Modelling the chloroquine chemotherapy of falciparum malaria: the value of spacing a split dose

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SUMMARY

We have attempted to provide a rational basis for improving the protocols for chemotherapy of malaria. We model the regression of parasitaemia by *Plasmodium falciparum*, its subsequent elimination from the body, or recrudescence, for populations of cells treated with chloroquine. Our model assumes that the drug forms a complex with some receptor in the parasite and that parasites possessing this complex die at a defined rate. We take into account that chloroquine is eliminated exponentially from the body. We show how the parameters of the model can be derived from observations in the field. The model correctly predicts the effects of drug dose, degree of initial parasitaemia, rate of parasite multiplication and degree of drug resistance to chloroquine chemotherapy. The level of parasitaemia will reduce to a minimum at sufficiently high concentrations of chloroquine, but only if the parasitaemia is reduced to below that of 1 parasite per infected person will a cure of malaria be obtained. Otherwise, recrudescence will, sooner or later, occur. We show that, even for drug-resistant malaria, if 2 doses of chloroquine are given to a patient with an interval of some 10 days between them, parasites can be eliminated from the body without toxic levels of chloroquine being reached.

Key words: malaria, *Plasmodium falciparum*, chloroquine, chemotherapy, drug resistance.

INTRODUCTION

Malaria still constitutes one of the most important infectious diseases, affecting 300–500 million people and causing the death of 1·5–2·5 million people annually, many of whom are young children (WHO, 1997). The total population at risk represents about 40% of the world's inhabitants. A major reason for this devastating situation is the emergence of drug resistance to classical and affordable anti-malarial drugs. In most areas where chloroquine resistance has emerged, this drug has been substituted by others which are more expensive and do not always provide long-term efficacy because of a rapid evolution of resistance (Foster, 1994; Watkins, 1995). Some attention has been devoted to possible improvement of drug action through rational regimens and improved treatment protocols, but these were restricted to relatively short durations (Karbwang & Harinasuta, 1992; Pussard & Verdier, 1994; Winstanley *et al*. 1994).

There is a large body of empirical clinical data on dosage and scheduling for various anti-malarial drugs. Pharmacokinetic and pharmacodynamic data (albeit, rather imprecise; White & Krishna, 1989) are available for most drugs and some drug combinations, and the stage-dependence of drug action is known for most drugs (White & Krishna, 1989; White, 1995, 1997; Krishna & White, 1996; Meshnick, Taylor & Kamchonwongpaisan, 1996). In the present work, we have combined the available data on pharmacokinetics and pharmacodynamics of the chemotherapy of *Plasmodium falciparum* by chloroquine, to form a mathematical model that describes the time-course of parasite killing as a function of drug dose, scheduling of treatment, and drug resistance. The model is designed to use the minimal number of parameters, and to yield information that will be useful to the clinician. Simulations of drug treatment suggest alterations in currently used treatment schedules so as significantly to improve the efficacy of chloroquine, even in cases of drug resistance. This approach may possibly allow reinstating this drug in the pharmacopoeia, in areas where its use has been discontinued. The model can be extended to other drugs and possibly also to drug combinations.

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MATHEMATICAL MODEL FOR CHEMOTHERAPY OF MALARIA BY CHLOROQUINE

We shall develop a model which will describe the progression of malaria in the human body in the presence of added chloroquine (CQ). We assume that therapy of malaria is effected by reversible binding of the drug CQ to some site in the parasite P to form a complex B, with dissociation constant K. In the presence of the complex the parasite dies with

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rate constant k_1 (to produce a form that we denote C):

$$\mathrm{CQ} + P \overset{K}{\leftrightarrow} B \overset{k_1}{\rightarrow} C. \quad (1)$$

We have omitted any consideration of the known stage-dependence of chloroquine's action, since in most of our calculations the times used are far longer than the parasite generation time. In addition, most *P. falciparum* infections are asynchronous (White & Krishna, 1989), so that at any time a substantial fraction of parasites will be at a sensitive stage.

We assume that the parasites (whether bound to CQ or free) multiply with rate constant a. For an initial parasitaemia of TP_0, the population at time t ($TP_t = P + B$) will be (see Appendix):

$$TP_t = TP_0 \times \exp\left(\left(a - \frac{k_1}{1 + (1/K \times \mathrm{CQ})}\right)t\right). \quad (2)$$

This expression is valid for the *in vitro* culture of parasites in the presence of drug. The growth or decay of parasite number is thus exponential in time (this is true only to a certain limit until growth-limiting factors come into play). *In vivo*, however, we have a more complex behaviour, for the concentration of the drug itself drops exponentially in time with a measurable pharmacokinetic half-time $t_{1/2}$. (We prefer to use the exponential form of the time-constant $t_e = t_{1/2}/0{\cdot}693$.) Thus the drug concentration at time t is $d_t = d_0\, \mathrm{e}^{-t/t_e}$, where d_0 ($= C_{\max}$) is the initial dose concentration. The resulting total parasitaemia is then (see Appendix):

$$TP_t = TP_0 \left(\frac{1 + \mathrm{e}^{-t/t_e} \times dok}{1 + dok}\right)^{k_1 t_e} \times \mathrm{e}^{at}. \quad (3)$$

We write the initial drug concentration in the normalized form; that is, we multiply the concentration of drug by the dissociation constant K, defining $dok = d_0 K$. Equation 3 expresses the parasitaemia at any given time t, as a function of the growth rate constant of the parasites a, the killing rate k_1, the drug elimination time t_e and the initial drug dose *dok*. To apply this expression to real data we have to find the values of these parameters for a given individual and a specific drug.

First we estimate the value of the growth rate constant a, the degree of the multiplication of the parasite population per day, in the absence of medication. Kwiatkowski & Nowak (1991) analysed the development of parasitaemia in naive infected subjects and found that a single parasite produced 20 viable daughter-parasites in a cell cycle of 2 days. This gives a growth rate of $\sqrt{20} = 4{\cdot}47$/day. The growth rate constant a, in exponential terms, is thus approximately $\ln 4{\cdot}5 = 1{\cdot}5$.

Next, consider the killing rate k_1. With a high dose we find that the kill rate is 99 %/cycle or 90 %/day (White, 1997). For high concentrations (i.e. putting $dok \to \infty$ in equation 3) $TP_t = TP_0\, \mathrm{e}^{(a-k_1)t}$. Thus after 1 day $TP_1/TP_0 = 0{\cdot}1 = \mathrm{e}^{(a-k_1)t}$. Thus $\ln 0{\cdot}1 = a - k_1$ and $-2{\cdot}3 = 1{\cdot}5 - k_1$ or $k_1 = 3{\cdot}8$.

Consider now the estimation of the drug elimination time t_e. This major pharmacokinetic datum is available from numerous studies (Krishna & White, 1996). Drug elimination is often described as a polyexponential process (see Pussard & Verdier (1994) and Krishna & White (1996) for reviews). It is convenient to dissect the process into 3 phases, although this may not be fully justified. During the rapid initial fall in CQ concentration often noted, there is no time for much parasite killing to occur. The relevant phase of CQ elimination is the second, longer-enduring phase, which is still in the range for significant killing to be occurring. During the third phase, the CQ concentration would be below the minimum required to kill the parasites, hence, is insignificant for our purposes. The characteristic time t_e during a phase is the time in which the concentration drops to 1/e of its value during 1 day, and it is the value required in equations 2 and 3. We took a value of 12 days, based on $t_{1/2} = 8$ days, within the range reported by Krishna & White (1996).

To calculate *dok*, we need to find the value of the dissociation constant K. This may be found from the following consideration. When the drug concentration is equal to the minimal inhibitory concentration (MIC), the parasitaemia is neither increasing nor decreasing, so that from the Appendix (equation A9) $\mathrm{MIC} = \mathrm{a}/((\mathrm{k}_1 - \mathrm{a})\mathrm{K})$.

With the calculated values of a and k_1, we find $K^{-1} = 1{\cdot}5 \times \mathrm{MIC}$. Using the estimate of MIC as 44 μg/l (Krishna & White, 1996), K^{-1} would be 68 μg/l. Thus the normalized CQ concentration would be the concentration in μg/l divided by 66. In the simulation presented in the Results section, CQ concentrations are recorded as multiples of MIC = 0·65 *dok*. Krishna & White (1996) reported that for a typical CQ dose of 600 mg total dose, $C_{\max}$ is 300–1500 μg/l, i.e. *dok* is between 5 and 25. Accordingly, for the simulations we chose *dok* values in the range of 0·65 ($1 \times$ MIC) and 8 ($12 \times$ MIC).

Results of the simulations are displayed either as percentage parasitaemia as a function of time, or as plasma drug concentration in multiples of MIC as a function of time. Where relevant, we have indicated, with a dotted line, the level of parasitaemia which is just detectable (50 parasites/1 μl blood) or a parasitaemia of 1×10^{-3} %. We have also indicated, with a solid line, the level of complete cure, which is less than 1 parasite/body, or 10^{-11} %.

RESULTS

Treatment with single doses of chloroquine

Figure 1 depicts the time dependence of dis-

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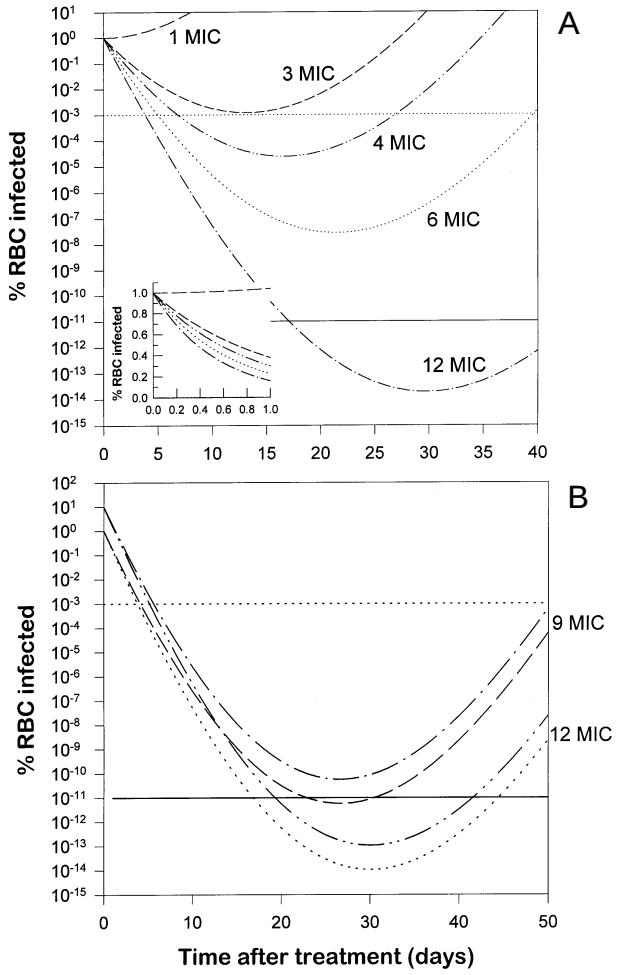

Fig. 1. Simulation of time-course of parasitaemia progression at different single doses of CQ. (A) Parasitaemia as a function of time at CQ doses of 1, 3, 4, 6 and 12 times MIC. Ordinate, percentage parasitaemia; abscissa, time in days post-treatment. Inset: progression of parasitaemia during the first day. (B) Time-course of parasitaemia decrease at 2 different levels of initial parasitaemia and 2 different drug concentrations.

appearance of parasites from the infected patient following treatment with 5 initial doses of CQ. The uppermost curve is for an initial dose equal to 1 × MIC (equal to 44 μg/l in a typical case). Here the parasite number in the host is maintained for almost 24 h (see inset to Fig. 1A) before increasing exponentially to 10-fold in 10 days. This is equivalent to RIII resistance (WHO, 1973). The next lower curve for a dose of 3 × MIC shows a measurable drop in parasite count preceding a subsequent rise (RII resistance). In the curve 4 × MIC we see a reduction in the number of parasites to about half in 12 h (inset to Fig. 1A); the number falls to below the detection limit at 10 days, but 2 weeks later infection recrudesces exponentially. This behaviour is consistent with that of a patient who is apparently cleared of parasites, released from the clinic, only to appear 2 weeks later with a recrudescence of malaria (delayed RI resistance). At 6 × MIC, the number of parasite drops but not below an effective zero, recrudescence takes place and it is obvious by 45 days. This patient, with recrudescence after 45 days, would be generally considered to have been re-infected. But the model suggests that he has all the time been harbouring occult parasites. We label this as RI, very delayed resistance. On the other hand, at a dose of 12 × MIC (equal to 528 μg/l), the number of parasites falls below an effective zero i.e. to less than a single parasite in the body. Thus the general behaviour of the model describes well the different clinical observations (Kilimali & Mkufya, 1985; Khoromana *et al*. 1986; Hellgren *et al*. 1989).

These same simulations allow us also to understand the course of the disease in a patient presenting with a resistant malaria. In this case, the dose which was previously sufficient to give the MIC is now insufficient. Assume that the resistance is 3-fold, that is, the MIC is now 3 times higher or 132 μg/l. Thus the previous highest dose of 528 μg/l is now only 4 times the effective MIC which would give the behaviour of that of the curve labelled 4 × MIC in Fig. 1A, namely a rapid recrudescence of active disease, defined as RII response. A dose of 18 times MIC of the sensitive strain would still give a very delayed RI response, and one would have to give 36 times MIC of the sensitive strain for radical cure, a dose that will possibly be toxic to the patient.

Fig. 1B compares the time-course of disappearance of parasites from the blood of 2 patients, one of whom has a parasitaemia of 10%, the other of 1%. A plasma concentration of 9 × MIC will cure a patient presenting with 1% parasitaemia (reducing its parasitaemia to less than 1 parasite per body) but would not cure the patient presenting with 10% parasitaemia who would need a plasma level of 12 × MIC for radical cure. The inverse correlation between cure rate and efficacy with initial parasitaemia, has been observed in the clinic (Filippov & Galzunova, 1989; Gernaat, Verhagen & Woods, 1990; Lokman-Hakim *et al*. 1996).

Effects of parameter variation on model prediction

We thought it advisable to study how the predictions of the model depended on the values of the chosen parameters. The choice would reflect natural variations of these parameters in the parasite and in the human population. We consider first the parameter *a*, the rate constant of parasite growth in the absence of drug. We originally took this value from Kwiatkowski & Nowak (1991) for naive adults. A smaller value of *a* would be expected for semi-immune populations, for example for people living in areas where transmission is seasonal (White, 1992). Fig. 2A illustrates the time-course of the drop in parasitaemia for a patient receiving exactly the same doses as were depicted in Fig. 1A, but now for

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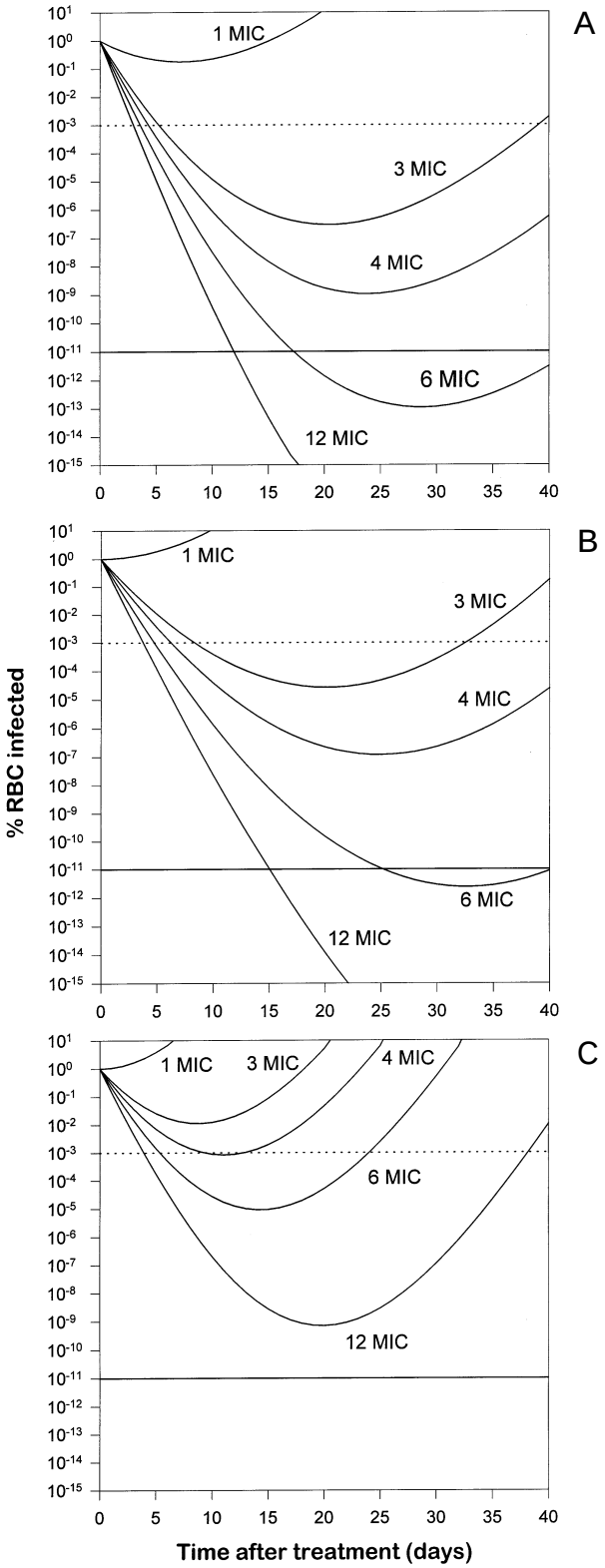

Fig. 2. Effect of variation in rate of parasite growth and drug elimination time on the progression of parasitaemia decrease. (A) Effect of a reduced growth rate $a = 1$ (7·5 parasites produced per growth cycle). (B) Effect of drug elimination time of 18 days and (C) of 8 days with a growth rate of 20 parasites/growth cycle.

$a = 1$ (7·5 parasites produced/growth cycle rather than $a = 1{\cdot}5$ or 20 parasites/growth cycle). For such a patient a dose of $6 \times$ MIC would now be curative,

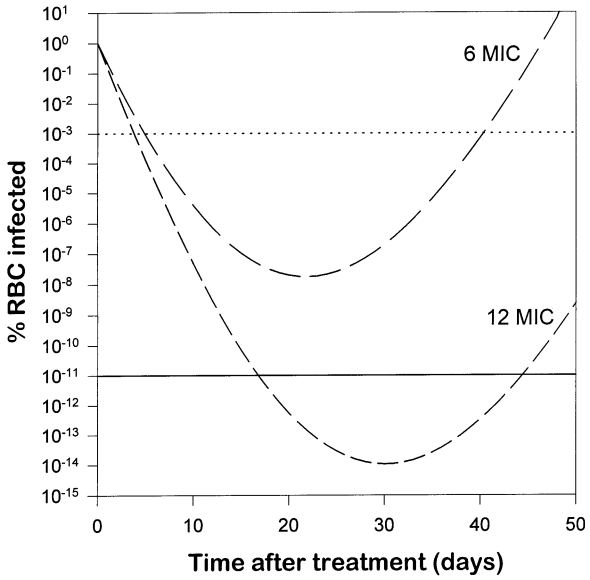

Fig. 3. Parasitaemia repression at 2 different doses of CQ.

although such a dose led to recrudescence with a resistant RI response in the non-immune patient (Mvondo, James & Campbell, 1992).

We also tested the effect of variation in the parameter t_e, the drug elimination time, i.e. the time taken for the concentration of drug to drop to $1/e$ of the C_{max} value. This parameter varies in different studies and has been shown to be subject to individual variation within the population (up to 6-fold between minimal and maximal values (Krishna & White, 1996). In Fig. 2B and C we show the simulation for values of t_e of 18 and 8 days as compared to the value of 12 days in Fig. 1A. As could have been expected, the slow elimination results in a more efficient cure: even $6 \times$ MIC is sufficient to give a full cure, a dose that was not sufficient for the simulation of Fig. 1A. In contrast, we see that for the rapid elimination time (Fig. 2C), even the highest curative dose used previously now led to recrudescence.

Treatment with double or fractionated doses of chloroquine

We have just shown that to effect a cure of a resistant malaria, or of a patient presenting with a high level of parasitaemia, high, possibly toxic doses of chloroquine are required. In this section we shall compare the effectiveness of smaller doses given at different times. Fig. 3 shows the time-course of parasitaemia in a patient given a dose of $6 \times$ MIC or this dose doubled at $12 \times$ MIC. Fig. 4B presents the simulated levels of chloroquine in the plasma of these patients given $4 \times$ MIC and $8 \times$ MIC. Six times MIC will give a very delayed RI response and $12 \times$ MIC will radically cure the disease but with corresponding doubling of plasma drug level. Fig. 4 shows what

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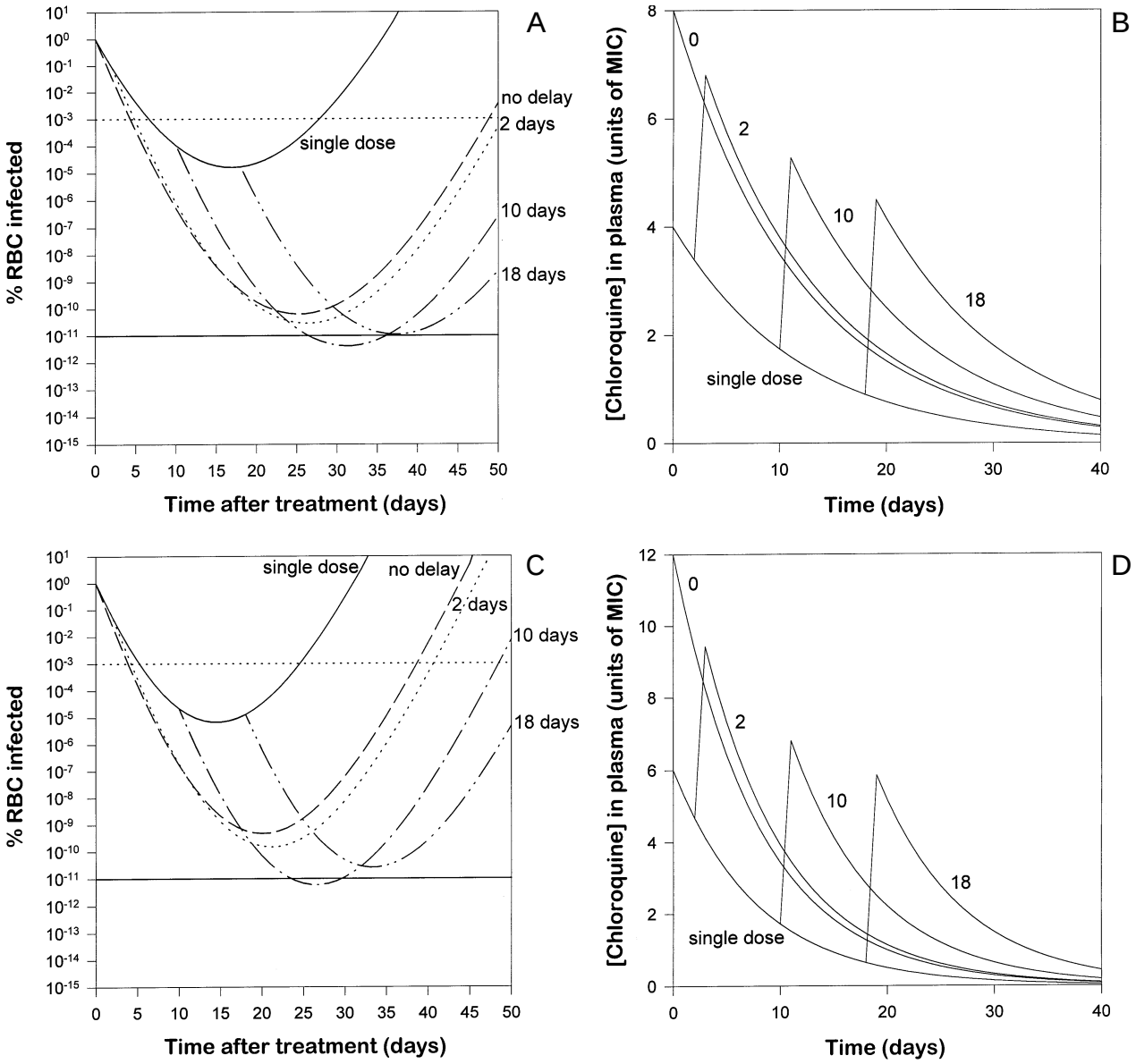

Fig. 4. Effect of fractionating the dose of CQ on repression of parasitaemia and pharmacokinetics at 2 different drug elimination times. (A) Drug elimination time 12 days. Two doses of 4 × MIC each are given at 4 different time-intervals. (B) Pharmacokinetics corresponding to (A). (C) Drug elimination time 8 days. Two doses of 6 × MIC each are given at 4 different time-intervals. (D) Pharmacokinetics corresponding to (C).

happens when instead of giving a double dose, the additional dose is given at various times after the first dose. The corresponding levels of chloroquine in the blood are depicted in the simulation of Fig. 4B. In Fig. 4A we see the surprising result that one can totally clear the parasites from the patient if the second dose is given with a delay of 10 days, but not if the delay is either 2 days or 18 days. The fractionated dose clearly gives an effective cure, and the pharmacokinetics suggest that this cure may be achieved without undesirable high maximal chloroquine levels, with their possible toxic side-effects.

When the same approach is used under conditions where the drug elimination time is shorter (Fig. 4C and D where $t_e = 8$ days), the advantage of fractionated dose is even more striking. For such a rapid elimination time, neither an initial dose of 6 × MIC nor of 12 × MIC gives a cure. But if 6 × MIC is given on admission and a second dose of 6 × MIC is given 10 days later, total cure is achieved, with a minimal increase in the maximal plasma drug level.

We have also modelled an example of polyclonal infection where we take the case of 2 strains of parasites, one of which is resistant and the other is not, and are present in the patient at very different initial abundances. Fig. 5 depicts such a case where the two strains differ 2-fold in resistance, with the resistant strain being initially present at an abundance only 1 % of that of the sensitive strain. As the figure shows, treatment of this population with a CQ dose of 12 × MIC (referred to the sensitive strain) clears the population of the sensitive strain but

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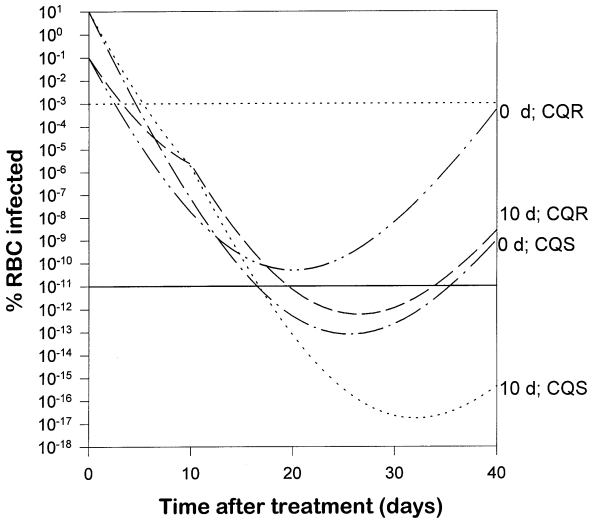

Fig. 5. Effect of drug dosage and timing on repression of parasitaemia in a polyclonal infection. A CQ-sensitive (CQS) clone and a CQ-resistant (2-fold; CQR) clone were present at 100:1 ratio at the beginning of treatment. Two doses of 12 × MIC were given at different time-intervals in days (*d*). Drug elimination time: 8 days. The differential effect of the 2 regimens on the sensitive and the resistant clones are depicted.

allows the resistant strain to recrudesce so that it takes over the whole population, as has been observed in the clinic (Duraisingh *et al*. 1997; Al-Yaman *et al*. 1997). In contrast, if this dose is divided into 2 equal doses of 6 × MIC, the second dose given 10 days after the first, this protocol will successfully eliminate both strains.

DISCUSSION

The model presented here for the chemotherapy of malaria by chloroquine is simple, miminalistic, and uses only parameters derived from observations on human patients. Yet it appears to successfully simulate a variety of clinical observations. First, the half-time of parasite elimination (8–12 h; Inset Fig. 1A) accords well with data from the field (see for example Brasseur *et al*. (1995) for a recent report). Second, the model predicts that at doses lower than those needed for full cure, recrudescence will occur. Third, the model allows for the phenomenon of resistance in that when the MIC of the drug increases (as in resistance), higher doses of chloroquine are required to give a curative response. At lower doses, the RI, RII and RIII responses found in the field, are predicted. Fourth, the model predicts a very steep dependence of cure success on the dose of chloroquine administered. Thus in Fig. 3, doubling of the dose of CQ administered is sufficient to give 7 orders of magnitude reduction in parasitaemia, sufficient to make the difference between cure and failure. This is consistent with the fact that doubling of the dose of CQ to a patient with resistant parasites can be sufficient for radical cure (Karbwang & Harinasuta, 1992; Pussard & Verdier, 1994). Fifth, our simulation of the behaviour of a population of lower growth rate (Fig. 2A compared with Fig. 1A), such as might be expected for a semi-immune population (Verdrager, 1986, 1995; White, 1992), is consistent with the successful relief of parasitaemia in such patients with lower CQ doses. This is also in line with the practice of self-treatment in fully immune populations. Sixth, the simulation of fractionated dosing is consistent with the clinical finding that giving a fractionated dose within 24–48 h is of no clear benefit in reducing parasitaemia. However, the second and surprising prediction of Fig. 4, that 1–2 weeks delay between the first and second doses of CQ will give an improved response has not, as far as we know, been documented, although suggested (Filippov & Glazunova, 1989). Finally, the model successfully simulates the situation of a mixed infection of sensitive and resistant strains (Fig. 5) and shows that the resistant strain becomes dominant at dosages of CQ insufficient to destroy it. The simulation does predict, however, that a well-timed second dose should provide an effective treatment even of a mixed infection.

A further insight into parasite chemotherapy that arises from these simulations, is that a small increase in the administered dose of CQ, in an appropriate range, can result in large (orders of magnitude) decrease in parasitaemia. Thus in Fig. 3 at day 10 after treatment, doubling of the dose of CQ from 6 × MIC to 12 × MIC, resulted in a 100-fold decrease in parasitaemia, and this ratio increased considerably with time after treatment. This behaviour is far from the naive perception of dose–response in which a doubling of dose results at most in the doubling of the response. The phenomenon arises, of course, from the fact that we are here dealing with an exponential rate of growth and the cumulative effect of killing.

Our most significant finding is that it would appear that there is a great advantage in giving a patient a well-spaced fractional dose. In the field, one already gives an initial dose which is as high as permissible and yet avoids toxicity. Doubling of such a dose would be unthinkable. There have already been attempts to give a fractionated double dose dispersed over up to 48 h. Our simulation shows that this is not nearly as effective as giving the second dose in the region of 10 days after the first dose. With such a delayed second dose, one gets effective clearance of the parasites without subjecting the patient to excessively high, potentially toxic, levels of drug. This counter-intuitive approach (White, 1992) has not, as far as we are aware, yet been applied in the field, but its use is demanded by our simulations.

An important implication of our simulations suggests that chloroquine might be able to be

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reinstated in the pharmacopoeia of anti-malarial drugs, at the previously used lower doses, holding in reserve higher doses of CQ if resistance to this drug continues to evolve. Obviously, the application of the proposed 2 doses protocol requires the patient's compliance. Without it, recrudescence is bound to occur and further selection of resistant parasite strains under drug selection is predicted.

The predictive value of this model, and its promising potential for clinical application, call for its further examination with specifically collected clinical data. What one needs to test the model further are closely spaced measurements of parasitaemia and of plasma drug concentration in the same patient over several weeks, both during the phase of clearance of parasites from the bloodstream and during the subsequent recrudescence (if present), prior to further medication. Most beneficial in these experiments would be the identification of the various parasite strains by specific PCR probes to establish the population dynamics during therapy. There are certain data which it is essential to determine. These are: initial parasitaemia, the maximal drug concentration ($C_{\max}$), drug elimination half-time, the time taken for the parasitaemia to decrease to detection limits (and the value of this limit) and the drug concentration at this time. In the case of recrudescence, we need values for the time taken for parasitaemia to rise again to the same detection limit, and the drug concentration at this time.

We have in this paper simulated only the behaviour of the parasite in the presence of 1 or 2 doses of chloroquine. We intend to extend this approach to other anti-malarials, each with its known pharmacokinetics and pharmacodynamics, and then to extend it further to combination of such drugs, in the hope that we can improve existing protocols for combination therapy, or suggest new ones. We also intend to simulate slow drug release protocols.

We thank Drs N. J. White, S. Krishna and R. Price, for their helpful suggestions and comments.

APPENDIX

(1) *Derivation of the mathematical model*

Consider equation 1 of the text: $\mathrm{CQ}+P \overset{K}{\leftrightarrow} B \overset{k_1}{\rightarrow} C$. We assume that the reactions by which B is formed and breaks down are much faster than the reaction in which it transforms to C, so that we can treat the B state as metastable. We denote the total parasitaemia as TP in

$$TP = P + B. \tag{A 1}$$

From the equilibrium in equation (A 1):

$$K = \frac{B}{\mathrm{CQ} \times P}. \tag{A 2}$$

By substituting P from (A2) into (A1)

$$TP = B\left(1 + \frac{1}{K \times \mathrm{CQ}}\right). \tag{A 3}$$

This is a transformation of the Michaelis–Menten equation. It is clear from here that, if not for the breakdown of B to C, we would receive half dissociation at a concentration of $\mathrm{CQ} = 1/K$. This would be known as the IC_{50}. We assume, with White (1997), that parasite killing is a first-order process, i.e. that the complex B decays into stage C at rate k_1, that is, there are $k_1 B$ parasites dying per day. On the other hand, the living parasites are multiplying at a rate a, or $a \times TP$ new parasites per day. (It is unimportant in principle whether only parasites in the form P multiply, or those in the form B as well. This only changes the numerical values of k_1.) We denote the time-dependent value of TP as TP_t. We can write the differential equation:

$$\frac{dTP_t}{dt} = aTP_t - k_1 B = aTP_t - k_1 \frac{TP_t}{1 + (1/K \times \mathrm{CQ})}$$
$$= TP_t\left(a - \frac{k_1}{1 + (1/K \times \mathrm{CQ})}\right). \tag{A 4}$$

For the standard *in vitro* conditions, with CQ constant, this is simple to integrate and we receive (cf. (2) above):

$$TP_t = TP_0 \times \exp\left(\left(a - \frac{k_1}{1 + (1/K \times \mathrm{CQ})}\right)t\right).$$

It is easy to obtain from here the value of the IC_{50}.

For the clinically more interesting case of a drug, with maximal concentration d_0, being eliminated from the body with rate $1/t_e$, or $\mathrm{CQ} = d_0 \times \mathrm{e}^{-t/t_e}$, we have a somewhat more complex equation. The integration may be done manually, or by symbolic machine integration (using Maple V2) to yield (cf (3) above):

$$TP_t = TP_0\left(\frac{1 + \mathrm{e}^{-t/t_e} \times dok}{1 + dok}\right)^{k_1 t_e} \times \mathrm{e}^{at}.$$

Here $dok = d_0 K$, this being the initial dose in units of the IC_{50}. To find the minimum of the concentration curve for given medication, we need to simply differentiate equation (3) by time t and equate to 0.

$$t_{\min} = t_e \ln\left[\left(\frac{k_1}{a} - 1\right) dok\right]. \tag{A 5}$$

The depth of the minimum is obtained on substituting in equation 3:

$$TP_{\min} = TP_0\left[\frac{1}{(1 - k_1/a)(1 + dok)}\right]^{k_1 t_e}$$
$$\times \left[(k_1/a - 1) \times dok\right] \mathrm{e}^{at_e}. \tag{A 6}$$

Thus we can check whether a given dose is curative, which would be the case if the curve descends below the level of 1 parasite/body. From this we can decide the minimal dose required to bring about a cure.

As discussed in the text, there is another possibility for treatment, and that is by splitting the dose. Here the differential equation is identical, but the solution consists of 2 parts, for 2 different doses, the initial dose, and after t_δ

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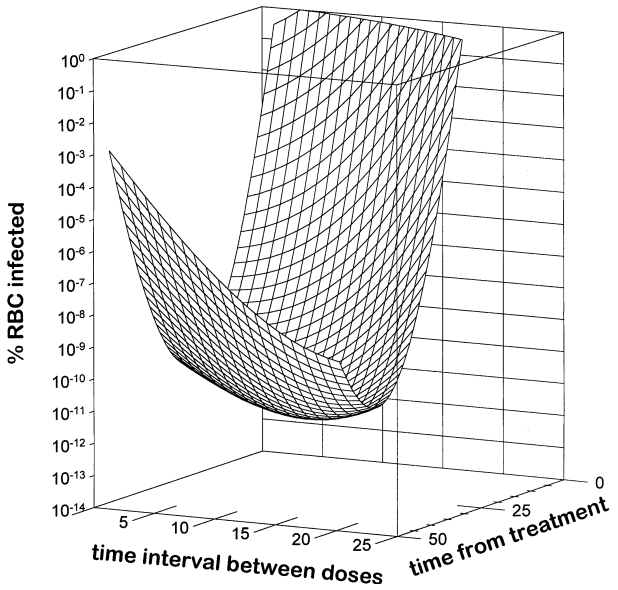

Fig. 6. Parasitaemia as a function of time at different time-intervals between 2 consecutive doses of CQ. x-axis is t_δ, the time-interval between doses; y-axis is the time from initial dose; z-axis is log(percentage parasitaemia).

(the time of the second dose) an additional dose. The first part has the same solution. The second part demands 2 changes: (i) The initial dose is the sum of the new dose and the present value of the plasma concentration $CQ = d_0(1+\exp(-t_\delta/t_e))$. (ii) The initial condition of the differential equation is the plasma concentration at time t_δ, namely TP_{t_δ}. The solution for the second section of the time-course is:

$$TP_t = TP_0\left(\frac{1+e^{-t_\delta/t_e}\times dok}{1+dok}\right)^{k_1 t_e} \times\left(\frac{1+e^{-(t-t_\delta)/t_e}\times dok(1+e^{-t_\delta/t_e})}{1+dok(1+e^{-t_\delta/t_e})}\right)^{k_1 t_e} e^{at} \quad \text{or}$$

$$TP_t = TP_0\left(\frac{(e^{t/t_e}+dok+e^{t_\delta/t_e}\times dok)\,(dok+e^{t_\delta/t_e})}{(1+dok)\,(e^{t_\delta/t_e}+dok(1+e^{t_\delta/t_e}))}\right)^{k_1 t_e} e^{(a-k_1)t}. \tag{A 7}$$

This equation gives us the time dependence of the parasitaemia. It is obviously dependent on t_δ. It is this value that is to the discretion of the clinician. We are interested in the minimal parasitaemia for all times for various values of t_δ. To find this minimum we find the point of zero slope in a 3-dimensional graph $(t, t_\delta, TP(t, t_\delta))$ (Fig. 6) or, in mathematical language, the point at which

$$\nabla TP = \left(\frac{\partial TP_t}{\partial t}, \frac{\partial TP_t}{\partial t_\delta}\right) = (0,0).$$

The analytical solution of this equation for t_δ is, by differentiation and elimination of t:

$$t_\delta = \ln \frac{\left(\frac{k_1}{a}-1\right)\times dok^2 - 2dok + dok\sqrt{\left[\left(\frac{k_1}{a}-1\right)^2\times dok^2 + 4\frac{k_1}{a}\right]}}{2(dok+1)}. \tag{A 8}$$

This gives the ideal time for administering the second half of the dose. The value of the actual depth of the minimum can be obtained readily, graphically, in a straight-forward method by looking in a 3-D graph for this value. Figure 7 is the silhouette of the 3-D graph. We can see clearly the envelope reflecting the t dependence, and the lowest point (along the t_δ axis) is the minimum. A table of the timing of the best t_δ can easily be prepared for clinical application.

(2) *Obtaining the relevant pharmacokinetic and pharmacodynamic parameters*

For the purpose of fitting to our model, we need to extract, from the literature, values for the parameters d_0 and t_e. In many cases, we find instead the values of the AUC (area under concentration-time curve). However, killing rate depends on the concentration in terms of the Michaelis–Menten equation (A 3). Hence, the number of parasites in the B state will not be influenced greatly if the dose is changed from, say, 10 to 20 times K^{-1} (although the AUC would be much larger). At high CQ concentrations, the number of parasites killed per day is almost independent of CQ. The importance of the size of the original dose is that it determines the length of the killing time. Thus we should be more interested in the time the concentration is above K^{-1}, rather than in the overall area under the curve. What is called for is an integration of the Michaelis–Menten equation with time. This will be treated separately elsewhere.

The second term we would like to comment on is the minimal inhibitory concentration (MIC) that is, the concentration at which the parasite population growth is checked. There is no standard method for obtaining MIC, but a common experimental procedure is to report the average between the concentration just before detection of recrudescence, and just after the detection. This, however, is not the MIC but merely the concentration at which the re-emerging parasitaemia can be detected (and depends on the sensitivity of detection methods). The true MIC is the concentration at which $dTP_t/dt = 0$ (momentarily there is no change in parasitaemia, until the concentration drops further). This is where (from equation (A 4)) $a = k_1/[1+(1/K\times CQ)]$. Thus $K\times CQ = a/(k_1-a)$ and so

$$\text{MIC} = \frac{a}{k_1-a}1/K. \tag{A 9}$$

From basic calculus, this is the minimum of the parasitaemia graph, not its intersection with the detection limit. A better estimate of MIC would be the average of the concentration at disappearance c_d and at detection of recrudescence c_r. Of course, for consistency purposes, we should measure both either just below detection, or just above. Since the drug concentration drops exponentially, the correct average would be a geometric average of these concentrations, so that

$$\text{MIC} = \sqrt{c_d\times c_r}. \tag{A 10}$$

As the parasitaemia curve in this region is not far from being symmetrical through $t_{\min}$, this estimate would be close to the true value. In the absence of a better estimate we have in this paper used the standard published estimates.

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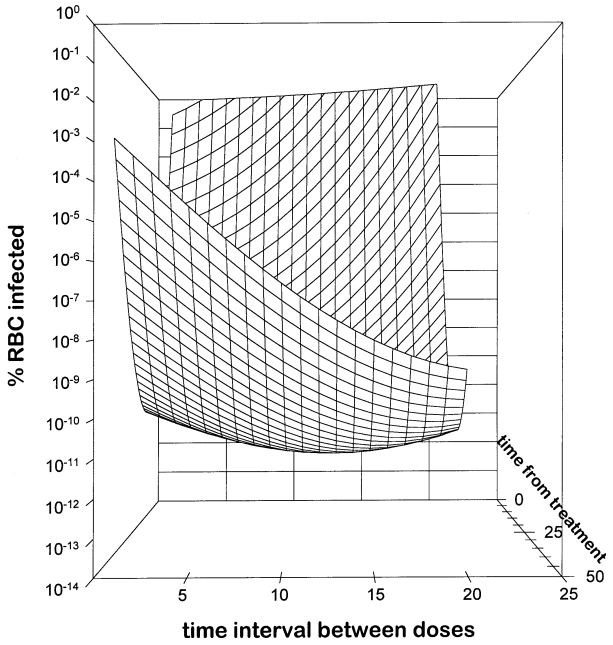

Fig. 7. Same as Fig. 6, showing silhouette of the time dependence.

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