Genetic and Dietary Predictors of CYP2E1 Activity: A Phenotyping Study in Hawaii Japanese Using Chlorzoxazone

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Abstract
Cytochrome P4502E1 (CYP2E1) is considered to play an important role in the metabolic activation of procarcinogens such as N-nitrosamines and low molecular weight organic compounds. An RsaI polymorphism is present in the 5′-flanking region of the CYP2E1 gene, which could possibly affect its transcription. However, the relationship between genotype and the phenotypic catalytic activity of the enzyme has not been defined. Also, the effects in humans of specific dietary factors, other than ethanol, which have been shown in animal and in vitro studies to modulate CYP2E1 activity, are unknown. Accordingly, the CYP2E1-mediated metabolism of chlorzoxazone to its 6-hydroxy metabolite was investigated in 50 healthy Japanese of both sexes in Hawaii. The oral clearance of the in vivo probe, the trait measure of CYP2E1 activity, was smaller than that reported in European-Americans. Significantly, after adjustment for age and sex, the oral clearance of chlorzoxazone decreased with the number of variant c2 alleles, and its mean in the c2/c2 genotype (147 ml/min) was statistically lower (P ≤ 0.05) than that for either the homozygous wild-type (238 ml/min) or the heterozygote (201 ml/min) genotypes. Stepwise multiple regression analysis indicated that body weight was a major contributor to the interindividual variability in the oral clearance of chlorzoxazone, accounting for 43% of the variance. Consumption of lettuce, broccoli, and black tea explained additional components of the variability (7, 5, and 6%, respectively), as did medication use (3%), age (4%), and CYP2E1 genotype (5%). Overall, 73% of the variance could be accounted for by these variables. Body weight, lettuce, and use of medications were associated with increased CYP2E1 activity, and the other covariates were associated with reduced enzyme function. Because of the role that CYP2E1 plays in procarcinogen activation, especially of N-nitrosamines involved in lung cancer, the identified factors may account in part for observed differences in individual susceptibility to disease and may also have implications for cancer prevention.

Introduction
Among the various human cytochrome P450s, CYP2E13 is of particular interest because of its involvement in the metabolic activation of many carcinogenic and toxic chemicals, such as environmental N-nitrosamines present in tobacco smoke and the diet, which are considered to play a significant role in the induction of certain types of cancer (1–3), butadiene, vinyl chloride, benzene and many of its derivatives, and halocarbons (4, 5). CYP2E1 is also involved in the oxidation of ethanol (6), as well as the production of reactive oxygen species that may initiate lipid peroxidation (5).

Considerable interindividual variability in human CYP2E1 activity has been observed both in vitro using liver microsomes (7, 8) and, to a somewhat lesser extent, in vivo based on the 6-hydroxylation of chlorzoxazone as a probe (9, 10). Both pathophysiological determinants, such as obesity (11), fasting (11), liver dysfunction (12), and environmental factors including induction by ethanol (10) and inhibition by drugs, such as disulfiram (13) and chlormethiazole (14, 15), or by dietary isothiocyanates (16), have been shown to modulate CYP2E1 activity and contribute to this variance. In addition, genetic factors may also be involved because RFLPs exist in the 5′-flanking region (RsaI) and in intron 6 (DraI) of the CYP2E1 gene (17). Determination of putative relationships between these partially linked variant alleles and the resulting phenotype have, however, been largely limited both in vitro (18, 19) and in vivo (9, 20, 21) to Caucasian subjects, where the rarity of the variant alleles makes studies of sufficient statistical power difficult. Nevertheless, there is suggestive evidence that the variant c2 allele of the RsaI polymorphism may be associated with reduced CYP2E1 activity (18–21), despite the fact that in vitro this allele was found to increase expression of a reporter gene construct (22, 23).

The importance of variability in CYP2E1 activity lies in its possible relationship to individual susceptibility to the carcinogenic and/or toxic effect of its substrates following activation by the enzyme. For example, the lower CYP2E1 level in Japanese men (21) could be a contributing factor to the reduced risk of lung cancer in this population compared with Caucasians with a similar smoking history (24, 25). Similarly, it may be a factor in explaining the lower lung cancer risk in individuals carrying the RsaI or DraI variant allele compared with those with a wild-type genotype, observed among Japanese, Hawaiians, and African-Americans (26–29).

To further explore the genotype:phenotype relationship for CYP2E1, a study was undertaken in healthy men and women of...
pure Japanese ancestry residing in Hawaii. In addition, possible relationships between CYP2E1 activity and certain dietary constituents were also investigated.

Materials and Methods

The clinical protocol used in this study was reviewed and approved by the Committee on Human Studies of the University of Hawaii and by the Institutional Review Board of the Kapiolani Health Research Institute. Written informed consent was also obtained from all participants.

About half of the 50 subjects studied were identified from individuals who had participated as controls in case-control studies of lung and colorectal cancers in Hawaii (29, 30). The remainder were separately recruited through newspaper advertisement. Potential subjects were genotyped for the CYP2E1 Rsal polymorphism to recruit an approximately equal number of individuals of each genotype (c1/c1, c1/c2, and c2/c2). All subjects were of pure Japanese ancestry (i.e., with four Japanese grandparents) and healthy on the basis of medical history and routine laboratory tests including those indicative of renal and hepatic function. None of the individuals smoked tobacco products or regularly drank ethanol, and the female subjects were not pregnant. Subjects were instructed to abstain from ethanol, acetaminophen, and any other nonessential medications during the week prior to the phenotyping and also to keep a record of all foods, medications, and vitamin supplements taken during the last 3 of the 7 prestudy days.

After an overnight fast, 250 mg of chlorzoxazone (PAR Pharmacy, New York, NY) was administered p.o., and fasting was maintained for an additional 3 h. Venous blood samples were obtained using heparin as an anticoagulant from an arm vein at 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h after drug administration. Plasma was separated and stored at −80°C until analysis. Urine was also collected during the 0–4- and 4–8-h periods and stored at −80°C. Chlorzoxazone and its 6-hydroxy metabolite in plasma and urine were determined by a reverse-phase HPLC procedure, subsequent to appropriate hydrolysis of conjugated metabolite, as described previously (11). These assays were done blinded as to the subject’s genotype. Quality control measurements, during the period that the samples were analyzed, indicated an interday coefficient of variation <12% for both chlorzoxazone and its 6-hydroxy metabolite at a concentration of 1 μg/ml. The area under the plasma concentration-time curve for each compound was determined trapezoidally and extrapolated to infinity using a log-linear estimation of the terminal rate constant. The oral clearance of chlorzoxazone was determined from the ratio of the administered dose to the area under the curve of the drug.

Subjects were genotyped for the CYP2E1 Rsal polymorphism after isolation of DNA from peripheral lymphocytes by using the primers 5′-TTCTACTGTCTTCACACTGG-3′ and 5′-CCAGTGTGATCTTATGTCA-3′ to PCR amplify a region containing a base substitution in the 5′ flanking region of the gene (9, 29). Cycling conditions included an initial denaturation at 94°C for 4 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, and final annealing and extension at 55°C for 1 min and 72°C for 7 min. Rsal digestion of the PCR products and electrophoresis on an agarose gel identified the homozygous wild-type genotype (c1/c1; 360- and 50-bp fragments), homozygous variant genotype (c2/c2; single 410-bp fragment), and heterozygous c1/c2 genotype (all three fragments).

Dietary intakes, averaged over the 3 days of record, were used to investigate possible effects of diet on the oral clearance of chlorzoxazone. The food composition data used to compute nutrient intake were primarily based on the United States Department of Agriculture’s nutrient database (31) and were supplemented with data from other research and commercial publications. Nutrient intakes were adjusted for calories by the method of residuals (32). These food intake and nutrient values, along with the pharmacokinetic parameters of chlorzoxazone, were log-transformed for their distributions to approximate normality.

Analysis of covariance was used to compare means of continuous variables among the CYP2E1 genotypes, adjusting for covariates. The unadjusted results comparing oral clearance across genotype were confirmed by a nonparametric method (Mann-Whitney U test). A forward stepwise procedure was used to determine the importance of each variable in explaining the variance in the oral clearance of chlorzoxazone after controlling for other covariates. A variable was retained in the model when the P for the association of the variable with the independent variable was <0.05. The explanatory variables selected for potential inclusion in the regression were those shown previously to affect levels of CYP2E1 or other xenobiotic-metabolizing enzymes and/or to act as confounders of an association of CYP2E1-mediated activation with cancer. Genotype was parameterized by two dummy variables representing the heterozygous and homozygous variant genotypes. The procedure was repeated with genotype entered as number of variant alleles to test for a genetic trend. The model assumptions were met, as verified by residual analysis. Pearson’s correlation coefficients were computed to investigate the relationships between the oral clearance and continuous variables of chlorzoxazone, such as age and weight.

Results

Complete phenotyping data were obtained in all 50 subjects (33 men and 17 women) ranging in age from 20 to 78 years and weighing 46 to 87 kg. Urine collection in one individual was incomplete, and he was excluded from analyses involving the urinary excretion of 6-hydroxychlorzoxazone. The oral clearance of chlorzoxazone ranged from 77 ml/min to 850 ml/min. The geometric mean oral clearance of chlorzoxazone was modestly higher in men (218 ml/min) than in women (179 ml/min). However, this difference was not statistically significant (P = 0.17) and was not present after normalizing for body weight (3.4 ml/min/kg versus 3.3 ml/min/kg, P = 0.97). Accordingly, the data from men and women were combined in subsequent analyses. Oral clearance was found to be correlated with weight (r = 0.66, P < 0.001), cruciferous vegetable intake (r = 0.45, P = 0.01), and to a lesser extent, age (r = −0.27, P = 0.06). No statistically significant differences were found across genotypes for age, body size, total calories, and intakes of fat, ethanol, total vegetables, soy products, total fruits, and a number of other nutrients and foods (Table 1). On the basis of their relatively high fat intake (~60 g/day) and low soy intake (~7 g/day), the diet of the Japanese subjects could clearly be characterized as “westernized.”

Significantly higher chlorzoxazone plasma concentrations were observed in the c2/c2 group compared with that of the c1/c1 genotype after oral administration (Fig. 1). The initial plasma levels were also greater in heterozygotes than homozygous c1/c1 subjects, but concentrations were similar in the postabsorptive phase. By contrast, the plasma concentration-
time profiles of 6-hydroxychlorzoxazone were similar to all three genotypes (data not shown), as were the 0–8-h urinary recoveries of the metabolite (44.7% for c1/c1, 37.4% for c1/c2, and 42.3% for c2/c2). Accordingly, the geometric mean oral clearance showed intergenotypic differences (Table 2), being greater for the homozygous wild-type genotype (c1/c1) and decreasing with the number of variant c2 alleles. Although only a small number of women were studied, these differences were similar when the data were stratified by sex. The exclusion of one c1/c1 individual with a high oral clearance value, or subjects who reported using alcohol, acetaminophen, other medication or nutritional supplements during the 3 days preceding phentypotyping with chlorzoxazone, did not materially change the findings (Table 3).

By far, the largest contributor to the variance in the oral clearance of chlorzoxazone was body weight, which entered the stepwise regression first and explained 43% of the variance, with increasing size being associated with greater enzyme activity (Table 4). Lettuce, broccoli, black tea, use of medications other than acetaminophen, age, and CYP2E1 genotype explained an additional 7, 5, 6, 3, 4, and 5% of the variance in the oral clearance of chlorzoxazone, respectively. Collectively, the selected variables explained 73% of the variation in the oral clearance of chlorzoxazone, with weight, lettuce consumption, and use of medication being associated with increased chlorzoxazone 6-hydroxylation, whereas the other factors were linked to reduced metabolism. The limited sample size did not permit a detailed analysis of the contributions of specific medications. Exclusion of the possible c1/c1 outlier did not substantially affect the stepwise regression, with genotype entering the model at the 0.09 significance level.

**Discussion**

The disposition of chlorzoxazone in healthy subjects has been well described (9, 10, 21, 33), as have factors affecting its metabolism (10–15, 34). Available evidence in humans indicates that in vivo the 6-hydroxylation of the drug is predominantly, if not exclusively, mediated by CYP2E1, and that this pathway of metabolism is the major determinant of the clearance of the drug after oral administration (9). Accordingly, observed differences in the oral clearance of chlorzoxazone reflect overall CYP2E1 activity within the body. On the basis of this in vivo probe approach, the interindividual variability of CYP2E1 in young, medication-free, healthy Caucasians has been shown to be unimodally distributed with a 4- to 5-fold range in activity (9). The present findings in healthy subjects of Japanese ancestry residing in Hawaii are generally consistent with this magnitude of variability. Moreover, the clearance of the in vivo probe in these subjects was lower than that reported in Caucasians (9), which is similar to an earlier finding that the oral clearance of chlorzoxazone was 30–40% lower in Japanese men residing in Tokyo than in European-Americans, even after taking into account differences in body size (21). Because environmental factors are quite distinct between Japan and Hawaii, and in particular, subjects in the present study consumed a “western” diet, this suggests that this difference between the two ethnic groups probably has a genetic basis.

It was also of interest that the ~25% lower oral clearance of chlorzoxazone found for Japanese women compared with men was of similar magnitude as to the sex difference observed previously in Caucasians (9, 11). However, in our data, this difference was explained by body weight. Indeed, this factor was identified as a major predictor of the oral clearance of chlorzoxazone, supporting the notion that CYP2E1 activity is regulated in some fashion by body mass. This association could simply reflect a relationship with liver size (35); alternatively, it may be indicative of a more fundamental mechanism involving the regulation of CYP2E1 by nutritional and physiological factors (5). In this regard, it is noteworthy that dietary fat intake and its composition, despite being inducers of CYP2E1 in animals (36, 37), were not identified as statistically significant covariates in the regression analysis.

The identification of RFLPs in the CYP2E1 gene (17) has resulted in considerable speculation as to their possible functional significance and possible involvement in individual susceptibility to disease including lung cancer. Efforts to address such issues have been complicated by the fact that the frequencies of these polymorphisms vary substantially according to the racial background of the population. In the case of the RsaI point mutation in the 5’-flanking region, the c2 allelic variant is relative rare (2–8%) in Caucasian and African-American populations (38, 39). As a result, association studies of this polymorphic locus in these groups have had low statistical power (27, 40–44). The c2 mutation is more prevalent (24%) in Japanese and Taiwanese (38, 39), and investigations in these populations have detected significant associations, with lung cancer for example (26, 29).

Similar power considerations also apply to attempts to relate genotype to phenotype on the basis of the 6-hydroxylation of chlorzoxazone. For example, in vitro studies of CYP2E1 activity in human liver microsomes (18, 19) have collectively included samples from 134 wild-type homozygotes, 16 heterozygotes, and only 1 homozygous variant individual. In general, previous differences were identified between c1/c1 and c1/c2 microsomal activity; however, the single c2/c2 sample had a V_max of ~50% that of all of the microsomes studied, whereas the K_M exhibited no intergenotypic differences (19). In vivo studies in uninduced individuals (9, 20, 21) have had a similar genotypic frequency distributions (154 c1/c1, 8 c1/c2, and 1 c2/c2) but are, in general, consistent with the in vitro findings, i.e., no statistical difference was found between homozygous wild-type and heterozygote groups, although the suggestion of lower activity was present for the latter; again, a substantially reduced chlorzoxazone 6-hydroxylation was observed in the single c2/c2 individual (20). Additionally, patients with the c2 allele appeared to have less induction of CYP2E1 than wild-type individuals after ethanol administration (20).

The present study in Hawaii Japanese was designed to overcome the above sampling problems by specifically recruit-
ing individuals of known genotype so that all three genotypic groups would be adequately represented. Although no significant difference was observed in the oral clearance of chlorzoxazone between homozygous wild-type and heterozygous groups, a highly significant lower value was found in the homozygous variant group. Moreover, the stepwise regression analysis showed a statistically significant inverse relationship between CYP2E1 activity and the number of variant RsaI alleles \( (P < 0.02) \), after adjusting for the other covariates. However, it should be noted that the magnitude of the difference between the two homozygous groups was modest \((<2\text{-fold})\). Nevertheless, a number of studies have suggested that the risk of developing cancers of the lung, liver, and esophagus, for example, is reduced in individuals who have a \( c2 \) allele \((27, 29, 41, 43–45)\), consistent with a reduced level of procarcinogen activation by CYP2E1. Other studies, however, have not found such an association \((40, 46, 47)\), which probably reflects power limitations and possibly the fact that other contributors of CYP2E1 variability were not adjusted for in these studies. Also, it should be noted that the CYP2E1 genotype:phenotype relationship determined in human hepatic tissue or in vivo is contrary to that suggested by in vitro 5'-flanking region deletion studies \((22, 23)\). Such gene experiments have suggested that the \( c2 \) allele is associated with increased tran-

![Fig. 1. Mean plasma concentration-time curves of chlorzoxazone after an oral 250-mg dose according to genotype.](image)

| Table 2 | Geometric mean\(^a\) (and 95% confidence interval) for CYP2E1 activity as assessed by the oral clearance (ml/min) of chlorzoxazone according to the CYP2E1 RsaI genotype |
|---|---|---|---|---|---|---|---|
| | c1/c1 | c1/c2 | c2/c2 | \( p^b \) |
| All | 238 (193–293) | 201 (166–243) | 147 (115–188) | 0.004 | 0.24 | 0.05 |
| Male | 234 (186–296) | 210 (163–271) | 171 (127–231) | 0.07 | 0.32 | 0.36 |
| Female | 227 (160–321) | 195 (156–244) | 118 (85–164) | 0.03 | 0.49 | 0.03 |

\(^a\) Adjusted for age, and sex when appropriate, by multiple covariance analysis. See Table 1 for numbers of subjects.

\(^b\) \( P \)s for differences between genotypes \((1, c1/c1; 2, c1/c2; 3, c2/c2)\).

| Table 3 | Geometric mean\(^a\) for oral clearance of chlorzoxazone (CL; ml/min) after exclusion of a possible “outlier” or subjects who used alcohol, medications, or supplements during the previous 3 days |
|---|---|---|---|---|---|---|
| Exclusions | n\(^b\) | c1/c1 | c1/c2 | c2/c2 | \( P^c \) |
| Possible “outlier” | 17 | 223.9 | 19 | 201.1 | 13 | 147.6 | 0.008 | 0.42 | 0.04 |
| Alcohol | 16 | 220.6 | 18 | 199.3 | 12 | 155.0 | 0.04 | 0.49 | 0.12 |
| Acetaminophen | 16 | 221.7 | 18 | 199.1 | 13 | 146.4 | 0.01 | 0.47 | 0.06 |
| Any medications | 9 | 221.7 | 8 | 199.1 | 8 | 146.4 | 0.01 | 0.47 | 0.06 |
| Supplements | 6 | 276.8 | 6 | 276.6 | 5 | 120.8 | 0.03 | 0.99 | 0.03 |

\(^a\) Adjusted for age and sex by multiple covariance analysis.

\(^b\) Number of subjects included.

\(^c\) \( P \)s for differences between genotypes \((1, c1/c1; 2, c1/c2; 3, c2/c2)\).
had not consumed any significant amount of alcohol during the 7 days prior to phenotyping, a period of time sufficient to remove any ethanol induction of CYP2E1 (10, 20).

Because of the relatively large number of environmental variables considered in our analysis, some of the statistically significant associations may have occurred by chance. Consequently, and also because of the cross-sectional design, the observed dietary relationships require confirmation prospectively, preferably by intervention studies. Finally, it should be emphasized that, although the overall genotypic distribution of the study subjects was purposely different from that in the general population, the dietary findings should be generalizable to the general population because similar relationships were observed when the analyses were stratified according to genotype.

In summary, this is the first study to demonstrate a genetic component in the interindividual variability in CYP2E1 activity in humans. Additionally, the findings have identified body weight as an important determinant of the variance and suggest that certain dietary constituents may up- or down-regulate the activity of the enzyme. Because of the role that CYP2E1 is considered to play in the activation of procarcinogens, especially N-nitrosamines involved in lung cancer, such factors may account in part for differences in individual susceptibility to disease. Furthermore, the dietary findings may have implications for cancer prevention.

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References


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