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The relationship of the pharmacokinetics of chloroquine to dose in the rabbit

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Four albino rabbits were treated with 4 different doses of chloroquine phosphate (30–50 mg kg^{-1}) given intragastrically and 10 others were given the hydrochloride (2·5–12·5 mg kg^{-1} i.v.). The i.v. doses were given as bolus into the median ear vein over 5–8 min. Samples (3–5 mL) of whole blood were subsequently collected at intervals for up to 6 weeks and analysed by HPLC. From the results, log concentration–time curves were drawn and the i.v. data fitted by computer to 3-compartment (8 rabbits) or 2-compartment curves (2 rabbits). The area under the curve (AUC) and half-lives were calculated for the different doses. A plot of AUC versus dose yielded a straight line but the half-lives showed wide inter-individual variation from the same doses.

Frisk-Holmberg et al (1979), in a study on rheumatoid patients, presented evidence for possible dose-dependence of chloroquine kinetics. In their study, half-lives of 3·1, 42·9 and 312 h, respectively, were obtained for doses of 250, 500 and 1000 mg administered to volunteers. The fluorimetric assay methods used in the above study were not sensitive enough to measure plasma concentrations beyond 48 h after oral doses of 250 and 500 mg. They were however able to measure plasma drug levels for as long as 1000 h after a dose of 1 g. The terminal half-lives were therefore measured from non-identical portions of the multiexponential log concentration–time curves. Since the log-concentration versus time plot is non-linear, the $t_{\frac{1}{2}}$ will vary over the entire time period even for a single dose and will increase the further the $t_{\frac{1}{2}}$ is measured from the time of treatment. While this in itself would not indicate dose-dependence, a way to detect dose-dependence would be to determine $t_{\frac{1}{2}}$ and AUCs at the same point in time for the different doses. This has been done in the following experiments.

Materials and methods

Albino rabbits bred in the College of Medicine animal house and whenever possible belonging to the same litter, had free access to food until the eve of the experiment and again 6 h after the start of the experiment. The liquid chromatographic system employed was that described by Alvan et al (1982).

On the morning of the experiment each rabbit was weighed and its ears shaved. Chloroquine sulphate (Nivaquine M & B lot No. K203) was given as tablets crushed in 10–20 mL of water and administered via an intragastric tube in four doses: 30, 40, 45 and 50 mg kg^{-1}. Chloroquine hydrochloride injection (Winthrop Laboratories, USA) was used for the i.v. doses of 2·5, 5, 7·5 and 12·5 mg kg^{-1}. The chloroquine was drawn up into a syringe to which sterile normal saline was added to 10 mL. This was then given as a bolus slowly over 5–8 min through a cannula into the central ear vein. The end of treatment constituted zero time. Collection of 2–3 mL blood samples began 15 min later and lasted for at least 6 weeks according to the following schedule: 15, 30, 60 min, 1, 2, 3, 4, 6, 24 h, and 2, 3, 4, 5, 7, 21, 28, 35 and 42 days. The blood samples were stored frozen (−70 °C) until analysed.

Pharmacokinetic data analysis

Whole blood concentration–time data were analysed for model-dependent pharmacokinetic parameters using a non-linear least squares regression program. Data for 8 rabbits were fitted to the polyexponential equation:

$$C_B = C_1e^{-\alpha t} + C_2e^{-\beta t} + C_3e^{-\gamma t}$$

and in 2 rabbits to:

$$C_B = C_1e^{-\alpha t} + C_2e^{-\beta t}$$

Weighting with 1/concentration and incorporation of a lag time did not improve precision of parameter estimates or the quality of fits.

The area under the curve (AUC) was obtained using the linear trapezoidal rule up to the last data point. The AUC beyond the last data point was estimated using the equation:

$$\int_{t_{last}}^{\infty} Cdt = \frac{C_{last}}{2} \cdot \frac{1}{\beta}$$

where β is the terminal rate constant. Plasma clearance

Table 1. Oral chloroquine data.

Rabbit no.	Wt (kg)	Dose (mg kg^{-1})	$AUC_{0\rightarrow\infty}$ (ng mL^{-1} h^{-1})
1	2·08	31·6	86940
2	1·34	37·3	97440
3	2·10	46·3	109120
4	2·02	49·5	111190

* Correspondence.

Table 2. Pharmacokinetic parameters for i.v. chloroquine.

Rabbit no.	Wt (kg)	Dose (mg kg^{-1})	$AUC_{0-168\,h}$ (ng mL^{-1} h^{-1})	Cl (L kg^{-1} h^{-1})	VD (L kg^{-1})	Terminal $t_{\frac{1}{2}}$ (h)	Corr. coeff.
1	1·9	2·6	12 111·6	0·1	18·0	20·9	0·9339
2	1·7	2·9	12 665·0	0·0	22·0	45·1	0·9375
3	2·3	4·3	9 996·8	0·1	30·0	37·8	0·9624
4	1·5	4·8	12 085·6	0·1	30·0	39·8	0·9546
5	1·5	5·0	17 812·9	0·1	40·0	319·7	0·9510
6	1·8	7·0	24 615·0	0·1	19·0	365·6	0·9828
7	2·2	7·0	23 637·0	0·1	696·0	32·2	0·9882
8	2·5	10·0	34 359·0	0·1	194·0	243·7	0·9946
9	2·3	10·0	54 675·8	0·0	176·0	330·6	0·9650
10	1·8	10·0	27 711·2	0·1	173·1	1510·5	0·9798

(Cl) was calculated from the model-independent equation below:

$$Cl = \frac{Dose}{AUC_{0\rightarrow\infty}}$$

The terminal half-lives ($t_{\frac{1}{2}}$) were calculated by linear regression of the log concentration–time curve using values from 168 h and beyond, a minimum of 4 observations being used for such calculations. Values are given as means. Differences between means were evaluated using Student's *t*-test and *P* values less than 0·05 were taken as significant.

Results and discussion

The oral blood chloroquine concentration-time profile was not adequately described by a single absorption and two elimination terms as was the i.v. dosing. Plots of AUC against dose for the i.v. and oral dosing showed a linear increase in AUC with dose whether the AUC was calculated at 168 h or extrapolated to infinity (Table 1). The correlation between the AUC and dose of chloroquine was r = 0·8441 and 1·0404, respectively, for the i.v. and oral doses. The half-life of chloroquine varied from 60 to 330 h (disregarding the half-life of rabbit 10). The $t_{\frac{1}{2}}$ was dependent on the time of sampling rather than the dose of the drug since the higher doses were detectable in the blood for longer than the lower doses. Nevertheless there was also inter-individual variation (Table 2). Other pharmacokinetic parameters calculated from the i.v. dosing are also shown in Table 2.

AUCs have been compared at the same point in time for several different doses. AUCs measured at 168 h showed a linear increase with dose in all of the rabbits. $AUC_{0\rightarrow\infty}$ also showed a linear increase with dose in all of the 10 rabbits. The inter-individual variability observed in the terminal $t_{\frac{1}{2}}$ of animals who had the same doses of chloroquine has also been observed in man (Gustafsson et al 1983), and in dogs (Aderounmu & Fleckenstein 1983).

In rabbits,the AUC of chloroquine increases linearly with dose. Furthermore, the $t_{\frac{1}{2}}$ shows a wide inter-individual variation even for equal doses, and there is a general trend for an increase in $t_{\frac{1}{2}}$ with dose, due to the longer sampling period for the higher doses.

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