Effects of the CYP Oxidoreductase *Ala503Val* Polymorphism on CYP3A Activity In Vivo: A Randomized, Open-Label, Crossover Study in Healthy Chinese Men

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ABSTRACT

Background: Cytochrome P450 (CYP) oxidoreductase (POR) is the electron donor for microsomal CYP enzymes. The *POR Ala503Val* (*POR*28* C > T) polymorphism has been reported to influence CYP3A activity in vivo in a white population. The influence of this polymorphism on CYP3A activity in vivo in the Chinese population currently unknown.

Objective: This study was designed to assess the influence of the *POR*28* polymorphism on the CYP3A activity in vivo in healthy Chinese men using midazolam (MID) as a probe drug.

Methods: The *POR*28* polymorphism was genotyped in healthy Chinese men. A randomized, 2-phase, open-label, crossover study was performed to assess in vivo CYP3A activity after both oral and intravenous MID administration, which reflect both intestinal and hepatic CYP3A or only hepatic CYP3A activity, respectively. The plasma concentrations of MID and 1-hydroxy-midazolam (1-OH-MID) were determined by liquid chromatography–tandem mass spectrometry.

Results: A total of 73 healthy Chinese men were enrolled (CC genotype, 21 subjects; TT genotype, 11; CT genotype, 41), 22 of whom were selected for additional phenotyping of the *CYP3A5*3* polymorphism (CC, 7; TT, 8; CT, 7). The mean (range) age, weight, height, and body mass index of the 22 subjects were 23 (20–28) years, 65.0 (57–75) kg, 1.74 (1.63–1.80) m, and 22.01 (19.27–24.46) kg/m^2, respectively. The frequency of the *POR*28* T (503V) allele was 43.2%. No significant differences in the demographic characteristics of the subjects were observed between the *POR*28* genotype groups. All of the *POR*28* CC and TT homozygotes and 2 of the *POR*28* CT heterozygotes carried the *CYP3A5*3/*3* genotype (CYP3A5 low expressors); 6 CT heterozygotes carried the *CYP3A5*1* allele (CYP3A5 expressors). The mean (SD) 1-OH-MID AUC_{0-8} was significantly greater in the TT homozygotes compared with the CT heterozygotes after intravenous (86.15 [24.34] vs 53.21 [31.36] ng/mL/h; $P = 0.026$) but not oral (126.36 [31.60] vs 103.09 [31.00] ng/mL/h; $P = 0.159$) MID administration. Mean 1-OH-MID C_{max} was significantly greater in the TT homozygotes (51.40 [10.72] ng/mL) compared with the CC homozygotes (31.47 [11.54] ng/mL; $P = 0.002$) and CT heterozygotes (30.12 [9.21] ng/mL; $P = 0.001$) after intravenous MID administration. After intravenous MID injection, the MID metabolic ratio was significantly greater in the TT homozygotes compared with carriers of the C allele ($P = 0.031$). Based on these findings, no significantly differences in overall (hepatic plus intestinal) CYP3A in vivo activity were observed between the *POR*28* genotypes.

Conclusion: These findings suggest that individuals with the *POR*28* C > T polymorphism underwent an increase in 1-hydroxylation of MID after intravenous MID administration, and that the polymorphism was associated with increased hepatic, but not intestinal, CYP3A activity in these healthy Chinese volunteers. (*Clin Ther*. 2011;33:2060–2070) Crown Copyright © 2011 Published by Elsevier HS Journals, Inc. All rights reserved.

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INTRODUCTION

The cytochrome P450 (CYP) 3A isozyme is the most abundant CYP isoform in the adult human liver and intestine, composing up to 30% of the total CYP content.[1] It is involved in the metabolism of 45% to 60% of all drugs currently on the market.[2,3] Significant interindividual variation in CYP3A-mediated drug metabolism has been reported in the general population and may result in significant differences in the efficacy and tolerability of drugs administrated at standard doses.[4] Factors such as diet, concurrent medications, and genetics have been reported to influence CYP3A activity. Genetic factors are assumed to account for 70% to 90% of the interindividual variability in CYP3A activity.[5] The intronic *CYP3A5*3* polymorphism has been reported to result in a truncated protein with low CYP3A5 expression, which may partially account for the notable interindividual variation in CYP3A activity in vivo. However, the allele frequencies of most CYP3A4 polymorphisms are <1% in the general population[6]; thus, the contribution of the *CYP3A4* polymorphism on interindividual variation in CYP3A activity may be limited. Genetic variations in genes encoding CYP functional partners, such as nuclear receptors[7–9] and membrane transporters[10–14] have also been reported to contribute to the interindividual differences in CYP-mediated drug metabolism.

CYP450 oxidoreductase (POR) is a 78-kDa, membrane-bound protein containing 680 amino acids and acts as an electron donor for microsomal CYP enzymes.[15] The human *POR* gene is located on chromosome 7 and contains 16 exons.[16] Two sequential single-electron transfers are required during P450-mediated substrate oxidation. The source of these electrons is reduced nicotinamide adenine dinucleotide phosphate (NADPH).[17,18] The POR protein contains 2 flavins: adenine dinucleotide (FAD) and mononucleotide (FMN). These flavins are located in 2 separate lobes of POR.[19,20] Electrons pass from NADPH through FAD to FMN in the POR protein. At this point, a conformational change occurs in which the FMN-binding domain of the POR interacts with P450 enzymes and donates electrons to the P450 heme iron (Fe^{3+}).[19]

It has been reported that the liver-specific deletion of the *POR* gene in mice led to a profound disruption of hepatic drug metabolism.[21,22] Huang et al[15] identified 43 single-nucleotide polymorphisms (SNPs) in the human *POR* gene, with an allele frequency >1%. Among these SNPs, 15 were missense mutants, and some may have reduced the activity of P450 enzymes in vitro. However, the allele frequencies of most these SNPs were <5%. The *POR*28* C > T variation is an SNP that results in the *Ala503Val* substitution and is common in the general population. Huang et al[15] also reported that the frequency of the *POR*28* T (503V) allele is 36.7% in Chinese Americans, and that a previously published article had suggested that the *POR*28* C > T polymorphism plays an important role in the interindividual variability in drug response.

Huang et al[15] reported that the *POR*28* polymorphism may decrease the catalytic activity of POR, as suggested by different functional assays in vitro. The polymorphism has been reported to affect cytochrome C reduction and steroid 17α-hydroxylase activities[23] but has not been reported to have affect steroid 21-hydroxylase activity.[24,25] In addition, the *POR*28* polymorphism may affect the activities of particular CYP isozymes (ie, decreasing CYP1A2 activity and increasing CYP2C19 activity) in vitro.[26] It has also been reported that the contribution of the *POR*28* C > T polymorphism to the variation of CYP3A activity in vivo was greater than that of the functional genetic variants at the *CYP3A* gene locus in a white population.[27]

CYP3A is expressed in both the intestinal tract and in the liver.[28] Administration of the CYP3A probe drug midazolam (MID) through oral routes reflects the total in vivo CYP3A activity in the intestinal tract and liver, whereas assessment after intravenous MID dosing mainly reflects hepatic CYP3A activity. It is unclear whether the *POR*28* polymorphism has similar effects on intestinal and hepatic CYP3A activity.

In this study, the effect of the *POR*28* genetic polymorphism on CYP3A activity in vivo was evaluated in healthy Chinese volunteers by monitoring MID exposure after oral and intravenous administration.

SUBJECTS AND METHODS

Study Population

Healthy male volunteers were recruited from southwest China through advertisements by the Clinical Pharmacology Center of the Third Xiangya Hospital