

Chloroquine and desethylchloroquine concentrations during regular long-term malaria prophylaxis

L. ROMBO,¹ Y. BERGQVIST,² & U. HELLGREN³

The concentrations of chloroquine and desethylchloroquine in the blood of 10 healthy adult Swedish volunteers who had been taking 310 mg chloroquine base once a week for at least 8 months for malaria prophylaxis were measured. Samples of capillary whole blood from the volunteers were dried on filter-paper and the drug and its principal metabolite determined by a specific high-performance liquid chromatography (HPLC) method. The day after taking the drug, the mean concentration of chloroquine and desethylchloroquine in whole blood were 1305 nmol/l and 915 nmol/l, respectively, and immediately before the next weekly dose, 489 nmol/l and 384 nmol/l, respectively. These are considered to be greater than the minimum inhibitory concentrations for susceptible strains but less than the maximum tolerated concentrations. The dosage of chloroquine recommended roughly 40 years ago for regular long-term prophylaxis should therefore not be changed.

Chloroquine has been used in malaria prophylaxis for approximately 40 years. The recommended dosages (300 mg chloroquine base weekly for adults) are based on the results of extensive studies carried out in 1944-46 on volunteers and included therapy and prophylaxis (1). The concentration of chloroquine in the blood was determined at this time using the unspecific methods then available (2). From that time the dosages recommended have remained virtually unchanged.

Drug concentrations during short-term malaria prophylaxis with chloroquine were later studied by McChesney et al. (3), Brohult et al. (4), Rombo et al.^a and Chiluba.^b However, the concentration of chloroquine during prolonged prophylaxis has to our knowledge only been studied by Brohult et al. (4). Apart from the recent studies by Chiluba,^b all involved the use of unspecific spectrophotofluorometric methods that failed to separately detect chloroquine and its metabolites. In addition, McChesney et al. and

Chiluba used plasma samples, while Brohult et al. and Rombo used serum samples. As the concentration of chloroquine varies considerably between these different blood components (5, 6), the results of these studies are therefore not directly comparable.

Here, we report the results of a study that analysed the concentrations of chloroquine and its main metabolite, desethylchloroquine, in the whole blood of individuals undergoing prolonged prophylaxis, using high-performance liquid chromatography (HPLC). The levels found are discussed in relation to the recommended dosage.

MATERIALS AND METHODS

Subjects

Ten healthy Swedish adults of both sexes who had taken 310 mg chloroquine base once weekly for at least 8 months gave informed consent to participate in the study. All were working in Angola and used chloroquine for malaria prophylaxis. None took other drugs regularly during this period, and the intake of chloroquine^c was supervised during the month preceding the study. The mean weight of the volunteers was 65 kg (range: 48 kg to 80 kg) and the mean age, 39.5 years (range: 30 years to 55 years).

The study was approved by the ethical committee of the Karolinska Hospital, Sweden.

^c Chloroquine phosphate, from KABI-Vitrum, Stockholm, Sweden.

¹ Consultant, Department of Infectious Diseases, Karolinska Institute, Roslagstull Hospital, Box 5651, S-114 89 Stockholm, Sweden. Requests for reprints should be sent to this address.

² Clinical Chemist, Department of Clinical Chemistry, Falun Central Hospital, Falun, Sweden.

³ Registrar, Department of Infectious Diseases, Karolinska Institute, Roslagstull Hospital, Stockholm, Sweden.

^a ROMBO, L. *Chloroquine concentrations in suppression and treatment of malaria—clinical use and methodological considerations*. MD Thesis. Karolinska Institute, Stockholm, Sweden, 1984.

^b CHILUBA E. M. *Studies of chloroquine and proguanil during malaria prophylaxis*. M.Sc. thesis, University of Liverpool, England, 1985.

Table 1. Mean, median, standard deviation, and range of chloroquine and desethylchloroquine concentrations in samples of capillary whole blood from adults taking prolonged prophylaxis with 310 mg chloroquine base weekly

Days after drug administration	Chloroquine concentration (nmol/l)				Desethylchloroquine concentration (nmol/l)			
	Mean	Median	Standard deviation	Range	Mean	Median	Standard deviation	Range
1	1305	1372	283	637-1656	915	993	283	342-1156
2	924	932	213	482-1168	667	774	216	233- 889
3	821	847	250	446-1330	608	623	245	217- 958
4	667	704	141	438- 820	523	550	198	175- 853
5	554	586	133	351- 752	465	500	197	150- 796
6	544	586	146	280- 793	420	434	173	100- 679
7	489	481	129	292- 738	384	397	166	110- 679

Study design

Blood samples were taken the day after administering the regular weekly prophylactic dose of chloroquine and then daily for one week until the next dose. The blood was obtained by fingerprick in 100- μ l capillary tubes and dried on filter-paper.^d The filter-papers were put in an envelope and stored in the dark at room temperature for 2 months until analysis.

Drug analysis

The chloroquine and desethylchloroquine on the dried filter-papers were extracted and then assayed by high-performance liquid chromatography (HPLC) (5). The limit of the determination for both compounds was 100 nmol/l.

Calculations

The area under the curve (AUC) of whole blood concentration of chloroquine or desethylchloroquine versus time was estimated using the trapezoidal rule. The half-life was calculated by linear regression from the plot of log (concentration) versus time.

RESULTS

No side-effects were observed and all participants remained healthy during the study. Two volunteers were absent on one day each, and thus the results for the first and fourth days after drug intake are based on data for nine volunteers only. The mean, median, standard deviation, and range of chloroquine and

desethylchloroquine concentrations in whole blood are shown in Table 1. The AUCs for chloroquine were in the range 2848-6399 nmol·day/l and those for desethylchloroquine, 1272-5540 nmol·day/l. For chloroquine the correlation coefficient (r_{xy}) between the weight of participants and the AUC was -0.29, while the mean whole blood elimination half-life over the period 24-168 hours after drug intake was 4.5 days for chloroquine and 5.3 days for desethylchloroquine. The combined concentrations of chloroquine and desethylchloroquine were uniformly greater than 1000 nmol/l only on the first day after drug administration, and the minimum concentration of chloroquine was as low as 280 nmol/l in one patient. Inter-individual variations in the concentration of chloroquine measured on the same day were generally two- to threefold, while those for desethylchloroquine varied three- to sixfold.

DISCUSSION

The same dose of chloroquine was given to volunteers irrespective of whether there were considerable differences in their body weight. The dose used, approximately 5 mg chloroquine base/kg body weight/week, was chosen to reflect normal conditions, where the dosage of malaria prophylaxis is seldom adjusted according to the recipient's body weight. No blood samples were taken until 24 hours after drug administration and thus its whole absorption phase was not studied. Since the absorption time for chloroquine varies, adequate analysis of the absorption phase would have required making measurements at several additional times, which was not feasible and beyond the scope of the study.

^d Whatman No. 3.

Preferred concentrations of chloroquine and desethylchloroquine

Ideally, the concentrations of chloroquine and desethylchloroquine during malaria prophylaxis should be sufficiently high to make the drug uniformly effective but not reach such levels that dose-dependent side-effects occur.

Minimum inhibitory concentrations

The inhibition of *Plasmodium falciparum* parasites to such an extent that the host is asymptomatic requires a lower concentration of chloroquine than that required to eradicate all the parasites. This is exemplified by chloroquine treatment of RI-resistant *P. falciparum*. During and after such treatment the drug concentration in the host is clearly less than parasitocidal since the parasites survive and later reappear. The concentration is, however, higher than the minimum inhibitory concentration (MIC), as all parasites are suppressed, and the patient is thus asymptomatic (8).^e Subsequently, the drug is eliminated to such an extent that its residual concentration is less than the MIC and allows surviving parasites to multiply and start another period of symptomatic illness.

For prophylaxis it is probably sufficient to attain concentrations that are at least inhibitory, although such levels are poorly established. Using an un-specific spectrophotofluorometric method that co-determined chloroquine and desethylchloroquine, Rombo reported serum concentrations of chloroquine of up to 100 nmol/l *before* therapy in patients with chloroquine-susceptible malaria.^e These concentrations were clearly not inhibitory. Because serum concentrations are variable and represent only fractions of those in whole blood (6), and the biological activity of the metabolite, desethylchloroquine, has not been fully established *in vivo*, these results cannot be directly compared with those we have reported in the present study. All the same, the combined concentration of chloroquine and desethylchloroquine in whole blood was approximately 500 nmol/l, which is probably close to that below which there is a risk of ineffective prophylaxis also against chloroquine-susceptible strains.

Minimum parasitocidal concentrations

The minimum fully parasitocidal whole-blood concentration of chloroquine is also not well established. In the early studies of Berliner et al. (2), chloroquine concentrations were determined in plasma, and those

of the metabolites were co-determined. In addition, concentrations were reported as means over a number of days, and it was not stated when blood samples were taken in relation to drug administration. Nevertheless, the results form the basis for the often-cited minimum recommended plasma concentration of 90 nmol/l chloroquine (9–11) that is fully effective for therapy against chloroquine-susceptible strains of malaria parasites. The whole-blood concentrations of chloroquine during treatment are, however, much higher. For example, in two recent studies (12, 13) the reported whole-blood mean concentrations were at least 1000 nmol/l for 3 days of treatment with a standard total dose of 25 mg chloroquine base/kg body weight.

Maximum tolerated concentrations

The maximum tolerated chloroquine concentration is also not well established. Side-effects of chloroquine might be dose-dependent or idiosyncratic, i.e., not dose-dependent. Dose-dependent side-effects were noted already in 1948 by Berliner et al. (2) in 32 volunteers who were administered 50–400 mg chloroquine (presumably as base) in a step-wise increased fashion. Only one case of light-headedness was observed when less than 400 mg was given daily. Later, using a specific HPLC method, Gustafsson et al. (14) noted accommodation weakness in patients with peak serum concentrations greater than 1000 nmol/l. The corresponding whole-blood concentrations would probably have exceeded those reported in the present study.

Inter-individual variability of chloroquine concentrations

Even though the inter-individual variation in whole-blood concentration was generally two- to threefold for chloroquine and three- to sixfold for desethylchloroquine, this is less than what has been previously observed after a single dose or short period of treatment with the drug (12–14).^e It also differs from what would have been found if dose-dependent elimination operated, since inter-individual variations in steady-state concentrations would then have been greater than those after a single dose.

The number of participants in the study was small. Thus, concentrations outside the observed ranges are not proof of defaults in patient compliance but might well be observed in individual patients who had been taking regular prophylaxis. There is abundant clinical evidence that the standard dosage of 300 mg chloroquine base weekly is effective for malaria prophylaxis

^e See footnote a, p. 879.

against chloroquine-susceptible strains of *P. falciparum*. The results we have reported support this evidence and indicate that the 40-year-old recommended dosage of chloroquine for malaria pro-

phylaxis is still valid. However, even one week of forgetfulness might result in unsatisfactorily low blood concentrations of the drug.

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RÉSUMÉ

CONCENTRATIONS DE CHLOROQUINE ET DE DÉSÉTHYLCHLOROQUINE DANS LA PROPHYLAXIE ANTIPALUDIQUE AU LONG COURS

Les doses de chloroquine recommandées pour le traitement et la prophylaxie du paludisme sont basées sur les résultats d'études très complètes réalisées en 1944-1946, mais qui avaient utilisé les méthodes peu spécifiques d'analyse qui existaient à l'époque. Les concentrations de chloroquine dans le sang de sujets soumis à une prophylaxie prolongée n'avaient également été déterminées qu'au moyen de ces méthodes.

Lors de la présente étude, on a déterminé, au moyen d'une méthode spécifique, la chromatographie liquide à haute pression (HPLC), les concentrations de chloroquine et de son principal métabolite, la déséthylchloroquine, dans le sang total chez des sujets soumis à une prophylaxie antipaludique au long cours. Les résultats ont été confrontés aux doses actuellement recommandées pour la prophylaxie par la chloroquine.

Dix Suédois adultes des deux sexes (poids moyen 65 kg; âge moyen 39,5 ans) prenant régulièrement une dose hebdomadaire de 310 mg de chloroquine base depuis au moins huit mois, se sont portés volontaires pour l'étude. Des prélèvements de sang ont été effectués dans des capillaires de 100 µl le lendemain de la prise hebdomadaire du médicament puis une fois par jour pendant une semaine.

Aucun effet secondaire n'a été observé; la moyenne, la médiane, l'écart type et l'intervalle des concentrations de chloroquine et de déséthylchloroquine dans le sang total ont été déterminés. La somme des concentrations de chloroquine et de déséthylchloroquine ne dépassait systématiquement 1000 nmol/l que le lendemain de la prise du médicament, les concentrations moyennes de chloroquine et de déséthylchloroquine dans le sang total étant

respectivement de 1305 nmol/l et 915 nmol/l. Immédiatement avant la prise de la dose hebdomadaire suivante, ces concentrations étaient tombées à 489 nmol/l pour la chloroquine et de 384 nmol/l pour la déséthylchloroquine. Ces doses sont supérieures à la concentration minimale inhibitrice et inférieures aux concentrations maximales tolérées. La concentration la plus faible de chloroquine mesurée était de 280 nmol/l chez un sujet, et les variations inter-individuelles des concentrations de chloroquine étaient en général de l'ordre du simple au double ou au triple.

Dans l'idéal, les concentrations de chloroquine et de déséthylchloroquine au cours d'une prophylaxie antipaludique devraient être suffisamment élevées pour que le médicament soit uniformément efficace d'une prise à l'autre mais sans atteindre des concentrations telles que des effets indésirables liés à la dose surviennent. On ne connaît pas exactement la concentration minimale pleinement efficace de chloroquine dans le sang total; néanmoins, on considère qu'une concentration plasmatique de 90 nmol/l de chloroquine est la plus faible qui soit pleinement efficace dans le traitement des infestations à souches chloroquino-sensibles de *Plasmodium falciparum*. La concentration maximale tolérée de chloroquine n'est pas non plus établie avec précision, mais on sait que des concentrations sériques supérieures à 1000 nmol/l de chloroquine sont associées à des troubles de l'accommodation.

On dispose de nombreuses preuves cliniques de l'efficacité de la dose classique de chloroquine pour la prophylaxie du paludisme; nos résultats indiquent que la dose prophylactique recommandée depuis 40 ans est encore valable.

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