PHARMACOKINETICS AND *EX VIVO* ANTI-MALARIAL ACTIVITY OF SERA FOLLOWING A SINGLE ORAL DOSE OF DIHYDROARTEMISININ IN HEALTHY THAI MALES

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Abstract. The pharmacokinetics of dihydroartemisinin (DHA) was studied in eight healthy male Thai subjects after a single oral dose of 300 mg. Absorption of oral DHA was rapid, C_{max} of 679 (307-1000) ng/ml was observed at 1.5 (1-2.5) hours after dosing [median (range)]. Plasma concentrations declined monoexponentially and at 12 hours after administration, the levels were below the detection limit (3 ng/ml). A large variation in the AUC (approximately) 50% was observed. The median (range) AUC was 2010 (636-4079) ng h/ml. The lag time and absorption half-life ($t_{1/2a}$) were 0.169 (0.111-0.277) hours and 0.709 (0.367-1.118) hours respectively. $t_{1/2z}$ was 1.25(0.79-1.89) hours V_z/f and CL/f were 5.9 (3.5-8.2) l/kg and 45.3 (28.6-122.8) ml/min/kg, respectively. The pattern of its *ex vivo* serum activity coincided with the plasma concentrations of DHA.

INTRODUCTION

Artemisinin (qinghaosu) and its derivatives are the most promising antimalarials currently available for the treatment of multidrug-resistant *Plasmodium falciparum* (Bunnag *et al,* 1991; 1992, Harinasuta and Karbwang, 1994.). Dihydroartemisinin (DHA) is one of artemisinin derivatives, which is the intermediate product in the synthesis of all other derivatives, *eg* artemether, artesunate and arteether (Klayman, 1985; Luo and Shen, 1987). It is a very active antimalarial with potency approximately 4-5 times of that the parent compounds themselves (Alin *et al,* 1990; Basco and Le Bras, 1993;) and more importantly, as an *in vivo* metabolic product of all other derivatives (Luo and Shen, 1987), it definitely adds to their therapeutic efficacy. Administration of DHA as pharmaceutical formulated product may, therefore, offer therapeutic benefit in view of either pharmacodynamics or pharmaco-economics. Whether the pharmacokinetic profile of this formulated oral DHA would allow an adequate therapeutic efficacy has yet to be examined. In this study, we investigated the pharmacokinetics/plasma bioavailability of DHA, in association with its *ex vivo* blood schizontocidal activity, in healthy male Thais following the administration of a single oral dose of DHA.

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PATIENTS AND METHODS

Eight healthy male Thai volunteers aged between 21 and 37 years and weighing 43-64 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. They were admidtted to the Bangkok Hospital for Tropical Diseases on the first day and returned for follow-up daily until day 7. The study was approved by the ethics committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

Prior to administration of the drug, the subjects were hospitalized overnight; each underwent a physical examination, monitoring of heart rate, blood pressure, a twelve-lead electrocardiogram (ECG), routine blood examinations (hematology, clinical chemistry) and urinalysis. Dihydroartemisinin (DHA; Arenco nv, 100 mg per capsule) was given as a single oral dose of 300 mg with a glass of water following an overnight fast. Subjects were allowed to take meal 2 hours after drug administration.

Prior to, and following drug administration, a total of 21 blood samples (8 ml each) for quantification of DHA concentrations and for assessment of *ex vivo* antimalarial activity, were collected from an antecubital vein (through an indwelling catheter) at hours 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16, 20, 24 and on days 2, 3, 4, 5, and 7. Five ml blood samples were collected into heparinized tubes and centrifuged immediately at 1200g for 15 minutes. Plasma samples for drug analysis were transferred to plastic tubes and stored at -70°C. The remaining portion of 3 ml blood was allowed to completely clot, and serum samples (for assessment of *ex vivo* antimalarial activity) were separated and stored at-70°C until analysis.

Adverse effects on the gastrointestinal, central nervous and cardiovascular systems were monitored daily by means of questionnaire, recording frequency and the 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 7.

Plasma concentrations of DHA (α-anomer) were measured by high performance liquid chromatography with electrochemical detection (Karbwang *et al,* 1997a). The average recoveries of DHA in the concentration range 80-640 ng/ml, were 86-93% with coefficients of variation below 10%. The minimum detectable concentration was 3 ng/ml.

The pharmacokinetic calculations were performed using model-independent method (Gibaldi, 1991). The time at which maximum concentrations occurred (t_{max}) and the 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 of the last plasma concentration-time data. The areas under the curve from zero time to the last observed time were calculated using the linear trapezoidal rule for ascending data points and by the log trapezoidal rule for descending data points. The area under the curve extrapolated from the last data point to infinity (AUC), was estimated by dividing the concentration at the last time point by the elimination rate constant (λ_z). The apparent total body clearance and apparent volume of distribution associated with the teminal phase were calculated as $CL/f = dose/[AUC]$ and $V_z/f = [CL/f]/\lambda_z$, rspectively. To better characterize the absorption phase, a one-compartment open model with first-order input and first-order elimination was fitted to the data by iterative, weighted non-linear regression. The observed concentrations were weighted as the reciprocal of the analytical variance.

Significant change of laboratory and vital sign parameters, following drug administration, in relation to baseline were calculated using Wilcoxon Signed-Rank test at a statistical significance level of p=0.05.

Ex vivo blood schizontocidal activities of sera

Sera collected following drug administration was investigated for their schizontocidal activities by *in vitro* microtechnique of Rickmann *et al* (1978) with modification, using K_1 strain *P.falciparum* (resistant to chloroquine and pyrimethamine) as a test organism. Briefly, serum samples collected at each instance were two-fold serially diluted with normal AB serum to dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 and 1:256. The final volume in each well of the microtiter plate was 100 µl. The inoculum in each triplicate well consisted of 80 µl of the prepared parasite cell suspension (0.5-1% parasitemia, 5% cell suspension, > 90% ring stage), and 20 µl of the diluted sera from volunteers (or AB-serum in the control wells). Following 48 hours of serum exposure (37°C, an atmosphere of 96% N_2, 1% O_2 and 3% CO_2 in a candle jar), thin blood smear was made from each well, stained with Giemsa stain and examined for the number and morphology of the ring trophozoite, and schizont stages *P. falciparum*. The assay was considered successful if at least 4 to 5-fold increase in number of parasitized cells in the control wells was achieved. The number of infected RBCs with normal appearance were counted per 10,000 RBCs. Effect of drug-containing sera on the parasites were assessed microscopically by both a decrease in parasite density, and the viability of the remaining parasites. Blood schizontocidal activities of serum samples collected after administration was evaluated by using the maximum inhibitory dilution (MID) as an end-point. MID was determined by noting the maximum dilution of drug-containing serum at which complete inhibitory effect on parasite viability (at least 95% inhibition, $1C_{95}$) was produced.

RESULTS

DHA was well tolerated with no clinical adverse effects or drug-related changes in laboratory values, hematological, serum biochemical or vital signs (heart rate, blood pressure). No abnormality in electrocardiographic tracing was recorded.

Pharmacokinetics

Pharmacokinetic results are shown in Table 1. Fifteen minutes after administration, concentrations of DHA in plasma were detected in all subjects (Fig 1). The medians (range) of maximum balues (C_{max}) of 679 (307-1,000) ng/ml were observed 1.5 (1-2.5) hours after administration. Plasma concentrations declined rapidly and at 12 hours after administration, the levels were below the detection limit (5 ng/ml). A large variation in the AUC (approximately) 50% was observed. The median (range) AUC was 2,010 (636-4,079) ng hour/ml. The lag time and absorption half-life ($t_{1/2a}$) were 0.169 (0.111-0.277) hours and 0.709 (0.367-1.118) hours respectively. $t_{1/2z}$ was 1.25 (0.79-1.89) hours. V_z/f and CL/f were 5.9 (3.5-8.2) 1/kg and 45.3 (28.6-122.8) ml/min/kg, respectively.

Table 1

Pharmacokinetic parameters of DHA following a single oral dose in 8 healthy Thai males.

Subject	Vz/f (1/kg)	AUC (ng h ml^{-1})	$t_{1/2a}$ (h)	t_{max} (h)	C_{max} (ng/ml)	$t_{1/2z}$ (h)	Cl/f (ml/min/kg)
1	8.2	636	0.59	1.5	307	0.79	122.8
2	5.7	2,116	0.71	1.5	387	1.52	23.1
3	6.1	2,056	0.9	2	735	1.24	56.5
4	3.5	1,985	0.78	2	978	0.89	45.8
5	3.6	4,079	1.12	2.5	1,000	1.89	22.0
6	4.2	2,035	0.37	1.5	800	1.26	39.0
7	6.4	1,972	0.57	1.5	623	1.62	46.0
8	6.1	1,266	0.71	1	579	0.97	73.1
Median	5.9	2,010	0.71	1.5	679	1.25	45.3
Minimum	3.5	636	0.37	1	307	0.79	28.6
Maximum	8.2	4,079	1.12	2.5	1,000	1.89	122.8

Ex vivo blood schizontocidal activities of the sera

No inhibitory effect was found in the undiluted sera collected prior to DHA administration, as well as in the control wells. In the undiluted sera, complete inhibitory effect was observed starting from 0.25 until 12 hours in most cases. The pattern of MIDs (Fig 2a) varied markedly throughout the 0.5 to 8 hours investigation period, with the range of dilution between 1:1 and 1:64. During the first half an hour, the MIDs of individual samples were in the range of 1:1 to 1:4, whereas during the period of 1-4 hours, the MIDs shifted to higher range of dilution, *ie* 1:1 to 1:64. Subsequently, the trend of decreasing of the MIDs (decrease in potency of activities) was observed during 6-12 hours period.

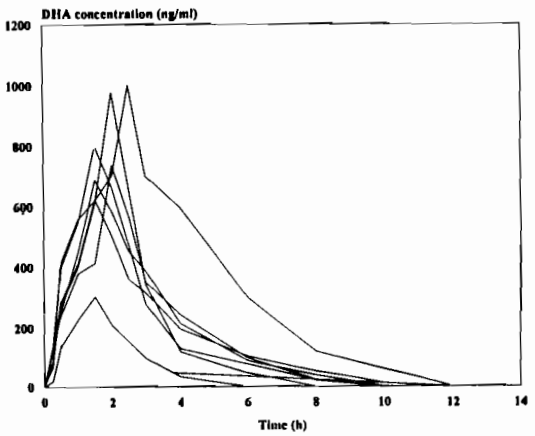

Fig 1–Plasma concentration of DHA in eight healthy Thai males after 300 mg oral administration of DHA.

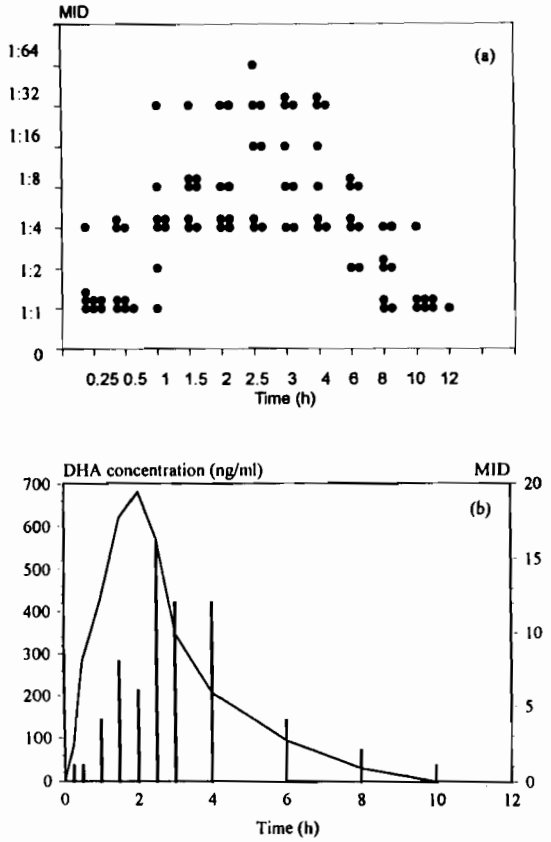

Fig 2–(a) Maximum inhibitory dilution (MID) of sera from 8 subjects after 300 mg of oral DHA (b) relationship between MIDs (bar) and plasma DHA concentrations (line) presented as median values.

DISCUSSION

Reductive mode high performance liquid chromatography (HPLC-EC) used in this study is considered the most sensitive, selective, accurate and reproducible assay method for the pharmacokinetic studies of artemisinin and derivatives (Karbwang *et al*, 1997a). The observed pharmacokinetics of DHA were generally in agreement with those reported in Chinese volunteers (Zhao and Song, 1993) where rapid absorption phase short systemic exposures were characterized. This kinetic profile generally coincided with its *ex vivo* serum antimalarial activity pattern. The activity could be observed at an early time when DHA was first detectable in plasma (15 minutes after dosing). Previous *in vitro* data has shown the IC_{50} of DHA against *P.falciparum* isolates to be in the range of 10^{-8}-10^{-7} mol/l or 3 to 30 µg/l (Klayman, 1985; Luo and Shen, 1987; Alin *et al*, 1990; Basco and Le Bras, 1993). The parasites would therefore, be expected to be killed almost immediately after drug administration (limit of quantification for the assay method = 3 ng/ml or 3 µg/l). The delay of maximum activity (0.5-1 hours) from the observed peak plasma concentration represents a lag peiod for the maximum effect of DHA. It is interesting to note that, certain degree of activity still persisted at the time when DHA was undetectable in plasma (lower than 3 ng/ml; Fig 2b). This activity is probably accounted for by either the potent activity of DHA itself, or activity responsible by other active metabolites that may be present in sera. Integrated information on kinetic and dynamic profiles would be worthwhile for designing of optimal therapeutic regimen(s) of oral DHA in falciparum malaria.

The most striking observation on the kinetics of DHA is a remarkable difference in the terminal phase elimination half-life of the drug. Following the administration of formulated DHA in this study, median (range) of value of 1.25 (0.79-1.89) hours was observed. Pharmacokinetics of DHA as an active plasma metabolite of artemether, artesunate have been investigated in previous studies using an equivalent oral dose of the parent drugs (a single oral dose of 300 mg) and analytical method (Karbwang *et al*, 1997b; Na-Bangchang *et al*, 1997). The half-life was found much longer after the administration of artemether, compared to artesunate or formulated DHA (3-6 *vs* 1-2 hours). This marked discrepancy is explained by the hybrid characteristic of this parameter, which is considerably influenced by the rate of transformation of artemether into DHA, and therefore, estimation of $t_{1/2z}$ for DHA would be more realistic after the intake of DHA.

Clinical experiences have shown that all artemisinin derivatives exert rapid and equipotent initial action . The rate of parasite and fever clearance time after artesunate, artemether and DHA were similar (Bunnag *et al*, 1991; Price *et al*, 1995), *ie* approximately 24 hours period. Prolonged action of these drugs reflects their pharmacokinetic profiles. The system bioavailability of the parent compounds themselves and DHA is greatest with artemether (Karbwang *et al*, 1997b), comparing to artesunate, and to a lesser extent, formulated oral DHA [C_{max}: 927, 724, 679 ng/ml; AUC: 2,602, 7,440, 2,010 ng h/ml; t_{max} 1.5, 3.6. 1.5 hours for

formulated artesunate, artemether and DHA, respectively]. Artemether may be superior to artesunate or formulated DHA with regard to its sustained (gradual increase of systemic exposure) and prolonged antimalarial action. Overall radical cure rate from this derivative, is governed largely by both the parent compound and active metabolite (DHA), whereas antimalarial action of artesunate and formulated DHA in particular, depends solely on DHA. Modification of pharmaceutical formulation of oral DHA to improve drug disintegration/dissolution, while offering high and sustained plasma concentration, *eg,* sustained release formulation could be an alternative approach to achieve immediate and prolonged therapeutic concentration of oral DHA.

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