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Inter-subject variability in the metabolism of proguanil to the active metabolite cycloguanil in man

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1 The metabolism of proguanil to the active metabolite cycloguanil has been evaluated in 135 British Troops and 26 Kenyan schoolchildren.

2 Large inter-subject variability was observed in both plasma and urinary concentrations of proguanil and cycloguanil after standard doses of drug.

3 Based on the ratio of proguanil to cycloguanil (P/C) in urine the British troops formed a non-normal distribution. 90% of the population formed a discrete distribution with P/C ranging from 0.5 to 9.0 while the remaining 10% were scattered throughout the distribution to an extreme value of 39. A similar pattern of variability was observed using P/C from a 6 h plasma sample.

4 This variability was due to differences in the ability of individuals to metabolise proguanil to cycloguanil.

5 Thirteen schoolchildren who had experienced malaria during prophylaxis with proguanil and thirteen matched controls each received proguanil (100 mg). We could not discriminate between the two groups based on P/C ratio in either a 6 h plasma or 0–6 h urine sample.

Keywords proguanil cycloguanil inter-subject variability

Introduction

Proguanil is an arylbiguanide which was introduced as an antimalarial agent in the 1940s, having been shown to be both efficacious and non-toxic in man (Maegraith *et al.*, 1946). Proguanil is relatively inactive as an antimalarial agent requiring metabolism to the active dihydrotriazine metabolite, cycloguanil (Carrington *et al.*, 1951), which is a potent inhibitor of the plasmodial enzyme, dihydrofolate reductase. This enzyme is essential for parasite viability being of vital importance in folate biosynthesis. The development of the 4-aminoquinoline antimalarials, in particular chloroquine, greatly reduced the clinical importance of proguanil as a prophylactic agent. However, the continuing emergence of strains of plasmodia resistant to the action of chloroquine has resulted in renewed interest in proguanil to the extent that a recent risk/benefit analysis study has stated that proguanil (200 mg daily) plus chloroquine (400 mg weekly) is the most valuable regimen for successful malaria prophylaxis (Peto & Gilks 1986).

Although proguanil has been available for more than 40 years, little is known concerning the disposition of this drug in man. Limited studies have been carried out aimed at measuring proguanil in biological fluids (Maegraith *et*

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al., 1946; Spinks & Tottey, 1946; Smith *et al.*, 1961). These methods however lacked both sensitivity and selectivity. Recently plasma and urinary concentrations of proguanil and cycloguanil have been determined by high performance liquid chromatography (Moody *et al.*, 1980; Edstein, 1986; Kelly & Fletcher, 1986). Watkins *et al.* (1987) used a bioassay technique to determine the plasma concentrations of cycloguanil after a single dose of proguanil (200 mg). These authors observed large inter-subject variability in circulating plasma cycloguanil concentrations although the cause of this variability was not apparent. In agreement with these findings Worsley (1975) and Kelly & Fletcher (1986) have also shown significant inter-subject variability in cycloguanil plasma concentrations following a standard oral dose of proguanil.

Inter-subject variability in drug disposition has been extensively studied. Genetic, environmental and pathophysiological factors have all been identified as potential sources of variability (Vessell, 1973). Such variability in drug disposition has obvious clinical implications with respect to both therapeutic efficacy and drug toxicity.

The aims of the present studies were first to determine whether the variability in circulating cycloguanil concentrations result from poor patient compliance, variable drug absorption or individual differences in the metabolism of proguanil to cycloguanil and second to evaluate whether this variability could result in prophylactic failure in some individuals due to low circulating concentrations of the active metabolite.

Methods

Subjects

Study I 135 male British troops (27 Gurkha, 108 British) took part in the study having given informed consent. All subjects had been based in Kenya for 1 month prior to the study and were receiving proguanil (200 mg daily) plus chloroquine (400 mg weekly) as part of an antimalarial prophylactic programme and were, therefore, assumed to be at steady state with respect to proguanil. Each subject received proguanil (200 mg orally – ICI Pharmaceuticals, Alderley Edge, UK) after an overnight fast and a single venous blood sample (5 ml) was taken at either 6 h (n = 69) or 10 h (n = 66). After centrifugation (1000 g for 10 min) the separated plasma was removed and stored at –20° C until the time of analysis. Each subject also provided a single spot urine sample collected just before the blood sample was taken. The urine volume was recorded and an aliquot (10 ml) was stored at –20° C until the time of analysis.

Study II 26 Kenyan school children (9–14 years) took part in the study, informed consent having been obtained from the school authorities and parents. All the children had previously taken part in a 3 month proguanil prophylaxis study (100 mg day^{-1}). Thirteen of these children had experienced at least one malarial attack during the prophylaxis study (treatment failure group). The remaining thirteen children represent age and sex matched controls who were completely protected from malaria during the prophylaxis study. Each subject received proguanil (100 mg orally) after an overnight fast. A single venous blood sample was removed at 6 h (2 ml). After centrifugation (1000 g for 10 min) the separated plasma was removed and stored at –20° C until the time of analysis. Urine was collected from time = 0 to time = 6 h. The volume was recorded and an aliquot (10 ml) was removed and stored at –20° C until the time of analysis.

Both Studies I and II had local research ethics permission. In addition Ministry of Defence permission was obtained for Study I and ethics permission from the Kenyan Medical Research Institute was granted for Study II.

Drug analysis

Plasma and urine concentrations of proguanil and cycloguanil were measured by reversed-phase h.p.l.c. The chromatograph consisted of an SP 8700 solvent delivery system with an SP 8750 organiser module (Spectra Physics, St. Albans, UK) equipped with a Rheodyne valve injection system. Chromatographic separation (based on the method of Taylor *et al.*, 1987) was carried out on a Bondapak 'Rad-Pak' phenyl reversed phase column housed in a 'z-module' and equipped with a 'CN – guard pak' precolumn (Millipore Waters, Hartford, Cheshire, UK). Detection was by U.V. absorption at 254 nm. The mobile phase consisted of water – containing octane sulphonic acid 0.5 mM/ acetronitrile/methanol (60/25/15 v/v/v) flowing at 3 ml min^{-1}.

Plasma samples, containing the internal standard chlorcycloguanil (250 ng ICI Pharmaceuticals, Alderley Edge, UK) were prepared by solid phase extraction using a 'Bond Elut' C_8 cartridge. Plasma samples (1 ml) were loaded onto the cartridge and washed with distilled water (1 ml) followed by methanol (1 ml). Proguanil and cycloguanil (P and C) were eluted

from the cartridge with 1% perchloric acid in methanol (250 μl) (Taylor *et al.*, 1987). The eluant was evaporated to dryness under nitrogen and samples were reconstituted in methanol (50 μl), 10 to 50 μl was injected on to the chromatograph. Urine samples were diluted 1/50 with distilled water after the addition of the internal standard (chlorcycloguanil 2 μg), 10 to 50 μl of sample was injected directly onto the chromatograph. Assay sensitivity representing a peak 4 × baseline noise at 0.005 AUFs was 1 ng ml^{-1} for cycloguanil and 2 ng ml^{-1} for proguanil. Inter and intra-assay coefficients of variation were less than 10% for both cycloguanil and proguanil at a plasma concentration of 50 ng ml^{-1}.

Data analysis

The concentration ratio of (P/C) in plasma and urine for each individual was used as a measure of the drug metabolising activity of each subject. All samples were taken after the reported times to peak concentration for both parent drug and metabolite (Wattanagoon *et al.*, 1987) and as such represent the ratio of drug to metabolite during the elimination phase.

Results

The frequency distribution profile of urinary P/C ratios in the British troops is shown in Figure 1. There was a non-normal distribution with P/C ratios ranging from 0.5 to 39. However, 90% of the study group did form a discrete distribution with P/C ratios between 0.5 and 9.0. The remaining 10% were scattered throughout the distribution. The Gurkha troops, when isolated from the rest of the study group, appeared as a very distinct distribution within the major mode of the frequency distribution profile (Figure 1).

Figures 2a and 2b show the frequency distribution profiles obtained from the 6 h and 10 h plasma samples respectively. The profiles obtained from the 6 h plasma samples reflect the results obtained from the urine data with about 90% of the population forming a tight distribution, with P/C ratios between 0.5 to 10, the remaining individuals being scattered along the distribution to an extreme P/C ratio of 30. However, based on the 10 h plasma sample (Figure 2b) all subjects appeared to fall into a discrete distribution with P/C ratios of 0.5 to 6 with one individual exhibiting a ratio of 11. Figure 3 shows the correlation between urinary and plasma P/C ratios for each individual. The r^2 value was 0.52 which was significant at the 0.1% level based on a sample size of 135.

The median plasma concentrations of proguanil and cycloguanil in plasma were 190 ng ml^{-1} (range 42–815 ng ml^{-1}) and 86 ng ml^{-1} (range 20–545 ng ml^{-1}) for those individuals in the major mode of the distribution and 390 ng ml^{-1} (range 82–1103 ng ml^{-1}) and 14 ng ml^{-1} (range 9–69 ng ml^{-1}) for those individuals with P/C >10. The corresponding urine data are median proguanil concentration 73 μg ml^{-1} (range 15–254 μg ml^{-1}) and cycloguanil concentration 34 μg ml^{-1} (range 3–192 μg ml^{-1}) for those individuals with P/C < 10 and 235 μg ml^{-1} (range 45–278 μg ml^{-1}) and 8 μg ml^{-1} (range 1–33 μg ml^{-1}) for those individuals with P/C > 10.

Figures 4a and 4b describe the P/C ratios in plasma and urine obtained from the school children from study II. The median P/C ratio in plasma was 1.1 (range 0.5–17) in the treatment failure group and 1.0 (range 0.5–37) in the treatment success group. The corresponding values

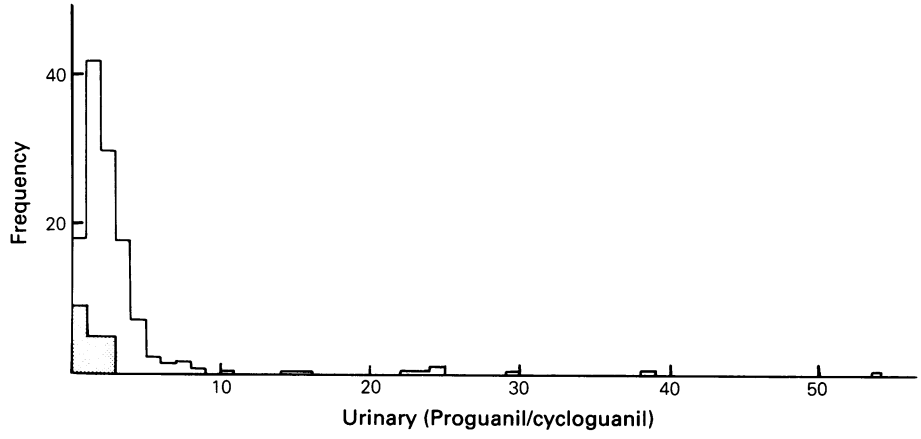

Figure 1 Frequency/distribution profile of urinary proguanil/cycloguanil concentrations in 135 troops after 200 mg proguanil hydrochloride. Hatched area represents Gurkha troops.

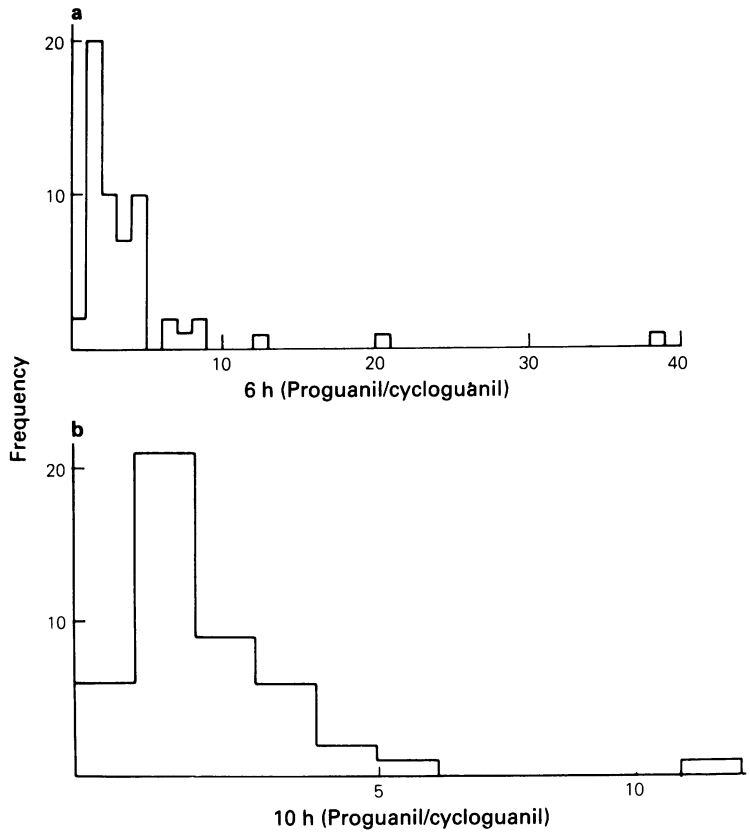

Figure 2 Frequency/distribution profile of 6 h, $n = 69$ (a) and 10 h, $n = 66$ (b) plasma proguanil/cycloguanil concentrations in troops after 200 mg proguanil hydrochloride.

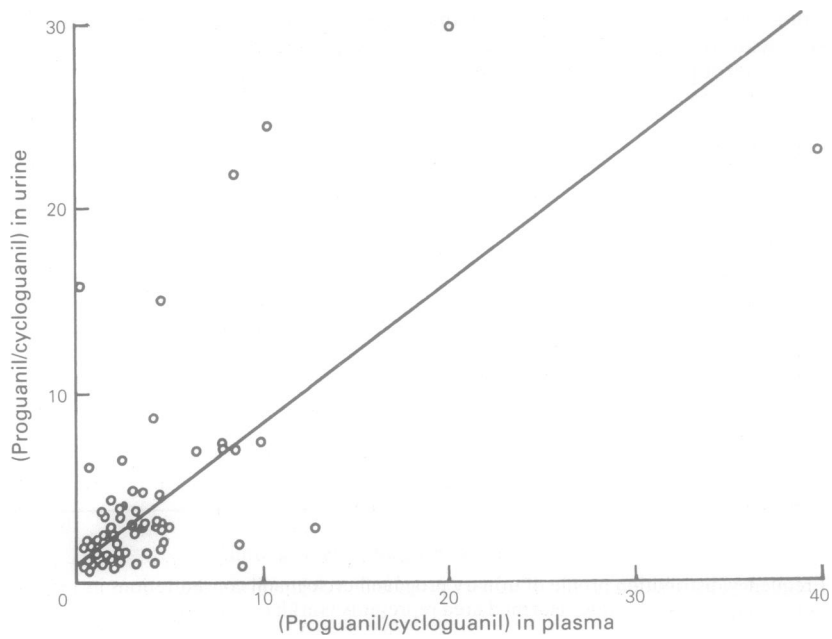

Figure 3 Plot of urinary *vs* plasma proguanil/cycloguanil ratios. $r^2 = 0.52$.

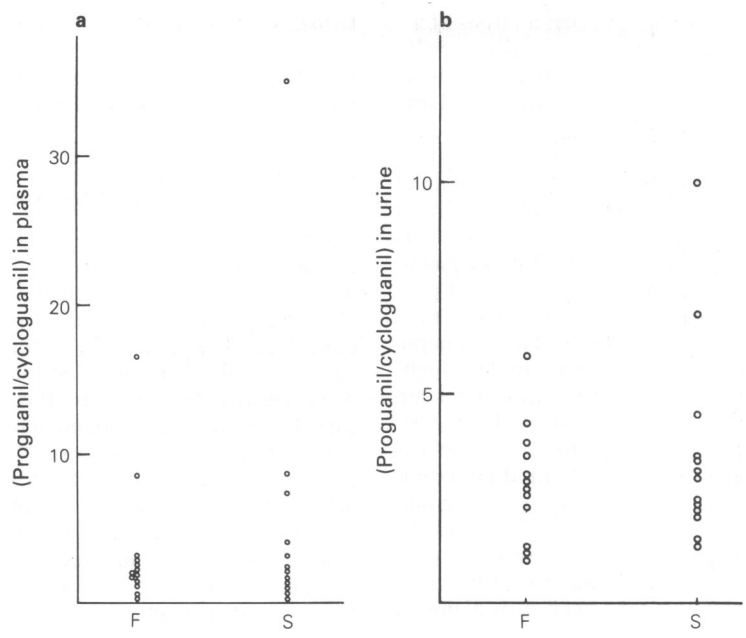

Figure 4 Proguanil/cycloguanil ratios in plasma (a) and urine (b) in school children 6 h after 100 mg proguanil hydrochloride. F = prophylactic failure group; S = prophylactic success group.

for urine were 2.7 (0.8–6.5) and 3.1 (1.1–10). There was no statistically significant difference between the two groups using the non-parametric Wilcoxan Rank sum test.

Discussion

Recent risk/benefit analysis has highlighted the importance of proguanil (Peto & Gilks, 1986) as the safest and most appropriate drug for malaria prophylaxis when used in combination with chloroquine. Despite these recommendations the clinical pharmacology of proguanil is poorly understood. The data presently available are limited to those obtained using analytical methods which lacked both selectivity and sensitivity. The observation that certain individuals have abnormally low circulating concentrations of cycloguanil after a standard dosage (Watkins *et al.*, 1987; Kelly & Fletcher, 1986; Worsley, 1975) is an intriguing one and raises a number of questions related not only to therapeutic failures associated with the use of this drug but also with respect to the promotion of parasite resistance to antifolate drugs. For these reasons it is important to determine the source of these inter-individual differences and to establish which factors contribute to the variability observed.

This study of British troops receiving proguanil as a prophylactic agent confirmed the finding of Watkins *et al.* (1987) that there are large inter-individual differences in circulating cycloguanil levels. This variability does not represent poor patient compliance as dosage administration was rigorously controlled and all individuals had measurable concentrations of proguanil and cycloguanil in plasma and urine. Variable absorption seems unlikely as the sum of concentrations of proguanil and cycloguanil for each individual in either plasma or urine resulted in a small range of values which did not exhibit the same degree of variability as seen with the P/C ratios. It would appear therefore that this variability is a result of intrinsic differences in the ability of these individuals to metabolise proguanil to cycloguanil. The Gurkha troops represent a genetically more homogeneous group in comparison with the rest of the study group. The observation that these individuals form a discrete distribution based on their urinary P/C ratio may indicate the importance of genetic factors in the metabolism of proguanil. However, due to the small number of Gurkha troops and the non-normality in the population distribution profile, the absence of P/C > 3 may be purely due to limitations in sample size. This observation requires further investigation.

The sampling schedule adopted in this study was restricted due to the size of the study group and the important limitation that the study could not interfere with the work schedules of the

troops. However, we believe that analysis of a 6 h and 10 h plasma sample does give a valid indication of an individual's ability to form cycloguanil. This is based on the data available which indicate that peak concentrations of drug and metabolite are achieved by 6 h post-dose after which plasma levels decline in parallel (Wattanagoon *et al.*, 1987). Additionally it would appear that cycloguanil elimination is formation rate limited and as a result cycloguanil concentrations fall in parallel with that of proguanil during the elimination phase (Smith *et al.*, 1961). The validity of a single spot urine sample collected just before the plasma sample is highlighted by the significant correlation between the plasma and urinary P/C ratios (Figure 3). Although less accurate than a total urine collection or a sample taken over a limited interval if we assume that the renal clearance of drug and metabolite are equivalent (Wattanagoon *et al.*, 1987) linear pharmacokinetics apply, metabolites are end products exhibiting formation rate limited kinetics and the 'well stirred' model of hepatic clearance applies (Wilkinson & Shand, 1975) then this urinary ratio is related to the clearance of proguanil to cycloguanil as follows:

$$Ae_{ss} = C_{ss} \cdot CL_R \quad (1)$$

$$Ae(m)_{ss} = C(m)_{ss} \cdot CL(m)_R \quad (2)$$

$$\left(\frac{\text{Drug}}{\text{metabolite}}\right) \text{ in urine} = \frac{C_{ss} \cdot CL_R}{C(m)_{ss} \cdot CL(m)_R} \quad (3)$$

If we assume that CL_R is equivalent to $CL(m)_R$ (Wattanagoon *et al.*, 1987)

$$\left(\frac{\text{Drug}}{\text{metabolite}}\right) \text{ in urine} = \frac{C_{ss}}{C(m)_{ss}} \quad (4)$$

At steady state

$$C(m)_{ss} = \frac{CL_m}{CL(m)_R} \cdot C_{ss} \quad (5)$$

Combining (4) and (5), assuming no first pass metabolism,

$$\frac{\text{Drug}}{\text{metabolite}} = \frac{CL(m)_R}{CL_m} \quad (6)$$

Ae_{ss} = The amount of drug excreted at steady state per unit time
C_{ss} = the steady state plasma concentration
CL_R = renal clearance of drug
$Ae(m)_{ss}$ = the amount of metabolite excreted at steady state per unit time
$C(m)_{ss}$ = the steady state plasma metabolite concentration
$CL(m)_R$ = renal clearance of metabolite
CL_m = clearance of drug to metabolite

(We assume that compared with metabolic clearance, renal clearance is relatively invariant in our population of healthy male troops.)

It is reasonable to assume on the basis of these findings that along the distribution profile there will be an area beyond which individuals will not receive adequate prophylatic cover from a standard dose of proguanil. In addition it has been suggested that parasite resistance can result from selective drug pressure. It is possible therefore that circulating cycloguanil levels below the concentrations required to kill the parasite will promote resistance to antifolates and seriously affect their usefulness as prophylatic agents. This would be of immense importance when we consider the limited number of agents available for malarial prophylaxis and the widespread resistance of parasites to most other classes of antimalarial agents.

The second study was carried out to assess whether prophylatic failures observed in school children resulted from impaired metabolism of proguanil to cycloguanil in these individuals. However, we could not distinguish between the prophylatic failure group and controls based on either their 0–6 h urinary or 6 h plasma P/C ratios. Although these findings do not contradict the belief that low circulating cycloguanil concentrations will result in prophylactic failure it does suggest that the treatment failures observed in the earlier study were the result of non-host dependent factors.

In summary, we have confirmed that there is large inter-subject variability in circulating cycloguanil levels resulting from differences in the ability of individuals to metabolise proguanil. This variability may lead to treatment failure or the promotion of parasite resistance to these drugs. It is unknown whether this variability is determined by genetic, environmental or pathophysiological factors nor whether an individual's ability to metabolise proguanil remains unchanged on subsequent occasions.

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