PHARMACOKINETIC INTERACTIONS OF ARTEMETHER AND PYRIMETHAMINE IN HEALTHY MALE THAIS

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Abstract. The pharmacokinetics of a single oral dose of artemether (300 mg) and pyrimethamine (100 mg) given as each individual drug alone or as a drug combination (artemether 300 mg plus pyrimethamine 100 mg), were investigated in 8 healthy male Thai volunteers. Both artemether and pyrimethamine were rapidly absorbed after oral administration. Elimination of pyrimethamine was however, a relatively slow process compared with artemether, and thus resulted in a long terminal phase elimination half-life (50-106 hours). Pharmacokinetics of artemether and dihydroartemisinin following a single oral dose of artemether alone or in combination with pyrimethamine were similar. In contrast, coadministration of artemether resulted in significantly increased C_{max} (medians of 818 *vs* 1,180 ng/ml) and contracted the apparent volume of distribution (medians of 3 *vs* 2.56 l/kg) of pyrimethamine.

INTRODUCTION

Malaria remains a major public health problem worldwide due to the emergence of parasite resistance to the available antimalarials (Wernsdorfer, 1994). Artemether is an artemisinin derivative currently plays significant role in coping with the situation. It has a rapid onset of action and destroys asexual parasites at an earlier stage of development (Li *et al*, 1983). Clinical trials conducted in several countries, advocate clinical use of this drug both in uncomplicated and severe falciparum malaria (Myint *et al*, 1989; Bunnag *et al*, 1991; 1992, Karbwang *et al*, 1992a, 1995b; Li *et al*, 1994; Hein *et al*, 1996). However, due to its short half-life, long course treatment of more than 5 days is required in order to achieve 100% cure rate. One strategic approach to improving the clinical effectiveness (efficacy, patient's compliance) of artemether is to use this drug in combination with other long half-life drug *eg*, mefloquine (Karbwang *et al*, 1995b; Bunnag *et al*, 1996; Na-Bangchang *et al*, 1997a). Pyrimethamine, a dihydrofolate reductase inhibitor, is another antimalarial with a long half-life, which has been used for therapy or prophylaxis of falciparum malaria for decades. The drug is active against several stages of human malaria, but due to its slow blood schizontocidal action, the use of pyrimethamine alone is limited in acute falciparum malaria (Karbwang and Harinasuta, 1992b). Although *Plasmodium falciparum* in Thailand is resistant to pyrimethamine (Karbwang and Harinasuta, 1992b), from pharmacokinetic and pharmacodynamic points of view, artemether and pyrimethamine are promising combination partners which might offer therapeutic synergistic effects. Regarding combination therapy however, drug interaction is an important consideration. To date there has been no interaction study between artemether and pyrimethamine in man. We have carried out a study in healthy Thai volunteers to investigate the possibility of this interaction(s).

MATERIALS AND METHODS

Subjects

Eight healthy male Thai volunteers, aged between 21 and 28 years and weighing 45 to 70 kg, with no history of liver or kidney disease participated in the study. None was a smoker or alcohol drinker or was on regular medication. No other drugs were taken during the study. Written informed consent was obtained from each volunteer. The study was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

Prior to administration of all antimalarial regimens, the subjects were hospitalized overnight; each underwent a physical examination, monitor-

ing of heart rate, blood pressure, a 12-lead electrocardiogram (ECG), routine blood examinations (hematology, clinical chemistry) and urinalysis.

Drug administration and study design

The study was of a three-way cross-over, randomized design, in which each subject attending the study on three separate occasions. On the first occasion, the subjects were randomized to receive one of the three drug regimens as follows,

Regimen-I: a single oral dose of 300 mg artemether (Arenco nv Belgium 50 mg per tablet)

Regimen-II: a single oral dose of 100 mg pyrimethamine (Daraprim®, Welcome: 25 mg per tablet)

Regimen-III: a single oral dose of 300 mg artemether, given concurrently with a single oral dose of 100 mg pyrimethamine

The tablets were administered with a glass of water under supervision following an overnight fast. Subjects were allowed to take meal 2 hours after drug administration. The wash-out period after *Regimen-I* was at least 7 days, while this period was extended to at least 14 days after *Regimen-II* and *Regimen-III*. Subjects were hospitalized in the Bangkok Hospital for Tropical Disease for 7 days following *Regimen-I*, and for 14 days following *Regimen-II* and *regimen III*.

Blood sample collection

Prior to, and following drug administration on all occasions, blood samples (5 ml each) for the assay of artemether and its active plasma metabolite, dihydroartemisinin, and/or pyrimethamine were collected from an antecubital vein, into sodium heparinized plastic tubes, at hours 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 18 and 24. For regimens containing pyrimethamine (*Regimen-II, III*), blood samplings were continued daily until day 7 and on days 10 and 14 after dosing.

Plasma samples were obtained through centrifugation within 5 minutes (1,500*g*, 10 minutes), and stored at -70°C until analysis.

Adverse effect monitoring

Adverse effects on the gastrointestinal, central nervous and cardiovascular systems were monitored daily by mean of questionnaires, recording the frequency, date and time at which they occurred and disappeared. Heart rate, blood pressure and ECGs were recorded at intervals during blood sampling and daily until day 14. Blood tests and routine blood biochemistry investigations were performed on days 0, 2, 7 and 14 after drug intake.

Drug analysis

Concentrations of artemether, dihydroartemisinins and pyrimethamine in plasma were measured by high performance liquid chromatography according to the methods of Karbwang *et al* (1997b) and Na-Bangchang *et al* (1997b). The average recoveries of artemether, dihydroartemisinin and pyrimethamine at the concentration range of 10-1,000 ng/ml were 90, 88 and 100%, respectively. The coefficients of variation for the assays were below 10%. The minimum detectable concentrations for artemether, dihydroartemisinin and pyrimethamine were 5, 3 and 3 ng/ml, respectively.

Pharmacokinetic and statistical analysis

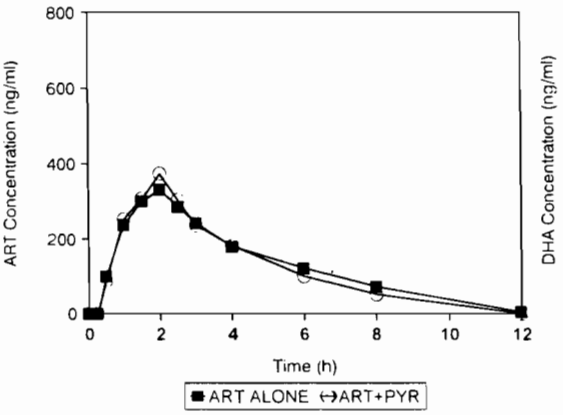
The pharmacokinetic calculations were performed by using model-independent methods (Gibaldi, 1991). The time at which maximum concentrations occurred (t_{max}) and maximum concentration (C_{max}) were obtained directly from the plasma concentration-time data. The terminal elimination half-life ($t_{1/2z}$) was calculated from log-linear regression of at least four last plasma concentration-time data. The areas under the curve from zero time to the last observed time (AUC) was calculated by log-linear trapezoidal rule for ascending and descending data points. Area under the curve extrapolated from the last data point to infinity was estimated by dividing the regressed concentration at the last time point by the estimated elimination rate constant (λ_z). The extrapolations contributed on average 4.2% (range 0-9.8%). The apparent total body clearance and apparent volume of distribution associated with terminal phase were calculated as CL/f=dose/AUC and $V_z/f = CL/f/\lambda_z$, respectively.

RESULTS

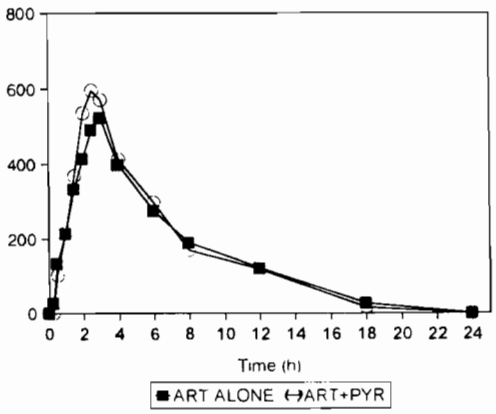
The pharmocokinetics of either artemether and its active plasma metabolite, dihydroartemisinin,

including pyrimethamine generally showed marked inter-individual variability. The greatest variation among subjects were noted for AUC, in which its coefficients of variation varied from 25 to 40%. Fig 1, 2 depict the plots of time-course of plasma concentrations of artemether, dihydroartemisinin and pyrimethamine following the administration of artemether or pyrimethamine alone and the combination of each drug. Tables 1 and 2 summarize their pharmcokinetic parameters.

Artemether was rapidly absorbed after oral administration and extensively biotransformed to dihydroartemisinin; significant plasma concentrations of both compounds were detectable as early as15 minutes after dosing. Rapid and monoexponential decline of artemether concentrations resulted in its low systemic exposure and short $t_{1/2z}$. The metabolite–dihydroartemisinin exhibited greater and more prolonged systemic exposure. Absorption of pyrimethamine from the gastrointestinal tract was rapid; C_{max} of approximately 631-1,500 ng/ml were achieved within 0.5-4 hours. Following the absorption phase, the drug concentrations in plasma decline bi-exponentially. Elimination of pyrimethamine was however, a relatively slow process compared with artemether, and thus resulted in a long terminal phase elimination half-life (50-106 hours).



(a)



(b)

Fig 1–Median plots of plasma concentration-time profiles of (a) artemether (ART) and (b) dihydroartemisinin (DHA) when given alone and in combination with pyrimethamine (PYR).

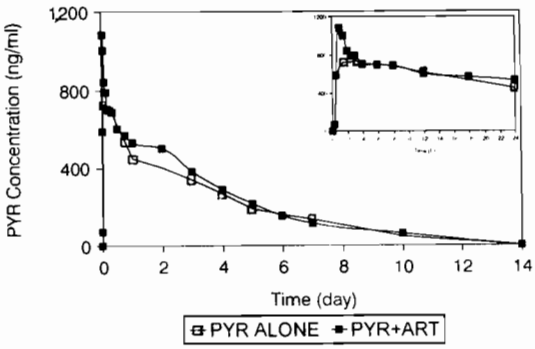


Fig 2–Median plots of plasma concentration-time profiles of pyrimethamine (PYR) when given alone and in combination with artemether (ART).

Pharmacokinetics of artemether and dihydroartemisinin when given as artemether alone or in combination with pyrimethamine were similar (Table 1). In contrast, coadminstration of artemether significantly altered the kinetics of pyrimethamine (Table 2). C_{max} of pyrimethamine was increased (medians of 818 *vs* 1,180 ng/ml) while the apparent volume of distribution was reduced (medians of 3 *vs* 2.56 l/kg) in the presence of artemether.

DISCUSSION

Pharmacokinetics of oral artemether observed in the present study are in broad agreement with that reported in other studies in healthy subjects, where rapid absorption and elimination kinetic characteristics of the drug were demonstrated (Karb-

Table 1

Pharmacokinetics of oral artemether (300 mg) when given alone and in combination with pyrimethamine (100 mg); data are presented as median (range) values.

Pharmacokinetic parameters	Artemether alone	Artemether plus pyrimethamine
Artemether		
C_{max} (ng/ml)	499 (287-648)	511 (301-700)
t_{max} (h)	2 (1.5-2.5)	1.8 (1.5-2.5)
AUC (μg h/ml)	2.16 (0.98-3.67)	1.74 (0.97-3.64)
$t_{1/2z}$ (h)	2.7 (1.8-3.8)	2.2(1.7-3.7)
CL/f (ml/min/kg)	37.7 (27.9-75.2)	48.5 (24.8-56.6)
V_z/f(l/kg)	9.6 (6.6-11.4)	9.1 (6.6-9.4)
Dihydroartemisinin		
C_{max} (ng/ml)	885 (654-1,250)	872 (644-1,570)
t_{max} (h)	2.8 (1.5-4)	3.5 (2-5)
AUC (μg h/ml)	6.5 (2.2-19.2)	7.68 (2.4-17.1)
$t_{1/2z}$ (h)	5.5 (3.6-8.4)	4.9 (2.2-8.2)

Table 2

Pharmacokinetics of oral pyrimethamine (100 mg) when given alone and in combination with artemether (300 mg); data are presented as median (range) values.

Pharmacokinetic parameters	Pyrimethamine	Pyrimethamine plus artemether
C_{max} (ng/ml)	818 (676-1,190)	1,180 (631-1,500)[a]
t_{max} (h)	1.5 (1-4)	1.25 (0.5-1.5)
AUC (μg h/ml)	63.8 (43.9-86.8)	75.7 (49.1-79)
$t_{1/2z}$ (h)	67.1 (58.6-106)	77 (49.7-90.5)
CL/f (ml/min/kg)	28.5 (16.7-31.1)	22.8 (21.2-34.2)
V_z/f(l/kg)	3 (1.83-4.02)	2.56 (1.88-4.16)[b]

[a]significantly different from pyrimethamine alone with p = 0.008 (CI = 122-475)
[b]significantly different from pyrimethamine alone with p = 0.008 (CI = 0.16-1.0)

wang *et al*, 1997a; Teja-isvadharm *et al*, 1996; Mordi *et al*, 1997). A wide variation of systemic exposure of artemether could be accounted for by the variable extent/rate of drug absorption from the gastrointestinal tract, and the extent of first-pass metabolism of the drug. Pharmacokinetics of its active plasma metabolite, dihydroartemisinin, was governed by the kinetic characteristics of the parent drug. Relatively longer terminal phase elimination half-life of dihydroartemisinin was observed following the administration of oral artemether compared with oral dihydroartemisinin administered as pharmaceutical formulation (Na-Bangchang *et al*, 1997c). With regard to pyrimethamine, absorption of the drug was as rapid as that of oral artemether, but elimination was much slower (half-lives of 2.7 *vs* 67.1 hours). Although disposition kinetics of pyrimethamine observed in our study was, in general, in accord with the previously reported studies (Weidekamm *et al*, 1982; Edstein *et al*, 1990) its absorption kinetics was noticeably different. The rate of drug absorption was relatively fast as evidenced by the shorter time to maximum plasma concentration (1.5 hours) compared with that previ-

ously published (3-4 hours). In most of those previous studies, kinetics of pyrimethamine was evaluated in the presence of other antimalarials which are commonly used with pyrimethamine as combined therapy (*eg* sulfadoxine, mefloquine, dapsone), and therefore drug interactions could not be ruled out. The present study is the first report that describes the kinetics of pyrimethamine when given alone and its interaction with artemether, one derivative of the promising group of antimalarials, artemisinin.

Our results showed that pyrimethamine had no influence on the kinetics of artemether. Both absorption and disposition phases of the drug were similar either when given alone or concurrently with pyrimethamine. In contrast, the presence of a single oral dose of artemether resulted in modification of pyrimethamine kinetics in some way. While the extent of drug absorption (represented by AUC) remained unchanged, the rate of drug absorption seems to be improved when artemether was coadministered, as indicated by the significantly higher C_{max}. In addition, t_{max} was more or less shortened, but due to large inter-individual variation in absorption, statistically significant difference could not be reached. The higher C_{max} of pyrimethamine however, did not result in augmentation of adverse effects of each individual drug alone. The change in absorption kinetics coincidentally occurred with the observed reduction of the apparent volume of distribution of pyrimethamine. Contraction of pyrimethamine volume of distribution of the drug as a consequence of the competition for tissue protein binding sites of artemether, pyrimethamine and their metabolites, might also in part, contribute to the immediately high plasma concentration of pyrimethamine.

In the treatment of malaria rapid onset and a relatively long duration of antimalarial action to cover up 3-4 parasite's life cycle are essential for radical therapy. On the basis of the results shown in this study, the increased maximum plasma concentration of pyrimethamine resulted from the kinetic interaction, in connection with the prolonged residence time of the active antimalarial moiety from the added dose of pyrimethamine, would be anticipated to offer therapeutic benefits. Results from our *ex vivo* assessment of antimalarial activity of the combination confirmed this assumption (Tanariya, unpublished observation). Evaluation of efficacy and tolerability, in conjunction with pharmacokinetic investigation in patients with malaria are required to define the most appropriate dose regimen of the combination--artemether/pyrimethamine for the treatment of multidrug resistant falciparum in Thailand.

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