PARTICIPATION OF CYP2C19, CYP3A4, AND CYP3A7 IN N-OXIDATED AND UNKNOWN METABOLITES FORMATION FROM VORICONAZOLE BY HUMAN LIVER MICROSONES

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Objective: Voriconazole is a triazole antifungal agent with a broad-spectrum. Hyland et al. (2003) have reported important roles of CYP2C19, CYP2C9, and CYP3A4 responsible for voriconazole N-oxidation in human liver microsomes. Previously human lymphoblastoid cells expressing human P450 isoforms were predominantly used as the sole enzyme source. To confirm the precise roles of P450 isoforms in voriconazole clearance, we investigated oxidative metabolism of voriconazole catalyzed by different recombinant P450 enzymes as well as human liver microsomes genotyped for the CYP2C gene.

Methods: Voriconazole (5-250 μM) was incubated with different recombinant P450 isoforms using E. coli or insect cell expression systems and human liver microsomes genotyped for the CYP2C gene.

Results: Among P450 isoforms tested, CYP2C19 and CYP3A4 had high voriconazole N-oxidation activities, but not CYP2C9. CYP3A4 and CYP3A7 yielded an unknown metabolite from voriconazole detected by HPLC-UV, with apparently low affinity but high capacity than the N-oxidation. The average of voriconazole N-oxidation activities at low substrate concentrations in human liver microsomes genotyped for the CYP2C19*1/*1 was approximately 9-fold to those harboring the CYP2C19*2/*2 or CYP2C19*2/*3 genotypes.

Conclusions: These results suggest that the CYP2C19 genotype, but not CYP2C9 genotype, would be evaluated as a factor in the pharmacokinetics of voriconazole. Together with the present results and reported findings, the unknown pathway of voriconazole may be important for individuals with genetically lacking the CYP2C19 catalytic function.

FUNCTIONAL CHARACTERIZATION OF SEVEN NOVEL ALLELES OF CYP2C9 FOUND IN JAPANESE

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Several genetic polymorphisms in CYP2C9 are already known to affect the metabolism of clinically important drugs such as glimepiride and contribute to the large interindividual and interethnic variability in response to these drugs. We previously identified seven novel nonsynonymous SNPs, Leu17Ile, Lys118ArgfsX9 (*25), Thr130Arg (*26), Arg150Leu (*27), Gln214Leu (*28), Pro279Thr (*29), and Ala477Thr (*30) by resequencing CYP2C9 in 263 Japanese subjects. In this study, to functionally characterize these novel alleles in vitro, the wild-type and each of the seven variant proteins were transiently expressed in COS-1 cells and their protein expression levels and enzymatic activities toward diclofenac 4'-hydroxylation were assessed. No CYP2C9 protein expression was detected by Western blotting for the Lys118ArgfsX9 variant, indicating that it was a null allele. The protein expression levels of other six variants were not significantly different from that of the wild-type. The *26 product showed a 90% decrease in the Vmax value but little change in the Km value toward diclofenac 4'-hydroxylation. Both *28 and *30 products showed two-fold higher Km values and three-fold lower Vmax values than the wild-type, suggesting the importance of Gln214 and Ala477 for substrate recognition. The kinetic parameters of other variants (Leu17Ile, Arg150Leu, and Pro279Thr) were similar to those of the wild-type. These results suggest that CYP2C9*25, CYP2C9*26, CYP2C9*28, and CYP2C9*30 influence metabolic phenotypes of CYP2C9 substrate drugs.