

Halofantrine pharmacokinetics in Kenyan children with non-severe and severe malaria

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- 1 Kenyan children with uncomplicated malaria given oral halofantrine (HF; non-micronised suspension; 8 mg base kg⁻¹ body weight 6 hourly for three doses) showed wide variation in the disposition of HF and desbutylhalofantrine (HFm).
- 2 Eight Kenyan children with severe (prostrate) falciparum malaria who were receiving intravenous quinine, were given the same HF regimen by nasogastric tube. One patient had undetectable HF and two had undetectable HFm at all times after drug administration.
- 3 The mean AUC(0,24 h) of HF in prostrate children was half (7.54 compared with 13.10 µg ml⁻¹ h) ($P = 0.06$), and that for HFm one-third (0.84 compared with 2.51 µg ml⁻¹ h) ($P < 0.05$) of the value in children with uncomplicated malaria.
- 4 Oral HF may be appropriate for some cases of uncomplicated falciparum malaria in Africa, but in patients with severe malaria, the bioavailability of HF and HFm may be inadequate.

Keywords falciparum malaria halofantrine treatment pharmacokinetics parasite chemosensitivity

Introduction

Halofantrine (HF) is an effective treatment for chloroquine-resistant strains of *Plasmodium falciparum* [1, 2]. Although expensive in Africa, HF is being employed increasingly in private clinics to treat chloroquine-resistant falciparum malaria. In severe malaria, the combination of quinine and HF might be clinically superior to quinine alone because HF, but not quinine, significantly reduces the viability of circulating ring-stage parasites [3]. The absence of a parenteral formulation would complicate drug administration in severe disease, although the suspension could be given by nasogastric tube. Both chloroquine [4] and mefloquine [5] have been administered successfully by this route to patients with severe malaria. However, plasma concentrations of HF following oral dosage vary considerably in healthy Caucasian adults [6] and in adult Thai with malaria, which may contribute to drug failure [7]. HF produces a lengthening of the ECG QT-interval [8], which is apparently dose-related [9], and may predispose to ventricular arrhythmias. We have studied

the pharmacokinetics of orally administered HF in uncomplicated and severe paediatric malaria to assess the potential for this drug as treatment for severe falciparum malaria.

Methods

Two consecutive studies were conducted, in children with non-severe, and with severe malaria. Approval for the study was given by the Ethics Committee of the Kenya Medical Research Institute (KEMRI).

Children with non-severe malaria

Patients and clinical care Children attending the outpatient department of Kilifi District Hospital, with a primary diagnosis of malaria, were recruited if they had *Plasmodium falciparum* infection only (greater than 10 000 but less than 250 000 asexual parasites

mm⁻³), and if written informed consent was obtained from the parent. Children were excluded if they had severe malaria [10], or concurrent disease. Criteria for exit from the study were: withdrawal of consent, vomiting following a dose of HF, and occurrence of a drug-related adverse event. Patients were nursed in the KEMRI Clinical Research Ward at Kilifi District Hospital. Patients were weighed and examined, and a Teflon[®] cannula was inserted into a forearm vein for blood sampling. Temperature was lowered by oral paracetamol and tepid sponging. Patients were observed closely for vomiting.

Dosing and sampling Patients were given HF (non-micronised oral suspension; 8 mg base kg⁻¹ body weight) 6 hourly for three doses; food and water were unrestricted. Blood (2 ml), for the measurement of plasma HF, was drawn through the cannula pre-dose, and at 3, 6, 9, 12, 15, 24, 36, 48 and 72 h after the first dose. Blood was centrifuged (1000 g for 10 min) within 1 h and plasma was stored at -70° C until transferred to Nairobi (on dry ice) for analysis. Plasma HF and HFm were estimated by the method of Mberu *et al.* [11].

Children with severe malaria

Patients and clinical care Children admitted to Kilifi District Hospital with a primary diagnosis of malaria were recruited if they were unable to drink from a cup, unable to sit unsupported (prostrate) [10] and if written consent was available. Patients were excluded if there was concurrent disease and criteria for exit were as described for the non-severe group. Patients were nursed in the KEMRI Clinical Research Ward, where they were examined at least 12 hourly by a physician until discharge. Teflon[®] cannulae were inserted into veins of both arms (one for i.v. fluids and drugs; one for sampling). A nasogastric tube was inserted; correct placement was determined by testing aspirate pH (Litmus paper), and by auscultation during air injection. Patients were observed closely for vomiting.

Dosing and sampling An intravenous loading dose of quinine (20 mg kg⁻¹) was given, followed by nine doses of 10 mg kg⁻¹ at 12 hourly intervals, as described elsewhere [12]. HF (non-micronised suspension: 8 mg base kg⁻¹ body weight; 6 hourly; three doses in total) was given by the nasogastric tube starting at the same time as the quinine. The nasogastric tube was flushed with water (10 ml) after each dose and then spigoted. Blood (2 ml) was drawn from the sampling cannula before treatment at the following times: 1, 2, 3, 4, 5, 6, 9, 12, 15, 24, 48, 72, 86 and 120 h. Aliquots of whole blood (100 µl in duplicate) were transferred by positive displacement pipette to filter paper strips for quinine assay [13]; the rest of the blood samples were then centrifuged (1000 g; 10 min). Plasma (200 µl in duplicate) was transferred onto filter paper strips, which were stored at room temperature out of sunlight. Plasma HF and HFm concentrations were measured as before [11].

In vitro chemosensitivity tests

The *in vitro* chemosensitivity to HF of 21 isolates of *P. falciparum* from the study area was measured by the Rieckman micro test [14]. For each test, seven wells of a 96-well microculture plate (Sterilin, UK) were loaded with drug solution (25 µl in culture medium) immediately before inoculation with parasitised blood. All other test conditions were as previously described [1].

Pharmacokinetic and statistical analyses

Peak plasma HF and HFm concentrations (C_{max}) and the corresponding times (t_{max}) were noted directly from the data. The elimination rate constant (λ_z) was calculated by linear regression of the terminal portion of the log plasma drug concentration-time curve (4–5 data points). The elimination half-life ($t_{1/2,z}$) was estimated from $\ln 2/\lambda_z$. AUC values were calculated by a combination of the linear trapezoidal method (rising concentrations) and the logarithmic trapezoidal method (falling concentrations), followed by extrapolation to infinity using C_{last}/λ_z .

The means of the derived pharmacokinetic parameters were compared using the Mann-Whitney U-test ($P < 0.05$ = significant difference), and 95% confidence intervals (CI) were calculated for differences between means.

Results

Clinical outcome

Non-severe malaria Ten children (aged 6 to 84 months) were recruited. Parasitaemia ranged from 26 to 250 × 10³ mm⁻³ (geometric mean 47 × 10³ mm⁻³), and haemoglobin was 8.7 ± 2.0 g% (mean ± s.d.). None of the children had Kwashiorkor and none was overtly marasmic. Weight for age was within the normal range for the locality. All children made uneventful recoveries and were aparasitaemic on day 7. In two patients blood slides became positive on days 14 and 23 after treatment; both children were febrile, with parasite counts of 2 and 660 asexual forms per 100 white blood cells. They were treated with pyrimethamine-sulphadoxine and paracetamol.

Severe malaria Eight children (aged 17 to 72 months) were recruited; all could localise pain, but none was able to drink or sit unsupported. Parasitaemia ranged from 6 to 93 × 10³ mm⁻³ (geom mean 29 × 10³ mm⁻³), and haemoglobin was 6.9 ± 2.4 g%. None of the children had Kwashiorkor and none was overtly marasmic. Weight for age was within the normal range for the locality. All children were aparasitaemic by day 7, and remained slide-negative during follow up. Geometric mean parasitaemias were lower in the severe malaria group than in the non-severe malaria group. There is no simple relationship between disease severity and peripheral parasitaemia, because of

sequestration of parasitised RBCs in deep vascular beds.

In vitro parasite chemosensitivity

The dose-response relationship for 21 isolates of *P. falciparum* is shown in Figure 1. Fifty per cent, 90% and 99% inhibitory concentrations (IC_{50} , IC_{90} and IC_{99}) were 1.6, 15.1 and 38.0 nmol l⁻¹ (0.0008, 0.0076 and 0.019 µg ml⁻¹), respectively. Since HF-resistant parasites from Africa exhibit IC_{50} values > 20 nmol l⁻¹ [15, 16], the population of parasites tested at Kilifi is regarded as essentially sensitive.

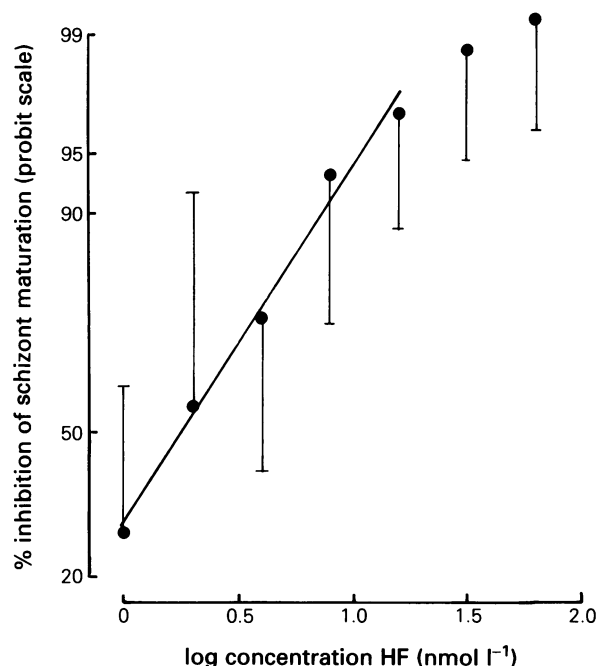


Figure 1 In vitro chemosensitivity in 21 isolates of *Plasmodium falciparum* to halofantrine (HF), Kilifi, Kenya Coast, 1991–93. Bars represent 1 s.d.

1/21 isolates had an IC_{50} > 8 < 16 nmol l⁻¹, which suggests intermediate sensitivity, but this was not from one of the clinical cases.

Pharmacokinetics

Pharmacokinetic parameters for HF and HFm, for both non-severe and severe malaria patients, are summarised in Table 1. Because some patients with severe malaria had undetectable plasma HF and HFm concentrations, pharmacokinetic parameters could not be estimated for all patients. Mean concentrations of HF and HFm in non-severe malaria are shown in Figure 2a; corresponding values in severe malaria patients are shown in Figure 2b.

Non-severe malaria Drug disposition was very variable, but both HF and HFm were detectable in every case. C_{max} ranged from 0.18 to 1.96 µg ml⁻¹ (0.94 ± 0.53 µg ml⁻¹; mean \pm s.d.), and that of HFm ranged from 0.06 to 0.36 µg ml⁻¹ (0.22 ± 0.09 µg ml⁻¹). In the two patients who developed parasitaemia 14 and 23 days after treatment the C_{max} of HF was 0.5 and 0.62 µg ml⁻¹, respectively, and that of HFm was 0.08 and 0.19 µg ml⁻¹, respectively.

Severe malaria HF disposition in these patients was even more variable than in the non-severe group. In three patients plasma concentrations of HF and HFm were particularly erratic. In one of these patients both HF and HFm were undetectable in all samples. In another patient, although the C_{max} of HF was 0.1 µg ml⁻¹, no HFm was detected in any sample. In the third patient, HF was not measurable until 12 h, and reached a C_{max} of only 0.03 µg ml⁻¹. In the same patient, HFm was not measurable until 48 h and reached a C_{max} of 0.005 µg ml⁻¹. In the severe malaria group, the C_{max} of HF ranged from undetectable to 1.31 µg ml⁻¹, and that of HFm ranged from undetectable to 0.1 µg ml⁻¹. Quinine data were available in seven patients; the C_{max} after the first

Table 1 Pharmacokinetic parameters for HF and HFm in children with severe and non-severe malaria. Data are expressed as mean (\pm s.d.), with the exception of t_{max} which is expressed as median (range). 95% CI = 95% confidence intervals of the differences between the means

	HF parameters			HFm parameters		
	Severe malaria	Non-severe malaria	95% CI	Severe malaria	Non-severe malaria	95% CI
t_{max} (h)	15 (n = 7) (3–24)	15 (n = 10) (9–24)	—	72 (n = 5) (24–120)	24 (n = 10) (12–48)	—
C_{max} (µg ml ⁻¹)	0.52 (n = 7) (0.51)	0.94 (n = 10) (0.53)	-2, 0.99	0.07 (n = 5) (0.02)	0.22 (n = 10) ^a (0.09)	0.05, 0.23
$t_{1/2}$ (h)	16.20 (n = 6) (4.60)	30.60 (n = 10) ^a (13.40)	2.14, 26.7	*	*	—
AUC(0–24) (µg ml ⁻¹ h)	7.54 (n = 7) (7.5)	13.10 (n = 10) (6.5)	-1.71, 14.5	0.84 (n = 5) (0.44)	2.51 (n = 10) ^a (1.32)	0.34, 3
AUC(0,∞) (µg ml ⁻¹ h)	16.80 (n = 4) (13.0)	32.20 (n = 10) (16.6)	-5, 35.8	*	*	—

^aStatistically significant difference, $P < 0.05$.

* = could not be determined.

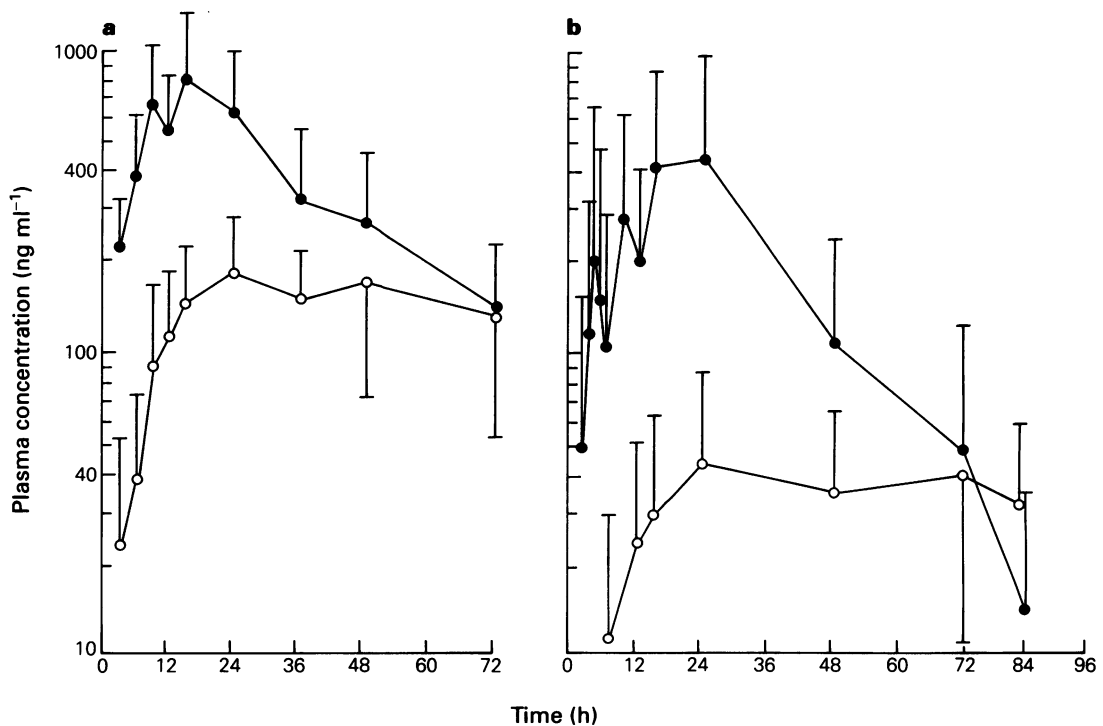


Figure 2 Mean plasma concentrations of HF (closed symbols) and HFm (open symbols) after oral HF treatment in Kenyan children; a): non-severe falciparum malaria; b): severe falciparum malaria. Bars represent 1 s.d.

dose was $20.1 \pm 3.3 \mu\text{g ml}^{-1}$, and after the second dose it was $17.1 \pm 2.1 \mu\text{g ml}^{-1}$.

Discussion

Quinine is the treatment of choice for severe malaria in Africa while parasites remain sensitive to the drug. However, even with optimal care, the mortality rate in severe malaria treated with quinine is 10 to 15% [10, 17], which has stimulated the search for improved therapy. The observation that HF rapidly reduces the viability of *P. falciparum* ring-forms [3] suggested a possible role for HF in the treatment of severe malaria, despite the lack of a parenteral dosage form. However, clinical trial of quinine plus HF in severe malaria would have been premature without definition of the relative bioavailability of HF when administered by nasogastric tube, which was the rationale for this study.

In the children recruited from outpatients with uncomplicated disease, drug disposition was variable, as reported by others, although HF and HFm were measurable in all cases and at all sampling times. In these patients, the IC_{99} for HF (38 nmol l^{-1} ; $0.019 \mu\text{g ml}^{-1}$) was exceeded in all patients for at least 48 h. The pharmacokinetic parameters for both drug and metabolite were similar to those reported by Karbwang *et al.* [7], with the exception of the elimination half-life ($t_{1/2,z}$) for HF which was considerably shorter in our study than the value ($> 100 \text{ h}$) reported for Thai adults with non-severe malaria. The $t_{1/2,z}$ of HFm could not be determined accurately in the present study because the sampling period was too short.

Even for HF it is possible that we have not defined the true terminal elimination rates in Kenyan children, since our data are incomplete beyond 72 h. Two of our patients became parasitaemic again on days 14 and 23 after treatment. Although their C_{max} values for HF and HFm, and HF $\text{AUC}(0,24)$ values were all lower than the group mean, plasma HF concentration exceeded the IC_{99} for the first 72 h, and parasite reappearance may have been due to reinfection. However, early reappearance of parasites following HF treatment warrants caution, and further study.

HF disposition after administration by nasogastric tube to patients with severe malaria was much more variable than in the non-severe group; in the most extreme case neither HF nor HFm could be detected at any time point up to 120 h. All children with severe malaria received standard quinine therapy in addition to HF, and achieved adequate plasma drug concentrations [12]. Since all these children were cured, it must be assumed that quinine was the effective treatment where HF was undetectable, and that reliance of HF alone would have had serious, possibly fatal, consequences.

The relative bioavailability of HF is a function of food intake and type; fatty food increases HF absorption [6]. Our results may also reflect the effect of clinical malaria on appetite. Children with uncomplicated malaria are often anorectic, but prostrate children cannot eat, drink or breast-feed at all. Further, patients with severe malaria are relatively immobile, and subject to malabsorption (including drug malabsorption) which may be due to changes in small bowel blood flow [18]. Thus, several factors may have contributed to the variability in plasma HF

and HFm concentrations in our study patients. We consider it likely that the differences in some estimated pharmacokinetic parameters between our study subjects reflect disease severity, although it is possible that they may have resulted from concomitant quinine (QN) treatment in the severely ill patients. However, no QN-HF drug interaction has been reported of which we are aware. Additionally, QN does not interfere with the h.p.l.c. assay of HF and HFm [11].

HF is being used increasingly in Africa as a treatment for chloroquine-resistant malaria, particularly in private clinics. The results of this study emphasise the potential dangers of the borderline case; progression from non-severe to severe malaria is often very rapid, occurring over a few hours. Although our study involved children who were severely ill, they were not in coma and oral medication could have been considered an appropriate option under some circumstances.

The therapeutic ranges of HF and HFm have not been defined. *In vitro* tests of parasite chemosensitivity, as recorded in this study, remain essential tools for assessing temporal and geographical trends, but are of limited value in estimating *in vivo* efficacy. One reason for this is the difference in protein concentration between plasma and culture medium, which produces different concentrations of unbound drug; the degree of plasma protein binding of HF and

HFm is not known. Similarly, although the QT prolongation produced by HF is thought to be concentration-dependent [9], there are insufficient data to estimate a toxic threshold. The one cardiac death which has been linked unequivocally to HF occurred in a patient with plasma HF and HFm concentrations of 0.31 and 0.18 $\mu\text{g ml}^{-1}$, respectively [8]; concentrations which are well within the ranges encountered during treatment without obvious toxicity.

HF remains a significant addition to a diminishing range of effective antimalarial drugs. However, the use of oral HF in severe malaria remains unproven; the negligible absorption in some patients is of considerable concern, and suggests that monotherapy with HF is inappropriate.

We thank the Director, Kenya Medical Research Institute, for permission to publish these results. The study was supported by the Wellcome Trust of Great Britain, SmithKline Beecham and the Kenya Medical Research Institute. The study would not have been possible without the work of the nurses and technicians of the KEMRI Unit. The authors wish to thank Dr N. Peshu and Mr P. A. Warn, Chief Technician, for their help. WMW is grateful to the Wellcome Trust for personal and research support. PW holds a Medical Research Council Special Training Fellowship in Tropical Medicine. KM holds a Wellcome Trust Senior Research Fellowship in Clinical Science. CRJCN holds a Wellcome Trust Advanced Training Fellowship in Tropical Medicine.

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(Received 12 May 1994,
accepted 18 October 1994)