PHAMACOKINETICS OF A SINGLE ORAL DOSE OF DIHYDROARTEMISININ IN VIETNAMESE HEALTHY VOLUNTEERS

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Abstract. Pharmacokinetics of a 240 mg single dose of oral dihydroartemisinin (DHA) was investigated in 8 healthy (5 males, 3 females) Vietnamese volunteers. Plasma concentrations were measured by high-performance liquid chromatography with electrochemical detection in the reductive mode. The concentration time profile of DHA was fitted with one-compartment model with a lag time. Pharmacokinetics of DHA is comparable between males and females even when adjusted with dosage. The median (range) values of pooled pharmacokinetics of oral DHA were:t_{lag} 0.41 (0.09-0.78) hours, $t_{1/2a}$ 0.58 (0.17-1.43) hours, t_{max} 1.6 (1.1-2.2) hours, C_{max} 466 (128-787) ng/ml. C_{max}/dosage 97.7 (27.2-124.6) ng/ml, $t_{1/2z}$ 2.0 (1.5-3.4) hours, AUC 1867 (420-3535) ng.h/ml, AUC/dosage 364.3 (89.3-559.7) ng.h/ml/dosage, Cl/f 45.8 (30.0-190.0) ml/min/kg, V_z/f 8.0 (5.5-29.9) l/kg. Interindividual variation was large, the coefficients of variation (CV) were 47.8% and 45.3% respectively to AUC and C_{max}. The t_{max} of DHA formulation was comparable with that of DHA metabolite of artemisinin derivatives. The $t_{1/2z}$ was longer and shorter than that of DHA metabolites of oral formulations of artesunate and artemether, respectively. For monotherapeutic regimen(s) of DHA, dosing frequency of at least twice a day is suggested. Combined regimen(s) of DHA with other potent, long half-life antimalarials may also be an alternative approach.

INTRODUCTION

Artemisinin and derivatives are now the most promising medicaments for malaria treatment in many areas of the world, especially in the South-east Asian region where multidrug resistant *P. falciparum* exists. Despite the widespread use, the pharmacokinetic informations of these drugs are limited. This is due to the lack of reliable analytical methods for the measurement of their concentrations in biological fluids following drug administration. Currently, high performance liquid chromatography with reductive electrochemical detection (HPLC-ECD) provides excellent sensitivity as well as specificity for this purpose (Edwards, 1994). Dihydroartemisinin (DHA) is the main acting blood schizontocidal metabolite of all the semi-synthetic artemisinin derivatives, *ie*, artemether, artesunate and arteether. With HPLC-ECD method, pharmacokinetics of artemisinin (Due *et al,* 1994; de Vries *et al,* 1997; Dien *et al,* 1997), including derivatives, *ie*, artemether, artesunate and DHA (Navaratnam *et al,* 1995; Teja-Isavadharm *et al,* 1996; Karbwang *et al,* 1997a; Mordi *et al,* 1997; Na-Bangchang *et al,* 1998a) were investigated. The production of DHA is simple because this compound- - a reduced lactol derivative of artemisinin, is the intermediate product in the synthesis of other derivatives (Haynes *et al,* 1994; Webster *et al,* 1994). Thus, the yield of DHA is higher than that of artemether (75% *vs* 59%, respectively) (Haynes *et al,* 1994). In addition, it was shown that 57% of artemether was metabolized back to DHA in rat liver microsomes (Leskovac *et al,* 1991). Oral DHA formulation has been produced in China for malaria treatment. Pharmacokinetics of oral DHA in Chinese subjects was reported using radioimmuno assay method (Zhao *et al,* 1993). Recently, kinetics of oral DHA (Arenco nv, Belgium), has been studied in healthy Thai males using HPLC-ECD (Na-Bangchag *et al,* 1997). Differences in ethnics, pharmaceutical formulations and analytical methods can influence the kinetics of the drug. We reported here the pharmacokinetics of a single oral dose of DHA (Cotecxin®, China) in Vietnamese healthy volunteers using HPLC-ECD as the assay method.

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METERIALS AND METHODS

Subjects

The study was approved by the Scintific Committee of Cho Ray Hospital, Ho Chi Minh City,

Vietnam. Eight healthy Vietnamese volunteers (5 males and 3 females, aged 21-41 years, weighing 37-52kg, height 145-170 cm) with no history of liver, kidney and mental disorders, were recruited into the study. All gave their written informed consents for participation prior to the study. Smokers, alcohol drinkers and persons on regular medication were excluded. No other drugs were taken during the study. Subjects were admitted to the Tropical Diseases Clinical Research Center (TDCRC), Cho Ray Hospital, HCM City, Vietnam for drug administration and blood sample collection during the first 24 hours, and returned for follow-up on days 2 and 7. Clinical examination and a 12-lead electrocardiogram (ECG) were done on admission, followed by 12 hourly during the first 24 hours, and then on days 2 and 7. Routine blood examinations (hematology and biochemistry) and urinalysis were performed on days 0, 2, and 7.

Drug administration and blood sampling

Each volunteer received 12 tablets of 20 mg DHA (240 mg total dose) (Cotecxin®, Beijing Cotec New Technology Corp PRC, Beijing Sixth Pharmaceutical Factory, People's Republic of China) with a glass of water under supervision following an overnight fast. A normal breakfast was given 3 hours after drug administration. Venous blood (4 ml) for the assay of DHA was collected into lithium-heparinized plastic tubes through an indwelling catheter kept patent with heparinized saline inserted into an antecubital vein of the arm. Samples were taken before dosage, and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hours after the dosage. Plasma was separated immediately by centrifugation (1,500*g*, 15 minutes) and stored at -80°C until analysis.

Quantification of dihydroartemisinin concentrations

All samples were transported to Bangkok in dry ice and kept at -80°C until analysis. DHA (α-auomer) in plasma samples were measured by HPLC-ECD according to the method of Na-Bangchang *et al*, 1998a). The calibration curves were linear with a mean ± SD correlation coefficient of 0.997 ± 0.003 (n=5), with negligible intercepts and a very small variation of slopes. The average recoveries of α-DHA and artemisinin (internal standard) at the concentration range of 10-800 ng/ml were 88.2-101.1%, with coefficients of variation below 10%. The limit of quantification for α-DHA was 3 ng/ml.

Pharmacokinetic analysis

The pharmacokinetic calculation was performed using model-independent method (Gibaldi, 1991). The terminal phase elimination half-life ($t_{1/2z}$) was calculated from log-linear regression of at least four of the last plasma concentration-time data. The area under the curve from zero time to the last observed time (AUC_{0-t}) was calculated using the linear trapezoidal rule for ascending data points and the log linear trapezoidal rule for descending data points. The area under the curve, extrapolated from the last data point to infinity, was estimated by dividing the concentration at the last time point by the elimination rate constant (λ_z). The extrapolations contributed on average 0.27% (0.01-0.9%) of the total *AUC*. The apparent total body clearance and apparent volume of distribution associated with the terminal phase were calculated as Cl/f=dose/*AUC* and $V_z/f=[Cl/f]/\lambda_z$, respectively.

To better characterize the absorption phase, an oral one-compartment open model with first-order input and first-order elimination was fitted to the data by an iterative, unweighting non-linear regression using TopFit® program (version 2). Akaike's inclusion criterion (AIC) was used to select the best fit curves (Yamaoka *et al*, 1978). The maximum concentration (C_{max}), time of the maximum concentration (t_{max}), absorption half-life ($t_{1/2a}$), lag time (t_{lag}) were obtained from the fitted curve.

RESULTS

Oral DHA was well tolerated with virtually no clinical side effect as well as abnormality in laboratory findings or electrocardiographs on the follow-up days after drug administration in all subjects. Total body fluid volume, blood volume, hematocrit, hemoglobin and red blood cell tended to be larger in males than in females (Table 1). Other routine laboratory tests were in normal ranges and were comparable between the two sex groups.

Plasma concentrations were below the limit of quantification (3 ng/ml) in one male subject at 12 hours and in all subjects at 24 hours. Lag time, t_{lag}, [0.35(0.09-0.45) *vs* 0.59 (0.44-0.78) hours, median (range)] tended to be shorter in males than in females but the time to maximum concentration, t_{max}, was comparable between the two sex groups. Fig 1A and 1B present respectively, observed plasma concentrations and dosage adjusted plasma concentrations after administration of a single dose of 240 mg DHA in females and males. The profiles appear similar but the concentrations tended to be higher in females even when adjusting with dosage per

kilogram of body weight (Fig 1B). Maximum plasma concentrations (C_{max} and C_{max}/dosage) in males and females were 401 (128-754) *vs* 537 (467-787) ng/ml and 78.5 (27.2-116.2) *vs* 99.2 (96.2-124.6) ng/ml per dosage, respectively. No difference in $t_{1/2a}$, $t_{1/2z}$, Cl/f, V_z/f, *AUC* and *AUC*/dosage was found between males and females. The pooled values of pharmacokinetics of oral DHA is presented in Table 2. Interindividual variation was large with the coefficients of variation (CV) of 47.8% and 45.3% for *AUC* and C_{max}, respectively.

DISCUSSION

The concentration-time profile of oral DHA formulation was fitted with one compartment kinetic model, similar to those of DHA as a metabolite of oral artesunate, oral artemether and oral artemisinin in previous studies (Due *et al,* 1994; Karbwang *et al,* 1997a, b; de Vries *et al,* 1997; Dien *et al,* 1997; Benakis *et al,* 1997; Na-Bangchang 1998a,b). The lag time and C_{max} appeared to be longer and higher, respectively, in female than in male, but all other pharmacokinetic values of oral DHA were comparable between the two sex groups even when adjusted with dosage. No sex difference in kinetics of DHA metabolite of oral artesunate was found in Caucasian subjects (Benakis *et al,* 1997). Pharmacokinetics of oral artemisinin was also comparable between males and females in both healthy subjects and patients with uncomplicated falciparum malaria (Benakis *et al,* 1997; Sidhu *et al,* 1998; Hassan Alin *et al,* 1996).

The $t_{1/2z}$ of oral DHA (Cotecxin®, China) in Vietnamese subjects in this study was longer than that of oral DHA (Arenco nv, Belgium) in male Thai subjects (Na-Bangchang, 1997). The *AUC* was comparable between two studies and C_{max} tended to be higher in Thai subjects. These differences may reflect the bioinequivalence between these two pharmaceutical formulations and/or the effect of ethnic on the kinetics of DHA. Further study on bioequivalence of generic oral formulations of DHA is needed. Interindividual variation of bioavailability was large with the coefficients of variation (CV) of mean *AUC* of 47.8% and 48.6%, respectively, for Vietnam and Thai studies. The t_{max} and $t_{1/2z}$ of oral DHA measured with HPLC-ECD were similar to those reported with radioimmunosassay (RIA) (Zhao *et al,* 1993) but the C_{max} was higher with RIA method due to the lack of differentiation capacity between parent drug and its metabolite of RIA.

The t_{max} of oral DHA (in this study and Na-Bangchang *et al,* 1997) was not different from those of DHA metabolite of oral artmether or artesunate (Navaratnam *et al,* 1995; Teja-Isavadharm *et al,* 1996; Karbwang *et al,* 1997b; Mordi *et al,* 1997; Benakis *et al,* 1997; Na-Bangchang, 1998b). This implicates the extensive and rapid first-pass hepatic metabolism of these parent drugs. Oral artemisinin (500 mg, 1,773 nM) gave the *AUC* of 2,054 (455) and 2,611 (1,225) ng.h/ml in two studies in Vietnamese subjects (Duc *et al,* 1994, Dien *et al,* 1997) but oval DHA (240 mg, 845 nM), equal to half-dose of artemisinin, gave *AUC* of 1980 [942] ng.h/ml. Thus, the bioavailability of artemisinin could be lower than that of oral DHA, which may be related to the relatively low water solubility nature of artemisinin (Lin *et al,* 1992).

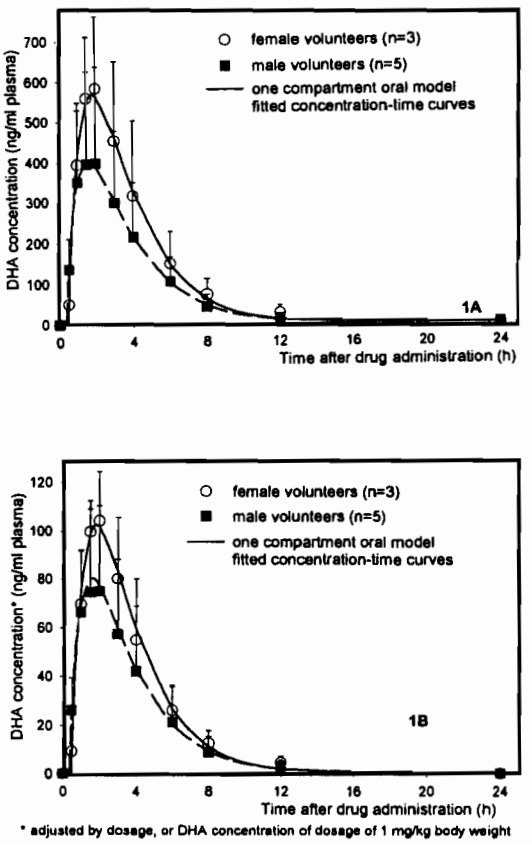

Fig 1–(A) Mean [SD bar] plasma dihydroartemisinin concentrations versus time profiles of a single oral dose of 240 mg dihydroartemisinin in female (○) and male (■) volunteers. (B) Mean [SD bar] of the profiles when adjusted with dosage in female (○) and male (■) volunteers.

Table 1

Baseline characteristics of the eight healthy Vietnameses; data are presented as median (range).

	Male volunteers (n = 5)	Female volunteers (n = 3)
DHA dosage (mg/kg)	4.7 (4.6-6.48)	5.58 (4.7 - 6.3)
Total body fluid (TBF[a]:1)	30.6 (22.2 - 31.2)	21.5 (19.0 - 25.5)
Blood volume (BV[b]:1)	3.82 (3.08 - 4.08)	2.81 (2.52-3.27)
Hematocrit (%)	46.4 (37.2 - 50.3)	37.2 (34-42.4)
Red blood cells (10^6/µl)	5.23 (4.1 - 5.5)	4.2 (3.7 - 4.7)
Hemoglobin (g/dl)	15.4 (13.4 - 16.4)	13.1 (12.5 - 14.1)

[a]TBF = 0.6 (l/kg)* weight (kg) (Ritschel, 1994).

[b]BV (blood volume); for male = 0.3669*height^3(m) + 0.03219*weight(kg) + 0.6041; for female = 0.3561*height^3(m) + 0.03308* weight(kg) + 0.1833) (Ritschel, 1994)

Table 2

Pharmacokinetics of single dose of oral dihydroartemisinin in Vietnamese healthy volunteers; data are presented as median (range).

Pharmacokinetics	Male volunteers						Female volunteers				Median (range) for pooled data
	Nb 1	2	3	4	5	Median (range)	Nb 1	2	3	Median (range)	
t_{lag} (h)	0.40	0.09	0.35	0.45	0.25	0.35(0.09-0.45)	0.44	0.59	0.78	0.59(0.44-0.78)	0.41(0.09-0.78)
$t_{1/2a}$ (h)	0.71	1.43	0.78	0.17	0.27	0.71(0.17-1.43)	0.25	0.61	0.54	0.54(0.25-0.61)	0.58(0.17-1.43)
$t_{1/2z}$ (h)	1.67	2.16	1.51	3.43	1.77	1.77(1.51-3.43)	2.39	2.24	1.69	2.24(1.69-2.39)	1.97(1.51-3.43)
t_{max} (h)	1.63	2.15	1.61	1.21	1.07	1.61(1.07-2.15)	1.3	2.04	1.87	1.87(1.3-2.04)	1.62(1.07-2.15)
C_{max} (ng/ml)	401	464	754	311	128	401(128-754)	537	787	467	537(467-787)	466*(128-787)
C_{max} /dosage (ng/ml/dosage)	78.5	100.5	116.2	67.4	27.2	78.5(27.2-116.2)	96.2	124.6	99.2	99.2(96.2-124.6)	97.7(27.2-124.6)
AUC (ng.h/ml)	1,426	2,546	2,610	1,594	420	1,594(420-2,610)	2,139	3,535	1,570	2,139(1,570-3,535)	1,867**(420-3,535)
AUC/dosage (ng.h/ml/dosage)	279.3	551.6	402.4	345.4	89.3	345.4(89.3-551.6)	383.2	559.7	333.6	383.2(333.6-559.7)	364.3(89.3-559.7)
Cl/f (ml/min/kg)	59.7	30.2	41.4	48.2	186.6	48.2(30.2-186.6)	43.5	29.8	50	43.5(29.8-50)	45.9(29.8-186.6)
V_z/f (l/kg)	8.6	5.7	5.4	14.3	28.7	8.6(5.4-28.7)	9.0	5.8	7.3	7.3(5.8-9.0)	7.95(5.4-28.7)

* mean [SD] : 481 [218] ng/ml, coefficient of variation (CV): 45.3%

** mean [SD] : 1980 [942] ngxh/ml, coefficient of variation (CV) : 47.6%

t_{lag}: lag time between drug administration and absorption, $t_{1/2a}$: absorption half-life, $t_{1/2z}$: elimination half-life; t_{max}: time to maximum concentration, C_{max}: maximum concentration, Cl: clearance, V_z: volume of distribution, h:hour, min: minute, f:bioavailability, *AUC*: area under the concentration-time course.

Oral artesunate (AS) is extensively metabolized to DHA. The AUC values of oral AS vary depending on the pharmaceutical formulations. Doses of 200 mg AS (493 nM) of Plasmotrim® (Mepha Ltd, Basel, Switzerland) (Benakis *et al,* 1997) and 300 mg AS (739 nM) of Guilin, People's Republic of China (Na-Bangchang *et al,* 1998b) biotransformed to DHA metabolite at the levels [AUC of DHA metabolites [mean (SD)] of 740 (240) and 1,630 (365) ng.h/ml, respectively. These levels were lesser than that of DHA fromulation [1980 (942) ng.h/ml] in this study. Inversely, 300 mg AS of Arenco, Belgium produced *AUC* of DHA metabolite [2,600 1,020) ng.h/ml, Na-Bangchang *et al,* 1998b] higher than that of DHA formulation. Oral artemether was mainly metabolized to DHA but not so extensively. The *AUC* of oral artemether were 29%, 45.4% and 89% of those of DHA metabolite in studies of Karbwang *et al,* (1997b), Teja-Isavadharm *et al* (1996) and Mordi *et al* (1997), repectively. Nevertheless, the total *AUC* of both artemether and its DHA metabolite was higher than that of oral DHA formulation at equivalent doses. Whether this favorable presence of both active moieties confers superior antimalarial activity from oral artemether compared to oral DHA remains to be proved in clinical trials.

The half life [mean (SD)] of oral DHA formulation was 2.11 (0.62) hours. This is longer than that of DHA metabolite of oral artesunate [mean (SD) 0.65 (0.21) hours (Benakis *et al,* 1997), and 1.77 (0.8) hours (Na-Bangchang *et al,* 1998b)], but shorter than those of DHA metabolite of oral artemether [mean (SD) 5.1 (0.8) hours (Karbwang *et al,* 1997b), median (range) 5.5 (3.6-8.4) hours (Tan-ariya *et al,* 1998)]. These differences reflect the difference in absorption among various artemisinin derivatives. The difference in absorption leads to difference in rates of drug disposition (distribution and biotransformation), and thus, influencing the terminal phase elimination half-lives of the drugs. High recrudescence rate, as seen with other artemisinin derivatives, when used in short course monotherapy in malaria treatment (Li *et al,* 1994) is associated with its short half-life. For monotherapeutic regimen(s) of DHA, dosing frequency of at least twice a day is suggested. Combined regimen(s) of DHA with other potent, long half-life antimalarials may also be an alternative approach.

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