Br. J. clin. Pharmac. (1993), **35**, 653–656

The multiple dose pharmacokinetics of proguanil

N. A. HELSBY,[1] G. EDWARDS,[1,2] A. M. BRECKENRIDGE[1] & S. A. WARD[1]
[1]Department of Pharmacology and Therapeutics, New Medical Building, Ashton Street, University of Liverpool, P.O. Box 147, Liverpool L69 3BX and [2]Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA

Proguanil, a prophylactic antimalarial agent, is metabolised by the polymorphic isoenzyme $CYP2C_{mep}$ in man. In this study the multiple dose pharmacokinetics of proguanil were investigated in subjects who were phenotyped previously as extensive ($n = 6$) or poor ($n = 2$) metabolisers of the drug. Steady-state plasma concentrations of proguanil were achieved within 48 h in extensive metaboliser subjects and chronic administration of the drug did not appear to alter the disposition of proguanil or that of its active metabolite, cycloguanil. The currently recommended dosage regimen appears to be appropriate for extensive metabolisers of proguanil. Poor metabolisers of proguanil had significantly lower plasma concentrations of the active metabolite cycloguanil compared with extensive metabolisers. Thus, even on multiple dose administration these subjects may not achieve adequate plasma concentrations of cycloguanil. Deficient metabolism of proguanil to cycloguanil leads to an increased appearance of the *N*-dealkylated metabolite *p*-chlorphenylbiguanide in the urine of poor metabolisers.

Keywords proguanil multiple dose genetic polymorphism

Introduction

Proguanil is metabolised by cytochrome P450 isoenzymes (Armstrong, 1973; Armstrong & Smith, 1974) to an active triazine metabolite, cycloguanil (CG), which is an inhibitor of dihydrofolate reductase in the malaria parasite *P. falciparum*, and to a minor extent to an *N*-dealkylated product, *p*-chlorophenylbiguanide, which has no antimalarial activity. Previous studies (Helsby *et al.*, 1990; Ward *et al.*, 1991) have indicated that the metabolism of the inactive prodrug proguanil to its active metabolite cycloguanil is mediated by the polymorphic cytochrome P450 isoenzyme $CYP2C_{mep}$. Consequently, approximately 3% of Caucasian populations will have a relative inability to convert proguanil to its active metabolite (Helsby *et al.*, 1990). Data from a single dose study (Helsby *et al.*, 1990) suggest that these individuals may be at risk of prophylactic failure. The expression of $CYP2C_{mep}$ displays pronounced inter-ethnic variation, with 18% of Japanese deficient in this isoenzyme (Nakamura *et al.*, 1985) and possibly 25% of Kenyans (Watkins *et al.*, 1990). Thus, variable metabolism of proguanil to its active metabolite cycloguanil may have profound clincial importance in Asian and African populations at risk of malaria infection. Prophylactic failure may be particularly relevant during multiple dose administration when poor metaboliser subjects (PM) may experience sub-therapeutic concentrations of cycloguanil. Therefore, we have investigated the pharmacokinetics of proguanil given orally over 14 days to a group of individuals characterised previously as extensive metabolisers and to two PM subjects.

Methods

Protocol

Approval for the study was obtained from the Ethics Committee of the Mersey Regional Health Authority.

Healthy volunteers were designated from the ratio of proguanil (PG) to cycloguanil (CG) in an 8 h urine sample obtained after a single dose of proguanil (200 mg) as either extensive (EM; $n = 6$) or poor metabolisers (PM $n = 2$; Helsby *et al.*, 1990). Individuals with a ratio of less than 10 were classed as EM and individuals with ratios greater than 10 as PM (Helsby *et al.*, 1990). The subjects gave full informed consent to participate in this study. EM subjects (5 male, 1 female) were aged between 24 and 33 years (weight 56–76 kg). PM subjects were aged 25 and 27 years and weighed 50 and 72 kg. The urinary PG/CG ratios were EM: 0.8–5.5 and PM: 23 and 27. Each subject received PG (2 × 100 mg, p.o.; ICI Pharmaceuticals, Alderley Park, Cheshire, UK) daily for 14 days. Blood samples (10 ml) were obtained on days 1 and 14 through an i.v. catheter at the following times: predose, 1, 2, 3, 4, 6, 8 and 12 h. Samples were

Correspondence: Dr S. A. Ward, Department of Pharmacology and Therapeutics, New Medical Building, Ashton Street, University of Liverpool, P.O. Box 147, Liverpool L69 3BX

also taken at 24, 36, 48 and 72 h after dosage on day 14. Further samples were removed by venepuncture predose and 2 h later on days 3, 6, 9 and 12. Blood samples were centrifuged (2000 *g*, 15 min), plasma was removed and stored at −20°C until analysis. Random samples were stored as whole blood for analysis of drug and metabolite concentrations.

Urine collections were made on days 1, 3, 6, 9, 12, 14, 15 and 16. Volume and pH were measured, and aliquots (10 ml) were stored at −20°C until analysis.

Drug analysis

Proguanil, cycloguanil and *p*-chlorophenylbiguanide (PBG) were measured in plasma and urine by h.p.l.c. as described previously (Helsby *et al.*, 1990). Inter- and intra-assay coefficients of variation were less than 10% for all compounds in plasma, whole blood and urine. The limit of determination for all compounds was 5 ng ml^{-1}.

Data analysis

Data are presented graphically as mean + standard error of the mean (s.e. mean) and tabulated as mean ± standard deviation (s.d.).

Maximum concentrations (C_{max}) of PG, PBG and CG were determined by visual inspection of the data. The terminal elimination rate constant (λ_z) was determined by least squares regression of the post absorptive and distributive log plasma drug concentration-time data. The terminal elimination half-life ($t_{½,z}$) was calculated from the ratio $0.693/\lambda_z$. AUC values from 0–24 h (AUC_τ) at day 14 were calculated by the linear trapezoidal rule (Gibaldi & Perrier, 1982). Clearance at steady state was calculated from dose/AUC_τ.

Five subjects who took part in this study had also participated in a single dose study 12 months previously (Helsby *et al.*, 1990). Data obtained from these subjects were compared with that obtained from the present study. Statistical comparisons between the single dose data and multiple dose data were made using the unpaired Student's *t*-test. Statistical significance was accepted when $P < 0.05$.

Results

The plasma concentrations of PG and CG in EM ($n = 6$) and PM subjects (individual data) over 14 days are shown in Figure 1. Steady state concentrations of the parent drug were achieved rapidly in EMs, with mean peak concentrations of 230 ± 50 ng ml^{-1} and mean trough concentrations of 76 ± 41 ng ml^{-1}. Maximum concentrations of PG were not statistically different on chronic administration compared with single dose administration in EMs (Table 1). The mean oral clearance of PG in EMs on day 14 was 1309 ± 251 ml min^{-1} and was not significantly different ($P > 0.05$) from that in subjects following single dose administration. The mean $t_{½}$ was also not significantly different after multiple and single dose administration.

The two PM subjects also achieved steady-state rapidly, with mean peak plasma PG concentrations of 315 ± 112 ng ml^{-1} and 301 ± 66 ng ml^{-1} and mean trough concen-

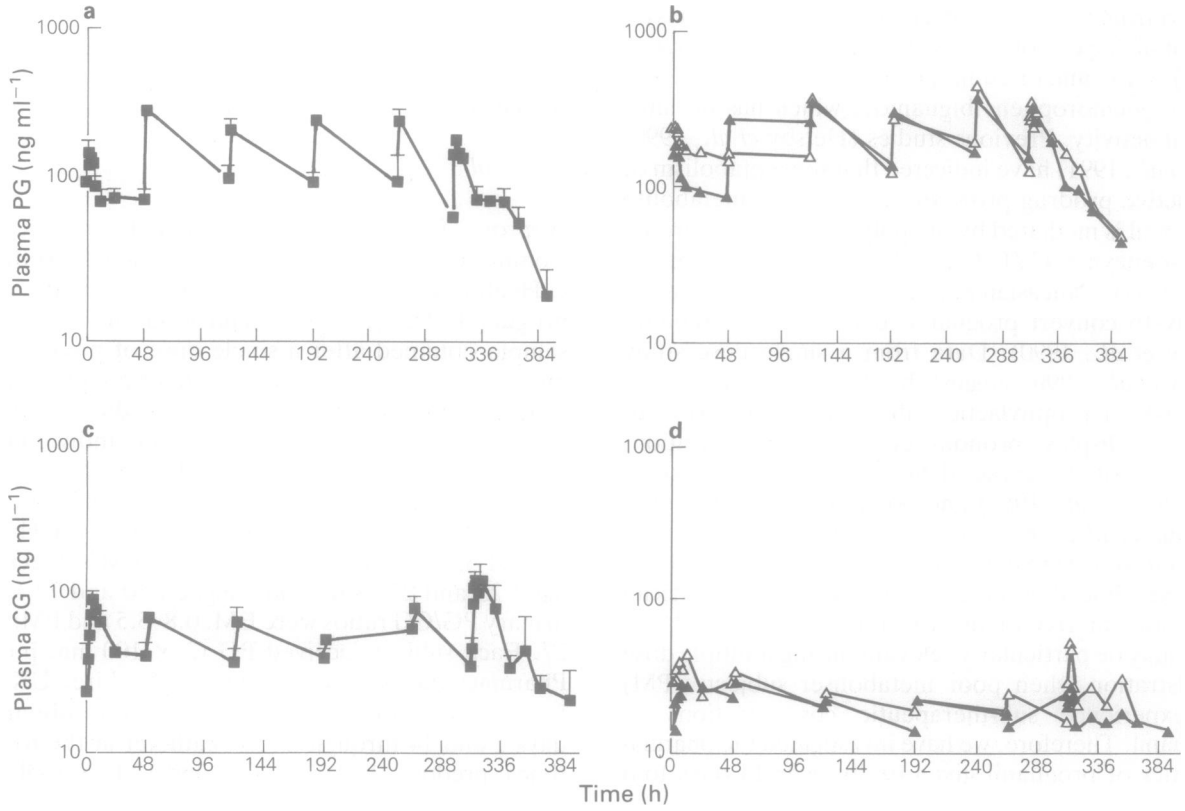

Figure 1 Mean (± s.e. mean) plasma concentrations of proguanil and cycloguanil in extensive metabolisers [■; (a) and (c)] and in two poor metabolisers [△,▲; (b) and (d)] during oral administration of 200 mg proguanil day^{-1} for 14 days.

Table 1 Pharmacokinetic parameters of proguanil in extensive and poor metabolisers on chronic administration compared with single dose administration.

	Dosage (200 mg, p.o.)	*EM mean (± s.d.) (n = 6)*	*PM 1*	*PM 2*
PG C_{max} (ng ml^{-1})	x1	209 (43)	289	256
	x14	190 (51)	348	327
CL_o (ml min^{-1} kg^{-1})	x1	858 (482)	534	245
	x14	1309 (251)	680	665
$t_{½,z}$ (h)	x1	18 (5)	31	40
	x14	21 (6)	25	25
CG AUC(0, 72 h) (μg min^{-1} ml^{-1})	x1	141 (24)	84	34
CG AUC(τ) (μg min^{-1} ml^{-1})	x14	190 (50)	74	69

trations of 125 ± 66 and 134 ± 87 ng ml^{-1}, respectively. Clearance appeared to increase, particularly in PM 2 and $t_{½}$ of PG decreased on chronic administration when compared with single dose administration.

The AUC for CG did not alter significantly on chronic administration of PG in either EM or PM subjects but was considerably lower in both PMs compared with the EMs (Table 1).

The *N*-dealkylated metabolite, *p*-chlorophenylbiguanide (PBG) could not be detected in the plasma of either EM or PM subjects. However, on days 3 to 16 it was the major metabolite in the urine of PMs. Although the appearance of PBG in the urine was highly variable it was consistently one order of magnitude greater in the poor compared with the extensive metabolisers.

Daily fluctuations in urinary pH had no effect on the PG/CG ratio. Chronic administration of PG did not effect the ratio of PG/CG.

Blood to plasma concentration ratios of PG were 2.7 ± 0.8 in EMs and 2.2 and 2.1 in the PMs. Unlike the parent compound, the active metabolite CG was not concentrated in whole blood compared with plasma, as the blood to plasma concentration ratios were 0.87 ± 0.22 in EMs and 1.3 and 0.95 in the PMs. PBG could not be detected in whole blood of either EMs or PMs.

Discussion

The metabolism of PG to the active metabolite CG is mediated by $CYP2C_{mep}$ and approximately 3% of Caucasian populations are phenotypically poor metabolisers of this drug (Helsby *et al.*, 1990; Ward *et al.*, 1991).

Following the recommended daily dose of 200 mg PG (Peto & Gilks, 1986), steady-state plasma concentrations of both the parent drug and cycloguanil, were reached within 48 h in EM subjects. Peak and trough concentrations were below levels associated with toxicity (1–2 g, Fairley, 1946) and concentrations of CG were above the minimum inhibitory concentration (10^{-9} M) for most malaria parasites sensitive to the actions of this compound (Ramanaiah & Ganjanana, 1988). The recommended dosage regimen would appear to be appropriate for EM subjects. The disposition of PG and CG appears to be similar after single and chronic dosage of PG and neither compound accumulates significantly in the plasma on multiple dosing in EMs.

In contrast to EMs, PMs developed sub-therapeutic concentrations of CG on chronic administration. Since one site of action of PG is at the erythrocytic stage of the parasite life cycle, concentrations of the active metabolite in whole blood are of interest. PG appears to be concentrated within blood cells, whereas the active metabolite CG is not. These findings are in agreement with those of Maegraith *et al.* (1946).

We conclude that the currently recommended dosage regimen for PG is suitable for EM subjects. However, the disposition of PG in PM subjects appears to be more complex and an interpretation of the data is compromised by the limited number of PM subjects available for study. Concentrations of the active metabolite in PM subjects are low in comparison with EM subjects even on chronic administration. The deficient metabolism of PG to CG in PM subjects appears to result in an increased production of the inactive *N*-dealkylated metabolite, PBG.

This work was supported by the UNDP/World Bank/World Health Organisation Special Programme in Research and Training in Tropical Diseases.

References

Armstrong, V. L. (1973). Metabolism of chlorguanide, a unique mixed function oxidase substrate. *Diss. Abstr.*, **33**, 3832B.

Armstrong, V. L. & Smith, C. C. (1974). Cyclisation and N-dealkylation of chlorguanide by rabbit and rat hepatic microsomes. *Toxicol. appl. Pharmac.*, **29**, 90.

Fairley, N. H. (1946). *Researches on paludrine (M4888) in malaria.* An experimental investigation undertaken by LHQ medical research unit (AIF), Cairns, Australia. *Trans. Roy. Soc. Trop. Med. Hyg.*, **40**, 105–151.

Gibaldi, M. & Perrier, D. (1982). Pharmacokinetics. In *Drugs and pharmaceutical sciences*, (2nd edition) ed. Swarbrick, J. L. New York: Marcel Dekker International.

Helsby, N. A., Ward, S. A., Edwards, I. G., Howells, R. E. & Breckenridge, A. M. (1990). The pharmacokinetics and activation of proguanil in man: consequences of variability in drug metabolism. *Br. J. clin. Pharmac.*, **30**, 593–598.

Maegraith, B. G., Tottey, M. M., Adams, A. R. D., Andrews, W. H. H. & King, J. D. (1946). The absorption and excretion of Paludrine in the human subject. *Ann. Trop. Med. Parasitol*, **40**, 493–506.

Nakamura, K., Goto, F., Ray, W. A., McAlister, C. B. &

Jacqz, E. (1985). Inter-ethnic differences in genetic polymorphism of debrisoquine and mephenytoin hydroxylation in Japanese and Caucasian populations. *Clin. Phamac. Ther.*, **38**, 402–408.

Peto, T. & Gilks, C. (1986). Strategies for the prevention of malaria in travellers; comparison of drug regimens by means of risk:benefit analysis. *Lancet*, **i**, 1256–1261.

Ramanaiah, T. V. & Ganjanana, A. (1988). Superior antimalarial activity of proguanil to cycloguanil after *in vitro* bioconversion. *Trans. Roy. Soc. Trop. Med. Hyg.*, **82**, 358–359.

Ward, S. A., Helsby, N. A., Skjelbo, E., Brosen, K., Gram, L. F. & Breckenridge, A. M. (1991). The activation of the biguanide antimalarial proguanil co-segregates with the mephenytoin oxidation polymorphism–a panel study. *Br. J. clin. Pharmac.*, **31**, 689–692.

Watkins, W. M., Mberu, E. K., Nevill, C. G., Ward, S. A., Breckenridge, A. M. & Koech, D. K. (1990). Variability in the metabolism of proguanil to its active metabolite cycloguanil in healthy Kenyan adults. *Trans. Roy. Soc. Trop. Med. Hyg.*, **84**, 492–495.

(Received 28 May 1992, accepted 25 January 1993)