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Concentration dependent stereoselectivity of propafenone N-depropylation metabolism with human hepatic recombinant CYP1A2

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Concentration dependency of stereoselective N-depropylation metabolism of propafenone was studied by using transgenic cell line expressing human CYP1A2. Enantiomers of propafenone and N-depropylpropafenone were separated and assayed simultaneously by RP-HPLC with precolumn GITC chiral derivatization. The experimental results showed that CYP1A2 was involved in enantioselective N-depropylation of propafenone and that the metabolic stereoselectivity depends on substrate concentration. For racemic propafenone, stereoselectivity was observed at low substrate concentration and was not seen at high substrate concentration. For individual isomers, *S*(+)-propafenone was metabolized faster than its antipode at higher enantiomer concentrations and *R*(-)-propafenone was eliminated faster than its antipode at lower enantiomer concentrations. There is interaction between *S*- and *R*-propafenone. *R*(-)-propafenone inhibited the metabolism of *S*(+)-propafenone with IC₅₀ 0.225 mmol/L for human CYP1A2.

1. Introduction

Pharmacokinetic differences involving stereoselective drug disposition have proved important in the clinical evaluation of some chiral drugs that are routinely administered in their racemic form. Propafenone (PPF) is a Ic antiarrhythmic drug and is marketed as the racemate. The two enantiomers of PPF are equipotent in terms of sodium channel-blocking activity, but the main side effect, β -adrenoreceptor-blocking action, resides in the *S*(+)-isomer [1, 2], and, therefore, information on stereoselective disposition of the racemate is of clinical relevance. There are three main metabolic pathways for propafenone, including 5-hydroxylation via CYP2D6, N-desalkylation via CYP3A4 and CYP1A2 and glucuronidation via glucuronosyltransferase [3]. N-depropylpropafenone has the same electrophysiological potency as 5-hydroxypropafenone and propafenone, and the plasma concentrations of N-depropylpropafenone are similar to those of 5-hydroxypropafenone during chronic administration in humans. Therefore, N-depropylpropafenone contributes to the antiarrhythmic effects of propafenone, especially in patients with the poor metabolizer phenotype with CYP2D6. Stereoselective propafenone 5-hydroxylation and glucuronidation have been reported [4, 5] and N-depropylation stereoselectivity in rat liver microsomes [6] and human hepatic combinant CYP3A4 [7] has been described by our lab.

In this study, we employed recombinant human CYP *in vitro* models to address the concentration dependency of stereoselectivity in N-depropylation in propafenone metabolism with human CYP1A2.

2. Investigations and results

2.1. Effect of racemate concentration on stereoselective metabolism of propafenone

We used transgenic Chinese hamster CHL cells expressing human liver CYP1A2 as drug metabolizing enzymes to study the concentration dependent oxidation metabolism of racemic PPF. No stereoselectivity was observed for the higher concentration of racemate (1.173 mmol/L) and significant metabolic stereoselectivity was observed for the lower concentration of racemate (0.0293 mmol/L) with 1A2 transgenic cell line. N-desalkylation metabolism via

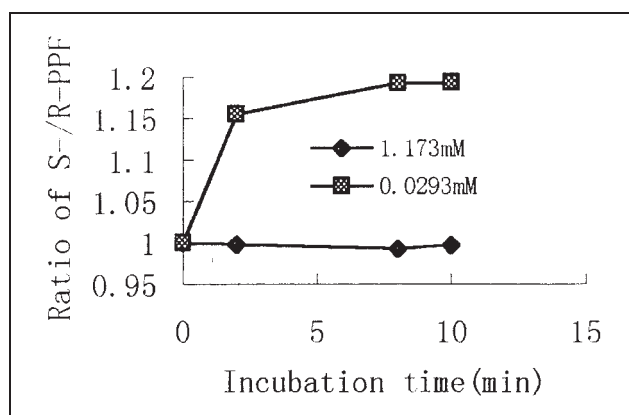


Fig. 1: The ratio of *S*(+)-/*R*(-)-PPF concentration remaining after incubation for 15 min with CYP1A2 transgenic cells at PPF racemate concentrations of 1.173 mmol/L and 0.0293 mmol/L.

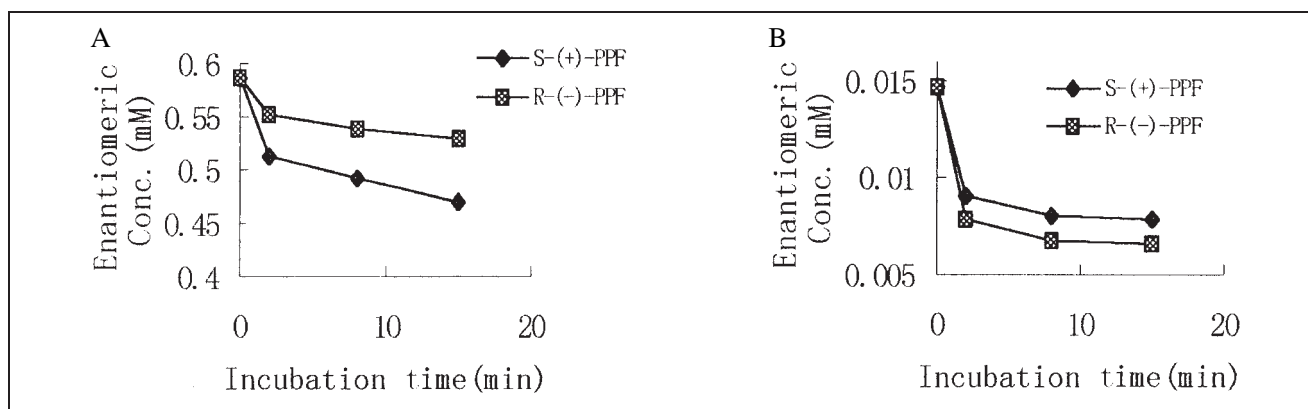


Fig. 2: Metabolic depletion of enantiomers of propafenone via *CYP1A2* expressed in transgenic Chinese hamster CHL cells at PPF enantiomeric concentrations of 0.5865 mmol/L (A) and 0.0147 mmol/L (B)

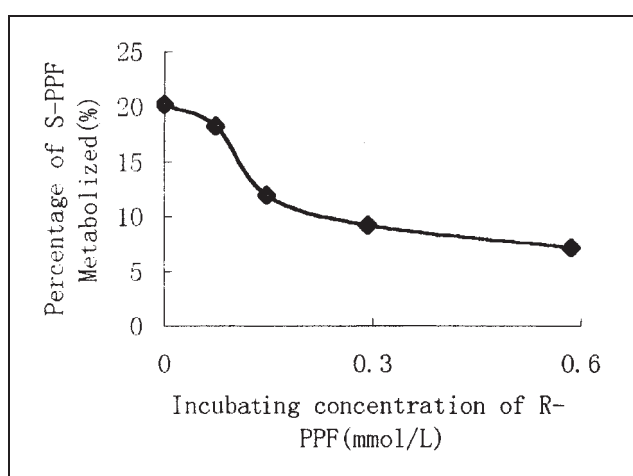


Fig. 3: Metabolism of *S*-PPF in dependence of *R*-PPF concentration

1A2 favors *R*(-)-PPF at 0.0293 mmol/L of racemate concentration (Fig. 1).

2.2. Effects of single enantiomer concentration on stereoselective metabolism of propafenone incubated with CHL-CYP1A2

The metabolic rate of *S*-(+)-PPF was faster than that of the *R*(-)-enantiomer at a substrate concentration of 0.5865 mmol/L (*S*-(+)/*R*(-)-PPF < 1, Fig. 2A) when an individual enantiomer of PPF was incubated with CHL-*CYP1A2*. However, the opposite stereoselectivity was observed at a substrate concentration of 0.0147 mmol/L (*S*-(+)/*R*(-)-PPF > 1, Fig. 2B).

2.3. Metabolism of *S*-(+)-PPF inhibited by *R*(-)-PPF

The metabolic rate of *S*-(+)-PPF was inhibited by the *R*(-)-enantiomer (Fig. 3). Calculated from plots of percentage activity remaining vs. $\log_{10}$ inhibitor concentration, the IC_{50} is 0.225 mmol/L for human *CYP1A2*.

3. Discussion

Chiral chromatography has been very useful and helpful for the study of enantioselective metabolism of racemic drugs *in vitro* and *in vivo* [8–10]. It is very important to establish an enantioselective method for the simultaneous analysis of *S*- and *R*-PPF in order to understand the interaction between *S*- and *R*-PPF enantiomers. The principle

of resolving two enantiomers of PPF is that the second amine function group in the PPF structure reacts with GITC and forms diastereomers that are separated on the ordinary analytical column. The derivatization reaction is simple and efficient for separation of enantiomers of PPF and its *N*-depropylation metabolite, *N*-depropylpropafenone.

When racemic PPF was used as a substrate and incubated with human liver *CYP1A2* transgenic cells, the *N*-depropylation of PPF showed stereoselective oxidation metabolism at a lower substrate concentration which disappeared at a higher substrate concentration. In contrast, when a single enantiomer of *S*- and *R*-PPF was used separately as the substrate, the *N*-depropylation of PPF showed stereoselectivity at both lower and higher substrate concentrations. It is interesting that for incubation of a single enantiomer of PPF with CHL-*CYP1A2* separately, the results indicated that the stereoselectivity favors *S*-(+)-PPF with a faster metabolic rate at a higher substrate concentration while there is a faster metabolic rate of *R*(-)-PPF at low concentration. The results of time-dependent studies performed with *CYP1A2* indicated that enantiomer/enantiomer interaction occurs at high concentrations of racemic PPF, resulting in the loss of stereoselectivity. However, there is no enantiomer/enantiomer interaction at low concentrations of racemic PPF, thus keeping *R*(-)-PPF the superiority in affinity with *CYP1A2*. This hypothesis was proved by enantiomeric inhibition experiments.

In this study, *CYP1A2* was confirmed as being involved in the enantioselective *N*-depropylation of PPF by using stable expressed cytochrome P450. The enantiomers of a racemic drug may differ in metabolic behavior as a consequence of stereoselective interaction with hepatic drug metabolizing enzymes. Both PPF enantiomers may mutually compete for the same catalytic site of the enzyme. It has been reported that metabolism of *S*-PPF seems to be retarded in the presence of *R*-PPF *in vivo* [4]. Furthermore, experiments also showed that *R*-PPF is a more potent inhibitor than the *S*-enantiomer with respect to *CYP2D6*-mediated 5-hydroxylation [5]. Our study also showed that *R*-PPF inhibited the *CYP3A4* and *CYP1A2*-mediated *N*-depropylation metabolism of *S*-PPF in rat microsomes and recombinant human cells [6, 7]. The underlying mechanism of stereoselectivity in metabolism, as many studies have shown, might be enantiomeric difference in enzymatic catalyzing ability and/or in enzyme affinity to the substrate. Considering the interesting finding that stereoselectivity depends on substrate concentration, we postulate that stereoselectivity at low substrate concentration was

mainly due to enantiomeric differences in enzyme affinity to the substrate, and that the insignificant enantiomeric difference in catalyzing ability resulted in the loss of stereoselectivity at high substrate concentration. Because the beta-blocking properties of propafenone reside in the *S*-enantiomer, inhibition of metabolism of this enantiomer by *R*-PPF may provoke side effects in patients who are intolerant of beta-blockade.

In conclusion, the stereoselectivity of oxidation metabolism of PPF depropylation via human hepatic *CYP1A2* is dependent on substrate concentration. *S*-(+)-PPF was metabolized faster than its antipode at higher enantiomer concentrations (*S*-/ *R*-PPF < 1) and *R*-(-)-PPF was eliminated faster than its antipode at lower enantiomer concentrations (*S*-/ *R*-PPF > 1). There is an interaction between *S*- and *R*-PPF. *R*-(-)-PPF inhibited the metabolic rate of *S*-(+)-PPF with IC_{50} 0.225 mmol/L for human *CYP1A2*.

4. Experimental

4.1. Chemicals and solutions

S(-)-propranolol, (*R,S*)-propafenone, *R*(-) and *S*(+)-propafenone, 2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl isothiocyanate (GITC), and NADPH, were purchased from Sigma Chemical Co (St. Louis, MO, USA). Tris-hydroxymethyl aminomethane (Gibco BRL) and bovine serum albumin (Serva) were purchased from Shanghai Reagent Store, China. *N*-Depropyl-propafenone was a generous gift from Prof. Tang YN (Xinhua Hospital, Shanghai). All other chemicals were obtained from the usual commercial sources.

Stock buffer (pH 7.4): 1 mol/L pH 7.4 Tris-HCl buffer 25 mL, 1 mol/L KCl 75 mL and 1 mol/L $MgCl_2$ 5 mL were mixed and diluted with H_2O to 500 mL. NADPH solution: dissolve NADPH in 10 g/L $NaHCO_3$ solution pre-cooled with ice to the desired concentration of 25 mmol/L. The solution should be freshly prepared just before the incubation.

4.2. HPLC procedure for determining propafenone enantiomer

The HPLC system comprised of an LC-10AT VP pump and an SPD-10A VP detector ($\lambda = 220$ nm). The analytical column was a 5- μ m reverse phase column (Shimpack CLC-ODS 15 cm $\times$ 4.6 mm). the mobile phase was a mixture of methanol-water-glacial acetic acid (67:33:0.05). The flow rate was 0.8 mL/min.

4.3. Preparation of *S*₉ from *CYP* transgenic cells

Transgenic Chinese hamster CHL cells expressing *CYP1A2* (CHL-*CYP1A2*) were established by the Department of Pathophysiology & Laboratory of Medical Molecular Biology (College of Medicinal Sciences, Zhejiang University, China), and cultured in MEM (Gibco, Uxbridge, UK) supplemented with 10% heat-inactivated fetal calf serum (Gibco), benzylpenicillin (100 mg/L), streptomycin (100 mg/L), kanamycin (100 mg/L)

and G418 (200 mg/L, Gibco) in 25 cm² tissue culture flasks at 37 °C in 5% CO₂ in air. Cells grown to confluence were washed twice with phosphate-buffered saline (PBS, pH 7.4), and harvested by scraping. The cells were sonicated in KCl 0.15 mol/L at 4 °C. The sonicates were centrifuged at 9000 $\times$ g for 20 min at 4 °C, and the supernatant (*S*₉) was stored at -70 °C. The protein content was estimated according to the method of Lowry et al. [11, 12].

4.4. Incubation of propafenone with *S*₉ fractions from *CYP* transgenic cells

The time-dependent study was performed in 250 μ L of incubation mixture containing racemic PPF (0.0293 or 1.173 mmol/L) or individual enantiomer (0.0147 or 0.5865 mmol/L), 0.5 mg *S*₉ protein and stock buffer (pH 7.4). Enantiomeric inhibition experiments were performed using *R*(-)-PPF as an inhibitor and *S*(+)-PPF as a substrate to study the interaction between *R*(-)-PPF and *S*(+)-PPF. The *R*(-)-PPF concentration was set at five different concentrations which were 0, 0.0733, 0.1466, 0.2933 and 0.5865 mmol/L. Oxygen was bubbled through the incubation mixture for 1 min before use. After pre-incubation in a shaking incubator bath at 37 °C for 5 min, reaction was started by adding 1 mg NADPH. Incubation of individual enantiomers was terminated by adding 1.0 mL chloroform. After centrifugation at 2000 $\times$ g for 10 min, 200 μ L was transferred to a clean tube and evaporated to dryness under a gentle stream of N₂ at 45 °C. The residue was dissolved with 100 μ L of GITC solution (1 mg/mL in CH₃CN). The tube was capped and allowed to stand at 35 °C for 10 min. The reaction mixture was evaporated to dryness under a gentle N₂ stream. The residue was reconstituted with 200 μ L mobile phase and an aliquot of 20 μ L was injected into the HPLC system.

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