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The disposition of chloroquine in healthy Nigerians after single intravenous and oral doses

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1 The pharmacokinetics of chloroquine after single oral (600 mg) and intravenous (2 mg kg^{-1}) doses were studied in healthy black Nigerian students.
2 The plasma concentrations of chloroquine and its desethylmetabolite were determined using a high performance liquid chromatography technique. Concentrations as low as 3 ng ml^{-1} were reproducibly determined.
3 After i.v. dosage, the half-life ranged between 144 and 298 h; the total plasma clearance was between 282 and 1130 ml min^{-1} and the volume of distribution between 142 and 398 l kg^{-1}. Renal clearance was about 52% of plasma clearance. The estimated total urinary recovery of chloroquine and desethylchloroquine was 77% of the oral dose.
4 There was no significant difference in the pharmacokinetics between the oral and the i.v. administration.
5 The pharmacokinetic properties of chloroquine, in these black subjects did not differ from those previously demonstrated in Caucasians.

Keywords pharmacokinetics chloroquine desethylchloroquine ethnic groups

Introduction

The recent development of sensitive and specific high performance liquid chromatographic (h.p.l.c.) methods for the determination of the concentrations of chloroquine and its main metabolite in body fluids (Alván *et al.*, 1982; Bergqvist & Frisk-Holmberg, 1980) has permitted a detailed characterisation of the kinetics of chloroquine in healthy Caucasian subjects (Gustafsson *et al.*, 1983) and in African patients with malaria (Walker *et al.*, 1983). Pharmacokinetic studies on chloroquine in healthy subjects are needed in different ethnic groups since interethnic differences in the disposition of chloroquine might occur.

Methods

Subjects

Fourteen healthy African subjects (13 males and one female) volunteered to take part in the study. They were mainly members of staff or students of the College of Medicine, University of Ibadan, Ibadan, Nigeria. Their age was between 19 and 55 years (mean 27.3 ± 11 s.d.) and their weight ranged from 42.0 kg to 92.6 kg (mean 56 ± 12.7 s.d). All of the subjects were non-smokers. Some were moderate drinkers of alcohol. Nobody took any medication regularly. None of the subjects took chloroquine within 3 months prior to the study. Measurements of

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haematological indices, liver enzymes, serum electrolytes and urea which were done at the start of the study were within normal limits in all the subjects. On the day of the study all the volunteers had their early morning urine tested for specific gravity, albumin and sugar. The tests were all normal. Their electrocardiograms were all within normal limits. The study was approved by the ethics committee of the Medical College. The committee did not approve that the same volunteer received chloroquine both i.v. and orally.

Study design

The subjects were divided into two groups. The first group of six volunteers was given chloroquine phosphate (Resochin, Bayer) at a dose corresponding to 2 mg kg^{-1} of the base. The dosage form was diluted with isotonic saline to 50 ml and given by intravenous infusion over 20 min. Blood samples were taken from a fore-arm vein via an indwelling butterfly needle with a heparin lock during the first 8 h of sampling. Venous blood (10 ml) was taken before the start of the infusion and at 0.25, 0.5, 1, 2, 4, 8 and 24 h after starting the infusion. Subsequently blood samples were taken by direct venepuncture on days 3, 5, 7, 14 and 21. Twenty-four hour urinary samples were collected from each volunteer on days 1, 7, 14 and 21. Blood pressure and pulse measurements were taken before and after chloroquine infusion.

The second group consisted of eight volunteers who took 600 mg of chloroquine base (Nivaquine, Embechem Nig, Ltd) orally. Blood and urinary sampling schedule was as for the first group except that no sample was taken at 0.25 h and additional samples were collected on days 28 and 35.

All blood samples were centrifuged at 1,200 *g* within 10 min of drawing the blood and the plasma was stored frozen at −70° C until analysed. This was done to minimise leakage of chloroquine from the cellular elements of blood into plasma while standing as some of them like platelets may contain several hundred times the corresponding plasma concentration (Bergqvist & Domeij-Nyberg 1983). Urinary volumes were carefully noted and 10 ml aliquots taken and stored frozen at −70° C until analysed.

Chloroquine determinations

Plasma, and urinary chloroquine and desethylchloroquine concentrations were analysed in duplicate using h.p.l.c. The extraction procedure was as described by Alván *et al.* (1982). The mobile phase consisted of acetonitrile and methanol in a ratio of 1.5:1 (v/v). Ammonia (25%, 0.8 ml) was added to every 100 ml of this solution. The column was an Ultrasphere-Si 5 μm 150 × 4.6 mm. The internal standard used was 7-chloro-4-(4-dimethylamino-1-methylbutylamino)-quinoline. A fluorescence detector with excitation wavelength set at 335 nm and emission at 380 nm was used. The compounds eluted from the column in the following order, internal standard, chloroquine and desethylchloroquine. The retention times were 3, 4 and 6 min respectively. The lower limit of sensitivity for chloroquine was 3 ng ml^{-1}. The intra-assay coefficient of variation was 8% at 12 ng ml^{-1}.

Pharmacokinetic calculations

The concentration data obtained were analysed assuming a two-compartment open model. The 'terminal' half-life ($t_{½}$) in plasma was calculated by linear regression of the log concentration-time plots, using the last four or five time points. The area under the plasma concentration-time curve (AUC) was estimated using the trapezoidal rule. The area to infinite time was added by integration ($C_{tn/\beta}$), where C_{tn} is the last value of chloroquine concentration on the calculated β slope, and β is the slope of the least squares linear regression line of the log concentration-time plots. The plasma clearance (CL_p) for the intravenous dose was calculated from equation 1:

$$CL_p = \frac{F \cdot D}{AUC} \quad (1)$$

where D is the dose given, and F for 1 for intravenous administration.

The apparent volume of distribution (V) was calculated according to equation 3 using i.v. data only:

$$V = \frac{CL_p}{\beta} \quad (2)$$

Renal clearance was calculated from urinary and plasma data from days 1, 7, 14 and 21 for each dose according to equation 2:

$$CL_R = \frac{\text{Excretion rate}}{C_p} \quad (3)$$

where C_p is the plasma concentration at the midpoint of the urinary collection interval. The means for the four urinary collection days are presented in the results.

The urinary recovery of chloroquine (CQ) and desethylchloroquine (DCQ) up to day 35 was approximately estimated by calculating the area under the curve obtained by plotting the

rate of urinary excretion against time and using the trapezoidal rule. Excretion to infinite time was estimated using the *k*-values obtained between days 14 and 35. The sum of the total areas under the excretion curves of CQ and DCQ is an estimate of the total amount of CQ and DCQ recovered in urine.

Values are given in the text and Tables as means ± s.d. Differences between means were tested for significance using Student's *t*-test and p values less than 0.05 are taken as significant.

Results

Intravenous administration

The log concentration-time curves for CQ and DCQ, after a slow i.v. infusion of 2 mg kg^{-1} of chloroquine are shown in Figure 1. A summary of the data is shown in Table 1. Peak concentrations of CQ were reached within 15 min of starting the infusion, with the peak values ranging between 113 and 417 ng ml^{-1} (mean 249 ± 121 ng ml^{-1}). The concentrations declined rapidly on the first day but subsequently slowly and CQ was still detectable in plasma 21 days after drug administration. The apparent terminal half-life varied between 144 and 298 h with a mean of 250 ± 62 h. The coefficient of regression for the calculation of half-life was 0.90 ± 0.06. In spite of a relatively wide interindividual variation in peak concentration the terminal slopes of the individual log concentration-time curves were similar, except for one subject (subject No. 2, Table 1). Similarly, the extrapolated AUC was 20% or less of the total AUC except for subject No. 2 in whom the extrapolated AUC was 37.6% of the total AUC. The apparent volume of distribution varied between 147 and 398 l kg^{-1} (mean 261 ± 108 l kg^{-1}). The total plasma clearance was between 282 and 1130 ml min^{-1} (mean 664 ± 320 ml min^{-1}) of which approximately 52% was due to renal clearance which had a mean of 346 ± 277 ml min^{-1} (range 165–896 ml min^{-1}).

Desethylchloroquine was detectable in plasma from 15 min after starting the CQ infusion. Peak plasma concentrations of this metabolite varied between 7 and 34 ng ml^{-1} (mean 15 ± 10 ng ml^{-1}) and the peak was reached between 0.25 and 8 h after starting the infusion. As with CQ, the decline phase of the DCQ log concentration-time curve was slow and the metabolite continued to be detectable in plasma for up to 21 days (Figure 1). The plasma concentration of DCQ in individual subjects towards the end of the sampling period varied between 6 and 45%

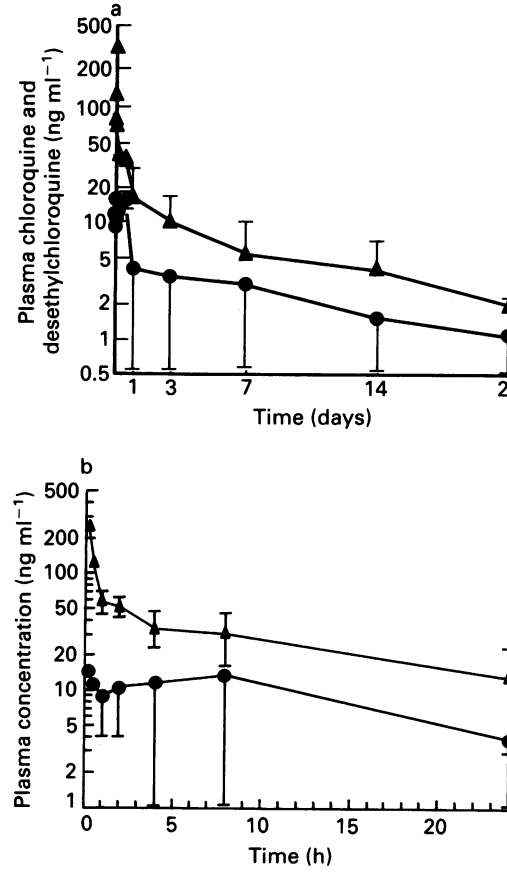

Figure 1 (a) Mean plasma chloroquine (▲) and desethylchloroquine (●) log concentration-time curves after intravenous infusion over 20 min of 2 mg kg^{-1} chloroquine in six healthy Nigerian adults. (b) First 24 h mean plasma chloroquine (▲) and desethylchloroquine (●) log concentration-time curves after intravenous infusion over 20 min of 2 mg kg^{-1} chloroquine in six healthy Nigerian adults. The standard deviations are shown as bars.

of the corresponding concentration of CQ with a mean of 29%.

Oral administration

Figure 2 shows the mean CQ and DCQ plasma log concentration-time curves for all eight subjects given one oral dose of 600 mg chloroquine base. The pharmacokinetic parameters derived from the individual concentration-time data are detailed in Table 2. Peak plasma concentrations ranged between 308 and 442 ng ml^{-1} (mean 374 ± 56 ng ml^{-1}) and were reached in 2 to 8 h (mean 5 ± 3 h). Plasma CQ concentrations declined

Table 1 The pharmacokinetics of chloroquine in normal Africans after an i.v. infusion of 2 mg kg^{-1} of chloroquine base

Subject	*Weight (kg)*	*Peak chloroquine concentration (ng ml^{-1})*	*Peak metabolite concentration (ng ml^{-1})*	*Time for metabolite to reach peak concentration (h)*	*Last time point used calculation (h)*	*$t_{½}$ (h)*	*V (l kg^{-1})*	*CL_p (ml min^{-1})*	*CL_R† (ml min^{-1})*	*Total AUC (ng ml^{-1} h)*	*Extrapolated AUC as % of total*
1	42.2	113	8	2	528	260	398	1130	329	1535	20.0
2	92.0	170	7	9	528	463*	263*	605*	896	2572*	37.6*
3	53.5	151	12	2	504	298	330	677	165	2086	18.5
4	40.0	321	16	0.25	744	261	266	469	168	2578	9.2
5	53.6	324	34	4	672	144	147	761	230	2992	10
6	41.7	417	15	0.25	960	287	168	282	288	4527	3.2
Mean ± s.d.	54 ± 20	249 ± 121	15 ± 10	3 ± 3	656 ± 177	250 ± 62	261 ± 108	664 ± 320	346 ± 277	2744 ± 1136	12 ± 7

† = mean of 4 determinations on day 1, 7, 14 and 21
* = not used in calculating mean due to inconsistent decline of concentrations

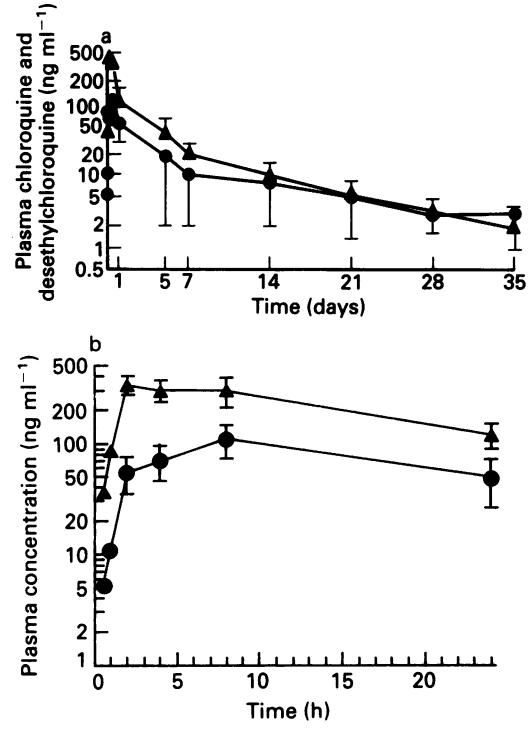

Figure 2 (a) Mean plasma chloroquine (▲) and desethylchloroquine (●) log concentration-time curves after single oral dose of 600 mg chloroquine base in eight healthy Nigerian subjects. (b) First 24 h mean plasma chloroquine (▲) and desethylchloroquine (●) log concentration-time curves after a single oral dose of 600 mg chloroquine base in eight healthy Nigerian subjects. The standard deviations are shown as bars.

slowly after reaching peak level and the drug was still detectable in plasma 35 days after its administration. The apparent terminal half-life varied between 157 and 248 h (mean 192 ± 28 h). The coefficient of regression for the calculation of half-life was 0.97 ± 0.02. Renal clearance ranged between 320 and 456 ml min^{-1} with a mean of 378 ± 45 ml min^{-1}.

Desethylchloroquine was detectable in plasma from 30 min after the oral dose. Peak concentrations varied between 71 and 163 ng ml^{-1} (mean 113 ± 31 ng ml^{-1}) and were attained 2–8 h (mean 7 ± 2 h) after the dose. Peak DCQ concentrations were reached at approximately the same time as peak CQ concentration with the DCQ C_{max} being about 30% of that of CQ. The decline phase of the concentration-time curve was relatively flat and DCQ was detectable in plasma at the last sampling 35 days after the intake of CQ.

Table 2 The pharmacokinetics of chloroquine in normal Africans after an oral intake of 600 mg of chloroquine base

Subject	*Weight (kg)*	*Peak chloroquine concentration ($ng\ ml^{-1}$)*	*Time for chloroquine to reach peak concentration (h)*	*Peak metabolite concentration ($ng\ ml^{-1}$)*	*Time for metabolite to reach peak concentration (h)*	*Last time point used for calculation (h)*	$t_{½}$ *(h)*	CL_R† *($ml\ min^{-1}$)*	*Total AUC ($ng\ ml^{-1}\ h$)*	*Extrapolated AUC as % of total*
1	60.8	370	4	95	4	840	184	333	18725	2.4
2	66.2	314	2	84	8	672	191	320	14755	3.5
3	56	313	4	129	8	672	198	456	13965	3.6
4	56	431	8	134	8	840	163	406	24403	1.7
5	56	410	2	71	8	840	187	397	22622	2.8
6	53	401	8	163	8	840	157	397	20302	1.3
7	55	442	2	95	2	672	207	371	21098	5.7
8	58.5	308	8	129	8	840	248	347	12998	5.4
Mean ± s.d.	58 ± 4	374 ± 56	5 ± 3	113 ± 31	7 ± 2	777 ± 87	192 ± 28	378 ± 45	18609 ± 4254	3.3 ± 1.6

† = mean of 6 determinations on day 1, 7, 14, 21, 28, 35

Urinary excretion

Analysis of the excretion rate of CQ and DCQ in 24 h urine collections showed an exponential decline of both compounds with time (Figure 3). The total CQ and DCQ excretion in the first 24 h after oral drug administration was 83 ± 18 mg which was approximately 14% of the administered dose (Table 3). The total estimated urinary CQ and DCQ recovery in individual subjects varied between 57 and 104% (mean 77 ± 13%) of the administered dose. Calculating from the estimated total excretion 81% of the recovered quinoline was due to the parent compound and only 19% to the metabolite giving a ratio of approximately 4.3 (Table 3). Urinary concentrations of CQ markedly exceeded those in plasma as at 35 days after administering CQ, its mean urinary concentration was 680 ± 335 ng ml^{-1}.

The two groups of subjects did not report any side effects and there was no significant change in blood pressure measurements at the start and end of infusion.

Discussion

The present knowledge about the pharmacokinetics of CQ is largely based on studies in healthy Caucasians (McChesney *et al.*, 1967; Gustafsson *et al.*, 1983). Chloroquine is probably more extensively used in Africa than anywhere else in the world and complementary studies of its pharmacokinetics in black Africans are there-

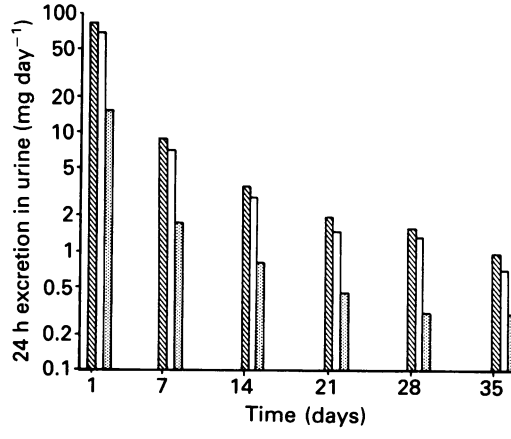

Figure 3 Mean daily excretion of chloroquine and desethylchloroquine in eight healthy Nigerian subjects given a single oral dose of 600 mg chloroquine. Urine collection on days 1, 7, 14, 21, 28 and 35. □ chloroquine alone, ▨ chloroquine + desethylchloroquine and ▩ desethylchloroquine alone.

Table 3 Mean urinary recovery and estimated total excretion of chloroquine and desethylchloroquine in eight subjects given 600 mg chloroquine orally as a single dose

	Amount recovered (mg)						*Estimated total recovery (mg)*	*Total recovery as % of dose given*
Day	1	7	14	21	28	35		
Chloroquine	68	7.0	2.7	1.5	1.3	0.7	372	62
Desethylchloroquine	15	1.7	0.8	0.4	0.3	0.3	88	15
Total recovery of CQ + DCQ	83	8.7	3.5	1.9	1.6	1	460	77

fore needed. We have employed the same analytical method and much of the design used in a recent study of Swedish healthy volunteers (Gustafsson *et al.*, 1983).

Administration of chloroquine i.v. (2 mg kg^{-1}) over 20 min did not produce any untoward effect in the subjects. Gustafsson *et al.* (1983) who administered approximately 5 mg kg^{-1} CQ i.v. over the same length of time observed transient adverse effects such as diplopia, difficulty in swallowing, disturbances in accommodation, fatigue or dizziness. In their subjects peak plasma CQ concentrations ranged between 478 and 1200 ng ml^{-1} and the adverse effects generally occurred at concentrations above 250 ng ml^{-1}. In our subjects, mean peak plasma CQ concentrations were less than 250 ng ml^{-1}. Adverse effects following i.v. chloroquine may thus be avoided if toxic levels of plasma CQ are not reached. A more recent study has also demonstrated the correlation between chloroquine plasma concentrations and adverse reactions (Looareesuwan *et al.*, 1986). However, in the treatment of severe malaria requiring intravenous CQ, a dose of 2 mg kg^{-1} i.v. is not enough. The required dosage regimen may be 5 mg kg^{-1} i.v. over 2–4 h repeated if necessary every 12 h but not exceeding a total of 25 mg kg^{-1} over a period of 3 days (WHO Technical Report; 1984).

Earlier studies using less sensitive methods for the assay of CQ and sampling for usually less than 10 days have reported half-lives of CQ between 2 and 7 days (McChesney *et al.*, 1967; Brohult *et al.*, 1979; Adelusi *et al.*, 1982). Using the more sensitive h.p.l.c. method of CQ assay, Gustafsson *et al.* (1983) found a mean half-life of 12 days which is in good agreement with this study giving a mean half-life of 10 days after i.v. infusion ($P > 0.76$). These two most recent studies therefore show that CQ has a much longer half-life than originally assumed.

Our study confirms the exceptionally large apparent volume of distribution of chloroquine. Interestingly, the V (mean 261 l kg^{-1}) in our Nigerian subjects was comparable ($P > 0.3$) to that (mean 204 l kg^{-1}) in the Swedish subjects (Gustafsson *et al.*, 1983). This large apparent volume of distribution is due to the high concentrations of CQ in tissues and organs like the skin, liver, kidney, spleen and lungs (Adelusi & Salako, 1982; Lindquist, 1973). Slow redistribution of CQ between tissue stores and blood accounts for the slow elimination and resulting long half-life.

CQ is eliminated both by metabolism and renal excretion. Metabolism is incomplete but occurs rapidly and the main metabolite, DCQ, appears in the blood within 15 min of starting an i.v. infusion and within 30 min of administration an oral dose. Peak plasma concentration of the metabolite after i.v. infusion was only 6% of the peak concentration of the parent compound whereas the peak metabolite concentration after oral dose was about 30% of the peak concentration of the parent compound.

Both CQ and DCQ are removed from the body mainly via the kidney as a mean urinary recovery of 77% of the administered dose was estimated. The renal clearance of CQ after i.v. administration was approximately 52% of the total plasma clearance and did not differ from the renal clearance after the oral route. The clearance data in these African subjects are essentially similar to those reported in Caucasian subjects (Gustafsson *et al.*, 1983) in which renal clearance was 57% of total clearance. Since the renal clearance is substantially greater than the glomerular filtration rate, renal excretion of chloroquine probably takes place by both glomerular filtration and tubular secretion. About 14% of the oral dose of CQ was excreted in the first 24 h as CQ and DCQ in a ratio of 4.5 which remained relatively constant over time. The importance of the renal route for the elimi-

nation of CQ and DCQ clearly indicates the need for studying the influence of renal disease on the pharmacokinetics of CQ.

Our study has thus confirmed in Nigerians the unusual pharmacokinetic properties of chloroquine already demonstrated in Caucasians. These properties include an extremely large apparent volume of distribution and consequently a long half-life and slow elimination although the drug is efficiently cleared from plasma. The study has not revealed any systematic difference between the pharmacokinetics of CQ in black Africans as compared to Caucasians. We are then referring to the data by Gustafsson *et al.* (1983) who used similar protocols and analytical techniques. Recent unpublished observations in Ghanians also support the notion that Africans and Caucasians do not differ markedly with respect to chloroquine kinetics (Adjepon-Yamoah *et al.*, 1986).

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