Oxidative activation of proguanil and dapsone acetylation in Thai soldiers

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The prevalence of putative poor metaboliser (PM) phenotypes of proguanil oxidation in Caucasian populations is 3–10%. The PM frequency in Oriental populations is unknown. In this study the plasma metabolic ratios of proguanil and dapsone to their principal metabolites cycloguanil and monoacetyldapsone were determined in Thai soldiers receiving antifolate drug combinations for malaria prophylaxis. The distribution ratio of proguanil to cycloguanil (PROG/CYC) was highly skewed with no evidence of bimodality. Assuming subjects with a PROG/CYC ratio greater than 10 are PMs from studies in Caucasians, the incidence of PMs in the soldiers would be 18% (30 of 170). The mean PROG/CYC ratio for PMs in the Thai soldiers was 31.2 ± 28.9 ($n = 30$) compared with 25.5 ± 2.5 ($n = 3$) in a study of Caucasians. The corresponding values for putative EMs were 5.4 ± 2.1 ($n = 140$) and 2.4 ± 0.2 ($n = 134$). Similar to other Oriental populations, Thais were found to be predominantly (76%, 173 of 228) rapid acetylators of dapsone.

Keywords polymorphism proguanil dapsone malaria

Introduction

Recently, a polymorphism in the oxidative activation of the antimalarial pro-drug proguanil to its active metabolite, cycloguanil, was reported in two Caucasian populations. Using an arbitrary proguanil to cycloguanil ratio greater than 10:1 the incidence of putative poor metabolisers (PMs) was found to be 3% [1] and 10% [2]. Applying the same ratio in a group of healthy Kenyans, 35% were identified as PMs of proguanil [3]. Thus, the incidence of PMs of proguanil appears to vary between ethnic groups. The large inter-individual variability in plasma concentrations of cycloguanil suggests that the oxidative polymorphism of proguanil could be of clinical importance in the chemoprophylaxis of malaria.

Recent *in vitro* studies indicate that the activation of proguanil is mediated by mephenytoin hydroxylase [4]. The prevalence of mephenytoin PMs in Caucasian populations is about 3% [5] compared with about 20% in Orientals [6]. These findings suggest that there may also be differences in the incidence of proguanil PMs between Oriental and Caucasian populations.

Over the past 7 years combinations of proguanil with either sulphonamides or dapsone have been used for malaria prophylaxis in Thailand [7, 8]. Since the individual components of these drug combinations inhibit the folic acid pathway of the malaria parasite synergistically, knowledge of the frequency distribution of proguanil

oxidative activation and dapsone acetylation in Thais may be of clinical relevance. Thus, the aim of the present investigation was to determine the frequency distribution of the various phenotypes in Thai soldiers taking proguanil plus dapsone and pyrimethamine plus dapsone.

Methods

Royal Thai Marine Militia ($n = 228$) on duty along the Thai-Cambodian border participated in the study. Soldiers received either daily 200 mg proguanil hydrochloride (Paludrine, ICI Pharmaceuticals, UK) combined with dapsone (4 mg or 12.5 mg, Jacobus Pharmaceutical Company Inc, NJ, USA, $n = 170$) or a weekly tablet of 100 mg dapsone plus 12.5 mg pyrimethamine (Maloprim, Wellcome Foundation Ltd, UK, $n = 58$). Informed consent was obtained from all participants. Ethical approval for the study was obtained under the guidelines of the Surgeons General of the United States Army and Royal Thai Navy.

After being on proguanil plus dapsone (P/D) for 40 days heparinised venous blood samples (10 ml) were collected from uninfected soldiers ($n = 156$) at either 12

h or at 24 h following the last dose. Uninfected soldiers ($n = 22$) on Maloprim were bled at 168 h after their last dose. For soldiers who failed malaria prophylaxis ($n = 14$ on P/D; $n = 36$ on Maloprim), blood samples were obtained at least 6 h after their last dose of medication. Samples were centrifuged at 1000 g for 10 min and the plasma separated. All plasma samples were stored at -70°C until the time of analysis.

Proguanil (PROG) and cycloguanil (CYC) were measured by ion-paired high-performance liquid chromatography (h.p.l.c.) [9]. The inter- and intraassay coefficients of variation for both PROG and CYC at 10 ng ml^{-1} were less than 10% ($n = 6$). The limit of detection was 1 ng ml^{-1} for both compounds. Ion-suppression h.p.l.c. was used for the analysis of dapsone (DDS) and monoacetyldapsone (MADDS) [10]. The inter- and intraassay coefficients of variation for DDS and MADDS at 25 ng ml^{-1} were less than 10% ($n = 5$). The detection limit was 2 ng ml^{-1} for both compounds.

Individuals with a PROG/CYC ratio ≤ 10 were classified as putative extensive metabolisers (EM), while those with ratios > 10 were identified as putative PM phenotypes [2]. Slow acetylators of DDS were defined as subjects with MADDS/DDS ratios of < 0.30 and rapid acetylators with ratios of > 0.35 [11]. Subjects with ratios in between were classified as intermediate acetylators. Statistical comparisons were made by Student's paired t -test with a significance level of 5%.

Results

The frequency distribution of plasma PROG/CYC ratios in Thai soldiers is shown in Figure 1. Of the 170 soldiers examined, 18% ($n = 30$) were assigned as putative PMs of proguanil with a mean ratio of 31.2 ± 28.9 (range

Figure 1 Frequency distribution of plasma PROG/CYC concentration ratios in Thai soldiers ($n = 170$) following administration of proguanil and dapsone. □ putative extensive metaboliser, ■ putative poor metaboliser.

10.1–135). The remaining putative EMs had a mean ratio of 5.4 ± 2.1 (range 1.9–9.9). Of the 14 soldiers who failed proguanil/dapsone prophylaxis, 21% (3/14) were PMs which is comparable with the frequency (17%) in uninfected soldiers. In five individuals, no significant differences were found between the metabolic ratios determined at the time of failure and either at 12 h or 24 h post-dose.

The frequency distribution of plasma MADDS/DDS ratios in Thai soldiers is shown in Figure 2. Seventy-six percent (173/228) were classified as rapid acetylators of dapsone, 21% (47) as slow acetylators and the remaining eight as intermediate acetylators. The median MADDS/DDS ratios for rapid and slow acetylators were 0.58 (range 0.36–1.96) and 0.20 (range 0.10–0.29), respectively.

Discussion

Thai soldiers exhibited a highly skewed distribution of PROG/CYC ratios. As in Caucasian populations the majority of Thais would be classified as putative EMs of proguanil (Figure 1). However, the frequency of putative PMs of proguanil was higher in Thais (18%) compared with previous reports of 3–10% in Caucasian populations [1, 2]. Although the mean PROG/CYC ratio for PMs was similar in the Thai soldiers (31.2 ± 28.9 , $n = 30$) and in a population of Caucasians (25.5 ± 2.5 , $n = 3$) [1], the range of values was larger in the Thais. The corresponding mean ratios for EMs were 5.4 ± 2.1 ($n = 140$) and 2.4 ± 0.2 ($n = 134$).

The oxidative activation of proguanil has also been shown to cosegregate with mephenytoin oxidation [12]. No study on the oxidation polymorphism of mephenytoin has been reported in Thai subjects. Unfortunately,

Figure 2 Frequency distribution of plasma MADDS/DDS concentration ratios in Thai soldiers ($n = 228$) following administration of dapsone combined with proguanil or pyrimethamine. ▨ slow acetylator, □ intermediate acetylator, ■ rapid acetylator.

because of military operational commitments whilst on duty and the redeployment of soldiers after border duty, there was no opportunity to determine the phenotypic status of mephenytoin oxidation in the soldiers. Nonetheless, in this study, the incidence of PMs of proguanil in Thais is in good agreement with the frequency of PM phenotypes of mephenytoin oxidation in other Oriental populations (23% in Japanese and 17% in Chinese) [6].

The metabolic ratio of drugs can be altered due to drug-drug interactions. However, dapsone and pyrimethamine have been shown not to alter PROG/CYC [13] and MADDS/DDS ratios [14], respectively, and therefore the coadministration of drugs was unlikely to influence the results of this study.

Selection of 12 h and 24 h post-dose plasma samples to determine the metabolic ratios of the individuals was based upon previous observations. Studies in healthy Thais have shown that peak plasma proguanil and cycloguanil concentrations are reached between 2 and 7 h post-dose [15]. Thus, a 6 h sample, as used in Caucasian studies [1, 2], would not have been as appropriate. Also, because the elimination of both proguanil and cycloguanil was observed to be biphasic and their plasma concentrations declined in parallel with half-lives of about 16 h, we expected that the metabolic ratio would be relatively constant beyond 12 h.

In contrast to EMs, in three Caucasians of PM phenotype plasma concentrations of proguanil and cycloguanil did not decline in parallel [1]. This finding

suggests that during the elimination phase the metabolic ratio would vary. Although phenotype status was not determined in the Thai study by Wattanagoon *et al.* [15], three of the nine subjects appeared to be PMs, based on the PROG/CYC AUC ratio, and two of nine based on the urinary PROG/CYC ratio (N. J. White, personal communication). Recently, it was found that plasma proguanil and cycloguanil concentrations fell in parallel in 10 Thai patients identified as PMs following administration of proguanil alone (M. D. Edstein, unpublished data). Thus, in addition to Thais having a higher incidence of PMs, the kinetics of proguanil in Thai PMs appear to differ from those observed in Caucasian PMs.

Similar to other Oriental populations [16], Thais were found to be predominantly rapid acetylators (76%) of dapsone. The high incidence of rapid acetylators (76%) observed in this study is in agreement with the figures of 72% reported by Sunahara *et al.* [17] and 77% by Kukongviriyapan *et al.* [18] using isoniazid and sulphadimidine as the test compounds.

In summary, the incidence of apparent poor metabolisers of proguanil and of rapid acetylators of dapsone is higher in Thais than in Caucasians. Further studies are indicated to determine the relationship between drug metabolism and efficacy of antimalarial prophylaxis.

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