

Genetic polymorphism of cytochrome P450 2C19 in healthy Malaysian subjects

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Aims

Impaired S-mephenytoin 4'-hydroxylation is a well-described genetic polymorphism affecting drug metabolism in humans. Although ethnic differences in its distribution of polymorphism has been described, it is not known whether there is an ethnic heterogeneity of the structure and expression of the CYP2C19 enzyme in the Malaysian population.

Methods

Study subjects were 142 healthy, unrelated Malaysians aged 18–29 years. Baseline omeprazole and 2-h post-ingestion omeprazole and 5'-hydroxymeprazole concentrations were measured for CYP2C19 phenotype determination. Identification of CYP2C19 genotypes was performed with the use of polymerase chain reaction.

Results

Phenotyping of CYP2C19 revealed that the prevalence of poor metabolizers (PMs) in the Malaysian population was 14.1%, whereas prevalence of PMs in genotyping was 12.6%. The PM genotypic prevalence rate was 5.6% in Malays, 19.1% in Chinese and 10.0% in Indian subjects. There were significant differences in PM genotypic prevalence rates among the three primary ethnic groups ($P \leq 0.05$).

Conclusions

Phenotyping and genotyping revealed significant differences in the prevalence rates among the three ethnic groups in Malaysia, with Chinese recording highest prevalence.

Introduction

The polymorphic expression of cytochrome P450 2C19 (CYP2C19) is known to be one of the determinants responsible for the pronounced interethnic and interindividual differences in response and disposition of clinically important drugs such as omeprazole, diazepam and proguanil [1]. On the basis of their ability to metabolize (S)-mephenytoin or other CYP2C19 substrates, individuals can be classified as extensive metabolizers (EMs) or poor metabolizers (PMs). Eight variant alleles (CYP2C19*2 to CYP2C19*8) that predict PMs have been identified. To date, CYP2C19*2

and CYP2C19*3 have been shown as the main mutations contributing to the PM phenotype. CYP2C19*2 arises from the G→A transition in exon 5 at position 681 of CYP2C19, creating an aberrant splice site. CYP2C19*3 arises from a G→A transition at position 636 in exon 4 of CYP2C19, which produces a truncated protein [2].

Several studies indicate that the distribution of these common alleles is disparate among different populations. For example, the frequency of CYP2C19*2 has been reported to be ~17%, 30% and ~15% in African-Americans, Chinese and Caucasians, respectively, and

the CYP2C19*3 allele was shown to be more frequent in Chinese (~5%) than in Caucasians (0.04%) and Blacks (0.4%) (reviewed by Xie *et al.* [3]). These two alleles account for almost all PMs in Asian and Black African populations. The main defective allele, CYP2C19*2, accounts for some 75–85% of CYP2C19 alleles responsible for PMs in Orientals and Caucasians [4]. Although CYP2C19*3 is extremely rare in Caucasian populations, it accounts for almost all the remaining defective alleles in Orientals [5].

Most of the CYP2C19 polymorphism studies in Oriental populations were performed in East Asian populations (i.e. Chinese, Japanese or Korean). Studies of the CYP2C19 polymorphism in south-east Asian populations are limited. Also, there have been no studies conducted in the Malay population. This study aimed to determine the genetic polymorphism in the Malaysian population that consists of Malays, Chinese and Indians.

Methods

Study population

A total of 142 unrelated, healthy Malaysian subjects 18–29 years of age were recruited for this single-dose, open-label outpatient study. The subjects comprised 54 Malays, 68 Chinese and 20 Indians and were recruited from University of Malaya Medical Centre. A brief medical history was obtained, smoking history documented, and fasting state verified. Criteria for exclusion included a history of renal insufficiency, liver disease, cardiovascular disease, cancer, any chronic illness, drug or alcohol abuse, omeprazole use within the previous 7 days, and the use of any medication within 2 weeks of enrolment. Ethics Committee approval and written informed consent of the volunteers were obtained before the study.

Phenotyping procedures

Predose 6 ml whole blood were collected between 10 and 30 min prior to dosing for DNA extraction and genotype determination as well as for baseline omeprazole sample. An additional 6 ml were taken 2 h after administration of 20 mg omeprazole. The time of maximum concentration of omeprazole and 5'-hydroxyomeprazole in plasma was found in previous work to occur at 2 h after dose, which was the justification for the 2-h collection time [6]. The plasma concentrations of omeprazole and hydroxyomeprazole were analysed by the method of Kobayashi *et al.* [7] with some modifications. The molar ratio of omeprazole over hydroxyomeprazole in plasma was used to assess the individual capacity of CYP2C19.

Baseline and 2-h omeprazole blood sample tubes were centrifuged at 3000 g for 15 min; plasma was then withdrawn and stored at –70 °C until analysis for phenotype determination based on omeprazole and metabolite concentrations. Whole blood was transferred into EDTA tubes and stored at –70 °C until analysis to ascertain genotype status. The metabolic ratio of omeprazole over hydroxyomeprazole in plasma was used to assess the individual capacity of CYP2C19.

*Genotyping procedures: polymerase chain reaction-based allele-specific analysis of the CYP2C19*2 and CYP2C19*3 alleles*

Genotyping for CYP2C19*1, CYP2C19*2 and CYP2C19*3 alleles was performed by the polymerase chain reaction (PCR) method described by Goldstein and Blaisdell [8]. PCR amplification of CYP2C19*2 was done using the forward and reverse primers: 5'-CAGAGCTTGGCATATTGTATC-3' and 5'-GTAAA CACACA ACTAGTCAATG-3'. The primers used for analysis of CYP2C19*3 mutant allele were: 5'-AAAT TGTTTCCAATCATTAGCT-3' and 5'-ACTTCAG GGCTTGGTCAATA-3'. The PCR products of CYP2C19*2 and CYP2C19*3 were digested with SmaI and BamH1 enzymes, respectively, and separated on 3% agarose gel. Since the restriction site is absent in the mutant alleles, the PCR products are not digested by restriction enzymes. In the CYP2C19*1 (wild-type) allele the restriction enzymes SmaI and BamH1 splice the 321-bp DNA fragments into 212 bp, 109 bp, and the 271-bp DNA fragments into 175 bp, 96 bp, respectively. Positive and negative controls were always included in each PCR analysis and digestion of samples with restriction enzymes. The observed genotype frequencies of CYP2C19 were compared with expected genotype frequencies according to the Hardy–Weinberg law.

Statistical analysis

Frequency distribution analysis was performed on the log omeprazole hydroxylation index. The cut-off point to separate between PM and EM was determined to be at the metabolic rate of 6.0, meaning those with metabolic ratios <6.0 were classified as EMs and those with a ratio >6.0 as PMs. Standard statistical analyses with Student's *t*-test, χ^2 test, Man–Whitney, Wilcoxon and Kruskal–Willis, where appropriate, were used to evaluate potential differences in the demographic characteristics and log omeprazole hydroxylation index between PMs and EMs. *P* < 0.05 was the criterion for statistical significance. Goodness of fit between observed and estimated genotype frequencies according to the

Total number n	EM genotypes			PM genotypes			PMs %
	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3	
Malay	27	20	4	2	1	0	5.6
Chinese	25	26	4	4	8	1	19.1
Indian	7	11	0	2	0	0	10.0
Total	59	57	8	8	9	1	

Table 1

Distribution of CYP2C19 genotypes in healthy Malaysian population

Hardy–Weinberg equilibrium was determined by the χ^2 test.

Results

CYP2C19 phenotype

The omeprazole hydroxylation ratio ranged from 0.07 to 20.08. Twenty subjects (14.1%) were phenotyped as PMs with hydroxylation ratios >6.13 and 119 subjects as EMs with metabolic ratios ranging from 0.07 to 5.46. However, the metabolic ratio in three of the 142 subjects was undetectable. This may have arisen from poor absorption of omeprazole, since low oral bioavailability has been reported previously [6].

CYP2C19 genotype

CYP2C19*1 was the most frequently identified allele in the Malaysian population. The frequencies of CYP2C19*1 (*wt*), *2 (*m1*) and *3 (*m2*) alleles in healthy Malaysian subjects were 0.66 [confidence interval (CI) 0.60, 0.71], 0.28 (CI 0.23, 0.33) and 0.06 (CI 0.04, 0.01), respectively. Genotype determination revealed that 57 of the EMs were heterozygous for CYP2C19*1/*2, right were heterozygous for CYP2C19*1/*3 and the remaining 59 were homozygous for CYP2C19*1/*1. Of 142 subjects, 18 (12.7%) were PMs with heterozygous (*2/*3, $n = 9$) and homozygous for a single base pair mutation (*2/*2, $n = 8$; *3/*3*, $n = 1$) (Table 1). The PM genotypic prevalence rate was 5.6% in Malays, 19.1% in Chinese and 10.0% in Indian subjects.

Table 2 shows the allele frequency between different ethnic groups in the Malaysian population. The most common mutant allele in the Malaysian population was CYP2C19*2. The frequency of normal CYP2C19*1 and defective CYP219*2 and CYP2C19*3 alleles appeared highest in the Chinese population compared with other ethnic groups. CYP2C19*3 allele was absent in the Indian population. However, there was no statistically significant difference between the allele distributions among the three major ethnic groups when analysed by Kruskal–Wallis test. There were no significant differ-

Table 2

CYP2C19 allele frequencies and 95% confidence intervals (95% CI) in respective ethnic groups in Malaysian population

Ethnic group	Allele frequency		
	*1	*2	*3
Malay	0.72 [0.64 to 0.80]	0.23 [0.15 to 0.31]	0.05 [0.02 to 0.10]
Chinese	0.59 [0.51 to 0.67]	0.31 [0.23 to 0.39]	0.10 [0.05 to 0.15]
Indian	0.63 [0.46 to 0.77]	0.38 [0.22 to 0.54]	0 [0.00 to 0.88]

Data in parentheses represent 95% CI.

ences in allele distribution between the male and female subjects in all the ethnic groups. Based on Hardy–Weinberg equilibrium, no significant differences in the expected and observed frequencies of the three Mendelian genotypes exist.

Discussion

This study is the first to characterize the phenotype and genotype of CYP2C19 in the Malaysian population. The results revealed that 14.1% of Malaysian subjects were found to be PM phenotypes and 12.7% PM genotypes for drug metabolism through the CYP2C19 enzyme system. The phenotyped PM frequencies predicted correctly 90% of the genotyped PM frequencies due to inconsistency in the results of two subjects. A larger proportion of the EM heterozygous genotypes (especially *1/*2) in the EM subpopulation, and a higher allelic frequency of the CYP2C19*2 in the population are two putative explanations for the possible decreased CYP2C19 activity in the Malaysian population, specifically the Chinese.

The PM genotypic prevalence rate was 5.6% in

Malays, 19.1% in Chinese and 10.0% in Indian subjects. The prevalence of PMs in Chinese was significantly higher compared with Malays ($P \leq 0.05$) and Indians ($P \leq 0.05$). There were also significant differences in PM genotypic prevalence rates between Malays and Indians ($P \leq 0.05$). Nevertheless, the Indian subgroup ($N = 20$) in our study may be too small to draw a firm conclusion. To the best of our knowledge, this is the first study reporting PM genotypic and phenotypic prevalence rates in the Malays. In conclusion, the distribution of CYP2C19 alleles varies significantly among Malaysian subjects of different ethnic backgrounds, indicating that there is a possible genetic heterogeneity in the structure and expression of the CYP2C19 gene in the Malaysian population.

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