0145-6008/99/2301 2-1868$03.00/0
ALCOHOLISM: CLINICAL AND EXPERIMENTAL RESEARCH

Vol. 23, No. 12
December 1999

CYP2E1 Expression in Human Lymphocytes From Various Ethnic Populations

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Background: Monitoring CYP2E1 levels in alcoholic individuals holds inherent appeal because such determinations might indicate individuals at increased risk for alcoholic liver disease. We previously demonstrated that lymphocyte CYP2E1 expression reflects in vivo activity of the hepatic enzyme.

Methods: To further validate this approach, the current investigation compared lymphocyte CYP2E1 content and chlorzoxazone pharmacokinetics in 51 alcoholic and nonalcoholic White, Navajo, and Mexican American subjects. After an oral dose of chlorzoxazone, blood samples were collected and lymphocytes isolated.

Results: Alcoholics exhibited a 2-fold elevation in lymphocyte CYP2E1 messenger ribonucleic acid (mRNA) and protein compared to nonalcoholics. Chlorzoxazone clearance rates were 1.9-fold higher and area under the concentration curve (AUC) values 1.8-fold lower in alcoholic individuals compared to nonalcoholics. Furthermore, chlorzoxazone clearance rates correlated ($r = 0.55, p < 0.01, n = 38$) with lymphocyte CYP2E1 mRNA content, and transcript levels further correlated ($r = 0.52, p < 0.001, n = 38$) with CYP2E1 protein content. To compare phenotype with genotype, restriction fragment length polymorphism analyses on deoxyribonucleic acid samples were performed to identify polymorphisms in the CYP2E1 gene. No subjects were homozygous for rare alleles c2 or C. Nonetheless, 27% of the Navajos and 15% of the Mexican Americans were heterozygous for the c2 allele. Two White subjects appeared heterozygous (c1/c2) when *Rsa*I was used to characterize CYP2E1 genotype but homozygous (c1/c1) at the *Pst*I locus. Fifteen percent of Mexican American subjects, 20% of Navajo subjects, and 6% of White subjects were heterozygous for the C allele. Neither CD nor c1/c2 genotypes were associated with alcoholism.

Conclusions: Human lymphocyte CYP2E1 mRNA levels may be useful predictors of alcohol-mediated alterations in hepatic CYP2E1 activity. Moreover, ethnicity does not appear to play a major role in the levels of expression of lymphocyte CYP2E1.

Key Words: CYP2E1, Chlorzoxazone, Ethnicity, Genotype, Lymphocyte.

CYP2E1, THE MAJOR ethanol-inducible P-450, has received much attention because of its potential toxicological role. This enzyme is important in the activation of many hepatotoxins such as acetaminophen or hepatocarcinogens such as nitrosamines. It is also involved in the generation of reactive oxygen species leading to oxidative stress. Thus, it follows that individuals with increased CYP2E1-mediated activity would be at greater risk from adverse effects due to enhanced activation of toxins. Although greater enzymatic activity is generally associated with higher hepatic CYP2E1 protein concentrations, the cause for elevated levels of this P-450 is complex. Both chemically mediated induction of CYP2E1 and a genetic component lead to large variations in hepatic CYP2E1 expression. With regard to variation in CYP2E1 activity due to genetic factors, recent reports describe restriction fragment length polymorphisms (RFLPs) for this P-450 (Nedelcheva et al., 1996). One RFLP is defined with either *Pst*I or *Rsa*I restriction sites and is located in the transcription regulation region of CYP2E1 (Hayashi et al., 1991). This polymorphism is associated with altered gene expression. The wild-type or more common allele, designated c1, possesses a lower level of expression compared to the mutant allele (c2) when tested in vitro. The frequency of the rare c2/c2 genotype is about 4% in White subjects and 20% in Japanese subjects. A second polymorphism (designated C) located in intron 6 was revealed by using the *Dra*I restriction enzyme, and about 10% of Whites and 25% of Japanese are homozygous for this variant (Uematsu et al., 1991). Unlike the c2 mutation, a direct relationship of the C allele to CYP2E1 expression and activity has not been established (Ingelman-Sundberg et al.,1993; Nedelcheva et

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Received for publication February 11, 1999; accepted September 21, 1999.

This work was supported by Department of Health and Human Services Grants AA08990 (J.L.R.) and NCRR-GCRC RR00997 (Clinical Research Center, University of New Mexico).

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al., 1996). However, both polymorphisms are associated with various forms of cancer and chemically mediated hepatotoxicities (Uematsu et al., 1991; Wu et al., 1997, 1998). Furthermore, it is hypothesized that these may play a role in altering the inducibility of this P-450, thereby enhancing susceptibility of certain subpopulations to CYP2E1-related diseases, such as hepatocarcinogenesis or alcoholic liver disease (ALD). Heightened susceptibility to ALD has been described for one particular ethnic subpopulation, namely Native Americans (National Institute on Alcohol Abuse and Alcoholism, 1993), which could be caused by elevated constitutive expression of CYP2E1 or by greater enzyme induction by ethanol.

To clarify the toxicological role of this enzyme in particular diseases, phenotyping and genotyping assays have been developed over the last few years to study determinants of CYP2E1 activity in humans. CYP2E1 induction is primarily assessed by monitoring in vivo metabolism of the drug chlorzoxazone (CZX) (Bachmann and Sarver, 1996; Dreisbach et al., 1995; Lucas et al., 1993), whereas direct quantitation of the enzyme requires liver biopsy specimens. A recent report (Raucy et al., 1997) suggested that expression of the enzyme in peripheral blood lymphocytes (PBLs) can reflect changes in the content of hepatic CYP2E1. Thus, PBL CYP2E1 levels may serve as a surrogate to liver biopsies for assessing expression of the hepatic enzyme. Animal studies further substantiate results obtained in humans by demonstrating that starvation of rats or ethanol administration to rabbits increases the expression of both PBL and hepatic CYP2E1 simultaneously (Raucy et al., 1995; Soh et al., 1996). In the present study, in vivo CZX metabolism was compared to PBL expression of CYP2E1 protein and its corresponding messenger ribonucleic acid (mRNA) in alcohol abusers and nonabusers. In addition, ethnic variability in expression of PBL CYP2E1 and CZX metabolism was assessed in three separate populations including Whites, Navajos, and Mexican Americans. Comparisons were also made between the three ethnic groups regarding ethanol-mediated induction of PBL CYP2E1 and its corresponding in vivo activity. Finally, the occurrence of two rare alleles, C and c2, was determined, and these genotypes were compared to PBL CYP2E1 expression and inducibility by ethanol.

METHODS

Volunteers

Fifty-one subjects, 30 males and 21 females, ranging in age from 20 to 51 years participated in this investigation. Approval for studies involving human subjects was granted by the University of New Mexico Research Review Committee (HRRC 90–213). Volunteers, 26 alcoholic and 25 control participants, were within 20% of ideal body weight and were screened for participation as previously described (Raucy et al., 1997). Individuals with diabetes or obesity, those receiving prescription medications, or those diagnosed with a substance abuse codependency were eliminated from the study. At the time of recruitment, informed consent was obtained, questionnaires were completed, and blood samples were drawn for routine blood evaluations (Reference Laboratory, Inc., Albuquerque, NM). Based on a physician's evaluation of laboratory results, subjects who demonstrated abnormal liver function values or those diagnosed with hepatic disease were discharged. Volunteer subjects were admitted to the University of New Mexico Clinical Research Center where they received thorough physical examinations. All alcohol-abusing individuals had been recently drinking, with BACs >100 mg/dl. Subjects remained overnight in the Clinical Research Center to prevent ethanol abusers access to alcoholic beverages and to allow blood alcohol concentrations (BACs) to return to zero. A BAC of <5 mg/dl was needed to ensure that competitive inhibition of CZX metabolism did not occur. During the overnight stay, volunteers were placed on caffeine-free diets, and alcoholics were carefully monitored for any signs of withdrawal. At 0730 hr, a blood sample was obtained to determine BACs; all subjects had values <5 mg/dl. Blood (320 ml) was drawn at 0800 hr for lymphocyte isolation from all but 13 subjects; in these cases, the large sample required was unobtainable by venous puncture. After the blood draw, 500 mg of CZX was orally administered. Blood samples (10 ml) were subsequently taken at 0, 0.25, 0.50, 0.75, 1.0, 1.5, 2, 3, 4, 5, 6, 8, 10, and 12 hr. Plasma was separated by centrifugation at 2300 rpm for 15 min at 4°C and immediately frozen and stored at −20°C until assayed.

Clinical Evaluations

Alcohol consumption habits were evaluated by using two types of questionnaires as previously described (Raucy et al., 1997). First, subjects completed a survey developed by our laboratory containing questions adapted from CAGE (cut, annoyed, guilty, and eye opener), a common screening instrument for identifying controls (Ewing, 1984; Mayfield et al., 1974). Second, a more detailed personal interview was employed to characterize alcohol consumption patterns in both control and alcoholic individuals. This profile incorporates physiological, psychological, and social components and was determined by posing questions compiled in the National Longitudinal Alcohol Epidemiological Survey (NLAES). The NLAES is a diagnostic questionnaire similar to the *Diagnostic and Statistical Manual of Mental Disorders,* fourth edition (DSM-IV), but was primarily developed for epidemiological research (Grant and Hasin, 1990). Both surveys, NLAES and that adapted from CAGE, were subjectively scored using a protocol developed by an epidemiologist in our laboratory and as described and validated previously (Raucy et al., 1997).

Lymphocyte Isolation and Microsome Preparation

Lymphocytes were immediately separated from 320 ml whole blood (Raucy et al., 1997) and a portion resuspended in 5.0 ml of homogenizing buffer (0.1 M Tris-HCl, pH 7.4) containing 0.5 mM phenylmethylsulfonyl fluoride (PMSF, Sigma Chemical Co., St. Louis, MO) for microsome preparation. A separate aliquot of isolated white cells was mixed with Trizol™ (Gibco BRL Products, Gaithersburg, MD) for RNA isolation. Samples were then frozen in liquid nitrogen and stored at −70°C until use. Microsomes were isolated from thawed white cells by previously published procedures (Raucy et al., 1997). Briefly, lymphocytes were sonicated on ice for 3 × 60 sec to disrupt cells. Microsomes were subsequently prepared and pellets resuspended in sucrose assay buffer (Raucy and Lasker, 1991). Lymphocyte microsomes were stored at −70°C until use. Protein values were determined by the bicinchoninic acid procedure with bovine serum albumin as a standard (Carpenter et al., 1996).

Immunoblot Analysis

Microsomal proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrophoretically transferred to nitrocellulose filters. Filters were subsequently blocked in 5% nonfat dry milk/tris-buffered saline with Tween-20 (TBST) (20 mM Tris buffer, pH 7.6, 137 mM NaCl, and 0.1% Tween-20) for 1 hr at 37°C and allowed to react overnight at 4°C in 5% milk/TBST containing a previously characterized antihuman CYP2E1 immunoglobulin G (IgG; 5 μg/ml) (Raucy et al., 1997). Filters were then incubated for 60 min with biotinylated goat anti-rabbit IgG (Calbiochem, La Jolla, CA) (1:2000 in TBST), followed by

a 60 min incubation with streptavidin-conjugated horseradish peroxidase (Calbiochem, La Jolla, CA) (1:2000 in TBST) at room temperature. Immunochemical staining was performed by reacting the filters with 10 ml of enhanced chemiluminescence (ECL) detection reagents (Amersham, Arlington Heights, IL) for 1 min at room temperature and exposure to Amersham Hyperfilm for 10 to 30 sec. Immunoreactive CYP2E1 content in lymphocyte microsomes was quantified with a Microtek Scanmaker IIHR scanner interfaced to ImageQuant software. Various amounts (5 to 75 μg) of lymphocyte microsomal protein were applied to the gel to determine the linear range of signal intensity of immunoblots. The concentration of microsomal protein (25 μg) used for all samples and subsequent immunoblot analyses was within the linear portion of that curve.

Quantitative Reverse Transcriptase Polymerase Chain Reaction

Total RNA from lymphocytes was isolated using Trizol® reagent (Gibco BRL Products, Gaithersburg, MD) and quantified by measuring its absorbance at 260 nm; purity was assessed by determining the 260/280 nm ratio and by agarose gel electrophoresis. To facilitate quantification of CYP2E1 mRNA by polymerase chain reaction (PCR), we constructed a "standard" plasmid as previously described (Jung et al., 1995). Briefly, primer pairs were designed that amplified a 579 base pair (bp) fragment spanning exons 4 and 6 of CYP2E1. Additional primers to the same region of CYP2E1 were then modified to amplify a region of deoxyribonucleic acid (DNA) that contained a section of mouse Cα-constant complementary DNA (cDNA) and the CYP2E1 primer sites as well as *Hin*dIII and *Xba*I restriction sequences for cloning. The cDNA amplified by these primers was cloned into a modified pBluescript II SK (−) vector, pBAT-NOT, that contains a T7 promoter site. During in vitro transcription, RNA molecules were synthesized by run-off transcription from the DNA template and quantitated by measuring absorbance at 260 nm. Serial dilutions of the transcribed RNA ranging from 10 to 10^5 molecules were prepared for the reverse transcriptase PCR (RT-PCR). Reverse transcription of lymphocyte RNA was performed as previously described (Raucy et al., 1997) with the following modification. A constant amount of cellular target RNA was mixed with varying numbers (10 to 10^5) of standard mRNA transcripts and the DNA was amplified. PCR products were visualized in 1% agarose gels, and the ratios of the band intensities of the PCR products from the standard mRNA and target mRNA were plotted against the starting number of standard mRNA molecules by using a double logarithmic scale (Jung et al., 1995).

Analytical Methods

CZX and 6-hydroxychlorzoxazone (6-OH-CZX) concentrations in plasma were determined by a reverse-phase high-performance liquid chromatography (HPLC) based assay as previously described by our laboratory (Raucy et al., 1997). Briefly, plasma samples (250 μl) were subjected to 20 μl (200 activity units) of *Helix pomatia* type H-2 β-glucuronidase (Sigma Chemical Co., St. Louis, MO) and incubated overnight at 37°C. After incubation, 200 μl of 0.2 M theophylline was added to each tube for use as an internal standard, and the plasma was extracted with 2 ml of ethyl acetate. The organic layer was removed and the aqueous phase subjected to a second ethyl acetate extraction. The extracts were pooled and evaporated to dryness under nitrogen with low heat. The residues were resuspended in 100 μl of mobile phase [20% acetonitrile (v/v) and 80% of 0.5% phosphoric acid (v/v)] and 20 μl aliquot of extract was subsequently analyzed by use of a model 504 Autosampler (Beckman Instruments, Palo Alto, CA), a model 600 solvent delivery system (Waters Millipore, Milford MA), and a 486 detector (Waters Millipore, Milford, MA) set at 287 nm. Chromatographic separation was achieved at a flow rate of 1 ml/min with a 5 μm C8 Ultrasphere 4.6 × 250 mm column (Beckman Instruments, Palo Alto, CA).

Standard curves of area ratios for CZX/theophylline and 6-OH-CZX/theophylline were constructed from plasma containing various amounts, 0.20 to 8.0 μg/ml, of CZX (Sigma Chemical Co., St. Louis, MO) and 6-OH-CZX (McNeal Consumer Products, Fort Washington, PA). Recoveries of the 6-OH-CZX and CZX were 77 and 70%, respectively, by use of this extraction method. Values for each sample were extrapolated from linear calibration curves (r = 0.99) of the standards. The interday coefficient of variation (n = 51), determined by adding CZX and 6-OH-CZX to a plasma sample, was approximately 15% for each at concentrations of 2, 4, and 8 μg/ml. The limits of detection for both CZX and 6-OH-CZX were 0.09 ± 0.01 μg/ml.

Pharmacokinetic Analysis

The area under the plasma concentration-time curves (AUC) for CZX and its metabolite were determined using the trapezoid rule. Noncompartmental analysis was used to compare the oral clearance of CZX in control and alcoholic subjects and was determined from the ratio of the administered dose to the total AUC.

CYP2E1 Genotyping

DNA was isolated from lymphocytes using Trizol™ reagent and quantified by measuring its absorbance at 260 nm. Oligonucleotide primers were synthesized to regions of CYP2E1 DNA that flanked the polymorphic regions characterized by either the *Dra*I (CD loci) or the *Pst*I/*Rsa*I (c1/c2 loci) restriction enzymes. The genotypes of CYP2E1 were identified as previously described by restriction enzyme digestion with *Rsa*I and *Pst*I or *Dra*I (Hayashi et al., 1991; Uematsu et al., 1991). Briefly, samples of DNA (1 μg) were added to PCR reaction mixtures and amplification was performed by 25 thermal cycles under the following conditions: 1 min at 95°C, 1 min at 55°C, and 1 min at 72°C. The PCR-amplified DNA fragments were digested with *Rsa*I, *Pst*I, or *Dra*I and subjected to electrophoresis on 2% agarose gels.

Statistical Analysis

Three separate statistical tests were employed to determine significance or whether any correlations existed between study populations. Group comparisons were performed using two-tailed unpaired t test. A one-way ANOVA or Games/Howell post hoc test was used to determine statistical significance between more than two distributions or sample groups. Correlation analyses were also performed, and a curve fit determined the association of the two fields. Statistical calculations were made using Statview Version 5.0 software. Statistical significance was set at $p < 0.05$. Values were expressed as mean ± SE.

RESULTS

Study Subjects

Volunteers from three different ethnic groups were recruited: 16 White individuals, 15 Native Americans of Navajo descent, and 20 Mexican Americans. Subjects ranged in age from 20 to 51 years, with a mean age of 32.8 ± 1.2 years. Of the 51 subjects involved in this study, 30 were men and 21 were women. Participants were divided into two groups: 25 controls (11 men and 14 women) and 26 alcoholics (19 men and 7 women). Ethanol consumption habits were based on subjective scores from a screening survey as previously described (Raucy et al., 1997). Survey scores, the sum of raw scores for individual questions in each survey, did not differ based on ethnicity (109 ± 9 for Mexican Americans, 105 ± 20 for Whites, and 116 ± 14 for Navajo subjects). Body heights (men, 176 ± 2.4 cm; women, 161.4 ± 1.9 cm) and weights (men, 85.3 ± 3.5 kg; women 65.4 ± 2.8 kg) were within the normal ranges for healthy individuals and did not vary with ethnicity.

Table 1. Chlorzoxazone Pharmacokinetic Parameters

Subjects	CZX clearance (liters/hr)	AUC CZX (μg/ml)hr
Control	20.6 ± 1.4 (25)	26.9 ± 2.2 (25)
Alcoholic	39.0 ± 3.4 (26)*	14.8 ± 1.0 (26)*
Control		
White	21 ± 3 (9)	28.7 ± 4.4 (9)
Navajo	22.4 ± 2.4 (8)	23.8 ± 2.6 (8)
Mexican American	20.5 ± 2.5 (8)	27.9 ± 4.6 (8)
Alcoholic		
White	39.9 ± 6.8 (7)*	14.0 ± 1.5 (7)*
Navajo	43.3 ± 9.4 (7)*	14.5 ± 2.6 (7)*
Mexican American	36.0 ± 3.7 (12)*	15.5 ± 1.6 (12)*

CZX, chlorzoxazone; AUC, area under concentration curve.
Values represent mean ± SEM.
Numbers in parentheses denote sample size.
* Significant difference from corresponding control subjects, $p < 0.05$.

Chlorzoxazone Pharmacokinetics

Study participants were given a 500 mg single oral dose of CZX to assess CYP2E1-mediated metabolism in vivo, and pharmacokinetic parameters were determined for each volunteer (Table 1). CZX clearance rates were significantly ($p < 0.05$) elevated 1.9-fold in alcoholic subjects compared to controls (Table 1). Moreover, there was a statistically significant ($p < 0.05$) decrease (1.8-fold) in the AUC for CZX in alcohol abusers compared to nonalcoholic subjects. When CZX pharmacokinetic parameters were examined based on ethnicity, alcohol abuse caused statistically significant ($p < 0.05$) increases of 1.9-, 2.0-, and 1.7-fold in CZX clearance rates in White, Navajo, and Mexican American subjects, respectively. There were no significant differences between alcohol abusing men (37 ± 4 liter/hr) and women (36 ± 3.9 liter/hr). A 1.7- to 2.0-fold decrease in AUC for CZX was also observed among all three ethnic groups (Table 1).

Detection of Lymphocyte CYP2E1

Detection of CYP2E1 protein and mRNA in lymphocytes was performed by immunoblot analysis of isolated microsomes and by quantitative RT-PCR of RNA, respectively (Table 2). For these determinations, fewer samples were analyzed due to the inability to obtain an adequate quantity of blood from certain individuals. When alcoholics were compared to control subjects, a significant 2.0-fold ($p < 0.05$) increase in PBL CYP2E1 was observed, which was similar to the fold increase observed for CZX clearance (Table 1). Furthermore, CYP2E1 mRNA levels were significantly ($p < 0.05$) increased 1.8-fold in alcoholics compared to nonalcoholics. When segregated by ethnicity, alcohol abuse caused a significantly greater, 2.6-fold elevation, of PBL CYP2E1 in Navajo subjects than Mexican American subjects (2.1-fold). An ethanol-mediated elevation in CYP2E1 was also noted in the White population; however, due to the small sample of alcoholics (four), this was not statistically significant (Table 2). CYP2E1 mRNA levels were also compared among ethnic groups and between alcoholic and control subjects. Navajo and Mexican American alcohol abusers exhibited 2.5-fold and 2.1-fold higher levels of CYP2E1 mRNA in their lymphocytes, respectively, than their control counterparts ($p < 0.05$, Table 2). The lack of a significant difference in CYP2E1 mRNA levels between alcoholic and nonalcoholic Whites was in all likelihood due to the small sample size within the alcohol abuser group.

Table 2. CYP2E1 and mRNA Content in Human Peripheral Blood Lymphocytes

Subjects	Lymphocyte CYP2E1 (OD/μg microsomal protein)	Lymphocyte CYP2E1 mRNA (10^4 molecules/μg RNA)
Control	144 ± 18 (21)	$1.2 \times 10^4 \pm 1.3 \times 10^3$ (21)
Alcoholic	291.2 ± 41 (17)*	$2.2 \times 10^4 \pm 3.5 \times 10^3$ (17)*
Control		
White	177 ± 25 (8)	1.3 ± 0.2 (8)
Navajo	156 ± 38 (6)	1.1 ± 0.3 (6)
Mexican American	120 ± 32 (7)	0.9 ± 0.3 (7)
Alcoholic		
White	232 ± 31 (4)	2.4 ± 0.1 (4)
Navajo	398 ± 105 (6)*§	2.7 ± 0.4 (6)*§
Mexican American	251 ± 44 (7)*	1.9 ± 0.1 (7)*

RNA, ribonucleic acid; mRNA, messenger RNA.
Values represent mean ± SEM.
Numbers in parentheses denote sample size.
* Significant difference from control subjects, $p < 0.05$.
§ Significant difference in the extent of induction from that of Mexican Americans.

Correlation Analyses

Comparisons were subsequently determined between PBL CYP2E1 protein content, mRNA levels, and CZX clearance rates. Lymphocyte CYP2E1 microsomal protein levels correlated with CZX clearance rates ($r = 0.54$, $p < 0.01$). Moreover, CYP2E1 mRNA concentrations were significantly correlated with CZX clearance rates ($r = 0.55$, $p < 0.01$). These findings suggest that hepatic CYP2E1 activity in vivo can be monitored by white cell expression of the enzyme or its corresponding mRNA. Lymphocyte CYP2E1 protein and mRNA from subjects in which both parameters were measured ($n = 38$) were also correlated ($r = 0.52, p < 0.001$) (Fig. 1), which suggests that CYP2E1 protein and mRNA in PBLs are coordinately elevated by ethanol.

CYP2E1 Genotyping

To compare phenotype to genotype, we examined the c1/c2 and CD loci. None of the subjects participating in this study were homozygous for either rare mutant allele. However, 14% of the participants were heterozygous for the C or c2 alleles (Fig. 2A and 2B). More control subjects than alcohol abusers exhibited the C (16% vs. 12%) (Fig. 2A) or c2 (16 vs. 12%) alleles, but this was not statistically significant (Fig. 2B). Gender did not play a role in the frequency of the CD genotype (Fig. 2A); however, men (20%) more commonly exhibited the c1/c2 polymorphism than did women (5%) (Fig. 2B). When segregated by ethnicity, 1 of the 16 White subjects (6%) was heterozygous for the C

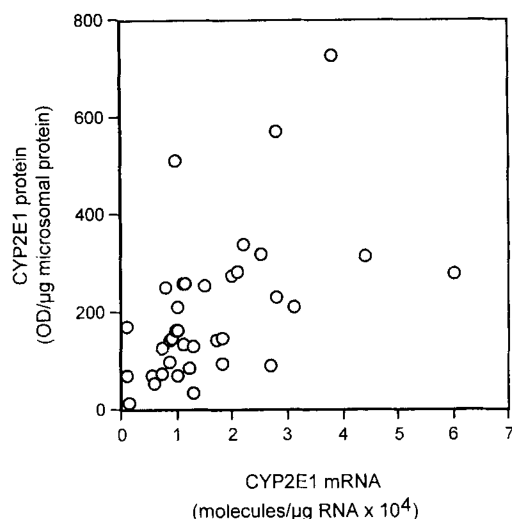

Fig. 1. Scattergram of lymphocyte CYP2E1 mRNA and apoprotein content. Each point on the scattergram represents the lymphocyte content of CYP2E1 mRNA and apoprotein from a single subject. Correlation analysis was performed on CYP2E1 content of lymphocyte microsomes and CYP2E1 mRNA levels for 38 subjects (18 alcoholics and 20 control subjects). A correlation coefficient of $r = 0.52$ ($p < 0.001$) was obtained.

allele, whereas 20% of the Navajo subjects and 15% of the Mexican Americans possessed the C allele. Only Mexican American and Navajo subjects exhibited the c1/c2 genotype with a frequency of 15 and 27%, respectively. However, two female White subjects seemed to be c1/c2 heterozygous when defined by the *Rsa*I restriction enzyme but were homozygous for the c1 allele when genotyped by *Pst*I digestion. The majority of the c1/c2 heterozygotes (71%) were also CD heterozygotes. When genotype was compared to phenotype in control subjects, the c1/c2 heterozygotes did not exhibit significantly higher CZX clearance rates or lower AUC for CZX (Table 3) when compared to the c1/c1 homozygotes. Alcohol-mediated induction of CYP2E1 protein and mRNA in lymphocytes occurred in c1/c2 heterozygotes to a similar extent as that in c1/c1 individuals (Table 3). In alcoholic individuals exhibiting the c1/c1 genotype, CZX clearance rates were significantly higher (1.9-fold) than control subjects, as was CYP2E1 protein (1.9-fold) and mRNA (1.9-fold), whereas AUC for CZX was 2-fold lower. Similarly, in subjects heterozygous for the c1/c2 mutation, CZX clearance rates were 2.2-fold higher than controls, and CYP2E1 protein and mRNA were 2.7- and 1.6-fold higher, respectively, than for nonalcoholics. The AUC for CZX was 1.8-fold lower in alcohol abusers (Table 3).

DISCUSSION

The present investigation describes the effects of chronic alcohol consumption on CYP2E1 levels in PBLs and on in vivo CZX metabolic activity. Additionally, genotype was compared to phenotype among control and alcoholic subjects of three different ethnic groups, namely Whites, Navajos, and Mexican Americans. Constitutive levels of PBL

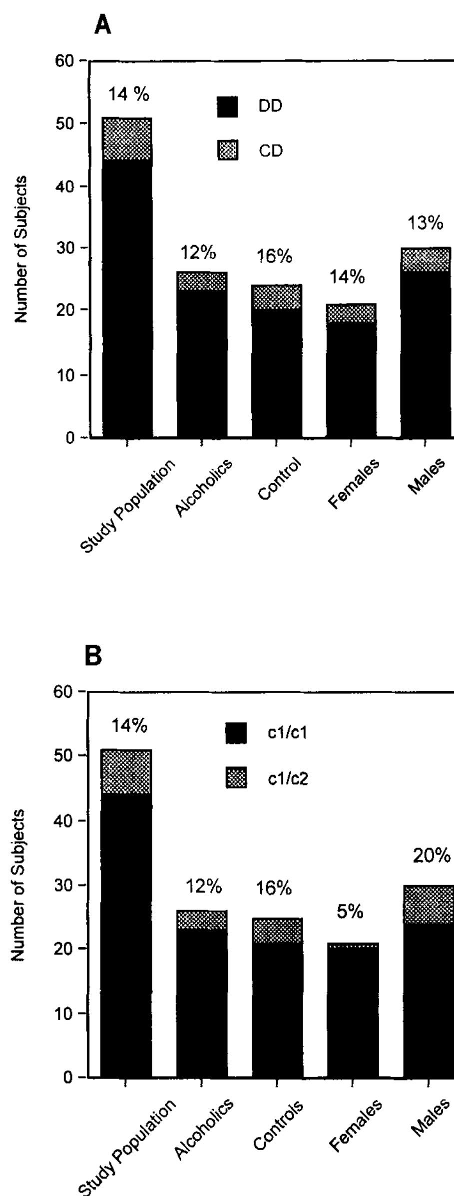

Fig. 2. Distribution of CYP2E1 polymorphisms among the study populations, among alcoholics and controls, and among males and females. Panel A: CD polymorphism. Panel B: c1/c2 polymorphism. Comparative analysis was performed for the C and c2 polymorphisms among 51 individuals. The dotted bars represent the number of heterozygous individuals exhibiting either the C or c2 rare alleles, and the solid bars are the number of individuals homozygous for the D or c1 alleles. The percentages refer to frequency of the heterozygous state.

CYP2E1 protein, mRNA, and CZX pharmacokinetic parameters were similar among the three ethnic groups (Tables 1 and 2). Of alcoholic subjects, Navajos exhibited a significantly ($p < 0.01$) greater increase (2.6-fold) in PBL CYP2E1 protein than values observed in Mexican Americans (2.1-fold) or Whites (1.3-fold) (Table 2). Similar increases were observed in CYP2E1 mRNA levels. However, the significant 2-fold elevation in CZX clearance rates caused by alcohol ingestion was the same in all three ethnic groups (Table 1). This is the first investigation to determine CZX pharmacokinetic parameters in control and alcoholic individuals from White, Navajo, and Mexican American

Table 3. Chlorzoxazone Pharmacokinetic Parameters and Human Peripheral Blood Lymphocyte CYP2E1 mRNA and Protein Levels

Subject	CZX clearance (liter/hr)	AUC CZX (μg/ml)hr	Lymphocyte CYP2E1 (OD/μg protein)	Lymphocyte CYP2E1 mRNA (10^4 molecules/μg RNA)
Subjects homozygous for CYP2E1 c1/c1 genotype				
Control	21.8 ± 1.5 (19[a])	26 ± 2.4 (19)	155 ± 21 (17)	1.2 ± 0.2 (17)
Alcoholic	41 ± 3.3 (23)*	13 ± 0.8 (23)*	295 ± 49 (14)*	2.3 ± 0.4 (14)*
Subjects heterozygous for CYP2E1 c1/c2 genotype				
Control	24 ± 3 (4)	22 ± 2 (4)	101 ± 33 (4)	0.9 ± 0.2 (4)
Alcoholic	53 ± 19 (3)	12 ± 4 (3)	274 ± 32 (3)*	1.4 ± 0.7 (3)*

CZX, chlorzoxazone; AUC, area under concentration curve; OD, optical density; RNA, ribonucleic acid; mRNA, messenger RNA.
Values represent mean ± SEM.
Numbers in parentheses are sample sizes.
* Significant difference from control subjects, $p < 0.05$.
[a] The two white subjects homozygous for the c1/c2 genotype as defined by the *Pst*I enzyme only were not included.

ethnic groups. It is also the first report to suggest an ethnic variation in alcohol-mediated induction of PBL CYP2E1. Indeed, previous studies have primarily focused on genotyping ethnic subpopulations. One investigation compared genotype with phenotype, using in vivo CZX metabolism as an indicator of CYP2E1 levels; however, studies were performed exclusively in White subjects (Lucas et al., 1995). A more recent investigation examined CZX pharmacokinetics in African Americans, but the extent of alcohol-mediated induction of CZX activity in this subpopulation was not compared to that of any other ethnic group (McCarver et al., 1998).

To compare phenotype with genotype, DNA samples from all three ethnic groups were isolated and examined for the cl/c2 and CD polymorphic loci. Of the 51 subjects examined, none were homozygous for either c2 or C. However, the sample size was small, which may explain why homozygotes for either polymorphism were not identified. Heterozygotes for the C polymorphism were discovered in all three ethnic groups. However, White subjects exhibited a lower frequency of heterozygotes, only 1 of 16, compared to a previous study where frequencies were 19% in White subjects for the C allele (Lucas et al., 1995). These contrasting results may be due to the small sample size used in the present investigation. Interestingly, the frequency for heterozygotes of the C polymorphism was much higher in the Mexican American and Native American populations than Whites. In fact, frequencies in these two ethnic groups were similar to those observed in the Japanese population (Nedelcheva et al., 1996). Herein, three Navajo subjects (20%) and three Mexican American subjects (15%) displayed the CD genotype.

Among the White subjects, none exhibited the rare c2/c2 genotype and those heterozygous for the c2 allele were mainly male subjects (20%). Only one female subject (5%) possessed this trait. However, if the two White women exhibiting the cl/c2 genotype as defined by *Rsa*I but not *Pst*I were included, the number of women and men with the c2 allele would be similar (14% vs. 20%). A gender-specific CYP2E1 genotype has not been previously described, and its significance to expression and activity of this P-450 is not apparent by the present investigation. Of those subjects with the cl/c2 genotype, three were Mexican Americans and four were Navajo subjects. Furthermore, DNA samples from four individuals displayed both the cl/c2 and the CD genotypes. Other reports have described similar frequencies (Kato et al., 1992; Lucas et al., 1995); however, the significance underlying the occurrence of both polymorphisms is unclear. Of the subjects examined here, frequencies of c2 and C alleles in alcoholics were less than those of control subjects (Figure 2), suggesting that a predisposition toward alcoholism is not associated with either polymorphism.

Surprisingly, we found a correlation between CYP2E1 protein levels and mRNA content in PBLs of alcoholics and nonalcoholics, indicating that both are elevated by ethanol. Exposure to ethanol is known to induce both hepatic and extrahepatic CYP2E1 by complex mechanisms that involve both increased enzyme synthesis (Tsutsumi et al., 1989) and protein stabilization (Powell et al., 1998; Roberts et al.,1995). Studies described here did not differentiate the mechanism of ethanol-mediated induction of CYP2E1. Nevertheless, results herein agreed with those that demonstrate increases in both CYP2E1 protein and mRNA in hepatic samples from alcoholic subjects (Tsutsumi et al., 1989). Because mRNA and protein were elevated concordantly in PBLs, it may be unnecessary to measure CYP2E1 protein in these white cells. Instead mRNA levels may indicate the extent of alcohol-mediated induction of hepatic CYP2E1 activity. This finding could be clinically important, because smaller amounts of sample are needed to assess mRNA levels than protein content, which would alleviate the need to draw large quantities of blood from subjects.

In conclusion, induction of both PBL CYP2E1 and its mRNA occurred in chronic ethanol abusers. Considerable attention has been given to CYP2E1 genotype as a cause for variation in expression. However, we found that cl/c2 or CD genotypes did not affect constitutive or induced levels of CYP2E1 among the three ethnic groups examined here. In response to alcohol consumption, Navajo subjects displayed significantly greater increases in white cell CYP2E1 levels when compared to Whites or Mexican Americans. However, due to the small sample size, the results are

inconclusive, and more studies are needed to confirm such a finding. We also found that CYP2E1 mRNA in lymphocytes correlated with the activity of the hepatic enzyme (as estimated from CZX pharmacokinetics) and PBL CYP2E1 protein levels in blood. This suggests that CYP2E1 mRNA could be used as an indicator of CYP2E1 levels in lymphocytes, alleviating the need for determining CYP2E1 protein content. This in turn, would allow for collection of less blood from each subject when phenotyping individuals.

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