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Declining Concentrations of Dihydroartemisinin in Plasma during 5-Day Oral Treatment with Artesunate for Falciparum Malaria

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Six patients with uncomplicated falciparum malaria received artesunate for 5 days. Plasma concentrations of artesunate and dihydroartemisinin were determined by high-performance liquid chromatography with electrochemical detection. The concentrations of dihydroartemisinin in plasma 2 h after a dose showed a time-dependent decline. Concentrations of artesunate in plasma especially after the last dose, were very low. Despite this, all patients responded with a fast recovery.

Artesunate is the water-soluble derivative of artemisinin and possesses strong antimalarial activity (7). An often-recommended treatment regimen for uncomplicated malaria is 3 days of artesunate with mefloquine (11, 12). The single-dose pharmacokinetics of artesunate have been described in a few reports (2, 5, 6). Multidose studies have not yet been reported.

Several techniques to measure concentrations of artemisinins are available. High-performance liquid chromatography with electrochemical detection (HPLC-ED) is highly sensitive and specific and is regarded as the reference technique for pharmacokinetic studies. In order to establish the multidose pharmacokinetics of artesunate by HPLC-ED, a pharmacokinetic study of patients with uncomplicated falciparum malaria was undertaken.

Patients with uncomplicated falciparum malaria were studied. Inclusion criteria were age of more than 14 years and a parasitemia level between 1,000 and 50,000 μl^{-1}. Exclusion criteria were pregnancy and lactation, mixed plasmodium infection, complications of malaria, administration of artemisinin or derivatives during the prior 3 days or allergy to one of these drugs, hepatic insufficiency, and a positive test for hepatitis B surface antigen.

Artesunate tablets (50 mg; Mediplantex, Medicinal Plant Company No. 1, Hanoi, Vietnam) were used. The patients received 150 mg at the start of the study (time zero) and at time (t) = 12 h and 100 mg at t = 24, 48, 72, and 96 h. At t = 120 h mefloquine (15 mg · kg^{-1}) was given for radical cure. Parasite counts were done every 8 h until two consecutive negative smears were obtained. Physical examination and routine laboratory tests, including hepatitis B surface antigen were done. An electrocardiogram was performed before treatment and at 48 h. Possible adverse effects were recorded.

Approval was obtained from the medical ethics committee of the Academic Medical Center, Amsterdam, The Netherlands, and the Board of the Institute for Clinical Research in Tropical Medicine, Bach Mai Hospital, Hanoi, Vietnam. Informed written consent was obtained from all subjects.

Blood samples were drawn before drug intake (time zero) and at 2, 12, 14, 24, 26, 96, 98, 100, 102, 104, 106, 108, 112, and 120 h. Handling of specimens and measurements were performed as reported before (6, 13). The precision and accuracy of the HPLC-ED assay have been specified for dihydroartemisinin in a previous report (13). For artesunate the interassay (day-to-day) coefficient of variation was 5 to 6% for the expected concentrations, 50 to 200 $\mu g \cdot liter^{-1}$, and approximately 18% for 10 $\mu g \cdot liter^{-1}$. The intra-assay (within 1 day) coefficient of variation was 4 to 5% for concentrations from 50 to 200 $\mu g \cdot liter^{-1}$ and 20% for a concentration of 10 $\mu g \cdot liter^{-1}$. By this technique artesunate and dihydroartemisinin plasma concentrations of 5 $\mu g \cdot liter^{-1}$ or higher could be detected in all samples. Under optimal chromatographic conditions lower concentrations could also be detected. Before and after assay of all samples of each patient, calibration lines were constructed by using four standard solutions of 10, 50, 100, and 200 $\mu g \cdot liter^{-1}$. For comparison of the parameters after administration of the respective dosages, analysis of variance (ANOVA) was used.

Six male patients with a mean (range) age of 30 (21 to 38) years and a mean (range) body weight of 54 (50 to 60) kg were included. The mean (range) dose of artesunate by body weight was 2.8 (2.5 to 3.0) mg · kg^{-1} for the 150-mg dose.

The concentrations of artesunate in plasma were very low. The values are shown in Table 1. Undetectable concentrations were entered as 0 $\mu g \cdot liter^{-1}$. There was no difference between the respective peak concentrations which were normalized to a 150-mg dose (P = 0.09 [ANOVA]). In patient 2 the artesunate concentrations did not decrease to below the detection limit at t = 120 h. The concentrations of dihydroartemisinin are shown in Table 2. In patients 1 and 2 the concentrations of dihydroartemisinin in plasma did not fall below the detection limit within the sampling period. The dihydroartemisinin concentrations normalized to a 150-mg dose of artesunate observed 2 h after each dose were significantly different (P = 0.024 [ANOVA]).

The mean (± standard deviation [SD]) parasite count at the start of treatment was 28,520 (±20,423) μl^{-1}. All patients experienced a rapid, uneventful recovery, with a parasite clear-

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TABLE 1. Plasma artesunate concentrations in six patients with uncomplicated falciparum malaria

Patient no.	Plasma artesunate concn ($\mu g \cdot liter^{-1}$) at sampling time (h)														
	0[a]	2	12[a]	14	24[b]	26[c]	96[b]	98	100	102	104	106	108	112	120
1	0	2	7	7	0	0	2	2	2	2	2	2	0	0	0
2	0	20	18	18	24	12	4	4	4	5	6	3	12	26	10
3	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0
4	0	9	7	10	4	4	3	0	0	0	0	0	0	0	0
5	0	—[d]	16	20	12	50	12	—	12	10	—	0	0	0	0
6	0	10	7	16	6	9	—	4	—	—	—	4	4	4	0
Mean	0	8	9	12	8	14	4	2	4	4	3	1	3	5	2
SD	0	8	7	8	9	18	5	2	5	4	3	2	5	11	4

[a] A 150-mg dose of artesunate was administered at this time.
[b] A 100-mg dose of artesunate was administered at this time.
[c] For the two subsequent dosing periods (48 and 72 h), 100-mg doses of artesunate were administered but concentrations in plasma were not determined.
[d] —, missing value.

ance time of 32 h in four and 40 h in two patients. There were no complaints indicative of side effects. Laboratory values remained within normal limits. The electrocardiograms were all normal before treatment and at $t = 48$ h; in particular, no changes of the QT interval were observed.

This study shows that after repeated oral administration of artesunate a time-dependent decline of artesunate and dihydroartemisinin concentrations in plasma occurs. The pharmacokinetics of artesunate and dihydroartemisinin have been addressed in a few recent reports (2–6, 15). After a single oral dose of artesunate to healthy Caucasians, the concentrations in plasma were very low (5). The dihydroartemisinin concentrations in plasma were much higher. In six patients with uncomplicated malaria who received 120 mg of artesunate intravenously, the concentrations in plasma were measured by HPLC with UV detection (detection limit, 50 $\mu g \cdot liter^{-1}$) (2). The estimated elimination half-life of artesunate was 0.06 h. The elimination half-life of dihydroartemisinin was 0.57 h. In a bioassay unable to discriminate between artesunate and dihydroartemisinin, the kinetics of a single oral dose (3 mg/kg) in children with uncomplicated malaria was studied (6). The maximum concentration of dihydroartemisinin concentration equivalents in serum (C_{max} of dihydroartemisinin) was 664 $\mu g \cdot liter^{-1}$, and the elimination half-life was 1 h. A single 2-mg $\cdot kg^{-1}$ intramuscular dose to severe malaria patients resulted in a C_{max} of 510 $\mu g \cdot liter^{-1}$, with an elimination half-life of 0.49 h (3). In this study HPLC-ED was used for measuring the artesunate concentrations. In the same study a 2-mg $\cdot kg^{-1}$ intravenous dose was studied. The same elimination half-life was found, but the C_{max} was much higher: 2,640 $\mu g \cdot liter^{-1}$.

In all these studies single dosages of artesunate were used. In the management of malaria however, multiple dosages of artesunate are often used (10, 12). Based on findings with artemisinin it is known that a time-dependent decline of plasma concentrations occurs after repeated dosing (1, 9). A similar effect was observed when artemether was administered orally to Chinese and European patients with uncomplicated falciparum malaria (14). The concentrations of dihydroartemisinin in plasma after the first dose were comparable to findings with single-dose administration of this compound (4, 15).

In our study dihydroartemisinin and to a lesser extent also artesunate are involved in the time-dependent decrease of plasma concentrations. The explanation of this phenomenon is not clear. The decline of the concentrations in plasma of both artesunate as well as its major metabolite suggests that this metabolization step is not affected, because this would have opposite effects on the two compounds. If artesunate is transformed into dihydroartemisinin very early after ingestion, a decrease of absorption or an increase of degradation could explain the declining concentrations of both in plasma. At present it remains unclear which mechanism explains the time-dependent decrease of concentrations in plasma. Especially after the last dose the concentrations of both compounds in plasma were very low. The reason for the relatively slow elimination of artesunate after the last dose in patient 1 and of

TABLE 2. Plasma dihydroartemisinin concentrations in six patients with uncomplicated falciparum malaria

Patient	Plasma dihydroartemisinin concn ($\mu g \cdot liter^{-1}$) at sampling time (h)														
	0[a]	2	12[a]	14	24[b]	26[c]	96[b]	98	100	102	104	106	108	112	120
1	0	38	0	233	3	0	14	10	17	10	13	7	11	15	2
2	0	44	0	348	0	86	3	9	21	17	21	26	27	24	18
3	0	109	7	9	5	127	0	34	4	4	0	0	0	0	0
4	0	230	3	202	4	163	0	0	5	0	0	0	0	0	0
5	0	—[d]	0	315	0	332	3	—	3	0	—	0	0	0	0
6	0	178	0	416	0	205	0	0	—	—	6	5	0	0	0
Mean	0	120	2	254	3	152	4	10	10	6	8	6	6	6	3
SD	0	84	3	143	2	113	6	14	8	7	9	10	11	10	7

[a] A 150-mg dose of dihydroartemisinin was administered at this time.
[b] A 100-mg dose of dihydroartemisinin was administered at this time.
[c] For the two subsequent dosing periods 148 and 72 h), 100-mg doses of dihydroartemisinin were administered but concentrations in plasma were not determined.
[d] —, missing dose.

dihydroartemisinin in patients 1 and 2 is not clear. Slow dissolution, slow absorption, assay artifacts, and other explanations are possible. The in vitro-determined MIC for *P. falciparum* is ~0.1 to 2 $\mu g \cdot liter^{-1}$ for both compounds (7). Although the in vivo situation is different, it is unlikely that the low concentrations after the last dose contribute much to the therapeutic effect. The artemisinin drugs induce such a strong reduction of the parasite biomass that a short treatment is enough to initialize cure. However, to prevent recrudescence, longer treatment is needed (7).

Our findings show that extending the duration of the treatment course with artesunate may not be rational. Because short courses initiate a very fast recovery but do not prevent recrudescence, the best option is to combine a short course of artesunate with another antimalarial agent.

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