Original Article
Effect of dextromethorphan on CYP450 isoforms activity of rats

Zhuyin Jia[1*], Shihui Bao[2*], Yanyan Xu[3*], Zezheng Liu[4], Yingying Lin[4], Suping Yang[4], Xueli Huang[4], Congcong Wen[4], Lufeng Hu[5], Xuezhi Yang[5], Xianqin Wang[4]

[1]Department of Cardiology, Wenzhou Central Hospital Wenzhou 325000, Zhejiang, China; [2]The Second Affiliated Hospital & Yuying Children's Hospital, Wenzhou Medical University, Wenzhou 325000, Zhejiang, China; [3]Department of Pharmacy, Lishui Central Hospital, Lishui 323000, Zhejiang, China; [4]Analytical and Testing Center of Wenzhou Medical University, Wenzhou 325035, Zhejiang, China; [5]The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang, China. *Equal contributors.

Abstract: Dextromethorphan at high doses has phencyclidine-like effects on the NMDA receptor system; recreational use of high doses has been found to cause mania and hallucinations. In order to investigate the effects of dextromethorphan on the metabolic capacity of cytochrome P450 (CYP) enzymes, a cocktail method was employed to evaluate the activities of CYP2B1, CYP2D1, CYP1A2, CYP3A2, CYP2C11. The rats were randomly divided into dextromethorphan group (Low, Medium, High) and control group. The dextromethorphan group rats were given 12, 24, 48 mg/kg (Low, Medium, High) dextromethorphan by continuous intragastric administration for 7 days. Five probe drugs bupropion, metroprolol, phenacetin, testosterone and tolbutamide were given to rats through intragastric administration, and the plasma concentrations were determined by UPLC-MS/MS. Statistical pharmacokinetics difference for metroprolol, phenacetin and testosterone in rats were observed by comparing dextromethorphan group with control group. Continuous 7 days-intragastric administration of dextromethorphan inhibits the activities of CYP2D1 of rats. Enzyme inhibition by co-administered drugs and genetic variations of their expression can increase the risk of adverse reactions. Additionally, continuous 7 days-intragastric administration of dextromethorphan may not cause hepatotoxicity.

Keywords: CYP450, dextromethorphan, cocktail, UPLC-MS/MS, rat

Introduction

Dextromethorphan is an over-the-counter antitussive medication present in many brands of cough, cold, and flu preparations, such as Robitussin, Coricidin, Benylin, and others [1]. It is also the subject of renewed therapeutic interest as an adjunct to opiate use in pain management [2] and has been proposed as a possible rapidly acting antidepressant. The antitussive effects are believed to be mediated through the sigma receptor group, which has affinity for opioid antitussives such as codeine [3]. When taken in significant excess, dextromethorphan produces hallucinogenic and dissociative effects similar in nature to phencyclidine and ketamine [4]. Dextromethorphan metabolism is mediated through the isoenzyme group CYP2D6, which is subject to genetic polymor-

phism, which may result in impaired metabolism and accumulation of the drug, leading to toxicity [5].

Cytochrome P450 (CYP) enzymes are responsible for most biotransformation steps of xenobiotics and endogenous molecules. Variations of their activity by inhibition or induction can influence the pharmacokinetics and thereby the effect of drugs (of abuse). Enzyme inhibition by co-administered drugs (of abuse) and/or genetic variations of their expression can increase the risk of adverse reactions [6] or reduce the desired effect [7]. Such drug-drug interactions were described as a major reason for hospitalization or even death [8].

So far, no study on the effects of dextromethorphan on the metabolic capacity of CYP enzyme

was reported. Therefore, in this study, five probe drugs were employed to evaluate effect of dextromethorphan on the metabolic capacity of CYP2B1, CYP2D1, CYP1A2, CYP3A2, CYP2C-11. The effects of dextromethorphan on rat CYP enzyme activity will be evaluated according to the pharmacokinetic parameters changes of five specific probe drugs (bupropion, metropro-lol, phenacetin, testosterone and tolbutamide).

Material and methods

Chemicals

Bupropion, metroprolol, phenacetin, testoster-one and tolbutamide (all >98%) and the inter-nal 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) pur-chased from Shanghai SLAC Laboratory Animal Co., Ltd. Animals were housed under a natural light-dark cycle conditions with controlled tem-perature (22°C). All thirty-two rats were housed at Laboratory Animal Research Center of Wen-zhou Medical University. All experimental pro-cedures were approved ethically by the Wen-zhou Medical University Administration Com-mittee of Experimental Animals.

Pharmacokinetics

Thirty-two rats (220±20 g) were randomly divid-ed into four different dosages of dextrometho-rphan groups (Low-group, Medium-group, High-group and control group with 8 rats in each group). Dextromethorphan was dissolved in corn oil as suspension at three different concentrations (12, 24, 48 mg/mL). Three dif-ferent dextromethorphan group (Low-group, Medium-group, High-group) were respectively give dextromethorphan 12, 24, 48 mg/kg one time by intragastric administration at every morning, and last for 7 days. Control group were give saline by same administration meth-od. At 8 days morning, five probe drugs bupro-pion, metroprolol, phenacetin, testosterone and tolbutamide were mixed in corn oil and given to the rats of three dextromethorphan groups and control group by intragastric admin-istration at a single dosage 10 mg/kg for bupro-pion, metroprolol, phenacetin, testosterone, 0.1 mg/kg for tolbutamide.

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 h after intragastric administration of five pro-be drugs. Plasma (100 μL) was obtained from blood sample 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 sample. After vortex-mixing for 1.0 min, the sample was centrifuged at 13000 g for 15 min. Then super-natant (2 μL) was injected into the UPLC-MS/MS system for analysis.

Concentration of plasma probe drugs versus time was analyzed by Version 3.0 Data Analysis System (Wenzhou Medical University, China). The main pharmacokinetic parameters of the dextromethorphan group and control group were analyzed by SPSS I8.0 statistical soft-ware; statistical significance was assessed by t-test ($P<0.05$ was considered as statistically significant).

UPLC-MS/MS determination of probe drugs

The concentration of bupropion, metroprolol, phenacetin, testosterone and tolbutamide in rat plasma were simultaneously determined by a sensitive and simple UPLC-MS/MS meth-od [9]. The compounds were analyzed by a UPLC-MS/MS with ACQUITY I-Class UPLC and a XEVO TQD triple quadrupole mass spectrome-ter that equipped with an electrospray ioniza-tion (ESI) interface (Waters Corp., Milford, MA, USA). Data acquisition and instrument control were performed on the Masslynx 4.1 software (Waters Corp., Milford, MA, USA).

The LLOQ for each probe drug in plasma was 2 ng/mL. The RSD of the five probe drugs were less than 14%. 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 93% to 114%. The matrix effects were more than 85% or less than 112%. The extraction recoveries were better than 86%.

Histopathology

After pharmacokinetic properties analysis, rats were deeply anesthetized with 10% chloral

Table 1. Pharmacokinetic parameters of probe drugs from control group and dextromethorphan group rats (mean ± SD, n=8)

Parameters		$AUC_{(0-t)}$ ng/mL*h	$AUC_{(0-\infty)}$ ng/mL*h	t1/2z h	CLz/F L/h/kg	Vz/F L/kg	C_{max} ng/mL
Bupropion (CYP2B1)	Control	394.0±153.2	438.9±165.2	1.5±0.5	25.9±9.9	56.6±34.1	194.0±102.9
	Low	241.0±88.1	282.1±63.8	1.7±1.5	37.5±11.1	89.5±77.0	79.0±35.2*
	Medium	459.2±220.4	519.4±294.9	1.4±0.7	24.1±11.4	48.0 ±28.2	156.6±71.0
	High	401.6±157.2	460.3±177.1	1.8±0.5	24.8±10.3	62.9±28.8	119.7±44.8
Metroprolol (CYP2D1)	Control	1002.4±270.0	1009.5±276.1	0.7±0.1	10.5±2.5	11.0±3.5	458.1±124.1
	Low	1479.0±334.8**	1505.0±335.3**	0.6±0.4	6.9±1.5**	6.3±4.6*	492.4±84.6
	Medium	1822.1±357.3**	1885.1±505.6**	0.8±0.6	5.5±1.1**	5.3±2.1**	631.9±79.8**
	High	1955.4±437.1**	2053.6±463.5**	1.1±0.3**	5.1±1.0**	8.1±2.1	650.1±193.4*
Phenacetin (CYP1A2)	Control	7481.4±2117.8	7525.9±2176.2	0.4±0.1	1.4±0.5	0.8±0.4	5398.5±1002.0
	Low	2418.3±1327.2**	2434.5±1336.5**	0.5±0.1	6.0±4.5*	4.3 ±3.2**	1821.9±1022.6**
	Medium	6357.5±2656.6	6396.3±2682.1	0.4±0.1	1.8±0.8	1.1±0.5	5479.9±4932.8
	High	3592.1±1890.0**	3606.3±1892.3**	0.5±0.1	5.1±6.3	3.8±5.1	2672.7±1358.8**
Testosterone (CYP3A2)	Control	114.2±41.9	121.8±44.6	0.9±0.3	93.1±35.7	118.8±59.2	83.2±35.7
	Low	82.3±46.6	83.4±46.9	0.7±0.1	150.1±64.0	146.9±73.6	72.2±52.3
	Medium	61.0±13.4**	67.9±16.5**	1.0±1.1	155.3±38.5**	197.5±159.7	48.6±20.9*
	High	107.3±42.7	117.9±43.0	1.1±0.8	96.0±40.3	141.0±84.2	86.8±51.9
Tolbutamide (CYP2C11)	Control	101950.5±12783.0	106587.1±13578.1	5.2±0.3	0.010 ±0.001	0.071±0.008	9556.1±1075.7
	Low	111493.2±10194.4	118672.4±11743.0	5.7±0.7	0.008±0.001	0.069±0.007	10518.8±1426.4
	Medium	113210.5±6280.4	123104.4±7378.1	6.4±0.7	0.008±0.001	0.075±0.009	10131.2±838.5
	High	109777.7±5647.2	117088.7±7042.9	5.9±0.3	0.009±0.001	0.072±0.003	10790.9±2483.7

Dextromethorphan group was compared with the control group, *P<0.05, **P<0.01.

hydrate (i.p., 20 mg/kg). The some liver of control group and dextromethorphan treated groups were rapidly isolated and immersed in freshly prepared 4% w/v formaldehyde (0.1 M phosphate buffer, pH 7.2) for 48 h, and then embedded in paraffin. Then 5 µm-thick histo-logic sections were prepared and stained with routine HE method (hematoxylin and eosin). The morphological changes of liver were observed under light microscope.

Results

Pharmacokinetics

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

From the **Table 1**, no difference in pharmacokinetic behaviors can be observed between dextromethorphan group and control group for bupropion and tolbutamide. While for metropro-

lol, compared with the control group, $AUC_{(0-t)}$ increased (P<0.01), CL decreased (Low, P>0.05; medium, P<0.01; high, P<0.01), C_{max} increased (low, P>0.05; medium, P<0.01; high, P<0.05). While for phenacetin, compared with the control group, $AUC_{(0-t)}$ decreased (Low, P<0.01; medium, P>0.05; high, P<0.01), CL increased (Low, P<0.05; medium, P>0.05; high, P>0.05), C_{max} decreased (Low, P<0.01; medium, P>0.05; high, P<0.01). There no difference in pharmacokinetic behaviors can be observed between dextromethorphan group (low and high) and control group for testosterone, while the pharmacokinetic behaviors of testosterone in medium dosage group compared with the control group, $AUC_{(0-t)}$ decreased (P<0.01), CL increased (P<0.01), C_{max} decreased (P<0.05).

Morphological changes of liver

There was no obvious morphologic change observed between control group, low dose group, medium dose group and high dose group according to pathological examination of liver stained with hematoxylin and eosin (HE). The pathology slice examination showed: in control group, the liver lobules were intact, hepatocytic
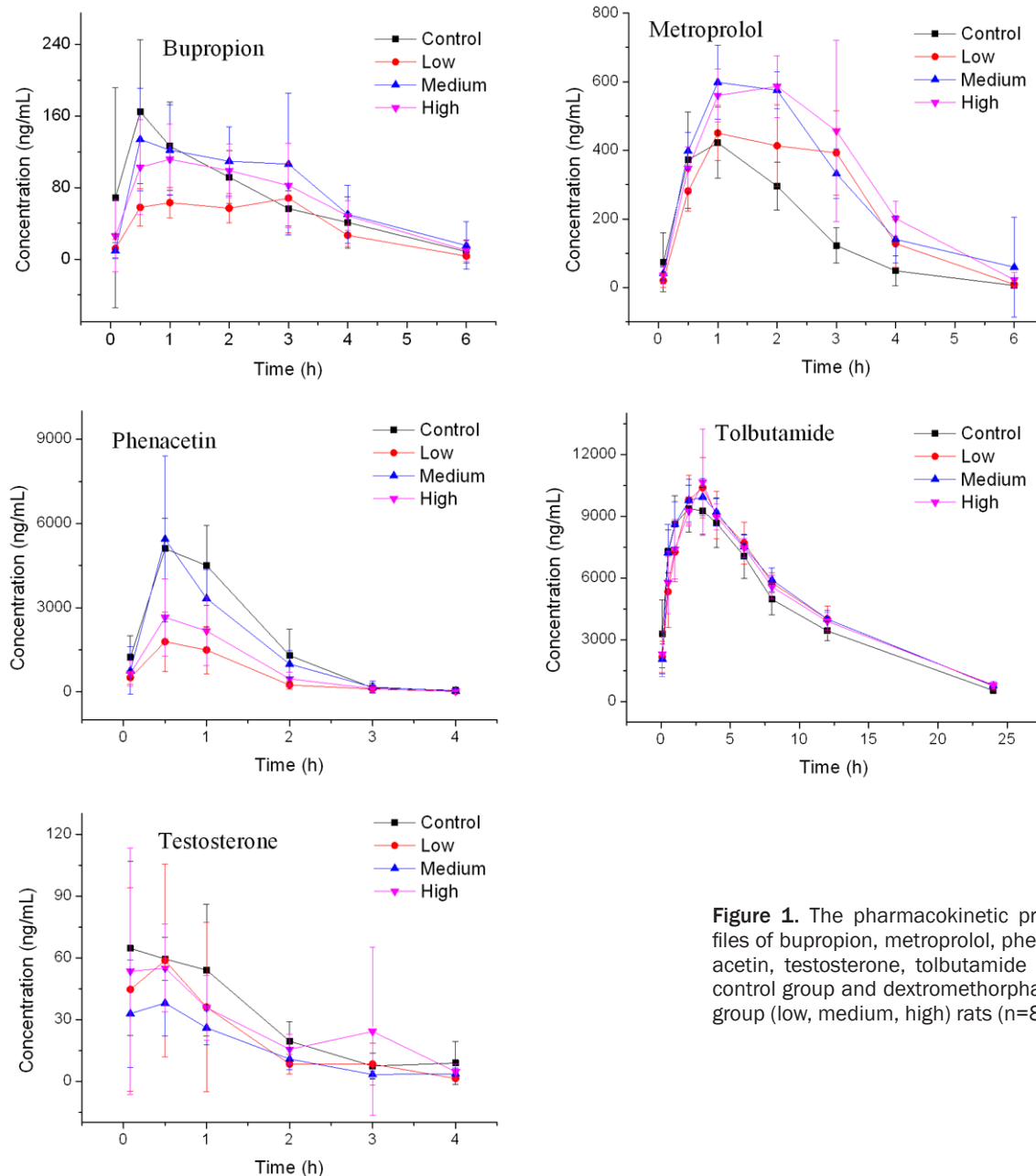
Figure 1. The pharmacokinetic profiles of bupropion, metroprolol, phenacetin, testosterone, tolbutamide in control group and dextromethorphan group (low, medium, high) rats (n=8).

plates separated by sinusoids, liver cells attached to each other tightly and arranged radially along with central veins. These normal histological structures hadn't damaged in three different dose groups after administrated dextromethorphan. In low dose group, medium dose group and high dose group, the hepatocytic plates were separated by sinusoids, liver lobules were intact and liver cells arranged tightly along with central veins which can be recognized clearly. There was no lobular inflammatory cell infiltration, liver cells swelling, dis-

solved, necrosis observed. The morphological changes of four groups were showed in **Figure 2**.

Discussion

In general, changes in pharmacokinetics are thought to be caused by drug-drug or drug-food interactions [10-15]. In pharmacokinetic interactions, approximately 65% of drug-drug interactions occur in metabolic sites, and drug metabolic enzymes are considered to be the most
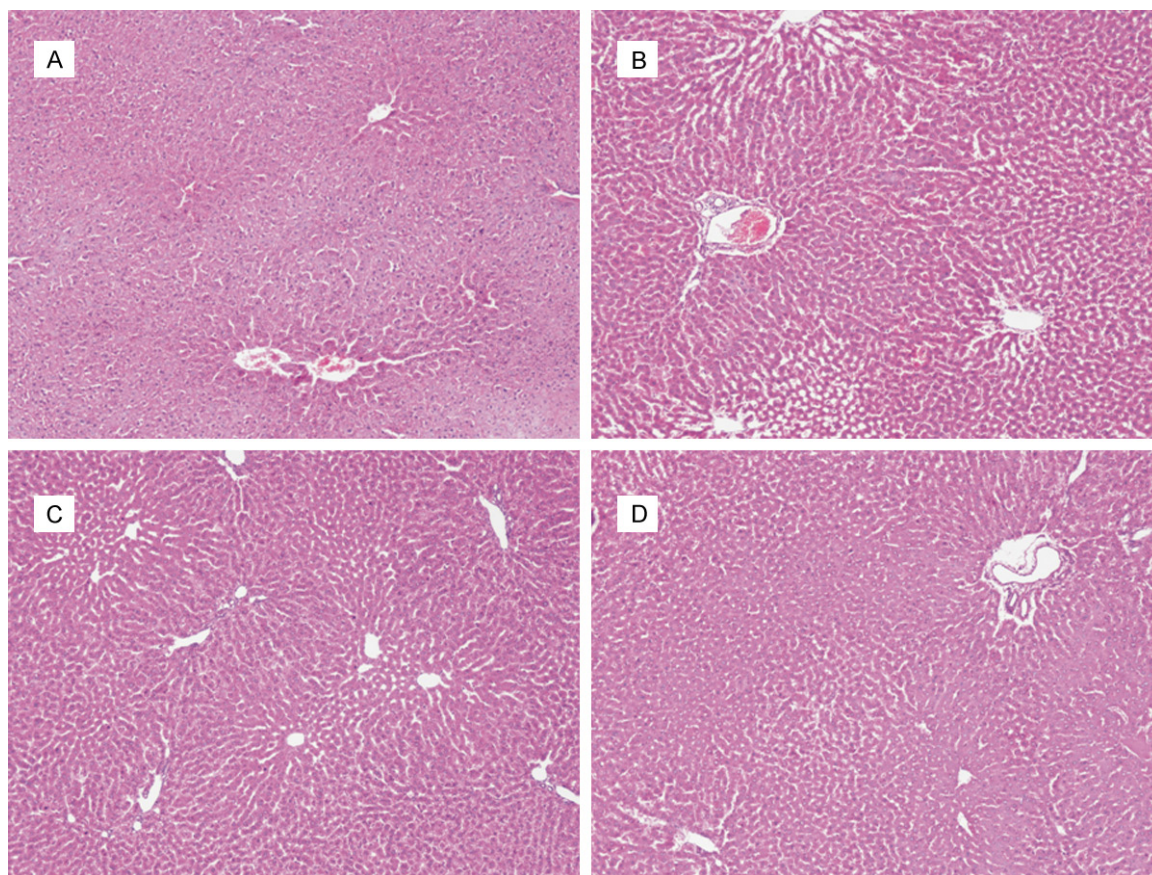
Figure 2. Morphological changes of liver in control-group (A) and low dosage group (B), medium dose group (C) and high dosage group (D) (hematoxylin-eosin, ×100).

important interactive sites. 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 [16, 17]. Similarly, supplement-drug interactions involving CYP activity are occasionally found to cause considerable adverse events. For these reasons, we evaluated the effects of dextromethorphan on the activity of CYP enzymes in *vivo*. We selected CYP isoforms CYP1A2, CYP2D1/CYP2D61, CYP3A2/CYP3A4, CYP2C11/CYP2C9 and CYP2B1/CYP2B6 because more than 90% of drugs are known to be metabolized by these 6 CYP enzymes [18, 19].

There no significant difference for AUC, CL and C_{max} of bupropion and tolbutamide ($P>0.05$) between the dextromethorphan group (low, medium, high) and control group was observed. It suggested that the dextromethorphan was not able to induce or inhibit the activity of CYP2B1 and CYP2C11 enzyme. The pharmaco-

kinetic parameters of metroprolol experienced obvious change with increased $AUC_{(0-t)}$ ($P<0.05$), C_{max} ($P<0.05$) and decreased CL ($P<0.05$) after the dosage increase. This result indicates that the 7 days-intragastric administration of dextromethorphan could inhibit the metabolism of metroprolol (CYP2D1) in rat. The pharmacokinetic parameters of phenacetin and testosterone experienced obvious change after 7 days-intragastric administration of dextromethorphan. However, pharmacokinetic results were not consistent with the dosage increase. It indicates that the 7 days-intragastric administration of dextromethorphan could not induce or inhibit the activity of the metabolism of phenacetin (CYP1A2) and testosterone (CYP3A2) in rat.

As dextromethorphan is always administrated in combination with other drugs, interactions between dextromethorphan and other drugs would increase the risk of either diminished efficacy or adverse effects. In our study, we

found that 7 days-intragastric administration of dextromethorphan inhibit the metabolism of metroprolol (CYP2D1). Therefore, the metabolism and elimination of drugs would change if they are administrated in combination with dextromethorphan.

After the pharmacokinetic profiles evaluation by cocktail method, we also investigated the hepatotoxicity of dextromethorphan by observing the pathological changes of liver after dextromethorphan administration. The pathological changes of liver were observed at three difference dosages with no changes, **Figure 2**. A more systematic and comprehensive study to investigate the hepatotoxicity of dextromethorphan will be carried out.

Conclusion

The results observed in this study would provide us valuable information regarding the interactions of dextromethorphan with other drugs. Inhibit of drug metabolizing enzyme CYP2D1 by dextromethorphan would increase the plasm concentration of other drug. Enzyme inhibition by co-administered drugs and genetic variations of their expression can increase the risk of adverse reactions.

Acknowledgements

Disclosure of conflict of interest

None.

Address correspondence to: Xuezhi Yang, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang, China. Tel: (86) 57755-579706; E-mail: yangxuezhi1977@163.com; Xianqin Wang, Analytical and Testing Center of Wenzhou Medical University, Wenzhou 325035, Zhejiang, China. Tel: (86) 57786699156; E-mail: lankywang@163.com

References

[1] Schwartz RH. Adolescent abuse of dextromethorphan. Clin Pediatr (Phila) 2005; 44: 565-568.

[2] Suski M, Bujak-Gizycka B, Madej J, Kacka K, Dobrogowski J, Woron J, Olszanecki R and Korbut R. Co-administration of dextromethorphan and morphine: reduction of post-operative pain and lack of influence on morphine metabolism. Basic Clin Pharmacol Toxicol 2010; 107: 680-684.

[3] Brown C, Fezoui M, Selig WM, Schwartz CE and Ellis JL. Antitussive activity of sigma-1 receptor agonists in the guinea-pig. Br J Pharmacol 2004; 141: 233-240.

[4] Logan BK, Yeakel JK, Goldfogel G, Frost MP, Sandstrom G and Wickham DJ. Dextromethorphan abuse leading to assault, suicide, or homicide. J Forensic Sci 2012; 57: 1388-1394.

[5] Takashima T, Murase S, Iwasaki K and Shimada K. Evaluation of dextromethorphan metabolism using hepatocytes from CYP2D6 poor and extensive metabolizers. Drug Metab Pharmacokinet 2005; 20: 177-182.

[6] Gasche Y, Daali Y, Fathi M, Chiappe A, Cottini S, Dayer P and Desmeules J. Codeine intoxication associated with ultrarapid CYP2D6 metabolism. N Engl J Med 2004; 351: 2827-2831.

[7] Stamer UM, Musshoff F, Kobilay M, Madea B, Hoeft A and Stuber F. Concentrations of tramadol and O-desmethyltramadol enantiomers in different CYP2D6 genotypes. Clin Pharmacol Ther 2007; 82: 41-47.

[8] Dinger J, Meyer MR and Maurer HH. Development of an in vitro cytochrome P450 cocktail inhibition assay for assessing the inhibition risk of drugs of abuse. Toxicol Lett 2014; 230: 28-35.

[9] Ma J, Wang S, Zhang M, Zhang Q, Zhou Y, Lin C, Lin G and Wang X. Simultaneous determination of bupropion, metroprolol, midazolam, phenacetin, omeprazole and tolbutamide in rat plasma by UPLC-MS/MS and its application to cytochrome P450 activity study in rats. Biomed Chromatogr 2015; 29: 1203-1212.

[10] Naramoto K, Kato M and Ichihara K. Effects of an Ethanol Extract of Brazilian Green Propolis on Human Cytochrome P450 Enzyme Activities in Vitro. J Agric Food Chem 2014; 62: 11296-302.

[11] Wang X, Han A, Wen C, Chen M, Chen X, Yang X, Ma J and Lin G. The effects of H2S on the activities of CYP2B6, CYP2D6, CYP3A4, CYP2C19 and CYP2C9 in vivo in rat. Int J Mol Sci 2013; 14: 24055-24063.

[12] Wang X, Chen X, Chen M, Hu G, Ma J, Pan J, Hu L and Lin G. Assessment of effects of chronic hydrogen sulfide poisoning on cytochrome P450 isoforms activity of rats by cocktail approach. Biol Pharm Bull 2013; 36: 1627-1633.

[13] Wang X, Chen M, Chen X, Ma J, Wen C, Pan J, Hu L and Lin G. The effects of acute hydrogen

sulfide poisoning on cytochrome P450 isoforms activity in rats. Biomed Res Int 2014; 2014: 209393.

[14] Cai J, Zhang Q, Lin K, Hu L and Zheng Y. The Effect of MGCD0103 on CYP450 Isoforms Activity of Rats by Cocktail Method. Biomed Res Int 2015; 2015: 517295.

[15] Lin K, Zhang Q, Liu Z, Yang S, Lin Y, Wen C and Zheng Y. Effects of suberoylanilide hydroxamic acid on rat cytochrome P450 enzyme activities. Int J Clin Exp Pathol 2015; 8: 5584-5590.

[16] Lin GY, Ma JS, Xu RA, Hu LF, Wang Z and Wang XQ. Effects of Ougan juice on P450 activities using a cocktail method. Pharmazie 2012; 67: 242-246.

[17] Qin CZ, Ren X, Tan ZR, Chen Y, Yin JY, Yu J, Qu J, Zhou HH and Liu ZQ. A high-throughput inhibition screening of major human cytochrome P450 enzymes using an in vitro cocktail and liquid chromatography-tandem mass spectrometry. Biomed Chromatogr 2014; 28: 197-203.

[18] Borkar RM, Bhandi MM, Dubey AP, Nandekar PP, Sangamwar AT, Banerjee SK and Srinivas R. Plasma protein binding, pharmacokinetics, tissue distribution and CYP450 biotransformation studies of fidarestat by ultra high performance liquid chromatography-high resolution mass spectrometry. J Pharm Biomed Anal 2014; 102: 386-399.

[19] Tan ML and Lim LE. The effects of Andrographis paniculata (Burm.f.) Nees extract and diterpenoids on the CYP450 isoforms' activities, a review of possible herb-drug interaction risks. Drug Chem Toxicol 2015; 38: 241-53.