PHARMACOGENETICS OF CYP2C SUBFAMILY IN A JAPANESE POPULATION

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Four members of the CYP2C subfamily, CYP2C8, CYP2C9, CYP2C18 and CYP2C19, have been identified in man, and a number of allelic variants of *CYP2C9*, *2C18* and *2C19* genes which are associated with the metabolic polymorphism have been reported. We examined the frequencies of known mutations in these three genes, the allelic linkage among CYP2C subfamily, and the effects of mutations on phenytoin (PHT) stereoselective hydroxylation in a medium-sized Japanese population. Based on these observations, we will discuss the relationship between the stereoselective hydroxylation of PHT and clinically observed side effects.

Frequencies of mutant alleles and genotyping results

Genotyping of two mutations (*2 and *3) in *CYP2C19* (n=140)(1), the amino acid variants (*2 and *3) in *2C9* (n=140)(2) and two mutations (m1 and m2) in *2C18* (n=175)(3,4) was carried out. Thirty-three subjects (23.6%) were genotypically identified as poor metabolizers (PMs) of *CYP2C19*, and the allele frequencies of the *2 and *3 were 0.35 and 0.11, respectively. All subjects were homozygous for Arg144Tyr358Ile359Gly417 in CYP2C9 (*1) except for 5 subjects (3.6%) who were heterozygous for Leu359 (*3), which corresponded well to the findings in the Chinese population(5). These observations suggest that the inter-individual variation in the *CYP2C9* sequence among Orientals is small, and Ile/Leu359 is one possible site showing inter-racial polymorphism. The very low frequency of the Cys144 allele (*2) in the Japanese and Chinese populations is different from the frequency found in Caucasian subjects (0.125-0.192)(6,7). The frequencies of mutant alleles, m1 (T→A, in exon 2) and m2 (T→C, in 5'-flanking region), and the genotyping results of CYP2C18 were completely consistent with those of *CYP2C19*: *CYP2C19* *1/*1, *2/*2 and *3/*3 homozygotes and *1/*2, *1/*3 and *2/*3 heterozy-

gotes were homozygous for CYP2C18 wt/wt, m2/m2 and m1/m1 and heterozygous for wt/m2, wt/m1 and m2/m1, respectively. These findings suggest that the poor metabolizer (PM) phenotype of CYP2C19 is also the PM phenotype of CYP2C18, and of interest the possibility of another genetic linkage in the CYP2C subfamily.

The stereoselective hydroxylation of PHT and CYP2C subfamily(8)

A single dose of PHT was administered to 6 healthy subjects in whom the genotype and phenotype of CYP2C19 had been determined previously. The urinary excretion profiles of (R)- and (S)-p-HPPH (major hydroxy metabolites of PHT) up to 36 h post-dose were compared between the two groups of PMs and extensive metabolizers (EMs) with respect to *CYP2C19*. *CYP2C9* genotype was also determined: all the alleles were found to be wild type (*1). The mean value for cumulative urinary excretion of unchanged PHT was not significantly different between the PMs and the EMs. However, recovery of (R)-p-HPPH at 36 h was 3.5-fold lower, and that of (S)-p-HPPH was 1.3-fold lower in PMs than in EMs. Although the mean urinary excretion values for both metabolites were significantly lower in the PMs than in the EMs, the difference between the two groups was larger for (R)-p-HPPH. A significant negative correlation was observed between the hydroxylation index of omeprazole and the log100-12 h urinary recovery of (R)-p-HPPH. In human, the 4'-hydroxylation of PHT is highly stereoselective towards formation of the (S)-enantiomer. Thus, (S)-hydroxylation by CYP2C9 might be the major determinant of the disposition of PHT. However, these results support the hypothesis that the stereoselective hydroxylation pathway of PHT to form (R)-p-HPPH cosegregates with the *CYP2C19* metabolic polymorphism. Recently, Bajpai et al.(9) and Krecic et al.(10) examined the stereoselective formation of (R)- and (S)-p-HPPH using cDNA expressed CYP2C9, 2C18, 2C19 and human liver microsomes, and reported that these

three CYPs catalyzed the formation of both enantiomers. However, CYP2C9 was highly (S)-enantioselective and CYP2C19 was highly (R)-enantioselective. These in vitro observations support our results in healthy volunteers. Thus, evidence collected in vitro and in vivo suggests that CYP2C9 will become a key enzyme when PHT is given to patients who have the PM phenotype of CYP2C19.

p-HPPH enantiomers and gingival hyperplasia: in vivo and in vitro study(II)

To assess the possible role of the (R)- and (S)-enantiomers of the PHT metabolite p-HPPH in the pathogenesis of gingival hyperplasia (GH), a clinical study was conducted. Clinical data were collected from April, 1998, to March, 1994, from 94 compliant, ambulatory, adult epileptic patients. Thirty-one patients with GH and sixty-three patients without GH, who had been receiving PHT for at least 6 months, took part in the study. In the total sample of 94 subjects, eight (9%) had (R)-p-HPPH serum concentrations lower than the limit of quantification (10 ng/ml) of the current HPLC system and had S/R enantiomeric ratios lower than 0.002. A bimodal distribution was found in log S/R enantiomeric ratio, suggesting that the stereoselective hydroxylation of PHT is polymorphically distributed. There were significant differences between these two groups in (R)-p-HPPH level, both enantiomer/racemate level ratios and R/S enantiomeric ratio; higher serum (R)-p-HPPH levels were observed in patients with GH. In a separate experiment, the effects of medium alone and in combination with different concentrations of p-HPPH enantiomers or PHT on the incorporation of 3H-thymidine into DNA and on the cell count were evaluated using a normal human dermal fibroblast cell line (NHDF). Increased uptake of 3H-thymidine was observed in the presence of (R)-p-HPPH compared to NHDF treated with medium alone ($p < 0.05$). However, there were no significant differences in incorporation of 3H-thymidine into DNA between control (medium only) and other drug-treated NHDF cultures. Similarly, the mean cell numbers were also significantly increased in (R)-p-HPPH-treated NHDF in comparison with controls ($p < 0.001$). The metabolites of PHT do not possess significant anticonvulsant properties. However, a contributory role of (R)-p-HPPH in the occurrence of one well-known side effect of chronic PHT, gingival hyperplasia, has been suspected.

The mechanisms of PHT-induced gingival hyper-

plasia has yet to be fully explained. It is characterized by the presence of inflammation and a marked fibrotic response. Gingival hyperplasia is induced by not only phenytoin but also cyclosporin and calcium channel blockers. Thus, development of gingival hyperplasia could result from the local metabolic activation of these agents to form active metabolites. Recently, Zhou et al. (12) examined phenytoin metabolism and CYPs contents in gingival tissues from 10 patients undergoing surgery for various periodontal conditions, and found that the microsomes obtained from the gingiva showed significant phenytoin hydroxylase activity, and also found that CYP1A1, 1A2, 2C9, 2E1 and 3A4 were present in these microsomes. Based on these findings, they speculated that proliferative inflammation observed with phenytoin may be initiated by the formation of reactive metabolites and that formation of these metabolites may be catalyzed by CYPs found in the gingiva. These metabolites may then cause cellular injury and induce a reactive inflammatory response, followed by fibroblastic hyperplasia.

The formation of (R)-p-HPPH cosegregates (S)-mephenytoin type polymorphism, and as a result, the urinary excretion and serum concentrations of (R)-p-HPPH were significantly higher in EM than in PM subjects. It is of interest whether drug concentrations in serum or urine reflect those in gingival tissues. However, if gingival hyperplasia is induced by (R)-p-HPPH, the frequency of gingival hyperplasia would be higher in EM or in Caucasian subjects than in PM or Oriental subjects.

REFERENCES

- I. Ieiri, T. Kubota, A. Urae, M. Kimura, Y. Wada, K. Mamiya, S. Yoshioka, S. Irie, T. Amamoto, K. Nakamura, S. Nakano, S. Higuchi: Clin. Pharmacol. Ther. 1996;**59**:647-53.
- M. Kimura, I. Ieiri, K. Mamiya, A. Urae, S. Higuchi: Ther. Drug Monit. (in press).
- K. Komai, K. Sumida, H. Kaneko, I. Nakatsuka: Pharmacogenetics 1996;**6**:117-9.
- Y. Tsuneoka, Y. Matsuo, E. Okuyama, Y. Watanabe, Y. Ichikawa: FEBS Letters 1996;**384**:281-4.
- S. Wang, J. Huang, M. Lai, J. Tsai: Pharmacogenetics 1995;**5**:37-42.
- H. Furuya, P. Fernandez-Salguero, W. Gregory, H. Taber, A. Steward, F. Gonzalez, J. Idle: Pharmacogenetics 1995;**5**:389-92.
- M. Stubbins, L. Harries, G. Smith, M. Tarbit, C. Wolf:

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Pharmacogenetics 1996;**6**:429-39.

- I.Ieiri, K.Mamiya, A.Urae, Y.Wada, M.Kimura, S.Irie,
T.Amamoto, T.Kubota, S.Yoshioka,
K.Nakamura, S.Nakano, N.Tashiro, S.Higuchi:
Br. J. Clin Pharmacol. 1997;**43**:441-5.
- M.Bajpai, L.Roskos, D.Shen, R.Levy: Drug Metab.
Dispos. 1996;**24**:1401-3.
- M.Krecic, D.Shepard, T.Chang, J.Collins, N.Gerber:
ISSX Proc. 1995;8;370.
- I.Ieiri, W.Goto, K.Hirata, A.Toshitani, S.Imayama,
Y.Ohyama, H.Yamada, K.Ohtsubo, S.Higuchi:
Eur. J. Clin. Pharmacol. 1995; **49**:51-6.
- L.Zhou, B.Pihlstrom, J.Hardwick, S.Park,
S.Wrighton, J.Holtzman: Clin. Pharmacol. Ther.
1996; **60**:191-8.