In Vivo and In Vitro Characterization of CYP2E1 Activity in Japanese and Caucasians

RICHARD B. KIM, HIROSHI YAMAZAKI, KAN CHIBA, DIARMUID O’SHEA, MAYUMI MIMURA, F. PETER GUENGERICH, TAKASHI ISHIZAKI, TSUTOMU SHIMADA and GRANT R. WILKINSON

Departments of Pharmacology (R.B.K., D.O’S., G.R.W.) and Biochemistry (F.P.G.), Vanderbilt University, Nashville, Tennessee; Osaka Prefectural Institute of Public Health, Osaka, Japan (H.K., M.M., T.S.); and Department of Clinical Pharmacology, International Medical Center of Japan, Tokyo, Japan (K.C., T.I.)

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ABSTRACT

Chlorzoxazone’s disposition after oral administration was determined in 20 young healthy Caucasian men and a similar group of Japanese men. The drug’s plasma concentrations were significantly higher and its rate of elimination slower in Japanese compared to Caucasians men. Accordingly, chlorzoxazone’s oral clearance was smaller (40%) in Japanese men and a similar difference (30%) was still apparent after normalizing for body weight (3.74 ± 1.23 versus 5.05 ± 1.41 ml/min-1·kg-1, P < .05). This slower elimination was associated with a reduced (fractional) clearance by 6-hydroxylation (2.34 ± 1.04 ml/min-1·kg-1 versus 3.23 ± 1.10, P < .05). Because such metabolism is mediated by cytochrome P4502E1 (CYP2E1), these findings suggest a lower level of the enzyme’s catalytic activity in Japanese men. This was confirmed by in vitro studies with microsomes prepared from livers of individuals representative of the two racial groups. CYP2E1 levels were lower (61% P < .002) and CYP2E1-mediated chlorzoxazone 6-hydroxylase (22%, P < .001) and aniline 4-hydroxylase (35%, P < .0001) activities were reduced in Japanese preparations compared to those from Caucasians. No relationships were found between measures of CYP2E1 activity, both in vivo and in vitro, and genomic polymorphisms in the CYP2E1 gene identified by Rsal/PstI and DraI restriction fragment length polymorphisms. Collectively, these data show an inter racial difference in CYP2E1 activity. Because this enzyme is importantly involved in the activation of environmental procarcinogens, such a difference may account, in part, for the lower rate of some cancers, e.g., lung cancer, in Japanese compared to Caucasians men.

CYP consist of a superfamily of heme-thiolate proteins that play an important role in the metabolism of a wide variety of xenobiotics, including drugs, carcinogens and environmental agents, as well as endogenous compounds such as steroids and fatty acids (Gonzalez, 1989). The individual isoforms have different regulatory and functional characteristics including selective substrate specificities. CYP2E1 is of particular interest because it is involved in the metabolic activation of many low molecular weight chemicals associated with cancer and other toxic effects, e.g., N-nitrosamines present in tobacco smoke and derived from the diet (Yang et al., 1990; Yamazaki et al., 1992), benzene, vinyl chloride and other halocarbons (Guengerich et al., 1991; Koop, 1992). The enzyme also metabolizes ethanol (Lieber, 1994) and it has been postulated to play a role in the pathogenesis of alcoholic liver disease (Morimoto et al., 1993). In addition, a number of drugs such as acetaminophen (Raucy et al., 1989), chlorzoxazone (Peter et al., 1990) and halogenated general anesthetic agents (Kharasch and Thummel, 1993; Thummel et al., 1993) are also CYP2E1 substrates. Considerable inter individual variability is present in human CYP2E1 activity irrespective of whether measured in vitro in hepatic microsomes (Yoo et al., 1988; Hunt et al., 1990; Tassaneeyakul et al., 1993) or in vivo using the 6-hydroxylation of chlorzoxazone as a phenotypic probe (Girre et al., 1994; Kim et al., 1995). Several factors including obesity and fasting (O’Shea et al., 1984), alcoholism (Gilger et al., 1994) and enzyme induction or inhibition by concomitantly administered drugs (Kharasch et al., 1993; Zand et al., 1993) have been shown to modulate human CYP2E1 activity. A number of other determinants are demonstrable in animals; and, in general, such factors appear to involve posttranslational mechanisms (Koop and Tierney, 1990); however, it has been suggested that transcriptional regulation may also be important. This notion is based on the presence of several RFLPs in the CYP2E1 gene including two linked, 5'-flanking region polymorphisms, associated with Rsal and PstI endonucleases, and demonstration that the

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ABBREVIATIONS: AUC, area under the plasma concentration-time curve; CYP, cytochrome P450; RFLP, restricted fragment length polymorphism.
mutant alleles are associated with increased in vitro transcription of model construct reporter genes (Hayashi et al., 1991; Watanabe et al., 1994). A DraI RFLP is also present in intron 6 of the CYP2E1 gene (Uematsu et al., 1991).

Recently, these CYP2E1 genomic polymorphisms have been of interest because of their suggested association with lung cancer risk. For example, in Japanese subjects the presence of the minor (mutant) C allele of the DraI polymorphism appeared to be less common in lung cancer patients compared to control subjects (Uematsu et al., 1991, 1994). By contrast, studies in European-Americans (Kato et al., 1994) or Europeans (Hirvonen et al., 1993; Persson et al., 1993) have not detected such an association. The frequency of the mutant c1 RsaI allele was also reported to be lower in Swedish lung cancer patients (Persson et al., 1993), but this was not found in other Caucasian populations residing in Finland (Hirvonen et al., 1993) or the United States (Kato et al., 1992). Similarly, no association is apparently present in the Japanese population (Watanabe et al., 1995). The problem with these molecular epidemiological studies is that marked interracial differences are present in the frequencies of the various mutant alleles. For example, both the mutant DraI C and RsaI c1 alleles are about 2- to 5-fold more common in Japanese people than in Caucasians; thus, studies in Caucasians generally have lower statistical power than those in Japanese people. More significantly, however, is the lack of any direct evidence relating these allelic mutations to CYP2E1 in vivo expression and catalytic activity.

Allelic variants of particular CYP enzymes have been shown to result in differences in catalytic functions, as demonstrated by the null phenotypes associated with genetic polymorphisms such as those involving CYP2C19 and CYP2D6 (Bertilsson et al., 1995). Furthermore, the frequency of such mutations may be dependent on the racial origin of the population (Bertilsson et al., 1995). In addition, the plasma concentration-time profiles of several drugs whose metabolism is continuously rather than discontinuously distributed have been found to be dependent on the racial background of the study population (Ahsan et al., 1993; Castañeda-Hernández et al., 1993). Such differences may be genetically determined and/or reflect environmental factors, e.g., diet, but little data are available on these possibilities, especially regarding individual CYP isoforms. Accordingly, our study was undertaken to determine whether CYP2E1 activity is different in Japanese compared to Caucasian subjects, using chloroxazzone 6-hydroxylation as a probe. In addition, associations between such activity and the RFLP polymorphisms present in the CYP2E1 gene were investigated.

Methods

Clinical studies. Investigations were undertaken in 20 Caucasian and 20 Japanese men residing in middle-Tennessee or around Tokyo, respectively, who were judged to be healthy on the basis of medical history, physical examination and laboratory tests indicative of normal cardiac, renal and liver function. Although all of the subjects were aged between 18 and 35 yr, the Japanese were of smaller body size than the Caucasians (body weight, 61.6 ± 8.1 versus 76.4 ± 8.7 kg (mean ± S.D.); body mass index, 21.1 ± 2.1 versus 23.8 ± 2.2 kg·m⁻², respectively). None of the subjects were tobacco-users or were taking any medications; in addition, they abstained from ethanol use for at least 3 days before the study. After an overnight fast, 250 mg chloroxazzone (Geneva Pharmaceutical Inc., Bloomfield, CO) was orally administered and fasting maintained for at least an additional 3 hr. Venous blood samples, using EDTA as anticoagulant, were obtained from an arm vein at 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 hr after drug administration. Plasma was harvested and stored at -20°C before analysis, and urine was collected over a 0- to 24-hr period.

Chloroxazzone and its 6-hydroxy metabolite in plasma and urine were determined by a reversed-phase HPLC procedure, subsequent to appropriate hydrolysis of conjugated metabolite, as previously described (O'Shea et al., 1994). The area under the plasma concentration-time curves (AUC) for each compound was determined trapazoidally and extrapolated to infinity using a log-linear estimation of the terminal rate constant (k), that also allowed calculation of a terminal half-life (0.693/k). Chloroxazzone's oral clearance was determined from the ratio of the administered dose to the drug's AUC. The fractional clearance rate reflective of 6-hydroxylation was estimated from the product of oral clearance and the fraction of the administered dose recovered as the metabolite in the 0 to 24 hr urine.

An unpaired Student's t test, Mann-Whitney U test or analysis of variance with repeated measures were used to compare the various pharmacokinetic parameter estimates between the two racial groups; P < .05 was taken as indicative of a statistically significant difference.

On a separate occasion from the above study period, usually within 3 to 5 days, a 20-ml peripheral blood sample was obtained from each subject and white blood cells were prepared using sucrose-Triton X and centrifugation. Subsequently, genomic DNA was extracted, after proteinase-K digestion. Mutations in the 5'-flanking region of the CYP2E1 gene (PstI/RsaI) and in intron 6 (DraI) were determined by PCR-amplification/RFLP analysis, as previously described (Kim et al., 1995).

In vitro metabolism studies. Liver samples were obtained from 30 Caucasian organ donors through Tennessee Donor Services (Nashville, TN). By contrast, the 30 Japanese liver samples were obtained from patients undergoing liver resection and represented portions of the organ without particular pathohistological changes. Additional characteristics of these tissues and preparation of liver microsomes, which were suspended in 10 mM Tris-HCl buffer (pH 7.4) containing 0.1 mM EDTA and 20% v/v glycerol before use, have been previously described (Shimada et al., 1994). Incubations were carried out at 37°C for 15 min with a mixture containing liver microsomes (0.5 mg protein/ml), and substrate (0.5 mM chloroxazzone, Sigma Chemical Co., St. Louis, MO), or 1.0 mM aniline (Wako Pure Chemical Co., Osaka, Japan) in a final volume of 0.5 ml 100 mM potassium phosphate buffer (pH 7.4) containing a NADPH-generating system (Peter et al., 1990). After stopping metabolism, by the addition of either 50 μl 43% w/v H₂PO₄ and 1.5 ml CH₃Cl, when chloroxazzone was the substrate or 0.5 ml 10% trichloracetic acid in the aniline studies, the incubate was analyzed to determine the amount of metabolite formed: either 6-hydroxychloroxazzone (Peter et al., 1990) or 4-hydroxyniline (Imai and Sato, 1966). Protein, total CYP and cytochrome b₅ concentrations were measured by well-described methods (Lowry et al., 1951; Omura and Sato, 1964). Microsomal NADPH-cytochrome c reduction activity was also determined (Williams and Kamin, 1962) as a measure of NADPH reductase. The CYP2E1 level was estimated by coupled sodium dodecyl sulfate-polyacrylamide gel electrophoresis/immunochemical development using rabbit anti-CYP2E1 antibody (Guengerich et al., 1982). A Mann-Whitney U test, with P < .05 as the minimum level of statistical significance, was used to compare the findings between the two racial groups.

The kinetics of chloroxazzone 6-hydroxylation were determined in microsomes from selected liver samples using a range of substrate concentrations. Based on the assumption of a single enzyme system, estimates of the Michaelis-Menten parameters (Kₘ and V_max) were obtained by nonlinear regression analysis using the Kaleida Graph program (Synergy Software, Reading, PA).
In most cases, all of the liver tissues were used in the production of microsomes; however, sufficient material was left over from eight Caucasian and six Japanese samples to extract genomic DNA, after homogenization and incubation with protease-K. PCR-RFLP analysis was then performed in the same fashion as described for the peripheral white blood cell-derived DNA.

**Results**

After administration of a single oral dose, chlorzoxazone was rapidly absorbed and subsequently eliminated in a monoexponential fashion in both racial groups (fig. 1). However, the drug's plasma concentrations were significantly higher and its rate of elimination slower in Japanese compared to Caucasians subjects. Accordingly, chlorzoxazone's oral clearance in the former group was on average 40% smaller than in Caucasians, and a similar weight-adjusted (30%) was still apparent after normalizing for body weight (table 1). 6-Hydroxychlorzoxazone also appeared rapidly in the plasma (fig. 1) and its elimination half-life was similar or slightly longer than that of chlorzoxazone (table 1). In contrast to parent drug, there was no difference between the two racial groups in the metabolite's plasma AUC; also, the 0- to 24-hr urinary recovery of 6-hydroxychlorzoxazone was similar in both groups (table 1).

The lower oral clearance of chlorzoxazone in the Japanese subjects was associated with a reduced (fractional) clearance by 6-hydroxylation, and this racial difference was also significant on both an absolute and body weight adjusted basis (table 1, fig. 2). Other measurements reflective of CYP2E1-mediated metabolism, such as the ratio of the 6-hydroxychlorzoxazone to chlorzoxazone plasma AUC values of the analogous plasma level ratio at 4 hr after drug administration, similarly indicated lower activity in the Japanese (fig. 2). All of the estimated pharmacokinetic parameters appeared to be continuously distributed, with the range of values being generally 2- to 5-fold.

Considerable variability was found in the in vitro catalytic activities of liver microsomes from both racial groups, and similar variability in the total CYP and CYP2E1 levels was also noted (fig. 3). Nevertheless, statistically significant lower mean levels of chlorzoxazone 6-hydroxylation (22%, \( P < .001 \)), aniline 4-hydroxylation (35%, \( P < .0001 \)) and NADPH-cytochrome c reduction (65%, \( P < .002 \)) activities, and total CYP (61%, \( P < .0001 \)) and CYP2E1 levels (61%, \( P < .002 \)) were present in the Japanese preparations compared to those from Caucasians. Such differences were apparent regardless of whether the activity was normalized with respect to microsomal protein or CYP2E1 content. By contrast, the cytochrome \( b_5 \) content was not different between the two racial groups. The 7-ethoxycoumarin O-deethylation and \( N \)-nitrosodimethylamine \( N \)-demethylation activities of the microsomes (data not shown) were consistent with those reported earlier (Shimada et al., 1994).

Comparison of the Michaelis-Menten parameters in two selected sets of microsomes (Caucasian HL-11 and 51 and Japanese HL-4 and 46) indicated similar \( K_m \) values (63 and 68 versus 57 and 63 \( \mu \)M), respectively. However, the \( V_{max} \) values differed among the preparations (1.4 and 0.17 versus 0.11 and 0.18 nmol/min/mg protein).

Genomic DNA was obtained in all Caucasian individuals and 19 of the 20 Japanese who participated in the clinical study. The linked 5'-flanking region \( PstI/RsaI \) RFLP poly-

**Table 1**

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Caucasian (mean ± SD)</th>
<th>Japanese (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral clearance ( \text{ml} \cdot \text{min}^{-1} )</td>
<td>( 385 ± 111 )</td>
<td>( 232 ± 83^a )</td>
</tr>
<tr>
<td>Oral clearance ( \text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} )</td>
<td>( 5.05 ± 1.41 )</td>
<td>( 3.74 ± 1.23^b )</td>
</tr>
<tr>
<td>Fractional clearance by 6-hydroxylation ( \text{ml} \cdot \text{min}^{-1} )</td>
<td>( 246 ± 82 )</td>
<td>( 146 ± 71^a )</td>
</tr>
<tr>
<td>Fractional clearance by 6-hydroxylation ( \text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} )</td>
<td>( 3.23 ± 1.10 )</td>
<td>( 2.34 ± 1.04^a )</td>
</tr>
<tr>
<td>Chlorzoxazone half-life, hr</td>
<td>( 0.90 ± 0.16 )</td>
<td>( 1.09 ± 0.20^a )</td>
</tr>
<tr>
<td>Chlorzoxazone half-life, hr</td>
<td>( 1.17 ± 0.18 )</td>
<td>( 1.28 ± 0.23 )</td>
</tr>
<tr>
<td>6-Hydroxychlorzoxazone excreted in 0-24 hr urine, % dose</td>
<td>( 63 ± 11 )</td>
<td>( 61 ± 11 )</td>
</tr>
</tbody>
</table>

**Notes:**

- \( a \) \( P < .001 \) Japanese versus Caucasian.
- \( b \) \( P < .05 \) Japanese versus Caucasian.

**Fig. 1.** Mean (± SD) plasma concentration-time curves of chlorzoxazone (○) and its 6-hydroxy metabolite (□) in healthy Caucasian and Japanese subjects (\( n = 20 \) each group) after oral administration of 250 mg chlorzoxazone.
Discussion

A major finding of the clinical study was the marked difference in the disposition of chlorzoxazone between Caucasian and Japanese men; in particular, the drug's oral clearance. Because essentially no chlorzoxazone is excreted unchanged (Kharasch et al., 1993; Kim et al., 1995), this pharmacokinetic parameter reflects the combined effects of absorption from the gastrointestinal tract and first-pass metabolism, primarily by the liver. The urinary recovery of 6-hydroxycilorzoxazone was similar in both racial groups, indicating comparable absorption, therefore, the difference in oral clearance indicates a lower drug-metabolizing ability of Japanese compared to Caucasians. Furthermore, partitioning this parameter into the 6-hydroxylation component should indicate metabolism by this specific pathway and by inference, a reduced catalytic level of CYP2E1. This interracial difference was confirmed by other measures of metabolism (Girre et al., 1994; Kim et al., 1995), for example, the 4-hr plasma concentration ratio and the AUC ratio of chlorzoxazone to its 6-hydroxy metabolite. Moreover, after weight normalization of the estimated clearance values, the difference was still present, suggesting that it reflects an intrinsic factor rather than one related to differences in body size between the two populations. This interpretation is supported by the in vitro findings using liver microsomes obtained from individuals of the same racial groups, but different from the subjects investigated in the clinical study.

Despite considerable interindividual variability, the content of total CYP enzymes and immunoreactive CYP2E1 were substantially lower in microsomes obtained from Japanese livers compared to Caucasian ones; however, the fraction of CYP2E1 relative to total CYP protein was similar in the two groups. Additionally, the ability to mediate the spe-
cific oxidations of two established CYP2E1 substrates was lower in the Japanese samples, and to an extent comparable to that observed in the clinical study. These differences were present regardless of whether catalytic activity was expressed in terms of either protein or cytochrome P450, but, because of the total amount of the hemoprotein per mg protein was lower in the Japanese microsomes (~60%), the racial difference in catalysis was greater on a protein basis. It is, therefore, likely that the in vitro interracial difference in metabolism primarily reflects a difference in the amount of hepatic CYP2E1 protein; an interpretation consistent with the limited enzyme kinetic data indicating a higher $V_{\text{max}}$ value in Caucasian microsomes compared to those derived from Japanese individuals. However, it is not entirely possible to rule out that a qualitative difference may be present in the enzyme, so that its catalytic activity is reduced in the Japanese; for example, microheterogeneity in the amino acid sequence. An alternative explanation is suggested by the reduced level, on a protein basis, of cytochrome $b_5$ and also NADPH-cytochrome $c$ reduction activity, a measure of NADPH-P450 reductase, and a determinant of CYP2E1-catalyzed reactions (Yamazaki et al., 1996). A difficulty with this interpretation is that the ratio of NADPH-P450 reductase, and cytochrome $b_5$, which is another important factor in CYP2E1 metabolism (Yamazaki et al., 1995, 1996), to total CYP and, also, CYP2E1 were similar in the Japanese and Caucasian samples. Another explanation for the interracial difference is that the in vitro differences are simply a result of variability in sample handling and subsequent deterioration. This possibility cannot be dismissed, because the liver samples were obtained from different types of patients in two countries by a number of individuals using different experimental procedures. The larger variance of the in vitro measures, especially CYP2E1-mediated metabolism, compared to
TABLE 2
Restriction fragment length polymorphisms of CYP2E1 in Caucasian and Japanese subjects and their relationship to the metabolism of chlorzoxazone

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of Subjects</th>
<th>Oral Clearance (ml·min⁻¹·kg⁻¹)</th>
<th>Fractional Clearance (ml·min⁻¹·kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>20</td>
<td>5.0 ± 1.4</td>
<td>3.2 ± 1.0</td>
</tr>
<tr>
<td>PstI/RsaI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c1c1</td>
<td>18</td>
<td>5.1 ± 1.5</td>
<td>3.2 ± 1.2</td>
</tr>
<tr>
<td>c1c2</td>
<td>2</td>
<td>4.8 ± 0.8</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>c2c2</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drai</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DD</td>
<td>15</td>
<td>5.3 ± 1.5</td>
<td>3.4 ± 1.2</td>
</tr>
<tr>
<td>CD</td>
<td>5</td>
<td>4.3 ± 0.7</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>CC</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japanese</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>19</td>
<td>3.7 ± 1.2</td>
<td>2.3 ± 1.0</td>
</tr>
<tr>
<td>PstI/RsaI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c1c1</td>
<td>4</td>
<td>4.5 ± 1.9</td>
<td>2.8 ± 1.3</td>
</tr>
<tr>
<td>c1c2</td>
<td>12</td>
<td>3.7 ± 0.8</td>
<td>2.4 ± 1.0</td>
</tr>
<tr>
<td>c2c2</td>
<td>3</td>
<td>2.6 ± 0.6</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>Drai</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DD</td>
<td>4</td>
<td>4.1 ± 1.9</td>
<td>2.5 ± 1.3</td>
</tr>
<tr>
<td>CD</td>
<td>11</td>
<td>4.0 ± 0.8</td>
<td>2.6 ± 0.9</td>
</tr>
<tr>
<td>CC</td>
<td>4</td>
<td>2.6 ± 0.5</td>
<td>1.5 ± 0.4</td>
</tr>
</tbody>
</table>

chlorzoxazone's in vivo clearance, a phenomenon that has also been noted with other CYP isoforms, may also result from this factor. Regardless, the interracial difference in chlorzoxazone’s in vivo disposition appears to be attributable to an intrinsically lower catalytic activity of hepatic CYP2E1.

Factors involved in CYP2E1 expression and the resulting amount of enzyme are not well understood. One suggestion, based on reporter gene construct experiments with elements of the CYP2E1 5'-regulatory region, is that the mutations responsible for the PstI/RsaI genomic polymorphisms result in enhanced expression (Hayashi et al., 1991; Watanabe et al., 1994). However, a study comparing genotype and CYP2E1 activity using chlorzoxazone as an in vivo probe was unable to demonstrate this in a population of 70 healthy Caucasians (Kim et al., 1995). This has recently been confirmed in a study involving more than 100 subjects (Lucas et al., 1995), and an in vitro study using more than 90 Caucasian liver samples has also failed to find a relationship between genotype and phenotype (Carrière et al., 1996). The lack of association between the mutant c2 allele and chlorzoxazone’s 6-hydroxylation in the 20 Caucasians in our study is, therefore, consistent with these earlier studies. The statistical power of these findings is low, however, because the mutant c2 allele is extremely uncommon in this racial group (Kato et al., 1992; Hirvonen et al., 1993; Persson et al., 1993). It is far higher in Japanese (Kato et al., 1992; Watanabe et al., 1995) and, in fact, 3 homozygote (c2c2) individuals were present in the 20 subjects in whom chlorzoxazone’s metabolism was determined. Nevertheless, neither these individuals nor those who were heterozygotes appeared to have different CYP2E1 activities from the homozygous wild-type subjects. Also, the overall lower CYP2E1 activity in Japanese is the opposite to that expected on the basis of the mutant allele’s frequency and its speculated up-regulatory effect on expression. Overall, therefore, the 5'-region genomic polymorphisms in CYP2E1 do not appear to have any functional consequence at the enzyme level.

The frequency of the Drai polymorphism of the CYP2E1 gene is also racially determined (Kato et al., 1992, 1994; Hirvonen et al., 1993; Persson et al., 1993), which accounts for the absence of homozygous mutant individuals in the Caucasian group but four such individuals in the Japanese. Again, with these few numbers, interpretation of the findings must be cautious. Nevertheless no relationship appeared to

![Fig. 4. Comparison of CYP2E1 genotypes and CYP2E1 content, aniline 4-hydroxylase and chlorzoxazone 6-hydroxylase activities in microsomes from Caucasian (●) and Japanese (▲) livers. Data reflect the PstI/RsaI 5'-flanking region (c1, c2 and c1c1) and intron Drai 6 (CD and DD) restriction fragment length polymorphisms.](image-url)
be present between the genotype and chlorozoxazone's 6-hydroxylation, again confirming an earlier in vivo (Kim et al., 1995; Lucas et al., 1995) and in vitro (Carrière et al., 1996) findings in larger groups of Caucasians. This is not surprising because the Dral-associated mutation is in intron 6, and, unless linked to another regulatory or exonic mutation, would not be expected to affect expressed CYP2E1 activity. Nevertheless, it has been speculated that an apparent association between the C2 mutant allele involved in this polymorphism and susceptibility to lung cancer in Japanese subjects could involve increased CYP2E1-mediated activation of a tobacco-related procarcinogen (Uematsu et al., 1991 and 1994). However, our findings indicate that the epidemiological association between Dral and lung cancer does not appear to involve a difference in CYP2E1 metabolism.

CYP2E1 is the major enzyme involved in the activation of various nitrosamines and other procarcinogens present in the diet, tobacco smoke and the environment (Yang et al., 1990; Guengerich et al., 1991; Yamazaki et al., 1992). In particular, it is important in the activation of the potent, tobacco-specific procarcinogen 4-(methyl-nitrosamino)-1-(3 pyridyl)-1-butane (NNK), which is considered to contribute to lung cancer in smokers, along with arylamines, polynuclear aromatic hydrocarbons and other chemicals (Hecht, 1994). Thus, a lower level of CYP2E1 activity would be expected to reduce susceptibility to such chemical-induced carcinogenicity, as occurs in animals (Hecht, 1994). Such modulation is also the basis for an anticancer chemoprevention strategy (Hecht, 1984). It is noteworthy, therefore, that the lung cancer rate in male Japanese smokers is strikingly lower than that in European-American men (Japanese Statistics and Information Department, Vital Statistics, Vol. 1. Japanese Ministry of Health and Welfare, Tokyo, 1989).


Send reprint requests to: Dr. G. R. Wilkinson, Department of Pharmacology, Vanderbilt University, Nashville, TN 37232-6600.