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Research Article

Evaluation of cytochrome P4502E1 polymorphisms in healthy adult Western Indians and patients with antituberculous drug-induced hepatotoxicity

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ABSTRACT

Objectives: Cytochrome P4502E1 (CYP2E1) is involved in the metabolism of isoniazid and the mediation of its hepatotoxicity. It exhibits genetic polymorphism in humans. This study evaluated the polymorphism of CYP2E1 in adult healthy Western Indians and patients on antituberculous drugs by phenotyping and genotyping.
Methods: A 500 mg single dose of chlorzoxazone (CZX) was administered to 136 healthy adult Western Indian participants. Venous blood samples 2 h postdose were analyzed for the levels of CZX and 6-hydroxy CZX, and the metabolic ratio (MR) was calculated to determine the extent of rapid and poor metabolizers using probit plot analysis. Patients on antituberculous drugs who had raised the liver enzymes or clinical symptoms of hepatotoxicity were also recruited. Genotyping for CYP2E1 * 5B allele was performed by polymerase chain reaction – rapid fragment length polymorphism technique.
Results: A total of 139 healthy participants were enrolled, of which the final analysis consisted of data from 136 participants for genotyping and 137 for phenotyping. Only 1 participant had reported mild drowsiness 2 h postdose, and no other adverse events were observed. The median (range) MR of population was 0.2 (0.1–4.0), and no polymorphisms were detected using phenotype data. A total of 134/136 (98.5%) had c1/c1 genotype and 1/136 each (0.75%) had c1/c2 and c2/c2 genotypes, respectively. Of the 2/136 participants harboring c2 allele, one had MR of 0.1 (c1/c2) and another had 0.5 (c2/c2). A total of 25 cases of antituberculous drug-induced hepatotoxicity and 50 control patients were recruited, of which finally 22 cases and 49 controls were available for evaluation. All the cases had c1/c1 genotype while 42/49 (85.7%) controls had c1/c1, 6/49 (12.2%) had c1/c2, and 1/49 (2.1%) had c2/c2 genotype and the crude odds ratio was 7.9 (0.4, 145.6).
Conclusions: A background prevalence of CYP2E1*5B polymorphism and their activity in Western Indian population was observed. The study suggests no association between the CYP2E1 genotyping with antituberculous drug-induced hepatotoxicity.

KEY WORDS: Chlorzoxazone, cytochrome P4502E1, genotyping, hepatotoxicity, Indians, isoniazid, phenotyping

Introduction

India reports the highest incidence of tuberculosis globally. Untill date the first line antituberculous agents include isoniazid (INH), rifampicin, pyrazinamide, ethambutol, and streptomycin. Although effective in

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treating disease, adverse reactions to these drugs occur at an incidence of around 45% and still remains a major challenge.[1] Among them, drug-induced liver injury (DILI) is the most common and serious adverse reaction that may limit their use either in the prophylaxis or treatment of tuberculosis. INH, rifampicin, and pyrazinamide are the most hepatotoxic agents among them.

The incidence of DILI with INH ranges from 2% to 29% when used alone (e.g., for prophylaxis) and around 3–9% discontinue therapy due to this.[2] The irreversible damage to the hepatocytes that is seen in some patients after exposure to INH occurs due to free radicals produced from hydrazine and acetyl hydrazine, which are metabolites of INH generated through cytochrome P450 2E1 (CYP2E1) enzyme.[3] Although the gene encoding this enzyme is located on chromosome 10 and several polymorphisms have been reported,[4] an increased risk of DILI with the c1/c1 genotype of CYP2E1*5B (RsaI polymorphism) as compared to c1/c2 and c2/c2 genotypes has been reported.[3,5] This is attributed to an enhanced activity of c1/c1 genotype resulting in increased formation of reactive metabolites.

Polymorphisms of CYP2E1*5B have been evaluated in various ethnic groups of Indian population[6-8] but none of them have assessed the activity of different polymorphisms. Hence, this study was conducted to assess the prevalence of CYP2E1*5B polymorphisms in adult healthy Western Indian population and correlate it with the activity using chlorzoxazone (CZX) as the probe drug.[9] We also have done genotyping of CTP2E1*5B genotyping in patients who were receiving antituberculous medications.

Methods

The study was conducted between January 2007 and December 2008 after obtaining the Institutional Review Board approval and written informed consent from participants. Key eligibility criteria were healthy, unrelated adults (>18 years) of either gender adjudged normal by history, physical examination, and laboratory investigations including HIV and hepatitis B surface antigen serology and who were either nonsmokers or had refrained from smoking for the past 3 months. Exclusions were chronic alcoholics, alcohol or caffeine consumption in the past 24 h or paracetamol consumption in the past 7 days. Participants who satisfied the eligibility criteria were administered CZX (500 mg/oral) with 200 ml of water. Physical activity and food were restricted for 4 h postdose. Two hours postdose, single venous blood sample of 10 ml was collected for phenotyping and genotyping.

Patients receiving antituberculous medications were assessed through clinical history and were evaluated with the liver function tests. Those patients with symptoms/signs such as anorexia, nausea, vomiting, malaise, icterus, and raised serum aminotransferase levels exceed 2 times the upper limit of normal (ULN) value or more than 5 times the ULN without clinical symptoms were considered to have hepatotoxicity. Only those who never had consumed alcohol and whose hepatitis B serology was negative were included as cases. Controls were chosen as those on antituberculous medications which never had any clinical symptoms or abnormal liver function tests suggestive of hepatotoxicity. Five milliliter of blood was drawn for genotyping of CYP2E1 from both the cases and controls.

Laboratory Methodology

Phenotyping was done by analysis of CZX and 6-OH CZX by reverse phase high-performance liquid chromatography using a minor modification of the method described by Lucas *et al*.[10] The metabolic ratio (MR) was calculated by 6-OH-CZX/CZX (2 h concentrations), which gave the extent of CZX hydroxylation in an individual. Genotyping was done by the method as described by Yu *et al*.[11]

Statistical Analysis

Demographic data were represented using descriptive statistics. Normality of MR was assessed using Kolmogorov–Smirnov test. A frequency histogram plot was prepared with various MR values. Log MR was placed on X-axis and with the probit values on Y-axis, a probit plot was prepared. Antimode was generated as described by Varshney *et al*.[12]

The allelic frequency of c1 and c2 was represented using percentages and 95% confidence intervals (CIs) in parenthesis. The crude odds ratio was determined for the association of c1 allele with hepatotoxicity in patients. All the statistical tests were performed using GraphPad Instat 3.0 (GraphPad Software Inc., San Diego, CA, USA) and Microsoft Excel (Microsoft version 2003, Microsoft Excel, Redmond, Washington, USA) As no previous data were available on the prevalence of variant genotypes in Western India, no formal sample size calculations were performed.

Results

Demographics

A total of 153 participants were screened. Of which, 139 were found eligible and enrolled. DNA could not be extracted for 3/139 participants and phenotyping could not be performed for 2 participants (due to interference), and therefore, the final analysis consisted of data from 136 participants for genotyping and 137 for phenotyping. Mean (standard deviation) age of the study participants was 21.86 ± 2.66 years and the male:female ratio was 2:1. Only 1 participant reported mild drowsiness 2 h postdose. No other adverse events were seen.

A total of 25 cases of antituberculous drug-induced hepatotoxicity and 50 controls were recruited. Median (range) of age (years) of the cases were 32 (19–62) and male:female ratio was 3:2. Median (range) of age (years) of the controls were 26 (18–60) and male:female ratio was 3:2. Of these, DNA could not be extracted in 3 cases and 1 control leaving a total of 22 evaluable cases and 49 controls.

Phenotyping in Healthy Participants

The median (range) MR of the population was 0.2 (0.1–4.0). Log MR was rounded off to one decimal, and the histogram for log MR scale was plotted [Figure 1]. The probit values were obtained, and the probit plot [Figure 2] gave antimodes of −1.8 and 3.3, both of which are far apart from the original dataset indicating no polymorphisms.

Genotyping in Healthy Participants

The ladder pattern of different genotypes is illustrated in Figure 3. A total of 134/136 (98.5%) healthy participants

had c1/c1 genotype and 1/136 each (0.75%) had c1/c2, and c2/c2 genotypes, respectively. The allelic frequency of c1 was 98.9% (97.2, 100%) and c2 was 1.1% (0, 2.85%).

Genotyping in Patients

Genotyping of all the cases of antituberculous drug-induced hepatotoxicity revealed c1 alleles while 42/49 (85.7%) controls had c1/c1, 6/49 (12.2%) had c1/c2, and 1/49 (2.1%) had c2/c2 genotype, and the crude odds ratio was 7.9 (0.4, 145.6) ($P > 0.05$).

Genotype and Phenotype Correlation in Healthy Participants

Of the 2/136 participants harboring c2 allele, one had MR of 0.1 (c1/c2), and another had 0.5 (c2/c2).

Discussion

This study evaluated the polymorphisms of CYP2E1 by phenotyping and genotyping in healthy participants and genotyping in patients with antituberculous drug-induced hepatotoxicity. Our study with a sample of 136 healthy participants did not find any polymorphism by phenotype. By genotyping, only 2 out of 136 were found to have c2 allele, and the rest had a wild-type allele. Similarly, all the 22 cases had wild allele while only 7/49 controls had c2 allele. Because of such a low prevalence of the mutant allele, we were unable to find an association between the genotype and phenotype of CYP2E1*5B and literature in this regard is conflicting.[13,14] The 95% CI for the frequency of different genotypes of CYP2E1*5B among the healthy participants in this study relative to various ethnic groups is represented in Table 1. The genotype frequency was found to be similar to North and South Indians and Caucasians whereas lower than that observed in Kashmiris, Chinese, Taiwanese, and Thai populations.

The risk of DILI with antitubercular medications (AT DILI) ranges from 2% to 28% worldwide. The etiological basis of AT DILI includes older age, malnutrition, alcohol, concomitant hepatotoxic drugs, and c1/c1 genotype.[3,5] The presence of c1/c1 genotype in CYP2E1*5B increases the formation of the reactive metabolite in the hepatocytes as compared to

Figure 1: Frequency histogram plot of metabolic ratio. Metabolic ratios were determined as the ratio of the concentration of metabolite to parent drug for each patient, and the histogram of metabolic ratio for each study participant is plotted here. The median metabolic ratio obtained was 0.2, and the distribution looks such as normal distribution suggesting the absence of any polymorphism in the cytochrome P450 2E1 gene

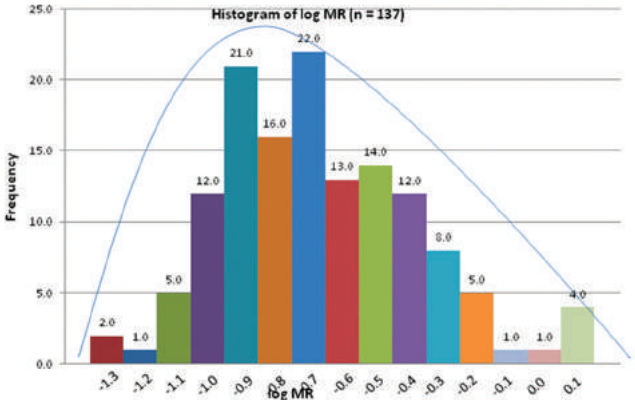


Figure 2: Probit plot of logarithm of metabolic ratios. A quadratic equation was obtained with the metabolic ratio values of study participants, and a probit plot was created. The values in the plot following the equation obtained in the figure fetches the antimodes of −1.8 and 3.3, both of which are far apart from the original dataset indicating no polymorphisms

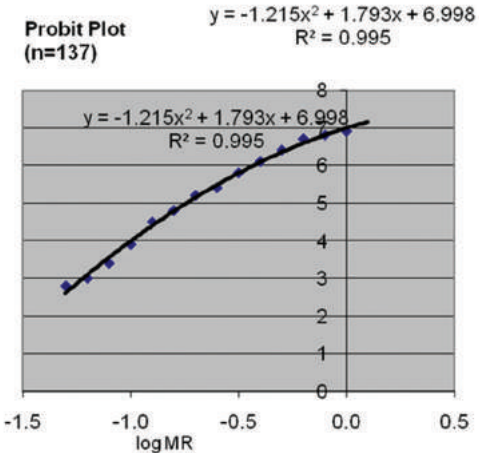


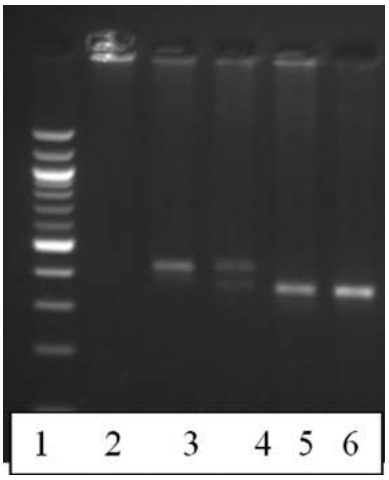
Table 1:

Comparison of prevalence of polymorphisms of CYP2E1*5B in different ethnic groups (*n* (%) (95% CI))

Genotype of CYP2E1*5B	Healthy Western Indians (present study) (n=136)	South Indians[6] (n=123)	North Indians[7] (n=50)	Kashmiri[8] (n=160)	Caucasians[15] (n=1454)	Taiwanese[16] (n=320)	Thai[17] (n=99)	Chinese[17] (n=98)
c1/c1	134 (98.5)	122 (99.2)	50 (100)	112 (70)	1344 (92.4)	198 (61.9)	70 (70.8)	43 (43.9)
	(96.5-100)	(97.6-100)	(100-100)	(63-77)	(91-93.8)	(56.6-67.2)	(61.8-79.8)	(34.1-53.7)
c1/c2	1 (0.75)	1 (0.8)	0	20 (12.5)	109 (7.5)	113 (35.3)	28 (28.2)	51 (52)
	(0-2.2)	(0-2.4)		(7.4-17.6)	(6.2-8.9)	(30-40.5)	(19.3-37.1)	(42-62)
c2/c2	1 (0.75)	0	0	28 (17.5)	1 (0.1)	9 (2.8)	1 (1)	4 (4.1)
	(0-2.2)			(11.6-23.4)	(0-0.3)	(1-4.6)	(0-3)	(0-8)
c1/c2 or c2/c2	2 (1.5)	1 (0.8)	0	48 (30)	110 (7.6)	122 (38.1)	29 (29.2)	55 (56.1)
	(0-3.5)	(0-2.4)		(23-37)	(6.2-8.9)	(32.8-43.4)	(20.2-38.2)	(46.3-66)

95% CI was calculated with the point estimate and total number of study participants recruited in each of the above study including this study. Ethnic difference was observed with a similar genotype frequency to North and South Indians and Caucasians whereas lower than that observed in Kashmiris, Chinese, Taiwanese, and Thai populations. CI=Confidence interval, CYP2E1=Cytochrome P450 2E1

Figure 3: Gel picture of cytochrome P450 2E1. Well 1 – Ladder (100 base-pair [bp]). Well 3 – Homozygous mutant (c2/c2) (410 bp). Well 4 – Heterozygous mutant (c1/c2) (410 and 360 bp). Well 5 – Homozygous wild (c1/c1) (360 bp). (1) The well number 1 represents the 100 bp ladder as a marker. (2) In well numbers 5 and 6 the 360 bp fragment found after RsaI digestion represents the wild allele (c1) i.e., wild-type genotype (c1/c1) and that of well number 3 the 410 bp is the rare allele (c2), i.e., homozygous mutant genotype (c2/c2). (3) The well number 4 represents the heterozygous mutant genotype, i.e., (c1/c2). In this two fragments (410 bp + 360 bp) are observed after digestion



c1/c2 or c2/c2 genotype following administration of INH.[18] Various studies in patients of other ethnic population have evaluated the association of c1/c1 genotype with INH-induced hepatotoxicity and have shown an increased risk.[3,5] However, this study did first in Indian population reveal no such association. This study gives an indication for population prevalence of c1/c1 genotype but given the fact that the vast majority of the participants have genotype; it may not be cost-effective to do genotyping in patients prior to initiating antitubercular therapy.

CYP2E1*5B is also involved in the conversion of various procarcinogens to carcinogens. The presence of c1/c1 genotype has been shown in different ethnic groups to increase the risk of various cancers such as squamous cell carcinoma of the esophagus,[19] hepatocellular carcinoma,[11] and lung cancer[20] relative to the presence of c2 allele. In contrary, an increased risk of gastric,[21] colorectal,[8] nasopharyngeal[17] was shown with c2 allele relative to c1. All the above studies are limited by a small sample size (n = 30–250) due to very low frequency of c2 variant allele and had included patients of various stages of cancer. Perhaps studies which evaluate the association of CYP2E1*5B with the above cancer states in Indian population will generate knowledge on association with these cancers. Furthermore, Pinto *et al.* have shown that the polymorphism is protective in inhibiting the bacterial growth in leprosy patients, and there are inconclusive reports with non-alcoholic steatohepatitis.[22] To conclude, the findings from this study in normal healthy participants have to be corroborated in different kinds of patients. No association exists between the mutant allele of CYP2E1 with antituberculous drug-induced hepatotoxicity.

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Conflicts of Interest

There are no conflicts of interest.

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