ABSTRACT

In Thailand, multidrug resistant strains of falcipaurm malairia are now increasing and spreading, particularly on the borders of Thai-Myanmar and Thai-Cambodia. With this deterioration situation, one of the strategies aiming at controlling the disease is through modification of antimalarial drug regimens or the use of drug combinations. The combination of artemisinin and derivatives (artemether, artesunate, dihydroartemisinin, *etc*) with drugs with long half-lives, *i.e.*, mefloquine, pyrimethamine, doxycycline and azithromycin could be promising alternative regimens to improve patient compliance and cure rate. The rational of applying these combination lies both in the enhancement of efficacy as compared to each individual component drug when given alone, and in the retardation of development of resistance of *Plasmodium falciparum* malaria against drugs. The present study aimed to provide the basic knowledges on pharmacokinetics, pharmacodynamics, as well as, pharmacokinetic/pharmacodynamic interaction(s) of these combination regimens, both in healthy subjects and in acute uncomplicated falciparum malaria. Rational and effective combination regimens for coping with the situation of multidrug-resistant *P. falciparum*) would hopefully, be achieved from the studies. Furthermore, *e.g.*, susceptibility of the parasite to antimalarials which could contribute to treatment outcome were also investigated

Pharmacokinetic and pharmacodynamic interactions between dihydroartemisinin (DHA) and mefloquine (MQ) were investigated in eight healthy Thai males. The study was of a three-way cross over design. Subjects were randomised to receive three drug regimens on three separate occasions as follows; *regimen-I*: a single oral dose of 300 mg DHA; *regimen-II*: a single oral dose of 750 mg MQ; *regimen-III*: a single oral dose of 300 mg DHA, given concurrently with a single oral dose of 750 mg MQ. All regimens were well tolerated. Oral DHA was rapidly absorbed and disappeared from systemic circulation within 8-10 h. MQ

absorption and disposition were relative slow processes. Pharmacokinetics of DHA and MQ when given concurrently were similar, except for the absorption rate of MQ which was faster in the presence of DHA ($t_{1/2a}$ 2.18 *vs* 2.65 h, $p = 0.04$). Pharmacodynamically, combination of DHA and MQ resulted in the greatest antimalarial effect. Both maximum activity (A_{max}) and area under effective-time curve (AUEC) of DHA and MQ were increased approximately 1.7- and 7-fold *vs* 865- and 49-fold, respectively, compared to each individual drug alone. AUEC of MQ during the first 24 h ($AUEC_{24h}$) was increased approximately 202-fold in the presence of DHA.

The pharmacokinetics of the combination DHA/MQ were investigated in 20 patients with acute uncomplicated falciparum malaria after oral administration of (a) a single oral dose of 300 mg DHA given concurrently with 750 mg MQ (b) initial dose of 300 mg DHA plus 750 mg MQ, followed by 500 mg MQ at 24 h apart. Both regimens were well tolerated. No significant clinical adverse effects were observed in any of patients during acute malaria infection. Pharmacokinetics of MQ and DHA were markedly influenced by malaria infection. Significant changes in the pharmacokinetic parameters of both MQ and DHA were observed in patients with malaria compared with healthy subjects in the previous study (Chapter III). C_{max} and AUC of MQ when normalised with dose (C_{max}/mg dose, AUC/mg dose) were significantly higher in patients [(2.33 *vs* 1.94 *vs* 1.73 ng/ml/mg dose) (regimen-I *vs* regimen-II *vs* healthy subjects) and (0.77 *vs* 0.62 *vs* 0.56 μg.h/ml/mg dose)]. Furthermore, t_{max} was prolonged, V_z/f and Cl/f were reduced, and $t_{1/2}$ was shortened. [(12 *vs* 12 *vs* 4 h), (8.99 *vs* 10.69 *vs* 14.26 l/kg), (0.38 *vs* 0.42 *vs* 0.56 ml/min/kg), and (10.3 *vs* 12.6 *vs* 15.04 d)]. For DHA, C_{max}, AUC, V_z/f and Cl/f were changed in the same direction as MQ, but $t_{1/2}$ was prolonged (1.62 *vs* 1.91 *vs* 1.11 h).

The pharmacokinetics of a single oral dose of artemether (ARM) (300 mg) and pyrimethamine (PYR) (100 mg) given as each individual drug alone or as a drug combination (ARM 300 mg plus PYR 100 mg), were investigated in eight

healthy male Thai volunteers. ARM was rapidly absorbed after oral administration and extensively biotransformed to DHA; significant plasma concentrations of both compounds were detectable as early as 15 min after dosing. Rapid and monoexponential decline of ARM concentrations resulted in its low systemic exposure and short half-life. The metabolite-- DHA exhibited greater and more prolonged systemic exposure. Absorption of PYR from gastrointestinal tract was rapid; C_{max} of approximately 631-1500 ng/ml were achieved within 0.5-4 h. Following the absorption phase, the drug concentrations in plasma decline bi-exponentially. Elimination of PYR was however, a relatively slow process compared with ARM, and thus results in a long terminal phase elimination half-life (57-106 h). Pharmacokinetics of ARM and DHA following a single oral dose of ARM alone or in combination with PYR were similar. In contrast, coadministration of ARM resulted in significantly increased C_{max} (median of 818 *vs* 1180 ng/ml) and contracted V_z/f (median of 3 *vs* 2.56 l/kg) of PYR.

Clinical efficacy, the status of *in vitro* susceptibility of multidrug resistant *P. falciparum* to PYR, the incidence of unregulated use of PYR (as Fansidar®) and the relevance of pharmacodynamic (intrinsic activity of PYR in resistant parasite) and pharmacokinetic (plasma concentrations of PYR) factors in determining the treatment outcome in the three combination ARM/PYR were investigated in patients with acute uncomplicated falciparum malaria. The majority of patients (50.6%) had concentrations between 1-100 and only (34.8%) more than 100-500 ng/ml, while concentration of more than 500 ng/ml were found in only 1.1%. Sixty patients were ramdomised to receive 3 oral regimens of combination ARM/PYR as follow; *regimen-I*: ARM (300 mg) plus PYR (100 mg) on the first day, then placebo on the two consecutive days; *regimen-II*: ARM (300 mg) plus PYR (100 mg) on the first day, then ARM (150 mg) plus PYR (50 mg) on the second day, and placebo on the third day; *regimen-III*: ARM (300 mg) plus PYR (100 mg) on the first day, then ARM (150 mg) plus PYR (50 mg) on the second and third days. All patients had a rapid initial response to treatments with 95% of parasitaemia being cleared within the first 24 h. $PCT_{24hours}$ and $PCT_{48hours}$ were similar among the three drug regimens

(11 *vs* 4, 6 *vs* 12, and 9 *vs* 11 patients for a 1-day, 2-day, and 3-day combination regimen, respectively). Fever was cleared within 48 h in all patients in either group. Transient mild nausea, vomiting and loss of appetite were found in a few patients during the first 2 d of treatment. The cure rate of the combination following *regimen-I*, *regimen-II* and *regimen-III* were 0, 27.8 and 75%, respectively. All of the isolates were highly resistant to PYR, with the in *vitro* MIC (minimum inhibition concentration) of 10^{-5} M. No association was found between treatment outcome and the presence of baseline plasma PYR concentrations nor plasma PYR concentrations on day-1 and day-2.

The efficacy of the combination of ARM with doxycycline (DOX) or azithromycin (AZM) was evaluated in 60 patients with acute uncomplicated falciparum who attended Malaria Clinic in Mae-Sot, Tak province (Thai-Myanmar border). Patients (30 each) were randomised to receive (a) 300 mg ARM together with 100 mg DOX as initial doses, followed by 100 mg ARM plus 100 mg DOX at 12 h later, then 100 mg DOX every 12 h for another 4 d, or (b) 300 mg ARM together with 500 mg AZM, followed by 250 mg AZM at 24 and 48 h. The follow-up period was 28 d. Patients in either group had a rapid initial response to treatment with comparable PCT and FCT. The cure rate of ARM/AZM regimen was significantly lower than that of ARM/DOX regimen (14.8 *vs* 53.3%).

Monitoring of *in vitro* sensitivity were carried out with a total of 103 fresh isolates of *P. falciparum.* A total of 296 individual tests both before and after treatment with the three regimens of CGP 56697 were performed with five different antimalarial drugs. One-hundred and five isolates were tested for their susceptibility to artemisinin (ARN) (83 and 22 isolates on the day of admission and recrudescence, respectively). The number of isolates (admission and recrudescence) tested for susceptibility to benflumetol (BF), quinine (QN), MQ and chloroquine (CQ) were 100 (79, 21), 40 (31, 9), 41 (34, 7), and 10 (10, 0) isolates, respectively. The number of tests with paired isolates (day of admission and recrudescence) were accomplished with 21 isolates, 10 for ARN and 11 for BF.

Based on the cut-off levels, all of the pretreatment of *P. falciparum* isolates were considered highly resistant to CQ and markedly resistant to MQ. Low grade resistance was defined for the susceptibility of the parasite isolates to QN. The mean IC_{50} and IC_{99} values for ARN and BF were 32 *vs* 1477 and 25 *vs* 1385 nmol/l, respectively. There were no significant difference in the susceptibility of *P. falciparum* isolates collected prior to treatment and at the time of recrudescence to all drugs (MQ, QN, ARM and BF). Sensitivity of the paired *P. falciparum* isolates (collected on the admission and recrudescence days in the same patient) to ARN and BF were accomplished with 21 isolates. Similar findings with the unpaired isolates were found. Although there was a trend of declining in susceptibility of the parasites to both drugs in post-treatment compared with the pretreatment isolates, this did not reach a statistical significant difference ($p > 0.05$).