Proguanil metabolism in relation to S-mephenytoin oxidation in a Turkish population

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The oxidation of proguanil was studied in 89 unrelated healthy Turkish volunteers after administration of proguanil (single dose, 200 mg, orally). Based on the distribution of the ratio of proguanil to cycloguanil excreted in urine, and using an antimode value of 15, the prevalence of poor metabolizers in a Turkish population was estimated to be 5.6% (95% confidence interval 2.0%–17.3%) which was similar to that in the other Caucasian populations. The relationship between the oxidative capacities of CYP2C19 for the two substrates, proguanil and mephenytoin, was studied in 39 subjects (two poor and 37 extensive metabolizers of proguanil). The two poor metabolizers of proguanil were also identified as poor metabolizers of S-mephenytoin and no misclassification by the two phenotyping methods was observed. The correlation between the metabolic ratio of proguanil to cycloguanil and the S/R-mephenytoin ratio as assessed by Spearman's rank test, was statistically significant ($r_s = 0.50$, $P < 0.001$).

Keywords proguanil mephenytoin oxidation polymorphisms Turkish population

Introduction

Proguanil (PG) is an antimalarial drug used for prophylactic treatment of the infection caused by *Plasmodium falciparum*. As a prodrug [1] it requires cytochrome P450-mediated bioactivation to cycloguanil [2]. The parent compound is also excreted unchanged and converted to a minor inactive metabolite, 4-chlorophenylbiguanide (4-Ch). Studies have indicated large inter-subject variability in the plasma concentrations of cycloguanil (CG) in man [3, 4]. Based on the population studies using the urinary PG to cycloguanil (PG/CG) ratio, it was suggested that the oxidative activation of PG shows a polymorphic distribution and individuals were classified as extensive (PG/CG < 10) (EM_{pro}) or poor (PG/CG > 10) (PM_{pro}) metabolizers of PG [5, 6]. In a subsequent study the oxidative activation of PG was shown to co-segregate with the mephenytoin oxidation polymorphism [7] mediated by CYP2C19 [8]. Although, there is direct evidence that CYP2C19 catalyses the bioactivation of PG, CYP3A also contributes to the reaction [9].

The prevalence of poor metabolizers of PG ranges from 2.2 to 10% in Caucasians [5, 6] and from 18 to 35% in Far-Eastern and other ethnic groups [10–13]. The present study was undertaken to investigate the

pattern of oxidation of PG in a Turkish population and to ascertain whether PG metabolism co-segregates with the genetically determined metabolism of mephenytoin in Turkish subjects.

Methods

Eighty-nine unrelated healthy subjects, 88 men and 1 woman, with a median age of 20 years (range: 19–21 years) were studied. All were students or staff members of Hacettepe University and Gülhane Military Medical Academy, who were born in Turkey with Turkish parents. None of the subjects were regular alcohol users and had no history of liver or kidney disease. The study was approved by the Ethics Committees of Hacettepe University Medical Center and the Ministry of Health of Turkey.

Each subject took 200 mg proguanil orally. At least 2 weeks later, 39 subjects from this group (37 EM_{pro} and 2 PM_{pro}) were given orally 100 mg mephenytoin. No other drug was taken for at least 1 week before and during the study. Drugs were given immediately after emptying the bladder and a 0–8 h urine sample was collected. A 10 ml aliquot was kept frozen at -20°C

for up to 1 month and 3 months before mephenytoin and PG analysis, respectively.

PG and CG were determined by h.p.l.c. with u.v. detection [14]. The limits of determination for CG and PG were 20 and 100 ng ml⁻¹, respectively. Coefficients of variation for within-day and day-to-day precision were less than 10%. S- and R-mephenytoin were measured by gas chromatography [15]. In the urine samples in which R-mephenytoin but not S-mephenytoin was detected, the S/R-mephenytoin ratio was given a value (0.1) based on the lower level of detection of the S-enantiomer. Subjects with an S/R-mephenytoin ratio above 0.8 were phenotyped as poor metabolizers of mephenytoin (PM_{meph}) [15].

Correlations were examined using the Spearman's rank test.

Results

The frequency distribution profiles and probit plots for urinary PG/CG ratios obtained from 89 healthy volunteers are shown in Figure 1. The PG/CG ratios ranged from 1.49 to 50.80. In previous studies in Caucasian populations, subjects with PG/CG ratios greater than 10 were classified as PM_{pro} [5, 6]. However, in the present study, the antimode value for PG/CG ratio in this Turkish population is 15 by visual inspection (Figure 1). Thus, five subjects with PG/CG ratios >20.8 were classified as PM_{pro} (5.6%; 95% confidence interval 2% to 17.3%) and the remaining 84 subjects with PG/CG ratios <12.6 were classified as EM_{pro}.

The S/R-mephenytoin ratios obtained from 39 volunteers previously phenotyped with PG (2 PM_{pro} and 37 EM_{pro}) showed a statistically significant correlation with the PG/CG ratio (Figure 2, $r_s = 0.50$, $P < 0.001$). Both PM_{pro} were poor metabolizers of mephenytoin. Within the subgroup of 37 EM_{pro} subjects, there was also statistically significant correlation between the S/R-mephenytoin and PG/CG ratios ($r_s = 0.41$, $P < 0.01$).

No subjects participating in the study experienced any reported side effect from PG or mephenytoin.

Figure 1 Frequency distribution and probit plot of the 0–8 h urinary PG/CG ratios of 89 subjects given 200 mg oral proganil. Closed bars indicate PM_{pro}; (△) EM_{pro}, (■) PM_{pro}.

Figure 2 The correlation between urinary S/R-mephenytoin ratios and PG/CG ratios in 39 subjects ($r_s = 0.50$, $P < 0.001$) given both proganil (200 mg, orally) and mephenytoin (100 mg, orally) 2 weeks apart. (△) EM_{pro}, (■) PM_{pro}.

Discussion

We found a bimodal distribution of the PG/CG ratio in this Turkish population (Figure 1). The prevalence of putative PM_{pro} (5.4%; 95% confidence interval from 2.0% to 17.3%) is in the same range as that reported in Caucasian populations, 2.2–10.0% [5, 6], but lower than in Vietnamese (22%) [10], Thai (18%) [11], Khmer (19%) [12] and Kenyan (35%) [13] populations.

The results of this study showed that an antimode ratio of PG/CG of 10 [5, 6] is not applicable to the Turkish population. This conclusion is based on visual inspection of the PG/CG distribution (Figure 1) and supported by the observation that three of the five subjects with PG/CG ratios between 10 to 15 were EM_{meph} according to their S/R-mephenytoin ratios. Helsby *et al.* [6] used a PG/CG ratio of 10 to separate poor and extensive metabolizers of PG, but in two recent studies [10, 12] in which this antimode value was used, it could not clearly separate two phenotypes of PG on reanalysis of the data. Further evidence that the most appropriate antimode is a PG/CG ratio of 15 is the finding that the five subjects with values between 10 and 15 were genotyped as EM with respect to CYP2C19 (unpublished data).

In agreement with the results of previous studies in Caucasian [7, 16] and other populations [10, 14], our data show that the oxidative activation of PG co-segregates with the 4-hydroxylation of S-mephenytoin in Turkish subjects. This is based on a significant but modest correlation between the S/R-mephenytoin ratio and the PG/CG ratio in 39 Turkish subjects and in the subgroup of 37 EM_{pro} subjects when the 2 PM_{pro} were excluded. Phenotyping the whole population with mephenytoin was not possible because of the unwillingness of many of the volunteers to take both drugs. However, these limited data indicate that PG can be used to phenotype but not as a quantitative measure of

CYP2C19 activity. There was no discordance between PMs and EMs, whatever drug was used. Some [7, 10, 14] but not all [16, 17] investigators have found correlation between the mephenytoin hydroxylation index and PG/CG ratio. This discrepancy could be explained by the fact that the systemic clearance of PG depends on more than one enzyme, whereas that of mephenytoin is more specifically controlled by CYP2C19. Recently, Birkett *et al.* [9] reported that activation of PG to CG by human liver microsomes was mediated both by CYP2C19 and CYP3A isoforms.

In conclusion, PG is an additional and safe probe that can be used to classify subjects according to their CYP2C19 phenotypes.

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