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An improved formulation of chloroquine for intramuscular administration: absorption kinetics in rabbits

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Abstract—Intramuscular chloroquine is rapidly absorbed, even in severe falciparum malaria, and may cause potentially lethal hypotension. Less rapidly absorbed formulations should be safer. A chloroquine phosphate solution containing 2% methylcellulose 1500 released chloroquine 2·6 times more slowly than a commercial aqueous solution in an in-vitro absorption simulator. There was a log linear relationship between viscosity and release rate. The absorption pharmacokinetics of the more viscous chloroquine phosphate solution were then compared with those of a commercial solution after intramuscular injection to eight rabbits in an open cross over comparison. The rate of absorption was over three times slower with the viscous solution; median time to peak whole blood concentration with the commercial aqueous solution was 10 (range 5–20) min compared with 30 (range 10–60) min for the more viscous formulation ($P < 0.05$). Peak whole blood concentrations were 66% (95% CI 50–82%) of those with the commercial preparation, but the acute bioavailability of the two solutions was similar. This simple new formulation may be safer than currently available chloroquine preparations and should now be evaluated in man.

Chloroquine is still the most widely used antimalarial in the world (WHO 1984). For nearly forty years parenteral chloroquine has been given for the treatment of severe malaria, particularly in children. In 1984 the World Health Organization concluded that parenteral chloroquine was dangerous, and should no longer be used (WHO 1984). This recommendation was based on anecdotal reports of sudden death following intramuscular chloroquine administration (Harris 1955; Toboku-Metzger 1964; Williams 1966; Geddes 1970; O'Holohan 1973), and uncertainties over the disposition and toxicity of the parenterally administered drug. Recent pharmacokinetic studies provide a plausible explanation for the apparent toxicity of parenteral chloroquine (Looareesuwan et al 1986, White et al 1987, 1988). The compound has unusual pharmacokinetic properties with a central apparent volume of distribution that is several orders of magnitude smaller than the total distribution volume (Looareesuwan et al 1986; White 1988). Furthermore, chloroquine is rapidly absorbed after intramuscular or subcutaneous injection even in sick children (White et al 1987; 1988). As a consequence, transiently high blood concentrations follow intramuscular or subcutaneous administration. These levels may cause a fall in blood pressure which may be lethal in vulnerable subjects (i.e. children with severe disease who are dehydrated, inadvertently given a large dose without being weighed, and are nursed upright following injection). Reduction in dose and more frequent administration improve safety (White et al 1987, 1988). If chloroquine absorption after intramuscular or subcutaneous injection could be slowed slightly without significantly reducing bioavailability, then toxicity would be considerably reduced and dose regimens simplified. We have developed a simple formulation which attempts to fulfil these criteria and report here preliminary studies in-vitro and in rabbits.

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Materials and methods

In-vitro absorption simulations. Chloroquine phosphate solutions (1·0 mg mL^{-1}) of various viscosities were prepared and their in-vitro release kinetics studied. The solutions were prepared by dispersing weighed amounts of methylcellulose 1500 (0, 0·5, 1·0, 1·5 or 2·0 g) in 75 mL of distilled water warmed to 70°C. The solutions were allowed to cool and 100 mg of chloroquine phosphate salt (Rhone-Poulenc lot No. CA 822 9902), and 250 mg of sodium chloride were then added. After mixing, the solutions were made up to 100 mL with distilled water and allowed to stand overnight. The following morning the viscosity of the solution was measured at $37 \pm 0{\cdot}1$°C using a high shear rate synchrolectric viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, USA).

The release rate of chloroquine from the prepared solutions (after standing overnight) was determined using an absorption

simulator (Sartorius GmbH, Gottengen, W. Germany). A membrane barrier of cellophane with barrier area of 40 cm^2 was used to separate the drug solutions from phosphate buffer solutions (pH 7·5). The buffer solution was prepared by mixing 20·5 g of Na_2HPO_4 $12H_2O$ and 2·8 g of KH_2PO_4 and then making up to 1 L with distilled water. After equilibration at 37°C, magnetic stirrers and the peristaltic pumps were started simultaneously and samples of the buffer solution were collected at 0, 5, 10, 15, 20, 30, 60 and 90 min. Chloroquine was assayed spectrophotometrically (Beckman Acta CIII, Beckman Instruments Inc, Fullerton, USA) at 343 nm. The amount of chloroquine released was plotted against time, and the release rates were calculated from the initial slopes by linear regression.

In-vivo pharmacokinetics study. Eight healthy adult male albino rabbits weighing between 2·5 and 3·1 kg were placed in restraining boxes. The central vein of one ear was cannulated with a fine (24G) Teflon catheter and patency maintained with heparin saline. No sedation was given.

Formulations. The formulations used were a commercial preparation of chloroquine phosphate injection (Resochin: Bayer) 250 mg/5 mL (formulation A), and a viscous preparation of chloroquine diphosphate (250 mg/5 mL) in 2·0% w/v of MC 1500 (formulation B) prepared by the method as described earlier. The viscous preparation was filled into 5 mL amber vials and sterilised by autoclaving.

Procedure. A simple open cross over design was used; rabbits were randomly allocated to receive either formulation A then B, or B then A, with a wash out period of two weeks. The dose of chloroquine used was 10 mg base kg^{-1} in each case. The drugs were drawn up in a 1 mL syringe and administered by deep intramuscular injection to the lateral thigh. After clearance of the catheter dead space, blood (0·5 mL) was drawn at 0, 5, 10, 15, 20, 30, 40 and 60 min then at 2, 3, 4, 6, and 24 h. Whole blood taken into heparinized plastic tubes was stored at −70°C until analysis.

Chloroquine analysis. Chloroquine in whole blood was measured by high performance liquid chromatography with fluorescence detection using a previously described modification (Edwards et al 1988) of the method of Alvan et al (1982). All calibration curves were linear over the range 0–1000 ng mL^{-1} ($r > 0·995$)

Data analysis. Whole blood concentration time data were plotted graphically and the areas under curves (AUCs) were calculated by the trapezoid rule (Gibaldi 1984).

Statistical methods. Pharmacokinetic parameters were compared by the paired Wilcoxon's rank sum test.

Results

In-vitro absorption simulation. The chloroquine release rates, and the viscosity of the different methylcellulose solutions are presented in Table 1. The release rate of chloroquine fell as the viscosity of the solution increased. At a methylcellulose concentration of 2%, chloroquine was released 2·6 times slower than that released from the solution which contained no methylcellulose. There was a linear relationship between log release rate and log viscosity (Fig. 1) with a regression equation of:

$$\log J = -0·115 \log \eta - 2·362$$

where, J is the release rate of chloroquine in mg of diphosphate salt cm^{-2} min^{-1}, and η is the viscosity of chloroquine solution. The viscosity of the commercial chloroquine phosphate solutions for parenteral use (Resochin: Bayer Pharmaceuticals) was similar to that of water.

Table 1. Release rate of chloroquine phosphate from solutions of increasing viscosities.

Methylcellulose (% w/v)	Viscosity (mPa.s)	Release rate (mean ± s.d.) (mg cm^{-2} min^{-1}) × 10^3
0·0	0·679	4·90 (0·65)
0·5	20	2·78 (0·15)
1·0	112	2·42 (0·10)
1·5	668	2·11 (0·23)
2·0	2427	1·88 (0·08)

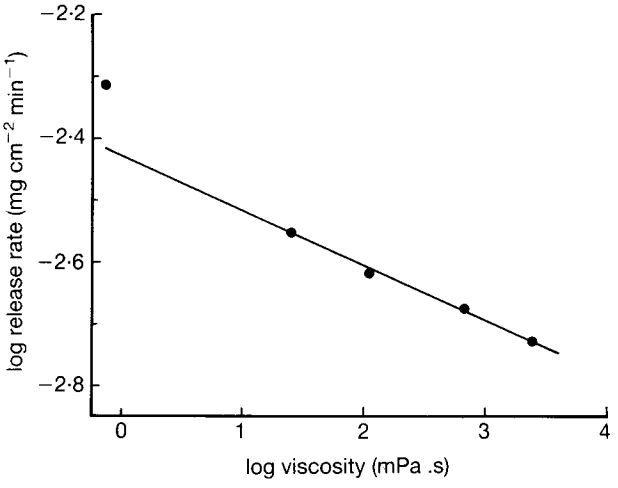

FIG. 1. Relationship between release rate of chloroquine phosphate (mg of salt) in an absorption simulator, and the viscosity of the methylcellulose 1500 solution.

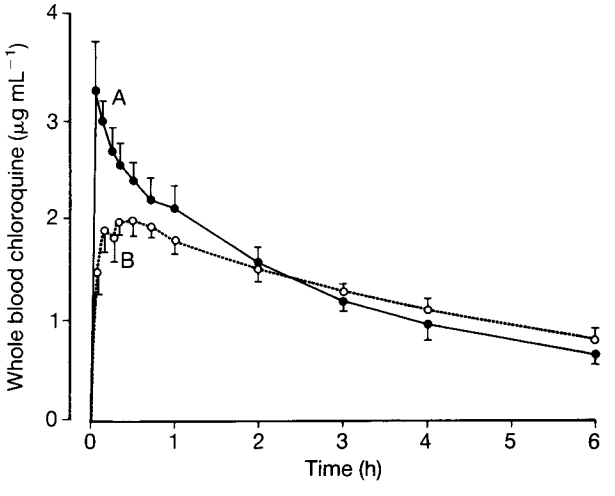

FIG. 2. Profile of whole blood chloroquine concentrations in 8 rabbits (mean ± s.e.) following intramuscular injection of chloroquine phosphate 10 mg base kg^{-1} in either aqueous solution (A) or in a 2% solution of methycellulose 1500 (B).

In-vivo absorption pharmacokinetics. The profiles of whole blood concentrations with the two formulations are shown in Fig. 2 Chloroquine absorption was significantly slower with the new

Table 2. Pharmacokinetic parameters with mean (s.d.).

	Commercial solution	Viscous formulation	Statistical significance
K_{abs} (min^{-1})	>0·5	0·154 (0·085)	<0·001
Peak blood concn ($\mu g\ mL^{-1}$)	3·41 (0·61)	2·15 (0·38)	0·004
AUC_{0-24} ($\mu g\ mL\ min^{-1}$)	1021 (305)	975 (461)	0·6 (NS)

Pharmacokinetic parameters derived from the whole blood concentration time profile following intramuscular administration of chloroquine (10 mg base kg^{-1}) in two different formulations to eight rabbits. K_{abs}: First order absorption rate constant. AUC_{0-24} = area under the whole blood concentration time curve in the first 24 h.

formulation (Table 2). The median time to peak concentration with the commercial aqueous solution was 10 min (range 5–20) compared with 30 min (range 10–60) with the new formulation ($P<0·05$). The absorption half-times were all less than two minutes with the commercial solution, compared with a median value of 6 min (range 2–8) with the new formulation. Peak concentrations were two thirds of those with the commercial solution (66%; 95% confidence interval 50–82%). Acute bioavailability estimated from the AUC_{0-24} values was very similar with the two formulations (Table 2). There was no obvious local or systemic toxicity.

Discussion

This simple new formulation of chloroquine phosphate was less rapidly absorbed than the commercial aqueous formulation following intramuscular injection to rabbits. Methylcellulose and carboxymethylcellulose are safe, parenterally acceptable hydrocolloids which can be sterilized by autoclaving (Macek 1963, Groves 1973). They were used originally as dispersion stabilizers for surfactant polysorbate 80 in parenteral formulations of the hydrophobic water insoluble cortisone acetate. Suspension of chloroquine in 2% methylcellulose 1500 was sufficient to reduce peak blood concentrations by one third, but did not alter acute bioavailability. The reduction in rate of chloroquine absorption (>three fold) by methylcellulose was close to that predicted by the in-vitro simulation studies in which release-rates were reduced by a factor of 2·6. The relationship between release-rate and viscosity was log-linear which suggests that release rates can be predicted over the range of viscosities studied, and therefore parenteral formulations can be prepared according to required absorption characteristics.

Intramuscular chloroquine is considered dangerous because it may produce a potentially lethal fall in systemic arterial blood pressure. Hypotension following intramuscular chloroquine administration in man is related both to the actual blood concentration, and also to the rate at which it is achieved (Looareesuwan et al 1986; White et al 1987, 1988). Provided that blood concentrations change relatively slowly, adaptive circulatory responses are able to compensate and blood pressure does not fall. Consequently the size of the central distribution volume, and the rates of chloroquine transfer into and out from this compartment are the principal determinants of cardiovascular toxicity. If absorption can be slowed then larger doses can be given safely as there will be sufficient time for distribution from the central compartment and blood concentrations will not rise to dangerous levels (as after slow intravenous or oral administration). Doses could therefore be given less frequently. This is a major practical consideration in the rural tropics.

The pharmacokinetic properties and toxicity of this simple new formulation should now be evaluated in man. In particular it will be important to determine whether absorption is adequate in severely ill children. If the profile of blood concentrations in man is similar to that observed in the rabbit, and there is no unpredictable local or systemic toxicity, then this will represent a significant improvement in the safety and practicability of parenteral chloroquine treatment.

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