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A dose-ranging study of the pharmacokinetics of hydroxychloroquine following intravenous administration to healthy volunteers

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1 The pharmacokinetics of hydroxychloroquine were studied in five healthy volunteers following an intravenous infusion of 155 mg (2.47 ± 0.25 mg kg^{-1}) racemic hydroxychloroquine. Four of these volunteers also received a further 310 mg (4.92 ± 0.45 mg kg^{-1}) infusion of hydroxychloroquine and evidence of nonlinearities in the pharmacokinetics of hydroxychloroquine were sought.

2 No nonlinear elimination or distribution processes appeared to be operating at the doses of hydroxychloroquine used in this study, supporting the hypothesis that in the therapeutic dosing range the pharmacokinetics of hydroxychloroquine are linear.

3 Half-life and mean residence time were long (around 40 days) and large volumes of distribution were calculated (5,522 l from blood, 44,257 l from plasma). Sequestration into the tissues is an important feature of the disposition of hydroxychloroquine. The persistence of hydroxychloroquine in the body is due primarily to this extensive tissue distribution, rather than to low clearance (667 ml min^{-1} based on plasma data, 96 ml min^{-1} based on blood data).

4 Plasma data were more variable than blood data. Blood to plasma concentration ratios were not constant (mean ± s.d.: 7.2 ± 4.2). The data indicate that it is preferable to measure whole blood concentrations of hydroxychloroquine, rather than plasma concentrations, in pharmacokinetic studies.

5 The pharmacokinetics of hydroxychloroquine are similar to those of chloroquine.

Keywords hydroxychloroquine pharmacokinetics desethylhydroxychloroquine desethylchloroquine dose-dependence

Introduction

Pharmacokinetic parameters of hydroxychloroquine, a slow acting antirheumatic drug, have not previously been reported. Hydroxychloroquine is usually administered orally, either 155 mg or 310 mg (200 mg or 400 mg of the racemic sulphate salt) daily. Clinical response to hydroxychloroquine takes up to 6 months to become apparent. It is unknown whether pharmacokinetic or pharmacodynamic factors are responsible for the delayed action. The early studies of McChesney *et al.* (1961, 1962) indicated that hydroxychloroquine had a long half-life (3 days) and was extensively distributed into the tissues. Steady-state concentrations of hydroxychloroquine would be expected in 15 or 18 days if this half-life were correct, however if the half-life were similar to that reported in recent publications for chloroquine (45 days, Frisk-Holmberg

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et al., 1984), clinically significant delays in the achievement of steady-state concentrations would be expected.

One study gave preliminary indications of possible nonlinearities in the pharmacokinetics of hydroxychloroquine (McChesney *et al.*, 1962). Dose-dependent kinetics of chloroquine have been proposed and discussed in the literature (Frisk-Holmberg *et al.*, 1979, 1984, 1985; Gustafsson *et al.*, 1983a), however it has been shown that these reported nonlinearities are probably an artefact of the experimental design, due to short sampling protocols and/or assays of low sensitivity for chloroquine (Tett & Cutler, 1987).

In the present study the pharmacokinetics of hydroxychloroquine, following intravenous administration of a single 155 mg dose, were investigated in five healthy volunteers. Four of these volunteers also received a second intravenous dose of 310 mg of hydroxychloroquine. This dose-ranging intravenous study of hydroxychloroquine was completed to establish the pharmacokinetic parameters of hydroxychloroquine and also to investigate whether or not the pharmacokinetics of hydroxychloroquine are dose-dependent. The doses administered are those used in the therapy of rheumatoid diseases therefore any nonlinearities apparent in the pharmacokinetics of hydroxychloroquine at these doses will be of importance clinically.

Methods

Volunteers

Five healthy volunteers were enrolled in the study, three females and two males. The mean age of the volunteers was 22.6 years (range: 19–27) and the mean weight was 63.5 kg (range: 55–68). Volunteer 1 smoked (15 per day), was taking oral contraceptives and had had a cholecystectomy 5 years before the trial commenced. The other volunteers were taking no medication.

Informed consent was obtained from all volunteers, after full medical, haematological and biochemical tests were performed. Ophthalmological examination, including visual fields, was performed on each volunteer before and after receiving each dose. Haematological and biochemical examinations were also repeated after each dose. Standard audiological testing was performed at the beginning and at the end of each study for all the volunteers.

The studies received the approval of the ethics and research committee of St Vincent's Hospital, Darlinghurst, Australia.

Assay

The samples were assayed for hydroxychloroquine and metabolites using the h.p.l.c. assay described previously (Tett *et al.*, 1985, 1986). The limit of sensitivity of the assay is 1 ng ml^{-1} for all of the compounds (relative standard deviation 10%).

Dose administration

Volunteers 1–4 received, on separate occasions, two intravenous infusions, the first containing 155 mg of hydroxychloroquine, the second 310 mg of hydroxychloroquine. Volunteer 5 received the 155 mg infusion only.

The infusion, formulated to be isotonic and at pH 7.4, was manufactured using aseptic techniques approximately 1 h prior to administration. The hydroxychloroquine sulphate used was found to be pyrogen free (National Biological Standards Laboratory, Canberra). The whole solution was sterilized after manufacture by filtration. The infusions were weighed before and after administration to assess the exact dose delivered (assuming the solution density to be 1 g ml^{-1}).

The volunteers remained in the intensive care ward of St Vincent's Hospital for the duration of the infusion (0.5 h) and 0.5 h following infusion. ECG was monitored continuously and heart rate and blood pressure were recorded every 5 min during the first hour. A medical practitioner and a senior nursing sister were present throughout the infusion.

The infusion was administered at a constant rate over 0.5 h using a Terumo syringe pump, Model STC-521. Subjective effects experienced during infusion were monitored by questioning the volunteers.

The volunteers fasted (no food or liquid) from 22.00 h on the night prior to each dose. The infusion commenced approximately 11 h later. The volunteers received 200 ml of water immediately prior to the infusion and received lunch 4 h after the end of the infusion. The volunteers were lying down during, and for 30 min after the infusion, and thereafter were ambulatory.

Blood sampling

Blood (20 ml) was collected into siliconized Vacutubes (Johns Mallinckrodt, Sydney, Australia), with 125 iu heparin added manually, at the following times (via an indwelling cannula over the first day and subsequently by venepuncture): 0 (blank), 0.25, 0.5 (end of infusion),

0.75, 1, 1.25, 1.5, 2, 2.5, 3.5, 4.5, 6.5, 8.5, 13, 24, 32, 48, 72, 96, 120, 168 h, then once a week thereafter until the limit of sensitivity of the assay was reached (5 months following the 155 mg dose, 6 months or more following the 310 mg dose).

To obtain the plasma sample, 10 ml of the collected blood was centrifuged at 1200 *g* for 20 min within 30 min of collection and the plasma aspirated, using a Filtrona plasma filter sampler FS416, into a 10 ml Plain Tube (Disposable Products). All samples were kept frozen at −22° C until analysis.

Urine sampling

Following the 155 mg infusion dose an aliquot of urine was collected from each void for 72 h following the dose, then once weekly for a period of 24 h, commencing 12 h before each blood sampling time.

Data treatment

Concentration-time data (blood and plasma) were fitted using the equations

$$C_{inf} = (C_1/\lambda_1)(1-e^{-\lambda_1 t}) + (C_2/\lambda_2)(1-e^{-\lambda_2 t}) + (C_3/\lambda_3)(1-e^{-\lambda_3 t})$$

during infusion and

$$C_{inf} = (C_1/\lambda_1)(1-e^{-\lambda_1 \tau})(e^{-\lambda_1 (t-\tau)}) + (C_2/\lambda_2)(1-e^{-\lambda_2 \tau})(e^{-\lambda_2 (t-\tau)}) + (C_3/\lambda_3)(1-e^{-\lambda_3 \tau})(e^{-\lambda_3 (t-\tau)})$$

after the infusion (τ is the duration of the infusion) to obtain the parameters of the equation

$$C = \sum_{i=1}^{3} C_i e^{-\lambda_i t}$$

C is the concentration predicted if the infusion dose had been administered as a bolus dose at time (t) zero. The nonlinear regression program Funfit was used. Upon examination of the grouped data, it was estimated that the error in the data was approximately proportional to the concentration, indicating that the reciprocal of concentration squared is the appropriate weighting scheme.

The area under the concentration-time curve (AUC) was calculated by integration of the fitted equation and also using the trapezoidal rule with an extrapolation correction (Gibaldi & Perrier, 1982). Steady-state volume of distribution (V_{ss}) and mean residence time (MRT) were calculated using statistical moment theory (Gibaldi & Perrier, 1982). The area under the first moment curve (AUMC) was obtained from the fitted triexponential equation and using the trapezoidal rule with an extrapolation correction.

Terminal elimination half-life ($t_{½}$), total clearance (CL) and renal clearance (CL_R) were calculated using standard pharmacokinetic formulae (Gibaldi & Perrier, 1982). The amount excreted unchanged in the urine was calculated by summing the amounts excreted in each collection interval and interpolating between the intervals using the trapezoidal rule.

Statistical analysis

Unless otherwise indicated parameters are expressed as mean ± s.d. Analysis of variance and Student's *t*-test were performed using the Epistat microcomputer statistical package. Probability values for a difference between tested means, reported as ($P = \ldots$), were considered statistically significant if $P < 0.05$.

Plasma protein binding

Plasma protein binding of hydroxychloroquine was measured using a Dianorm equilibrium dialyser (Spectrum Medical Industries Inc, California, USA). Teflon half-cells (1 ml) were separated by a semi-permeable Visking cellulose membrane and equilibrium was achieved within 3 h. The protein solution was dialysed against Krebs buffer solution (pH 7.4) and samples were taken from both sides of the membrane after 3 h for measurement of hydroxychloroquine.

Results

Materials in contact with hydroxychloroquine

Geary *et al.* (1983) reported binding of chloroquine to glass surfaces and to some plastics. Preliminary studies with hydroxychloroquine indicated substantial losses onto glass. Therefore all glassware was silanised using Aquasil (Pearce Chemicals, Rockford IL, USA) prior to each use. All infusion equipment and collection apparatus were tested prior to use to ensure that hydroxychloroquine was not bound to the surfaces. Certain evacuated blood collecting tubes have been shown to alter the blood to plasma ratios of some basic drugs (Cotham & Shand, 1979), possibly by the leaching of plasticisers from the lids of the tubes. The blood collecting tubes used in this study, siliconized Vacutubes, with heparin 125 i.u. added manually, were

tested and found to neither adsorb hydroxychloroquine nor alter blood/plasma ratios. The plastic syringes and apparatus used to manufacture and dispense the infusions, and the short plastic tubes and indwelling cannulae used to collect blood over the first day of each study were also found not to adsorb hydroxychloroquine.

Pharmacokinetic analysis

The mean parameters of the polyexponential equations, with standard deviations, are shown in Table 1 for the blood and plasma data.

Figure 1 shows the blood drug concentration-time plots for one volunteer. Peak blood concentrations of hydroxychloroquine were measured in the samples collected at the end of the infusion and ranged from 1161 ng ml^{-1} to 2436 ng ml^{-1} (mean 1918 ng ml^{-1}) following the 155 mg infusion, and from 2290 ng ml^{-1} to 4211 ng ml^{-1} (mean 3312 ng ml^{-1}) following the 310 mg infusion. In order to prevent artefacts arising from the experimental design, samples must be collected for a sufficient time to characterise adequately the terminal elimination phase (Tett & Cutler, 1987). Using the sensitive h.p.l.c assay developed for these studies (Tett *et al.*, 1985) hydroxychloroquine could be detected in whole blood for 5 months following the 155 mg infusion and more than 6 months following the 310 mg infusion (Figure 1).

Plasma drug concentrations were appreciably lower and more variable than blood drug concentrations, being measurable for 2.5 months (155 mg infusion) and 4.5 months (310 mg infusion) depending on the dose. Peak plasma concentrations of hydroxychloroquine determined at the end of the infusion ranged from 427 ng ml^{-1} to 1080 ng ml^{-1} (mean 793 ng ml^{-1}, 155 mg infusion) and from 1378 ng ml^{-1} to 2440 ng ml^{-1} (mean 1747 ng ml^{-1}, 310 mg infusion).

In both blood and plasma, the calculated terminal elimination half-life of hydroxychloroquine was very long: 44 ± 12 days from blood data following the 155 mg infusion and 43 ± 22 days following the 310 mg infusion (Table 1). From plasma data, the half-life following the 155 mg infusion and was 26 ± 10 days, and following the 310 mg infusion, 53 ± 22 days (Table 1).

The model-independent pharmacokinetic parameters calculated from the blood and plasma data are shown in Table 2 together with the ratios of the areas under the concentration-time curves, corrected for dose. The ratios are not statistically different from 1. Analysis of variance indicates that the variability of these ratios is accounted for by interindividual differences in the volunteer group, rather than a difference between the methods used to calculate the areas under the curves, or the biological fluid used. Furthermore analysis of variance indicates that the variability in clearance, steady-state volume of distribution and mean residence time is also attributable to the subjects rather than differences between the doses or the method used to calculate the statistical moments (Table 2).

Table 1 Mean parameters of the exponential equations fitted to the concentration-time data following the intravenous infusions of hydroxychloroquine

Dose	C_1	λ_1	C_2	λ_2	C_3	λ_3
Blood						
155 mg	706	0.261	55	0.0076	11.2	0.00065
s.d.	±134	±0.061	±18	±0.0053	±4.5	±0.00018
310 mg	971	0.219	101	0.0053	16.4	0.00067
s.d.	±164	±0.045	±25	±0.0046	±11.9	±0.00035
P =	0.03†	0.30*	0.80*	0.52*	0.47*	0.93*
Plasma						
155 mg	112	0.471	6.51	0.0185	2.80	0.00110
s.d.	±52	±0.229	±3.35	±0.0158	±0.70	±0.00041
310 mg	425	1.19	25.3	0.0129	4.32	0.00054
s.d.	±143	±0.53	±5.6	±0.0028	±2.34	±0.00022
P =	0.04†	0.03†	0.02†	0.51*	0.41*	0.04†

* Student's *t*-test indicates that these means are not statistically different.
† Student's *t*-test indicates that these means are statistically different at the 5% significance level.
s.d. is the standard deviation of the parameter.
$C_{1,2,3}$ in ng ml^{-1}; $\lambda_{1,2,3}$ in h^{-1}.

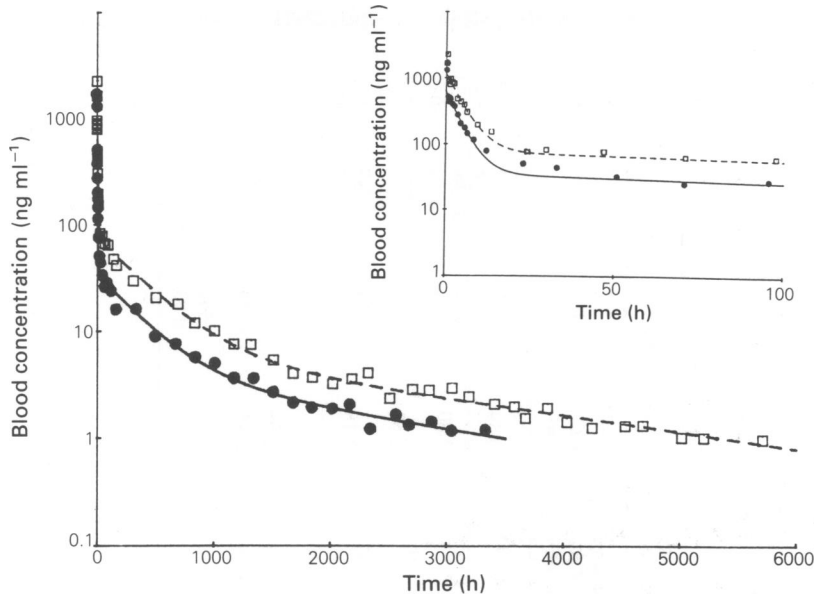

Figure 1 Log blood concentration-time plots for volunteer 1 following the 155 mg (•) and 310 mg (□) infusions of hydroxychloroquine. The solid (155 mg) and broken (310 mg) lines indicate the nonlinear least squares regression fitted curves. The inset shows an expanded time scale.

The distribution of blood to plasma ratios for all the samples from all volunteers are displayed in the bar graph in Figure 2. A wide range of blood to plasma ratios was observed, from 1 to 21, with a mean of 7.2 (±4.2).

Binding to a 4% human serum albumin solution was found to be constant over the concentration range 0.050–2.8 μg ml^{-1}, total hydroxychloroquine concentration, with a mean of 40 ± 6% bound (n = 48). Binding was investigated using the plasma of one healthy human and was found to be constant over the concentration range 0.050–1.0 μg ml^{-1}, with a mean of 45 ± 3% bound (n = 12).

Renal clearance was calculated following the 155 mg infusion. The mean amount excreted unchanged in the urine was 41 mg, which is 27% of the dose. The mean renal clearance of hydroxychloroquine from blood was 19.9 ml min^{-1} (22% of total blood clearance). The mean renal clearance from plasma was 211 ml min^{-1} (34% of total plasma clearance).

Representative blood concentration-time plots for two metabolites of hydroxychloroquine, desethyldroxychloroquine and desethylchloroquine, are shown for one volunteer in Figure 3 following the 155 mg and in Figure 4 following the 310 mg infusions of hydroxychloroquine. Peak concentrations of the metabolites were found in the sample collected at the end of the infusion.

Table 3 shows the terminal elimination half-lives calculated for hydroxychloroquine and the two desethylated metabolites of hydroxychloroquine from the blood data of each volunteer. The half-lives of the metabolites are longer than the half-life calculated for hydroxychloroquine.

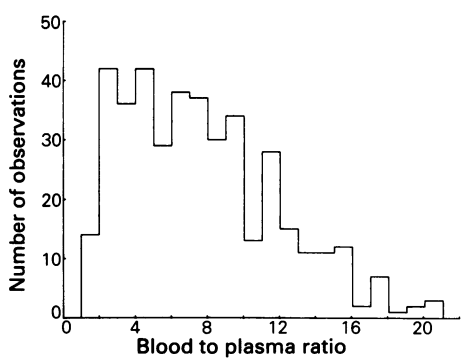

Figure 2 Blood to plasma hydroxychloroquine concentration ratios, 407 matched pairs. Mean (± s.d.) ratio = 7.2 (±4.2).

Table 2 Pharmacokinetic parameters of hydroxychloroquine in blood and plasma, calculated using the fitted equations

Subject	Dose ($mg\ kg^{-1}$)	Blood CL_R ($ml\ min^{-1}$)	AUC_B† ratio	CL_B ($ml\ min^{-1}$)	V_{ss},B (l)	MRT (h)	Plasma CL_R ($ml\ min^{-1}$)	AUC* ratio	CL ($ml\ min^{-1}$)	V_{ss} (l)	MRT (h)
1	2.51	22.94	1.09	112	8346	1241	115	0.59	309	38724	2090
	4.99			103	7867	1272			521	93998	3010
2	2.34	16.15	0.86	100	6178	1026	205	2.80	1061	40288	633
	4.47			117	4876	698			379	24674	1085
3	2.88	21.74	1.15	104	8024	1291	193	1.52	762	26698	584
	5.52			90	5315	980			500	52039	1735
4	2.39	25.95	1.09	93	4006	716	374	2.18	1314	52547	667
	4.69			86	2957	576			604	36317	1002
5	2.24	12.72		54	2402	747	168		719	25526	592
Mean	2.47	19.90	1.05	93	5791	1004	211	1.77	833	36757	913
s.d.	±0.25	±5.36	±0.13	±23	±2566	±268	±97	±0.95	±379	±11102	±659
	4.92			99	5254	882			501	51757	1708
	±0.45			±14	±2021	±310			±93	±30311	±928

† AUC_B ratio = $(AUC_{B,310}.Dose_{155})/(AUC_{B,155}.Dose_{310})$
* AUC ratio = $(AUC_{310}.Dose_{155})/(AUC_{155}.Dose_{310})$

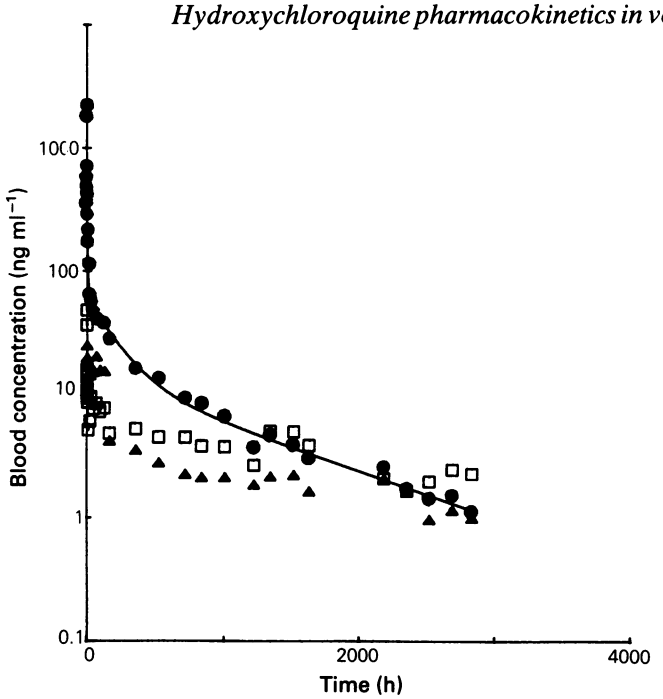

Figure 3 Log blood concentration-time plots for volunteer 4 showing the hydroxychloroquine (●), desethylhydroxychloroquine (□) and desethylchloroquine (▲) concentration-time points and the nonlinear least squares regression fitted curve of the hydroxychloroquine data following the 155 mg infusion of hydroxychloroquine.

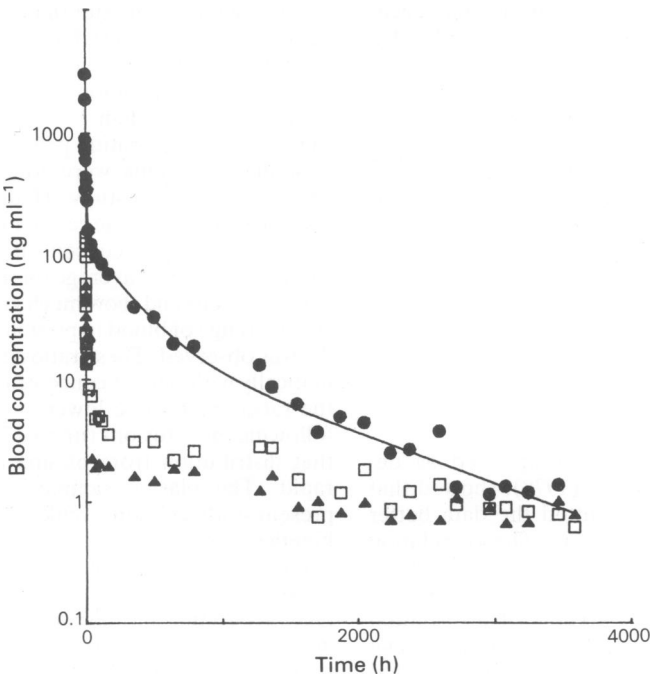

Figure 4 Log blood concentration-time plots for volunteer 4 showing the hydroxychloroquine (●). desethylhydroxychloroquine (□) and desethylchloroquine (▲) concentration-time points and the nonlinear least squares regression fitted curve of the hydroxychloroquine data following the 310 mg infusion of hydroxychloroquine.

Table 3 Terminal elimination half-life estimates (± s.d.) calculated from blood data for hydroxychloroquine (HCQ), desethylhydroxychloroquine (HCQM) and desethylchloroquine (CQM) following infusion of hydroxychloroquine (HCQ)

Subject	Dose of HCQ (mg kg^{-1})	Half-life (days) HCQ	HCQM	CQM
1	2.51	70 ± 38	170 ± 50	262 ± 143
	4.99	80 ± 9	131 ± 24	103 ± 15
2	2.34	38 ± 3	138 ± 79	100 ± 28
	4.47	29 ± 3	181 ± 57	825 ± 1084
3	2.88	52 ± 7	193 ± 90	545 ± 792
	5.52	78 ± 27	451 ± 261	152 ± 32
4	2.39	33 ± 5	78 ± 15	67 ± 9
	4.69	30 ± 6	61 ± 8	103 ± 15
5	2.24	45 ± 13	69 ± 3	111 ± 13

Side effects

Volunteer 2 reported a 'warm' feeling lasting for the duration of both infusions. This feeling gradually resolved over the ensuing 2 h. Volunteers 1 and 3 reported a metallic taste in the back of the mouth shortly after commencement of the higher dose (310 mg) infusion. This disappeared about 5 min after the end of the infusion. Volunteer 5 reported a feeling of faintness after becoming ambulatory an hour after commencement of the 155 mg infusion. This resolved with a further period of rest.

No significant changes were noted in the ECG, blood pressure or heart rate of any of the volunteers with either dose.

No abnormalities were reported from the ophthalmological, audiological, haematological and biochemical tests on the volunteers following each dose.

Discussion

Blood and plasma data

Akaike's Information Criterion, used as described by Yamaoka *et al.* (1978), showed that triexponential equations fitted the data better than biexponential equations. The correlation coefficients were all greater than 0.98 for the blood data fitted by triexponential equations. Clearance, steady-state volume of distribution and mean residence time were estimated using the polyexponential equations which were fitted to the data. These parameters were also calculated using the trapezoidal rule (values not reported). Statistical analysis showed that equivalent results were obtained using both methods.

Plasma concentrations of chloroquine are thought to be altered with increasing time between blood sample collection and plasma separation by centrifugation, and also with the centrifugation speed (Bergqvist & Domeij-Nyberg, 1983; Rombo *et al.*, 1985; Verdier *et al.*, 1983). This discrepancy has been attributed to redistribution of chloroquine out of blood cells. Bergqvist & Domeij-Nyberg (1983) suggested a separation procedure to overcome this problem (centrifugation at greater than 1000 *g* for 15 min, within 2 h of collection). However, Gustafsson *et al.* (1983b) reported a large range of blood cell to plasma concentration ratios for chloroquine (range: from less than 1 to 25), even following the suggested separation procedure.

Similar problems were found for hydroxychloroquine in this study. Hydroxychloroquine also concentrates in the cellular fraction of blood (Figure 2). Plasma concentrations of hydroxychloroquine are on average one seventh of blood concentrations and show much greater variability. A wide range of blood to plasma ratios, from 1 to 21, was observed. These ratios do not vary systematically with time or concentration, except that the ratio tends to be lower at very early times following the infusions (up to 0.75 h), suggesting that distribution from plasma into cells is not rapid. The plasma samples collected in the present study of hydroxychloroquine pharmacokinetics were centrifuged at 1200 *g* for 20 min within 30 min of collection. However, this separation procedure is still inadequate for complete separation of plasma from blood cells, as indicated by the variability in the blood to plasma ratio. From the results of the present study, it appears that hydroxychloroquine accumulates in blood cells in a similar manner to chloroquine and that similar problems of plasma separation have been experienced. It is recommended that,

until a reliable separation procedure becomes available, pharmacokinetic data on chloroquine and hydroxychloroquine be based on whole blood determinations.

It is apparent from the long half-life and mean residence time values and the large volumes of distribution calculated from the data (Table 2) that hydroxychloroquine distributes extensively into the tissues. However, clearance of hydroxychloroquine based on plasma data is high (667 ml min^{-1}). Total clearance calculated from blood (96 ml min^{-1}) is about one seventh plasma clearance, consistent with the mean value of 7.2 obtained for the blood to plasma ratio. Similarly the steady-state volume of distribution calculated from the blood data is about one seventh of that calculated from plasma data. The long half-life of hydroxychloroquine can be attributed to extensive tissue uptake rather than to an intrinsic inability to clear the drug. The expected delay in the attainment of steady-state concentrations (3 to 4 months) may be in part responsible for the slow therapeutic response observed with hydroxychloroquine.

The lower mean plasma half-life observed following the 155 mg dose is most probably an artefact due to inadequate characterisation of the terminal elimination phase in plasma following the lower dose. Despite the use of the sensitive assay, the sampling time possible, 2.5 months, is inadequate for a drug with a terminal elimination half-life of around 40 days.

Parameter estimates based on blood concentrations exhibited no evidence of dose-dependence in the concentration range in this study. Dose-adjusted areas under the concentration-time curves were not statistically different when calculated from the blood data (Table 2). No saturable elimination or distribution processes appear to be operating at the therapeutically relevant doses of hydroxychloroquine used in this study (Table 2).

The plasma protein binding studies indicate that hydroxychloroquine is mainly bound to albumin. Binding to a 4% albumin solution accounted for 90% of the binding estimated in plasma. However, hydroxychloroquine is not extensively protein bound in plasma, therefore alterations in protein binding, for example by disease states or the presence of other drugs, is not expected to have a significant effect on the clinical response.

Renal clearance

Mean renal clearance of hydroxychloroquine for each volunteer is shown in Table 2. Renal clearance accounts for approximately 22% of total blood clearance and about 34% of total plasma clearance. Mean renal clearance from plasma (211 ml min^{-1}) is 3 to 4 times greater than the glomerular filtration rate corrected for plasma protein binding, suggesting that hydroxychloroquine is secreted into the renal tubules. The low proportion of hydroxychloroquine eliminated unchanged through the kidneys indicates that no dosage adjustment is necessary in patients with mild or moderate renal impairment.

Metabolite data

Bisdesethylchloroquine, reported to be a metabolite of chloroquine and expected as a metabolite of hydroxychloroquine was not detected in any samples following either dose. This metabolite was present in the one patient, who had received hydroxychloroquine for 6 months, investigated during development of the assay (Tett *et al.*, 1985).

The desethylhydroxychloroquine and desethylchloroquine concentrations in plasma were difficult to quantitate, as concentrations were lower than 1 ng ml^{-1} by the end of the first day following the 155 mg infusion, and before the end of the first week following the 310 mg dose.

The metabolites accumulate in blood cells as does hydroxychloroquine. Higher concentrations are detected in whole blood than are found in plasma. There is some indication that desethylhydroxychloroquine accumulates to a higher degree in blood cells than desethylchloroquine, as desethylhydroxychloroquine concentrations in blood are approximately twice those of desethylchloroquine, but in plasma the concentrations of the two metabolites are approximately the same.

Peak blood metabolite concentrations, about 1 to 5% of the peak hydroxychloroquine blood concentrations, were found in the samples collected at the end of the infusion time. This indicates a capacity for rapid metabolic conversion of the parent drug, limited by tissue uptake. Thereafter, metabolite concentrations decreased more slowly than hydroxychloroquine concentrations.

From the metabolite data collected from whole blood, terminal elimination rates of desethylchloroquine and desethylhydroxychloroquine were calculated by linear regression analysis of the log concentration-time data for times greater than 168 h (Table 3). The standard deviations of the terminal elimination half-lives were large, an indication of the variability of the slowly decreasing concentrations quantitated at around the sensitivity limit of the assay. Examination of the metabolite concentration-time data did not

show disproportionate changes in concentrations of metabolites following the higher dose infusion of hydroxychloroquine. The data indicate that the metabolites may accumulate with chronic dosing. The clinical implications of this are uncertain as it has not been established whether the metabolites are of importance in efficacy or in the development of toxicity. However, preliminary evidence from one patient (Tett *et al.*, 1985) indicates that the metabolite concentrations achieved with chronic dosing do not exceed those of the parent drug.

The mean renal clearance of desethylhydroxychloroquine is about 20% that of hydroxychloroquine while desethylchloroquine mean renal clearance is about 40% that of the parent drug.

Comparison with chloroquine

The terminal elimination half-life of hydroxychloroquine, mean 43 days, is of the same order of magnitude as that of chloroquine, calculated using the data of Frisk-Holmberg *et al.* (1984, 1985) to be 45 days. The half-lives reported by other authors for chloroquine (Gustafsson *et al.*, 1983b; Edwards *et al.*, 1986; Aderounmu *et al.*, 1986; Adjepon-Yamoah *et al.*, 1986; Walker *et al.*, 1987) are shorter, around 10 days, but, as discussed by Tett & Cutler (1987), these are most likely an artefact of study design as samples were not collected for sufficient time and/or insensitive chloroquine assays were used.

The mean residence times calculated by Frisk-Holmberg *et al.* (1984, 1985) for chloroquine have a mean value of 990 h. Again, this is similar to the mean value of 943 h estimated for hydroxychloroquine from blood data in this study. Steady-state volumes of distribution are also of the same order of magnitude for both drugs, 60,000 l for chloroquine (Frisk-Holmberg *et al.*, 1984) and 44,000 l for hydroxychloroquine estimated from plasma data, and 7,725 l for chloroquine and 5,522 l for hydroxychloroquine, estimated from blood data.

Similar clearances are estimated for both drugs. A mean plasma clearance value of 1122 ml min^{-1} for chloroquine may be calculated from the study of Frisk-Holmberg *et al.* (1984). The mean plasma clearance of hydroxychloroquine in the present study was 667 ml min^{-1}. Blood clearance of chloroquine, calculated from the study of Frisk-Holmberg *et al.* (1984) is 131 ml min^{-1}. Blood clearance of hydroxychloroquine in this study was 93 ml min^{-1}.

The addition of the hydroxyl group to chloroquine does not therefore appear to greatly alter the pharmacokinetic parameters. Extensive tissue binding and hence slow elimination, even though the intrinsic clearance is high, are characteristic features of the disposition of both hydroxychloroquine and chloroquine. Clinical response to both drugs will be delayed as a period of some months is to be expected before attainment of steady-state levels.

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