PHARMACOKINETICS OF PROGUANIL IN MALARIA PATIENTS TREATED WITH PROGUANIL PLUS ATOVAQUONE

MD Edstein[1], S Looareesuwan[2], C Viravan[2] and DE Kyle[3]

[1]Army Malaria Research Unit, Sydney, Australia; [2]Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; [3]Division of Experiment Therapeutics, Walter Reed Army Institute of Research, Washington DC, USA

Abstract. Clinical studies have shown atovaquone (ATQ), a new blood schizontocidal drug, in combination with proguanil (PROG) to be very effective in the treatment of acute multidrug-resistant falciparum malaria. The multiple dose pharmacokinetics of PROG were determined in Thai patients with acute falciparum malaria given PROG alone (200 mg PROG twice a day for 3 days, n = 4) and concurrently PROG and ATQ (200 mg PROG and 500 mg ATQ twice a day for 3 days, n = 12). There were no statistical differences ($p > 0.05$) in the area under the plasma drug concentration-time curve (AUC), apparent oral clearance (CL/F) and elimination half-life ($t_{1/2}$) of PROG between patients given PROG alone and PROG/ATQ. The median (range) kinetic values of PROG in patients given PROG alone and PROG/ATQ were respectively: CL/F = 1.25 l/h/kg (0.99-1.45) and 0.95 (0.73-1.32) l/h/kg, and $t_{1/2}$ = 14.2 hours (9.3-16.8) and 13.6 hours (9.1-17.6). The CL/F and $t_{1/2}$ of PROG in the Thai patients treated with the 2 treatment regimens were also comparable to values reported in healthy Thai volunteers given a standard prophylactic dose (200 mg PROG). The results of this preliminary study suggest that ATQ is unlikely to affect the pharmacokinetics of PROG to a clinically important extent at an ATQ dosage of 500 mg twice a day for 3 days in malaria infected patients.

INTRODUCTION

Recently, proguanil (PROG) has been reported to potentiate the schizonticidal action of atovaquone (ATQ, formally 566C80), a new hydroxynaphthoquinone. PROG combined with ATQ is being developed by the Wellcome Research Laboratories (UK) for the treatment of acute multidrug-resistant falciparum malaria. Phase II studies have shown combinations of PROG/ATQ to be both effective and safe in the treatment of acute multidrug-resistant falciparum malaria (Looareesuwan *et al*, 1996). It has been reported that PROG/ATQ cured a patient with 4 episodes of falciparum malaria who had previously failed conventional treatment with quinine/mefloquine, quinine/tetracycline, and quinine/halofantrine (Blanchard *et al*, 1994).

PROG and ATQ act synergistically against the erythrocytic stages of the malaria parasite (Canfield *et al*, 1995). PROG mode of action is through its dihydrotriazine metabolite cycloguanil (CYC, Carrington *et al*, 1951) which blocks the folic acid pathway of the malaria parasite by inhibition of *Plasmodia* dihydrofolate reductase. ATQ acts by binding to the parasite's ubinquinol-cytochrome *c* reductase region of the mitochondrial respiratory chain, thus resulting in blockade of pyrimidine biosynthesis (Fry and Beesley, 1991).

PROG activation to CYC is by hepatic cytochrome P450 (CYP) isoenzymes. It has been demonstrated that PROG metabolism is mediated both by S-mephenytoin hydroxylase and isoforms of the CYP3A subfamily (Ward *et al*, 1991; Birkett *et al*, 1994). In contrast to PROG, there is no evidence that ATQ is metabolized in man with removal primarily via enterohepatic recirculation and elimination through the feces (Haile and Flaherty, 1993). When drugs are co-administered there is a need to determine whether a drug-drug interaction occurs and, if so, does it lead to differences in the therapeutic response. As part of a series of dose-finding and efficacy studies of PROG/ATQ combinations we determined the disposition of PROG in malaria patients given concurrently PROG and ATQ,and compared these findings in patients given PROG alone.

Correspondence: Dr MD Edstein, Army Malaria Research Unit, Liverpool Military Area, NSW 2174, Australia. Fax: 61-2-618-4596

MATERIALS AND METHODS

Phase II studies of PROG given alone and in combination with ATQ were approved by the Ethical Committee of Mahidol University, Bangkok. Thailand (Looareesuwan *et al*, 1996). Three hundred and seventeen adult Thai male patients with acute falciparum malaria (confirmed by examination of peripheral blood smear) gave written informed consent to participate in the series of dose-ranging studies. All patients had no history of liver or kidney diseases.

As part of the series of dose-ranging studies (Looareesuwan *et al*, 1996), blood samples were collected from 2 cohorts of patients treated with PROG alone and PROG/ATQ. Group 1 patients (aged 20-40 years; weighing 46-57 kg; n = 4) received 200 mg PROG (2 Paludrine® tablets, ICI Pharmaceuticals UK, each tablet containing 100 mg PROG hydrochloride) twice a day for 3 days. Group 2 patients (aged 18-40 years; weighing 40-59 kg; n = 12) were selected from 30 patients given concurrently 200 mg PROG and 500 mg ATQ (Wellcome Foundation Ltd, UK, each tablet containing 250 mg ATQ) twice a day for 3 days. The drugs were given 45 minutes after ingestion of liquid nourishment, usually consisting of Ovaltine®,fruit drink or soya milk. The initial median asexual parasite count per µl of blood were 316 (range 60-4, 130) for Group 1 and 9,782 (range 2,800-28,620) for Group 2.

Drug administration and blood collections

The patients were admitted to the Bangkok Hospital for Tropical Diseases, Thailand. During the first 24 hours after onset of treatment an indwelling cannula was inserted into a forearm vein and kept patent with heparinized saline. Subsequent blood samples were collected by venepuncture into heparinized tubes. Whole blood samples (10 ml) were collected immediately prior to and at 1, 2, 4, 8, 16, 24, 48, 72, 96 and 120 hours after commencing drug administration Blood samples were centrifuged at 1,200*g* for 10 minutes, plasma separated and stored at -70° until analysed.

Drug analysis

Plasma PROG and CYC concentrations were measured by high performance liquid chromatography (HPLC) by the method of Shanks and others (1994). The inter-assay coefficients of variation for PROG and CYC were 11% at 10 ng/ml (n = 6), with the limit of detection of 1 ng/ml for both compounds. ATQ concentrations were also measured by HPLC (Rolan *et al*, 1994). The inter-and intra-assay coefficients of variation of ATQ at 25 ng/0.25 ml (n = 5) were less than 14.5%. The limit of detection was 10 ng/0.25 ml for ATQ.

Pharmacokinetics and statistical analysis

Pharmacokinetic analysis was by non-compartmental methods (Gibaldi and Perrier, 1982). The elimination rate constant (k_{el}) was determined by least-squares regression analysis of the plasma drug concentration-time curve and the elimination half-life ($t_{1/2}$) from ln $2/k_{el}$. The area under the concentration-time curve ($AUC_{0\rightarrow t}$) was calculated to the last sample point (t) using trapezoidal rule integration. The $AUC_{t\rightarrow\infty}$ was calculated as C_t/k_{el}, where C_t was the last measured concentration. Total $AUC_{0\rightarrow\infty}$ was then determined as the sum of $AUC_{0\rightarrow t}$ and $AUC_{t\rightarrow\infty}$. The apparent oral clearance (CL/F), expressed as a function of bioavailability (F), was calculated as the dose divided by AUC. For statistical analysis the Mann-Whitney *U*-test was used and a value of $p < 0.05$ was taken as significant. Data are presented graphically as mean ± standard error of the mean (SEM) and in the text as mean ± standard deviation (SD).

RESULTS

The mean (± SEM) plasma concentration-time profiles of PROG in patients given PROG alone and PROG/ATQ are presented in Fig 1. The plasma profiles of PROG were similar for patients administered the 2 PROG treatment regimens. The pharmacokinetic parameters of PROG for the individual patients and the median values are listed in Table 1. For comparison purposes, the 4 hours post-first dose plasma concentration of PROG for each Thai patient was included as this time closely corresponds to the maximum plasma concentration of PROG following a single dose of 200 mg PROG in healthy Thai volunteers (Wattanagoon *et al*, 1987). There were no statistical differences ($p > 0.05$) in the 4 hours post-first dose concentration, oral clearance and elimination half-life of PROG between patients administered PROG alone and

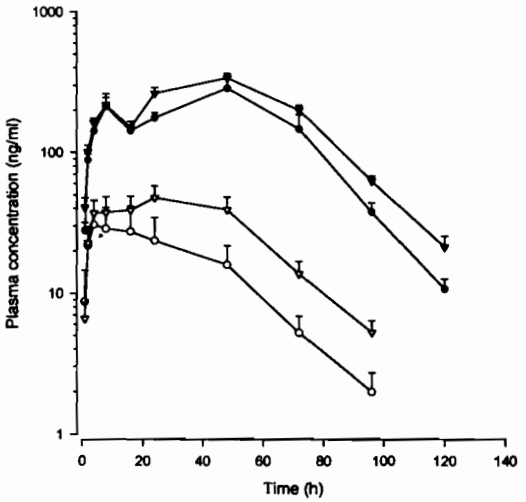

Fig 1–Mean (± SEM) plasma PROG concentrations in 4 Thai patients with falciparum malaria following administration of PROG (200 mg twice daily for 3 days) alone (PROG - ●; CYC - O) and in 12 patients after co-administration of PROG (200 mg) and ATQ (500 mg) twice daily for 3 days (PROG - ▼; CYC - ∇).

PROG/ATQ. The elimination half-life of PROG was estimated from 18 hours after the last dose of PROG. Although the median AUC of PROG in patients given PROG/ATQ was higher than PROG alone (21.7 *vs* 16.8 μg h/ml), the difference was not statistically significant ($p = 0.11$).

All patients given PROG/ATQ had measurable plasma concentrations of ATQ at 4 and 72 hours after commencement of treatment. The inter-individual variability in plasma ATQ concentrations was large, with median concentrations of 908 ng/ml (range 576-3,024) at 4 hours and 2,830 ng/ml (range 875-5,884) at 72 hours after onset of treatment.

DISCUSSION

Of the 2 regimens reported in the present study, the PROG/ATQ combination was the most efficacious with a cure rate of 93% compared with no cures in patients given PROG alone (Looareesuwan *et al*, 1996). Comparison of the pharmacokinetic parameters of PROG in patients administered PROG alone versus those who received concurrently PROG and ATQ revealed no statistical differences ($p > 0.05$) with respect to the 4 hours post-first dose concentration of PROG, AUC, oral clearance and elimination half-life. These findings suggest that ATQ does not appear to alter the kinetics of PROG.

At 4 hours post-first dose, the median PROG concentrations for the 2 groups of patients were similar (152 ng/ml for PROG alone *vs* 170 ng/ml for PROG/ATQ) and accord with the median level of 170 ng/ml (range 150-220) observed in healthy Thai volunteers at 2-4 hours after a standard prophylactic dose of 200 mg PROG (Wattanagoon *et al*, 1987). It is also interesting to note that after adjusting for dose differences, the mean AUC of PROG in the 4 patients given 1,200 mg PROG (17.6 μg h/ml) was comparable to that estimated in healthy Thai volunteers administered 200 mg PROG (2.98 μg.h/ml, Wattanagoon *et al*, 1987). Due to the experimental design of the efficacy study the maximum concentration of PROG after the last dose could not be determined. The mean oral clearance of PROG was similar in patients receiving the 2 regimens (PROG-1.23 l/h/kg; PROG/ATQ-0.98 l/h/kg) and in close agreement to that estimated in healthy Thai volunteers (1.15 l/h/kg, Wattanagoon *et al*, 1987). The mean elimination half-life of PROG in the malaria patients following PROG alone (13.6 ± 3.2 hours) and PROG/ATQ (13.4 ± 2.2 hours) were comparable to healthy Thai volunteers (16.1 ± 2.9 hours, Wattanagoon *et al*, 1987). No comparison was made between the 2 groups CYC data as metabolism of PROG to CYC is highly variable both between and within poor and extensive metabolisers of PROG (Ward *et al*, 1989).

Plasma concentrations of ATQ were measured to confirm absorption of the drug in the patients administered PROG/ATQ. ATQ is a lipophilic compound with extremely low aqueous solubility (< 100 ng/ml) and, because of its physicochemical properties, ATQ exhibits considerable variation in bioavailability which is both limited and sensitive to food intake (Rolan *et al*, 1994; Hughes *et al*, 1991). This variation in absorption was reflected in the wide range of plasma ATQ concentrations measured in the patients. Substantial improvement in the absorption of ATQ has been noted in healthy volunteers after a fatty meal, but not all fats increase absorption equally (Rolan *et al*, 1994). The effect of dietary fat on the absorption of ATQ in healthy and malaria infected Thai patients is unknown.

Table 1

Pharmacokinetic parameters of PROG in Thai patients with falciparum malaria following treatment with PROG alone and PROG/ATQ.

Patient	4 h (ng/ml) PROG	$AUC_{0\rightarrow\infty}$ (μg h/ml) PROG	CL/F (l/h/kg) PROG	$t_{½}$(h) PROG
PROG Alone:				
1	139	18.38	1.12	13.4
2	79	13.49	1.37	15.0
3	164	23.19	0.99	9.3
4	188	15.30	1.45	16.8
Median	152	16.84	1.25	14.2
PROG/ATQ:				
1	177	19.02	1.06	11.4
2	178	17.76	1.21	14.0
3	104	20.92	0.93	15.4
4	211	27.97	0.74	13.2
5	130	19.92	1.32	12.6
6	114	16.37	1.26	9.1
7	144	32.23	0.73	15.1
8	95	22.05	0.96	13.8
9	237	21.25	0.84	11.2
10	179	30.29	0.72	13.7
11	220	23.19	1.03	13.5
12	163	22.60	0.91	17.6
Median	170	21.65	0.95	13.6

Differences between the kinetic parameters following administration of PROG alone and PROG/ATQ are not significant ($p > 0.05$).

To date, no drug-drug interaction has been reported between PROG and ATQ. *In vitro* studies of hepatic microsomes from various species, including human microsomes, and time-course studies against *P. yoelii* in mice showed that ATQ is a metabolically stable compound, with no evidence of ATQ being rapidly oxidatively metabolized in man (Hudson *et al*, 1991). ATQ is extensively plasma protein bound (> 99.9%, Haile and Flaherty, 1993) and may compete with other highly protein-bound drugs for protein-binding sites. Unlike ATQ, PROG is not highly protein bound (75%, Pharmaceutical Codex, 1979) and plasma concentrations of both PROG and CYC are considerably lower than ATQ levels. Thus, the likelihood of a drug-drug interaction between PROG and ATQ appears to be remote.

Ideally to assess for a drug-drug interaction a randomized two-period cross-over design with an appropriate washout period between treatments is required. Although such studies are normally done in healthy volunteers, it is most difficult to perform in malaria infected individuals. This study, being part of an investigation into the efficacy of PROG/ATQ was opportunistic in that the sampling schedule, although not ideal, was still adequate to compare the pharmacokinetic parameters of PROG in malaria patients given concomitantly PROG and ATQ, and in individuals administered PROG alone.

We conclude that at a ATQ dosage of 500 mg twice a day for 3 days, ATQ does not appear to interact kinetically with PROG. However, the pos-

sibility of a metabolic or pharmacodynamic interaction with PROG cannot be excluded. When compared with healthy Thai volunteers, the oral clearance and the elimination half-life of PROG in the Thai patients were similar which tends to imply that the disease state may not affect the clearance of PROG.

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