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Single dose disposition of chloroquine in kwashiorkor and normal children—evidence for decreased absorption in kwashiorkor

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1 The single dose disposition of chloroquine was studied in five children with kwashiorkor and six normal control children after an oral dose of 10 mg kg^{-1} of chloroquine base.
2 Plasma concentrations of chloroquine and its main metabolite were assayed by high performance liquid chromatography (h.p.l.c.). Chloroquine was detectable for up to 21 days in all the subjects.
3 Chloroquine was detectable in all the subjects within 30 min after giving the drug except in one subject. Peak levels were reached between 0.5 and 8 h in all the subjects (with no significant difference in the t_{max} between the two groups of children).
4 Peak plasma chloroquine concentrations in the children with kwashiorkor varied from 9 ng ml^{-1} to 95 ng ml^{-1} (mean 40 ± 34 ng ml^{-1}). Peak chloroquine concentrations in the controls varied between 69 ng ml^{-1} and 330 ng ml^{-1} (mean 134 ± 99 ng ml^{-1}). The mean AUC in the kwashiorkor children was significantly lower than the mean AUC in the control children ($P < 0.001$).
5 Peak plasma desethylchloroquine concentrations in the children with kwashiorkor varied between 3 and 13 ng ml^{-1} (mean 6 ± 9 ng ml^{-1}) while in the controls the concentrations varied between 14 and 170 ng ml^{-1} (mean 50 ± 61 ng ml^{-1}). There was no significant difference in the half-life of chloroquine between the kwashiorkor children and the normal control children.
6 The possible influence of a different binding and distribution pattern of chloroquine in kwashiorkor could not be assessed in this study. However a decreased absorption seems to be the most likely explanation for the very low drug and metabolite concentrations obtained in the patients. It is concluded that the low concentrations obtained in kwashiorkor after a standard dose of chloroquine might be of clinical importance.

Keywords chloroquine desethylchloroquine kwashiorkor

Introduction

The development of highly specific and sensitive methods for the assay of chloroquine and its main metabolite desethylchloroquine, in biological samples (Alván *et al.*, 1982; Bergqvist & Frisk-Holmberg, 1980), has enabled a reevaluation of the pharmacokinetics of chloroquine

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both in adult Caucasian volunteers (Gustafsson *et al.*, 1983) and more recently in adult African volunteers (Adjepon-Yamoah *et al.*, 1986; Walker *et al.*, 1987). However, the majority of those who suffer from malaria for which chloroquine is prescribed are children. This led Walker *et al.* (1983b) to measure the plasma levels of chloroquine and desethylchloroquine in Nigerian children during and after treatment for malaria. In African patients, malaria quite commonly occurs together with kwashiorkor. This investigation was designed to compare the pharmacokinetics after a single oral dose of chloroquine in children with kwashiorkor with those in normal children.

Kwashiorkor is a malnutrition syndrome mainly caused by protein intake deficiency and affects children living in poor communities. It is most common between weaning and the age of 5. The disease is characterised by apathy, low body weight and oedema, sparse discoloured fluffy hair and dermatosis (Coward & Lunn, 1981; Hendrickse, 1984; Jelliffe & Stanfield, 1978; Waterlow, 1984). There is hypoalbuminaemia as well as hypokalaemia with depleted cellular potassium levels. Serum enzymes like aminotransferases and lipids are also decreased (Alleyne *et al.*, 1976; Scrimshaw & Béhar, 1965). Villous atrophy of the intestine and fatty liver have been reported (Scrimshaw & Béhar, 1965).

Methods

Subjects

The study was approved by the ethics committee of the College of Medicine Ibadan. The investigation was carried out in Nigerian children. Each child was admitted into the study after the aims and procedures had been carefully explained to the parents and informed consent obtained. All the participants were free to withdraw their children any time they wished from the study. A negative history of chloroquine intake in the 3 months preceding the study was obtained from the parents.

Twelve children in whom a clinical diagnosis of kwashiorkor was made using the Wellcome Trust Working Group classification (1970) were initially recruited into the study. Seven parents withdrew their children after sampling for between 24 and 72 h. The reasons given by the parents were that the procedure was too bothersome and time consuming. The ages of the patients ranged between 2 and 3.5 years (mean 2.5 ± 0.7 years) and their weights ranged from 6.1–8.6 kg (mean 7.5 ± 1 kg). The weights then ranged between the 5th and 10th percentile of the Boston reference standard. Their plasma albumin ranged from 16–19 g l^{-1}. However the serum concentrations of their liver enzymes were normal. No one had albuminuria or glycosuria. The normal control children were children of members of staff attending the malaria clinic. They were aged 2–6 years (mean 4 ± 2 years) and their weights ranged from 7.6–27.2 kg (mean 16.7 ± 7.2 kg). The control children all had normal haematological indices, liver function and had no albuminuria or glycosuria.

Study design

Each volunteer was given a dose corresponding to 10 mg kg^{-1} body weight of chloroquine base orally. A chloroquine sulphate preparation (Nivaquine syrup, M & B) from one batch was used. The drug was administered by one of the investigators (OW). The normal controls were admitted into a side ward for the first 8 h of sampling and then discharged home. Further samplings were done on out patient basis. The kwashiorkor children were admited into the paediatric ward.

Sampling procedure

During the first 8 h of sampling, blood was taken from a forearm vein using a 0.80 mm butterfly needle with a heparin lock, and thereafter by direct venipuncture. The heparin had a strength of 500 iu ml^{-1}. Venous blood samples of 3 ml were withdrawn before and at 0.5, 1, 2, 4, 8 and 24 h after the oral intake of chloroquine. Subsequently, blood was drawn on days 5, 7, 14 and 21. In the normal control children additional blood samples were taken on days 3 and 28. In two of the control children, sampling was extended to day 35. The blood was put into lithium heparin tubes and centrifuged at 1200 *g* for 10 min. The plasma was separated from the red cells and stored at −70° C until analysed.

Chloroquine determinations

The concentrations of chloroquine and desethylchloroquine in plasma were assayed in duplicates using a high performance liquid chromatography (h.p.l.c.) method that is a modification of the method of Alván *et al.* (1982). The parent compound and its main metabolite, desethylchloroquine, were extracted with diethyl ether. The mobile phase consisted of acetonitrile and methanol in a ratio of 1.5:1 (v/v). To 100 ml of this phase was added 0.8 ml of a 25% ammonia solution. The internal standard was 7-chloro-4-

(dimethylamino-1-methylbutylamino)-quinoline. The column was an Ultrasphere-Si, 5 μm. A Shimadzu (RF 530) fluorescence detector with excitation wave length at 335 nm and emission wave length at 380 nm was used. The compounds were eluted in the following order, internal standard, chloroquine and desethylchloroquine. The retention times were 3, 4 and 6 min respectively. No interfering peaks cold be detected in the chromatograms. The intra-assay coefficient of variation was 8% at 12 ng ml^{-1}.

Calculations

The plasma concentration data were interpreted according to a two compartment open model. The terminal half-life ($t_{½}$) of chloroquine in plasma was calculated by linear regression of the terminal phase of the log concentration-time plots using the last four or five data points. The area under the plasma concentration–time curve (AUC) was calculated by the trapezoidal rule. The area to infinite time was conventionally added as Ct_n/β. In cases of positive blank samples total AUC was corrected by subtracting Cx/β, where Cx is the plasma concentration of chloroquine in the blank sample and β is the elimination rate constant of the elimination phase.

Values are given in the text and tables as means ± s.d. Differences between kwashiorkor and control data were tested for significance using Student's *t*-test. Probability (P) values less than 0.05 were considered statistically significant.

Ethics

As much as possible, the study was not allowed to interfere with the management of the children with kwashiorkor which essentially consisted of high protein diet and bed rest. Kwashiorkor children with haematocrit of less than 20% did not take part in the study. The haematocrit of the kwashiorkor children was monitored constantly throughout the study to ensure that the sampling did not have any adverse effect on the patients. Withdrawal from the study did not interfere with the management of the patients.

Results

Children with kwashiorkor

After oral intake, chloroquine could be detected in plasma within 30 min. The mean plasma chloroquine concentration at 0.5 h was 4 ± 4 ng ml^{-1} (range 1–8 ng ml^{-1}). This rose to a peak plasma concentration (C_{max}) of 40 ± 34 ng ml^{-1} (range 9–95 ng ml^{-1}). The time to reach peak plasma concentration (t_{max}) was 4 ± 3 h (range 2–8 h). The plasma concentration at 24 h after dose (Figure 1) was 34 ± 36 ng ml^{-1} (range, 12–86 ng ml^{-1}). Chloroquine could still be detected in plasma 21 days after administration (Figure 2). The mean plasma concentration on day 21 was 2 ± 2 ng ml^{-1} (range, 1–5 ng ml^{-1}). The half-life was 180 ± 73 h (range, 108–285 h). The area under the chloroquine concentration against time curve (AUC) ranged from 1360–6670 ng ml^{-1} h (mean, 3310 ± 2100 ng ml^{-1} h). The mean metabolite concentration 30 min after chloroquine intake ws 1.9 ± 1 ng ml^{-1} (range, 1.8–2 ng ml^{-1}). Peak metabolite concentration was 6 ± 4 ng ml^{-1} (range 3–13 ng ml^{-1}) and occurred between 1 and 9 h after taking chloroquine. The mean desethylchloroquine concentration on day 21 was 1 ± 1 ng ml^{-1} (range 1–2 ng ml^{-1}). The

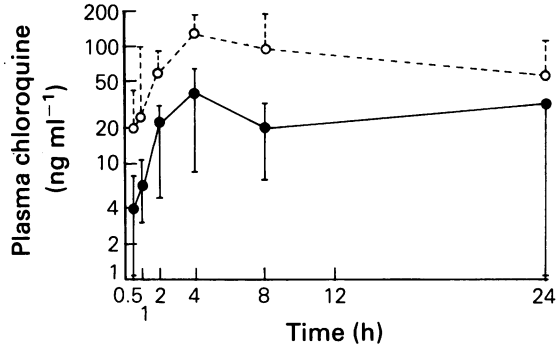

Figure 1 Mean plasma chloroquine log concentration-time disposition in five children with kwashiorkor (•) and six normal control children (○) after a single oral dose of 10 mg kg^{-1}, in the first 24 h. Each point represents the mean ± s.d. (The sixth normal control child with a blank chloroquine concentration of 268 ng ml^{-1} has been excluded from this figure).

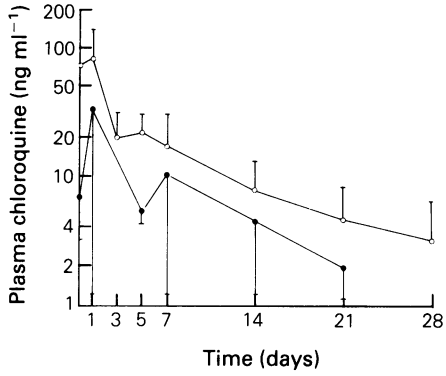

Figure 2 Mean plasma chloroquine log concentration-time disposition in five children with kwashiorkor (•) compared with six normal control children (○) after a single oral dose of 10 mg kg^{-1}. Each point represents mean ± s.d.

ratio between concentrations of the parent compound and the metabolite was 1.6 on day 21. The pharmacokinetic data from the children with kwashiorkor are presented in Table 1.

Normal control children

The plasma concentration of chloroquine at 0.5 h after administration was 68 ± 129 ng ml^{-1} (range, 0–331 ng ml^{-1}). This rose to a mean C_{max} value of 134 ± 99 ng ml^{-1} (range, 69–331 ng ml^{-1}). The t_{max} was 5 ± 3 h (range, 0.5–8 h). Thereafter, the plasma concentration of chloroquine at 24 h after dose was 82 ± 66 ng ml^{-1} (range 30–194 ng ml^{-1}) (Figure 1). The fall in plasma chloroquine concentration then became gradual and chloroquine could still be detected in plasma at day 28 (Figure 2). The mean plasma concentration of chloroquine on day 21 was 4 ± 4 ng ml^{-1} (range 1–10 ng ml^{-1}) and on day 28 it was 3 ± 3 ng ml^{-1} (range 1–3 ng ml^{-1}). The half-life was 195 ± 31 h (range, 130–231 h). The mean AUC was 9230 ± 3130 ng ml^{-1} h (range 5900–13100 ng ml^{-1} h). The plasma desethylchloroquine concentration at 0.5 h was 29 ± 69 ng ml^{-1} (range 0–171 ng ml^{-1}). The peak plasma desethylchloroquine level was 50 ± 61 ng ml^{-1} (range 14–171 ng ml^{-1}). This occurred between 0.5 and 24 h (mean 9 ± 8 h). The mean desethylchloroquine plasma concentration on day 21 was 4 ± 4 ng ml^{-1} (range 1.3–4.6 ng ml^{-1}) and on day 28 mean plasma desethylchloroquine concentration was 3 ± 2 ng ml^{-1} (range, 1–7 ng ml^{-1}). The ratio of parent compound to the metabolite at the end of day 21 was 1.1. The pharmacokinetic data for the normal control children are presented in Table 2.

There were no side effects attributable to chloroquine administration in the two groups of subjects. One subject each, from the two groups had chloroquine in the blank samples taken before dosing.

Discussion

The plasma concentrations of chloroquine in the kwashiorkor children were much lower than those obtained in the normal control children throughout the sampling period (Figures 1 and 2). Consequently, the mean AUC in the children with kwashiorkor was significantly lower than that in the normal children ($P < 0.001$) despite the wide interindividual variation. This suggests that the bioavailability of chloroquine might be

Table 1 Pharmacokinetic parameters of chloroquine in children with kwashiorkor

Subject	*Peak concentration (ng ml^{-1})*	*Time to reach peak concentration (h)*	*Peak metabolite concentration (ng ml^{-1})*	*Time for metabolite to reach peak concentration (h)*	$t_{1/2}$ *(h)*	** *AUC (ng ml^{-1} h)*
1	23	9	4	9	220	2240
2	25	2	3	4	168	2320
3	9	3	3	1	285	1360
4	48	2	7	8	108	6670
5	95*	9	13	9	121	3940
Mean	40	5	6	6.2	180	3310
± s.d.	34	3.7	4.2	3.6	73	2100

*Patient no 5 had 76 ng ml^{-1} of chloroquine in the zero sample. The estimated AUC without correction for positive blank sample was 17900 ng ml^{-1} h.
**$P < 0.001$ when compared to normal controls.

Table 2 Pharmacokinetic parameters of chloroquine in normal children

Subject	*Peak concentration (ng ml^{-1})*	*Time to reach peak concentration (h)*	*Peak metabolite concentration (ng ml^{-1})*	*Time for metabolite to reach peak concentration (h)*	$t_{1/2}$ *(h)*	** *AUC (ng ml^{-1} h)*
1	99	5	27	5	231	5900
2	70	8	18	24	202	13100
3	130	8	20	8	130	9250
4	103	5	51	9	206	6410
5	331	0.5	171	0.5	202	28200*
6	69	4	14	8	198	11506
Mean	134	5	50	9	195	9230
± s.d.	99	3	61	8	31	3130

*Patient no 5 had 268 ng ml^{-1} of chloroquine in the zero sample. This high value made it impossible to correct the AUC value which was not used in the calculation of the mean AUC.

decreased in the children with kwashiorkor. In previous studies in adults, the bioavailability of chloroquine was shown to be almost complete (Gustafsson *et al.*, 1983). Adelusi *et al.* (1982) came to similar conclusions in healthy Nigerian children. Some degree of intestinal malabsorption is usually present in kwashiorkor (Dossetor & Whittle, 1975; Scrimshaw & Béhar, 1965) and this would be consistent with reduced absorption and bioavailability of some drugs in this condition. Eriksson *et al.* (1983) found a markedly reduced absorption of chloramphenicol in severely malnourished children. The therapeutic implication of the decreased absorption of chloroquine in kwashiorkor is that in some patients with a severe form of the disease, enough chloroquine may not be absorbed from an oral dose to suppress an acute attack of malaria.

Chloroquine half-life after 21 days of sampling was 7.5 ± 3 days in the children with kwashiorkor. This was not significantly different from the half-life of 8 ± 1.4 days in the control children. It thus appears that the mild renal impairment in kwashiorkor (Coward & Lunn, 1981; Hendrickse, 1984) does not affect the half-life of chloroquine, although chloroquine is eliminated from the body largely by renal excretion (Gustafsson *et al.*, 1983), and chronic renal failure has been suggested to prolong the half-life of chloroquine (Salako *et al.*, 1984).

The present study could not assess whether a different drug distribution and decreased protein binding of chloroquine and desethylchloroquine in kwashiorkor could partly explain the low concentrations observed in the patients. Walker *et al.* (1983b) found protein binding of chloroquine to be about 60% in plasma from healthy, adult, Caucasian volunteers. Chloroquine was mostly bound to albumin, and to a lesser extent to orosomucoid. A small fraction of the binding could be ascribed to globulins and lipoproteins. Thus, even under the unlikely assumption that there would be no binding at all in the blood of kwashiorkor children this would not explain the finding that the total plasma concentration was about one third of that in the controls, Also, the chloroquine/desethylchloroquine ratio that was obtained in the kwashiorkor children on day 21 (1.6) was somewhat higher than that obtained in the controls (1.1). This may suggest that there is some impairment of the deethylation of chloroquine in kwashiorkor. This finding is in keeping with the result of a previous study by Waterlow (1961) who found evidence of reduced activity in some of the oxidative enzymes in the fatty livers of children with kwashiorkor. In a later study, Wharton & McChesney (1970) measured the urinary excretion of desethylchloroquine in the urine of kwashiorkor and control children and suggested that chloroquine metabolism might be impaired in kwashiorkor.

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