

Response of *Plasmodium falciparum* to chloroquine treatment: relation to whole blood concentrations of chloroquine and desethylchloroquine

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A standard treatment with 25 mg chloroquine base per kilogram body weight was given to 39 semi-immune asymptomatic Tanzanian schoolchildren with *Plasmodium falciparum* parasitaemia. Whole blood chloroquine and desethylchloroquine concentrations were monitored 12 times during 30 days of follow-up using 100 µl capillary blood dried on filter-paper.

All but three children had detectable amounts of chloroquine (≥ 10 nmol/l) in their blood before treatment. The interindividual variations in concentrations during the first week were 3.3 to 5.1-fold for chloroquine and 3.5 to 6.3-fold for desethylchloroquine. In seven children with RII response *in vivo*, the highest determined chloroquine concentration was lower ($P = 0.029$) than in the others. After treatment, a rough approximation of the minimum inhibitory concentration *in vivo* was made by calculating the average of the chloroquine concentrations before and after the time when parasites increased or reappeared again. RII-resistant parasites increased in number when the median residual whole blood concentration in the children was approximately 790 (range, 444–869) nmol/l. Parasites reappeared when the median residual whole blood concentration was approximately 147 (range, 44–673) nmol/l.

We conclude that interindividual variations of chloroquine concentrations have an impact on the outcome of treatment and the classification of resistance *in vivo*.

The response of *Plasmodium falciparum* to equal doses of chloroquine varies in different individuals. When a small dose of the drug is administered in an area with chloroquine-susceptible parasites, the parasitaemia is cleared in some children but not in others (1). In areas with chloroquine resistance, a standard treatment dose of chloroquine causes the parasites to disappear temporarily in some patients while there is only a reduction in others or no response at all (2, 3).

The varying efficacy of standard treatment with chloroquine is considered to be due to local variations in susceptibility of different co-existing strains of *P. falciparum* (4). This is in accordance with the reports of *in vitro* tests where resistant strains multiplied in the presence of higher drug concentrations than susceptible ones from the same area (5).

Although varying parasite susceptibility to chloroquine is important, other factors might also affect

the outcome of treatment. For example, we have found pronounced interindividual variability in chloroquine and desethylchloroquine concentrations in whole blood during and after supervised standard treatment (6). The main aim of this study was to relate the interindividual variability in chloroquine concentrations to treatment outcome. We also investigated the chloroquine concentrations in whole blood when parasites reappeared and thus were no longer inhibited *in vivo*.

Subjects and methods

In September 1986, 524 schoolchildren aged 7–14 years and attending Kinondoni primary school in a suburb of Dar es Salaam, United Republic of Tanzania, were screened for malaria parasites. Parasites were detected in 98 (19%) children and 42 of them who had at least 16 asexual *P. falciparum* parasites per 200 leukocytes were asked to participate in the study. All were asymptomatic. They were given uncoated tablets, containing either 155 mg or 100 mg of chloroquine base (Klorokinofosfat, Kabi-Vitrum, Sweden), and supervised while swallowing and for 30 minutes afterwards. Tablets were divided into halves or quarters if necessary. The children were given approximately 10 mg of chloroquine base per kg bodyweight during the first and second day and approximately 5 mg on the third day. The total

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dose varied between 25.0 to 25.3 mg per kg body weight. The children were instructed not to take any other drugs during the study without contacting the research team.

Capillary blood samples for drug analysis and thick films were collected on twelve occasions: immediately before each dosing, daily during the rest of the first week, and then once weekly for 3 weeks, beginning one week after termination of medication. Blood samples for *in vitro* studies were obtained immediately before the onset of medication.

In this voluntary study, no specific measures were undertaken to search for those who did not attend at the time of a blood sample.

Handling of samples

100 μ l of whole blood samples were obtained in capillary tubes and applied on filter-papers (Whatman No. 1). The blood was left to dry, after which the papers were stored in an envelope at room temperature for 2–4 months until analysed. Strict routines were followed in order to avoid chloroquine contamination (7).

Analysis

Thick blood films for malaria parasites were stained with Giemsa. During the initial screening procedure, the number of parasites were calculated against 200 leukocytes and during the actual study against 1000 leukocytes (assuming a leukocyte count of $8.0 \times 10^9/l$).

The Rieckmann micro technique was applied to determine the *in vitro* susceptibility of *P. falciparum* parasites to chloroquine (5). A RPMI-1640 medium was used to which 6.0 g/l of Hepes, 2.0 g/l NaHCO_3 , and 26 mg/l of gentamycin were added.

A high performance liquid chromatographic method (8) was used to determine chloroquine and desethylchloroquine concentrations. The limit of determination for chloroquine and desethylchloroquine was 10 nmol/l. The coefficients of variation at 40 nmol/l and 10 nmol/l were 5% and 15%, respectively.

Calculations

Statistical comparisons were done with the one-tailed Mann-Whitney U-test.

The area under the blood concentration versus time curve (AUC) was estimated using the trapezoidal rule.

Using a probit analysis of logdose/response, the effective drug concentrations for 50%, 90% and 99% inhibition of schizont maturation (EC_{50} , EC_{90} and EC_{99}) were calculated.

Results

Three children did not attend to receive all medications and were thus excluded. None of the 39 remaining children vomited during 30 minutes of observation after medication and no adverse reactions were observed.

In order to detect accidental chloroquine contamination of the filter-papers, the concentration ratio between chloroquine and desethylchloroquine was calculated. The ratio was within the normal range in all samples and thus no significant contamination had occurred.

Parasite density

The median *P. falciparum* density before treatment was $1510/\text{mm}^3$ (range, 490–22 400/ mm^3). In seven of the 39 children, parasites were still detected in thick films taken one week after initiation of therapy but in all of them the parasite density had decreased to less than 25% of the initial count indicating RII resistance.

The median parasite clearance time for the other 32 children was 3 days (range, 2–5 days). Out of them, 7 remained free from parasites during the rest of the study period indicating susceptibility *in vivo*. In 22 children the parasites reappeared, i.e., indicating RI resistance or reinfection. In 3 children, parasite reappearance could not be excluded in the end of the study owing to poor attendance.

In vitro susceptibility

In vitro microtests for chloroquine susceptibility were successful in 23 out of 32 children. Only 3 isolates were fully susceptible. The effective chloroquine concentration for 50% inhibition (EC_{50}) was calculated to 0.66×10^3 nmol/l, for 90% inhibition (EC_{90}) 2.6×10^3 nmol/l, and for 99% inhibition (EC_{99}) 8.2×10^3 nmol/l.

There were only two successful *in vitro* microtests in the seven children with RII response *in vivo* and thus a comparison between *in vitro* resistance, *in vivo* susceptibility and drug concentrations was not possible.

Drug concentrations

Before therapy. Already before treatment, chloroquine was detected in the blood of all but three children and desethylchloroquine in all but seven children. The median chloroquine concentration in those with detectable amounts before therapy was 56 nmol/l (range, 10–1325 nmol/l) and the corresponding median desethylchloroquine concentration was 48 nmol/l (range, 10–1003 nmol/l). In a total of twelve children, chloroquine concentrations exceed-

Fig. 1. Median (middle curve) and range (upper and lower curves) of whole blood concentrations of chloroquine (A) and desethylchloroquine (B) in 27 Tanzanian schoolchildren given chloroquine base in doses of 10, 10 and 5 mg/kg body weight on days 0, 1 and 2, respectively. In the abscissae, the top figures are the days after initiation of treatment; the bottom figures are the number of samples.

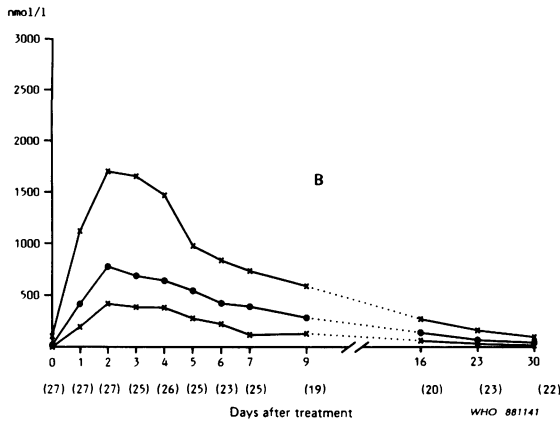
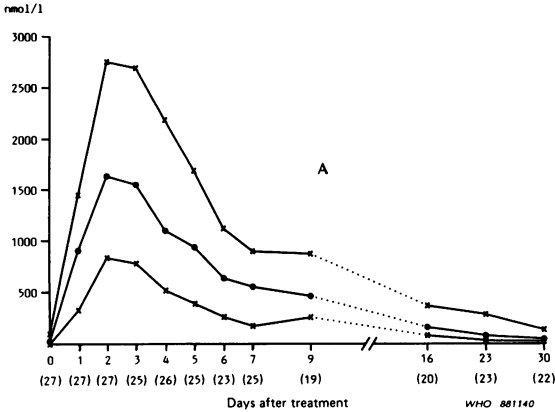


Table 1: Highest observed chloroquine (CQ) and desethylchloroquine (DECQ) concentrations in whole blood and the AUC for days 0-7 in 27 Tanzanian schoolchildren given a total of 25 mg chloroquine base/kg body weight

	Mean	Median	Range
Highest CQ concentration (nmol/l)	1689	1682	836-2749
Highest DECQ concentration (nmol/l)	848	777	417-1702
AUC (days 0-7): CQ (nmol × day/l)	7134	7102	3259-12234
AUC (days 0-7): DECQ (nmol × day/l)	3777	3797	1242-7935

Table 2: Relation between chloroquine (CQ) and desethylchloroquine (DECQ) concentrations in whole blood and outcome *in vivo* after treatment of Tanzanian schoolchildren with a total of 25 mg chloroquine base/kg body weight

	No parasites detected on day 7 (n = 29)		Parasites detected on day 7 (n = 7)		P value
	Mean	Median	Mean	Median	
Highest CQ concentration (nmol/l)	1799	1778	1473	1461	0.029
Highest DECQ concentration (nmol/l)	926	817	847	713	0.21
AUC (days 0-7): CQ (nmol × day/l)	7767	7699	6439	6088	0.058
AUC (days 0-7): DECQ (nmol × day/l)	4327	3931	3587	3446	0.24

ing 100 nmol/l indicated more recent chloroquine intake. Only three children stated that they had taken chloroquine within two weeks before therapy. The chloroquine concentrations of these children were 26, 37 and 163 nmol/l which was comparable to those who denied recent chloroquine intake.

During and after therapy. Only the 27 children with chloroquine concentrations below 100 nmol/l before therapy were included in this part of the study. The median and range of whole blood chloroquine and desethylchloroquine concentrations during and after therapy of these children are shown in Fig. 1. Chloroquine concentrations were generally above 1000 nmol/l at day 2 and 3, and most children had again less than 100 nmol/l at day 23. The inter-individual variabilities of drug concentrations during the first seven days were 3.3 to 5.1-fold for chloroquine and 3.5 to 6.3-fold for desethylchloroquine, which is in reasonable agreement with previous reports (6, 9).

The highest concentrations of chloroquine and desethylchloroquine as well as the AUC for the whole first week (days 0-7) are shown in Table 1. In all but three children the highest chloroquine concentrations were registered before the third dose. The actual peak concentration could not be assessed with the sampling scheme used.

Relation between drug concentrations and outcome *in vivo*

The relation between drug concentrations and outcome *in vivo* is shown in Table 2. The maximum observed chloroquine concentrations in whole blood

were lower in 7 children with parasitaemia on day 7 after initiation of treatment (RII) compared with 29 children without parasites on the same day ($P = 0.029$).

The AUCs for chloroquine during the first week after initiation of treatment were also smaller in children with persistent parasitaemia on day 7. The differences were close but not statistically significant ($P = 0.058$). For the major metabolite desethylchloroquine, corresponding differences were less pronounced.

Chloroquine concentrations when RII-resistant parasites increased or parasites reappeared

The concentrations of chloroquine in the whole blood of children who attended regular samplings immediately before and after the reappearance of parasites are shown in Fig. 2 ($n = 19$). When the parasites did not disappear (RII), the concentration on the day of the lowest parasite density and the following day were chosen ($n = 7$). RII-resistant parasites increased in number when the median chloroquine concentration decreased from 883 nmol/l to 646 nmol/l. Parasites reappeared when the median chloroquine concentration decreased from 195 nmol/l to 83 nmol/l.

A rough approximation of the minimum inhibitory concentration (MIC) *in vivo* in a single individual can be made by calculating the average of the chloroquine concentration before and after the day when parasites increased or reappeared. Using this approximation we found a median MIC of 790

(range, 444–869) nmol/l for RII-resistant strains and 147 (range, 44–673) nmol/l for parasites that reappeared.

Discussion

In semi-immune asymptomatic children there is spontaneous parasite clearance (10), which is illustrated by the fact that two children with an RII response later cleared the parasites without additional medication. The actual drug efficacy was thus overestimated to some extent.

The detection of chloroquine and desethylchloroquine in most of the children even before therapy confirms the widespread use of chloroquine in parts of Africa (11). No reliable information was obtained by questioning. Similar results have also been obtained in Nigeria when the parents were asked about the drug intake of the children (12).

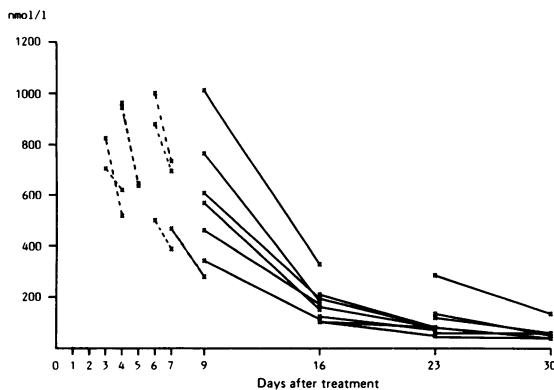
Relation between drug concentrations and outcome in vivo

The parasitocidal concentrations of chloroquine needed for eradication of *P. falciparum* parasites are higher than the inhibitory concentrations (13). In this study the parasites were at least temporarily inhibited in all children, but the obtained drug concentrations were not parasitocidal in the children with an RII response and in most of the children with reappearing parasites. (The transmission during this period was low and most of the reappearing parasites were thus recrudescences.)

It has not been established whether a high peak concentration, a large area under the curve, or a long time period with exposure of the parasite to chloroquine concentrations above the MIC are equally important for eradication of malaria parasites. *In vitro*, it was found in 1979 that 48 hours' exposure to a high drug concentration was enough for permanent eradication as no parasite growth was observed even when chloroquine was later washed away from the culture (14). This suggests that a high concentration during a short time is sufficient. However, there may be other explanations of the results of this study, e.g., irreversible binding of the drug to the parasites or other cell components with persistent biological activity. In addition, the parasites were exposed to chloroquine in 5 to 10-fold increased steps during 48 hours and thus other results might have been obtained with intermediate concentrations and/or different drug exposure periods.

Chloroquine accumulation in erythrocytes *in vitro* is less if the cells are infected with a resistant

Fig. 2. Whole blood chloroquine concentrations when RII-resistant parasites ($n = 7$) increased in number (broken lines) and when parasites ($n = 19$) reappeared (solid lines) in Tanzanian schoolchildren given chloroquine base in doses of 10, 10 and 5 mg/kg body weight on days 0, 1 and 2, respectively.



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compared to a susceptible strain (15). The reason for this has in a recent study been shown to be a 40 to 50-fold increase of chloroquine release from erythrocytes infected with resistant parasites (16). A high peak concentration provides a higher concentration gradient and might be the best way of attaining an effective concentration in the parasitized erythrocyte. Thus, high peak concentrations might be essential for the outcome of therapy in areas with chloroquine resistance.

In this study, interindividual variabilities of the pharmacokinetics of chloroquine were important for the outcome of therapy. Both maximum observed concentrations and AUC were smaller in children with RII-resistant strains. Contrary to the maximum observed concentrations, differences in AUCs were close to but not statistically significant. This must not be taken as proof that peak concentrations are "more important" for the outcome of therapy than the AUC. The fact that less differences were found between desethylchloroquine concentrations in children with persistent parasitaemia and others is to be expected as desethylchloroquine is less active *in vitro* against chloroquine-resistant plasmodia than the parent drug (17).

Drug concentrations when the parasite densities increased (RII) or parasites reappeared

Chloroquine concentrations obtained before the reappearance of recrudescing parasites can be considered to be higher than the MIC as parasite multiplication is still prevented or inhibited to such an extent that the resulting parasitaemia is subpatent. Drug concentrations immediately after parasite reappearance are evidently less than the MIC since the parasites are able to multiply despite the residual drug concentrations.

The median MIC concentrations in this study can be compared with another study where whole blood concentrations were obtained in 10 adults taking 310 mg chloroquine base weekly for regular long-term prophylaxis (13). Immediately before the weekly dose, the median whole blood chloroquine concentration was 481 nmol/l (range, 292–738 nmol/l), which is above the concentrations at which the majority of parasites reappeared in this study. This is in line with clinical observations that ongoing prophylaxis protects against most RI but not against RII strains.

We conclude from this study that interindividual differences in chloroquine pharmacokinetics were important for the classification of parasite resistance *in vivo*. Interindividual differences have probably an impact on the classification of sus-

ceptibility in areas with less pronounced chloroquine resistance as well. The same phenomenon might be valid for other antimalarials.

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Résumé

Réponse de *Plasmodium falciparum* au traitement par la chloroquine : relation avec les concentrations de chloroquine et de déséthylchloroquine dans le sang total

Un traitement standard de 25 mg de chloroquine base par kilogramme de poids corporel a été administré à 39 enfants tanzaniens d'âge scolaire, semi-immuns et asymptomatiques, présentant une parasitémie à *Plasmodium falciparum*. Les concentrations de chloroquine et de déséthylchloroquine dans le sang total ont été surveillées à 12 reprises pendant les 30 jours de suivi, dans des prélèvements de 100 µl de sang capillaire séché sur papier filtre.

Tous les enfants sauf trois présentaient des concentrations décelables de chloroquine (10 nmol/l) dans le sang total avant même le début du traitement. Les variations interindividuelles de la concentration au cours de la première semaine suivant le traitement s'échelonnaient entre 3,3 et 5,1 fois pour la chloroquine et entre 3,5 et 6,3 fois pour la déséthylchloroquine. Chez sept enfants présentant une réponse de type RII *in vivo*, la plus forte concentration de chloroquine déterminée était inférieure ($P = 0,029$) à celle mesurée chez les autres enfants. Après traitement, on a évalué grossièrement la concentration minimale inhibitrice (CMI) *in vivo* en faisant la moyenne des concentrations de chloroquine avant et après le moment auquel les parasites réapparaissaient ou devenaient plus nombreux. Le nombre de parasites résistants de type RII augmentait lorsque la concentration résiduelle médiane dans le sang total était d'environ 790 (intervalle: 444–869) nmol/l et les parasites réapparaissaient lorsque la concentration résiduelle moyenne dans le sang total était d'environ 147 (intervalle: 44–673) nmol/l.

Nous en concluons que les variations interindividuelles des concentrations de chloroquine ont une influence sur l'issue du traitement et sur la classification de la résistance *in vivo*.

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