Oral bioavailability of dihydroartemisinin in Vietnamese volunteers and in patients with falciparum malaria

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Aims To obtain comprehensive bioavailability data for artesunate (ARTS) and its active metabolite dihydroartemisinin (DHA) following their separate oral administration to Vietnamese volunteers and to patients with acute, uncomplicated falciparum malaria.

Methods Volunteers were randomized to receive either i.v. ARTS (120 mg) followed by oral ARTS (150 mg) 8 h later (Group 1, $n=10$), or i.v. ARTS (120 mg) followed by oral DHA (120 mg) 8 h later. Patients, also received oral ARTS (150 mg; Group 3, $n=8$) or DHA (120 mg; Group 2, $n=7$), in a randomized cross-over study design. Multiple blood samples were collected after each administration and plasma ARTS and/or DHA concentrations were determined by h.p.l.c. Pharmacokinetic descriptors were obtained from noncompartmental analysis and bioavailability was calculated from AUC data. In the patients, the time to 50% parasite clearance (PCT_{50}) and fever clearance time (FCT) also were measured.

Results In Group 1 (volunteers), the mean (95% CI) absolute bioavailability of oral ARTS was 80% (62,98%), while in Group 2 (volunteers), the bioavailability of oral DHA was 45% (34,56%). In the patients (Group 3), the bioavailability of oral DHA relative to oral ARTS was 88% (49,127%). The median PCT_{50} and FCT were 2.3 and 28 h, respectively.

Conclusions The study shows that the absolute bioavailability of DHA was significantly lower than that for ARTS in healthy volunteers. The bioavailability of ARTS in volunteers was consistent with previous studies in patients with uncomplicated falciparum malaria. The dose-normalized C_{max} and AUC$(0,\infty)$ for DHA were significantly greater in patients with falciparum malaria than in healthy volunteers. The high relative bioavailability of DHA in the patients may have been due to lower first-pass clearance. We conclude that, for the treatment of malaria, DHA is likely to be a suitable oral substitute for ARTS. Based on our mean AUC measurements, it appears that equal doses of DHA and ARTS (mg basis) should give equivalent systemic exposure to bioactive DHA in uncomplicated falciparum malaria.

Keywords: artesunate, bioavailability, dihydroartemisinin, falciparum malaria, pharmacodynamics, pharmacokinetics

Introduction

Artesunate (ARTS) is a water-soluble hemisuccinate derivative of artemisinin that is widely used in the treatment of both uncomplicated and severe falciparum malaria [1–3]. To date, most treatment regimens have used

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either i.v. or oral ARTS. Since ARTS is rapidly de-esterified to its pharmacologically active metabolite dihydroartemisinin (DHA) [4] [5–7], it may be equally acceptable to administer DHA itself. DHA has been developed for clinical use only relatively recently compared with other artemisinin derivatives. This largely may be due to its poor water solubility which means that it can only be administered orally or rectally. Whilst there are several reports of the pharmacokinetics of DHA after oral administration [5, 8–11], its absolute bioavailability is unknown. Since antimalarial efficiacy is dependent on adequate systemic concentrations of active drug, the present study was undertaken to ascertain the bioavailability of DHA following oral ARTS or DHA in volunteers and also in patients with falciparum malaria.

Methods

Subjects

Nineteen healthy adult volunteers were recruited from Bao Loc village, Lam Dong Province, Vietnam in September 1996. All volunteers were slide-negative for malaria before study. In addition, 12 patients with uncomplicated falciparum malaria were recruited from the same region in 1997, either following admission to Bao Loc Hospital or on referral from neighbouring primary care health facilities. In these patients, the diagnosis was confirmed by microscopic examination of thick and thin blood films, and a complete clinical assessment and routine laboratory tests were performed. Patients were excluded from the study if they had any signs or symptoms of severe malaria, specifically, impaired consciousness, jaundice (serum bilirubin $>50\ \mu mol\ l^{-1}$), renal impairment (serum creatinine $>250\ \mu mol\ l^{-1}$ after rehydration), anaemia (venous haematocrit $<20\%$), or hyperparasitaemia ($>150\,000$ asexual forms per μl whole blood from thick film analysis), or if they had been treated with ARTS or DHA in the previous 8 h, artemisinin in the previous 12 h or artemether in the previous 24 h. These latter criteria encompassed at least five times the established elimination half-life of the drug (40 min for DHA, 2.2–2.3 h for artemisinin and 4.2 h for artemether).

All patients and controls gave informed consent to participate in the study which was approved by the Ministry of Health, Vietnam and the University of Western Australia Human Rights Committee.

Study design and procedures

All volunteers were given i.v. ARTS 120 mg diluted in 10 ml 5% w/v dextrose and given as a bolus over 2 min followed by either oral ARTS (Group 1, $n=10$; 150 mg as 3×50 mg tablets) or oral DHA (Group 2, $n=9$; 120 mg as 2×60 mg tablets) 8 h later. Both i.v. and oral ARTS formulations were obtained from the Guilin no. 2 Pharmaceutical Factory, Guanxi, China. The sequential study design was used because previous data showed complete elimination of ARTS and DHA within 6 h [6, 7], and because there was no information on the pharmacokinetics of oral DHA.

Patients were randomised by a computer generated predetermined randomization schedule to receive either oral DHA (120 mg as 2×60 mg tablets) or oral ARTS (150 mg as 3×50 mg tablets), with the alternative preparation given 8 h later in an open crossover design. ARTS tablets from a different batch to those used in the volunteer studies were obtained from the Guilin no. 2 Pharmaceutical Factory, Guangxi, China, while DHA was obtained from Beijing Cotec New Technology Corp, PRC 6th Pharmaceutical Factory, China. A single dose of mefloquine (750 mg) was administered 24 h after admission to the study.

Venous blood samples (15 ml immediately prior to dosing and 3 ml thereafter) were obtained from the arm opposite to that used for drug administration. In the case of i.v. ARTS, sampling times were at 0, 5, 7, 9, 12, 15, 20, 30, 45, 60, 90 min and 2, 2.5, 3, 4 and 8 h after dosing. For oral doses in volunteers, sampling was at 0, 15, 30, 40, 50, 60, 75, 90, 105 min and 2, 2.5, 3, 3.5 and 4 h after dose, while for patients, sampling was at 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 105 min and 2, 2.5, 3, 3.5 and 4 h after dose. The greater frequency of sampling during the first 2 h in patients was used as a result of experience gained in the volunteer study. Blood was collected into fluoride-oxalate tubes and chilled immediately at 4°C to prevent ARTS degradation by plasma esterases. Samples were centrifuged within 30 min to minimize haemolysis and the separated plasma was stored below −20°C until analysed. In the patients, thick and thin blood films were prepared from the hourly samples to 4 h, and 4 hourly thereafter, until parasite clearance. Vital signs including oral temperature and urine output were monitored every 4 h. Patients were discharged when afebrile and aparasitaemic, while volunteers were discharged as soon as blood sampling was completed.

Pharmacokinetic and pharmacodynamic analyses

ARTS and DHA tablets were assayed by high performance liquid chromatography (h.p.l.c.) [12]. The mean (95% CI) ARTS content of the 50 mg ARTS tablets in the volunteer study was 44.8 (42.8, 46.7) mg and in the patient study 46.0 (43.7, 48.3) mg. The mean (95% CI) DHA content of the 60 mg DHA tablets used in both studies was 65.7 (61.9, 69.4) mg.

Plasma samples were assayed by a previously validated h.p.l.c. assay [12]. Briefly, following solid phase extraction,

ARTS and DHA were separated on a C8 reversed phase column and detected by u.v. absorption after postcolumn derivatization with alkali. Intra-and interassay coefficients of variation were $<14\%$ for ARTS (0.3–5.3 μmol l^{-1}) and $<11\%$ for DHA (0.915–5.6 μmol l^{-1}). The limits of detection were approximately 0.08 μmol l^{-1} for ARTS and 0.07 μmol l^{-1} for DHA. Stability of ARTS (0.78 and 4.56 μM) and DHA (1.06 and 6.16 μM) in plasma has been assessed for up to 12 months at -25°C and found to be within $\pm$ 7.6% of replicate samples stored at -80°C [13].

Pharmacokinetic parameters (AUC$(0,\infty)$, $t_{1/2}$, CL, V_z, C_{max} and t_{max}) were determined from the plasma concentration-time data using noncompartmental methods [14]. In view of the fact that doses encompassed a range from 312 to 460 μmol, values for AUC$(0,\infty)$ and C_{max} normalized to a 312 μmol dose were also calculated for each treatment group. Bioavailability was calculated as $F=(AUC_{oral}/AUC_{iv}) \times (Dose_{iv}/Dose_{oral})$ and relative bioavailability as $F=(AUC_{DHA}/AUC_{ARTS}) \times (Dose_{ARTS}/Dose_{DHA})$, with appropriate correction for tablet potency as above. Pharmacokinetic parameters derived for DHA assumed complete bioconversion from ARTS [15].

Blood films were stained (Giemsa) within 12 h of preparation and examined by the same microscopist. Thick films were used to determine parasite density in all patients. The time to reach 50% of the original parasite count (PCT_{50}) was determined by simple linear interpolation of the parasite count – time data. Fever clearance time (FCT) was taken to be the time from drug administration to the first one of three oral temperatures $<37.5^{\circ}$C.

Statistical analysis

All analyses were performed using SigmaStat for Windows, Version 2.03, SPSS Inc, Chicago, IL. Data are summarized as mean (95% CI) or median (interquartile range) as appropriate. Group differences between pharmacokinetic parameters were determined by Student's *t*-test, or by the Mann Whitney *U*-test where groups with unequal variances were encountered.

Results

Clinical course

Details of the patients and volunteers are given in Table 1. Two volunteers in the group who received oral DHA were excluded from data analysis as they had an insufficient number of plasma concentration measurements for calculation of pharmacokinetic parameters. All biochemical measurements in the control subjects were within normal reference ranges.

Although none of the 12 patients initially admitted to the study had an identifiable history of treatment with an artemisinin derivative, DHA (0.9–2.6 μM) was detected by h.p.l.c. in the predose samples in four cases. Since this suggested recent treatment with either ARTS or artemether, data from these patients were excluded from further analysis. The remaining patients had similar demographic characteristics to those of the volunteers, with the exception of higher mean serum glucose and total bilirubin concentrations (see Table 1). All patients responded to antimalarial and supportive therapy, and were discharged from hospital 2–5 days after presentation. The median PCT_{50} and FCT values in the patients were 2.3 h and 28 h, respectively.

Pharmacokinetic analysis

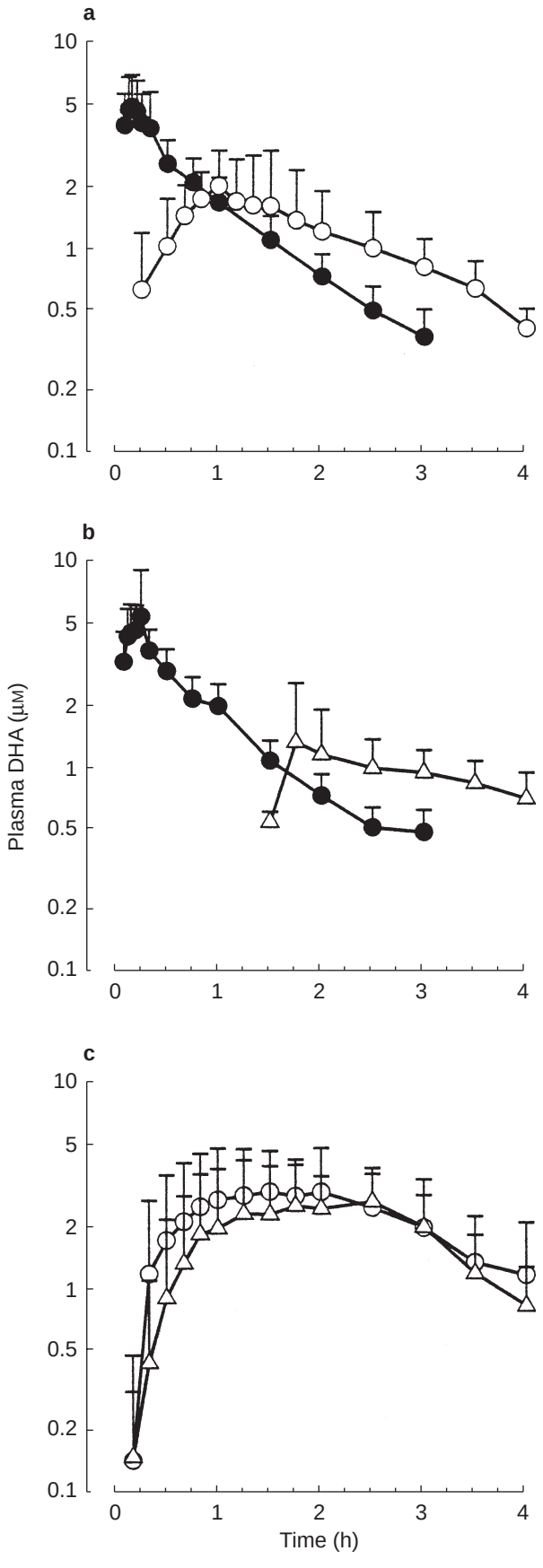
The concentration-time profiles for DHA following ARTS and DHA administration in volunteers (panels a and b) and malaria patients (panel c) are shown in Figure 1, and derived pharmacokinetic parameters are summarized in Table 2. For the volunteer studies, there were no significant between-group differences for any of the ARTS or DHA pharmacokinetic parameters following i.v. ARTS and the data were similar to previous studies from our laboratory [6, 7]. As in previous studies of oral administration of ARTS to either volunteers or patients, plasma ARTS itself was below the limit of quantification in almost all samples and its pharmacokinetic descriptors were therefore not obtainable.

For Group 1 volunteers given oral ARTS, the mean C_{max} for DHA (normalized to 312 μmol dose = 120 mg ARTS) was significantly ($t=4.7$, $P<0.001$) lower than after after i.v. administration (difference between means = 3.4 μmol l^{-1} h (95% CI = 1.9,5.0 μmol l^{-1} h)) as was the dose-normalized mean AUC (U = 133, $P=0.04$). Similarly, in Group 2 volunteers, after oral DHA, the C_{max} for DHA (normalized to 312 μmol dose) was significantly

Table 1 Demographic data for volunteers and patients. Data are presented as means (95% CI) or medians (interquartile range)⋆ as appropriate.

	Volunteers (Group 1 & 2 combined; n = *17)*	*Patients (Group 3;* n = *8)*
Age (years)	29 (26,32)	31 (20,42)
Weight (kg)	50 (48,53)	52 (48,56)
Haematocrit (%)	43 (41,45)	39 (36,43)
Glucose (mmol l^{-1})	4.5 (3.8,5.0)§	7.1 (6.3,8.0)§
Creatinine (μmol l^{-1})	100 (82 127)§	84 (65 103)§
Bilirubin (μmol l^{-1})	9.2 (7.1, 11.3)⋆	13.9 (11.4,30.3)⋆
PCT_{50} (h)	–	2.3 (0.9,8.1)⋆
FCT (h)	–	28 (18,42)⋆

§$P<0.05$.



(U = 76, $P = 0.001$) lower than after i.v. administration, as was the dose-normalized AUC (difference between means = 2.7 μmol l^{-1} h (95% CI = 1.6, 3.9 μmol l^{-1} h; $t = 5.2$, $P < 0.001$)). DHA (Group 2) was not measurable in plasma until 1 h after its oral administration, with a t_{max} that did not occur until 150 min (Table 2 and Figure 1b). This finding was unexpected and prompted a significant alteration to the sampling schedule used in the subsequent patient study.

In Group 3 patients, where oral ARTS or oral DHA was studied, median t_{max}, and mean $t_{1/2}$ for DHA were similar to values in the volunteers who received these same drugs orally. For oral DHA in the patients, the dose normalized (to 312 μmol) mean C_{max} was significantly lower ($t = 2.4$, $P = 0.03$) than for oral ARTS in these patients (difference between means = 1.5 μmol l^{-1} (95% CI = 0.13,2.8 μmol l^{-1})). However, the mean dose normalized AUC values after either DHA or ARTS in this group were not significantly different.

Pharmacokinetic parameters for DHA were also compared between patients and volunteers to identify differences attributable to malaria infection. Median t_{max} and mean $t_{1/2}$ were similar for both oral ARTS and oral DHA. For oral ARTS, the mean dose-normalized C_{max} of DHA (to 312 μmol), was significantly ($t = 3.7$, $P = 0.002$) higher in patients than in volunteers (difference between means = 2.2 μmol l^{-1} (95% CI = 0.9,3.3)). Similarly, the mean AUC was two-fold greater ($t = 3.3$, $P = 0.005$) in patients than in volunteers (difference between means = 4.2 μmol l^{-1} h (95% CI = 1.5,7.0). For DHA administration, the mean dose-normalized C_{max} (to 312 μmol), also was significantly ($t = 3.3$, $P = 0.006$) higher in patients than in volunteers (difference between means = 1.4 μmol l^{-1} (95% CI = 0.5 −2.3)). Similarly, the mean AUC was 2.6-fold greater ($t = 4.5$, $P < 0.001$) in patients than in volunteers (difference between means = 3.5 μmol h l^{-1} (95% CI = 1.8 − 5.2). DHA dose-normalized AUC (to 312 μmol) for oral DHA in Group 2 volunteers was similar that for oral ARTS in Group 1 volunteers.

In Group 1 (volunteers), the mean (95% CI) absolute bioavailability of oral ARTS was 80% (62,98%), while in Group 2 (volunteers), the bioavailability of oral DHA was 45% (34,56%). However, in the patients (Group 3), the bioavailability of oral DHA relative to oral ARTS was 88% (49,127%).

Figure 1 Plasma concentration-time profile for dihydroartemisinin following 312 μmol i.v. artesunate (●), 391 μmol oral artesunate (○) or 422 μmol oral dihydroartemisinin (△) in group 1 volunteers (a), group 2 volunteers (b) and group 3 patients (c). Data shown as mean ± s.d.).

Table 2 Pharmacokinetic parameters for artesunate and dihydroartemisinin following administration of i.v. artesunate, oral artesunate or oral dihydroartemisinin in volunteers and patients. Data are presented as means (95% CI) or medians (interquartile range)[a] as appropriate.

	Group 1, Volunteers (n = 10)		*Group 2, Volunteers (n = 7)*		*Group 3, Patients (n = 8)*	
Route/Drug (dose)	*i.v. ARTS (312 μmol)*	*Oral ARTS (350 μmol)*	*i.v. ARTS (312 μmol)*	*Oral DHA (462 μmol)*	*Oral ARTS (360 μmol)*	*Oral DHA (462 μmol)*
ARTS						
$t_{1/2}$ (min)	2.6 (2.1, 3.0)	–	3.3 (2.3, 4.3)	–		
CL (l h^{-1} kg^{-1})	3.0 (2.5, 3.6)	–	2.2 (1.5, 2.7)	–		
V_z (l kg^{-1})	0.19 (0.14, 0.25)	–	0.16 (0.09, 0.22)	–		
AUC (μmol l^{-1} h)	2.2 (1.7, 2.8)	–	3.3 (2.2, 4.4)	–		
DHA						
C_{max} (μmol l^{-1})	5.3 (3.8, 6.8)	2.0 (1.3, 2.7)	5.9 (2.1, 9.8)	1.7 (0.7, 2.7)	4.6 (3.1, 6.1)	3.7 (2.6,4.8)
C_{max} (μmol l^{-1})★	5.3 (3.8, 6.8)	1.8 (1.2, 2.4)†	5.9 (2.1, 9.8)	1.1 (0.5, 1.7)†	4.0 (2.7, 5.3)+	2.5 (1.7, 3.3)#,★★
t_{max} (min)[a]	9 (7, 12)	70 (50, 149)	15 (9, 19)	150 (109, 173)	95 (50, 120)	127 (90 151)
$t_{1/2}$ (min)	53 (39, 67)	46 (36, 57)	47 (43, 51)	61 (40, 82)	53 (39, 67)	53 (35, 72)
AUC (μmol l^{-1} h)	4.8 (3.9, 5.6)	3.9 (2.5, 5.3)	4.9 (3.8, 6.0)	3.3 (2.4, 4.2)	8.9 (5.9, 11.9)	8.5 (6.1, 10.9)
AUC (μmol l^{-1} h)★	4.8 (3.9, 5.6)	3.5 (2.3, 4.7)†	4.9 (3.8, 6.0)	2.2 (1.6, 2.8)†	7.7 (5.1, 10.3)+	5.7 (4.1, 7.3)§
Bioavailability (%)	–	80 (62, 98)	–	45 (34, 56)	–	88 (49, 127)

★C_{max} and AUC data normalized to a dose of 312 μmol (= 120 mg ARTS); †$P < 0.05$ compared with i.v. ARTS in same group; #$P = 0.03$ compared with oral ARTS in Group 3; +$P < 0.01$ compared with oral ARTS in Group 1; §$P < 0.005$ compared with oral DHA in Group 2; ★★$P < 0.001$ compared with oral DHA in Group 2.

Discussion

Our initial study in healthy volunteers was designed primarily to provide valid estimates of the oral bioavailability of ARTS and DHA. This was an important first step as DHA was essentially an untried treatment when the volunteer study was carried out and there were no published data. By comparison, both i.v. and oral ARTS were widely available in countries such as Vietnam and the pharmacokinetics of ARTS were well documented [1, 2]. Since there is no i.v. formulation of DHA, it is reasonable to calculate DHA oral bioavailability by assuming complete bioconversion of ARTS to DHA after i.v. ARTS administration [15]. Our results showed that mean values for absolute DHA bioavailability after oral ARTS and oral DHA were 80% and 45%, respectively. The second part of the study in patients with uncomplicated malaria was a direct comparison of ARTS and DHA with both drugs given orally in the same doses as those used in the volunteer study. We aimed to determine whether malaria infection *per se* influenced the bioavailability and pharmacokinetics of DHA. The patients had significantly higher peak plasma concentrations of DHA following ARTS and DHA administration than the volunteers for each of the two respective oral regimens.

In the volunteers, oral administration of DHA gave $t_{1/2}$ values that were in agreement with those in previously published studies regardless of whether oral ARTS [6, 7] or oral DHA [9, 10] was administered. However, the bioavailability of orally administered DHA was only 45% relative to DHA from i.v. ARTS. These data suggest either that DHA undergoes greater first pass clearance after oral administration than ARTS or it is less well absorbed when both drugs are given by mouth. Since the major metabolic clearance pathway for DHA is glucuronidation [16–18], it is likely that clearance occurs in the liver and possibly also in the intestinal wall. Thus, our results are consistent with the hypothesis that the hemisuccinate ester on ARTS protects the molecule from first pass metabolism at these sites, whereas the unprotected hydroxyl at the 12-position in DHA is subject to significant first pass metabolism and hence has a lower oral bioavailability.

In the patient group, the bioavailability of DHA was 88% relative to DHA from an orally administered dose of ARTS in the same patients. The most likely explanation for this higher bioavailability compared with that in the volunteers is a decreased hepatic clearance of DHA as a result of the malaria. Consistent with this hypothesis, we have previously shown that *P. berghei* infection in rats results in a significant decrease in DHA metabolism to the glucuronide [16]. Supporting evidence also comes from a recent study by Newton *et al.* [19] in which the oral bioavailability of ARTS (from AUC for DHA plus ARTS measured by bioassay) in patients with uncomplicated falciparum malaria was shown to decrease from 61% in the acute phase to 31% during convalescence. Nevertheless recent data from patients with severe malaria suggest that malaria *per se*, rather than the presence of complications is the prime determinant of DHA clearance [20].

Overall, our data show that both C_{max} and AUC$(0,\infty)$ for DHA were significantly higher in patients with malaria than in healthy volunteers. In the patients, plasma DHA

concentration-time profiles for oral DHA (462 μmol, 120 mg) and oral ARTS (360 μmol, 150 mg) were similar. Thus, on pharmacokinetic grounds, we suggest that oral DHA should be a suitable substitute for oral ARTS in the treatment of falciparum malaria. However, since we had only eight patients in our clinical pharmacokinetic study, further studies are desirable. If dose equivalence is confirmed, the choice between the pro-drug ARTS and its active metabolite DHA for oral treatment of falciparum malaria would be made on pharmacoeconomic grounds.

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