Pharmacokinetic-pharmacodynamic modelling of the anti-malarial activity of mefloquine

M. B. HOSHEN, W. D. STEIN *and* H. D. GINSBURG*

Department of Biological Chemistry, Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

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SUMMARY

Treatment protocols for the chemotherapy of malaria are usually acquired through clinical trials. Once pharmacokinetic and pharmacodynamic information becomes available, it is possible to use mathematical modelling for testing these protocols and, possibly, for improving them. In this report the case of monotherapy by mefloquine is analysed. Published pharmacokinetic and clinical results are used to derive the essential model parameters such as kill rate, parasite growth rates, drug sensitivity and the pharmacokinetic parameters. Good agreement is obtained between clinical results and simulated parasite numbers using the derived parameters. It is demonstrated that the 2 exponential kinetics of mefloquine elimination can be reduced to an operational single exponent for pharmacodynamic modelling by educated choice of sampling times of plasma drug concentration. It is deduced that a second drug dose, at a properly chosen time-interval, results in radical cure even when resistant parasites are present and at maximal parasite growth rates such as those found in non-immune patients. Finally, a table is provided for guiding the optimal choice of dosing intervals under different values of population pharmacokinetics, drug resistance and individual immunity parameters.

Key words: malaria, mefloquine, pharmacokinetics, pharmacodynamics, mathematical model.

INTRODUCTION

A main target of modern malaria research is to plan more effective chemotherapeutic regimens, which will enable radical cure, thus helping the individual patient and also preventing selection of drug-resistant strains (Wernsdorfer, 1994). Treatment failures in the past have resulted in the emergence of drug resistance to chloroquine (Wongsrichanalai *et al*. 1992), and to mefloquine and sulfadoxine/pyrimethamine (Price *et al*. 1997; Ronn *et al*. 1996).

Mefloquine (MQ) was first developed in the early 1970's and clinical trials began in Thailand in 1974 (Hall *et al*. 1977). The drug was employed there as Fansimef (a combination with pyrimethamine and sulfadoxine) from 1984. By 1990 resistance had developed, and doses had to be increased. From 1994 onwards, administration of the drug in this form was halted, and MQ was applied only in addition to qinghaosu derivatives (Price *et al*. 1999).

When a new drug is introduced, optimal chemotherapeutic protocols for achieving radical cure are developed semi-empirically, assessing the protocol in terms of its clinical success or failure. The clinician gives the maximal permitted dose, and may follow it up by additional doses when the drug concentration drops below curative levels. The number and spacing of doses are empirical, based on failure rates. A problem is that a patient with a subdetection level of parasitaemia (that is parasite burden) will be released as 'cured', although these parasites will eventually recrudesce (White, 1997). In many cases, the determination of the therapeutic range and the accurate measurement of the pharmacokinetics (PK) of the drug are performed *a posteriori* (Weidekamm *et al*. 1982). If such data are available, however, a more effective drug procedure could be developed if there was a mathematical model which would be able to predict total elimination of parasites i.e. radical cure. Development of such a model depends on precise knowledge of the drug's PK in the body and of its pharmacodynamics (PD) – the concentration dependence and stage dependence of the drug's effect on the parasite number. For slow-acting drugs, such as MQ, and unlike qinghaosu, the stage-dependence is probably less important because this dependence is smoothed by the long period during which it remains active in the body. In this paper a mathematical model is developed to account for the action of MQ. The model suggests a considerable improvement in the administration protocol for MQ, so that radical cure rates could be improved, even in cases of drug resistance, and thus reduce the spread of resistant parasites.

* Corresponding author: Tel: +972 2 6585539. Fax: +972 2 6585440. E-mail: hagai@vms.huji.ac.il

METHODS

Modern pharmacology suggests the use of mathematical methods for the evaluation of the efficacy of a proposed protocol, and for planning novel protocols. The methodology, known as pharmacokinetic-

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pharmacodynamic (PK–PD) modelling, consists of the following stages. First the pharmacokinetic profile of the drug (the concentration C at measurement site as function of time, (t), is determined by measurement. This profile is generally fitted to a single or double-exponential curve (single or 2 compartment model). Then the dependence of the drug's efficacy on its instantaneous concentration is estimated. Assuming first-order processes, the progress of parasitaemia may be written as:

$$dTP/dt = \boldsymbol{a}TP - kTP \quad (1)$$

where 'TP' here stands for the total parasitaemia, '$\boldsymbol{a}$' stands for the intrinsic growth rate (in absence of drug) and 'k' the drug-dependent kill rate, which is governed, it is assumed, by the conventional Michaelis–Menten equation

$$k = k(C) = k_1 * C/(C+K), \quad (2)$$

where k_1 is the maximal kill rate and K is the concentration at which half this maximal kill rate is obtained. The overall killing is thus dependent on the value of K, which could be deduced, in principle, from the dose-response curve of the drug. Integration of equation (1) assuming equation (2) and exponential PK, $C = C_{\max} e^{-mt}$ ($C_{\max}$ is the peak concentration and m is the elimination rate constant ($= \ln(2)/t_{\frac{1}{2}}$).

Writing $dok = C_{\max}/K$ results in

$$TP_t = TP_0 \left(\frac{1 + e^{-mt} \cdot dok}{1 + dok} \right)^{k_1/m} \cdot e^{at}. \quad (3)$$

Of the parameters in equation (3), one needs to find estimates of $\boldsymbol{a}$, m, and dok (which will be computed from an estimate of K). The concentration of zero growth, where $\boldsymbol{a} = k(C)$, is known as the minimal inhibitory concentration (MIC), and is given by $\mathrm{MC} = \boldsymbol{a}\, K/(k_1 - \boldsymbol{a})$. A problem is that $k(C)$ cannot be measured directly, *in vivo*. Thus 2 options are open: either the measurement of $k(C)$ *in vitro*, attempting to extrapolate to *in vivo* clinical data, or else the deduction of this function from clinical reports. The *in vitro* approach is difficult, since the effective concentration of MQ *in vivo* is unknown, it being, to a great extent, protein bound and having a huge apparent volume of distribution $V_d = dose/C$ in the body (Karbwang & Na-Bangchang, 1994). Considering the assumed non-linearity of the dose-response and of the protein-binding function, one concludes that only qualitative concepts may be obtained from the laboratory, and one must, therefore, deduce the values of the PD parameters from clinical data.

Clinical data are any set of pharmacokinetic and drug response results for single patients or for averages. Ideally, such results would be individually matched PK–PD results for individual patients, in whom both plasma drug concentration and parasitaemia are measured frequently. If these are not available, an understanding of the PK and PD for populations must suffice.

The actual benefit of modelling is not, however, in the simulating of existing protocols but in (1) establishing which PK–PD parameters are essential for the theoretical determination of clinical results and (2) the testing of novel, hitherto untested regimes.

For the statistical analysis, we used standard curve-fitting, as supplied in the SigmaPlot package analysis, which gives standard errors of the derived parameters. We used the unweighted fitting option of this package (equivalent to uniform weighting), due to the indeterminacy of the lower measurements. Alternative weighting methods did not make any significant difference to the results. Each parameter was accepted or rejected as significantly different from zero according to the Student's t-test at the $P < 0{\cdot}05$ level. The existence or non-existence of a second compartment was evaluated by use of the F-test at $P < 0{\cdot}05$.

RESULTS

Estimation of the PK parameters

The above methodology was applied to reports by Boudreau *et al.* (1990) on PK and cure rates for Thai patients who received either 750 or 1500 mg MQ as oral doses. (Although it would, in principle, be better to fit data from individual patients, the published information is on means and these must perforce be used.) Of the former, 10/16, and of the latter, 9/11, were cured. Resistance, as reflected by recrudescence and by high ID_{50} (50% growth inhibition) *in vitro* values, was detected in the two 1500 mg failure cases and in 2 of the 750 mg failure cases. The initial parasitaemia and regular measurements of average drug concentration were reported for all 4 groups. The data for the 4 groups are presented in Fig. 1. A best fit for a 2 compartment PK model for the post-absorption phases is presented for each group as a solid line in this figure, and the fit for a single compartment model as a dotted line. The derived PK parameters, based on the least square fits of the average concentration values to a 2-compartment model, are recorded in Table 1.

Estimation of the PD parameters

As the parameter k_1 is the maximal kill rate, its value may be estimated by measuring the kill rate when high, presumably saturating, concentrations of MQ are detected. Thus the value of k_1 may be obtained readily from *in vivo* human clinical trials. The elimination rate of parasites by MQ is extremely slow, compared to chloroquine and state-of-the art drugs such as artesunate, halofantrine, etc. Typical estimates are a maximal reduction of 100-fold per

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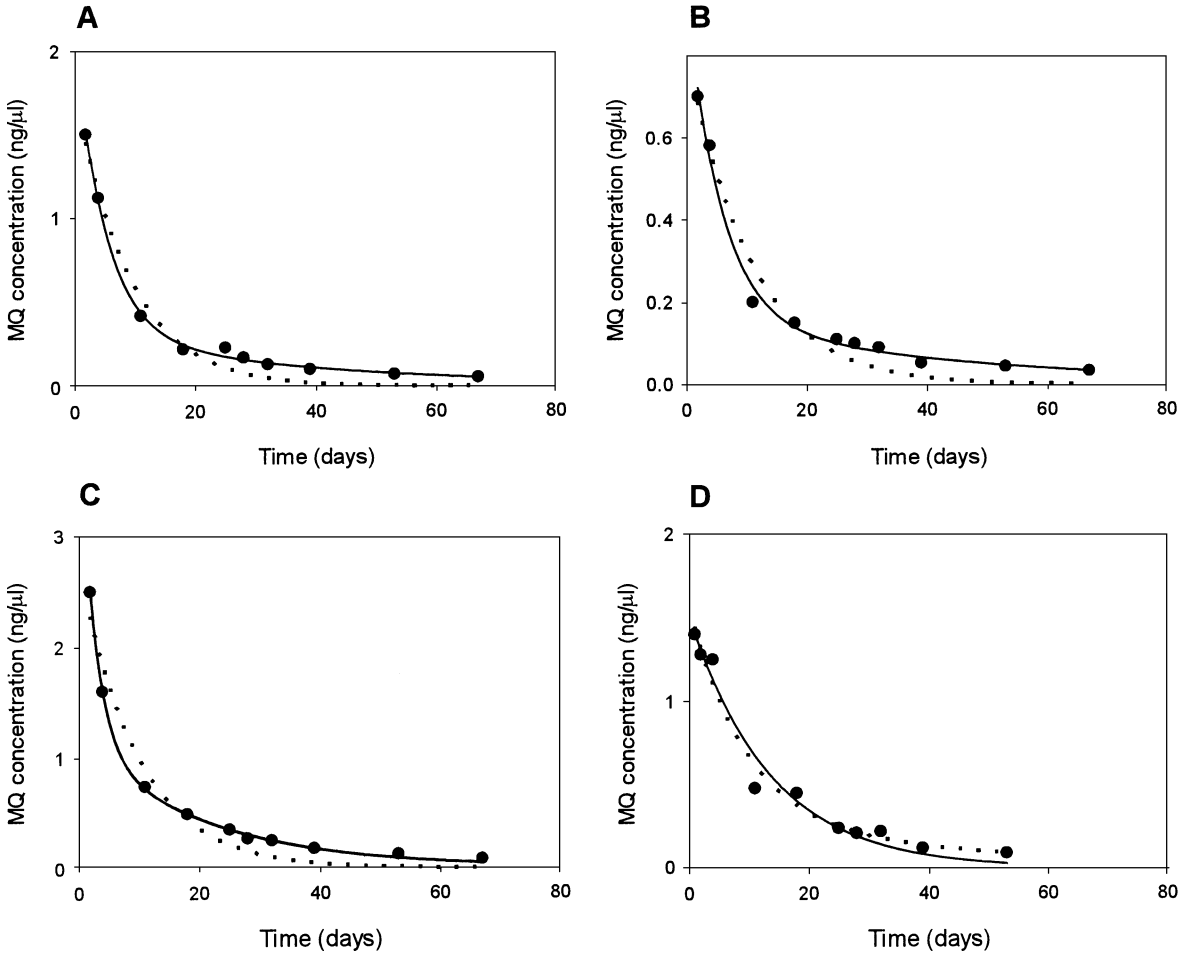

Fig. 1. Clinical plasma mefloquine profile as a function of time for *Plasmodium falciparum* patients as reported by Boudreau *et al*. (1990). (●) Clinical averages for groups. Solid line 2-compartment model, best unweighted fit for the points, by SigmaPlot regression option. Dotted line best unweighted fit for single-compartment model. (A) 10 patients (group A) cured by 750 mg MQ. (B) 6 patients (group B) who showed recrudescence after 750 mg MQ. (C) 9 patients (group C) cured by 1500 mg MQ. (D) 2 patients (group D) who showed recrudescence after 1500 mg MQ.

Table 1. Pharmacokinetic parameters (± standard error) for 2-compartment modelling of mefloquine single dose chemotherapy of falciparum malaria

$C(t) = Ae^{-k_a t} + Be^{-k_b t}$

Parameter/ Group	A 750 mg cured	B 750 mg failed	C 1500 mg cured	D 1500 mg failed*
Number of patients	10	6	9	2
A (μg/ml)	1·80 ± 0·10	0·80 ± 0·08	3·23 ± 0·16	1·32 ± 0·79
B (μg/ml)	0·33 ± 0·11	0·17 ± 0·09	1·15 ± 0·09	0·25 ± 0·85
k_a (1/day)	0·20 ± 0·03	0·18 ± 0·04	0·40 ± 0·03	0·10 ± 0·07
k_b (1/day)	0·03 ± 0·01	0·023 ± 0·015	0·047 ± 0·003	0·02 ± 0·07

* Second compartment not significant $P < 0{\cdot}05$.

48-h parasite cycle for MQ and 10^4 for artesunate and 10^3 for chloroquine (White, 1997). As clinically symptomatic parasitaemia is at least of the order of $10^3/\mu$l, and radical cure is a reduction to the order of $10^{-7}/\mu$l, cure requires at least 10 days of a sufficiently high drug concentration (Hall *et al*. 1977), and usually longer.

The value of k_1 can be estimated from Fig. 2 which shows as solid circles the fall in parasitaemia (means of 91 patients) as a function of time after treatment with 2 doses of MQ (750 mg + 500 mg) spaced 6 h as reported by Looareesuwan *et al*. (1995). The estimate is taken as the solid line fitted to the decrease in parasitaemia during the period from 20 to 55 h. This

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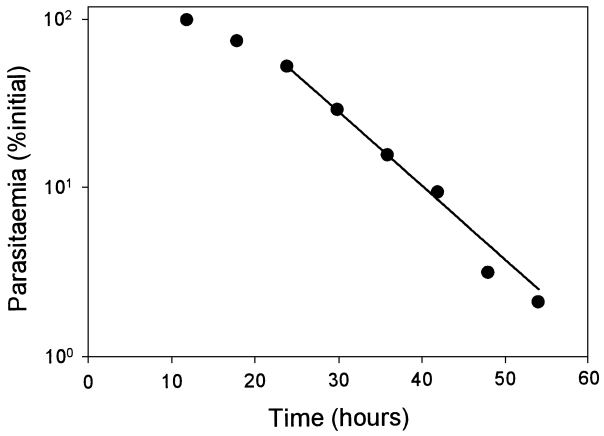

Fig. 2. Percentage of initial parasitaemia (mean of 91 patients) as a function of time for patients receiving MQ (Looareesuwan *et al*. 1995). (●) Clinical data, mean values from 12 h post-medication. (—) Best exponential through points for post-lag times > 20 h $594e^{-0{\cdot}1t}/\mu$l.

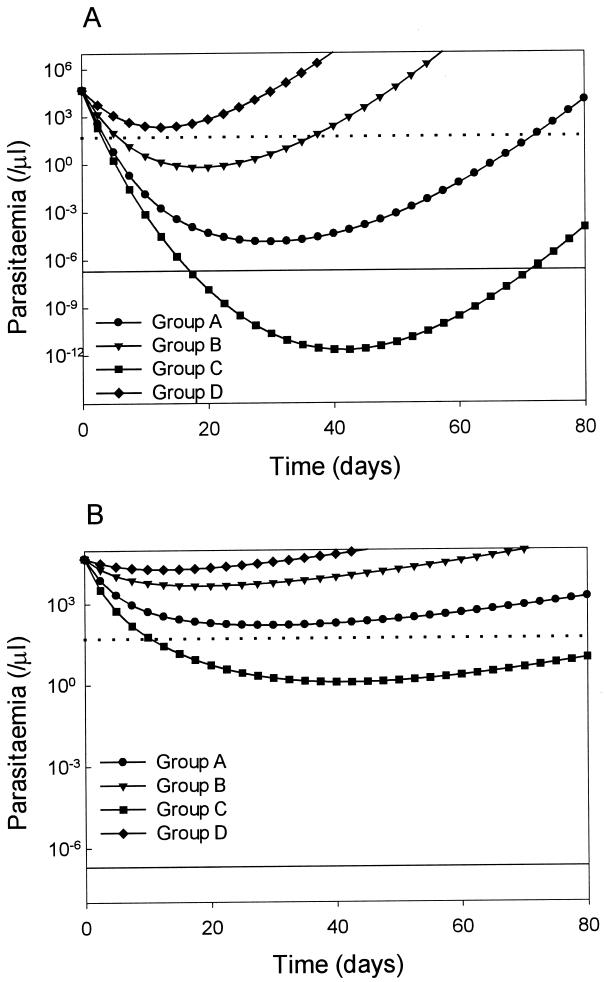

Fig. 3. Predicted parasitaemia as a function of time in days after medication, assuming either (A) $\boldsymbol{a} = 1$/day or (B) $\boldsymbol{a} = 0{\cdot}1$/day; $k_1 = 3{\cdot}3$/day. Groups A (●) and B (▼) received 750 mg MQ. Groups C (■) and D (◆) received 1500 mg. Groups A, B, C were sensitive $K =$ 375 (ng/μl). Group D resistant $K = 1150$ (ng/μl). (···) Detection limit; (—) total clearance (1 parasite/body).

is because MQ affects essentially mature trophozoites (Geary, Divo & Jensen, 1989), so that a lag in the effect of the drug is predicted and found, and the earlier values are unsuitable for a determination of the kill rate. The data give a parasitaemia reduction rate of $\boldsymbol{a} - k_1 = -0{\cdot}102 \pm 0{\cdot}003$/h $= -2{\cdot}4$/day. The daily survival is thus $e^{24*(\boldsymbol{a}-k_1)} = e^{-2{\cdot}4} \approx 0{\cdot}09$ of the initial parasite population being cleared per day, or approximately 91% clearance per day. This tallies with other estimates, such as that reported by White (1997), who estimated 10 to 10^3-fold reduction per cycle. To find k_1 from $\boldsymbol{a} - k_1$ the value of $\boldsymbol{a}$ needs to be determined.

The value of $\boldsymbol{a}$ (the intrinsic growth rate of the parasite) is population dependent (Hoshen, Stein & Ginsburg, 1998). It varies from 0 per day for an endemic population to a maximum where a multiplication rate of 16-fold per cycle (Kwiatkowski & Nowak, 1991) gives $\boldsymbol{a} = \ln 4 = 1{\cdot}38$ per day for uninhibited growth (such as in a non-immune infant, or artificially inoculated adults). To represent 3 levels of immunity (White, 1999), values of $\boldsymbol{a} = 0{\cdot}1$, $0{\cdot}69$ ($= \ln 2$) and 1 (per day in each case) for immune, semi-immune and partly immune patients (such as the Thai data of Fig. 1) were used respectively. Thus, taking $\boldsymbol{a} = 1$, $k_1 = 3{\cdot}4$. If $\boldsymbol{a} = 0{\cdot}69$ is taken for these patients, the value of k_1 would be correspondingly decreased to 3·09.

To obtain the value of parameter K, estimates of the value of MIC, the Minimum Inhibitory Concentration of the drug, are needed. MIC for resistant strains is estimated at 500 ng/ml (Price *et al*. 1999; Simpson *et al*. 1999), while for low resistance strains, it is approximately 1/3 of this value, or approximately 150 ng/ml. As MIC $= K\boldsymbol{a}/(k_1 - \boldsymbol{a})$, with $\boldsymbol{a}$ taken as equal to 1 and k_1 equals 3·4, estimates of $K = 350$ ng/ml for sensitive parasites and 1150 ng/ml for resistant strains are obtained.

Numerical simulations using PK/PD estimations

Based on this information, a numerical simulation of the course of parasitaemia for each of the 4 pharmacokinetic profiles described in Table 1 was performed. As high resistance was reported for EC_{50} measurements *in vitro* for group D, the higher K value for this group was used, but the 'sensitive' K value was used for the other groups. The parasitaemia as a function of time, as predicted by the analysis, is presented in Fig. 3, for a value of $\boldsymbol{a} = 1$ in Fig. 3 A and for $\boldsymbol{a} = 0{\cdot}1$ in Fig. 3B. The predictions for $\boldsymbol{a} = 0{\cdot}5$ do not differ greatly from those for $\boldsymbol{a} = 1$ (not shown). The dotted horizontal line (here and throughout) represents the typical parasite detection limit (50/μl) and the solid horizontal line represents a residual level of 1 parasite per body, below which a complete cure is defined. The relevant MQ concentrations were depicted in Fig. 1.

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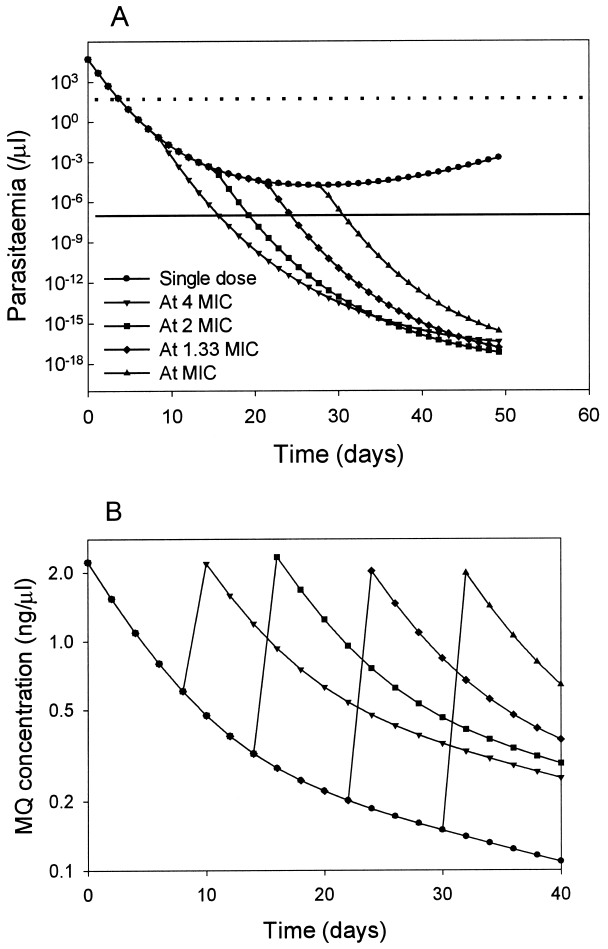

Fig. 4. Modelling the pharmacodynamics (A) and pharmacokinetics (B) for a hypothetical partly-immune patient with 'true' pharmacokinetics as determined for group A $C(t) = 1{\cdot}8e^{-0{\cdot}2t} + 0{\cdot}33e^{-0{\cdot}03t}$ ng/μl, $\boldsymbol{a} = 1$/day, $k_1 = 3{\cdot}3$/day, MIC = 150 ng/μl, if given a second 750 mg dose when concentration had dropped to C_δ. (●) Single dose; (▼) $C_\delta = 4$ MIC; (■) $C_\delta = 2$ MIC; (◆) $C_\delta = 1{\cdot}33$ MIC; (▲) C_δ = MIC. (···) Detection limit; (—) total clearance (1 parasite/body).

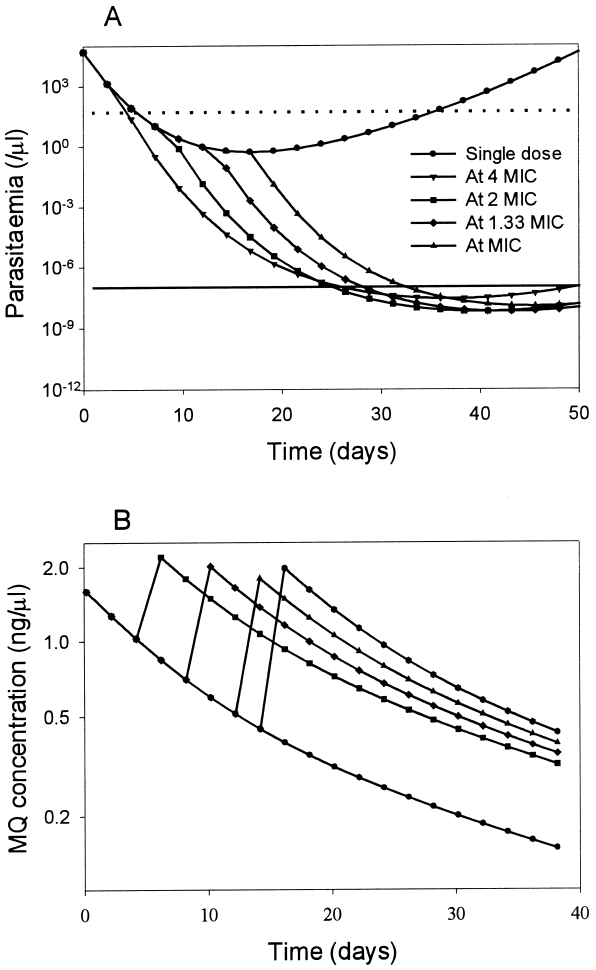

Fig. 5. Modelling the pharmacodynamics (A) and pharmacokinetics (B) for a partly-immune hypothetical patient with 'true' pharmacokinetics as determined for group B $C(t) = 0{\cdot}72e^{-0{\cdot}3t} + 0{\cdot}24e^{-0{\cdot}04t}$ ng/μl, $\boldsymbol{a} = 1$/day, $k_1 = 3{\cdot}3$/day, MIC = 150 ng/μl, if given a second 750 mg dose when concentration had dropped to C_δ. (●) Single dose; (▼) $C_\delta = 4$ MIC; (■) $C_\delta = 2$ MIC; (◆) $C_\delta = 1{\cdot}33$ MIC; (▲) C_δ = MIC. (···) Detection limit; (—) total clearance (1 parasite/body).

Simulations of repeated-dose protocols

Following the protocol of Hoshen *et al.* (1998) who showed the benefit of applying a second dose of chloroquine, the effect of administering a second dose of mefloquine, once, after a defined interval, was simulated. This was done in order to determine the time at which it would be most advantageous to apply the second dose. Consider the re-treatment of groups A, B and D above. The numerical simulations for different times of the second dose are depicted in Figs 4, 5 and 6, for groups A, B and D respectively, taking a value of $\boldsymbol{a} = 1$ and the pharmacokinetic parameters taken from Table 1. (There is no need to re-treat Group C because they were fully cured by the first dose.) From Figs 4–6, it is seen that a second dose is fully curative for the group A, and borderline for group B and a very late recrudescence is expected for group D. These computations are valid for a non-immune population. For a semi-immune population (adults, possibly exposed in the distant past, such as in areas of seasonal transmission), the relevant value of the parameter $\boldsymbol{a}$ will be lower than the value of 1 used in Figs 4–6. Fig. 7 presents a computation using a value of $\boldsymbol{a} = 0{\cdot}69$, appropriate for a semi-immune population and PK parameters appropriate for group B of Table 1. In this case, even in spite of the high resistance of the parasites such as those of group D, re-administering MQ would prevent late recrudescence. Delaying the second dose increases its efficacy and also reduces the danger of dose-related toxicity.

Approximating 2-compartment kinetics by 1-compartment models

PK and PD parameters may depend to a great extent on the particular ethnic and/or geographical setting. To arrive at a clinically useful treatment protocol for such a setting, it is important to ascertain the

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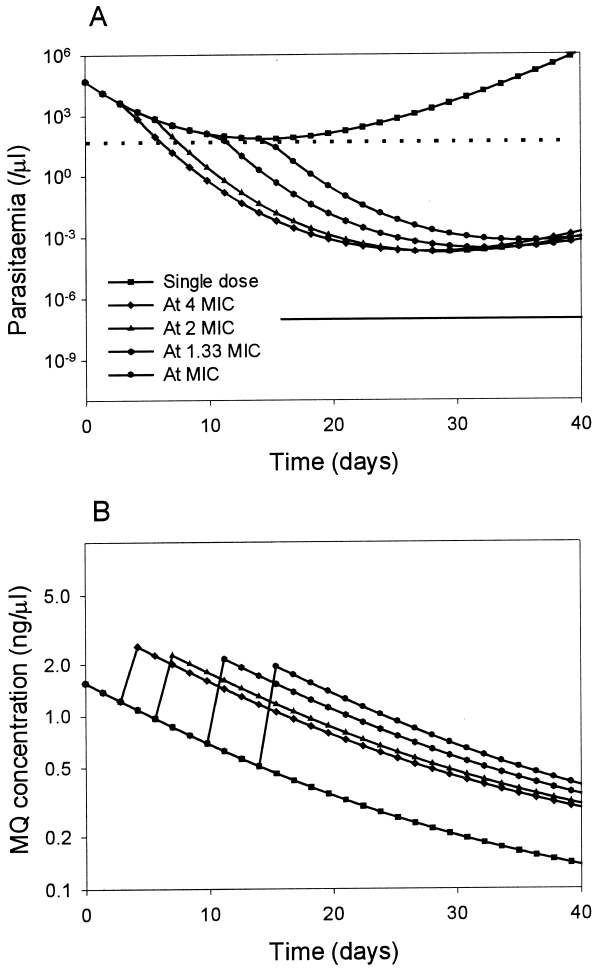

Fig. 6. Modelling the pharmacodynamics (A) and pharmacokinetics (B) for a hypothetical partly-immune patient with 'true' pharmacokinetics as determined for group D $C(t) = 1{\cdot}32e^{-0{\cdot}1t} + 0{\cdot}25e^{-0{\cdot}02t}$ ng/μl, $\boldsymbol{a} = 1$/day, $k_1 = 3{\cdot}3$/day, MIC = 500 ng/μl, if given a second 750 mg dose when concentration had dropped to C_δ. (■) Single dose; (◆) $C_\delta = 4$ MIC; (▲) $C_\delta = 2$ MIC; (⬣) $C_\delta = 1{\cdot}33$ MIC; (●) $C_\delta = 1$ MIC. (···) Detection limit; (—) total clearance (1 parasite/body).

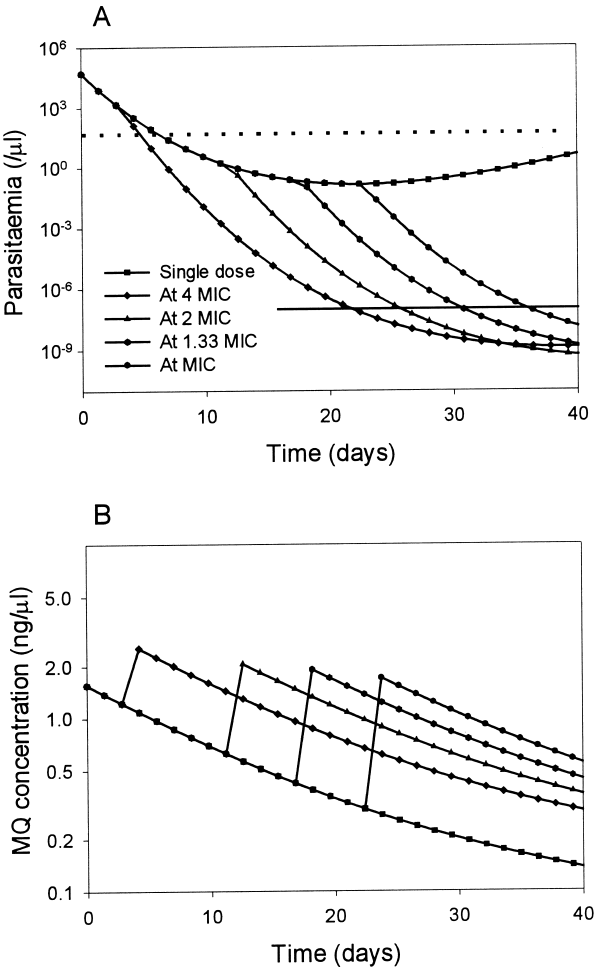

Fig. 7. Modelling the pharmacodynamics (A) and pharmacokinetics (B) for a hypothetical semi-immune patient with 'true' pharmacokinetics as determined for group D $C(t) = 1{\cdot}32e^{-0{\cdot}1t} + 0{\cdot}25e^{-0{\cdot}02t}$ ng/μl, $\boldsymbol{a} = 0{\cdot}69$/day, $k_1 = 3{\cdot}3$/day, MIC = 500 ng/μl, if given a second 750 mg dose when concentration had dropped to C_δ. (■) Single dose; (◆) $C_\delta = 4$ MIC; (▲) $C_\delta = 2$ MIC; (⬣) $C_\delta = 1{\cdot}33$ MIC; (●) $C_\delta = 1$ MIC. (···) Detection limit; (—) total clearance (1 parasite/body).

appropriate PK and PD parameters in an economical fashion. This section presents a possible approach to this.

For a full solution, equation (3) must be solved. Fig. 1 showed that the 2 exponential PK best fits the data. To obtain a valid fit needs many time-points, which may be difficult to obtain in the field. The present section explores the possibility of obtaining a good enough single exponential fit that requires fewer data points and suggests a general method for choosing good sampling times.

High drug concentrations (those dominant in the first exponential phase of parasite reduction) cause near maximal kill, so that change in concentration at the beginning of treatment will cause very little influence on the minimal parasitaemia. The sub-MIC concentrations (those in the latter half of the second exponential phase) will, at most, have influence on the post-minimal parasitaemia values. Thus the need is to model the concentration only for intermediate times, around the time at which the concentration has dropped to K, forming a single exponential which will give approximations to the correct MQ concentration values at t_{MIC} and at $t_{1{\cdot}5\,K}$ (the time at which the concentration drops to $1{\cdot}5 \times K$). These values can be obtained, digitally or manually, from a plot of the 2-compartment model. Analytically, one solves for m the single-compartment rate constant, $m = \ln(1{\cdot}5\,K/\mathrm{MIC})/(t_{\mathrm{MIC}} - t_{1{\cdot}5\,K})$, and substitutes m and $t = t_{\mathrm{MIC}}$ into equation (3), obtaining an approximation of the minimal parasitaemia. The plot of the parasitaemia course will be close to that of the 'true' 2-compartment model (not shown).

The next task is to establish the ideal timing for measuring MQ concentration, so as to acquire only 6 points (which should be enough to include a component from the first exponent) for a best 1-

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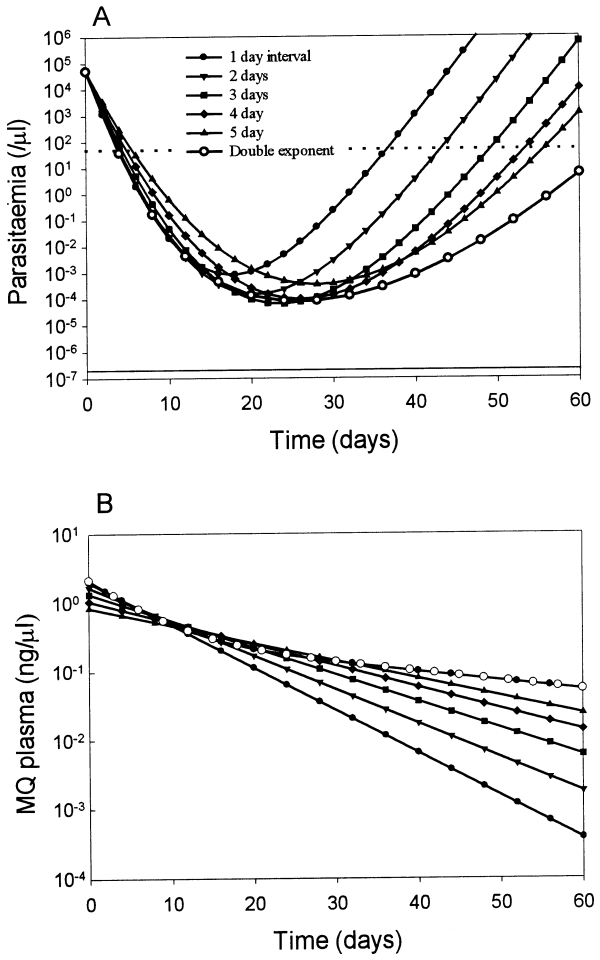

Fig. 8. Modelling the pharmacodynamics (A) and pharmacokinetics (B) for a hypothetical patient with 'true' pharmacokinetics as determined for group A $C(t) = 1{\cdot}8e^{-0{\cdot}2t} + 0{\cdot}33e^{-0{\cdot}03t}$ ng/μl, $\boldsymbol{a} = 1$/day, $k_1 = 3{\cdot}3$/day, MIC = 150 ng/μl. (A) X-axis: time in days post-medication; Y-axis: parasitaemia/μl. (B) X-axis: time in days post-medication; Y axis: plasma concentration ng/μl. (○) 'True' model. One-compartment models, as solid objects, determined by 6 samples, measured every 1 day (●), 2 days (▼), 3 days (■), 4 days (◆) or 5 days (▲). Symbols are used to identify the lines and do not depict sampling times. (···) Detection limit; (—) total clearance (1 parasite/body).

compartment estimator of pharmacodynamics. This is done by measuring at sampling times of 1 × Interval to 6 × Interval (Interval in days), and fitting the PK curve to a single exponential. The 2-compartment model is used as the 'true' model and is sampled at 6 times, for each interval length. The progress of the parasitaemia and the plasma concentration according to the model, are presented in Fig. 8, where the line defined by the open circles is the 2-compartment fit to the data of group A in Fig. 1, using the PK parameters listed in Table 1 and MIC = 150 ng/μl. Table 2 presents the values of the basic parameters which describe the fits for the chosen parameters. The result which best approximates the true 2-compartment model is the curve which has a t_{MIC} and a minimal parasitaemia, P_{MIC}, closest to that of the true model. In Fig. 8, the 3 and 4-day intervals are closest to the true fit as can also be seen in Table 2 where the t_{MIC} and P_{MIC} values are tabulated. It would appear that, to a good approximation, the appropriate 1-compartment fit parameters are $C_{\max} = 1{\cdot}2 \pm 0{\cdot}15$ ng/μl, $t_{\frac{1}{2}} = 8 \pm 1$ day.

The single-compartment model must be based on fairly long times (long intervals) of PK determination, so as to enable full consideration of the second compartment of drug elimination, which would not be sufficiently accounted for if the sampling period lasts only 6–12 days (as for 6 measurements at 1–2 day intervals). However, if the MIC (or K) is high, as for high resistance, or if the initial plasma concentration were low (unfavourable PK), very long sampling intervals would be undesirable, since sufficient concentrations above MIC would not appear in the data set. Thus a 3–5 day interval is probably the most suitable sampling protocol for a wide range of PK/PD values. The use of simplified models thus enables minimization of the number of clinical data points, reducing the number of tests, reducing expenses and effort associated with excess MQ concentration evaluations and patient discomfort.

Timing the second dose using the 1-compartment simulation

One can solve equation (1) analytically to find the best time for administering a second dose, if one uses the single-compartment simplification. As established by Hoshen *et al.* (1998), the second dose should be administered at time t_δ, as given by equation (4), where $dok = C_0/K$

$$t_\delta = (1/m)\ln\{[(k_1/a-1)dok^2 - 2dok + dok\sqrt{((k_1/a-1)^2 dok^2 + 4k_1/a)}] \div 2(dok+1)\}. \quad (4)$$

The correct use of a second dose would be curative even for high resistance, for an immune or semi-immune patient.

One possible strategy of use of this method is to form a table of ideal times for applying the second dose for each of many different values of the basic PK/PD parameters. Such a table is presented as Table 3. It is formed by application of equation (4) to an appropriate set of clinical parameters. It presents the ideal time for the maximal effect possible by a double dose. LA and HA in the Table are low- and high-absorbing patients (groups B and D and groups A and C of Table 1, respectively), given doses of either 750 or 1500 mg MQ. They present with $\boldsymbol{a}$ values of 1, 0·69 and 0·1 in the top, middle and bottom sets of predictions, with K values appropriate to either sensitive or resistant parasites. For each category, Table 3 lists the minimum parasitaemia reached with a second dose, given at its most effective time.

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Table 2. PK and predicted PD results (t_{MIC} and minimal parasitaemia P_{MIC}) as obtained by fitting single exponential curves to the 2-exponential PK curve at different sampling intervals

(Data of group A are used, and assuming $\boldsymbol{a} = 1$, $K = 0{\cdot}375$, $k_1 = 3{\cdot}3$.)

Interval (days)	C_{max} (ng/μl) $\pm$ S.E.	m(/day) $\pm$ S.E.	$t_{1/2}$(days)	t_{MIC} (days)	P_{MIC} (/10^{-5} μl)
1	1·99 $\pm$ 0·05	0·14 $\pm$ 0·005	5	17·6	82
2	1·68 $\pm$ 0·12	0·11 $\pm$ 0·01	6·3	20·6	15
3	1·35 $\pm$ 0·15	0·090 $\pm$ 0·01	7·7	23·6	6·5
4	1·06 $\pm$ 0·14	0·072 $\pm$ 0·01	9·6	26·1	9·9
5	0·84 $\pm$ 0·11	0·059 $\pm$ 0·008	12	28·0	35
Two exponent				25·8	7·9

Table 3. Time in days for application of second dose, for: (i) non-immune population ($\boldsymbol{a} = 1$), (ii) semi-immune ($\boldsymbol{a} = 0{\cdot}69$) and (iii) immune populations ($\boldsymbol{a} = 0{\cdot}1$) using $k_1 = 3{\cdot}3$, for various values of K, after either 750 or 1500 mg, and high (HA) or low (LA) initial absorption, and minimal parasitaemia achieved

(In μl assuming initial parasitaemia of 50000/μl.)

(i)

				Minimal parasitaemia (μl)	
Group	PK type	MIC (ng/μl)	Time of second dose (days)	Single dose	Two doses
A	750 HA	0·15	10	$3{\cdot}6 \times 10^{-6}$ (N.C.)	$2{\cdot}5 \times 10^{-19}$
A	750 HA	0·5	4	210·0 (N.C.)	0·025 (N.C.)
B	750 LA	0·15	12	0·5 (N.C.)	$5{\cdot}6 \times 10^{-9}$
B	750 LA	0·5	2	$1{\cdot}6 \times 10^{4}$ (N.C.)	250·0 (N.C.)
C	1500 HA	0·5	10	1·3 (N.C.)	$2{\cdot}0 \times 10^{-8}$
D	1500 LA	0·15	12	$4{\cdot}4 \times 10^{-10}$	$5{\cdot}9 \times 10^{-26}$
D	1500 LA	0·5	6	94·0 (N.C.)	$3{\cdot}2 \times 10^{-4}$ (N.C.)

(ii)

				Minimal parasitaemia (μl)	
Group	PK type	MIC (ng/μl)	Time of second dose (days)	Single dose	Two doses
A	750 HA	0·15	12	$9{\cdot}0 \times 10^{-12}$	$7{\cdot}8 \times 10^{-29}$
A	750 HA	0·5	10	3·1 (N.C.)	$1{\cdot}6 \times 10^{-6}$
B	750 LA	0·15	7	$2{\cdot}2 \times 10^{-4}$ (N.C.)	$1{\cdot}5 \times 10^{-16}$
B	750 LA	0·5	3	$2{\cdot}0 \times 10^{3}$ (N.C.)	1·8 (N.C.)
C	1500 HA	0·5	10	$4{\cdot}1 \times 10^{-4}$ (N.C.)	$4{\cdot}3 \times 10^{-15}$
D	1500 LA	0·15	12	$2{\cdot}5 \times 10^{-17}$	$2{\cdot}0 \times 10^{-37}$
D	1500 LA	0·5	12	0·27 (N.C.)	$9{\cdot}5 \times 10^{-10}$

(iii)

				Minimal parasitaemia (μl)	
Group	PK type	MIC (ng/μl)	Time of second dose (days)	Single dose	Two doses
A	750 HA	0·5	10	$1{\cdot}8 \times 10^{-9}$	$3{\cdot}2 \times 10^{-23}$
B	750 LA	0·5	10	$1{\cdot}8 \times 10^{-3}$ (N.C.)	$9{\cdot}0 \times 10^{-13}$
D	1500 LA	0·5	10	$1{\cdot}7 \times 10^{-13}$	$2{\cdot}2 \times 10^{-29}$

N.C., Not curative (parasitaemia did not decrease below 2×10^{-7}/μl).

DISCUSSION

PK–PD analysis of MQ levels and parasitaemia data. Fig. 1 showed that a two-compartment model, based on 9 time-points per curve, gave a significantly better fit ($P < 0{\cdot}05$), for the data of groups, A, B and C (but not for group D), than did a 1-compartment model. Boudreau *et al.* (1990) themselves reported 2 well-defined compartments, for all 4 cases, using early time-points that were omitted in the present analysis. A similar analysis (Simpson *et al.* 1999), which utilized many individual measurements but at only 5 time-points to form a population PK model, failed to define a 2-compartment model, presumably

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because a wide enough spread of time-points was not available. The population model presented by Simpson *et al*. (1999) does, however, reflect the strong inter-patient variation, which must be considered for practical application, since the individuality of the PK values can lead to indeterminacy in the resulting predictions. Inspection of the PK plots using the 2-compartment model suggests that at around 10 days the fast and the slow component are contributing equally to the concentration of MQ. There is considerable variation in the relevant PK values, rate-constants and apparent volume of distribution recorded in Table 1. Low values of compartment maximal concentration (denoted here as $C_{\max}$) are associated with treatment failure (comparing group A with B or C with D). However, the PK is not the only determinant. For example, groups A and D display PK parameters that are hardly different, although the outcome of treatment was totally different. Turning now to Fig. 3, one sees that the numerical analysis in the case of $\boldsymbol{a} = 1$, largely accords with the clinically observed results. Group C is radically cured. Treatment of Group B fails, with a long recrudescent time (> 20 days), and that for Group D fails at the RII level (RII is defined as a treatment which fails to cure resulting in medium term recrudescence), presumably due to the high resistance displayed by the parasites. Strict scrutiny of the graph shows that Group A should show very late recrudescence after > 70 days, but this would have been missed in the clinical follow-up and would have been described as a real cure. In the case of $\boldsymbol{a} =$ 0·1, all treatments fail, contrary to the clinical experience. Apparently, the appropriate value for the parameter $\boldsymbol{a}$ is greater than 0·1 for all these patients.

Timing the second dose. In a previous paper on the chemotherapy of chloroquine, which considered the timing of a second dose of that drug (Hoshen *et al*. 1998), it was demonstrated that the best time for the second dose is not when drug concentration has dropped to the MIC, but earlier. It was demonstrated in that paper that it is beneficial to achieve somewhat higher maximal drug levels, thus allowing higher cure rates, rather than attempting simply to keep the drug concentration above the MIC as long as possible (as opposed to the suggestion of Zhi, Nightingale & Quintiliani (1998) based on some simplifying assumptions). The precise time for applying the second dose can be found analytically for single exponential models or numerically for 2 exponential models.

Table 3 presents the best clinical protocols for the timing of the second dose of MQ in different settings of immunity and capacity to absorb the drug. Both the initial and the second treatments should preferably be given in 2 parts to increase bioavailability (Simpson *et al*. 1999). Of course, practical application requires intensive investigation of the precise data by population PK and PD methodology. As PK and PD characteristics vary between populations, results of identical protocols will not always give the same outcome. For example, most of the wealth of detailed PK and PD data is from Thai patients. A large part of the patients are from ethnic groups who are repeatedly inflicted by the disease and have therefore partial immunity. Use of mathematical modelling would allow rational individualization of treatment, as to dosage, by extrapolation from one subpopulation to another. Thus, one could use population PK and establish a new field of population PD to allow definition of personal or ethnic traits, which would require varied doses. For example, the use of high doses for indigenous populations would be undesired, because they would probably have low $\boldsymbol{a}$ values, and thus MIC would be much lower. Thus, they would unnecessarily risk the undesirable side-effects of protracted high concentrations of MQ. For the reverse case, of a traveller or infant, $\boldsymbol{a}$ would be higher, and so the patient would be inadequately treated, if depending on the Thai results. This would result in recrudescence and selection of resistant parasites. By using mathematical modelling one may establish *a priori* which dose to give for members of different populations. We acknowledge that whilst the modelling process which we have undertaken may be of considerable value, at present the approach is 'hypothesis generating' and needs to be evaluated in clinical practice.

Note that for low doses in cases of low absorption, even in areas of semi-immunity, a second low dose will not attain therapeutic concentrations, although 2 high doses will be effective. Since in the clinic it is impractical to establish whether a patient is a low absorber or a high absorber, all patients should be considered low absorbers and be given high doses. For non-immune cases, low absorber patients will not be cured even with 2 doses and clearly not with 1 dose. In non-immune cases, resistant strains cannot be cured, even by a second dose, except in the case of high doses and high absorption. Thus, for such strains, MQ monotherapy should not be used except for indigenous populations of holoendemic regions.

In the case of an immune population, as may be expected, there is no need for a second dose. A single small dose is sufficient to aid the body's immune system to overcome the slight surge in parasitaemia. In other cases too in which the parasitaemia achieved is well below the cure level, one might reduce the size of the dose, so as to limit the risk of toxicity. Our clinical colleagues have emphasized that compliance with the double dose may unfortunately not be practical in those geographical areas where such treatment would be most needed. While this may be true, the double dosing protocol may be still valuable for patients for whom more sophisticated health services are available.

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lication, an important paper dealing with MQ PK–PD modelling was published (Simpson *et al*. 2000). In that report the model of Hoshen *et al*. (1998) for chloroquine chemotherapy was applied using, *a priori*, single exponential PK. There are a few basic methodological differences between the present report and that of Simpson *et al*. (2000). The PD parameters that have been used in the 2 studies are different: K in the present work is derived from clinical data whereas the IC_{50} used by Simpson *et al*. (2000) was derived from *in vitro* drug tests. The PK parameters, too, are different. In the Simpson *et al*. (2000) study the analysis was based on long-term sampling data and thus disregards the early distribution and metabolism shown here to be essential for modelling. In the present report, PK modelling focuses on the therapeutic range, which is derived from clinical data. The paper of Simpson *et al*. (2000) focuses on the prevention of the evolution of drug resistance and does not deal with therapy in different settings of transmission and immunity. The present report emphasizes this latter important aspect.

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