Single dose pharmacokinetics of oral artemether in healthy Malaysian volunteers

M. N. Mordi,[1] S. M. Mansor,[1] V. Navaratnam[1] & W. H. Wernsdorfer[2]

[1] *Centre for Drug Research, Universiti Sains Malaysia, Penang, Malaysia and* [2] *Institute of Specific Prophylaxis and Tropical Medicine, University of Vienna, Vienna, Austria*

Aims To determine the pharmacokinetics of artemether (ARM) and its principal active metabolite, dihydroartemisinin (DHA) in healthy volunteers.

Methods Six healthy male Malaysian subjects were given a single oral dose of 200 mg artemether. Blood samples were collected to 72 h. Plasma concentrations of the two compounds were measured simultaneously by reversed-phase h.p.l.c. with electrochemical detection in the reductive mode.

Results Mean ($\pm$ s.d.) maximum concentrations of ARM, $310 \pm 153\ \mu g\,l^{-1}$, were reached 1.88 ± 0.21 h after drug intake. The mean elimination half-life was 2.00 ± 0.59 h, and the mean AUC $671 \pm 271\ \mu g\,l^{-1}$ h. The mean C_{max} of DHA, $273 \pm 64\ \mu g\,l^{-1}$, was observed at 1.92 ± 0.13 h. The mean AUC of DHA was $753 \pm 233\ \mu g\,h\,l^{-1}$. ARM and DHA were stable at $\leq -20^{\circ}$ C for at least 4 months in plasma samples.

Conclusions The relatively short half-life of ARM may be one of the factors responsible for the poor radical cure rate of falciparum malaria with regimens employing daily dosing. In view of the rapid loss of DHA in plasma samples held at room temperature (26° C) it is recommended to store them at a temperature of $\leq -20^{\circ}$ C as early as possible after sample collection.

Keywords: artemether, malaria, pharmacokinetics

Introduction

Artemisinin (ART), a sesquiterpene lactone occurring naturally in the annual composite plant *Artemisia annua* L., has marked blood schizontocidal activity against human and other mammalian Plasmodia [1]. Semisynthetic derivatives of ART, such as its methyl ether, artemether (ARM), show even higher activity. Originally, ARM was formulated for intramuscular injection as an oily solution [2] but an oral formulation has recently been developed [3].

Relatively high recrudescence rates after treatment with ARM and other ART derivatives have in part been ascribed to the inadequacy of current regimens [4]. This is due largely to the lack of appropriate analytical methods which could provide essential pharmacokinetic information [5]. Recently, a sensitive and reproducible assay for ARM has been developed using reversed-phase high-performance liquid chromatography (h.p.l.c.) with electrochemical detection (EC) in the reductive mode [6]. This report describes the pharmacokinetics of ARM and its active metabolite, DHA after a single oral administration of ARM to healthy Malaysian subjects. The stability of ARM and DHA in plasma at various temperatures was also investigated.

Methods

Subjects

Six healthy male Malaysian subjects of 29 to 40 years of age (mean $\pm$ s.d.; 32.7 ± 4.2 years) and weighing 48 to 70 kg (56.8 ± 7.8 kg) were studied. The study protocol was approved by the Institutional Ethics Committee. The investigation was conducted in accordance with the Helsinki Declaration, and informed written consent was obtained from all subjects. Physical and laboratory examinations showed that the subjects were healthy and free from cardiovascular, renal, hepatic or gastrointestinal disorders. No other drugs or alcohol were taken prior to or during the clinical trial. The subjects came from a non-malarious area and had not taken antimalarial drugs for several months.

Medication

After an overnight fast each subject was given a single oral dose of 200 mg artemether (4 tablets of 50 mg, Artenam, Profarma n.v., Belgium) administered with a glass of water. A standard breakfast was given 2 h after dosing.

Blood collection

Serial blood samples (5 ml) were taken immediately before drug administration and at 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0, 48.0 and 72.0 h after drug administration. They were collected into heparinized tubes, inverted slowly several times and centrifuged at 2073 *g* for 10 min. The plasma samples were transferred to polypropylene tubes and stored immediately at -70° C. Drug assays commenced the day after the last sample was taken.

Correspondence: Professor (Dr) V. Navaratnam, Centre for Drug Research, Universiti Sains Malaysia, 11800 Penang, Malaysia

Drug assays and pharmacokinetic analysis

Plasma concentrations of ARM and DHA were determined by h.p.l.c. with EC [6], with a limit of detection of 2.50 $\mu g\,l^{-1}$ for ARM and 1.25 $\mu g\,l^{-1}$ for DHA. For ARM the within-day coefficients of variation ($n=5$) were 5.3, 7.0, 1.7 and 1.5% at 15, 30, 60 and 120 $\mu g\,l^{-1}$ respectively, for DHA they were 8.1, 5.3, 2.9 and 4.8% at the same concentrations. The day-to-day CVs ($n=5$) for ARM were 8.4, 3.9 and 2.1% at 30, 60, and 120 $\mu g\,l^{-1}$. For DHA they were 6.3, 5.9 and 3.2% at the same concentrations. Pharmacokinetic parameters were determined by non-compartmental procedures [7].

Stability study

The stability of ARM and DHA in plasma spiked to 100 $\mu g\,l^{-1}$ was investigated at room temperature (RT, 26° C) 4° C, −20° C and −70° C. The samples were distributed into plastic scintillation vials and stored immediately at the scheduled temperature until analysis. One batch was analysed immediately to obtain baseline values (Day-0). The samples stored at RT were analysed on days 1, 3 and 7, those stored at lower temperatures on days 7, 14, 30 60 and 120. There were five replicates for each time point.

Results

The drug was well tolerated and no side effects were observed in any of the subjects.

The mean (±s.e.mean) plasma concentrations of ARM and DHA are presented in Figure 1. ARM (mean±s.d.) became detectable after 0.33±0.13 h and DHA after 0.29±0.10 h. For ARM C_{max}, 310±153 $\mu g\,l^{-1}$ was reached 1.88±0.21 h after drug administration, followed by a monoexponential decline of the concentration. AUC, apparant oral clearance (CL/*F*), apparant volume of distribution (*V*/*F*) and elimination half-life ($t_{1/2}$) were 671±271 $\mu g\,l^{-1}$h, 6.42±3.40 $lh^{-1}\,kg^{-1}$, 17.4±9.1 kg^{-1} and 2.00±0.59 h respectively. DHA reached C_{max}, 273±64 $\mu g\,l^{-1}$, at 1.92±0.13 h. The AUC(0,*t*) of DHA was 753±233 $\mu g\,l^{-1}$h.

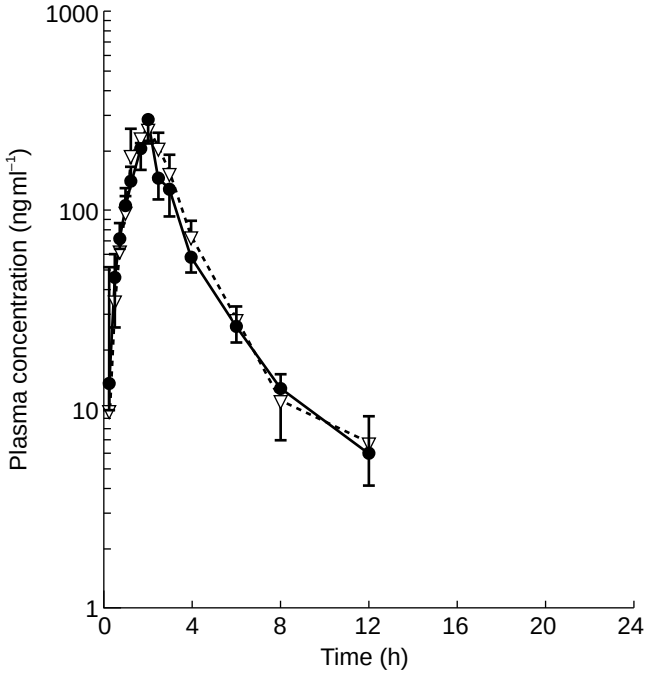

Figure 1 Plasma drug concentration-time profile of artemether (■) and dihydroartemisinin (▽) in healthy subjects ($n=6$) following a single oral dose of 200 mg artemether.

The recovery of ARM and DHA from spiked plasma stored at various temperatures is shown in Table 1. While ARM was not affected by storage under any of the conditions tested, DHA was found to be stable only when stored at −20 and −70° C. Storage at room temperature produced a rapid loss of DHA. At 4° C the recoverable quantities of DHA were reduced substantially within 1 day, and no DHA was detectable after 7 days.

Discussion

Oral administration of ARM has a definite advantage over the parenteral route when the drug is used in the treatment of uncomplicated falciparum malaria. It was therefore of interest to investigate the pharmacokinetics of ARM and its considerably more active metabolite DHA following oral administration of ARM, using a h.p.l.c./EC assay method giving more reliable estimates of AUC and terminal half-life compared with values obtained with earlier, less sensitive assay procedures.

Following oral administration ARM was rapidly absorbed.

Table 1 Stability of artemether (ARM) and dihydroartemisinin (DHA) in plasma at various temperatures and over various periods of time ($n=5$).

Duration of storage	*% of ARM recovered*		*% of DHA recovered*	
days	*mean*	*s.d.*	*mean*	*s.d.*
Room temperature				
Control	100.0	6.6	100.0	6.6
1	95.8	1.6	17.8[b]	4.4
3	102.2	107	1.7[b]	1.5
7	95.1	4.6	not detectable	
4° C				
Control	100.0	6.6	100.0	6.6
14	103.4	2.7	48.9[a]	9.1
30	106.0	2.8	30.1[a]	16.8
120	96.4	2.0	4.0[b]	4.6
−20° C				
Control	100.0	6.6	100.0	6.6
7	98.8	2.1	97.7	7.1
14	103.8	2.2	102.3	13.3
30	104.4	4.0	110.1	3.5
60	96.7	3.8	113.7	3.3
120	99.9	4.1	99.4	5.0
−70° C				
Control	100.0	6.6	100.0	6.6
7	104.3	5.4	107.6	9.1
14	105.6	3.4	97.0	3.0
30	101.1	4.8	101.7	4.0
60	103.4	2.4	110.0	1.3
120	95.2	3.1	99.6	1.8

a = significantly different from control group ($P<0.01$).
b = significantly different from control group ($P<0.001$).

Also, DHA became detectable very early. Both, ARM and DHA, reached C_{max} within 2 h and their concentration time profiles were similar. The C_{max} values showed considerable inter-individual variation, with ARM more variable than DHA. There was evidence of substantial and consistent conversion of ARM to DHA in all subjects.

In comparison with Thai subjects [8] t_{max} appears shorter in Malaysian subjects who had also relatively higher ARM and lower DHA concentration than healthy Thais. This may be due to ethnic factors although we have little direct evidence with which to substantiate that claim. Interestingly, t_{max} and $t_{1/2}$ determined in this study resemble those obtained with oral arteminisinin [9, 10].

The relatively short half-life of ARM (2.00 ± 0.59 h) may be one of the factors responsible for the poor radical cure rate of falciparum malaria with regimens employing daily or half-daily dosing. Such dose regimens entail pulsed exposure of the malaria parasites and long intervals with little or no drug, in contrast to the generally postulated maintenance of active drug levels over a time span of at least two or three cycles of blood schizogony.

The results of the stability study indicate that plasma samples for ARM and DHA assays may be stored, without appreciable loss of the drug, at $\leq -20^{\circ}$ C for at least 4 months. Further studies are needed to determine the maximum permissible duration of storage at -20° C and -70° C. In view of the rapid loss of DHA in plasma samples held at room temperature it is recommended to store them at a temperature of $\leq -20^{\circ}$ C earliest possible after sample collection.

The authors wish to thank Ms Hasliza Ali for typing the manuscript. M.N.M. is in receipt of a Postgraduate Fellowship of Universiti Sains Malaysia.

References

1 Klayman D. Qinghaosu (Artemisinin): an antimalarial drug from China. *Science* 1985; **228**: 1049–1054.

2 World Health Organization. *Practical chemotheraphy of malaria.* WHO Technical Report Series No. 805 WHO, Geneva 1990.

3 White NJ. Artemisinin: current status. *Trans Roy Soc Trop Med Hyg* 1994; **88**: S1/3-S1/4.

4 Bunnag D, Viravan C, Looareesuwan S, Karbwang J, Harinasuta T. Clinical trial of artesunate and artemether on multidrug resistant falciparum malaria in Thailand. *Southeast Asian J Trop Med Public Health* 1991; **22**: 380–385.

5 Edwards G. Measurement of artemisinin and its derivatives in biological fluids. *Trans Roy Soc Trop Med Hyg* 1994; **88**: S1/37-S1/39.

6 Navaratnam V, Mansor SM, Chin LK, Mordi MN, Asokan M, Nair NK. Determination of artemether and dihydroartemisinin in blood plasma by high-performance liquid chromatography for application in clinical pharmacological studies. *J Chromatogr* 1995; **669**: 289–294.

7 Rowland M, Tozer T. *Clinical Pharmacokinetics. Concepts and Applications*. Philadelphia: Lea and Fiebiger, 1989.

8 Na Bangchang K, Karbwang J, Thomas CG, *et al*. Pharmacokinetics of artemether after oral administration to healthy Thai males and patients with acute, uncomplicated falciparum malaria. *Br J Clin Pharmacol* 1994; **37**: 249–253.

9 Titulaer HAC, Zuidema J, Kager PA, Wetsteyn JCFM, Lugt CB, Merkus FWHM. The pharmacokinetics of artemisinin after oral, intramuscular and rectal administration to colunteers. *J Pharm Pharmacol* 1990; **42**: 810–813.

10 Duc DD, Vries PJ, Nguyen XK, Nguyen B, Kager PA, Boxtel CJ. The pharmacokinetics of a single dose of artemisinin in healthy Vietnamese subjects. *Am J Trop Med Hyg* 1994; **51**: 785–790.

(*Received 22 April 1996, accepted 2 December 1996*)