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World J Gastroenterol 2001;7(6):830-835
World Journal of Gastroenterology
 ISSN 1007-9327

• ORIGINAL RESEARCH •

Chiral metabolism of propafenone in rat hepatic microsomes treated with two inducers

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Supported by the National Natural Science Foundation of China (No39370805, No39770868) and Zhejiang Natural Science Foundation (No RC97016) of Zhejiang Province

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Received 2001-06-02 **Accepted** 2001-08-05

Abstract

AIM: **To study the influence of inducers of drug metabolism enzyme, β-naphthoflavone (BNF) and dexamethasone (DEX), on the stereoselective metabolism of propafenone in the rat hepatic microsomes.**

METHODS: **Phase I metabolism of propafenone was studied using the microsomes induced by BNF and DEX and the non-induced microsome was used as the control. The enzymatic kinetics parameters of propafenone enantiomers were calculated by regress analysis of Eadie-Hofstee Plots. Propafenone enantiomer concentrations were assayed by a chiral HPLC.**

RESULTS: **The metabolite of propafenone, N-desalkylpropafenone, was found after incubation of propafenone with the rat hepatic microsomes induced by BNF and DEX. In these two groups, the stereoselectivity favoring R(-) isomer was observed in metabolism at low substrate concentrations of racemic propafenone, but lost the stereoselectivity at high substrate concentrations. However, in control group, no stereoselectivity was observed. The enzyme kinetic parameters were: ①K_m. Control group: R(-) 83±6, S (+) 94±7; BNF group: R(-) 105±6, S(+)128±14; DEX group: R(-) 86±11, S(+) 118±16; ② υ_{max}. Control group: R(-) 0.75±0.16, S(+) 0.72±0.07; BNF group: R (-)1.04±0.15, S (+)1.0 7±14; DEX group: R(-) 0.93± 0.06, S(+) 1.04±0.09; ③ Cl_{int}. Control group: R(-) 8.9± 1.1, S(+) 7.6±0.7; BNF group: R(-)9.9±0.9, S(+)8.3± 0.7; DEX group: R(-) 10.9±0.8, S(+) 8.9±0.9. The enantiomeric differences in K_m and Cl_{int} were both significant, but not in υ_{max}, in BNF and DEX group. Whereas enanti omeric differences in three parameters were all insignificant in control group. Furthermore, K_m and υ_{max} were both significantly less than those in BNF or DEX group. In the rat liver microsome in duced by DEX, nimodipine (NDP) decreased the stereoselectivity in propafenone metabolism at low substrate concentration. The inhibition of NDP on the metabolism of propafenone was stereo selective with R(-)-isomer being impaired more than S(+)-isomer. The inhibition constant (Ki) of S(+)- and R(-)-propafenone, calculated from Dixon plots, was 15.4 and 8.6 mg·L^{-1}, respectively.**

CONCLUSION: ***CYP*1A subfamily (induced by BNF) and CYP3A4 (induced by DEX) have pronounced contribution to propafenone N-desalkylation which exhibited stereose lectivity depending on substrate concentration. The molecular base for this phenomenon is the stereo selectivity in affinity of substrate to the enzyme activity centers instead of at the catalyzing sites.**

Subject headings propafenone/metabolism; mitochondria; liver; rat; optical rotation

Zhou Q, Yao TW, Zeng S. Chiral metabolism of propafenone in rat hepatic microsom es treated with two inducers. *World J Gastroenterol*, 2001;7(6):830-835

INTRODUCTION

Propafenone, is a widely used antiarrhythmic agent administered as the racemic mixture of R(-) and S(+) enantiomers. The two enantiomers are equipotent in terms of sodium channel-blocking activity, but the main side effect, ie., β-adrenoreceptor-blocking action resides in the S (+)-isomer[1], and, therefore, information on stereoselective disposition of the racemate is of clinical relevance.

The main metabolic pathways of propafenone in vivo and *in vitro* involve CYP1A2 and CYP3A4 mediated N-desalkylation, CYP2D6 mediated 5-hydro xylation and UDPGT mediated glucuronidation[2-6]. N-desalkylpropafenone has the same electrophysi ological potency as 5-hydroxypropafenone and propafenone, and the plasma concentrations of N-desalkyl propafenone are similar to those of 5-hydroxypropafenone during chronic administration in human, therefore, N-desalkylpropafenone contributes to the antiarrhythmic effects of propafenone, especially in patients with poor metabolizer phenotype of CYP2D6[7-8]. Although stereoselectivities in 5-hydroxylation and glucuronidation *in vitro* have been reported[9-11], whether N-desalkylation exhibits stereoselectivity has not been addressed. Meanwhile, rat liver microsomes pretreated by specific inducers provide sound models to study metabolism *in vitro*[12-16]. Considering that β-nap hthoflavone (BNF) was a typical inducer of CYP1A subfamily and dexamethasone (DEX) was a typical inducer of CYP3A4[17-21], this experiment studied the stereoselective propafenone N-desalkylation in rat hepatic microsomes induced by BNF and DEX.

Material AND METHODS

Chemicals and solutions

Dexamethasone (DEX), β-naphthoflavone (BNF), 7-ethoxyresorufin (ER), triacety loleandomycin (TAO), NADPH, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocynate (GITC), (R,S)-propafenone, R(-) and S(+)-propafenone were supplied by Sigma Chemical Co (St. Louis, MO, US A). N-desalkylpropafenone was a generous gift from Prof. Tang YN (Xinhua Hospital, Shanghai). All other chemicals were obtained from the common commercial sources. Stock buffer (pH7.4): 1 mol·L^{-1} pH7.4 Tris-HCl buffer 25 mL, 1 mol·L^{-1} KCl 75 mL and 1 mol·L^{-1} $MgCl_2$ 5 mL were mixed and diluted with water to 500 mL. NADPH solution: dissolve NADPH in ice-cold 10 g·L^{-1} NaH CO_3

solution to the desired concentration of 25 mmol·L^{-1}. The solution should be freshly prepared just before the incubation.

Preparation of hepatic microsomes

Sprague-Dawley rats (male, 170-210 g) were divided into three groups. One group received three daily intraperitoned injection of 80 mg·kg^{-1} BNF (dissolved in oil); the second group received three daily DEX (132 mg·kg^{-1}·d^{-1}, ig) and the third group was used as the non-treated control. About 24 h after the last treatment and with no food supplied for 16 h before taking the livers, the rats were sacrificed by decapitation. Liver samples were excised and perfused by the ice-cold physiological saline to remove blood and homogenized in ice-cold Tris buffer. Hepatic microsomes were prepared with the ultracentrifugation methods[22,23]. All manipulations were carried out in cold bath. Pellets were re-suspended in sucrose-Tris buffer (pH 7.4)(95:5, mass to volume ratio) and immediately stored at -30℃. Protein and cy tochrome P450 contents were estimated according to the methods of Zeng *et al*[24]and Omura *et al*[25],respectively. Enzymatic activity of CYP1A was measured according to the method of Klotz *et al*[26], and expressed as initial velocity of O-deethylation of 7-ethoxyresorufin (activity of EROD). Enzymatic activity of CYP3A4 was determined according to the method of Wrighton *et al*[27], and expressed as the extent of P450-MI complex (absorbance difference per gram of protein between 456 nm and 510 nm) using triacetyloleandomycin as substrate. Incubation of propafenone with rat hepatic microsomes The incubation mixture contained microsomal protein (1.6 g·L^{-1}), stock buffer (pH 7.4) bubbled with oxygen for 1 min and racemic propafenone as substrate. After 5 min preincubation, reaction was started by adding 10 μL NADPH solution. The final volume was 250 μL. For kinetic experiments, racemic propafenone was used at concentrations of 10, 20, 40, 80, 160, and 320 mg·L^{-1} and the incubation time was 30 min. For the time dependent experiments, the substrate concentration used was 10 mg·L^{-1}. For inhibition experiments, nimodipine was used as inhibitors (at 0, 8, 16, 32 mg·L^{-1}) and incubated simultaneously with racemic propafenone (50, 100 mg·L^{-1}). After the indicated time, the reaction was terminated by adding 750 μL chlorform. The mixture was votexed for 3 min, then centrifuged at 2 000 g for 10 min. The organic layer was transferred to a clean tube and evaporated to dryness under a gentle stream of air. GITC solution (in acetonitrile) and methanol containing 14 g·L^{-1} triethamine were added and the tube was capped and allowed to react for 30 min a t 35℃. After evaporation of organic solvents, the residues were reconstutited with 100 μL methanol, and 20 μL was injected into HPLC system.

HPLC procedure for determining propafenone enantiomer in the rat hepatic incubates

Enantiomers of propafenone were quantitated with an HPLC system with UV detection (λ = 254 nm)[28]. A 5-ìm reverse phase column (Shimpack CLC- ODS 15 cm×4.6 mm) was used with a flow rate of 0.8 mL/min. The mobile phase was a mixture of methanol -water-glacial acetic acid (67:33:0.05).

Statistical analysis

The maximum velocity (υ_{max}) and Michaelis-Menten constant (K_m) values for propafenone enantiomer were determi ned by regress analysis of Edie-Hofstee plots. The $\bar{x}\pm s$ of three determinations of υ_{max} and K_m was calculated for each substrate and metabolic reaction. Intrinsic clearance was calculated by the ratio of υ_{max}/K_m. All statistical difference was tested by unpaired *t* test.

RESULTS

A baseline separation between the diastereomers of S(+) and R(-)-propafenone was achieved, with the retention time being 23 min and 28 min, respectively. The HPLC system also allowed monitoring the formation of N-desalkylpropafenone. The retention time was 8 min and 10 min for diastereomer of N-desalkylpropafenone, respectively. The amount of diastereomers of N-desalkylpropafenone were increasing while those of propafenone were decreasing during 30 min incubation with the rat hepatic microsomes induced by DEX and BNF. Typical chromatograms were showed in Figure 1. Quantitation was performed by external standardization. Calibration curves were linear at a range of 0.5 to 320 mg·L^{-1} for each enantiomer of propapfenone. The LOQ was 0.5 mg·L^{-1} (S/N = 10, *n* = 5) for each enantiomer. The inter-assay and intra-assay variability averaged 8.5% for both enantiomers. The method recovery averaged 77.1% for both enantiomers.

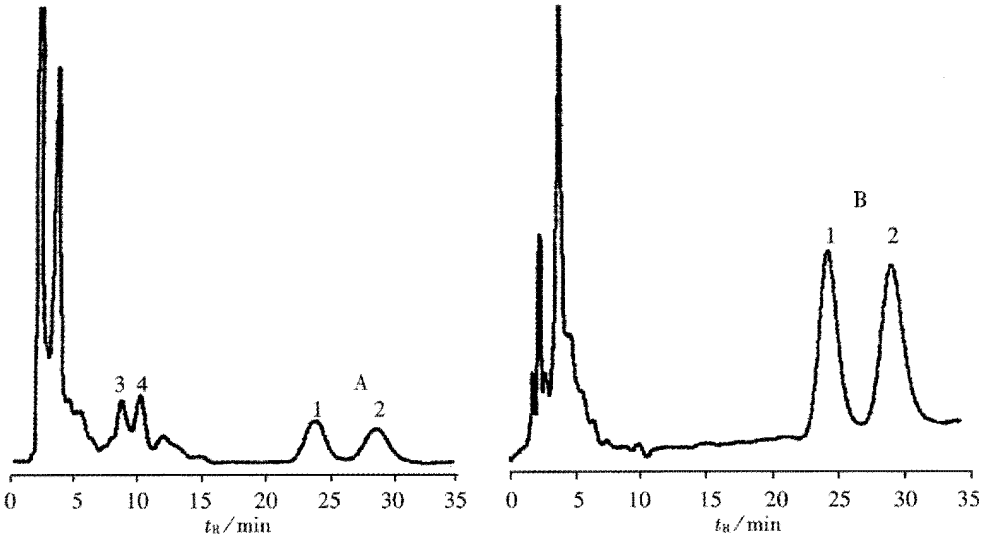

Figure 1 Chiral high performance liquid chromatogram of racemic propafenone in rat liver microsomal incubates after 30 min incubation. A:BNF pre-treated B:without incubation.

Peaks 1,2: Diastereomers of S(+)-propafenone and R(-)-propafenone; Peaks 3,4: Dia stereomers of metabolite (N-desalkylpropafenone)

Induction of rat hepatic metabolizingenzymes

In DEX group, the extent of P450-MI complex (an indicator of activity of CYP3A4) was significantly more than the control or BNF group (P<0.001, Table 1). In BNF group, the initial velocity of deethylation of 7-ethoxyresorufin (an indicator of activity of CY P1A) was significantly more than in the control or DEX group (about 20-fold, P<0.001). Therefore, CYP1A subfamily was successful inducted by BNF and CYP3A4 by DEX, which provided sound enzymatic sources for getting information on CYP1A and CYP3A4 mediated N-desalkylation of propafenone.

Table 1 The amount and activity of P450 in rat liver microsomes ($\bar{x}$±s, n = 3)

Pretreat	P450 in pro /μmol·g^{-1}	Extent of P450-MI complex△A	Activity of EROD /μmol·min^{-1}μg^{-1}
Control	0.95±0.15	0.5±0.2	0.22±0.04
BNF	1.42±0.21	2.2±0.4	3.87±0.20[b]
Dex	1.11±0.17	18.3±3.6[a]	0.18±0.02

[a]P<0.01,*vs* BNF or control, [b]P<0.01,*vs* Dex or control.

Impact of substrate concentration on stereoselective metabolism of propafenone

At 10 mg·L^{-1} concentration of racemic propafenone, stereoselectivity was observed in DEX and BNF group, but not in control group (Table 2). The depletion of R(-)-isomer was faster than that of S(+)-isomer. However, with the substrate concentration increasing, S/R ratios of prop afenone were not altered in control group (P>0.05), but in DEX and BNF group S/R ratios were decreasing from 1.18 to 1.00 (P <0.01), and 1.10 to 1.00 (P<0 .01), respectively.

Table 2 Ratio of S(+)/R(-) propafenone at different concentrations in rat liver microsomal incubates ($\bar{x}$±s, n = 3)

Enantiomer / mg·L^{-1}	Pretreat		
	Control	Dex	BNF
5	1.016±0.016	1.177±0.062[a,b]	1.104±0.019[a,b]
10	1.029±0.012	1.103±0.057	1.069±0.015
20	0.995±0.016	1.088±0.018	1.053±0.002
40	0.974±0.026	1.057±0.030	1.043±0.000
80	0.978±0.024	1.019±0.017	1.027±0.005
160	0.988±0.012	1.003±0.019	1.005±0.005

[a] P<0.01, *vs* control; [b]P<0.01,*vs* 160 mg·L^{-1}.

Concentration-time curves and ratio of S(+)/R(-) propafenone concentration

The ratio of S/R was in unity in control group from the incubation time of 0 to 30 min, whereas in DEX or BNF group, the ratio of S/R increased and was significantly different with the corresponding ratio in control group at 8 and 30 min (P<0.01, 0.05, Table 3). Moreover, the ratio of S/R in DEX group at incubation time of 30 min was significantly higher than that in BNF.

Table 3 Ratio of S(+)/R(-) propafenone concentration in rat liver microsomal incubates ($\bar{x}$±s, n = 3)

Group	t (incubation)/min				
	0	3	8	20	30 (min)
Control	1.000	1.017±0.010	0.997±0.016	1.006±0.012	1.016±0.016
Dex	1.000	1.007±0.003	1.044±0.011[d]	1.076±0.019	1.170±0.050[a,b,c]
BNF	1.000	1.005±0.002	1.031±0.012[d]	1.068±0.023	1.094±0.017[a,c]

[a]P<0.01, *vs* 8 min; [b]P<0.05, *vs* BNF group; [c]P<0.01, [d]P<0.05, *vs* control.

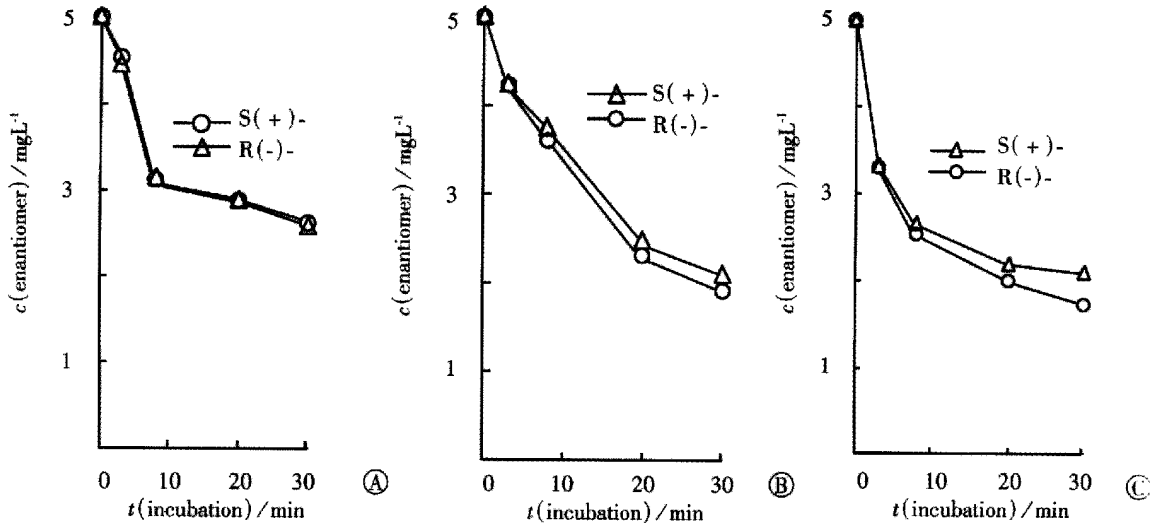

Figure 2 Concentration-time curves for S(+)- and R(-)-propa fenone metabolism in rat hepatic microsomes. A: Control; B: BNF; C: DEX.

Enzymatic kinetic parameters for propafenone metabolism in hepatic microsomes

Depletion of propafenone could be described by Michaelic-Menten kinetics. K_m had no statistical difference between the two enantiomers in control microsomes, whereas the enantiomeric difference in K_m was significant in the microsomes induced with DEX or BNF (S>R, P< 0.05, Table 4). There was signific ant difference for Clint between the two enantiomers (S<R, P<0.05, Table 4) in DEX or BNF group,

but not in control group. The K_m of S(+)-isomer in DEX, or S(+)- or R(-)-isomer in BNF group was significantly higher than the corresponding enantiomer in control group (P<0.05, 0.01, Table 4). The υ_{max} of S(+)-isomer in DEX group, or S(+)- or R(-)-isomer in BNF group, was significantly higher than the corresponding enantiomers in the control group (P<0.05, 0.01, Table 4). Difference for Clint between the two enantiomers in DEX or BNF group and the corresponding enantiomer in control group was insignificant. Moreover, the K_m of R(-)-propafenone in DEX group was significantly lower than that in BNF group (P <0.05, Table 4).

Table 4 Enzymatic parameters in propafenone enantiomer metabolism *in vitro* ($\bar{x}$±s, n = 3)

Pretreat	Enantiomer	K_m /μmol·L^{-1}	υ_{max} /μmol·g^{-1}·min^{-1}	Cl_{int} in prot /Lμmin^{-1}·g^{-1}
Control	S(+)	94±7	0.72±0.07	7.6±0.7
	R(-)	83±6	0.75±0.16	8.9±1.1
Dex	S(+)	118±16[a,b]	1.04±0.09[c]	8.9±0.9[a]
	R(-)	86±11[d]	0.93±0.06	10.9±0.8[b]
BNF	S(+)	128±14[a,c]	1.07±0.20[b]	8.3±0.7[a]
	R(-)	105±6[c]	1.04±0.15[b]	9.9±0.9

[a]P<0.05, *vs* R(-)-propafenone; [b]P<0.05, [c]P<0.01, *vs* corresponding enantiomer in control; [d]P<0.05, *vs* R(-)-isomer in BNF.

Stereoselective inhibition of propafenone metabolism by nimodipine

*K*i for S(+)- and R(-)-propafenone was 15.4 and 8.6 mg·L^{-1}, respectively, which suggested that nimodipine (specific substrate of C YP3A4) inhibited metabolism of propafenone enantiomer stereoselectively (Figure 3). With nimodipine amount increasing, the depletion of propafenone enantiomers and the S/R ratio of the remaining amount o f propafenone enantiomer were decreasing (Table 5).

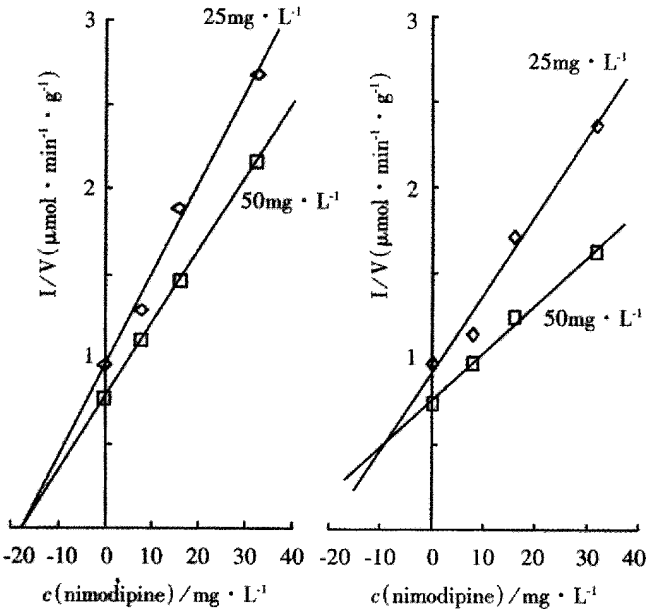

Figure 3 Dixon plot for S(+)-propafenone (Left) and R(-)-pro pa fenone (Right) wit nimodipine as inhibitor at three concentration. Ki for S(+)-and R(-)-PPF was 15.4, 8 mg·L^{-1}, respectively. Each data point represents the mean of duplicate determinations.

Table 5 The stereoselective effects of nimodipine on metabolic depletion of propafenone ($\bar{x}$±s, n = 3)

Group	Nimodipine /mg·L^{-1}	S(+)-propafenone	R(-)-propafenone /mg·L^{-1}	S/R
DEX	0	2.10±0.04	1.75±0.14[a]	1.20
DEX	8	2.32±0.26	2.10±0.21[b]	1.1
0DEX	16	3.81±0.11[c]	3.62±0.13[c]	1.0
6DEX	32	4.30±0.13[c]	4.17±0.26[c]	1.03

[a]P<0.01,*vs* S(+)-propafenone in DEX without nimodipine; [b]P<0.05, [c]P< 0.001, *vs* the corresponding enantiomer in DEX without nimodipine.

DISSCUSSION

Due to the capabilities of highly efficient separation and sensitive determination of enantiomers in microsome incubates, chiral chromatography is extremely valuable to study stereoselectivity of racemate met abolism[29-34]. So far as we are aware, we took the lead in acquiring the information on stereoc hemistry of propafenone metabolism by chiral HPLC method.

Previously, we observed that the glucuronidation of propranolol in rat hepatic microsome has stereoselectivity of S(-)-propranolol, and that the induction of phenobarbital reduced this stereoselectivity[35]. The phase I metabolic stereoselectivity of propranolol was reversed by the induction of BNF and increased by the induction of phenobarbital[36]. Phenobarbital instead of BNF inducted the stereoselective difference of Cl_{int} in glucuroniodation of ofloxacin[37]. However, the induction of DEX or BNF in this study vested propafenone metabolism with stereoselectivity in rat hepatic microsomes. It is thus clear that different inducers may have different impacts on some racemate metabolism.

The enantiomers of a racemic drug may differ in metabolic behavior as a consequence of stereoselective interaction with hepatic microsomes[38-42]. The underlying mechanism of stereoselectivity in metabolism, as many studies have shown, was enantiomeric difference in υ_{max} (an indice of enzymatic catalyzing ability) and/or in K_m (an index of enzyme affinity to the substrate). For example, the stereoselective N-demethylation of chlorpheniramine was due to enantiomeric differences in K_m[43]. Whereas there were little or no difference in K_m of the enantiomers of ofloxacin, the stereoselectivities in glucuronidation were caused by enantiomeric differences in υ_{max}[44]. The υ_{max} of the O-demethylation of (-)-tramadol was 1.6 times that of (+)-isomer, but the Km for both enantiomers was same, thus resulted in its stereos elective O-demethylation[45]. Recently, we have also proved that stereoselectivity of propranolol cytochrome P450 metabolism in the rat hepatic microsomes was due to the stereoselectivity of the catalyzing function in enzyme[35]. In this *in vitro* study, stereoselectivity of propafenone occurred in K_m and Clint in the rat hepatic microsomes induced by DEX or BNF, but not in υ_{max}. Combining with the interesting results of Table 2 that stereoselectivity depends on substrate conce ntration, we suppose that stereoselectivity at low substrate concentration was mainly due to the enantiomeric difference of the enzyme affinity to the substrate, and that insignificant enantiomeric difference in catalyzing abilities resulted in the abolished stereoselectivity at high subs trate concentration. Fujita *et al*[46] also reported that stereoselectivity of propranolol in rat liver microsomes was sometimes altered when the substrate concentration was varied. Augustijns *et al*[38] observed that the enantiomeric ratio (R/S) of desethylchloroquine was dependent on concentrati on, and ranged from 8 at 1 microM to 1 at 300 microM. Mutual enantiomer-enantiomer interacti on studies at low concentration (1-5 microM) revealed that the formation of (R)-desethylchlor oquine was strongly inhibited by (S)-chloroquine. In this *in vitro* metabolism,

enantiomer-enantiomer interaction at enzyme activity centers may also exist at low concentration, resulting in enantiomeric difference of the enzyme affinity to the substrate. This needs to be addressed by additional experiments.

Table 3 indicated that the stereoselectivity in DEX was stronger than in BNF. It maybe explained by the difference in K_m of R(-)-propafenone between DEX and BNF group and that the affinity of R (-)-PPF with CYP3A4 was higher than that with CYP1A, and that of S(+)-PPF with CYP3A4 was similar with CYP1A. Table 1 showed that CYP1A and CYP3A4 were significantly inducted by BNF and DEX, respectively, and this agreed with the well known documents. In BNF or DEX group, the υ_{max} was also significantly higher than that in the control group (about 1.5-fold), which indicated that CY P1A and CYP3A4 contributed to the metabolism of propafenone. This substantiated the methods used by Botsch *et al*[47]. In their study, CYP1A2 and CYP3A4 were identified involved in N-desalkylation using specific antibodies and inhibitors and stably expressed cytochrome P450. K_m in the control group was significantly lower than that in DEX or BNF group, which indicated that other enzyme with high affinity to substrate involved in metabolism of propafenone. CYP2D6 which had very low value of K_m might be one of such enzymes. Due to the lower value of both K_m and υ_{max} in control group, the Clint of propafenone enantiomer was not different from that in DEX or BNF group.

The competitive inhibition model (propafenone/nimodipine) suggested that propafenone and nimodipine were both substrates of the same coenzyme. Because nimodipine was as pecific substrate of CYP3A4[48,49], the results of inhibition experiment also proved that CYP3A4 contributed to propafenone metabolism. Drug interaction of enantiomer with specific inhibitor of P450 is an important tool in the search for detailed information on the stereoselective metabolism of xenobiotics[1]. Because fluoxetine impeded in vivo met abolism of R-methadone more than that of S-methadone, Eap *et al*[50] conclud ed that CYP2D6-mediated methadone metabolism exhibited stereoselectivity. The fact that the AUC ratio for the two enantiomers of reboxetine was minimally affected by ketoconazole treatment indicates similar affinities of the enantiomers for CYP3A4[51]. In the present study, the phenomenon that nimodipine in hibited S(+)-propafenone more than R(-)-isomer also implies that CYP3A4-mediated propafenone metabolism existed stereoselectivity.

REFERENCES

1 Zhou Q, Yao TW, Zeng S. Chiral drug interaction. *Zhongguo Yaoxue Zazhi*, 1999;34:721-724
2 Hofmann U, Pecia M, Heinkele G, Dilger K, Kroemer HK, Eichelbaum M. Determination of propafenone and its phase I and phase II metabolites in plasma and urine by high-performance liquid chromatography-electrospray ionization mass spectrometry. *J Chromatogr B Biomed Sci Appl*, 2000;748:113-123
3 Hemeryck A, De Vriendt C, Belpaire FM. Effect of selective serotonin reuptake inhibitors on the oxidative metabolism of propafenone: *in vitro* studies using human liver microsomes. *J Clin Psychopharmacol*, 2000;20:428-434
4 Tan W, Li Q, McKay G, Semple HA. Identification and determination of phase I metabolites of propafenone in rat liver perfusate. *J Pharm Biomed Anal*, 1998;16:991-1003
5 Bonato PS, de Abreu LR, de Gaitani CM, Lanchote VL, Bertucci C. Enantioselective HPLC analysis of propafenone and of its main metabolites using polysaccharide and protein-based chiral stationary phases. *Biomed Chromatogr*, 2000;14:227-233
6 Dilger K, Hofmann U, Klotz U. Enzyme induction in the elderly: effect of rifampin on the pharmacokinetics and pharmacodynamics of prop afenone. *Clin Pharmacol Ther*, 2000;67:512-520
7 Fonck K, Haenebalcke C, Hemeryck A, Belpaire F, Jordaens L, Calle P, Buylaert W. ECG changes and plasma concentrations of propafenone and its metabolites in a case of severe poisoning. *J Toxicol Clin Toxicol*, 1998;36:247-251
8 Yao TW, Zhou Q, Hu XY, Zeng S. Stereoselectivity of racemic propafenone in N -depropy lation with rat liver microsomes pretreated by different inducers. *Zhongguo Yaolixue Yu Dulixue Zazhi*, 2000;14:440-444
9 Chen X, Zhong D, Blume H. Stereoselective pharmacokinetics of propafenone and its major metabolites in healthy Chinese volunteers. *Eur J Pharm Sci*, 2000;10:11-16
10 Cai WM, Chen B, Cai MH, Chen Y, Zhang YD. The influence of CYP2D6 activity on the kinetics of propafenone enantiomers in Chinese subjects. *Br J Clin Pharmacol*, 1999;47:553-556
11 Cai WM, Chen B, Zhou Y, Zhang YD. Fluoxetine impairs the CYP2D6-mediated metabolism of propafenone enantiomers in healthy Chinese volunteers. *Clin Pharmacol Ther*, 1999;66:516-521
12 Nebbia C, Ceppa L, Dacasto M, Carletti M, Nachtmann C. Oxidative metabolism of monensin in rat liver microsomes and interactions with tiamulin and other chemotherapeutic agents: evidence for the involvement of cytochrome P-450 3A subfamily. *Drug Metab Dispos*, 1999;27:1039-1044
13 Yamazaki H, Komatsu T, Takemoto K, Shimada N, Nakajima M, Yokoi T. Rat cytochrome p450 1a and 3a enzymes involved in bioactiva tion of tegafur to 5-fluorouracil and autoinduced by tegafur in liver microsomes. *Drug Metab Dispos*, 2001;29:794-797
14 Fontaine F, de Sousa G, Burcham PC, Duchene P, Rahmani R. Role of cyto chrome P450 3A in the metabolism of mefloquine in human and animal hepatocytes. *Life Sci*, 2000;66:2193-2212
15 Watanabe M, Tateishi T, Asoh M, Nakura H, Tanaka M, Kumai T, Kobayashi S. Role of CYP3A in haloperidol N-dealkylation and pharmacokinetics in rats. *Fundam Clin Pharmacol*, 1999;13:337-342
16 Sarver JG, Bachmann KA, Zhu D, Klis WA. Ethosuximide is primarily metabolized by CYP3A when incubated with isolated rat liver microsomes. *Drug Metab Dispos*, 1998;26:78-82
17 Hansen T, Borlak J, Bader A. Cytochrome P450 enzyme activity and protein expression in primary porcine enterocyte and hepatoc yte cultures. *Xenobiotica*, 2000;30:27-46
18 Price RJ, Surry D, Renwick AB, Meneses-Lorente G, Lake BG, Evans DC. CYP isoform induction screening in 96-well plates: use of 7-benz y loxy-4-trifluoromethylcoumarin as a substrate for studies with rat hepatocytes. *Xenobiotica*, 2000;30:781-795
19 Shoda T, Mitsumori K, Onodera H, Toyoda K, Uneyama C, Takada K, Hirose M. Liver tumor-promoting effect of beta-naphthoflavone, a strong CYP 1A 1/ 2 inducer, and the relationship between CYP 1A1/ 2 induction and Cx32 decrease in its hepatocarcinogenesis in the rat. *Toxicol Pathol*, 2000;28:540-547
20 Zhao C, Duquet S, Zhou YX. Effects of combined use of diallyl disulfide and Nacetyl-cysteine on acetaminophen hepatotoxicity in beta -naphthoflavone pretreated mice. *World J Gastroenterol*, 1998;4:112-116
21 McCune JS, Hawke RL, LeCluyse EL, Gillenwater HH, Hamilton G, Ritchie J, Lindley C. *In vivo* and *in vitro* induction of human cytochrome P4503A4 by dexamethasone. *Clin Pharmacol Ther*, 2000;6 8:356-366
22 Gibson GG, Skett P. Introduction to drug metabolism (Second edition). *London: Blackie Aca demic & Professional*, 1994:217-221
23 Tang YN, Zhang SG, Li L, Zhang WL. Preparation of human liver microsomes and determination of cytochrome P450. *Zhongguo Yiyuan Yaoxue Zazhi*, 1998; 1 8: 535-536
24 Zeng S, Li Yan. Determination of stereoselectivity for phase I metabolism of S-(-)- and R-(+)-ofloxacin by RP-HPLC. *Zhejiang Daxue Xuebao (Yixue Ban)*, 1999;28:265-267
25 Omura T, Sato R. The carbon monooxide-binding pigment of live microsomes. I.Evidence for its hemoprotein nature. *J Biol Chem*, 1964; 239:2370-2378
26 Klotz AV, Stegeman JJ, Walsh C. An alternative 7-ethoxyresorufin O-deethylase activity assay: a continuous visible spectropho tometeic method for measurement of cytochrome P450 monooxygenase activity. *Anal Biochem*, 1984;140:138-145
27 Wrighton SA, Maurel P, Schuetz EG, Watkins PB, Young B, Guzelian PS. Identification of the cytochrome P450 induced by macrolide antibiotics in rat liver as the glucocorticoid responsive cytochrome P450p. *Biochemistry*, 1985;24:2171-2178
28 Zhou Quan, Yao TW, Zeng S. Chiral RP-HPLC assay for propafenone enantiomers in rat liver microsomal incubates and its application *in vitro* metabolism. *Yaoxue Xuebao*, 2000;35:370-373
29 Zeng S, Zhong J, Pan L, Li Y. HPLC separation and quantitation of ofloxacin enantiomers in rat microsomes. *J Chromatogr B*, 1999;728: 151-155
30 Li X, Yao TW, Zeng S. Reversed-phase high-performance liquid chromatographic analysis of atenolol enantiomers in rat hepatic microsome after chiral derivatizaton with 2,3,4,6,-tetra-O-acetyl-beta-D-glycopyranosyl isothiocyanate. *J Chromatogr B*, 2000;742:433-439
31 Zeng S, Mao HQ. Stereoselective determination of fenfluramine enantiom ers in rat liver microsomal incubates. *J Chromatogr B*, 1999; 727:107-112

32 Zeng S, Zhang L, Chen YZ. Chiral GC assay with flame ionization detection for amphetamine enantiomers in microsomal incubates. *Biomed Chromatogr*, 1999;13:33-36

33 Yao TW, Zeng S. Stereoselective determination of p-hydroxyphenylphenylhydantoin enantiomers in rat liver microsomal incubates by reversed-phase high-performance liguid chromatography using β-cyclodextrin as chiral mobile phase addictives. *Biomed Chromatogr*, 2001;15:141-144

34 Yao TW, Zhou Q, Zeng S. Stereoselective determination of propafenone enantiomers in transgenic Chinese hamster CHL cells expressing human cytochrome P450. *Biomed Chromatogr*, 2000;14:498-501

35 Li X, Liu Q, Zeng S. Influence of Phenobarbital induction the stereoselectivity of propranolol glucuronidation in rat hepatic microsome. *Zhejiang Daxue Xuebao (Yixue Ban)*, 1999;28:202-205

36 Li X, Zeng S. Stereoselective propranolol metabolism in two drug induced rat hepatic microsomes. *World J Gastroenterol*, 2000;6:74-78

37 Yao TW, Zeng S, Li X. Glucuronidation metabolism of ofloxacin enantiomers in rat microsomes treated with different inducers. *Zhongguo Yaolixue Yu Dulixue Zazhi*, 2000;14:268-272

38 Augustijns P, Peeters L, Croes K, Verbeeck RK.. Stereoselective deethylation of chloroquine in rat liver microsomes. *Eur J Drug Metab Pharmacokinet*, 1999;24:105-108

39 Echizen H, Tanizaki M, Tatsuno J, Chiba K, Berwick T, Tani M, Gonzalez FJ, Ishizaki T. Identification of CYP3A4 as the enzyme involved in the mono-N-dealkylation of disopyramide enantiomers in humans. *Drug Metab Dispos*, 2000;28:937-944

40 Desta Z, Soukhova N, Morocho AM, Flockhart DA. Stereoselective metabolism of cisapride and enantiomer -enantiomer interaction in human cytochrome P450 enzymes: major role of CYP3A. *J Pharmacol Exp Ther*, 2001;298:508-520

41 Wienkers LC, Allievi C, Hauer MJ, Wynalda MA. Cytochrome P-450-mediated metabolism of the individual enantiomers of the anti depressant agent reboxetine in human liver microsomes. *Drug Metab Dispos*,1999;27:133 4-1340

42 Li X, Zeng Su. Metabolic characteristics of the optical isomers of propranolol i n the cytochrome P450 of rat liver microsomes treated with different inducers. *Zhongguo Yaolixue Yu Duli Xue Zazhi*, 1999; 13:53-56

43 Nomura A, Sakurai E, Hikichi N. Stereoselective N-demethylation of chlorpheniramine by rat-liver microsomes and the involvement of cytochrome P450 isozymes. *J Pharm Pharmacol*, 1997;49:257-262

44 Okazaki O, Kurata T, Hakusui H, Tachizawa H. Stereoselective glucuronidation of ofloxacin in rat liver microsomes. *Drug Metab Dispos*, 1991;19:376-380

45 Paar WD, Frankus P, Dengler HJ. The metabolism of tramadol by human liver microsomes. *Clin Investig*, 1992;70:708-710

46 Fujita S, Umeda S, Funae Y, Imaoka S, Abe H, Ishida R, Adachi T, Masuda A, Kazusaka A, Suzuki T. Regio- and stereoseletive propranolol metabolism by 15 forms of purified cytochromes P-450 from rat live. *J Pharmacol ExpTher*, 1993;264:226-233

47 Botsch S, Gautier JC, Beaune P, Eichelbaum M, Kroemer H. Identification and characterization of the cytochrome P450 enzymes involved in N -dealkylation of propafenone: molecular base for interaction potential and variable disposition of active metabolites. *Mol Pharmacol*, 1993;43:120-126

48 Liu XQ, Ren YL, Qian ZY, Wang GJ. Enzyme kinetics and inhibition of nimodipine metabolism in human liver microsomes. *Acta Pharmacol Sin*, 2000; 21:690-694

49 Michalets EL. Update: Clinically significant cytochrome P-450 drug interactions. *Pharmacotherapy*, 1998;18:84-112

50 Eap CB, Bertschy G, Powell K. Fluvoxamine and fluoxetine do not interact in the same way with the metabolism of the enantiomers of methadone. *J Clin Psychopharmacol*, 1997;17:113-117

51 Herman BD, Fleishaker JC, Brown MT. Ketoconazole inhibitors the clearance of the enantiomers of the antidepressant reboxetine in humans. *Clin Pharmacol Ther*, 1999;66:374-379

Edited by Xu JY