ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Oct; 22 (10): 944-948
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Stereoselective metabolism of propafenone by human liver CYP3A4 expressed in transgenic Chinese hamster CHL cells lines[1]

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KEY WORDS propafenone; stereoisomers; metabolism; cytochrome P-450 CYP3A4

ABSTRACT

AIM: To investigate the stereoselective metabolism of propafenone (PPF) by human liver CYP3A4. **METHODS**: A chiral and an achiral HPLC were combined to determine the enantiomer of PPF in S_9 incubates prepared from transgenic Chinese hamster CHL cells lines expressing CYP3A4. The time-dependent study was performed using individual enantiomer or racemate at low or high substrate concentration. Kinetic parameters were determined employing individual enantiomers as substrates. Enantiomeric inhibition expriments were performed by using R(-)-PPF as an inhibitor and S(+)-PPF as a substrate. **RESULTS**: Stereoselectivity was found in metabolism of racemic PPF at low substrate concentration (10 mg/L) (S < R), and lost at high substrate concentration (400 mg/L). When an individual enantiomer of high concentration (200 mg/L) was used as substrate, S(+)-PPF was eliminated faster than its isomer (S > R). However, the opposite situation was observed at low concentration (5 mg/L) (S < R). The V_{max} of S(+)-PPF was significantly greater than that of R(-)-PPF [(2.66 ± 0.32) *vs* (1.71 ± 0.19) $\mu mol \cdot mg^{-1} \cdot min^{-1}$]. The K_m of R(-)-PPF was significantly lower than that of S(+)-PPF [(73 ± 11) *vs* (185 ± 17) $\mu mol \cdot L^{-1}$]. R(-)-PPF inhibited S(+)-isomer with an IC_{50} value of 125 $\mu mol \cdot L^{-1}$. **CONCLUSION**: It is concluded that stereoselectivity in metabolism of propafenone *via* CYP3A4 depend on substrate concentration. Enantiomer/enantiomer interaction of PPF occured at high concentration of substrate, and resulted in the loss of stereoselectivity. There maybe no enantiomer/enantiomer interaction at low concentration thus keeping the superiority of R(-)-PPF in metablism.

INTRODUCTION

Pharmacokinetic and pharmacodynamic differences involving stereoselective drug disposition have provided an important aspect in the clinical evaluation of some chiral compounds that are rountinely administered in their racemic form[1]. For propafenone (PPF), an Ic antiarrhythmic agent, 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[2].

It is generally accepted that drug metabolism introduces the greatest degree of stereoselectivity in drug disposition. Propafenone undergoes three main metabolic pathways, including *N*-desalkylation *via* CYP3A4 and CYP1A2, 5-hydroxylation *via* CYP2D6, and glucuronidation *via* glucuronosyltransferase[3]. A loss of substrate stereoselectivity in 5-hydroxylation of propafenone *in vitro* was observed due to enantiomer/enantiomer interaction[4], whereas glucuronidation *in vitro* exhibited stereoselectivity[3]. However, whether stereoselectivity or enantiomer/enantiomer interaction exists in *N*-desalkylation has not been addressed. Furthermore, considering that *N*-desalkylpropafenone has the same electrophysiological potency as 5-hydroxypropafenone and propafenone, and that the plasma concentrations of *N*-desalkylpropafenone are similar to those of 5-hydroxypropafenone during chronic administration in human[5], futher investigation in disposition *via* CYP3A4 is of clinical relevance.

Construction of cells lines expressing individual forms of human cytochromes P450 by means of genetic

[1] Project supported by the National Natural Science Foundation of China (№ 39770868 and № 39670801) and Zhejiang Natural Science Foundation (№ RC97016).
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Received 2001-07-02 Accepted 2001-08-08

engineering is an important tool in the search for detailed information on the oxidative metabolism of xenobiotics[6]. Stable expression of several cDNA-encoding human cytochromes P450 in Chinese hamster CHL cells has been achieved[7-9]. The present study was mainly to explore detailed information on stereoselective metabolism of PPF by CYP3A4 expressed in transgenic Chinese hamster CHL cells lines.

MATERIALS AND METHODS

Chemicals Racemic PPF hydrochloride (99.5 %) was obtained from Yiatai Pharmaceutical Company (Zhejiang, China), NADPH (98 %), S(+)-propranolol, S(+)-PPF, R(−)-PPF, 2,3,4,6-tetra-*O*-β-*D*-glucopranosyl isothiocyanate (GITC) were purchased from Sigma (St Louis, MO, USA). *N*-desalkylpropafenone was generously provided by Prof Tang Yao-Nian (Xinhua Hospital, Shanghai, China). All other chemicals and solvents were of analytical or chromatographic grade.

Preparation of S_9 Transgenic Chinese hamster CHL cells expressing CYP3A4 (CHL-3A4) were established by Department of Pathophysiology & Laboratory of Medical Molecular Biology (College of Medicinal Sciences, Zhejiang University, China), and cultured in MEM (Gibcol, Uxbridge, UK) supplemented with 10 % heat-inactiva ted fetal calf serum (Gibco), benzylpenicillin (100 mg/L), streptomycin (100 mg/L), kanamycin (100 mg/L), and G418 (200 mg/L, Gibco) in 25 cm^2 tissue culture flasks at 37 ℃ in 5 % CO_2 in air. Cells grown to confluence were washed twice with phosphate-buffered saline (PBS, pH7.4), and harvested by scraping. The cells were sonicated in KCl 0.15 mol/L at 4 ℃. The sonicates were centrifugated at 9000× *g* for 20 min at 4 ℃, the supernatant (S_9) was stored at −70 ℃. The protein contents was estimated according to the method of Lowry *et al*[10].

Incubation and determination of PPF enantiomers The time-dependent study was performed in a 250 μL incubation mixture containing racemic PPF (10 or 400 mg/L) or individual enantiomer (5 or 200 mg/L), 0.5 mg S_9 protein and stock buffer (pH 7.4). Kinetic parameters were determined employing individual enantiomers at five different concentrations ranging from 12.5 to 200 mg/L as substrates. Enantiomeric inhibition expriments were performed using R(−)-PPF as an inhibitor and S(+)-PPF as a substrate. Ketoconazole inhibition experiments were carried out at three different concentrations of PPF with three concentrations of inhibitor. The incubation mixture was bubbled with oxygen for 1 min before use. After pre-incubation in a shaking incubator bath at 37 ℃ for 5 min, reaction was started by adding 1 mg NADPH.

Incubation of individual enantiomer was terminated by adding 1.0 mL methanol. After centrifugation at 2000× *g* for 10 min, an aliquot (20 μL) was injected onto the Shim-pack CLC ODS (150 mm × 4.6 mm id, 5 μm). The HPLC system comprised of an LC-10AT VP pump and a SPD-10A VP detector (λ = 220 nm). The mobile phase was a mixture of 0.03 % triethylamine and 0.05 % phosphoric acid in water-acetonitrile-methanol (56:16:28, v/v/v). Incubation of enantiomer mixture of PPF was stopped by adding 1.0 mL chlorform. S(−)-propranolol was added as an internal standard and the sample was extracted at pH 10. The organic phase was taken to dryness and deriva tized for 30 min at 35 ℃ with GITC, followed by a chiral HPLC method described previously[11].

Statistics The maximum velocity (V_{max}) and Michaelis-Menten constant (K_m) values for PPF enantiomer were determined by regress analysis of Edie-Hofstee plots. The $\bar{x} \pm s$ of three determinations of V_{max} and K_m was calculated for each substrate and metabolic reaction. All statistical difference was tested by unpaired *t* test.

RESULTS

Characterization of CYP3A4 The established CHL-3A4 cell line did express CYP3A4 and was validated by the ability of metabolic activation for three genotoxic chemicals aflatoxin B_1, sterigmatocystin, and cyclophosphamide[7], and of metabolic deactivation for calcium antagonist, nifedipine, a specific substrate of CYP3A4[12]. Ketoconazole, a well known inhibitor of CYP3A4, can also inhibit the metabolism of PPF competitively ($K_i = 0.64$ μmol·L^{-1}, the Dixon plot was shown in Fig 1).

Substrate concentration-dependent stereoselective metabolism of PPF in CHL-3A4 S_9 incubates In time dependent study, at 400 mg/L concentration of racemic PPF, S/R was around unity. Whereas racemic PPF 10 mg/L was used as substrate, S/R was from 1.00 to 1.16 as the time of metabolic reaction was from 1 to 15 min (Tab 1). When an

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Oct; 22 (10)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

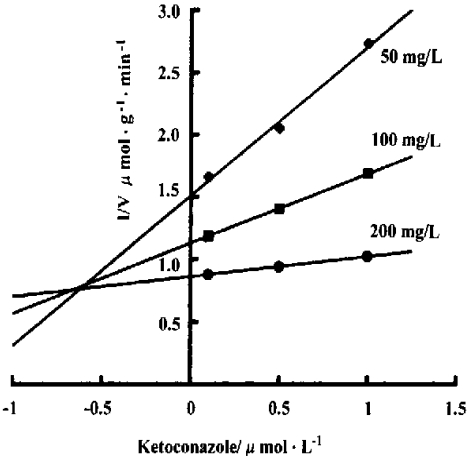

Fig 1. Competitive inhibition of propafenone metabolism (substrate concentration: 50, 100, and 200 mg/L) in CHL-3A4 S_9 incubates with increasing concentration of ketoconazole.

Tab 1. The metabolic depletion of racemic propafenone in CHL-3A4 S_9 incubates. $n=3$. $\bar{x} \pm s$.

Enantiomer/ mg·L^{-1}	Incubation time/min	Amount remaining/mg·L^{-1} S(+)	R(−)	Ratio S/R
200	1	185.4±0.7	185.9±1.2	1.00
	2	176±14	177±14	1.00
	5	172±10	172±10	1.00
	15	169±3	169±7	1.00
5	1	3.98±0.05	3.88±0.06	1.03
	2	3.50±0.05	3.05±0.05	1.15
	5	3.03±0.06	2.64±0.03	1.15
	15	2.92±0.21	2.52±0.17	1.16

individual enantiomer of PPF was incubated, the metabolic rate of S(+)-PPF was faster than that of R(−)-enantiomer at substrate concentration of 200 mg/L (S > R, Fig 2). However, the opposite situation was observed at substrate concentration of 5 mg/L (R > S, Fig 3).

Enzymy kinetic parameters Enzymy kinetic experiments showed that the ratio of the depleted amount of enantiomers (S/R) increased from 0.76 to 1.33 as the subsrate concentration from 12.5 to 200 μg/mL, ie, the stereoselectivity (R > S) was reversed with the increasing of substrate concentration (Tab 2). V_{max} and K_m were calculated according to Eadie-Hofstee equation. The linear correlation coefficient of the regression equation was 0.95 and 0.98 for S(+)- and R(−)-PPF, respectively. The V_{max} (μmol·mg^{-1}·min^{-1}) of S(+)-PPF was significantly greater than that of R(−)-PPF (2.66±0.32 *vs* 1.71±0.19, $P<0.01$). The K_m (μmol·L^{-1}) of R(−)-PPF was significantly lower than that of S(+)-PPF (73±11 *vs* 185±17, $P<0.01$).

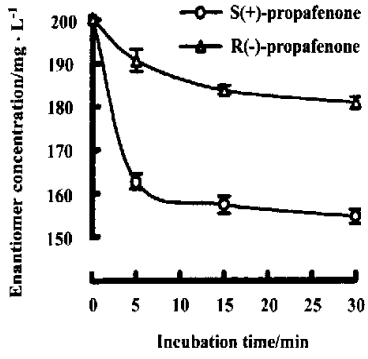

Fig 2. The metabolic depletion of individual enantiomer of propafenone at concentration of 200 mg/L in CHL-3A4 S9 incubates. $n=3$. $\bar{x} \pm s$.

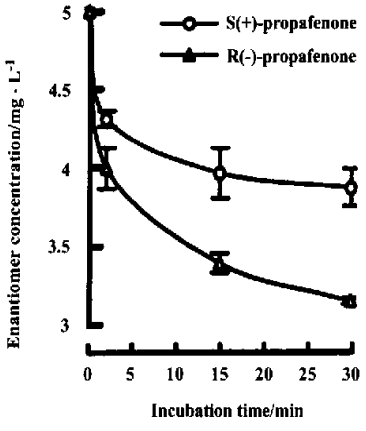

Fig 3. The metabolic depletion of individual enantiomer of propafenone at concentration of 5 mg/L in CHL-3A4 S9 incubates. $n=3$. $\bar{x} \pm s$.

Tab 2. Substrate dependency of propafenone after incubation of individual enantiomer with S_9 prepared from CHL-3A4 cell line. $n=3$. $\bar{x} \pm s$.

Enantiomer/ mg·L^{-1}	V/μmol·g^{-1}·min^{-1} S(+)	R(−)	Ratio S/R
12.5	0.412±0.055	0.541±0.007	0.761
25	0.66±0.04	0.76±0.03	0.866
50	1.06±0.12	1.14±0.07	0.925
100	1.66±0.13	1.35±0.11	1.230
200	1.98±0.27	1.49±0.14	1.334

Enantiomeric inhibition of PPF metabolism

via CYP3A4 The depletion of S (+)-PPF was inhibited by the addition of varying concentrations of R (−)-enantiomer (Tab 3). Calculated from plots of percentage activity remaining *vs* log10 inhibitor concentration, the IC_{50} is 125 $\mu mol \cdot L^{-1}$.

Tab 3. Inhibition of S(+)-propafenone metabolism in CHL-3A4 S_9 incubates by various concentrations of R(−)-propafenone. $n = 3$. $\bar{x} \pm s$.

Enantiomer/ $mg \cdot L^{-1}$		S(+)-propafenone	
S(+)	R(−)	Amount remaining/ $mg \cdot L^{-1}$	Percent reacted/%
200	0	148.5 ± 2.7	25.7
	25	168.8 ± 2.1	14.6
	50	173.8 ± 3.2	13.1
	100	179.2 ± 1.9	10.3
5	0	2.94 ± 0.17	41.3
	25	3.74 ± 0.15	24.5
	50	4.01 ± 0.09	19.8
	100	4.21 ± 0.10	15.8
	200	4.50 ± 0.11	10.0

DISCUSSION

The CYP3A4 isoenzyme is responsible for the metabolism of the widest range of drugs and endogenous compounds in humans. It accounts for 60 % of cytochrome enzymes in the liver[13]. Metabolic stereoselectivity resided in the enantiomeric difference in affinity or catalyzing ability of CYP3A4 and was found in metabolism of ifosfamide, verapamil, and citalopram[14-16]. Our results indicated that the depletion of S(+)-PPF was faster than that of R(−)-PPF when individual enantiomer was incubated at 200 mg/L. However, the opposite situation was observed at 5 mg/L. This partially resembled the previous finding by Fujita *et al* that stereoselectivity of propranolol in rat liver microsomes was sometimes altered when the substrate concentration was varied[17]. Fujita *et al* postulated that at high substrate concentration the stereoselectivity mainly depended on the enantiomeric difference in V_{max} whereas the enantiomeric difference in K_m resulted in the stereoselectivity at low substrate concentration. In this article, the V_{max} of S(+)-PPF was significantly greater than that of R(−)-PPF, thus probably resulting in the faster metabolic rate of S(+)-PPF at high concentration (200 mg/L). The K_m of R(−)-PPF was significantly lower than that of S(+)-PPF, thus being responsible for the priority in depletion of R (−)-PPF at low concentration (5 mg/L).

The results of time-dependent studies indicated that enantiomer/enantiomer interaction might occur at high concentration of racemic PPF, which resulted in the loss of stereoselectivity. This postulation was proved by enantiomeric inhibition experiment. However, there maybe no enantiomer/enantiomer interaction at low concentration of racemic PPF, thus keeping R(−)-PPF the superiority in affinity with CYP3A4. This also explained the results of our previous study using rat liver microsomes pretreated by specific CYP3A4 inducer dexmethasone as incubation media[18]. In that study, stereoselectivity was found in *N*-desalkylation of racemic PPF at the low substrate concentration with R(−)-PPF priority in depletion (S/R = 1.17), but lost at the high substrate concentration.

In summary, stereoselectivity in metabolism of propafenone via CYP3A4 expressed in transgenic Chinese hamster CHL cells lines depended on substrate concentration. Enantiomer/enantiomer interaction of PPF occurred at high concentration of substrate, but not at low concentration.

REFERENCES

1 Drayer DE. Pharmacodynamic and pharmacokinetic differences between drug enantiomers in humans: An overview. Clin Pharmacol Ther 1983; 34: 718-23.

2 Kroemer HK, Funck-Brentano C, Silberstein DJ, Wood AJJ, Eichelbaum M, Woosley RL, *et al*. Stereoselective disposition and pharmacological activity of propafenone enantiomers. Circulation 1989; 79: 1068-76.

3 Kroemer HK, Botsch S, Heinkele G, Schick M. *In vitro* assessment of various cytochromes P450 and glucuronosyltransferases using the antiarrhythmic propafenone as a probe drug. Methods Enzymol 1996; 272: 99-105.

4 Kroemer HK, Fischer C, Messe CO, Eichelbaum M. Enantiomer/enantiomer interaction of (S)- and (R)-propafenone for cytochrome P450 IID6-catalyzed 5-hydroxylation: *in vitro* evaluation of the mechanism. Mol Pharmacol 1991; 40: 135-42.

5 Botsch S, Gautier JC, Beaune P, Eichelbaum M, Kroemer HK. Identification and chracterization of the cytochrome P450 enzymes involved in *N*-dealkylation of propafenone: molecular base for interaction potential and variable disposition of active metabolites. Mol Pharmacol 1992; 43: 120-6.

6 Guengerich FP. *In vitro* techniques for studying drug metabolism. J Pharmacokinet Biopharm 1996; 24: 521-33.

7 Chen QF, Wu JM, Yu YN. Estabolishment of transgenic cell line CHL-3A4 and its metabolic activation. Chin J Prev Med

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Oct; 22 (10)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

1998; 32: 281 – 3.
8 Dong HT, Yu YN, Cai ZN. Construction of a human cytochrome P450 1A1 cDNA expression plasmid and estabolishment of its transgenic cells lines. Chin J Pathophysiol 1996; 12: 225 – 9.
9 Wu JM, Dong HT, Cai ZN, Yu YN. Stable expression of human cytochrome CYP2B6 and CYP1A1 in Chinese Hamster CHL cells; their use in micronuclear assays. Chin Med Sci J 1997; 12: 148 – 55.
10 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265 – 75.
11 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.
12 Qian YL, Zhuge J, Yu YN. Nifedipine induced reversal of MDR can be deteriorated by S_9 mix prepared from the transgenic cell line CHL-3A4. Chin J Pathophysiol 2000; 16: 385 – 88.
13 Wilkinson GR. Cytochrome P4503A (CYP3A4) metabolism: prediction of *in vivo* activity in humans. J Pharmacokinet Biopharm 1996; 24: 475 – 90.
14 Roy P, Tretyakov O, Wright J, Waxman DJ. Stereoselective metabolism of ifosfamide by human P-450s 3A4 and 2B6. Favorable metabolic properties of R-enantiomer. Drug Metab Dispos 1999; 27: 1309 – 18.
15 Tracy TS, Korzekwa KR, Gonzalez FJ, Wainer IW. Cytochrome P450 isoforms involved in metabolism of the enantiomers of verapamil and norverapamil. Br J Clin Pharmacol 1999; 47: 545 – 52.
16 Rochat B, Amey M, Gillet M, Meyer UA, Baumann P. Identification of three cytochrome P450 isozymes involved in *N*-demethylation of citalopram enantiomers in human liver microsomes. Pharmacogenetics 1997; 7: 1 – 10.
17 Fujita S, Umeda S, FunaeY, Imaoka S, Abe H, Ishida R, *et al*. Regio- and stereoseletive propranolol metabolism by 15 forms of purified cytochromes P-450 from rat live. J Pharmacol Exp Ther 1993; 264: 226 – 33.
18 Yao TW, Zhou Q, Hu XY, Zeng S. Stereoselectivity in *N*-desalkylation of racemic propafenone in rat liver microsomes pretreated by different inducers. Chin J Pharmacol Toxicol 2000; 14: 440 – 4.

普罗帕酮经转人肝 CYP3A4 基因中国仓鼠 CHL 细胞代谢的立体选择性

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关键词 普罗帕酮；立体异构体；代谢；细胞色素 P-450 CYP3A4

目的：研究普罗帕酮(PPF)经人肝 CYP3A4 代谢的立体选择性. **方法**：以转人肝 CYP3A4 基因中国仓鼠肺细胞 CHL 的 S_9 为酶源，考察了 PPF 消旋体及单个对映体在高、低底物浓度时的经时孵育代谢. 对映体抑制试验用于考察对映体之间的相互作用. 并进行了单个对映体的酶动力学试验. **结果**：PPF 消旋体高底物浓度(400 mg/L)时的代谢无立体选择性，低底物浓度 (10 mg/L)时 R(−)-体代谢快于 S(+)-体(R > S)；单个对映体孵育时在高底物浓度(200 mg/L)时的代谢呈现对 S(+)-体的立体选择性(S > R)，低底物浓度(5 mg/L)时呈现对 R(−)-体的立体选择性(R > S). S(+)-PPF 的 V_{max}(μmol·mg^{-1}·min^{-1})大于 R(−)-体(2.66 ± 0.32 *vs* 1.71 ± 0.19, $P < 0.01$). R(−)-PPF 的 K_m(μmol·L^{-1})小于 S(+)-体(73 ± 11 *vs* 185 ± 17, $P < 0.001$). R(−)-PPF 对 S(+)-体有代谢抑制作用(IC_{50} = 125 μmol·L^{-1}). **结论**：PPF 经人肝 CYP3A4 的代谢有底物浓度依赖性的立体选择性. 高底物浓度时两对映体有相互作用，相互作用的结果导致立体选择性丧失. 而低底物浓度时两对映体无相互作用，结果仍表现出 R 体代谢优先的立体选择性.

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