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The pharmacokinetics of chloroquine in healthy Thai subjects and patients with *Plasmodium vivax* malaria

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The pharmacokinetics of chloroquine (CQ) and desethylchloroquine (DECQ) were studied in seven male Thai patients with *P. vivax* malaria and seven healthy male Thais, after the standard oral dosage regimen of CQ (a total dose of 1500 mg given over 3 days). All patients showed a rapid initial response to the treatment with median (range) values of fever and parasite clearance times of 13.7 (2–47) and 58 (33–38) h, respectively. In the patients, the median range C_{max} value was significantly higher (1547 (996–2446) *vs* 838 (656–1587) ng ml^{-1}), and AUC(0,28d) was greater (281 (250–515) *vs* 122 (103–182) μg ml^{-1} h). In addition, the median (AUC(0,28d) of DECQ was significantly greater (170 (72–265) *vs* 77 (49–140) μg ml^{-1} h). The AUC(0,28d) ratio of DECQ to CQ in patients was significantly higher than that in healthy subjects (0.67 (0.43–0.90) *vs* 0.51 (0.29–0.61)).

Keywords chloroquine malaria pharmacokinetics

Introduction

Chloroquine (CQ) is the treatment of choice for vivax malaria. However, in the past 5 years there have been increasing reports of recurrence of *P. vivax* parasitaemia after therapeutic or prophylactic regimens of CQ in some parts of the world [1–7]. In Thailand, in spite of the total loss of efficacy of chloroquine in the treatment of *P. falciparum* malaria, the efficacy of the drug against *P. vivax* malaria has been complete [8–9], although declining susceptibility of the parasite to CQ was observed *in vitro* (Tan-ariya *et al.*, 1994, submitted for publication). The pharmacokinetics of CQ and its major plasma metabolite desethylchloroquine (DECQ) were compared in male Thai subjects with *P. vivax* malaria and healthy subjects, in order to define any differences which might account for altered response in patients.

Methods

Seven healthy male Thai subjects, and seven male Thai patients with *P. vivax* malaria, aged between 18 and 35 years and weighing 45 to 68 kg, were recruited for the study. Written informed consent for participation was obtained from each volunteer. The study was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

The healthy subjects were non-smokers, non-drinkers and had no previous history of liver or kidney diseases. None was on regular medication and no other drugs were taken during the study. They were admitted to the Bangkok Hospital for Tropical Diseases on the first day of drug administration and returned for blood sampling daily until day 14, and again on days 21 and 28. The patients were admitted to the Bangkok Hospital for Tropical Diseases for 28 days. It was not possible to keep the subjects in the hospital for longer than this time.

On admission, each subject had a physical examination, evaluation of vital signs, a 12-lead electrocardiogram, routine blood examinations (haematology, serum biochemistry), and urinalysis. Venous blood samples (2 ml) were drawn for measurement of baseline whole blood CQ and DECQ concentrations.

All subjects received CQ (Chloroquine phosphate, Government Pharmaceutical Organization of Thailand; 1 tablet of 250 mg salt contains 150 mg base) in a total dose of 1500 mg over 3 days (600 mg initially,

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followed by 300 mg at hours 6, 24 and 48). In healthy subjects, the drug was administered following an overnight fast and the subjects were allowed to take food 2 h after drug administration.

Following the first dose of CQ, a total of 18 blood samples (2 ml each) were collected at 6, 12, 24, and 48 h, daily until day 14, and on days 21 and 28. At 6, 24 and 48 h, blood samples were collected prior to the dose of CQ. Whole blood samples were collected into lithium-heparinized plastic tubes and stored at −70° C until analysis.

Adverse effects occurring after drug administration were monitored by daily questionnaires and physical examination for 7 days. Complete blood count, urinalysis and blood biochemistry investigations were performed on days 2, 4, 7 and then weekly until day 28. An electrocardiogram was recorded daily for 7 days then weekly for the remainder of the follow-up period.

In patients, a parasite count was performed every 6 h after the treatment until parasitaemia fell below the level of microscopic detection in a thick smear, then twice daily until day 28. Before discharge, an antirelapse drug primaquine phosphate (15 mg, o.d. Government Pharmaceutical Organization of Thailand), was given for 14 days to all patients.

CQ and DECQ concentrations in whole blood were measured by h.p.l.c. using the method of Alvan *et al.* [10] with modifications. In brief, whole blood samples containing 1000 ng primaquine as an internal standard were added to 1 ml of 0.1 M sodium hydroxide. After vortexing for 30 s, the mixture was extracted with 6 ml dichloromethane by mechanical tumbling for 30 min. The organic phase was separated by centrifugation (at 1000 *g*, 10 min, 4° C), and evaporated to dryness under a stream of nitrogen gas at 37° C. The residue was redissolved in the mobile phase (50 µl) and 20 µl was injected onto a reversed-phase C_{18} column (Techopak-10 C_{18}, 10 µm particles, 25 cm × 4.6 mm i.d., h.p.l.c. Technology, UK). The mobile phase consisted of acetonitrile and phosphate buffer pH 2.95 (25:75 v/v) containing perchlorate (200 mmol l^{-1}). The flow rate was 2.8 ml min^{-1} with u.v. detection at 254 nm. The limit of determination was 5 ng ml^{-1} for CQ and 10 ng ml^{-1} for DECQ. The intra- and inter-assay coefficients of variation were, respectively, 7.5% and 9.1% at 50 ng ml^{-1}, 5.9% and 1% at 500 ng ml^{-1} and 7.8% and 6.1% at 1000 ng ml^{-1} for CQ. The corresponding values for DECQ were 5.2% and 4.2% at 50 ng ml^{-1}, 2% and 1% at 500 ng ml^{-1} and 9.2% and 1.4% at 1000 ng ml^{-1}.

The maximum blood drug concentration (C_{max}) was defined as the highest concentration observed during the sampling time. An apparent elimination rate constant (λ_{8-28d}) was estimated by least squares regression anlaysis of at least 4 concentrations from day 8 onwards, and the corresponding half-life ($t_{1/2}$(8-28d)) from the ratio of $0.693/\lambda_{8-28d}$. The values of AUC(0,28d) were calculated using the linear trapezoidal rule.

The pharmacokinetic parameters of CQ or DECQ in healthy subjects and patients were analysed using the Mann-Whitney U test.

Results

The healthy subjects and patients were matched for age (median 28, range 27–35 *vs* median 21, range 18–41 years) and weight (median 59, range 55–68 *vs* median 54, range 45–65 kg). None of the healthy subjects had signs or symptoms or malaria parasitaemia in peripheral blood smears on admission. Median and range admission parasitaemia in patients was 757 (138–11480) μl^{-1}. The adverse effects of CQ in both groups of subjects were mild and self-limiting; one of the patients and two healthy subjects complained of dizziness. All except one patient complained of headache, five patients and one healthy subject had nausea and abdominal distress, but no vomiting. There were no noticeable drug-related haematological effects, biochemical or ECG changes during the course of follow-up.

All patients showed a rapid initial response to the treatment with median (range) values of fever clearance time (the time taken for the temperature to return to below 37.3° C and remain at that value for at least 24 h) and parasite clearance time (the time taken for the parasite count to fall below the level of microscopic detection) of 13.7 (2–47) and 58 (33–

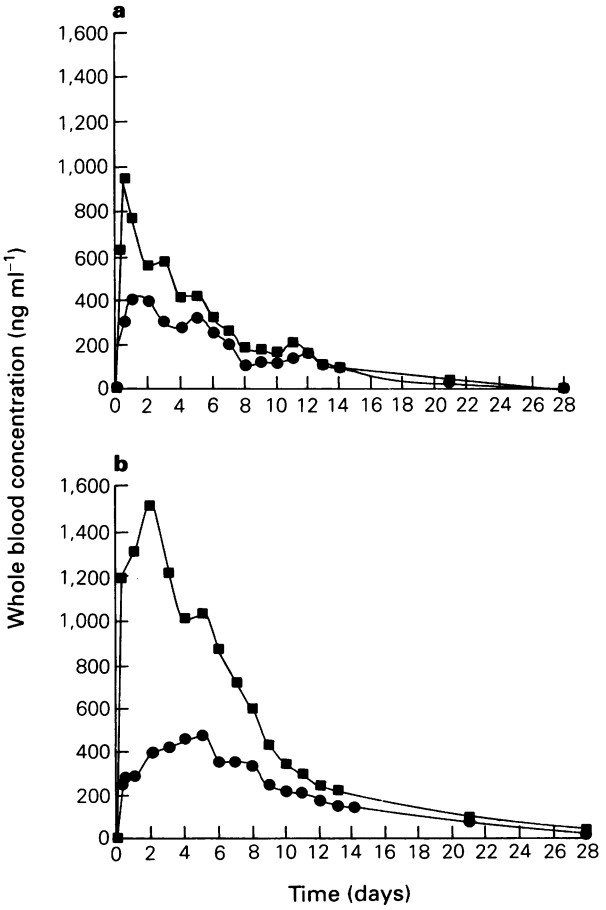

Figure 1 Median plots of whole blood concentrations of CQ (■) and DECQ (●) in a) healthy subjects and b) patients with *P. vivax* malaria.

88) h, respectively. No correlation between the rates of fever or parasite clearance and the concentration-time profiles of either CQ or DECQ was observed.

No subject had detectable levels of CQ and DECQ in their blood prior to treatment. Median plasma concentrations of CQ and DECQ in healthy subjects and patients are shown in Figure 1, and median (range) pharmacokinetic parameters derived from the 0–28 day data are listed in Table 1. The median (range) values of C_{max} and AUC(0-28d) of CQ were significantly higher in the patients, as was the AUC(0-28d) of DECQ. The ratio of AUC values of DECQ to CQ in patients was significantly greater than that in healthy subjects (0.67 (0.43–0.90) *vs* 0.51 (0.29–0.61)).

Discussion

Blood concentrations of both CQ and DECQ observed in the study were similar to those reported by others [11]. After multiple oral doses of CQ in both malaria patients and healthy subjects, systemic exposure of CQ was always higher than that of DECQ. The higher C_{max} and AUC values observed in the patients may reflect a change in the extent of absorption of the drug during the infection. The finding of no changes in the pharmacokinetics of CQ in patients infected with *P. vivax* after intravenous administration compared with healthy subjects [12], and the high variability in the fraction of dose absorbed after oral administration noted in various studies [13,14] support this possibility.

Despite these kinetic changes, no adverse reactions to CQ were seen. This should be advantageous as declining susceptibility of the parasite to the drug *in vitro* has been observed in Thailand (Tan-ariya *et al.*, 1994, submitted for publication). Blood concentrations of CQ or DECQ associated with adequate treatment of *P. vivax* malaria have not been established rigorously, although plasma or serum concentrations of CQ of 15–30 ng ml^{-1} or whole blood concentration of 90 ng ml^{-1} have been suggested [15, 16]. Even though a prominent trend in declining susceptibility of *P. vivax* has been observed *in vitro* [17, Tan-ariya *et al.*, 1994, submitted for publication), the results of a large clinical trial suggest that the drug is still very effective for the treatment of *P. vivax* malaria in Thailand (Karbwang *et al.*, 1994, submitted for publication).

Table 1 Pharmacokinetic parameters (median and range) of CQ and DECQ derived from data collected over 28 days in seven healthy Thai male subjects and seven patients after administration of 1500 mg chloroquine over 3 days

	Healthy subjects		*Patients* (P. vivax *malaria)*	
	CQ	*DECQ*	*CQ*	*DECQ*
C_{max} (ng ml^{-1})	838 (656–1587)[a]	428 (159–637)	1547 (996–2446)	591 (253–761)
AUC(0,28d) (μg ml^{-1} h)	122 (103–182)[b]	77 (49–140)[c]	281 (250–515)	170 (72–265)
$t_{1/2}$(8-28d) (h)	150 (103–266)	198 (132–329)	201 (155–224)	205 (190–276)

Significantly different from values in patients: (a) $P = 0.039$ (95% CI −234–1124); (b) $P = 0.002$ (95% CI 127–291); (c) $P = 0.013$ (95% CI 5–125).

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