Proguanil metabolism is determined by the mephenytoin oxidation polymorphism in Vietnamese living in Denmark

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- 1 A sparteine/mephenytoin phenotyping test was carried out in 37 Vietnamese living in Denmark. By visual inspection the urinary S/R-mephenytoin ratio appeared to show a bimodal frequency distribution. Eight putative poor metabolizers of mephenytoin, PMm (22%), had S/R-mephenytoin ratios from 0.79 to 1.12 and 29 putative extensive metabolizers of mephenytoin, EMm, had S/R-mephenytoin ratios ≤ 0.55 . All of the subjects were extensive metabolizers of sparteine with urinary metabolic ratios from 0.15 to 2.4.
- 2 The metabolism of the antimalarial prodrug proguanil was studied in 34 of the subjects after a single oral dose of 100 mg. The median 12 h urinary recoveries of the active metabolite cycloguanil and the minor metabolite 4-chlorophenylbiguanide were 5.8 and 1.9% of the dose, respectively, in 26 EMm compared with 1.6 and 0.4%, respectively, in 8 PMm ($P < 0.001$, Mann-Whitney U-test).
- 3 There was no statistically significant correlation (Spearman's r_s) between any index of proguanil metabolism and the sparteine metabolic ratio.

Keywords proguanil mephenytoin sparteine pharmacogenetics
Vietnamese malaria

Introduction

Proguanil, which was introduced as an antimalarial drug nearly 50 years ago, is an important prophylactic agent in Southeast Asia and East Africa where the frequency of chloroquine resistant strains of *Plasmodia* is high. Proguanil is a prodrug [1] requiring biotransformation to the active metabolite cycloguanil (Figure 1) [2]. The variability in the cytochromes P450 which catalyze the cyclization [3] is reflected by large interindividual differences in the plasma concentrations of cycloguanil during proguanil treatment [4]. The extremes of this variability are controlled by the mephenytoin oxidation polymorphism in white Caucasians [5, 6]. About 3% of this population have an autosomal recessive defect in their ability to hydroxylate (S)-mephenytoin [7, 8]. Thus the formation of cycloguanil is markedly impaired in poor metabolizers of S-mephenytoin (PMm). Although it may be hypothesized that proguanil is less effective against malaria in PMm compared with extensive metabolizers of mephenytoin (EMm), there is no clinical evidence for this [4].

The PMm frequency is much higher in Orientals than in whites. Thus, frequencies of about 20% have been

reported among Japanese and Chinese [9, 10, 12] and a frequency of 12.6% has recently been reported among Koreans [13]. Vietnamese have not previously been studied in this context. However, a PMm frequency and hence a frequency of poor metabolizers of proguanil of about 20% was to be expected in this population. To test this hypothesis we have performed a cross-over study of proguanil and mephenytoin metabolism in healthy Vietnamese living in Denmark.

Methods

Subjects of the study were 39 Vietnamese (31 men and 8 women) recruited in Odense and Svendborg. Their median age was 32 years (range: 20–60 years). No subjects had a history of heart, kidney or liver disease and none had a daily drug or alcohol intake.

Each subject took a combination of 100 mg racemic mephenytoin (Mesantoin, Sandoz) and 100 mg sparteine sulphate (Depasan, Giuliani) and collected urine for the

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Figure 1 Pattern of proguanil metabolism.

following 12 h [14]. The urine volume was recorded and an aliquot was kept frozen at -20°C for 1–3 months before analysis [15]. The ratio of the chromatographic peak areas of (R)- and (S)-mephenytoin was calculated [16, 17]. Ratios of less than 0.1 could not be differentiated.

Sparteine, 2,3-dehydrosparteine and 5,6-dehydrosparteine were assayed in urine by gas chromatography [18] and a sparteine metabolic ratio, MR, was calculated as sparteine/dehydrosparteines in the 12 h urine.

One week after the sparteine/mephenytoin test, each subject received an oral dose of 100 mg proguanil HCl (Paludrine, ICI), and collected urine for the following 12 h. The urine volume was recorded and an aliquot was kept frozen at -20°C until analysis. Proguanil and its metabolites cycloguanil and 4-chlorophenylbiguanide (Figure 1) were measured by h.p.l.c. [19]. The limit of quantitation was $1\text{ }\mu\text{M}$ for all compounds. The day-to-day variability was 2% for proguanil ($200\text{ }\mu\text{M}$), 2.6% for cycloguanil ($100\text{ }\mu\text{M}$) and 2.9% for 4-chlorophenylbiguanide ($50\text{ }\mu\text{M}$).

Owing to non-compliance, proguanil and its metabolites were undetectable in the urine of five men, and in two of these subjects sparteine, dehydrosparteines, (R)- and (S)- mephenytoin were also undetectable.

The Spearman's rank correlation test and the Mann-Whitney U-test were used for the statistical comparisons. All end points of proguanil metabolism are reported as medians and [95% confidence intervals of medians] (Table 1). The statistical tests were carried out using the MEDSTAT program package version 2.1 [20].

The protocol was approved by the Ethics committee of Vejle and Funen Counties and each subject consented to participate on the basis of written and verbal information in Vietnamese.

Figure 2 Frequency distribution of mephenytoin S/R ratio in 37 Vietnamese. □ Extensive metabolizers of mephenytoin. ■ Poor metabolizers of mephenytoin.

Results

The frequency distribution of the S/R-mephenytoin ratio appeared to be bimodal by visual inspection but the population was too small to be unequivocal about this (Figure 2). Thus, 26 putative EMm had S/R-mephenytoin ratios that ranged from < 0.1 to 0.55 and 8 putative PMm had ratios that ranged from 0.79 to 1.12. The sparteine MR ranged from 0.15 to 2.4 (median: 0.64) with no evidence of a bimodal distribution (data not shown). Accordingly, all subjects were classified as EM of sparteine (EMs) [21, 22]. There was no correlation between the S/R-mephenytoin ratio and the sparteine MR ($r_s = 0.01$, $P = 1.00$, $n = 37$).

The total urinary recovery of proguanil plus metabolites was about 21% of the dose in both phenotypes (Table 1). The median of the recovery of proguanil was approximately 30% lower in EMm than in PMm and the medians of the recoveries of cycloguanil and 4-chlorophenylbiguanide were about four times higher in EMm than in PMm (Table 1). Accordingly the proguanil/metabolite ratios were considerably higher in PMm than in EMm (Table 1, Figure 3) and all three ratios showed a statistically significant correlation with the S/R-mephenytoin ratio (Figure 3). However, the proguanil/metabolite ratios did not separate the two phenotypes completely. Two EMm with S/R-mephenytoin ratios of 0.11 and 0.24 respectively had proguanil/metabolite ratios within the PMm range (Figure 3). No indices of proguanil metabolism correlated with the sparteine MR (Table 1).

No adverse effects were reported spontaneously after either sparteine/mephenytoin or proguanil intake.

Discussion

In a small sample of 37 Vietnamese we found a PMm frequency of 21.6% (95% confidence interval: 9.8–38%). This is in agreement with the results of previous studies of other Oriental populations [9–13]. However, the S/R-mephenytoin ratio did not separate EMm and PMm as

Table 1 Proguanil metabolism in Vietnamese extensive (EMm) and poor metabolizers (PMm) of mephenytoin. Medians and [95% confidence intervals of medians]

	EMm (n = 26)	PMm (n = 8)	Mann-Whitney U-test EMm vs PMm P	Spearman's r_s S/R- mephenytoin	Spearman's r_s MR- sparteine
Proguanil ^a	14 [12–21]	20 [14–34]	0.035	0.21 ($P = 0.24$)	0.17 ($P = 0.35$)
Cycloguanil ^a	5.8 [3.0–8.1]	1.6 [0.7–2.6]	0.0008	–0.59 ($P = 0.00009$)	–0.065 ($P = 1.00$)
4-Chlorphenylguanide ^a	1.9 [1.0–2.5]	0.4 [0.1–0.5]	0.0001	–0.60 ($P = 0.00008$)	0.002 ($P = 0.99$)
Total ^a	21 [18–33]	22 [17–35]	0.858	–0.17 ($P = 0.32$)	0.11 ($P = 0.52$)
Proguanil/cycloguanil	2.8 [2.2–3.6]	12 [7.5–43]	0.00005	0.71 ($P < 0.00001$)	0.15 ($P = 0.41$)
Proguanil/ 4-chlorphenylbiguanide	9.8 [7.9–12]	68 [29–189]	0.0005	0.73 ($P < 0.00001$)	0.055 ($P = 0.76$)
Proguanil/cycloguanil + 4-chlorphenylbiguanide	2.1 [1.7–2.8]	11 [6.0–26]	0.00005	0.73 ($P < 0.00001$)	0.130 ($P = 0.46$)

^aMedian (range) % of a 100 mg dose of proguanil HCl (Paludrine®) in 12 h urine.
The individual data for each subject are available on request.

Figure 3 Relationships between S/R-mephenytoin ratios and 12 h urinary proguanil/metabolite ratios in 34 Vietnamese. ○ Extensive metabolizers of mephenytoin. ● Poor metabolizers of mephenytoin. r_s 0.71 and 0.73; $P < 0.00001$.

clearly as in Danes and Greenlanders [14, 24]. The reason for this discrepancy is not clear. Our study does not support the view that subjects from Southeast Asia are more prone to adverse effects after intake of 100 mg mephenytoin than whites [23]. No PMs were found in the Vietnamese and this is in agreement with the frequency of about 1% reported in Japanese and Chinese [9, 12]. The poor compliance in a few subjects corresponds to the observations made in Greenlanders [24].

In agreement with previous findings in Danish and French subjects [6, 25] our study shows that in Vietnamese the metabolism of proguanil and mephenytoin cosegregates. The proguanil/cycloguanil ratios found in the Vietnamese EMm and PMm (Table 1, Figure 3) appear to be of the same order of magnitude as in whites [6, 25], but differences in study design and drug assay make a direct comparison difficult. We show for the first time, that the dealkylation of proguanil (Figure 1) is deficient in Vietnamese PMm (Figure 3, Table 1).

The source of the mephenytoin polymorphism is a P450 in the 2C subfamily [26] which comprises more than seven closely related isoforms [27]. CYP2C18 appears to be the major (S)-mephenytoin 4-hydroxylase [27], but this has recently been questioned [28]. The molecular mechanism of the mephenytoin oxidation polymorphism has not yet been established. Thus, from our data it is not possible to tell with certainty whether the slow formation of cycloguanil and 4-chlorphenylbiguanide in the PMm is due to an alternative P450 in the absence of CYP2C18 or to an allelic variant of this isozyme with defective catalytic properties. The formation of cycloguanil via an alternative P450 could explain the overlap of the proguanil metabolic ratio with mephenytoin phenotypes (Figure 3) [29].

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