PLASMA CHLOROQUINE AND DESETHYLCHLOROQUINE CONCENTRATIONS IN CHILDREN DURING AND AFTER CHLOROQUINE TREATMENT FOR MALARIA

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- 1 Twelve children with acute falciparum malaria were treated with 25 mg/kg chloroquine orally in three divided doses at 24 h intervals.
- 2 Concentrations of chloroquine and its metabolite, desethylchloroquine, were measured in plasma from the beginning of treatment for up to 7 days using a high pressure liquid chromatography (h.p.l.c.) technique.
- 3 Chloroquine was detectable in plasma within 30 min of giving the drug. Peak level was reached in 1–8 h after the first dose of 10 mg/kg and the peak concentrations ranged between 65 and 263 ng/ml.
- 4 Chloroquine concentration declined slowly in plasma after stopping drug administration so that the concentration at the seventh day was 37.5% of the concentration on the third day. The apparent half-life was 3–4 days.
- 5 Desethylchloroquine was detectable in plasma within 30 min of giving chloroquine and peak levels were reached in 2–12 h. Peak concentration after the first dose of chloroquine ranged between 9 and 62 ng/ml.
- 6 Desethylchloroquine was also slowly cleared from plasma and mean concentration at the end of 7 days was 49% of the mean concentration at the end of 3 days.

Keywords chloroquine desethylchloroquine malaria

Introduction

Chloroquine was the safest and most effective of a large number of 4-aminoquinolines investigated for antimalarial activity under an extensive programme of research on antimalarial drugs instituted by the United States Government during the second world war (see Coatney, 1963, for review). It was introduced into therapeutics shortly after the war following the publication of a statement approved by the board for coordination of the malaria studies, affirming its therapeutic potentials in malaria (Loeb *et al.*, 1946). Since then it has been the most widely used drug in the treatment and lately, in the prophylaxis of malaria in addition to less important uses in hepatic amoebiasis and in collagen and chronic inflammatory diseases (Rollo, 1980).

In spite of its long and extensive use, its pharmacokinetics, particularly in the clinical situation, have not been studied to any great extent. Aderounmu *et al.*

(1980) measured chloroquine concentrations at intervals during treatment of children for acute malaria but their concentration-time data for chloroquine were not enough to determine pharmacokinetic parameters of the drug. In a follow-up to that study, Adelusi *et al.* (1982) determined the rates of uptake and elimination of chloroquine in malarial children and concluded that these did not differ significantly from values for healthy adult volunteers from other studies. The fluorimetric method of analysing chloroquine concentration used in the above studies (Adelusi & Salako, 1980) does not permit the differentiation of chloroquine from its metabolites and has a high coefficient of variation at concentrations approaching 5 ng/ml. With the recent development of h.p.l.c. method for analysing chloroquine (Alvan *et al.*, 1982) it has become possible to determine chloroquine and its main metabolites separately in any given

biological specimen. It was therefore decided to re-evaluate, using this new technique, basic pharmacokinetic parameters for chloroquine in malarial children and, in addition, determine possible relationships between the kinetics of chloroquine and those of its main metabolite, desethylchloroquine, in such subjects.

Methods

The investigation was carried out on twelve African children aged between 21 months and 6 years and suffering from acute falciparum malaria. They all had pyrexia of 2–3 days duration and malaria was diagnosed by finding the early trophozoite stage of the malaria parasites in appropriately stained thick or thin peripheral blood films. Patients with concomitant upper or lower respiratory tract infections, diarrhoea or measles were excluded as were also patients who were malnourished or were otherwise too ill to take part in a pharmacokinetic study. The protocol for the study was approved by the ethical committee of the medical school. Its purpose and nature were carefully explained to the parents of the children and only children whose parents gave their voluntary consent were admitted to the study. The children were admitted to the Paediatrics ward of the University College Hospital, Ibadan, Nigeria, during the study and they were under the care of a consultant paediatrician throughout.

Each child received a total of 25 mg/kg chloroquine base in three divided doses — 10 mg/kg body weight on days 1 and 2 and 5 mg/kg body weight on day 3. Chloroquine was given orally as Nivaquine syrup (May and Baker, Nigeria, Limited). Food was withheld from the children 1 h before and after giving the drug. None of the children vomited within 1 h of taking the drug. The only other drug administered during the study was aspirin which was given for its antipyretic effect.

Venous blood was taken before the first dose of chloroquine and at 0.5, 1, 2, 4, 8, 12 and 24 h after the dose. Subsequently, blood was taken at 24 h intervals for a total of 7 days. Approximately 3 ml blood was withdrawn on each occasion into a lithium heparin bottle and immediately centrifuged at 1000 g to separate the plasma from the red blood cells. The plasma was then stored frozen in screw-capped glass bottles at -20°C until analysed. Blood was withdrawn via an indwelling butterfly needle, gauge 18, during the first 8 h. Thereafter sampling was by direct venepuncture.

Duplicate thick and thin blood films were prepared from each blood sample and examined for malaria parasites by light microscopy using a $\times 100$ oil-immersion objective and $\times 6$ eye piece.

Plasma chloroquine samples were analysed by one of us (O.W.) at the Department of Clinical Pharma-

cology, Karolinska Institute, Huddinge Hospital, Huddinge, Sweden, using the h.p.l.c. method recently developed there (Alvan *et al.*, 1982). The analysis was done within 6 months of collecting the samples. Storage of specimens for such duration at temperatures between -20°C and 0°C has been shown not to affect the chloroquine measurements. The parent compound and its desethyl metabolite were extracted with glass distilled ether. The mobile phase for the h.p.l.c. was 80% acetonitrile, 19.26% methanol and 0.74% diethylamine. The internal standard was 7-chloro-4-(4'-dimethylamino-1'-methylbutylamino)-quinoline. The lower limit of sensitivity of the method was 1 ng/ml for chloroquine and 0.5 ng/ml for desethylchloroquine. The coefficient of variation for the determination of chloroquine was 3.5% at 50 ng/ml, and 5% at 25 ng/ml for desethyl-chloroquine. All samples were analysed in duplicate. Results are given in the text and Figures as mean $\pm$ s.d.

Results

Serial concentrations of chloroquine (CQ) and desethylchloroquine (DCQ) after single dose of chloroquine

Although a negative history of chloroquine ingestion in the preceding 1 week was elicited from all patients, analysis of the pre-treatment blood sample showed substantial chloroquine levels in five out of the 12 patients. The patients were therefore divided into two groups for analysis of the data. Group (a) comprised the seven children with no chloroquine in the pretreatment blood sample. Group (b) were those children with measurable pre-treatment plasma chloroquine.

Chloroquine was detectable in the plasma in all the group (a) patients 30 min after the oral drug. The mean half-hour plasma chloroquine concentration was 67.7 ± 46.8 ng/ml. The mean peak concentration was 143.9 ± 62.0 ng/ml and this was reached in individual patients between 1 and 8 h. Plasma chloroquine then fell gradually reaching a mean concentration of 67.5 ± 35.1 ng/ml at 24 h (Figure 1). Desethylchloroquine was detectable in plasma from 0.5 h. Peak concentration (mean 29.7 ± 16.0 ng/ml) was reached between 2 and 12 h after which the concentration fell, reaching a mean value of 19.1 ± 12.1 ng/ml at 24 h (Figure 1).

The pre-treatment chloroquine concentration in the group (b) patients varied between 21.9 and 287.8 ng/ml with a mean of 157.2 ± 106.3 ng/ml. Chloroquine concentration rose from this initial level to a mean peak concentration of 250.9 ± 61.7 ng/ml, peak level being reached between 1 and 8 h. The concentration subsequently declined reaching a mean level

Figure 1 Mean plasma chloroquine (●) and desethylchloroquine (○) log concentration-time curves in seven malarial children having no measurable chloroquine in the pre-treatment plasma samples, in the initial 24 h after a single oral dose of 10 mg/kg chloroquine. Each point represents the mean $\pm$ s.d.

of 165.3 ± 51.1 ng/ml at 24 h (Figure 2). Desethylchloroquine was similarly present in the pre-treatment blood sample from these patients (mean 39.6 ± 47.4 ng/ml). The concentration rose to a mean peak value of 81.9 ± 38.6 ng/ml with a $t_{\max}$ that varied between 4 and 12 h. At 24 h this concentration had fallen to 59.9 ± 44.7 ng/ml (Figure 2).

Changes in plasma CQ and DCQ concentrations after stopping multiple dose treatment

In the subjects in group (a) mean plasma chloroquine fell from 118.2 ± 52.8 ng/ml on day 3 to 38.6 ± 22.5 on day 7 (one and five days respectively after the last dose of chloroquine). Mean plasma desethylchloroquine fell during the same period from 34.9 ± 21.6 to 18.7 ± 11.2 ng/ml. Both chloroquine and desethylchloroquine plasma concentrations declined monoexponentially during the interval with a half-life of 74 ± 18 h for chloroquine and 72 ± 24 h for desethylchloroquine (Figure 3).

Plasma chloroquine concentration fell in the group (b) subjects from 148.0 ± 61.8 ng/ml on day 3 to 61.2 ± 22.6 ng/ml on day 7. Plasma desethylchloroquine fell from 69.5 ± 49.9 to 32.6 ± 15.2 ng/ml during the same period. Both the chloroquine and the desethylchloroquine plasma concentration-time curves were monoexponential during this interval with a half-life

Figure 2 Mean plasma chloroquine (●) and desethylchloroquine (○) log concentration-time curves in five patients having chloroquine in their pre-treatment plasma samples, in the initial 24 h after a single dose of 10 mg/kg chloroquine. Each point represents the mean $\pm$ s.d.

of 75 ± 21 h for chloroquine and 77 ± 28 h for desethylchloroquine (Figure 4).

Clinical and parasitological response to chloroquine

Clinical and parasitological response to treatment was rapid in the two groups. Temperature became

Figure 3 Decline of chloroquine (●) and desethylchloroquine (○) concentrations with time in seven malarial children, having no chloroquine in their pre-treatment plasma samples, after stopping 3-day treatment with 25 mg/kg chloroquine. Each point represents the mean $\pm$ s.d.

Figure 4 Decline of chloroquine (●) and desethylchloroquine (○) concentrations with time in five patients having chloroquine in their pre-treatment plasma samples, after stopping 3-day treatment with 25 mg/kg chloroquine. Each point represents the mean $\pm$ s.d.

normal and parasitaemia disappeared within 2 to 4 days in all patients.

Discussion

Almost half the patients had CQ and DCQ in their plasma before treatment was commenced. In some cases the concentration of CQ was so high that the drug must have been taken within the previous 24 h. Yet all denied having used CQ within a week of coming to hospital. This emphasises some of the problems of medical practice in Africa—the problem of self medication with potent drugs and that of unreliability of medical history obtained from patients and their relatives. Compounding these is an unrestricted sale of several brands of drugs which should normally be available only on prescription. Chloroquine for example, is available under diverse names either as single preparations or in combination with other drugs such as paracetamol and vitamins in 'fever medicines'. Hence a patient may well be taking a drug containing large amounts of chloroquine without knowing.

In these children with malaria, absorption of chloroquine was rapid as has been found in healthy adults (Gustafsson *et al.*, 1983) and other children (Adelusi *et al.*, 1982), but time to peak concentration was more variable and was on the average longer than has been found in healthy adults. This is probably a reflection of the mild gastrointestinal disturbance usually associated with malaria and similar acute infections. The rate of initial rise of CQ concentration

with time was too irregular to permit an estimation of the kinetics of absorption from our data. Similarly, the experimental points in the subsequent decline phase of the log concentration-time curve during the 24 h after the first dose of chloroquine were too few for estimation of the initial elimination half-life of the drug. However, from the decline curve after the last dose of chloroquine a half-life of between 3 and 4 days was obtained. This is in good agreement with the original data of McChesney *et al.* (1962) in adults and with our own recent data in children (Adelusi *et al.*, 1982).

It is now known that because of its high tissue binding, tissue/plasma equilibrium is not achieved for many weeks after a single dose of chloroquine (Adelusi & Salako, 1982; Gustafsson *et al.*, 1983). A multiexponential concentration-time curve is therefore obtained if the plasma concentration is followed long enough thus giving higher values for the terminal half-life (Frisk-Holmberg *et al.*, 1979; Gustafsson *et al.*, 1983).

In this study DCQ appeared in plasma within 0.5 h of the first administration of chloroquine. It was also, like CQ, eliminated very slowly, so that its time course after the last dose of CQ closely followed that of the parent compound. The two compounds thus had similar half-lives over the period of observation, with the concentration of DCQ varying between 35.2 and 56.8% of that of CQ. The only other study known to us in which plasma CQ and DCQ were monitored over a length of time after administering CQ was that of Gustafsson *et al.* (1983). These workers administered single doses of CQ orally and parenterally to adult volunteers and followed plasma concentrations of CQ and DCQ for up to 60 days. The time courses of the plasma CQ and DCQ concentration-time curves were approximately parallel for most of the sampling period after administration of 300 mg tablet of chloroquine. The concentration of DCQ was between 35 and 50% of that of CQ during most of the sampling period. This is similar to the percentage range obtained in this study. The terminal half-life of 208 h obtained in the Swedish study was however much longer than the value of 74 h obtained by us because of the known multiexponential nature of the chloroquine log concentration-time curve and the longer duration of sampling in the Swedish study.

Desethylchloroquine has activity against sensitive strains of *P. falciparum* comparable to that of chloroquine (Aderounmu & Fleckenstein, 1983). Since both drugs are cleared very slowly from the body and therapeutic concentrations are present in plasma for more than 1 week after a single oral dose of CQ, one would expect the parasites to be exposed to relatively high concentrations of the two compounds during treatment with CQ. What role this fact has played in the rate of development of resistance by different strains of *P. falciparum* to chloroquine is unknown. It

is however of interest to observe that although it has been possible to induce resistance to chloroquine by repeated *in vitro* culture of an African strain of *P. falciparum* in media containing low concentrations of chloroquine (Nguyen-Dinh & Trager, 1978) yet after about 35 years of unregulated use of the drug in West Africa, no authentic case of resistance has been found *in vivo* in the area even during intensive studies of large groups of naturally infected non-immune children (Aderounmu *et al.*, 1980, 1981; Olatunde *et al.*, 1981).

With the development of the sensitive and specific h.p.l.c. method for separately determining CQ and DCQ in biological samples it should now be possible

to investigate the relative roles played by the unchanged drug and its metabolite in the dissimilar profile of adverse reaction to CQ in different races and in the different rates of development of resistance to CQ by malaria parasites in different geographical areas.

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