Assessment of CYP2B6 activity in rats: A cocktail study with bupropion alone and in combined with tolbutamide

Ren-ai Xu, Lufeng Hu, Zhe Wang, Xiaoxiang Du, Haiyan Zhu and Xiuhua Zhang*
The First Affiliated Hospital of Wenzhou Medical College, Wenzhou, China

Abstract: A "cocktail"of numerous probe drugs to assess the metabolic activity of the corresponding cytochrome P450 enzymes requires that there is no problem of interaction among them. Some interactions among probe drugs can appear and may affect the rate of biotransformation of other ones. To develop a useful "cocktail" of probe drugs, our presented work was aimed on the influence of tolbutamide on cytochrome P450-mediated metabolism of bupropion. The biotransformation rates of bupropion administered either separately or in combined with tolbutamide were compared in this new study. The results revealed that tolbutamide had significantly decreased the rate of bupropion hydroxylation. Thus, due to shift in cytochrome P450 enzyme metabolic activity some extent differential results in the rate of P450-mediated metabolism can be observed when comparing assessment using combination of two model drugs with the common way (single marker use).

Keywords: Bupropion, tolbutamide, CYP, cocktail, drug-drug interaction.

INTRODUCTION

The activity of cytochrome P450 enzymes (CYP) is most often evaluated using specific probe drug of distinct P450 enzyme. In addition, there are often used more probe drugs together, so as the activity of multiple P450 enzymes could be determined simultaneously and the latter is the basis of many clinical studies in the field of drug metabolism and pharmacogenetics. CYP2C9 and CYP2B6 are two CYP isozymes for which activities are polymorphically expressed and that are therefore studied in clinical pharmacology researchs. Tolbutamide (TB) is a probe drug for CYP2C9 and its metabolite hydroxytolbutamide (HTB) is often used marker reactions for determining the metabolic activity of CYP2C9 *in vitro* (Tanaka *et al.*, 2003) as well as *in vivo* (Lee *et al.*, 2003). Bupropion (BUP), a second generation antide pressant agent with neurochemical properties different from common tricyclic antidepressants, is catalyzed almost exclusively by CYP2B6 (Faucette *et al.*, 2000; Parekh *et al.*, 2011). Hydroxybupropion (HBUP) is the major metabolite, since the plasma concentration of HBUP is neatly 4- and 6-fold greater and the AUC of HBUP is 10- to 16-fold greater compared to BUP (Faucette *et al.*, 2001). Thus BUP is recognized as a selective marker of CYP2B6 in vitro and *in vivo* (Faucette *et al.*, 2000; Kharasch *et al.*, 2008; Turpeinen *et al.*, 2004).

For practical reasons, cocktail approaches including TB and BUP were established to assess the activities of CYP2C9, CYP2B6 and other CYP isoforms to study whether other chemicals or drugs may induce or inhibit CYP system and predict the potential drug-drug interactions (Feidt *et al.*, 2010; Kim *et al.*, 2005; Otten *et al.*, 2011; Tolonen *et al.*, 2007; Turpeinen *et al.*, 2005). Unfortunately, the cocktail approach brings a question - can one marker influence the rate of biotransformation of another one? For example, interactions between chlorzoxazone (CYP2E1) and midazolam (CYP3A) (Palmer *et al.*, 2001), dextromethorphan (CYP2D6) and chloroguanide (CYP2C19) (Tenneze *et al.*, 1999) and caffeine (CYP1A2) and chlorzoxazone (CYP2E1) (Berthou *et al.*, 1995) have been reported. Thus, it is indispensable to undertake that the cocktail ingredients do not influence each other and that their metabolites do not produce any analytical interference in samples before using a probe cocktail.

The purpose of this article was aimed on the influence of TB on cytochrome P450-mediated metabolism of BUP. In the presented work, the biotransformation rates of BUP administered either separately or both simultaneously were compared.

MATERIALS AND METHODS

Materials

BUP (purity>98.0%), HBUP (purity>98.0%) and TB (purity>98.0%) were purchased from Sigma-Aldrich Company. Twelve male Sprague-Dawley rats, weighing between 200-250g, were all from Wenzhou Medical College Laboratory Animal Center (Wenzhou, China). The rats were housed into house cages at 20-24^{o}C and allowed free access to regular rodent diet and water. After the one week acclimatization period, all rats were utilized for official experiments and all efforts were made to minimize any animal suffering. All experimental procedures and protocols were reviewed and approved by the Animal Care and Use Committee of Wenzhou Medical College and were in accordance with the Guide for the Care and Use of Laboratory Animals.

**Corresponding author:* e-mail: wzzhangxiuhua@yahoo.cn

HBUP/BUP urinary metabolic ratio study

This was an open, randomized crossover study and the laboratory condition was kept standard at 23±2°C temperature and of 60% humidity. Twelve male SD rats were randomly divided into two groups (n=6), respectively. Before the study, diet was prohibited for 12h until 2 hours after drug administration, but water was freely available. In the first cycle, one group took BUP (15mg/kg) alone and another group received BUP (15mg/kg) and TB(3mg/kg) in combination after an overnight fast. Drugs were administered by gastric irrigation. Urine was collected during time intervals of 0 to 8 hours, 8 to 12 hours, and 12 to 24 hours after drug intake, and only 0.1mL supernatant urine was retained after centrifuged at 13,000 rpm for 10min. Before determination, each urine sample was diluted appropriate concentration.

Pharmacokinetic study

The above 12 rats were raised for a 2-weeks recovery stage for the secondary administration. In the secondary cycle, oral administration in the first cycle was repeated. Blood samples (0.15-0.2mL) from the tail vein were collected immediately into heparinized ploythene tubes before drug administration and 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 12 and 24h thereafter. The total volume of blood taken from each animal did not exceed 2.2 mL. After the samples centrifuged at 13,000 rpm for 10min, the plasma obtained (100μL) was transferred into 1.5mL heparinized ploythene tubes and stored at -20°C until analysis.

LC/MS analysis of BUP and its metabolite HBUP

For the analysis of BUP and its metabolite HBUP, a 1200 Series liquid chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a quaternary pump, a degasser, an autosampler, a thermostatted column compartment, and a Bruker Esquire HCT mass spectrometer (Bruker Technologies, Bremen, Germany) equipped with an electrospray ion source were used. Chromatographic separation was performed on a Agilent Zorbax SB-C18 column(150 mm × 2.1 mm, 3.5 μm particle) at 30 °C by using the gradient elution of 0.1% formic acid in water (mobile phase A) and acetonitrile (mobile phase B) as follows: 0-1.5 min (10-85% B), 1.5-6.0 min (85-85% B), 6.0-7.0 min (85-10% B), 7.0-10.0 min (10-10% B). The flow rate was 0.4 mL/min.

The quantification was performed by the peak-area method. Drying gas flow and nebuliser pressure was set at 6 L min^{-1} and 20 psi. Dry gas temperature and capillary voltage of the system were adjusted at 350 °C and 3,500 V, respectively. LC-MS was performed with SIM mode using target ions at m/z 239.9 for BUP and m/z 255.9 for HBUP in positive ion electrospray ionization interface. The lower limits of quantification (LLOQ) for BUP and HBUP were 10 ng/ml and 5 ng/ml, respectively. The accuracy (% bias) and precision (% CV) of the analytical method were less than 9% and 10% for both analytes at all concentrations.

Statistical analysis

The results were reported as a mean ± SD. All analyses for comparing the datas determined from BUP alone and in combination were performed with the SPSS software system version 16.0(SPSS Inc., Chicago) by use of Student's t-test. The level of significance was set at $P< 0.05$.

RESULTS

The mean BUP plasma concentrations (fig. 1) significantly increased ($P <0.001$) during coadministration of TB and area under the plasma concentration–time curve from zero to 24 hours [$AUC_{(0-24)}$], half-life, and peak concentration of BUP were significantly greater when BUP was given alone ($P<0.05$). However, mean HBUP concentrations (fig. 1) did not change significantly after co-administration of BUP and TB. Accordingly, there was significant difference in the pharmacokinetic parameters for BUP and for the plasma HBUP/BUP metabolic ratio in the presence of TB. In addition, the urinary HBUP/BUP metabolic ratio, an index of CYP2B6 activity, was significantly altered (table 1). When BUP was administered alone and in the presence of TB, there was a significant decrease ($P<0.001$) of the HBUP/BUP ratio between the urine collections from 8 to 12 hours and 12 to 24 hours, respectively. These results suggest that TB addition to BUP had significant effect on CYP2B6-mediated BUP hydroxylation.

Table 1: Urinary metabolic ratio of HBUP/BUP over 24 hours after administration of BUP alone or in the presence of TB

Time (h)	HBUP/BUP urinary metabolic ratio		P Value
	BUP alone	BUP + TB	
0-8	9.13 ± 4.32	8.77 ± 1.39	NS
8-12	111.64 ± 73.88	27.47 ± 9.67	P<0.001
12-24	1275.82 ± 505.64	151.10 ± 81.94	P<0.001

Values are expressed as mean ± SD. NS: Not significant.

DISCUSSION

The mechanisms underlying much of the inherited variability in drug response can be ascribed to variability in the activity of drug-metabolizing enzymes in partly. The differences of CYP enzyme activities can result in inter-individual differences in drug sensitivity and may cause serious side effects. Thus, the assessment of CYP enzyme activities becomes more and more important between individuals. A cocktail approach that simultaneously detects CYP enzyme activities following coinstantaneous administrations of different CYP-specific

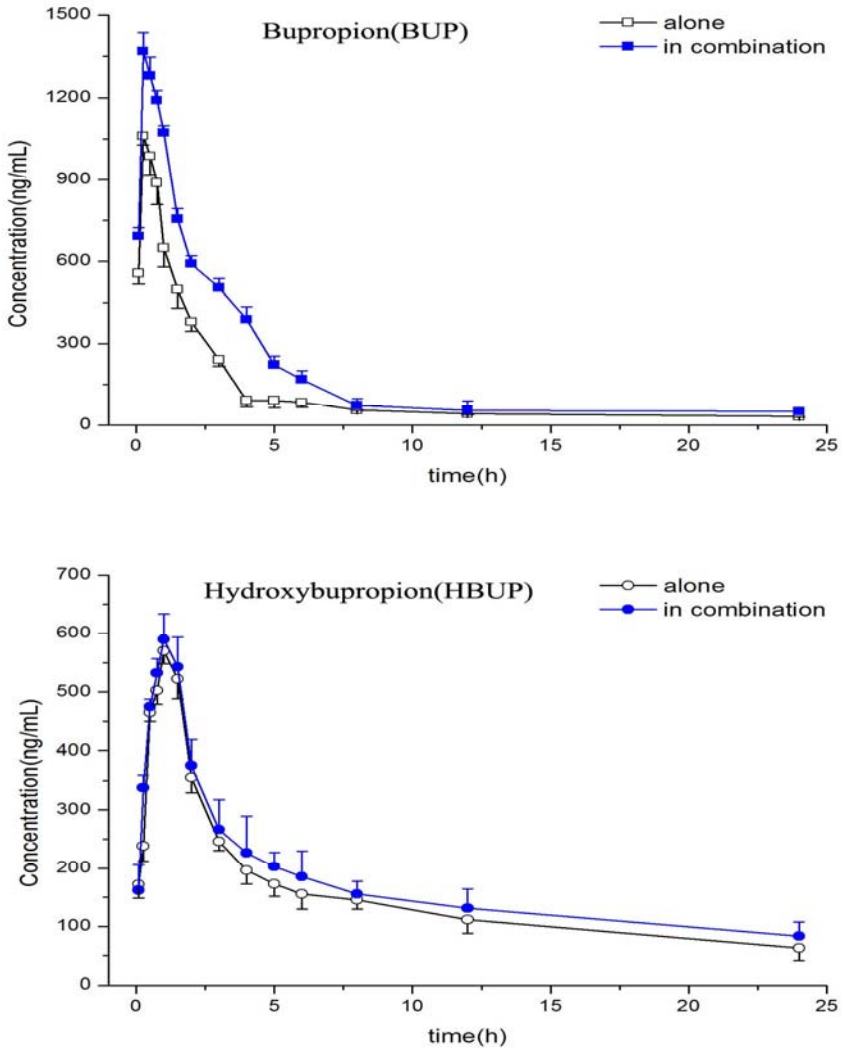

Fig. 1: Median plasma BUP and HBUP concentrations following BUP given alone and in combined with TB versus time profile.

probes is used to evaluate *in vivo* CYP enzyme activity (Li *et al.*, 2007; Liu *et al.*, 2009; Zhang *et al.*, 2008; Zhang *et al.*, 2010).

However, CYP metabolic activity may be influenced by many endogenous and exogenous factors, especially by co-administered specific drugs (Krizkova *et al.*, 2008). In our study, we observed that TB addition to BUP decreased significantly CYP2B6-mediated hydroxylation of BUP.

So far, detailed mechanism of this phenomenon is unclear. It could be hypothesized that acute decrease in the metabolic activity could not be explained by an enzyme inhibition (in its right sense - i.e. reducement of the expression of an enzyme), but more likely by some allosteric interaction. The possible explanation could be heterotrophic negative cooperativity (the third substance influences interaction of substrate and enzyme) of these substrates (or their metabolites or both), since similar effects have been already reported (Palmer *et al.*, 2001; Tenneze *et al.*, 1999). On the contrary, positive cooperativity also have been observed in cytochrome P450 enzymes, namely CYP1A2 (Isin *et al.*, 2008; Sohl *et al.*, 2008), CYP3A4 (Davydov *et al.*, 2008; Emoto *et al.*, 2001; Frank *et al.*, 2011; Frank *et al.*, 2009; Tang *et al.*, 2001), CYP3A5 (Okada *et al.*, 2009), CYP2B4 (Sulc *et al.*, 2008) and CYP2C9 (Liu *et al.*, 2005). The other explanation like enzyme inhibition can be eliminated due to the acute administration of substances, as well as the influence of tissue or solvent binding as the concentrations of marker and metabolite correspond in both experimental groups. Thus, we can see no other

possibility, how could other CYP enzymes (isoforms) decrease metabolic turnover of BUP to HBUP (reaction catalyzed selectively by CYP2B6).

Combination of the markers of metabolic activity in evaluating the CYP enzymes activity is quite often in practice. From the presented results may be suggested that TB had significantly decreased the rate of BUP hydroxylation.

ACKNOWLEDGMENT

This work was supported by Wenzhou Municipal Science and Technology projects, China, No. Y20110134.

REFERENCES

Berthou F, Goasduff T, Lucas D, Dreano Y, Le Bot MH and Menez JF (1995). Interaction between two probes used for phenotyping cytochromes P4501A2 (caffeine) and P4502E1 (chlorzoxazone) in humans, *Pharmacogenetics*, **5**(2): 72-79.

Davydov DR, Davydova NY, Tsalkova TN and Halpert JR (2008). Effect of glutathione on homo- and heterotropic cooperativity in cytochrome P450 3A4. *Arch. Biochem. Biophys.*, **471**(2): 134-145.

Emoto C, Yamazaki H, Iketaki H, Yamasaki S, Satoh T, Shimizu R, Suzuki S, Shimada N, Nakajima M and Yokoi T (2001). Cooperativity of alpha-naphthoflavone in cytochrome P450 3A-dependent drug oxidation activities in hepatic and intestinal microsomes from mouse and human. *Xenobiotica*, **31**(5): 265-275.

Faucette SR, Hawke RL, Lecluyse EL, Shord SS, Yan B, Laethem RM and Lindley CM (2000). Validation of bupropion hydroxylation as a selective marker of human cytochrome P450 2B6 catalytic activity, *Drug Metab. Dispos.*, **28**(10): 1222-1230.

Faucette SR, Hawke RL, Shord SS, Lecluyse EL and Lindley CM (2001). Evaluation of the contribution of cytochrome P450 3A4 to human liver microsomal bupropion hydroxylation. *Drug Metab. Dispos.*, **29**(8): 1123-1129.

Feidt DM, Klein K, Hofmann U, Riedmaier S, Knobeloch D, Thasler WE, Weiss TS, Schwab M and Zanger UM (2010). Profiling induction of cytochrome p450 enzyme activity by statins using a new liquid chromatography-tandem mass spectrometry cocktail assay in human hepatocytes. *Drug Metab. Dispos.*, **38**(9): 1589-1597.

Frank DJ, Denisov IG and Sligar SG (2011). Analysis of heterotropic cooperativity in cytochrome P450 3A4 using alpha-naphthoflavone and testosterone *J. Biol. Chem.*, **286**(7): 5540-5545.

Frank DJ, Denisov IG and Sligar SG (2009). Mixing apples and oranges: Analysis of heterotropic cooperativity in cytochrome P450 3A4. *Arch. Biochem. Biophys.*, **488**(2): 146-152.

Isin EM, Sohl CD, Eoff RL and Guengerich FP (2008). Cooperativity of cytochrome P450 1A2: Interactions of 1,4-phenylene diisocyanide and 1-isopropoxy-4-nitrobenzene. *Arch. Biochem. Biophys.*, **473**(1): 69-75.

Kharasch ED, Mitchell D and Coles R (2008). Stereoselective bupropion hydroxylation as an in vivo phenotypic probe for cytochrome P4502B6 (CYP2B6) activity *J. Clin. Pharmacol.*, **48**(4): 464-474.

Kim MJ, Kim H, Cha IJ, Park JS, Shon JH, Liu KH and Shin JG (2005). High-throughput screening of inhibitory potential of nine cytochrome P450 enzymes in vitro using liquid chromatography/tandem mass spectrometry, *Rapid Commun. Mass Spectrom.*, **19**(18): 2651-2658.

Krizkova J, Burdova K, Hudecek J, Stiborova M and Hodek P (2008). Induction of cytochromes P450 in small intestine by chemopreventive compounds. *Neuro. Endocrinol. Lett.*, **29**(5): 717-721.

Lee CR, Pieper JA, Frye RF, Hinderliter AL, Blaisdell JA and Goldstein JA (2003). Tolbutamide flurbiprofen and losartan as probes of CYP2C9 activity in humans *J. Clin. Pharmacol.*, **43**(1): 84-91.

Li X, Chen X, Li Q, Wang L and Zhong D (2007). Validated method for rapid inhibition screening of six cytochrome P450 enzymes by liquid chromatography-tandem mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **852**(1-2): 128-137.

Liu KH, Kim MJ, Jung WM, Kang W, Cha IJ and Shin JG (2005). Lansoprazole enantiomer activates human liver microsomal CYP2C9 catalytic activity in a stereospecific and substrate-specific manner. *Drug Metab. Dispos.*, **33**(2): 209-213.

Liu Y, Jiao J, Zhang C and Lou J (2009). A simplified method to determine five cytochrome p450 probe drugs by HPLC in a single run. *Biol. Pharm. Bull.*, **32**(4): 717-720.

Okada Y, Murayama N, Yanagida C, Shimizu M, Guengerich FP and Yamazaki H (2009). Drug interactions of thalidomide with midazolam and cyclosporine A: Heterotropic cooperativity of human cytochrome P450 3A5. *Drug Metab. Dispos.*, **37**(1): 18-23.

Otten JN, Hingorani GP, Hartley DP, Kragerud SD and Franklin RB (2011). An *in vitro*, high throughput, seven CYP cocktail inhibition assay for the evaluation of new chemical entities using LC-MS/MS. *Drug Metab. Lett.*, **5**(1): 17-24.

Palmer JL, Scott RJ, Gibson A, Dickins M and Pleasance S (2001). An interaction between the cytochrome P450 probe substrates chlorzoxazone (CYP2E1) and midazolam (CYP3A). *Br. J. Clin. Pharmacol.*, **52**(5): 555-561.

Parekh JM, Sutariya DK, Vaghela RN, Sanyal M, Yadav M and Shrivastav PS (2012). Sensitive selective and rapid determination of bupropion and its major active metabolite, hydroxybupropion, in human plasma by LC-MS/MS: Application to a bioequivalence study in

healthy Indian subjects. *Biomed. Chromatogr.*, 26(3)**:** 314-326.

Sohl CD, Isin EM, Eoff RL, Marsch GA, Stec DF and Guengerich FP (2008). Cooperativity in oxidation reactions catalyzed by cytochrome P450 1A2: Highly cooperative pyrene hydroxylation and multiphasic kinetics of ligand binding. *J. Biol Chem*, **283**(11): 7293-7308.

Sulc M, Hudecek J, Stiborova M and Hodek P (2008). Structural analysis of binding of a diamantoid substrate to cytochrome P450 2B4: Possible role of Arg 133 in modulation of function and activity of this enzyme. *Neuro. Endocrinol. Lett.*, **29**(5): 722-727.

Tanaka E, Kurata N and Yasuhara H (2003). How useful is the "cocktail approach" for evaluating human hepatic drug metabolizing capacity using cytochrome P450 phenotyping probes *in vivo*? *J. Clin. Pharm. Ther.*, **28**(3): 157-165.

Tang W and Stearns RA (2001). Heterotropic cooperativity of cytochrome P450 3A4 and potential drug-drug interactions. *Curr. Drug Metab.*, **2**(2): 185-198.

Tenneze L, Verstuyft C, Becquemont L, Poirier JM, Wilkinson GR and Funck-Brentano C (1999). Assessment of CYP2D6 and CYP2C19 activity *in vivo* in humans: A cocktail study with dextromethorphan and chloroguanide alone and in combination. *Clin. Pharmacol. Ther.*, **66**(6): 582-588.

Tolonen A, Petsalo A, Turpeinen M, Uusitalo J and Pelkonen O (2007). *In vitro* interaction cocktail assay for nine major cytochrome P450 enzymes with 13 probe reactions and a single LC/MSMS run: Analytical validation and testing with monoclonal anti-CYP antibodies. *J. Mass Spectrom.*, **42**(7): 960-966.

Turpeinen M, Nieminen R, Juntunen T, Taavitsainen P, Raunio H and Pelkonen O (2004). Selective inhibition of CYP2B6-catalyzed bupropion hydroxylation in human liver microsomes *in vitro*. *Drug Metab. Dispos.*, **32**(6): 626-631.

Turpeinen M, Uusitalo J, Jalonen J and Pelkonen O (2005). Multiple P450 substrates in a single run: Rapid and comprehensive in vitro interaction assay. *Eur. J. Pharm. Sci.*, **24**(1): 123-132.

Zhang S, Song N, Li Q, Fan H and Liu C (2008). Liquid chromatography/tandem mass spectrometry method for simultaneous evaluation of activities of five cytochrome P450s using a five-drug cocktail and application to cytochrome P450 phenotyping studies in rats. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.*, **871**(1): 78-89.

Zhang W, Han F, Guo P, Zhao H, Lin ZJ, Huang MQ, Bertelsen K and Weng N (2010). Simultaneous determination of tolbutamide, omeprazole, midazolam and dextromethorphan in human plasma by LC-MS/MS-a high throughput approach to evaluate drug-drug interactions. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.*, **878**(15-16): 1169-1177.