The pharmacokinetics and activation of proguanil in man: consequences of variability in drug metabolism

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1 Based on the ratio of drug to active metabolite excreted in urine approximately 3% of a healthy Caucasian population showed a reduced ability to convert proguanil to cycloguanil.

2 Pharmacokinetic analysis showed that this observation resulted from a reduced oral clearance of proguanil in these individuals (245, 534 and 552 ml min⁻¹) compared with the rest of the population (858 ± 482 ml min⁻¹).

3 Peak plasma concentrations of active metabolite were significantly lower in these subjects (54.2, 26.8 and 51.7 ng ml⁻¹) compared with the rest of the population (141 ± 45.2 ng ml⁻¹).

4 The observed variability may result from the polymorphic metabolism of proguanil in man.

Keywords proguanil activation variability

Introduction

Recent studies have suggested large inter-subject variability in the hepatic activation of proguanil (PG) to cycloguanil (CG) in man (Ward *et al.*, 1989; Watkins *et al.*, 1987). This could have important clinical consequences with respect to the therapeutic success of this extensively used antimalarial pro-drug. From the observation that the urinary proguanil to cycloguanil ratios in population studies exhibit a highly skewed frequency distribution profile (Ward *et al.*, 1989) it was suggested that the metabolism of proguanil may exhibit a genetic polymorphism in man. Additional support for this hypothesis is the finding that the anti-convulsant mephenytoin inhibits efficiently and competitively the formation of cycloguanil in *in vitro* human liver microsomes (Helsby *et al.*, 1990). It is well established that the metabolism of mephenytoin exhibits a genetic polymorphism in man (Küpfer & Preisig, 1984) and

in vitro observations may be indicative of linked routes of metabolism.

We have undertaken a population study in Liverpool to identify 'poor' and 'extensive' metabolisers of proguanil based upon their 0–8 h urinary PG/CG ratio. The plasma pharmacokinetics of proguanil and formation of cycloguanil have been determined in individuals selected from the population study to establish the pharmacokinetic consequences of an individual's phenotype.

Methods

Approval for both the population and pharmacokinetic studies was obtained from the Ethics Committee of the Mersey Regional Health Authority.

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Population study

Healthy volunteers ($n = 137$) took part in the study having given full informed consent. After an overnight fast each subject received proguanil (2×100 mg p.o. Paludrine; ICI Pharmaceuticals, Alderley Edge, U.K.). Urine was collected over 8 h, volume recorded and an aliquot (10 ml) stored at -70°C until the time of analysis.

Pharmacokinetic studies

Healthy volunteers previously designated as 'extensive' ($n = 7$) or 'poor' ($n = 3$) metabolisers of proguanil were included in the study having given full and informed consent. Each subject received proguanil (2×100 mg p.o.) after an overnight fast. Venous blood samples (10 ml) were removed into lithium: heparinised tubes pre-dose and at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48 and 72 h. Samples were centrifuged (3000 g, 15 min) and the plasma removed and stored at -70°C until the time of analysis.

Drug analysis

Concentrations of proguanil and cycloguanil in plasma and urine 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 μ Bondapak 'RadPak' phenyl column housed in a 'Z-module' and equipped with a 'CN-Guard Pak' pre column (Millipore Waters, Hartford, Cheshire, UK). Detection was by u.v. absorption at 238 nm. The mobile phase consisted of water containing 1% triethylamine phosphate (pH 3) : acetonitrile : methanol (82 : 8 : 1 v/v/v) flowing at 3 ml min⁻¹.

Plasma samples (0.5 ml), which contained the internal standard, chlorcycloguanil (200 ng, ICI Pharmaceuticals, Alderley Edge, UK) were diluted with an equal volume of water (0.5 ml) and prepared by solid phase extraction. A 'Bond-Elut' C₈ cartridge (Jones Chromatography, Hengoed, Mid-Glamorgan, UK) was preconditioned with methanol (1 ml) followed by water (1 ml). The plasma sample was then loaded onto the cartridge and washed with water (1 ml) and methanol (1 ml). Proguanil, cycloguanil and the internal standard were eluted from the cartridge with 1% perchloric acid in methanol (1 ml; Taylor *et al.*, 1987). The eluant was evaporated to dryness under

nitrogen and samples were reconstituted in mobile phase (65 μ l). 10 to 50 μ l was injected on to the chromatograph. Urine samples (10 μ l) diluted with distilled water (990 μ l) were prepared by solid phase extraction using a 'Bond Elut' C₈ cartridge as above. Assay sensitivity representing a peak $4 \times$ 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

Data are presented graphically as means $\pm$ s.e. mean and are tabulated as mean $\pm$ s.d. Proguanil and cycloguanil $C_{\max}$ were determined by visual inspection of the log proguanil concentration-time profiles. The terminal phase elimination rate constant (λ_z) was determined by least squares regression analysis of the post absorptive and distributive plasma concentration time data, the terminal phase elimination half-life ($t_{1/2}$) from the ratio $0.693/\lambda_z$.

The area under the plasma concentration time curve from time = 0 to time = t' was calculated by the trapezoidal rule (Gibaldi & Perrier, 1975) and from t' to ∞ by the ratio Ct/λ_z , where Ct was the plasma concentration at time t' . The area under the curve from $t = 0$ to infinity (AUC) was obtained from the sum of these areas. Oral clearance was calculated from the ratio dose/AUC and the apparent volume of distribution (expressed as a function of bio-availability, F) was calculated from the expression

$$\frac{V}{F} = \frac{CL_0 \times t_{1/2}}{0.693}$$

Statistical comparisons were made using Student's t -test. Statistical significance was accepted when $P < 0.05$.

Results

The frequency distribution profile for urinary PG/CG ratios obtained from 137 healthy volunteers after the administration of proguanil (200 mg p.o.) is shown in Figure 1. One hundred and thirty-four volunteers (97.8%) exhibited a PG/CG ratio less than 10 (mean 2.4 ± 0.20). However, in three individuals (2.2%) the ratio was more than ten standard deviations away from this mean (PG/CG, 22.6, 27.2 and 26.7). These PG/CG values > 20 did not appear to be due to changes in the bioavailability of

Figure 1 Frequency distribution profile for PG/CG ratios in 0-8 h urine samples.

Figure 2 Frequency distribution profile for total concentration of PG + CG in 0-8 h urine samples ($\downarrow$ indicates PM subject).

proguanil as the frequency distribution profile for PG + CG in those volunteers providing a complete 8 h urine collection did not show the same skewed distribution (Figure 2) and the three individuals with ratios > 20 were indistinguishable from the rest of the population.

Based upon the population data, individuals with PG/CG ratios < 20 have been tentatively classified as 'extensive metabolisers' of proguanil and those with ratios > 20 'poor metabolisers'. The mean plasma concentration time profiles for seven 'extensive metabolisers' of PG are shown in Figure 3a. The drug appeared to be rapidly absorbed achieving peak plasma concentrations of 271 ng ml^{-1} (± 118) within 3 h. Thereafter, plasma concentrations fell biexponentially with a terminal elimination half-life of $17.6 \pm 5 \text{ h}$. Individual plasma con-

centration-time profiles for three 'poor metabolisers' of proguanil are also shown in Figure 3a. There was no difference in the peak plasma concentrations of proguanil or time to peak concentrations compared to EMs. After achieving peak concentrations plasma proguanil concentrations again fell biexponentially. However, plasma proguanil concentrations and consequently AUC(0,72h) in these three subjects were higher than the mean values for the EMs and the elimination half-lives were significantly longer. The pharmacokinetic parameters derived from these data are shown in Table 1. It would appear that the longer half-life observed in PMs results from a significant reduction in the oral clearance of proguanil as there was no significant difference in the value V/F between our two groups.

Table 1 Pharmacokinetic parameters of EM (1-7) and PM (8-10) subjects

Subject	0-8 h PG/CG ratio	CL/F (ml min^{-1})	V/F (l)	$t_{1/2}$ (h)	Cycloguanil C_{max} (ng ml^{-1})
1	1.33	926	1158	14.4	167.0
2	0.8	626	1012	18.7	142.2
3	1.25	941	1256	15.4	140.2
4	3.3	639	1163	21.0	52.6
5	1.84	488	635	15.0	161.0
6	1.09	518	1200	26.8	125.2
7	1.28	1871	1923	11.9	197.4
Mean		858	1192	17.6	140.8
$\pm \text{s.d.}$		(± 482)	(± 384)	(± 5.02)	(± 45.2)
8	22.6	534**	1451	31.4**	54.2**
9	26.7	245**	845	40.0**	26.8**
10	27.2	552**	1058	22.1**	51.7**

** $P < 0.05$ (for PMs relative to EMs)

Figure 3a) Plasma proguanil concentration-time profile for EM ($n = 7$; ●) and PM subjects (■ □ △).

b) Plasma cycloguanil concentration-time profile for EM ($n = 7$; ●) and PM subjects (■ □ △).

The most striking difference between the two groups related to the circulating plasma concentrations of cycloguanil (Figure 3b). In the EM subjects peak plasma concentrations of 141 ± 45.2 ng ml⁻¹ were achieved within 4 h. Thereafter, plasma concentrations fell bi-exponentially such that at 72 h, plasma concentration of 19.9 ng ml⁻¹ (± 11.4) were still

detectable. In contrast, the peak plasma cycloguanil in the PMs were significantly lower than in the EMs ($54.2, 26.8, 51.7$ ng ml⁻¹; Table 1). The time to achieve peak concentrations was variable (1, 4 and 6 h) and unlike the biexponential decline observed in the EMs, plasma cycloguanil concentrations reached a plateau between 10 and 20 ng ml⁻¹ and remained at this level with little discernible decline in concentration for 48–72 h, in the PM subjects.

Discussion

The present studies have again highlighted the inter-subject variability associated with the activation of proguanil to cycloguanil in man. Based upon the proguanil/cycloguanil concentration ratio in a 0–8 h urine collection 97.8% of the population formed a distribution with ratios less than 10 in agreement with earlier observations (Ward *et al.*, 1989). The three individuals (2.2% of the population) not contained in this distribution exhibited urinary ratios more than ten standard deviations away from the mean of the major group. The absence of subjects in between suggests that this smaller group of individuals form a separate population rather than the tail of a skewed distribution. The 2.2% incidence of individuals with a high urinary ratio is lower than the earlier report of a 10% incidence (Ward *et al.*, 1989) which may imply differences in the composition of the two populations studied. Although several mechanisms could explain this variability, we have assumed that a large urinary ratio reflects a decreased ability of these subjects to metabolise proguanil to cycloguanil. This assumption is based on the observation that the frequency distribution profile for proguanil + cycloguanil does not exhibit a skewed distribution and the three subjects with large ratios were scattered within this profile. Therefore, variable bioavailability and potential errors associated with measurement if little of the drug was absorbed seem an unlikely explanation. Also, cycloguanil is a terminal metabolite which undergoes formation rate limited elimination with a renal clearance approximating GFR similar to that for proguanil (Wattanagoon *et al.*, 1987) and as such it would seem unlikely that variable renal elimination of cycloguanil can explain these observations. Based upon this information we suggest that the metabolism of proguanil to cycloguanil shows a polymorphic distribution in man. However, the relationship of this phenomenon to previously described polymorphisms is unknown at this time.

We have tentatively classified our subjects as 'extensive metabolisers' (EMs) of proguanil if their urinary ratios were less than 10 and 'poor metabolisers' (PMs) if this ratio was greater than 20. The plasma pharmacokinetics of proguanil and the formation of cycloguanil in our EMs is similar to data previously reported (Maegraith *et al.*, 1946; Wattanagoon *et al.*, 1987). Although peak proguanil concentrations were not significantly different in our PMs these subjects did show higher plasma proguanil concentrations in the post-distributive phase and a significantly longer elimination half-life. This resulted from a reduction in the apparent oral clearance of proguanil in these subjects. These data are not compromised by fluctuations in *F* as there was no difference in the parameter *V/F* in the two groups. It appears that this reduction in oral clearance reflects at least in part, a reduced ability to form cycloguanil, as peak metabolite concentrations in these PM subjects were significantly lower than those observed in EMs. *In vitro* sensitivity studies have indicated IC_{50} values for cycloguanil against *P. falciparum* of 5 to 150 ng ml⁻¹ (Watkins *et al.*, 1987). This would suggest that the metabolite concentrations achieved in PMs will be ineffective against many strains of *P. falciparum* which are considered totally sensitive to the biguanides.

A number of polymorphisms of oxidative drug metabolism have been demonstrated which have a genetic basis, i.e. the debrisoquine/sparteine polymorphism (Eichelbaum *et al.*, 1979; Mahgoub *et al.*, 1977) and the mephenytoin hydroxylase polymorphism (Küpfer & Preisig, 1984). The incidence of PMs in our population of 2.2% is similar to the

incidence of the mephenytoin hydroxylase polymorphism in a similar population (Wedlund *et al.*, 1984). Additionally, we have recently shown that mephenytoin can inhibit competitively the activation of proguanil to cycloguanil in human liver microsomes (Helsby *et al.*, 1990). These data indicate that the routes of metabolism for proguanil and mephenytoin cosegregate, with the observed variability in circulating cycloguanil concentrations resulting from the involvement of the mephenytoin genetic polymorphism. However, we have no direct evidence to confirm a genetic component to this variability as yet. However, it is of interest to note that proguanil possesses many of the structural requirements which have been suggested to be essential for a substrate for the mephenytoin hydroxylase enzyme (Hall *et al.*, 1987), although phenformin, itself a biguanide structurally related to proguanil, is a substrate for the enzyme involved in the debrisoquine/sparteine polymorphism (Oates *et al.*, 1981).

The present study has identified a 2.2% incidence of individuals with a reduced ability to metabolise proguanil to cycloguanil. This phenomenon has important consequences with respect to the pharmacokinetics of the parent drug and circulating concentrations of its active metabolite.

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