PHARMACOKINETICS OF PRIMAQUINE IN HEALTHY VOLUNTEERS

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Abstract. The pharmacokinetics of primaquine were investigated in 8 healthy subjects (4 males and 4 females). The volunteers received 15 mg base of primaquine daily for 14 days. The results showed that the concentration-time profiles in whole blood and in plasma were similar. The mean values (± SD) of area under the curve (AUC) of the last dose were significantly decreased when compared to the values of the first dose both in whole blood and in plasma (909.96 ± 603.07, 1,147.05 ± 684.8 ng.hr/ml respectively in whole blood with p = 0.007 and 1,255.11 ± 531.59, 1,603.66 ± 505.45 ng. hr/ml respectively in plasma with p = 0.023).

The decrease in the concentration-time profile of the last dose was due to enhancement of drug elimination with significant increase in clearance after the last dose (4.871 ± 1.741 and 6.443 ± 2.514 ml/min/kg respectively in whole blood with p = 0.007, 3.199 ± 1.197 and 4.422 ± 2.068 ml/min/kg respectively in plasma with p = 0.016).

INTRODUCTION

Primaquine, an 8-aminoquinoline derivative, has been selectively used as a tissue schizontocide for vivax malaria since 1950 (Hiser *et al,* 1971). It can also be used as a gametocidal drug for *Plasmodium falciparum* infections (Bunnag *et al,* 1980). In 1990, the emergence of mefloquine and halofantrine resistant *P. falciparum* parasites at Bo Rai District, Trat Province, Thailand, near the Thai-Cambodian border with the cure rates approximately 30 to 50% was recorded. Since 1991, the Ministry of Public Health has given primaquine at the dosage of 30 mg to all Thai workers who returned from Cambodia to prevent spreading of mefloquine and halofantrine resistant malaria (W Rooney, WHO Thailand, personal communication). Although primaquine has been widely used for a long time, studies of primaquine pharmacokinetics have only recently been developed and reported. Greaves *et al* (1980) and Fletcher *et al* (1981) studied the kinetics of a single dose and multiple doses of primaquine in healthy Thai and Caucasian volunteers using gas chromatography-mass spectrometry. Other studies of kinetic parameters of primaquine in volunteers and vivax malaria patients used reversed phase high performance liquid chromatography (HPLC) (Mihaly *et al,* 1984, 1985; Ward *et al,* 1985; Bhatia *et al,* 1986). We have found that primaquine can be measured by normal phase HPLC similar to the method used to determine pharmacokinetics of chloroquine, quinine and quinidine, and this method is simple and has less interfering peaks compared to reversed phase HPLC (Turk, 1985). This present study was conducted to examine the pharmacokinetics of primaquine in the therapeutic dosage of 15 mg daily for 14 consecutive days in healthy Thai volunteers of the same age group using simplified normal phase HPLC and UV spectrophotometry detection.

MATERIALS AND METHODS

Volunteers and drug regimen

Eight informed staff members of the Department of Tropical Pediatrics, Faculty of Tropical Medicine, Mahidol University (4 males and 4 females) aged 22 to 29 years and weight 43 to 65 kg gave consent and participated in the study. They

were taking no other drugs. Full clinical examination (blood pressure, pulse rate, body temperature and complete physical examination), complete blood count, electrolytes, blood sugar, liver function tests, renal function tests and screening for G-6-PD levels were performed before inclusion. All subjects were found to be healthy and had no abnormal laboratory findings. They were admitted to the Hospital for Tropical Diseases throughout 15 days of the study period. All of them were recruited at the same time and received similar meals. Each of them received a daily dose of 15 mg base of primaquine (Imperial Chemical Industries PLC Pharmaceutical Division, Macclesfield, Cheshire, UK) after overnight fast and before breakfast for 14 consecutive days. Fifteen minutes after drug administration, the volunteers were allowed to have breakfast. This was similar to the usual practice of primaquine administration to vivax malaria patients.

From each volunteer, venous blood was taken through an indwelling intravenous teflon catheter kept patent with heparinized saline. Blood samples were taken predose and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 18 and 24 hours after the first dose then daily before drug administration until day 13. After the last dose on day 13, blood samples were collected using the same schedule as on the first twenty four hours after the first dose, and again at hours 30 and 40. Each sample was transferred into a heparinized tube. Two ml of whole blood were kept in a plastic tube. The remaining blood was centrifuged (1,000 g for 10 minutes), plasma and red blood cells were collected separately and stored at -20°C until assayed. All blood samples were protected from light using aluminum foil. Side effects were observed and recorded during the study period.

Chromatography and assay methods

All assays were processed at the Laboratory of Pharmacokinetics, Paris using high performance liquid chromatograph and UV detection. The UV spectrophotometer (Lambda Max 480, Waters) was set at 254 nm. After hexane extraction of alkalinized whole blood, plasma and red blood cells, samples were chromatographied on a normal phase column (Zorbax 5 m Si spherical particles). Methanol, acetonitril and diethylamine were used as a mobile phase (60 : 39.5 : 0.5). Desmethylclomipramine (Ciba-Geigy, Switzerland), was used as internal standard and the peak height ratio for quantification of primaquine. The assay sensitivities were 2.5 ng/ml in plasma and 5 ng/ml in whole blood and in red blood cells. Due to unavailability of carboxylic acid, the metabolite of primaquine was not analyzed in this study.

Pharmacokinetic calculations

The peak concentration (Cmax), time at which it was reached (Tmax), mean concentration, area under the curve (AUC), elimination constant and elimination half life (t1/2) were calculated by conventional methods (Gibaldi, 1984). A noncompartmental approach was used for the estimation of kinetic parameters. Total body clearance (Cl) and volume of distribution (Vd) were determined assuming the bioavailability of the drug (F) is equal to 1 (Mihaly *et al,* 1984). Drug concentrations of the first dose (day 0, from hours 0 to 24) were used for estimation of pharmacokinetic parameters after a single dose. The concentrations after the last dose (day 13, from hours 0 to 40) were used for determination of parameters after multiple administration.

Statistical tests

Pharmacokinetic parameters expressed as mean ± SD. The Wilcoxon rank test was used for the statistical analysis of data after the first and the last dose administrations. The statistical significance was achieved when $p < 0.05$.

RESULTS

Primaquine was rapidly absorbed and reached mean maximum concentration (Cmax) of 123.75 ± 65.12 ng/ml and 195.63 ± 75.23 ng/ml within 2.75 and 2.97 hours in whole blood and plasma respectively. The mean elimination half lives (t1/2) after the first dose were 5.88 hours in whole blood and 5.65 hours in plasma. The mean values of area under the curve (AUC) were 1,147.05 ± 684.80 ng hr/ml in whole blood and 1,603.66 ± 505.45 ng hr/ml in plasma. Total body clearance was 16.56 l/hr and 10.65 l/hr in whole blood and plasma respectively. A higher volume of distribution was seen with whole blood (135.68 ± 56.72 l), compared to 88.53 ± 50.67 l in plasma.

Table 1

Means (± SD) of pharmacokinetic parameters of whole blood after the first dose and the last dose of primaquine.

	Tmax (hr)	Cmax (ng/ml)	t1/2 (hr)	AUC (ng.hr/ml)	MRT (hr)	total Cl (1/hr)	Cl/Bw (ml/min/kg)	Vd (l)
First dose	2.75 (0.71)	123.75 (65.1)	5.86 (1.63)	1,147.05 (684.8)	7.76 (1.4)	16.56 (7.45)	0.196 (0.074)	135.68 (56.72)
Last dose	2.25 (0.96)	122.50 (70.6)	5.33 (1.51)	909.96 (603.07)	7.64 (1.29)	22.09 (11.07)	0.264 (0.105)	163.84 (75.58)

Table 2

Means (± SD) of pharmacokinetic parameters of plasma after the first dose and the last dose of primaquine.

	Tmax (hr)	Cmax (ng/ml)	t1/2 (hr)	AUC (ng.hr/ml)	MRT (hr)	total Cl (1/hr)	Cl/Bw (ml/min/kg)	Vd (l)
First dose	2.97 (0.97)	195.63 (75.23)	5.65 (2.04)	1,603.66 (505.45)	7.95 (1.53)	10.65 (5.19)	0.128 (0.049)	88.53 (50.07)
Last dose	2.06 (0.62)	176.88 (86.43)	5.66 (1.78)	1,255.11 (531.59)	7.93 (1.96)	14.46 (7.16)	0.180 (0.087)	116.17 (56.68)

At the steady state, the mean trough concentrations were 7.35 ± 0.41 ng/ml in whole blood and 8.06 ± 0.33 ng/ml in plasma. After the last dose of primaquine, the mean Cmax of 122.50 ± 70.61 ng/ml and 176.88 ± 86.43 ng/ml were reached within 2.25 hours in whole blood and 2.06 hours in plasma. Mean t1/2 in both whole blood (5.33 hours) and plasma (5.66 hours) following the last dose were slightly shorter than those after the first dose. However the differences were not statistically significant ($p = 0.195$ and 0.937 respectively). When compared to the first dose, the mean values of AUC of the last dose were significantly lower both in whole blood and plasma (909.96 ± 603.07 and 1,255.11 ± 531.59 with $p = 0.007$ and 0.023 respectively). Higher Vd was noted after the last dose both in whole blood (163.83 ± 75.58) and in plasma (116.71 ± 56.68), but when comparing to the value after the first dose the statistical significant was only noted in plasma ($p = 0.02$).

Daily administration of primaquine for fourteen days resulted in a significant decrease in drug concentration and AUC in both whole blood and plasma. The finding suggests an increase in drug elimination as shown by the results of clearance of the drug after the first and the last administration both in whole blood and in plasma (16.56, 22.09 l/hr and 10.65, 14.46 l/hr respectively) (Tables 1 and 2).

Primaquine was undetected in red blood cells. This finding could also be suspected from the results of primaquine concentrations which were higher in plasma than in whole blood.

It was noted than after the first dose the Cmax in whole blood and in plasma in female volunteers were significantly higher than in male volunteers ($p = 0.03$, $p = 0.014$), but the differences in Cmax between males and females in whole blood and plasma after the last dose were not statistically

Table 3

Means (± SD) of pharmacokinetic parameters of whole blood after the first and the last doses of primaquine in male and female volunteers.

Means ± SD	Male volunteers		Female volunteers	
	First dose	Last dose	First dose	Last dose
Tmax (hr)	3.00 ± 0.82	1.88 ± 0.75	2.50 ± 0.58	2.62 ± 1.11
Cmax (ng/ml)	77.50 ± 15.54	88.75 ± 36.14	170.00 ± 62.85	156.25 ± 85.38
T1/2 (hr)	5.78 ± 1.34	4.92 ± 0.58	6.06 ± 2.08	5.85 ± 2.08
AUC (ng.hr/ml)	742.28 ± 211.40	552.15 ± 176.59	1,551.93 ± 782.64	1,267.84 ± 689.92
Vd (l)	121.51 ± 72.35	206.24 ± 57.48	100.32 ± 60.41	171.12 ± 22.74
Cl/BW (ml/min/kg)	0.166 ± 0.066	0.210 ± 0.094	0.240 ± 0.065	0.326 ± 0.089

Table 4

Means (± SD) of pharmacokinetic parameters of plasma after the first and the last doses of primaquine in male and female volunteers.

Means ± SD	Male volunteers		Female volunteers	
	First dose	Last dose	First dose	Last dose
Tmax (hr)	2.75 ± 0.96	2.00 ± 0.71	3.19 ± 1.07	2.12 ± 0.63
Cmax (ng/ml)	138.75 ± 37.50	148.75 ± 78.57	252.50 ± 56.35	205.00 ± 95.66
T1/2 (hr)	6.95 ± 1.94	6.89 ± 1.70	4.47 ± 1.40	4.50 ± 0.91
AUC (ng.hr/ml)	1,307.19 ± 522.29	1,153.49 ± 577.19	1,900.20 ± 298.18	1,356.82 ± 546.55
Vd (l)	87.36 ± 58.38	144.98 ± 43.29	50.79 ± 11.78	126.34 ± 43.67
Cl/BW (ml/min/kg)	0.149 ± 0.068	0.180 ± 0.092	0.11 ± 0.02	0.189 ± 0.094

significant. The mean AUC in whole blood after the first dose was also significantly higher in females when compared to male subjects ($p = 0.029$) (Tables 3 and 4).

No adverse clinical side effects of the drug were noted during the study.

DISCUSSION

The results of this study confirmed that primaquine is rapidly absorbed and reaches maximum concentration within 2 to 3 hours of administration (Mihaly *et al*, 1985; Ward *et al*, 1985). The drug is then distributed and almost completely eliminated from the body after 24 hours. The time to reach maximum concentration and the elimination half life were also similar to those reported previously.

The concentration-time profiles in whole blood and in plasma were similar with a decrease in drug concentration after fourteen days of administration. The AUC after the last dose showed a significant decrease compared to the AUC after the first dose both in whole blood and plasma ($p = 0.007$ and 0.023 respectively). Maximum concentrations after subsequent daily doses were reached faster than after the first dose and mean maximum concentrations of the first dose when compared to the last dose were slightly higher (Figs 1, 2). The change in the concentration-time profile suggested that after fourteen days of administration, elimination of the drug from the blood is enhanced resulting in a decrease of AUC after the last dose. As a consequence of this decrease, the total body clearances after the last dose were significantly higher than those after the first dose ($p = 0.007$ in whole blood and $p = 0.016$ in plasma). These findings differ from the results of Mihaly *et al* (1985) and Ward *et al* (1985) in which total body clearance after a single dose or chronic administration of 15 mg base of primaquine was similar. Ward *et al* (1985) found that daily administration had no effect on the pharmacokinetic parameters. Unfortunately, due to the lack of information about the actual value of F in our volunteers and unavailability of the metabolite, it was not possible to determine whether the huge differences observed were related to modifications in absorption, hepatic extraction, binding to tissues or decrease in drug metabolism.

The predose plasma concentrations at steady in this study varied from 0 to 35 ng/ml while Greaves *et al* (1980) found that all predose samples were negative. The difference is probably due to the different method for drug determination used in this study.

A wide range of drug concentrations in our subjects was noted leading to variable kinetic parameters. These variations were not only between subject but remarkable changes in the values of kinetic parameters were also found in the same subject after daily administration (Figs 3, 4 and 5).

The higher plasma concentrations in this study compared to those obtained from Mihaly *et al*

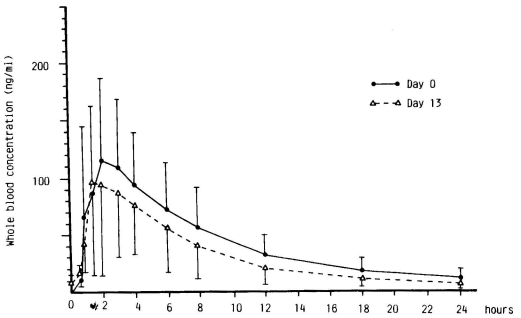

Fig 1—Mean kinetics profile (± SD) in whole blood after the first and the last administration of primaquine of all healthy volunteers.

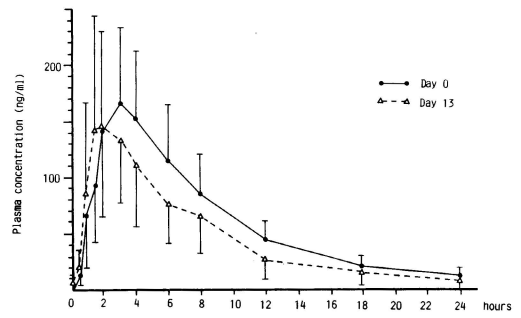

Fig 2—Mean kinetic profiles (± SD) in plasma after the first and the last administration of Primaquine of all healthy volunteers.

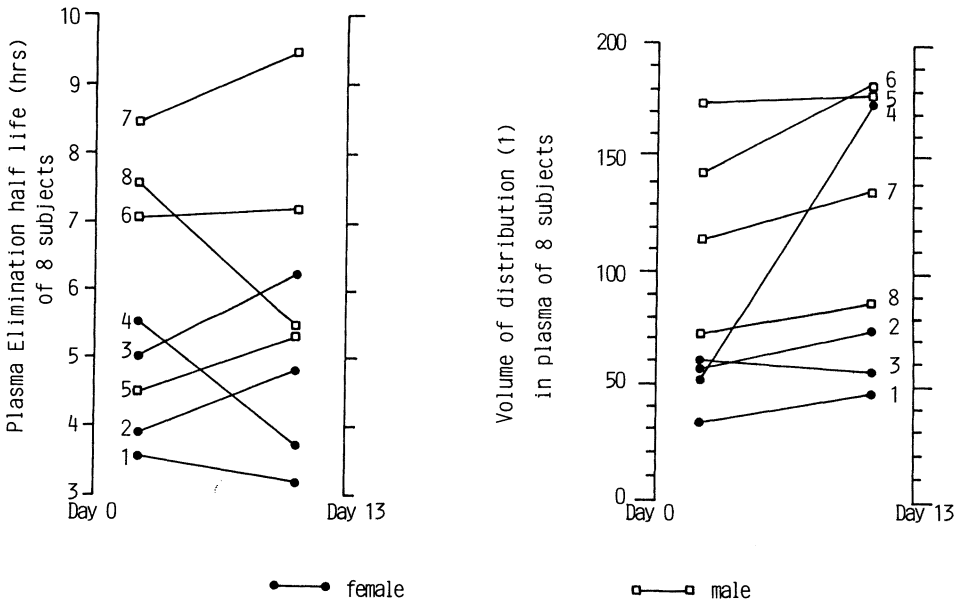

Fig 3—Individual variations in t½ Vd after the first and the last dose of primaquine.

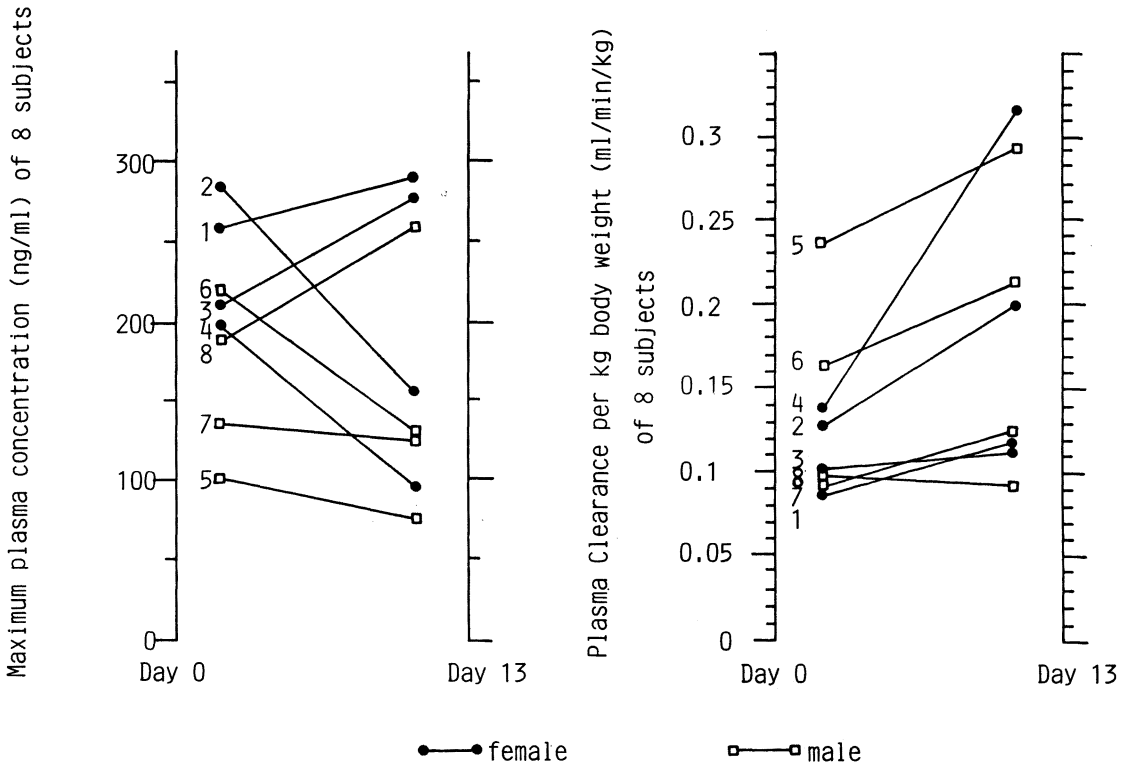

Fig 4—Individual variations in Cmax and Cl after the first and the last dose of primaquine.

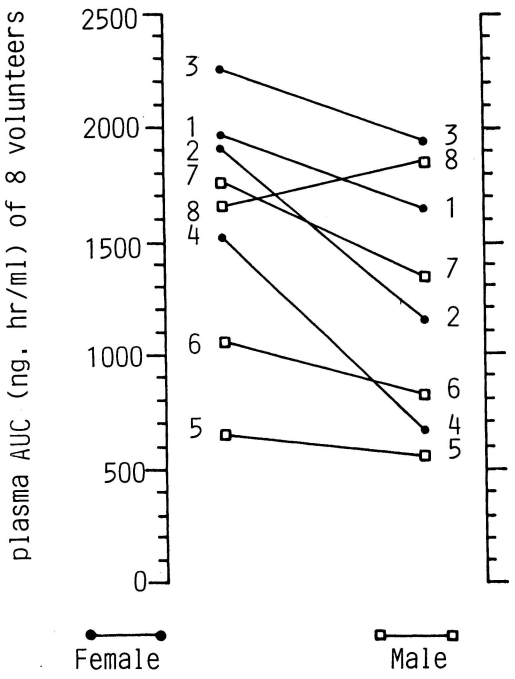

Fig 5—Individual variation of AUC after the first and the last dose of primaquine.

(1985) and Ward *et al* (1985) were probably due to the bioavailability of the drug as well as interindividual variation. Differences in the volunteers before and after drug administration could influence the absorption of the drug. For the study Greaves *et al* (1980) and Fletcher *et al* (1981), the fasting period was maintained for 3 hours after drug administration, but in this study, local food rich in carbohydrates was given to the volunteers fifteen minutes later as is general practice. These food intake could affect drug absorption since it has been reported for other antimalarials (Lagrave *et al,* 1985; Broom, 1989). Further studies with different kinds of food or duration of fasting and with determination of the carboxylic acid metabolite of primaquine should be carried out to confirm whether higher primaquine concentrations in this study were associated with food leading to the modification in drug absorption or with decrease in drug metabolism in our subjects.

A significant difference between males and females was found in Cmax and AUC after the first dose. Other kinetic parameters were similar. These findings should be confirmed in a larger study population.

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