed that *Flos Daturae* decoction may induce the activity of CYP2D6, but had no significant effects on activities of CYP2C19,

CYP3A4, CYP2C9, CYP1A2 and CYP2B6. The results provide a scientific basis for the safe clinical application of *Flos Daturae* in combination with other drugs, potentially preventing possible side effects induced by herb-drug interactions.

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Disclosure of conflict of interest

None.

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