Korean J Physiol Pharmacol
Vol 1: 597–602, October, 1997

Cytochrome P450 2E1 Activity in a Korean Population

Dou-Hyun Muhn, Ji-Min Chae, Jae-Yong Bahn, Hae-Jung Song, Hyung-Kee Kim, Jun-Tack Kwon, and Dong-Ryul Sohn

Department of Clinical Pharmacology, Soonchunhyang University College of Medicine, Chonan 330–090, Republic of Korea

Cytochrome P450 2E1 (CYP2E1) is involved in the toxicity and carcinogenicity of a number of solvents and xenobiotics. Like the various types of oxidation pharmacogenetics, the activity of the enzyme shows a discernible interindividual and interethnic variation. However, no pharmacogenetic information on CYP2E1 polymorphism has been available from a Korean population. The aim of this study was to explore the pharmacogenetics of CYP2E1 polymorphism in a native Koreans after an oral 400 mg dose of chlorzoxazone administered to 128 subjects. Urine samples were collected during the subsequent 8-hour period and urinary concentrations of chlorzoxazone and 6-hydroxychlorzoxazone were determined by a high performance liquid chromatography with an ultraviolet detector. The limit of detection in the samples was found to be 0.5 μg/ml. The mean value of the 6-hydroxychlorzoxazone excreted in 8 hr urine expressed as the percentage was 48.2 13.8%. The frequency distribution of percentage of the administered dose excreted as the 6-hydroxy metabolite was unimodally distributed in the subjects studied. However, the values showed wide (7-fold) interindividual difference, ranged from 11.6% to 79.8% of the dose of chlorzoxazone. Thus, it was considered that the pharmacogenetic characteristics of CYP2E1 in a Korean population did not represent multimodal distribution in the 6-hydroxychlorzoxazone excreted in 8-hr urine expressed as the percentage. And the activity of the CYP2E1 in a Korean population seemed to be less compared with that of the Caucasian subjects.

Key Words: Cytochrome P450 2E1, Chlorzoxazone, Koreans

INTRODUCTION

The importance of cytochrome P450 (CYP) enzymes in the oxidation of drugs, environmental pollutants, dietary chemicals, and endogenous compound is well established (Nelson et al, 1996). In recent years, particular interest has focused on CYP2E1 because of its involvement in the activation of many low molecular weight toxins and carcinogens, including benzene, styrene, and a number of halocarbons (Yang et al, 1990; Koop, 1992). Human CYP2E1 has also been shown to metabolize certain therapeutic drugs including chlorzoxazone, acetaminophen, and the volatile anesthetics (Raucy et al, 1989; Peter et al, 1990; Thummel et al, 1993). A marked interindividual variability of hepatic CYP2E1 activity in animals and humans have been shown in vitro studies (Wrighton et al, 1986; Guengerich & Turvy, 1991), and it has been speculated that this may be an important factor in individual susceptibility to CYP2E1-associated adverse effects. In vitro studies have shown a marked interindividual variability in the hepatic CYP2E1 levels of both animals and humans (Hunt et al, 1990; Guengerich & Turvy, 1991). In animals, several major determinants of CYP2E1 activity have been described (Yang et al, 1990). However, in humans for less information is available, and it is not known, for example, whether interspecies differences exist in CYP2E1 regulation. A major reason

Corresponding to: Dong-Ryul Sohn, Department of Clinical Pharmacology, Soonchunhyang University College of Medicine, Chonan 330-090, Republic of Korea. Tel (Office): 0417-570- 2450 Fax: 0417-572-8995

for this paucity of data has been lack of suitable approach for determining CYP2E1 activity in vivo. However, the 6-hydroxylation of chlorzoxazone by human liver microsomes has been to be primarily mediated by CYP2E1 (Peter et al, 1990).

Chlorzoxazone, a central nervous system depressant indicated for relief of painful musculoskeletal conditions, has had a resurgence of scientific interest recently on the basis of its disposition kinetics and metabolism (Lacy et al, 1996). Recently, Kim et al. (1994) demonstrated the population distribution of the activity of chlorzoxazone 6-hydroxylation after an oral administration of chlorzoxazone varied fourfold to fivefold within the population and were unimodally distributed in a visually normal fashion. More recently, Kim et al. (1996) revealed an interethnic difference in chlorzoxazone's disposition after an oral 250 mg dose of chlorzoxazone administered to 20 young healthy Caucasian men and a similar group of Japanese men. The drug's plasma concentrations were significantly greater and its rate of elimination slower in Japanese compared to Caucasian men. Whether these findings are applicable to other Oriental groups of similar ethnic origin is not known and no pharmacogenetic information on CYP2E1 polymorphism has been available from a Korean population. Thus the aim of this study was to explore the pharmacogenetic characteristics of CYP2E1 in a Korean subjects.

METHODS

Subjects

A total of 128 unrelated healthy native Korean subjects (79 men and 49 women; 19 to 41 years of age; 23.8±3.4 years, mean±s.d.) were studied. They were laboratory personnel and medical and nursing school students. They participated after having given written, informed consent. The use of human subjects was approved by the Ethical Committee of Soonchunhyang University Hospital (Chonan). Each was examined prior to being accepted into the study, and each was determined to be in good health on the basis of history, physical examination, and laboratory findings. The mean body weight (±s.d.) was 62.3±8.3 kg (ranged from 47.5 to 82.1 kg) and the mean body mass index (BMI) was 20.3±2.3 $kg \cdot m^{-2}$ (ranged from 11.9 to 35.2 $Kg \cdot m^{-2}$). They abstained from ethanol use for at least 3 days before the study.

Determination of phenotypes

After an overnight fast, each subject emptied his or her bladder and took a single oral dose of 400 mg of chlorzoxazone (Duksan Pharmaceutical Inc.) and fasting maintained for at least an additional 3 hours. Urine samples were collected over a 0- to 8-hr period after the drug administration. Immediately after each collection of urine, the volume was measured and the aliquot was stored at −20°C until analysed. The metabolic activity of CYP2E1 was expressed as a percentage of the administered dose of chlorzoxazone excreted as the 6-hydroxychlorzoxazone upto 8 hours.

Analytic methods

Chlorzoxazone and 6-hydroxychlorzoxazone were measured in urine by high-performance liquid chromatography (HPLC) by a minor modification of the Honiberg et al. (1979). In brief, samples of 200 μl of urine were added to 2-ml microcentrifuge tube containing 800 μl of 125 mM/l acetate buffer (pH 5.0) and 5 μl of β-glucuronidase/sulfatase. They were hydrolysed overnight at 37°C to liberate 6-hydroxychlorzoxazone from their conjugates. Proteins were then precipitated with 200 μl of 10% trichloracetic acid. After centrifugation for 10 min at 3500 g, chlorzoxazone and 6-hydroxychlorzoxazone were extracted from the supernatant. The supernatants were injected into the HPLC system. Analyses were performed with the following HPLC system: a model 307 pump, a model 118 UV/VIS detector set at a wavelength of 280 nm, a model 234 autoinjector (Gilson Inc., Middleton, WI, U.S.A.), a model HP 3395 integrator (Hewlett-Packard Co., Palo Alto, CA, U.S.A.) and a reversed-phase C_{18} column [10 mm, 3.9 mm×300 mm I.D., μBondapack, Waters (Allendale, NJ, U.S.A.)]. The mobile phase (pH 4.7) consisted of 30% aqueous acetonitrile to which 0.15% ammonium acetate (pH 4.7) were added, and was delivered at a flow rate of 1.0 ml/min. Chlorzoxazone and 6-hydroxychlorzoxazone were purchased from Research Biochemicals International (Natick, MA, U.S.A.). Benzoxazolone, β-glucuronidase/sulfatase, trichloracetic acid, acetonitrile for HPLC, and ammonium acetate were from Sigma (St. Louis, MO, U.S.A.). Other chemicals were

purchased from Merck (Darmstadt, Germany) as an analytical grade.

RESULTS

The chromatograms of blank urine, spiked urine and urine obtained from a volunteer who had received chlorzoxazone are shown in Fig. 1 A, B and C, respectively. The retention times were 16.3 min for chlorzoxazone and 6.3 min for 6-hydroxychlorzoxazone, and 7.8 min for benzoxazolone as an internal standard. The peaks of these compounds were sharp, symmetrical and well resolved among them. Although an unknown peak arising from urine had a retention time close to that of 6-hydroxychlorzoxazone, such a small overlapping did not interfere with the quantitation of 6-hydroxychlorzoxazone. No other interference peaks were observed in the chromatograms of blank urine samples. The lowest detection limits, defined as the lowest concentration with a signal-to-noise ratio of 3, were 0.5 μg/ml for all the analytes.

The mean recoveries of chlorzoxazone, 6-hydroxychlorzoxazone and benzoxazolone from urine ranged from 98.2 to 107.4%. The coefficients of variation were less than 1.8 for the analytes in the samples. The calibration curves for the analytes were linear over the ranging concentrations examined ($r>0.98$ for the analytes, $p<0.01$). The intra-assay and inter-assay coefficients of variation for the analytes were less than 9.2 and 9.8, respectively, in urine. Relative errors for the intra-assay and inter-assay ranged from 0.8 to 8.4% and 0.3 to 5.5%, respectively.

No clinically undesirable adverse signs and symptoms possibly attributed to the administration of chlorzoxazone were recognizable throughout the study period. Within the population the frequency distribution of percentage of the administered dose excreted as the 6-hydroxy metabolite upto 8 hrs was demonstrated in Fig. 2, exhibiting a wide (7-fold) interindividual difference in the excretion of the metabolite. It was unimodally distributed and ranged from 11.6 to 79.8% of the administered dose of chlorzoxazone. The mean value of the 6-hydroxychlorzoxazone excreted in 8-hr urine expressed as the percentage was 48.2±13.8% (95% confidence interval: 43.3 to 51.5%). There were no statistically differences

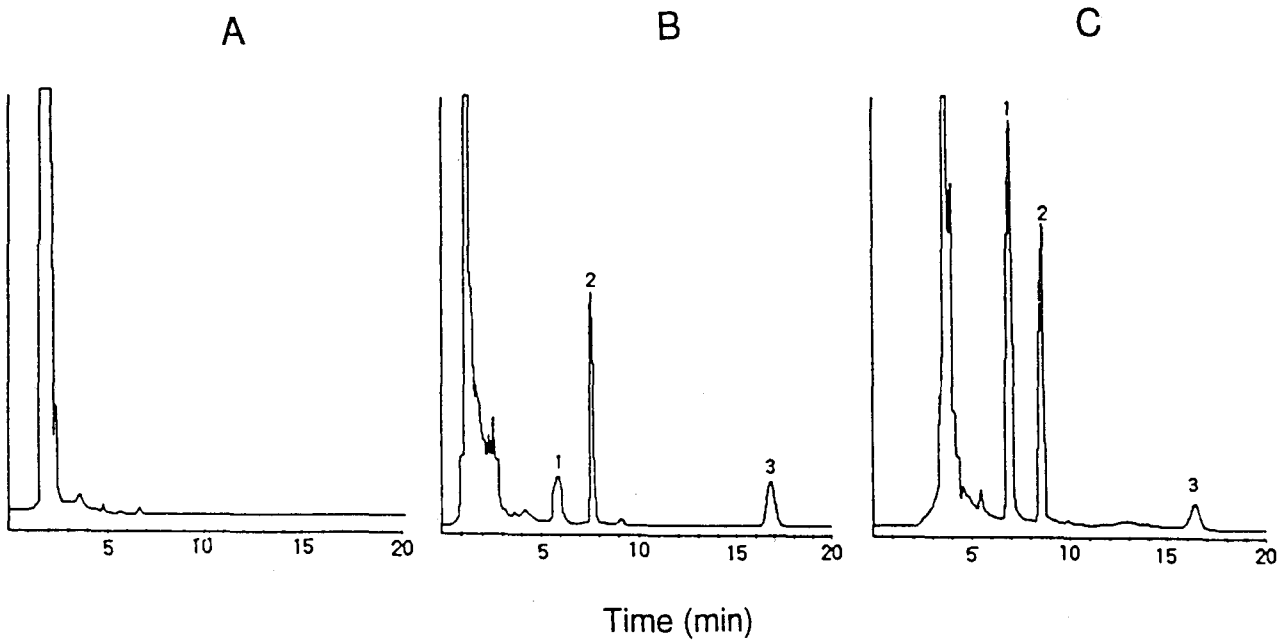

Fig. 1. Representative chromatograms obtained from blank urine (A), urine spiked with 6-hydroxychlorzoxazone, beonzoxazolone and chlorzoxazone to give a final concentration of 200 μ/ml each (B), urine obtained from a volunteer after an oral dosing of 400 mg of chlorzoxazone (C). Arabic numericals in B and C indicate the peaks of: 1=6-hydroxychlorzoxazone; 2=benzoxazolone (internal standard); and 3=chlorzoxazone.

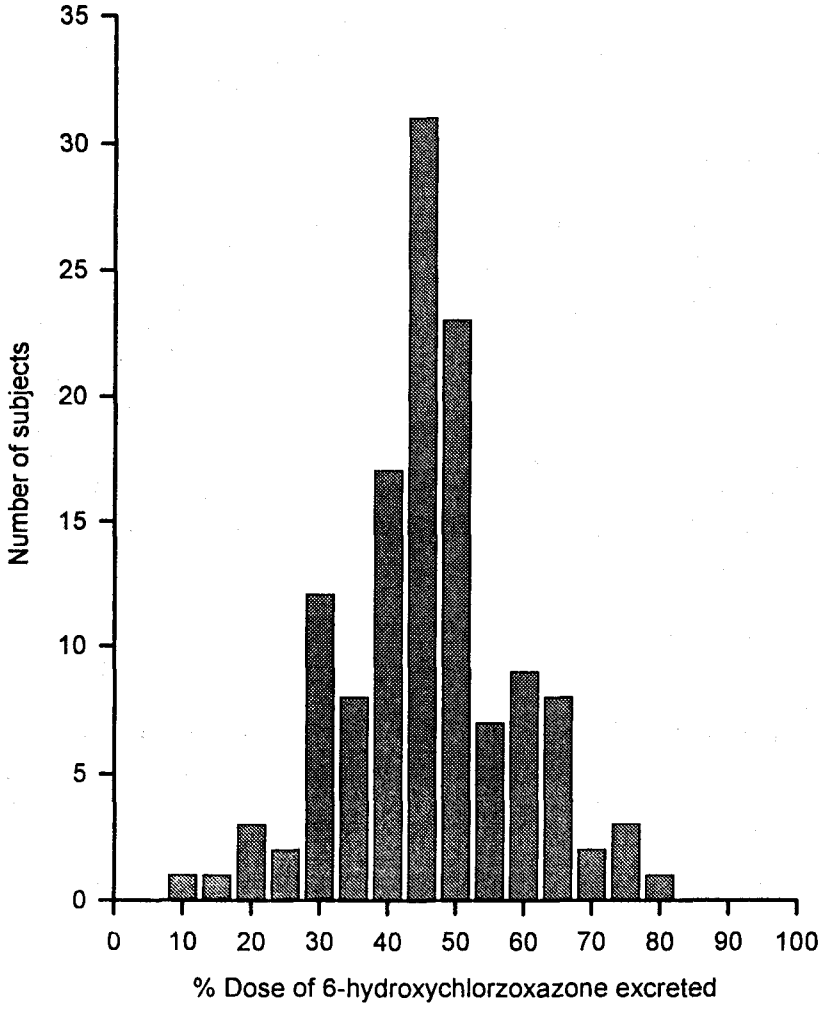

Fig. 2. Frequency distribution of the 0~8 hr urinary recovery of 6-hydroxychlorzoxazone after an oral administration of 400 mg of chlorzoxazone in 128 Korean subjects.

between male and female, and smoking status.

DISCUSSION

Recently the importance of CYP2E1 in the xenobiotic metabolism arises because of its likely role in the activation of important human procarcinogenes and protoxins (Yang et al, 1990; Yamazaki et al, 1992). The expression and regulation of this enzyme is unusual, if not unique, among the numerous CYP isoforms and also the enzyme's human liver microsomal content varies markedly (Yoo et al, 1988; Guengerich & Turvy, 1991). Such variability is consistent with the notion that susceptibility to environmental cancer and toxicity may reflect individual differences in the activity of enzyme(s) involved in the oxidative activation of the causative agent(s). The finding that 6-hydroxylation is the major pathway of chlorzoxazone metabolism and that this is mediated by CYP2E1 provides a means to evaluate this possibility in vivo and in individual subjects, inasmuch as measurement of the metabolite's formation rate should provide a probe of the enzyme's overall activity within the body. However, the validity of such an estimate depends on the assumption that CYP2E1 selectively mediates the 6-hydroxylation. Peter et al. (1990) provided strong evidence for such specificity

using human liver microsomes and a number of complementary experimental approaches. The currently available evidence indicates that the in vivo 6-hydroxylation of chlorzoxazone in humans is predominantly, in not exclusively, mediated by CYP2E1, and its measurement does indeed provide a valid estimate of the enzyme's activity.

Polymorphic distribution of certain drug-metabolizing enzyme activities has been described which has a genetic rather than environmental basis (Murray, 1992). No discontinuity was observed in the distribution of the percentage dose of 6-hydroxychlorzoxazone excreted in 0-8 hr urine after the administration of chlorzoxazone as a probe drug in the 128 subjects studied. In no instance has any individual been identified who might belong to a subpopulation with different in vivo CYP2E1 activity to that of the extent data. Thus, although a genetic polmorphism-arbitrarily defined as a frequency of about 1% to 2% of the less common phenotype cannot be excluded, its likelihood appears to be low. Despite substantial interindividual variations in CYP2E1 activity, clear relationships between any of the genetic polymorphisms (Rsa I and Dra I) and CYP2E1 activity were not reported. Thus, in humans, the level of CYP2E1 in different individuals markedly varies, probably due to differing induction levels caused by such environmental factors as alcohol consumption.

Interest in CYP2E1 genetic polymorphisms has arisen because of their possible association with individual susceptibility to lung cancer. For example, the genotypic distribution of the Dra I polymorphism has been reported to be different in Japanese patients with lung cancer relative to control subjects (Uematsu et al, 1994). However, such an association has not been observed in white populations, in which the mutant C allele is much rarer than in Japanese. Epidemiologic studies relating the presence of the Rsa I and Pst I polymorphisms and lung cancer have also been equivocal. In this regard, it is interesting that the relative lung cancer incidence rate in various racial groups, including white subjects and Oriental subjects.

The formation and subsequent excretion of the 6-hydroxychlorzoxazone was rapid with $48.2 \pm 13.8\%$ (mean $\pm$ s.d.) of the administered dose being recovered over the 0~8 hr collection period. Previous studies have shown that the metabolite is eliminated in a conjugated form and that urinary recovery is essentially complete within 8 hrs (Kharasch et al, 1993; O'Shea et al, 1994). In this regard it is important that the recovery value of the previous report in Caucasian subjects is much higher compared with this study. And this can be figured out another factor to explain the interethnic variation not only in the activity of CYP2E1 but the incidence of the lung cancer on the various ethnic origin.

In summary, the results reported herein, in subjects residing in Korea, the ability to 6-hydroxylate chlorzoxazone was continuously distributed in an apparently normal fashion. Because this group of subjects were all healthy and not known to be receiving any agents that might modulate CYP2E1 activity, the sevenfold range of interindividual variability is likely to primarily reflect genetic determinants.

ACKNOWLEDGEMENTS

This study was supported in part by a Grant-in-Aid from the Ministry of Human Health and Welfare, Seoul, Korea

REFERENCES

Guengerich FP, Turvy CG. Comparison of levels of several human microsomal cytochrome P450 enzymes and epoxide hydrolase in normal and diseases state using immunochemical analysis of surgical liver samples. *J Pharmacol Exp Ther* 256: 1189−1194, 1991

Hunt CM, Strater S, Stave EM. Effect of normal aging on the activity of human hepatic cytochrome P450 IIE1. *Biochem Pharmacol* 40: 1666−1669, 1990

Kharasch E, Thummel K, Mhyre J, Lillibridge J. Single-dose disulfiram inhibition of chlorzoxazone metabolism: A clinical probe for P450 2E1. *Clin Pharmacol Ther* 53: 643−650, 1993

Kim RB, O'Shea D, Wilkinson GR. Relationship in healthy subjects between CYP2E1 genetic polymorphisms and the 6-hydroxylation of chlorzoxazone: a putative measure of CYP2E1 activity. *Pharmacogenetics* 4: 162−165, 1994

Kim RB, Yamazaki H, Chiba K, O'Shea D, Mimura M, Guengerich FP, Ishizaki T, Shimada T, Wilkinson GR. In vivo and in vitro characterization of CYP2E1 activity in Japanese and Caucasians. *J Pharmacol Exp Ther* 279: 4−11, 1996

Koop DR. Oxidative and reductive metabolism by cytochrome P4502E1. *FASEB J* 6: 724−730, 1992

Lacy C, Armstrong LL, Ingrim NB, Lance LL: Chlorzoxazone. *Drug Inform Handbook* 4: 255, 1996

Murray M. P450 enzymes: inhibition mechanisms, genetic regulation and effects of liver disease. *Clin Pharmacokinet* 23: 132–146, 1992

Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R, Waxman DJ, Waterman MR, Gotoh O, Coon MJ, Estabrook RW, Gunsalus IC, Nebert DW. P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics* 6: 1–42, 1996

O'Shea D, Davis SN, Kim RB, Wilkinson GR. Effect of fasting and obesity in humans on the 6-hydroxylation of chlorzoxazone: A putative probe of CYP2E1 activity. *Clin Pharmacol Ther* 56: 359–367, 1994

Peter R, Böker R, Beaune PH, Iwasaki M, Guengerich FP, Yang CS. Hydroxylation of chlorzoxazone as a specific probe for human liver cytochrome P450 IIE1. *Chem Res Toxicol* 3: 566–573, 1990

Raucy JL, Lasker JM, Lieber CS, Black M. Acetaminophen activation by human liver cytochromes P450 IIE1 and P450IA2. *Arch Biochem Biophys* 271: 270–283, 1989

Thummel KE, Kharasch ED, Podoll T, Kunze K. Human liver microsomal enflurane defluorination catalyzed by cytochrome P-450 2E1. *Drug Metab Dispos* 21: 350–357, 1993

Uematsu F, Ikawa S, Kikuchi H, Sagami I, Kanamura R, Abe T, Satoh K, Motomiya M, Watanabe M. Restriction fragment length polymorphism of the human CYP2E1 (cytochrome P450 IIE1) gene and susceptibility to lung cancer: possible relevance to low smoking exposure. *Pharmacogenetics* 4: 58–63, 1994

Wrighton SA, Thomas PE, Molowa DT, Haniu M, Shively JE, Maines SL, Watkins PB, Parker G, Mendez-Picon G, Levin W, Guzelian PS. Characterization of ethanol-inducible human liver N-nitrosodimethylamine demethylase. *Biochemistry* 25: 6731–6735, 1986

Yamazaki H, Inui Y, Yun CH, Guengerich FP, Shimada T. Cytochrome P450 2E1 and 2A6 enzymes as major catalysts for metabolic activation of N-nitrosodialkylamines and tobacco-related nitrosamines in human liver microsomes. *Carcinogenesis* 13: 1789–1794, 1992

Yang CS, Yoo JSH, Ishizaki H, Hong J. Cytochrome P450IIE1: roles in nitrosamine metabolism and mechanisms of regulation. *Drug Metab Rev* 22: 147–159, 1990

Yoo JSH, Guengerich FP, Yang CS. Metabolism of N-nitrosodialkylamines by human liver microsomes. *Cancer Res* 48: 1499–1504, 1988