Influence of CYP2D6*10B genotype on pharmacokinetics of propafenone enantiomers in Chinese subjects

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ABSTRACT

AIM: To study the relationship between genotype of CYP2D6*10B and pharmacokinetics of propafenone enantiomers. **METHODS:** Genotype of 17 healthy Chinese HAN subjects was determined by an allele specific amplification method. The blood samples (0-15 h) of the subjects were taken after oral administration of a single dose (400 mg) of propafenone hydrochloride. Concentrations of propafenone enantiomers in plasma were measured by a reverse-phase HPLC with precolumn derivatization. **RESULTS:** Seventeen subjects characterized for CYP2D6*10B genotype included (*1/*1) (n= 4), (*1/*10) (n=5) and (*10/*10) (n=8). The metabolic ratios (lg MR) of the three genotypes were -2.68±0.23, -2.2±0.7, and -1.1±0.5, respectively. The AUC of the three groups were (1534±334), (1891±793), (3171±1075) μg·h·L^{-1} for *S*-enantiomer and (1136±345), (1467±817), (2277±745) μg·h·L^{-1} for *R*-enantiomer, respectively. The AUC of propafenone enantiomers in *10/*10 is about 1.5-2 times of that of *1/*10 group or *1/*1 group, and the CL of both enantiomers in *10/*10 is only half of that of *1/*10 group or *1/*1 group (P<0.05). **CONCLUSION:** CYP2D6*10B alleles induce the declined activity of CYP2D6 and impair the metabolism of propafenone.

INTRODUCTION

Debrisoquine 4-hydroxylase (CYP2D6) is an important cytochrome P450 enzyme which catalyzes the metabolism of more than 40 drugs, including tricyclic anti-depressants, neuroleptic drugs, lipophilic β-blockers, opioid drugs, and anti-arrhythmic drugs, *etc*. Previous studies have proven that about 7 % of Caucasians are poor metabolizers (PM)[1]. The occurrence of PM in Orientals is only about 1 %. However, some of the other studies found that metabolic ratios (MR) of Orient extensive metabolizers (EM) were shifted to the right compared with Caucasians[2,3]. Our previous study found that MR of a population of 120 Chinese subjects showed a bimodal distribution and about 36 % of the subjects were classified as intermediate metabolizers (IM)[4]. Further study suggested that IM were mainly homogenous for CYP2D6*10B mutant allele[5], which contains C/T 188 mutation in CYP2D6 gene. C/T188 mutation causes the crucial amino acid substitution of Pro^{34}→Ser in CYP2D6 and this substitution has been proven to produce an unstable enzyme with diminished catalytic activity[6]. The gene frequency of CYP2D6*10B alleles is about 58.4 % in a 119 Chinese population among whom 27.7 % subjects were homogenous for CYP2D6*10B mutant[5].

Propafenone (PPF) is a widely used anti-arrhythmic agent, which is mainly metabolized by CYP2D6. PPF is clinically used as recemate. However, it is actually a mixture of two enantiomers: *S*-PPF and *R*-PPF.

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The two enantiomers are different in pharmacokinetics and pharmacodynamics[7]. We found that pharmacokinetic parameters of PPF enantiomers correlated well with MR value measured with dextromethorpan as probe[8]. In this article we determined the pharmacokinetic parameters of PPF enantiomers and related these parameters to CYP2D6 genotypes.

MATERIALS AND METHODS

Subjects Seventeen healthy Chinese (HAN) volunteers consisted of 11 men and 6 women (age: 33±6, weight: 63 kg±7 kg). They all gave written consent to participate. The study was approved by the Ethics Committee of Jinling Hospital. The volunteers were all recruited from our previous study of a Chinese population consisting of 120 subjects who have had the phenotyping of CYP2D6[4]. They were selected according to their MR value. Seventeen subjects were classified as 7 VEM (lg MR≤-1.81), 9 IM (-1.81<lg MR≤ -0.52) and 1 PM (lg MR>-0.52). Before study, the healthy status of volunteers was confirmed by routine lab tests conducted in our hospital. All subjects were non-smokers and drug free for at least 2 weeks before and during the study.

Genotyping Blood samples of 17 volunteers were drawn and leukocyte DNA was extracted using a modified phenol-chloroform extraction technique. An allele specific amplification (ASA) method was used to determine CYP2D6*10B genotypes [9].

Pharmacokinetic study[8] After fasting overnight, each subject was given a single dose of 400 mg propafenone hydrochloride tablets (Xingyi Pharmaceutical Company, Shanghai, China). Blood samples were drawn at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, and 15 h after administration. Plasma concentrations of *S*- and *R*-propafenone were determined by a reversed-phase HPLC method established in our lab[10]. Briefly, the plasma was extracted with hexane-isopentanol (99:1) after basification, the organic form was separated and evaporated. A precolumn derivation was performed using 2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyransoyl isothiocyanate (GITC, sigma chemical Co, USA) as derivative reagent. The resulting diastereoisomers were eluted with acetonitrile-water-acetic acid (60:40:0.1) at a flow rate of 1 mL/min on an achiral hypersil ODS column and detected under UV 208 nm. The pharmacokinetic parameters of *S*-PPF and *R*-PPF were simulated with a PKBP-N1 computer program. Area under concentration-time curve (AUC) was estimated by means of trapezoidal method and extrapolation of the area to infinite time. The apparent oral plasma clearance (CL) was calculated for *S*-PPF and *R*-PPF as dose divided by AUC.

Data analysis Data obtained from our study were expressed as mean±SD. Pharmacokinetic parameters and MR among groups of different genotypes were compared with ANOVA. When the null hypothesis was rejected, the differences between two groups were compared with the two-tailed *t* test.

RESULTS

There were 4 wild-type for CYP2D6*10B (*1/*1), 5 heterozygous (*1/*10), and the other 8 subjects were all homogenous for mutant (*10/*10) in 17 subjects. The lg MR values of three groups obtained by dextromethorphan phenotyping were -2.68±0.23, -2.2±0.7, and -1.1±0.5, respectively. The MR of *10/*10 subjects were significantly higher than *1/*1 (P<0.01) and *1/*10 subjects (P<0.05, Fig 1).

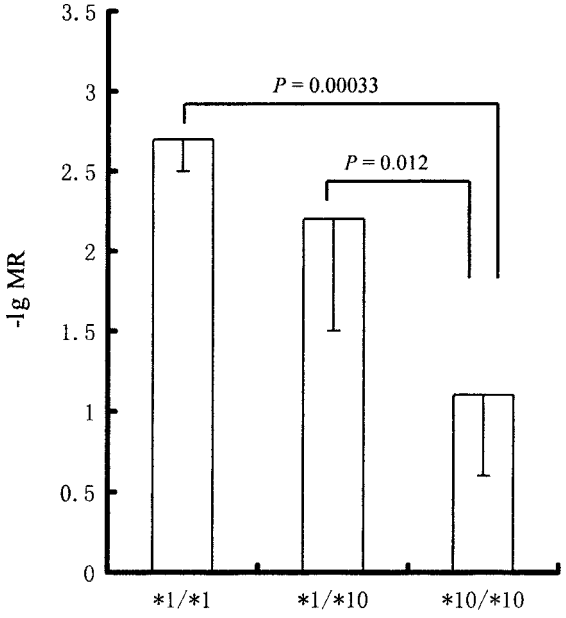

Fig 1. Influence of CYP2D6 genotype (*1/*1, *1/*10, *10/*10) on MR of dextromethorphan in 17 Chinese subjects. Mean±SD.

The plasma concentration-time curves of *10/*10 group were significantly different compared with *1/*10 and *1/*1 group in either *S*-PPF or *R*-PPF (Fig 2). AUC and C_{max} of *10/*10 group were about 1.5 to 2 times of those of *1/*10 and *1/*1 group in both enantiomers. At the same time, CL of *10/*10 group were about half of that of *1/*10 and *1/*1 group in *S*-

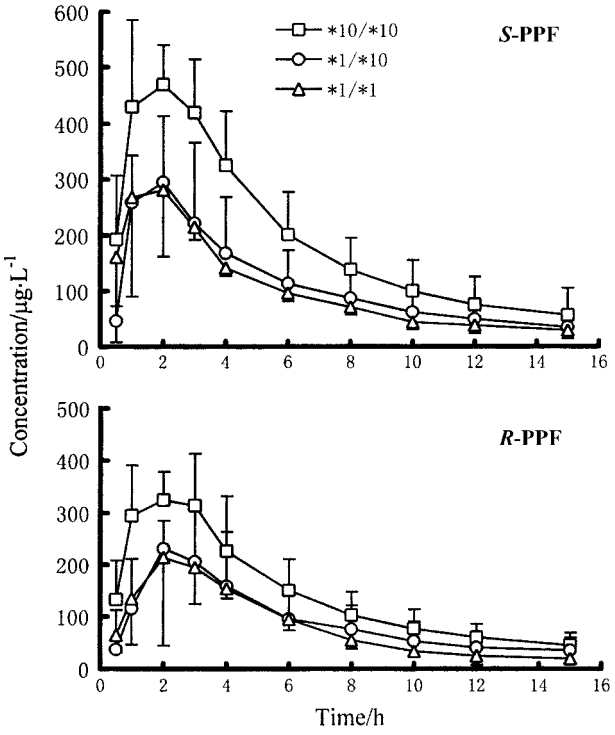

Fig 2. Plasma concentration of *S*-PPF and *R*-PPF after oral administration of a single dose of the recemate propafenone (400 mg) in 17 Chinese subjects of 3 different C/T188 genotypes.

PPF (P<0.05, Tab 1).

Tab 1. Pharmacokinetic parameters of propafenone enantiomers of 17 healthy Chinese subjects. Mean±SD. ^{b}P<0.05, ^{c}P<0.01 *vs10/*10. ^{e}P <0.05 *vs* *R*-PPF.**

Genotype	*n*		C_{max}/μg·L^{-1}	CL/L·h^{-1}	AUC/μg·h·L^{-1}
*1/*1	4	*S*-PPF	346±159^{be}	137±30^{c}	1534±334^{be}
		R-PPF	270±130^{b}	193±93^{c}	1136±345^{b}
*1/*10	5	*S*-PPF	316±132^{ce}	107±38^{b}	1891±793^{be}
		R-PPF	212±110^{c}	162±96^{b}	1467±817^{b}
*10/*10	8	*S*-PPF	520±71^{e}	61±18^{e}	3172±1075^{e}
		R-PPF	368± 64	85±25	2277±745

The C_{max} and AUC of *S*-PPF were higher than *R*-PPF (P<0.05 in all three genotyping groups), and CL of *S*-PPF was significantly lower than *R*-PPF in *10/*10 group (P<0.01, Tab 1). The *S*/*R* ratio of C_{max} and AUC were 1.30-1.56 and 1.30-1.42, and the *S*/*R* ratio of CL was about 0.70-0.80 in the three genotype groups. There were no statistical differences of *S*/*R* ratio among three groups. This suggested that genotype of CYP2D6*10B had no remarkable effect on the stereoselective metabolism of PPF.

DISCUSSION

The defection of CYP2D6 gene results in the polymorphism of CYP2D6. It has been proven that the PM of Caucasians are mainly caused by the defective genes such as CYP2D6*3, CYP2D6*4, and CYP2D6*5, *etc*. On the other hand, there are ultrarapid metabolizers (UM) with multicopy of functional CYP2D6*2 genes[11]. Dalen *et al* studied the pharmacokinetics of nortriptyline of a group of subjects with 0, 1, 2, 3, and 13 CYP2D6 functional genes, and found that ratios of AUC and CL were 36:25:10:4:1 and 1:1:4:5:13 respectively[12]. Dalen's study suggested that the metabolizing capacity of CYP2D6 was consistent with the number of active genes. This phenomena is called gene dose effect.

Studies on Oriental subjects showed the gene dose effect of CYP2D6*10B genotype on the pharmacokinetic parameters of some CYP2D6 substrates, such as propranolol, nortriptyline, metoprolol, and paroxetine[13-16]. Huang *et al*[15] found that AUC of *S*-metoprolol and *R*-metoprolol in *1/*1, *1/*10, and *10/*10 group of CYP2D6*10B were (1411±116), (1899±120), (3588±435) nmol·h·L^{-1}, and (901±80), (1304±105), (2848±394) nmol·h·L^{-1}, respectively. There were significant differences among the three groups. Our study is the first research about gene dose effect of propafenone in mainland Chinese. The result confirmed that CYP2D6*10B mutation was associated with diminished metabolic activity of CYP2D6 in Chinese subjects. However, differences of pharmacokinetic parameters between *1/*10 and *1/*1 group were not significant. This can be attributed to that the diminished activity of the enzyme produced by CYP2D6*10B alleles is not so remarkable as those produced by CYP2D6*3, CYP2D6*4 and CYP2D6*5 alleles. Therefore, metabolizing activity of *1/*10 subjects, who contain effective genes, has no statistical difference compared with *1/*1 subjects.

Our previous study on phenotype of CYP2D6 found that C_{max}, AUC, and CL of propafenone correlated well with MR of dextromethorphan[8]. When the subjects of EM were subdivided as VEM and IM group, we found that there were significant differences between two groups[17]. According to the present study, C_{max}, AUC, and CL of propafenone in *10/*10 group

are consistent with those in IM group, which confirmed that IM were caused by *10/*10 genotype. Although the phenotyping was widely used in the study of polymorphism of CYP2D6, it had disadvantages such as the needs of taking probe medicine, collecting urine, and controlling factors which may interfere with the results. Genotyping overcomes the disadvantages of phenoyping and the result is reliable. Therefore, genotyping of CYP2D6*10B is useful in interpreting the individual difference of pharmacokinetics of propafenone and other substrates of CYP2D6, and provides useful information on the use of some drugs with low therapeutic index and high incidence of adverse effect.

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