Interactions among primaquine, malaria infection and other antimalarials in Thai subjects

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- 1 The pharmacokinetics of *rac*-primaquine (45 mg base) and its principal plasma metabolite, carboxyprimaquine have been investigated in healthy Thai adults prior to and following a single oral dose of mefloquine (10 mg kg⁻¹).
- 2 Primaquine was rapidly absorbed, attaining peak plasma concentrations (median and range) of 167 (113–532) µg l⁻¹ in 2 (1–4) h. Thereafter, concentrations declined rapidly with an apparent terminal half-life of 6.1 (1.7–16.1) h and an oral clearance (CL_{po}) of 33.1 (17.6–49.3) l h⁻¹. Administration of mefloquine had no effect on the values of any of these parameters at the 5% level of significance [*C*_{max} 229 (114–503) µg l⁻¹; *t*_{max} 3 (2–4) h; *t*_{1/2,z} 3.9 (1.7–13.5) h; CL_{po} 34.0 (21.7–49.0) l h⁻¹].
- 3 The carboxylic acid metabolite of primaquine achieved maximum concentrations (median and range) of 890 (553–3634) µg l⁻¹ at 6 (3–16) h. Thereafter, plasma concentrations of carboxyprimaquine declined to 346 (99–918) µg l⁻¹ at 24 h. AUC (0,24 h) was 12737 (6837–27388) µg l⁻¹ h. Administration of mefloquine had no effect on the plasma concentrations of this metabolite [*C*_{max} 1035 (174–3015) µg l⁻¹; *t*_{max} 8 (2–24) h; AUC(0,24) 13471 (2132–17863) µg l⁻¹ h].
- 4 The effect of falciparum malaria and treatment with quinine (10 mg salt kg⁻¹ p.o.) on the pharmacokinetics of primaquine (45 mg base p.o.) has been investigated in adult Thai patients during and after infection with falciparum malaria. Acute falciparum malaria was associated with a statistically significant (*P* < 0.05) reduction in oral clearance (l h⁻¹; median and range) of primaquine from 21.3 (15.9–73.0) to 19.4 (9.3–24.7).
- 5 The area under the curve (AUC(0, 24 h); µg l⁻¹ h) for the carboxylic acid metabolite of primaquine (median and range) was significantly greater (*P* < 0.05) following the administration of primaquine alone (7533; 4876–18545) relative to the combination of quinine and primaquine (3831; 2144–15882).

Keywords primaquine malaria antimalarials

Introduction

Since its introduction forty years ago primaquine [(±8-4-amino-1-methylbutylamino)-6-methoxyquinoline] has been the drug of choice for the treatment of exo-erythrocytic stages of *Plasmodium vivax* and *Plasmodium ovale*. Primaquine is gametocytocidal against *Plasmodium falciparum* and often is given with either mefloquine or quinine to interrupt transmission of the infection. All of

these compounds have the propensity to inhibit hepatic drug metabolising enzyme systems (Mihaly *et al.*, 1985a; Murray, 1987; Riviere & Back, 1985; Suphakawanich & Thithapandha, 1987) and there is therefore the possibility of a drug interaction of clinical importance. In addition, malaria infection *per se* has been shown to alter hepatic drug metabolism and pharmacokinetics *in vitro* (Alvares

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et al., 1984), in the rat isolated perfused liver (Mansor *et al.*, 1990) and in man (White *et al.*, 1982). We have evaluated the pharmacokinetic properties of primaquine, and its conversion to carboxyprimaquine, the major plasma metabolite in man (Mihaly *et al.*, 1984) in healthy adult Thai volunteers given oral mefloquine, and in adult Thai patients treated with quinine for acute falciparum malaria.

Methods

The studies were carried out at the Faculty of Tropical Medicine, Mahidol University, Bangkok and Polphaholpayuhasera Hospital, Kanchanaburi, Thailand. Each of the patients and volunteers gave informed consent to the procedure which was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University.

Study 1: Investigation of the effect of mefloquine on the pharmacokinetics of primaquine in healthy volunteers

Nine healthy fasting adult male Thai volunteers (age range 21–38 years; weight 53–65 kg) were admitted to the study after a full physical examination and routine haematological and biochemical screens. Each received a single oral dose of *rac*-primaquine (45 mg base; ICI Pharmaceuticals, Alderley Park, Cheshire, UK). Venous blood (10 ml) was collected into heparinised tubes before drug administration and subsequently at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16 and 24 h. Plasma was removed immediately following centrifugation (1200 g; 15 min) and frozen (-20°C) prior to transportation to Liverpool for analysis. One week later, the study was repeated, except that on this occasion, each volunteer received a single oral dose of mefloquine (10 mg kg^{-1} Lariam[®], Roche) at the same time as the primaquine.

Study 2: Effect of falciparum malaria and treatment with quinine on the pharmacokinetics of primaquine

Fourteen adult male Thais with acute falciparum malaria were admitted to the study. However, owing to withdrawals and difficulties in following up subjects, only seven individuals completed the course. All those admitted underwent a full physical examination and each had routine blood chemistry analysis and a biochemical screen. Each received primaquine (45 mg p.o.) as a single dose in addition to routine treatment with oral quinine sulphate (10 mg salt kg^{-1} three times daily; Government Pharmaceutical Organisation of Thailand). One month later, each subject received, after recovery from infection, a further single oral dose of primaquine (45 mg) with quinine (10 mg kg^{-1} three times daily) given simultaneously. Finally, 10 days later, the same single dose of primaquine was given without quinine. On each occasion, the blood sampling schedule was as described above.

Analysis of primaquine and carboxyprimaquine in plasma

Primaquine and carboxyprimaquine were assayed by h.p.l.c. methods using ultraviolet detection (Mihaly *et al.*, 1984). Inter- and intra-assay variation (i.e. assay precision) for each procedure was $< 10\%$ and the limits of detection were $1 \mu\text{g l}^{-1}$ (primaquine) and $75 \mu\text{g l}^{-1}$ (carboxyprimaquine).

Pharmacokinetic calculations and data analysis

Peak plasma drug concentration (C_{max}) and the time at which it was reached (t_{max}) were noted directly. The apparent terminal phase elimination rate constant (λ_z) was determined by least squares regression analysis of the post-absorptive and distributive log plasma drug concentration vs time data using at least four points, and the terminal phase elimination half-life ($t_{1/2,z}$) was calculated from the ratio $0.693/\lambda_z$.

The area under the plasma drug concentration vs time curve up to the last data point (C_{last}) was calculated by the linear trapezoidal rule and where necessary was extrapolated to infinity using the ratio ($C_{\text{last}}/\lambda_z$). Oral clearance (CL_{po}) was calculated from the ratio of dose/AUC.

Statistical comparisons were made using the Wilcoxon signed rank test (Bolton, 1984) accepting a difference at the 5% level as significant.

Results

The effect of oral mefloquine on the pharmacokinetics of primaquine in healthy volunteers

The mean ($\pm$ s.d.) plasma concentrations of primaquine and carboxyprimaquine in the presence and absence of mefloquine are shown in Figure 1 (a and b). After oral dosage, peak plasma primaquine concentrations (median and range) of 167 (113 – 532) $\mu\text{g l}^{-1}$ were reached within 2 (1–4) h. Thereafter, drug concentrations declined rapidly with an apparent terminal elimination half-life of 6.1 (1.7–16.1) h and an oral clearance of 33.1 (17.6 – 49.4) l h^{-1} . After administration of a single oral dose of mefloquine (10 mg kg^{-1}) these plasma primaquine concentrations and associated parameters were similar to those seen after the control dose (Figure 1b; Table 2).

The carboxylic acid metabolite of primaquine was detected in plasma within 2 h of dosage of the parent drug and maximum concentrations of this metabolite (median and range) of 890 (553 – 3634) $\mu\text{g l}^{-1}$ were

Table 1 Admission details of those patients completing Study 2 [$n = 7$; mean $\pm$ s.d. (range)]

Age (years)	39.8 ± 5.7	(35–48)
Male/Female	6/1	
Weight (kg)	51.0 ± 4.9	(43–52)
Temperature ($^{\circ}\text{C}$)	39.1 ± 1.4	(37.0–40.5)
Haematocrit (%)	36.6 ± 7.6	(21–44)
Parasite count* (μl^{-1})	32028	(960–912610)

* Median.

Figure 1 Plasma concentrations (mean $\pm$ s.d.) of primaquine ($\square$) and carboxyprimaquine ($\triangle$) in nine healthy Thai male volunteers receiving either primaquine alone (45 mg p.o., Figure 1a) or in combination with mefloquine (10 mg kg⁻¹ p.o., Figure 1b).

reached at 6 (3–16) h. Thereafter, plasma concentrations of carboxyprimaquine declined slowly to 346 (99–918) $\mu\text{g l}^{-1}$ at 24 h. Administration of mefloquine had no effect on the plasma concentrations of this metabolite and there was no significant change in the values of any of the pharmacokinetic parameters for carboxyprima-

Table 2 Pharmacokinetic parameters (median and range, $n = 9$) for primaquine and carboxyprimaquine following oral administration of primaquine (45 mg) with and without mefloquine (10 mg kg⁻¹ p.o.)

Pharmacokinetic parameter	Treatment	
	Primaquine	Primaquine and mefloquine
Primaquine		
$C_{\max}$ ($\mu\text{g l}^{-1}$)	167 (113–532)	229 (114–503)
$t_{\max}$ (h)	2 (1–4)	3 (2–4)
CL _{po} (l h^{-1})	33.1 (17.6–49.3)	34.0 (21.7–49.0)
$t_{1/2,z}$ (h)	6.1 (1.7–16.1)	3.9 (1.7–13.5)
Carboxyprimaquine		
$C_{\max}$ ($\mu\text{g l}^{-1}$)	890 (553–3634)	1035 (174–3015)
$t_{\max}$ (h)	6 (3–16)	8 (2–24)
AUC(0,24) ($\mu\text{g l}^{-1} \text{h}$)	12737 (6837–27388)	13471 (2132–17863)

Figure 2 Plasma concentrations (mean $\pm$ s.d.) of primaquine ($\square$) and carboxyprimaquine ($\triangle$) in seven (primaquine) and six (carboxyprimaquine) adult male Thais a) with acute falciparum malaria, b) when fully recovered together with quinine (10 mg kg⁻¹ three times daily) and c) in convalescence.

quine calculated after the second phase of the study (Table 2).

Clinical response to treatment in malaria patients

A summary of admission clinical details is given in Table 1. There were no biochemical abnormalities. All patients made a full recovery as assessed by complete clearance of fever and parasitaemia. There were no complications and no adverse effects were apparent.

The effect of malaria infection and treatment with quinine on the pharmacokinetics of primaquine in patients with acute falciparum malaria

Mean plasma concentrations of primaquine and carboxyprimaquine ($\pm$ s.d.) during each phase of the study are shown in Figure 2 (a, b and c). Seven patients attended for all of these studies. Estimates of the pharmacokinetic

Table 3 Pharmacokinetic parameters for primaquine and carboxyprimaquine (median and range) following oral administration of primaquine a) in malaria infection, b) when fully recovered together with quinine (10 mg kg⁻¹ three times daily) and c) convalescence, without quinine

Pharmacokinetic parameter	Treatment		
	(a)	(b)	(c)
Primaquine (n = 7)			
C _{max} (µg l ⁻¹)	233 (144–489)	295 (64–308)	271 (147–431)
t _{max} (h)	4 (2–8)	2 (1.5–4)	3 (1.5–4)
CL _{po} (l h ⁻¹)	19.4 (9.3–24.7)	21.3 (15.9–73.0)	24.8 (12.6–48.4)
t _{1/2,z} (h)	4.9 (2.8–9.1)	5.1 (1.4–11.6)	3.5 (2.7–7.9)
Carboxyprimaquine (n = 6)			
C _{max} (µg l ⁻¹)	345 (145–580)	343 (185–875)	600 (380–1055)
t _{max} (h)	4 (0.5–8)	4 (1.5–24)	8 (3–24)
AUC(0,24) (µg l ⁻¹ h)	4306 (1680–12030)	3831 (2144–15882)	7533 (4876–18545)

variables for primaquine and carboxyprimaquine (median and range) are given in Table 3. Acute falciparum malaria was associated with a reduction in the oral clearance of primaquine. While *t*_{1/2} was unaffected, there was a decrease in *t*_{max} in five out of six subjects and in four of the subjects *C*_{max} was decreased. When primaquine alone was administered, there were no significant changes in any of the pharmacokinetic parameters compared with those obtained after the combination of quinine and primaquine. The median of AUC(0,24) for carboxyprimaquine was significantly greater following the administration of primaquine alone compared with primaquine and quinine in combination. The effect of quinine on the pharmacokinetics of the carboxylic acid metabolite is supported by the observation that *C*_{max} for carboxyprimaquine was greater in five out of six individuals than the value obtained during malaria or following its treatment with quinine. Generally, there was a greater degree of intersubject variability in drug disposition in this second study than was apparent from the results of Study 1 (Table 3, Figure 2).

Discussion

Primaquine, in contrast to other available quinoline antimalarials, can kill the gametocytes of *P. falciparum* and can interrupt malaria transmission. There is, therefore, a potential benefit to the community. However, there is no benefit to the patient and some risk to the individual from oxidant haemolysis or other primaquine toxicity which may relate in part to the plasma concentrations of the drug (White & Krishna, 1989). In any situation where the patient is exposed to higher circulating concentrations of primaquine there is potential for bio-

transformation to oxidising metabolites and toxicity. It has been demonstrated that the metabolic profile of primaquine is a balance between activation to various hydroxylated derivatives (Strother *et al.*, 1981, 1984, 1987), which are probably responsible for the observed pharmacological and toxicological properties of the drug, and conversion of the pharmacologically inactive carboxyprimaquine (Peters & Robinson 1987). This compound has been shown to be the major plasma metabolite in man (Mihaly *et al.*, 1984) although it is not excreted in urine, and the unchanged drug represents a relatively minor proportion of total radioactivity excreted following the administration of [¹⁴C]-primaquine to a panel of human subjects (Mihaly *et al.*, 1985b). Malaria infection alters the pharmacokinetics and metabolism of a number of drugs in animals (Alvares *et al.*, 1984, Mansor *et al.*, 1990) and in man (White *et al.*, 1982). Thus it is conceivable that alterations in the clinical condition of patients during treatment of *P. falciparum* malaria might influence the pharmacokinetics and pharmacodynamics of primaquine. Although Bhatia *et al.* (1986) could not demonstrate any effect of infection with *Plasmodium vivax* on the pharmacokinetics of primaquine, their study did not define either a level of parasitaemia or the severity of the infection. The present investigation has demonstrated that the oral clearance of primaquine is reduced significantly during infection with *P. falciparum* malaria and we can be 95% confident that the true value of the ratio CL_{po} (infection):CL_{po} (recovery) lies between 0.42 and 0.90. While the withdrawals reduced the power of the study, it was still capable of detecting, with 70–80% power at the 5% level of significance, changes in CL_{po} which might be expected to occur based on the findings of White *et al.* (1982) from investigations into the effect of malaria on the pharmacokinetics of quinine in Thai patients. Although the most plausible

explanation for this change in the oral clearance of primaquine is an impairment in hepatocyte function and a deterioration in the activity of drug metabolising enzyme systems, a reduction in absorption across the gut wall cannot be excluded. In addition, it should be noted that unbound concentrations of primaquine were not measured in this study. The fraction unbound of primaquine is not documented. Since it is a basic drug, it might be expected to bind to α_1 -acid glycoprotein, an acute phase protein the plasma concentrations of which are elevated during infection with malaria (Mansor *et al.*, 1991). An increased plasma binding would be expected to result in increased total plasma concentrations of primaquine but not necessarily any increase in free drug concentrations.

Drug-drug interactions may also contribute to alterations in pharmacokinetics and response. While mefloquine failed significantly to influence the conversion of primaquine to its carboxylic acid metabolite [mean ratio CL_{po} (mefloquine):CL_{po} (control) = 1.09; 95% confidence interval, 0.73–1.45] it appeared that the production and/or elimination of this compound was influenced by treatment with quinine [mean ratio AUC(0,24) (convalescence, no quinine):AUC(0,24) (recovery, with quinine) = 3.23; 95% confidence interval 1.18–5.28]. It is known

from investigations in the isolated perfused liver rat preparation (Nicholl *et al.*, 1987) that the formation of the carboxylic acid metabolite of primaquine is enantio-selective. (–)primaquine is cleared more extensively to carboxyprimaquine than is the (+)enantiomer.

We conclude that the pharmacokinetics of primaquine and its carboxylic acid metabolite are altered during malaria infection. Treatment with mefloquine, in agreement with findings from studies in animals (Parkhurst *et al.*, 1987) had no statistically significant effect on the pharmacokinetics of primaquine. Although the power of this study was not calculated *a priori*, it was possible based upon the results obtained, to estimate that, at the 5% level of significance, a difference of 40% in CL_{po} could confidently be detected in nine subjects with a power of approximately 70–80%. Finally, there appears to be an influence of quinine treatment on the plasma concentrations of carboxyprimaquine that is statistically significant but the mechanism(s) involved and its clinical importance remain to be elucidated.

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