Effects of salidroside on rat CYP enzymes by a cocktail of probe drugs

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A R T I C L E I N F O	A B S T R A C T
	Objective(s): In this study, we aimed to evaluate the effect of salidroside on the activities of the different drug-metabolizing enzymes CYP1A2, CYP2B6, CYP2C9, CYP2D6 and CYP3A4 in rats, in which a specific probe drug was used for each enzyme. ***Materials and Methods:*** After pretreatment with salidroside, five probe drugs were simultaneously administered to rats by gavage. The given dose was 2.0 mg/kg for phenacetin (CYP1A2 activity), 4.0 mg/kg for bupropion (CYP2B6 activity), 2.0 mg/kg for losartan (CYP2C9 activity), 8.0 mg/kg for metoprolol (CYP2D6 activity) and 1.0 mg/kg for midazolam (CYP3A4 activity). Then, an ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was used to analyze the concentrations of rats' blood, which were collected at different corresponding times. ***Results:*** Our data showed that salidroside exhibited an inductive effect on CYP1A2, CYP2B6, CYP2C9 and CYP3A4 activities by changing the main pharmacokinetic parameters (t1/2, CL/F, Cmax and AUC(0-∞)) of the four probe drugs in rats. However, no significant changes in CYP2D6 activity were observed. ***Conclusion:*** In a word, the results displayed that salidroside could induce the activities of CYP1A2, CYP2B6, CYP2C9 and CYP3A4, which may influence the disposition of the drugs that are mainly metabolized by these pathways. Our research can provide the basis for the study of related herb-drug interactions in clinic.

Introduction

Cytochrome P450 (CYP) enzymes, consisted of multiple isoforms, play a major role in drug metabolism (1). Among them, five CYP enzymes (CYP1A2, CYP2B6, CYP2C9, CYP2D6 and CYP3A4) are important and precious (2, 3). Although specific probe drugs have been widely employed for assessing various CYP activities, the main disadvantage of such phenotypes is inability to evaluate the activities of several enzymes simultaneously (4). As a result, a cocktail approach has been developed, which involves the administration of multiple probe drugs simultaneously. Recently, many developed cocktails have been used for the prediction of the influence of different clinical drugs on the activities of CYP isoforms *in vivo* (5-10).

Salidroside is an active ingredient isolated from *Rhodiola rosea*, which has long been used as an adaptogen in traditional Chinese medicine (11, 12). Salidroside is known to possess a lot of pharmacological properties, such as anti-inflammation, neuroprotective, immune regulatory and strong antioxidative activities (13-17). Moreover, evidences showed that salidroside also plays an antitumor role *in vivo* and *in vitro* (18-20). However, the effect of salidroside on CYP has not been reported in the previous literatures.

In this paper, we used a newly developed five-probe drug cocktail (including phenacetin for CYP1A2, bupropion for CYP2B6, losartan for CYP2C9, metroprolol

for CYP2D6 and midazolam for CYP3A4) to evaluate the effect of salidroside on the CYP activities in rats by ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) (21). We hope that the results of our experiment may be helpful for the clinical safety evaluation of herb-drug interactions involving salidroside.

Materials and Methods

Chemicals materials

Phenacetin, bupropion, losartan, metroprolol, midazolam (all >98%) and diazepam (internal standard, IS) were purchased from Sigma (St. Louis, MO, USA). Salidroside was purchased from Baoji Chenguang Biotechnology Co., Ltd. (Shanxi, China). LC grade acetonitrile and methanol were obtained from Merck Company (Darmstadt, Germany). Water used for the LC-MS/MS analysis was from Milli Q water purification system (Millipore, Bedford, USA).

UPLC-MS/MS conditions

An Acquity UPLC system (Waters Corp., Milford, MA, USA) equipped with a XEVO TQD triple quadrupole mass spectrometer via an electro-spray ionization (ESI) source was used for separation and detection. The quantification was operated in the multiple reaction monitoring (MRM) mode with the transitions of m/z 180.0→109.9 for phenacetin, m/z 240.0→184.1 for bupropion, m/z 423.1→207.2 for losartan, m/z 268.1 →

Table 1. Effects of salidroside on the main pharmacokinetic parameters of the five probe drugs in rats (n=6, mean ± SD)

Probe drugs	Parameters	$t_{1/2}$ (hr)	T_{max} (hr)	CL/F (L/h/kg)	C_{max} (ng/ml)	$AUC_{0 \to t}$ (ng/ml•h)	$AUC_{0 \to \infty}$ (ng/ml•h)
phenacetin	control	2.15 ± 0.62	0.25 ± 0.03	58.84 ± 31.33	47.47 ± 15.53	31.95 ± 6.68	31.96 ± 6.68
	single	1.50 ± 0.76*	0.23 ± 0.15	70.96 ± 20.76*	27.10 ± 8.63*	30.52 ± 10.01	30.53 ± 10.01
	multiple	2.33 ± 0.84	0.29 ± 0.10	58.48 ± 20.26	40.76 ± 12.15	33.44 ± 9.33	33.46 ± 9.32
bupropion	control	0.79 ± 0.12	0.43 ± 0.28	34.27 ± 17.52	84.34 ± 37.76	135.86 ± 47.75	135.98 ± 47.81
	single	0.50 ± 0.10*	0.55 ± 0.27	41.87 ± 17.15*	41.90 ± 15.48*	113.12 ± 46.24*	113.18 ± 46.28*
	multiple	0.40 ± 0.15*	0.46 ± 0.10	42.43 ± 18.09*	36.43 ± 11.74*	111.31 ± 44.57*	111.33 ± 44.56*
losartan	control	0.66 ± 0.08	0.79 ± 0.13	7.10 ± 2.98	85.21 ± 52.81	434.77 ± 252.88	434.83 ± 252.91
	single	0.49 ± 0.13*	0.48 ± 0.17*	10.22 ± 4.33*	53.58 ± 21.68*	233.87 ± 121.93*	233.92 ± 121.93*
	multiple	0.47 ± 0.10*	0.36 ± 0.32*	11.04 ± 3.47*	40.91 ± 11.58*	196.36 ± 63.99*	197.76 ± 64.22*
metroprolol	control	1.53 ± 0.31	0.61 ± 0.36	39.07 ± 7.61	100.25 ± 24.17	210.35 ± 35.88	210.48 ± 35.87
	single	1.41 ± 0.27	0.65 ± 0.34	43.19 ± 5.39	88.31 ± 23.88	207.78 ± 84.34	207.99 ± 84.32
	multiple	1.14 ± 2.23	0.69 ± 0.40	31.59 ± 3.92	94.03 ± 18.84	205.74 ± 28.43	206.20 ± 28.27
midazolam	control	3.12 ± 2.57	0.20 ± 0.04	85.17 ± 39.64	4.74 ± 2.46	4.58 ± 1.51	5.54 ± 2.39
	single	2.07 ± 0.92*	0.28 ± 0.16	248.04 ± 96.97*	0.88 ± 0.30*	1.46 ± 0.65*	1.82 ± 0.67*
	multiple	2.25 ± 1.19*	0.20 ± 0.07	161.44 ± 72.06*	2.26 ± 0.96*	2.86 ± 1.37*	2.93 ± 1.34*

*Significantly different from control, *P*< 0.05

115.8 for metroprolol, *m/z* 326.0→290.9 for midazolam and *m/z* 285.1→193.0 for diazepam (IS). Data acquisition and instrument control were performed by the Masslynx 4.1 software (Waters Corp., Milford, MA, USA).

Chromatographic separation was employed on an Acquity BEH C18 column (2.1 mm × 50 mm, 1.7 μm) with gradient elution. The mobile phase consisted of acetonitrile (A) and 0.1% formic acid (B) as follows: 0-0.3 min (28-28% A), 0.3-0.5 min (28-95% A), 0.5-1.2 min (95-95% A), 1.2-1.5 min (95-28% A), and 1.5-2.0 min (28-28% A) (21). In the whole process, the flow rate was 0.40 ml/min, and the overall run time was 2.0 min.

Animals and treatment
Male Sprague-Dawley rats (180-220 g) were obtained from Laboratory Animal Center of Wenzhou Medical University (Zhejiang, China). Diet was prohibited for 12 hr before the experiment, but water was freely available. Eighteen rats were randomly divided into 3 groups (n=6 per group): the control group, the single dose of salidroside group and the multiple dose of salidroside group. The rats in the control group were given saline orally for 7 consecutive days, while in the multiple dose of salidroside group, oral administration of salidroside (30 mg/kg) were given for 7 consecutive days. On the eighth day, three groups were all orally given salidroside (30 mg/kg) simultaneously. After 0.5 hr, 2.0 ml/kg of probe cocktail solution, including phenacetin (2.0 mg/kg), bupropion (4.0 mg/kg), losartan (2.0 mg/kg), metroprolol (8.0 mg/kg) and midazolam (1.0 mg/

kg), were administrated to all rats by gavage. After oral administration of probe drugs, 0.3 ml blood samples were collected from the tail vein into heparinized 1.5 ml polythene tubes at different times (0.167, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6 and 8 hr). The samples were immediately centrifuged at 4000 g for 8 min, and the supernatant plasma (100 μl) was obtained and stored at -20 °C until analysis.

Sample preparation
To 100 μl of plasma sample in a 1.5 ml centrifuge tube, 200 μl of the IS working solution (30 ng/ml in acetonitrile) was added. After vortex mixing for 1.0 min, samples were centrifuged at 13,000 g for 10 min. Finally, the supernatant (2 μl) was injected into the UPLC-MS/MS system for analysis.

Statistical analysis
Drug and Statistics (DAS) software (Version 2.0, Shanghai University of Traditional Chinese Medicine, China) was used to analyze the plasma concentration versus time data for each rat. Statistical analyses were performed by *t*-test (SPSS 19.0, Chicago, IL), with *P*< 0.05 considered statistically significant.

Results
Effect of salidroside on rat CYP1A2 activity
The effect of salidroside on the main pharmacokinetic parameters of phenacetin in the control, single and multiple dose of salidroside groups were displayed in Table 1, and the mean plasma concentration-time curves
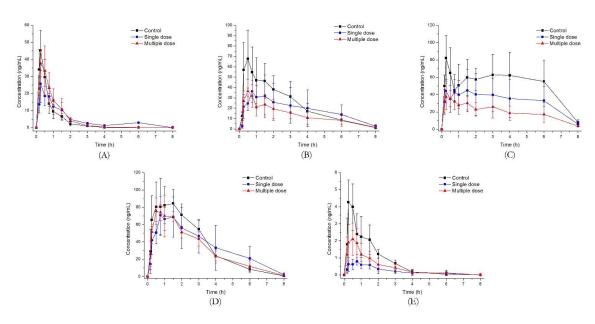
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Figure 1. Mean plasma concentration-time profiles of (A) phenacetin, (B) bupropion, (C) losartan, (D) metroprolol and (E) midazolam after oral administration in salidroside and control group rats (n=6)

of phenacetin were showed in Figure 1. Compared to the control group, the $t_{1/2}$ and C_{max} of phenacetin in the single dose of salidroside group were decreased significantly by 30.2% and 42.9%, respectively, while CL/F in the single dose of salidroside group was increased significantly by 20.6%. However, the main pharmacokinetic parameters ($t_{1/2}$, T_{max}, CL/F, C_{max} and $AUC_{(0-\infty)}$) of phenacetin in the multiple dose of salidroside group showed no significant change. Our results showed that salidroside in the single dose group could significantly speed up the metabolism of phenacetin, and salidroside had the potential to induce rat CYP1A2 activity *in vivo*.

Effect of salidroside on rat CYP2B6 activity

The pharmacokinetic behaviors and mean plasma concentration-time curves of bupropion were presented in Table 1 and Figure 1 after oral administration of salidroside. Compared to the control group, the $t_{1/2}$, C_{max} and $AUC_{(0-\infty)}$ of bupropion in the single dose of salidroside group were decreased significantly by 36.7%, 50.3% and 16.8%, respectively, while CL/F of bupropion was increased evidently by 22.2%. Moreover, in the multiple dose of salidroside group, the $t_{1/2}$, C_{max} and $AUC_{(0-\infty)}$ of bupropion were decreased significantly by 49.4%, 56.8% and 18.1%, respectively, while CL/F was increased significantly by 23.8%. These results showed that the activity of CYP2B6 in rats was induced by salidroside.

Effect of salidroside on rat CYP2C9 activity

Table 1 and Figure 1 displayed the pharmacokinetic profiles of losartan before and after oral administration of salidroside. Compared with the control group, the $t_{1/2}$ of losartan in the single dose of salidroside group significantly decreased from 0.66 hr to 0.49 hr, the T_{max} decreased from 0.79 hr to 0.48 hr, the C_{max} decreased from 85.21 ng/ml to 53.58 ng/ml, the $AUC_{(0-\infty)}$ decreased from 434.83 ng/ml to 233.92 ng/ml, and the CL/F significantly increased by 43.9%. In addition,

the $t_{1/2}$ of losartan in the multiple dose of salidroside group significantly decreased from 0.66 hr to 0.47 hr, the T_{max} decreased from 0.79 hr to 0.36 hr, the C_{max} decreased from 85.21 ng/ml to 40.91 ng/ml, the $AUC_{(0-\infty)}$ decreased from 434.83 ng/ml to 197.76 ng/ml, and the CL/F significantly increased by 55.5%. Our results showed that the activity of CYP2C9 could be induced by salidroside in rats.

Effect of salidroside on rat CYP2D6 activity

Table 1 and Figure 1 showed the results of the pharmacokinetic study of metroprolol after treatment with salidroside. Interestingly, no apparent changes were found on the main pharmacokinetic parameters of metroprolol in the control, single and multiple doses of salidroside groups. So, salidroside did not have effect on the activity of CYP2D6 in rats.

Effect of salidroside on rat CYP3A4 activity

As shown in Table 1 and Figure 1, $t_{1/2}$, C_{max} and $AUC_{(0-\infty)}$ of midazolam in the single and multiple dose of salidroside groups were decreased significantly compared with the control group, while CL/F of midazolam in these groups were increased significantly. Furthermore, the change in the single dose of salidroside group was more obvious. As a result, it was suggested that salidroside has the potential to induce the activity of CYP3A4 in rats.

Discussion

Salidroside, one of the main active ingredients from the root of *Rhodiola rosea*, has a variety of biological activities in clinical practice (13). However, there are no detailed reports on the metabolism of salidroside, especially the CYP drug metabolizing enzymes. In this study, we used a developed cocktail method to study drug metabolism and herb-drug interaction *in vivo* associated with five different CYP isoforms (21). After oral administration of a cocktail solution, which contained five different CYP probe drugs (i.e. phenacetin,

bupropion, losartan, metroprolol and midazolam), the concentrations of the probe drugs of various CYP isoforms were tested simultaneously. As the first time to evaluate the effect of salidroside on CYPs, our study may be helpful.

The present study demonstrated the inductive effect of the single dose of salidroside on CYP1A2 in rats, while the multiple dose of salidroside had no effect on this enzyme. It suggested that the inductive effect was not dose-dependent and accumulative. Our results implied that salidroside could temporarily induce CYP1A2, which is considered to be one of the important CYPs (22). Thus, the potential herb-drug interaction for salidroside should be considered when salidroside is used in combination with the substrate drugs of CYP1A2.

According to the results of the pharmacokinetic studies, salidroside has inductive effect on the activities of CYP2B6 and CYP2C9. As we know, CYP2B6, one of the most abundant CYP enzymes in the human liver, accounts for ~6% of hepatic total CYPs (23), and can metabolize approximately 8% of clinical drugs. Furthermore, CYP2C9 is largely expressed in the liver (~20% of total hepatic CYPs), where it metabolizes approximately 15% clinical drugs (24). In view of the above results, co-administration of salidroside with other drugs, which are metabolized by the CYP2B6 and/ or CYP2C9 enzyme, should be reevaluated.

In the present study, we also focused on the effect of salidroside on the rat CYP2D6 activity. As another investigated CYP enzyme, CYP2D6 accounts for 2-4% of total hepatic CYPs, and metabolizes different kinds of drugs that target the CNS and cardiovascular systems (25, 26). The results of this study showed that salidroside had no effect on the activity of CYP2D6 in rats, because there were no significant differences in the $t_{1/2}$, T_{max}, CL/F, C_{max} and $AUC_{(0-\infty)}$ of metroprolol among the control, single dose and multiple dose of salidroside-treated groups. Therefore, it can be speculated that a certain dose of salidroside has no clinically relevant effect on the disposition of CYP2D6-metabolizing drugs.

The results of this study showed that salidroside can significantly induce the activity of CYP3A4 in rats. Interestingly, one of the main findings of the study is that the metabolism of midazolam in the multiple dose of salidroside group was slower than those in the single dose of salidroside group in rats, which implied that the inductive effect could be alleviated. CYP3A4 metabolizes a large variety of clinical drugs, including many pediatric drugs; hence, special attention needs to be paid to the occurrence of herb-drug interaction when salidroside and CYP3A4-metabolizing drugs are used in combination.

Conclusions

Our results showed that salidroside had no significant effect on the activity of CYP2D6, but it could induce the activities of CYP1A2, CYP2B6, CYP2C9 and CYP3A4 in rats. It suggested that salidroside may accelerate biotransformation rates of CYP1A2, CYP2B6, CYP2C9 and CYP3A4 probe drugs, and reduce their pharmaceutical plasma concentration and clinical effects. These findings may provide some helpful information to support the clinical safety and effectiveness of salidroside.

Therefore, the CYP enzymes-mediated (CYP1A2, CYP2B6, CYP2C9 and CYP3A4) herb-drug interactions between salidroside and other drugs must be given high priority to avoid the occurrence of adverse reaction. It is of interest to further investigate the clinical significance in future studies.

Conflict of interest

None of the authors has any other conflict of interests related to this paper.

References

1. Zhou SF, Liu JP, and Chowbay B. Polymorphism of human cytochrome P450 enzymes and its clinical impact. Drug Metab Rev 2009; 41:89-295.
2. Wrighton SA, VandenBranden M, and Ring BJ. The human drug metabolizing cytochromes P450. J Pharmacokinet Biopharm 1996; 24:461-473.
3. Meyer UA. Overview of enzymes of drug metabolism. J Pharmacokinet Biopharm 1996; 24:449-459.
4. Breimer DD. Interindividual variations in drug disposition. Clinical implications and methods of investigation. Clin Pharmacokinet 1983; 8:371-377.
5. Zhou CJ, Qiao LM, Zhao LH, and Li ZY. Evaluation of the impact of cantharidin on rat CYP enzymes by using a cocktail of probe drugs. Fitoterapia 2015; 107:49-53.
6. Geng P, Wang S, Wang C, Chen J, Zhang L, Yang S, *et al.* Evaluation of the impact of Flos Daturae on rat hepatic cytochrome P450 enzymes by cocktail probe drugs. Int J Clin Exp Med 2015; 8:22310-8.
7. Wu Q, Zhang Q, Wen C, Hu L, Wang X, and Lin G. The effect of MS-275 on CYP450 isoforms activity in rats by cocktail method. Int J Clin Exp Pathol 2015; 8:9360-7.
8. Zhang YT, Zhang DF, Ge NY, Zhu GH, Hao C, Zhang Y, *et al.* effect of evodiamine on CYP enzymes in rats by a cocktail method. Pharmacology 2016; 97:218-223.
9. Xu RA, Xu ZS, and Ge RS. Effects of hydroxysafflor yellow A on the activity and mRNA expression of four CYP isozymes in rats. J Ethnopharmacol 2014; 151:1141-1146.
10. Huang Y, Zheng SL, Zhu HY, Xu ZS, and Xu RA. Effects of aescin on cytochrome P450 enzymes in rats. J Ethnopharmacol 2014; 151:583-590.
11. Hung SK, Perry R, and Ernst E. The effectiveness and efficacy of Rhodiola rosea L.: a systematic review of randomized clinical trials. Phytomedicine 2011; 18:235-244.
12. Spasov AA, Wikman GK, Mandrikov VB, Mironova IA, and Neumoin VV. A double-blind, placebo-controlled pilot study of the stimulating and adaptogenic effect of *Rhodiola rosea* SHR-5 extract on the fatigue of students caused by stress during an examination period with a repeated low-dose regimen. Phytomedicine 2000; 7:85-89.
13. Zhang H, Shen WS, Gao CH, Deng LC, and Shen D. Protective effects of salidroside on epirubicin-induced early left ventricular regional systolic dysfunction in patients with breast cancer. Drugs R D 2012; 12:101-106.
14. Shi TY, Feng SF, Xing JH, Wu YM, Li XQ, Zhang N, *et al.* Neuroprotective effects of salidroside and its analogue tyrosol galactoside against focal cerebral ischemia in vivo and H2O2-induced neurotoxicity *in vitro*. Neurotox Res 2012; 21:358-367.
15. Zhang JK, Yang L, Meng GL, Yuan Z, Fan J, Li D, *et al.*

Protection by salidroside against bone loss via inhibition of oxidative stress and bone-resorbing mediators. PLoS One 2013; 8:e57251.

16. Li D, Fu Y, Zhang W, Su G, Liu B, Guo M, *et al.* Salidroside attenuates inflammatory responses by suppressing nuclear factor-kappaB and mitogen activated protein kinases activation in lipopolysaccharide-induced mastitis in mice. Inflamm Res 2013; 62:9-15.

17. Guan S, He J, Guo W, Wei J, Lu J, and Deng X. Adjuvant effects of salidroside from Rhodiola rosea L. on the immune responses to ovalbumin in mice. Immunopharmacol Immunotoxicol 2011; 33:738-743.

18. Zhao G, Shi A, Fan Z, and Du Y. Salidroside inhibits the growth of human breast cancer in vitro and *in vivo.* Oncol Rep 2015; 33:2553-2560.

19. Sun KX, Xia HW, and Xia RL. Anticancer effect of salidroside on colon cancer through inhibiting JAK2/STAT3 signaling pathway. Int J Clin Exp Pathol 2015; 8:615-621.

20. Wang J, Li JZ, Lu AX, Zhang KF, and Li BJ. Anticancer effect of salidroside on A549 lung cancer cells through inhibition of oxidative stress and phospho-p38 expression. Oncol Lett 2014; 7:1159-1164.

21. Wei YL, Du HJ, Lin YP, Wu ML, Ying XQ, Ding MX, *et al.* Simultaneous Determination of Five Rat CYP450 Probe Drugs by UPLC-MS/MS Method. Lat Am J Pharm 2016; 35:1810-1815.

22. Shimada T, Yamazaki H, Mimura M, Inui Y, and Guengerich FP. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. J Pharmacol Exp Ther 1994; 270:414-423.

23. Stresser DM and Kupfer D. Monospecific antipeptide antibody to cytochrome P-450 2B6. Drug Metab Dispos 1999; 27:517-525.

24. Miners JO and Birkett DJ. Cytochrome P4502C9: an enzyme of major importance in human drug metabolism. Br J Clin Pharmacol 1998; 45:525-538.

25. Zhou SF. Polymorphism of human cytochrome P450 2D6 and its clinical significance: Part I. Clin Pharmacokinet 2009; 48:689-723.

26. Zhou SF. Polymorphism of human cytochrome P450 2D6 and its clinical significance: part II. Clin Pharmacokinet 2009; 48:761-804.