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Clearance of young parasite forms following treatment of falciparum malaria in humans: comparison of three simple mathematical models

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SUMMARY

To characterize post-treatment clearance of young forms of *Plasmodium falciparum* from the blood, three differential equation models, a linear decline, a linear then logarithmic decline, and the Michaelis–Menten (MM) kinetic equation, were fitted to log-transformed serial parasite counts from 30 semi-immune patients with synchronous parasitaemias allocated one of six antimalarial drug regimens. The first two equations were solved analytically. The MM equation was solved numerically using a fifth-order Runge–Kutta method. For each equation, parasite clearance was assumed stochastic and log-transformed parasite counts were assumed to be normally distributed at each time-point. Comparisons between models were by Minimum Akaike Information Criterion Estimate. A constrained MM equation fitted the data at least as well as the other two models in 5 of 6 drug groups and also when pooled data were analysed, providing a single index which could be used in drug efficacy studies in similar situations or as part of more complex models that encompass asynchronous, complicated infections.

INTRODUCTION

Several factors make falciparum malaria more readily quantifiable than other infections. Because *Plasmodium falciparum* develops within erythrocytes, sampling and microscopic identification of parasites can be done with relative ease. Progression of the infection can sometimes be assessed directly in humans, such as through the past controlled use of malaria fever therapy for syphilis and in drug-resistant cases. *In vitro* parasite culture is well established. Detailed observations of *P. falciparum* morphology and behaviour in these situations [1–3] have served as the basis for mathematical models of in vivo parasite development [4, 5]. These models demonstrate that changes in the density of *P. falciparum* in the peripheral blood of a patient with malaria depend on the distribution of parasites across their 48-h life-cycle.

In the first half of the life-cycle, parasites can be seen on stained blood smears as intracellular ring forms or early trophozoites which can be quantified. These young forms circulate with minimal clearance. However, as maturation continues, erythrocytes containing trophozoites adhere to microvascular endothelium and are thus removed from the peripheral circulation. These sequestered forms develop into schizonts which induce red cell rupture and the release merozoites. Merozoites quickly invade uninfected red cells to become the new generation of ring forms. In some patients, the majority of parasites remain at the same stage of development as the infection progresses

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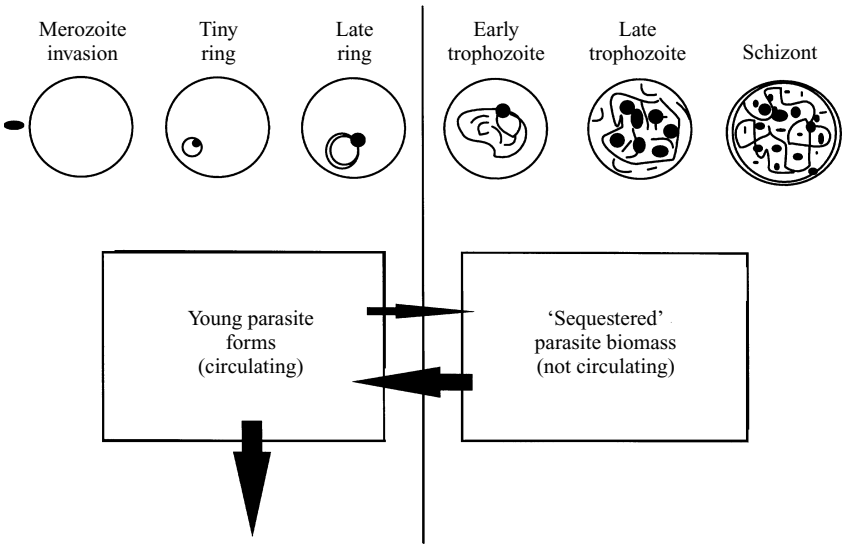

Fig. 1. Schematic diagram showing broad stages of parasite development across the 48-h life cycle of *Plasmodium falciparum*. The vertical line represents the stage at which parasites cytoadhere and disappear from the peripheral circulation. The simple models described in this report are based on clearance from the single compartment in the lower left panel; more complex models of asynchronous infections would need to include parasite fluxes to and from the sequestered biomass.

while in others the parasitaemia is relatively asynchronous.

Changes in the peripheral parasitaemia during treatment for falciparum malaria will, therefore, depend on fluxes to and from the cytoadherent, 'invisible' biomass as well as on drug effects which lead to the removal of young parasite forms from the circulation before they can sequester. Because these considerations suggest that the parasitaemia-time curve in both untreated and treated *P. falciparum* infections is complex, treatment response is commonly assessed using simple indices such as the time for clearance of 50, 95 or 100% of the initial parasitaemia. These measures ignore the overall pattern of parasite clearance which may be influenced by factors other than the drug under assessment.

Although some reports have displayed plots of serial parasite counts to show differences in drug efficacy, no mathematical analysis of these curves has been attempted. As a first step in this process, we have evaluated three simple models describing parasite clearance in patients whose partial immunity had synchronized their *P. falciparum* infections and who were treated when most parasites were early ring forms. In this situation, there is minimal appearance of new ring forms in the peripheral blood after initiation of treatment, while the disappearance of rings and early trophozoites should reflect drug effects or, where drug action is primarily in the second half of the life-cycle, sequestration of maturing parasite forms. The results show that clearance of young parasites can be described adequately by a modified Michaelis–Menten model which could have broader application in field studies of antimalarial drug efficacy.

SUBJECTS AND METHODS

Subjects and clinical procedures

A subset of the previously published data of Li and colleagues [6] was used to assess the models. The data were obtained from 30 untreated Chinese adults aged 14–60 years who had acute, uncomplicated falciparum malaria but no other apparent serious disease. All were long-term residents in the same endemic area. Clinical assessment and the appearances of smears taken from both peripheral blood and intradermal (tissue) sampling indicated that parasite development was clustered around one stage of the life-cycle, consistent with synchronization of the infection through partial immunity [2, 3]. At presentation, parasites were present in $< 5\%$ of red cells in all cases with a range of densities 4460–230 700 parasites/μl whole blood.

Equal numbers of patients were assigned at random to one of six oral antimalarial drug regimens: mefloquine alone, artemisin alone, sulfadoxine plus pyrimethamine (Fansidar®), mefloquine plus artemisinin, mefloquine plus Fansidar, or mefloquine plus

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Fansidar plus artemisin (Groups 1–6). Allocated treatment was started from 6–10 h of the parasite cycle as judged morphologically from a peripheral blood smear. Further smears were taken 4-hourly until two negative films had been obtained. Parasite densities were calculated from manual counts and the simultaneous haematocrit (thin blood films) or total white cell count (thick films). As in other bio-mathematical systems [7], changes in parasitaemia are measured in terms of orders of magnitude and parasite counts were transformed logarithmically before analysis.

Further assessment of parasite development and clearance during treatment was performed in two other previously-untreated Chinese patients with uncomplicated, synchronous falciparum malaria. To minimize drug pharmacokinetic effects on parasite clearance, both were given rapid intravenous therapy. The first patient received artesunate 120 mg by injection followed by further 60 mg doses at 6, 24 and 48 h. The other was treated with a split-infusion quinine loading dose of 17 mg/kg body weight over $4\frac{1}{2}$ h [8]. In both cases, blood smears were taken hourly for 8 h, then 4-hourly thereafter. In addition to total parasite counts, parasites were subdivided on morphological grounds (nuclear size, cytoplasmic content and presence of malarial pigment) as either small R1 rings (approximately 0–8 h of the cycle), medium R2 rings (9–16 h), or large R3 rings (17–24 h) at each time-point [9].

Definitions of models

In order to simplify the modelling process, the time at which drug-induced parasite clearance begins ($T = 0$) was shifted to the 4 h time-point immediately prior to a $> 5\%$ sustained fall in parasitaemia. To account for sources of noise such as errors made while measuring parasite densities, the clearance of parasites was assumed to be a stochastic process, with independent, additive noise normally distributed about a time-dependent mean (with respect to the log-transformed data). The models presented here describe the behaviour of the system in terms of the mean. The stochastic component of the models is presented subsequently.

The first model describes the log-transformed parasite count as a linear, decreasing function of time. That is, the untransformed data are modelled by an exponentially decreasing function $P(.)$, so that the mean proportion of parasites cleared per unit time is a constant:

$$\left.\begin{aligned} \mathrm{d}/\mathrm{d}t\, P(t) &= -aP(t) \\ P(0) &= P_0, \end{aligned}\right\} \tag{1}$$

where a and P_0 are positive constants, a having dimensions hours^{-1}. The second, log-linear model assumes that clearance mechanisms are limited. For the untransformed data, the clearance of parasites is piecewise linear-exponential, so that no more than a fixed number of parasites can be removed per unit time:

$$\left.\begin{aligned} \mathrm{d}/\mathrm{d}t\, P(t) &= -cb \quad \text{for } P(t) > b; \\ \mathrm{d}/\mathrm{d}t\, P(t) &= -cP(t) \quad \text{for } P(t) \leqslant b; \\ P(0) &= P_0. \end{aligned}\right\} \tag{2}$$

Here, b and c are positive constants with dimensions of parasites/μl and hours^{-1} respectively. The third model is the Michaelis–Menten (MM) equation used commonly in pharmacokinetic analysis [10]:

$$\left.\begin{aligned} \mathrm{d}/\mathrm{d}t\, Q(t) &= -k_1\, Q(t)/(k_2 + Q(t)) \\ Q(0) &= \ln(P_0), \end{aligned}\right\} \tag{3}$$

where $\ln(.) = \log_e(.)$. The log-transformed parasite counts are modelled by the function $Q(.)$, the parameters k_2 and P_0 are positive constants, and k_1 is a positive constant with dimension hours^{-1}. The MM differential equation is a saturation model as it describes a case in which the rate of parasite clearance is bounded in magnitude. The clearance of parasites increases with the parasitaemia to a maximum k_1. The parameter k_2 controls the curvature (second time-derivative) of the trajectory $Q(.)$ such that the smaller the value of k_2, the more rapidly parasites are cleared.

The linear model is easily solved, and is the log of the following equation:

$$P(t) = P_0 \exp(-at) \quad \text{for } t \geqslant 0. \tag{4}$$

To solve the log-linear model represented by the linear-exponential system (2), suppose that the switch between the linear and exponential parts occurs at time $\tau \geqslant 0$. On the time interval $[0, \tau)$, the parasite count is a linear function of time. That is, $P(t) = mt + \alpha$ for constants m and α. The boundary conditions $P_0 = \alpha$ and $P(\tau) = m\tau + \alpha = b$ can be used to solve for m and α:

$$\left.\begin{aligned} m &= (P(\tau) - P_0)/\tau \\ \alpha &= P_0. \end{aligned}\right\} \tag{5}$$

From eqn (2), the rate of parasite clearance is continuous at $t = \tau$. Thus:

$$P(\tau) = m = (P(\tau) - P_0)/\tau = -cb,$$

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so that the switching time becomes:

$$\tau = (P_0 - P(\tau))/(cb). \tag{6}$$

On the time interval $[\tau, \infty)$, it is true that

$$P(t) = b\exp(-c(t-\tau)).$$

Thus, the equation describing the parasite count for the log-linear model is the log of the following system:

$$\left.\begin{array}{l} P(t) = P_0 - cbt \quad \text{for } 0 \leqslant t \leqslant \tau = [P_0 - b]/(cb); \\ P(t) = b\exp(-c(t-\tau)) \quad \text{for } \tau \leqslant t. \end{array}\right\} \tag{7}$$

The MM differential equation cannot be solved analytically. For the numerical curve fitting that follows, eqn (3) was solved using a fifth-order Runge–Kutta method.

Selection of a model using MAICE

The Minimum Akaike Information Criterion Estimate (MAICE) is a systematic and general-purpose technique for choosing between several different models describing a set of data [11] which is used widely in biological systems [12–14]. Competing models can be nested or totally unrelated, and have different numbers of parameters. The best MAICE model is defined as the model and the corresponding maximum likelihood estimates (MLE) that minimize:

$$\text{AIC} = -2\ln(\text{MLE}) + 2p,$$

where p is the number of independently adjusted parameters within the model. When two models have the same MLE's, MAICE chooses the one with fewer parameters according to the so-called *Law of Parsimony*. In the MAICE technique, the MLEs are chosen to maximize the joint probability density corresponding to the data. At each sample time t, the log-transformed parasite counts are assumed to be distributed normally about the mean. A Gaussian model for noise was chosen because it is simple and adequately describes the variation of the (log-transformed) parasite counts. Although more complicated descriptions of noise may be used, the present results are not expected to be sensitive to the choice of model describing noise.

The variance of the distribution of parasite counts is σ^2 and is assumed to be the same at each sample time. The normal probability density function for the parasite data is:

$$f(y) = (2\pi\sigma^2)^{-1/2}\exp(-[\mathrm{y} - \mathrm{Q(t)}]^2/2\sigma^2),$$

where $Q(.)$ represents either the MM model of eqn (3), the linear model (log of eqn (4)), or the log-linear model (log of eqn (7)). The density $f(.)$ is time-dependent in the sense that its mean $Q(t)$ is a function of time.

For the linear model, the parameters P_0, a and σ are estimated by solving the following nonlinear programming problem:

$$\text{maximize } L(P_0, a, \sigma) = \prod_{i=1}^{N_p}\prod_{j=1}^{N_c(i)} f(y_j) \tag{8}$$

over $P_0 > 0$, $a > 0$, and $\sigma > 0$. Here, the number of patients used is N_p, while $N_c(i)$ denotes the number of parasite counts for patient i. Parasite count j is measured at time t_j and has value y_j, Π denotes product, and $L(.)$ denotes the likelihood function. For the log-linear model, the nonlinear programming problem is

$$\text{maximize } L(P_0, c, b, \sigma) = \prod_{i=1}^{N_p}\prod_{j=1}^{N_c(i)} f(y_j) \tag{9}$$

over $P_0 > 0$, $c > 0$, $b > 0$, and $\sigma > 0$. Three constraints need to be imposed when maximizing the likelihood function (9). The switching time τ defined in eqn (6) must be non-negative, the switching time may not exceed the last time at which a parasite count was taken, and $P_0 \geqslant b$. For the MM model, no constraints need to be imposed when maximizing the likelihood function

$$\text{maximize } L(P_0, k_1, k_2, \sigma) = \prod_{i=1}^{N_p}\prod_{j=1}^{N_c(i)} f(y_j) \tag{10}$$

over $P_0 > 0$, $k_1 > 0$, $k_2 > 0$, and $\sigma > 0$.

The nonlinear programming software FSQP [15] was used to solve the above minimization problems. FSQP is a FORTRAN subroutine that generates iterates that are feasible with respect to all inequality and linear equality constraints. The linear, log-linear, and MM models were fitted to parasitaemia-time data in each of the six drug groups individually, and to pooled data from all six drug groups.

Application of preferred model to individual data sets

After comparison of the three models using pooled data and MAICE, the preferred model was fitted to parasite clearance data from each patient to generate individual values of parameter k_2. Group medians for k_2 estimates were compared using the Kruskal–Wallis

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one-way analysis of variance [16]. The model of best fit was also applied, where possible, to clearance of ring forms subdivided by maturation stage in the two patients who received intravenous therapy.

RESULTS

Comparison of models

The maximum likelihood technique is a nonlinear optimization, where the unknown parameters correspond to hypotheses, and the optimal parameter values maximize the joint probability of data. The technique is valid only if the optimization problem is well-posed and possesses a unique maximum. If this is not the case, additional constraints can be applied so that the problem has a unique solution [17]. In terms of a numerical solution, the MM nonlinear programming problem (10) is ill-posed as evidenced from the following three observations. Firstly, the maximization routine FSQP stalled frequently during line searches and failed to converge. Secondly, maximizations that were started from different initial values of the likelihood parameters sometimes converged to points having different values of (k_1, k_2) but the same maximum likelihood (data not shown). However, the ratio k_1/k_2 at such points was similar. Finally, when the maximization did converge to a unique optimal point, the approximate 95% confidence intervals for k_1 and k_2 were very wide. These observations indicate that there exist regions in the parameter space over which the likelihood function is flat with respect to the likelihood parameters k_1 and k_2, so that the system has one too many independent optimization parameters.

In view of these observations, the constraint $k_1 = 1\ \mathrm{h}^{-1}$ was imposed on eqn (3) to eliminate one of its independent parameters, and the resulting equation

$$\mathrm{d}/\mathrm{d}t\, Q(t) = -Q(t)/(k_2 + Q(t))$$

was successfully refitted to the clearance data (see Table 1). The question of how to deal with optimization problems which have objective functions that are flat with respect to their minimization variables has been addressed in the context of other diseases and their treatment [6]. The goodness of fit of the MM differential eqn (3) with $k_1 = 1\ \mathrm{h}^{-1}$ compared to that of eqn (3) with k_1 unrestricted was the same or marginally worse across all treatment groups when measured using maximized likelihoods, and was superior with respect to the MAICE statistic (due to the elimination of one of the optimization parameters). This confirmed the validity of the decision to constrain the parameter k_1. At low parasite counts, clearance can be assumed as exponential with rate constant $-1/k_2$ under the modified MM model.

For 5 of 7 drug groups (including pooled data) the constrained MM model fitted the data best, measured using MAICE values, compared to the linear and log-linear models (see Table 1). It was sub-optimal only in the case of Group 2. For Group 6, the fits of the linear and MM models were comparable. Note that the linear and log-linear models are nested. That is, the log-linear model with a time switch at $\tau = 0$ becomes the linear model. For nested models, the MAICE technique is the same as a log-likelihood ratio test.

Model-derived parameter estimates

MM fits for parasite counts obtained after treatment with mefloquine plus Fansidar plus artemisinin (Group 6) and Fansidar (Group 3) are shown in Figures 2 and 3 respectively. In individual patients, the lag time (from first count until drug-induced clearance begins) varied from 4 h in both the artemisinin-treated groups (2 and 6) to 12–16 h in those who received mefloquine, Fansidar or mefloquine plus Fansidar (Groups 1, 3 and 5 respectively). Estimates of k_2 are shown in Table 1; those in patients treated with Fansidar (Group 3) were significantly less than in the mefloquine plus artemisinin and mefloquine plus Fansidar plus artemisinin groups (4 and 6 respectively).

The parasitaemia-time curves by parasite stage for the patients treated with intravenous artesunate and quinine are shown in Figures 4 and 5 respectively. In the case of artesunate, R1 forms cleared non-linearly but in parallel with the much smaller numbers of R2 forms present when treatment was started. Minimal numbers of R3 forms were seen from 16 h onwards, consistent with maturation of less than 0·1% of R1 and R2 forms to the R3 stage. The constrained MM model applied to clearance of R1 and R2 forms gave k_2 values of 14·7 and 15·8 $\ln/\mu\mathrm{l}$ respectively; the model could not be fitted to limited R3 data. In the case of quinine, peaks of R2 and R3 forms occurred at 8 and 16 h respectively, consistent with the morphometric grading system used and maturation of approximately 80% of the initial R1 and R2 rings into R3 forms. R3 forms declined non-linearly after 16 h, with an estimated k_2 of 8·0 $\ln/\mu\mathrm{l}$.

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Table 1. *MAICE scores derived from linear* (*lin*), *log-linear* (*l-l*) *and constrained Michaelis–Menten* (*MM*) *models fitted to log-transformed parasite density data in six treatment groups and to pooled data*

		MAICE			MM parameter estimates			
Group	*n*	lin	l-l	MM	$P_0 \times 10^{-4}$	k_2 (ln/μl)	σ	Individual patients k_2 (ln/μl)
1	50	124·6	126·6	116·9*	5·0 [2·9–8·6]	29 [23–35]	0·72 [0·47–0·96]	21·7 (15·2–44·7)
2	36	119·6	118·2*	123·3	3·5 [1·8–6·9]	19 [14–24]	0·97 [0·62–1·30]	19·0 (13·9–20·5)
3	59	133·8	135·8	114·5*	4·6 [2·9–7·2]	16 [14–18]	0·50 [0·35–0·66]	14·0 (12·9–15·6)**
4	44	110·2	112·2	108·2*	1·6 [1·0–2·7]	33 [25–40]	0·66 [0·43–0·89]	26·6 (15·3–35·5)
5	50	119·5	121·5	118·0*	2·4 [1·3–4·7]	28 [20–36]	0·95 [0·60–1·30]	21·2 (7·5–39·5)
6	42	133·8*	135·5	134·2	2·3 [1·3–4·1]	33 [25–40]	0·83 [0·56–1·10]	24·2 (19·8–38·4)
1–6	281	794·6	796·2	780·8*	3·1 [2·4–4·0]	24 [22–26]	0·92 [0·79–1·00]	—

Parameter estimates and approximate [95% confidence intervals] for MM fits are shown. The number of points (*n*) is the total for all subjects in each group and all time-points. Asterisked values are the lowest for the three models fitted. Median (range) k_2 for the MM model fitted to individual patient data are also shown. $^{**}P < 0{\cdot}05$ vs. Groups 4 and 6.

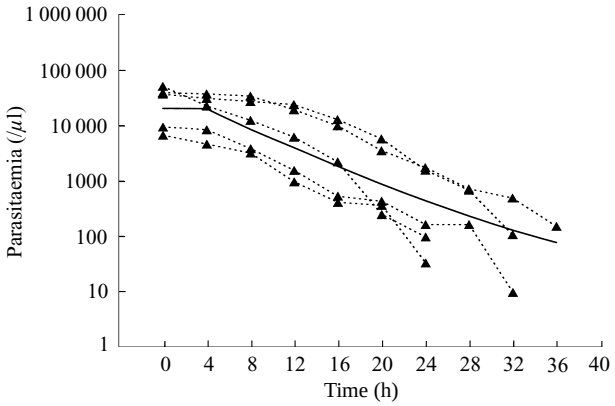

Fig. 2. Serial parasite counts from five patients who received mefloquine/sulfadoxine/pyrimethamine/artemisinin treatment at time 0 (dashed lines). The constrained Michaelis–Menten model fitted to the pooled data is shown as a solid line.

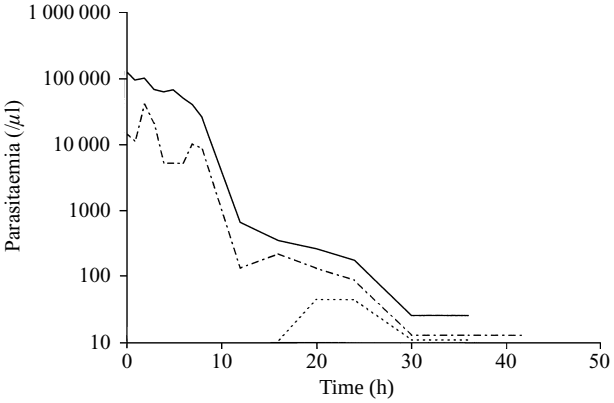

Fig. 4. Parasite densities for ring forms of *P. falciparum* assessed as at the R1 (——), R2 (— -) or R3 (----) stage of development after initiation of treatment with intravenous artesunate.

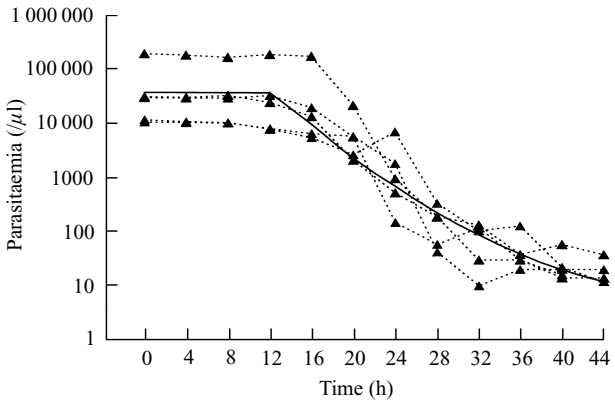

Fig. 3. Serial parasite counts from five patients who received Fansidar treatment at time 0 (dashed lines). The constrained Michaelis–Menten model fitted to the data is shown as a solid line.

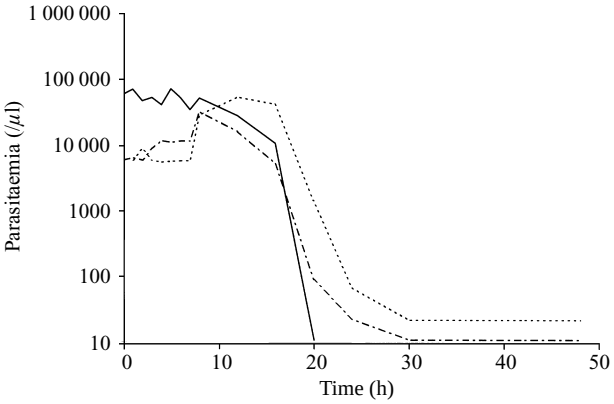

Fig. 5. Parasite densities for ring forms of *P. falciparum* assessed as at the R1 (——), R2 (— -) or R3 (----) stage of development after initiation of treatment with intravenous quinine.

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DISCUSSION

Although the sequestered biomass is an important determinant of some major complications of falciparum malaria [18], mature parasite forms cannot be quantified directly. Clearance of younger parasites from the peripheral circulation has, therefore, been used commonly as a general index of antimalarial drug efficacy. Nevertheless, *P. falciparum* rings and early trophozoites may themselves have a pathophysiological role especially if their density is high [19], whilst a logical aim of treatment is to damage and/or induce clearance of young forms so that they are no longer metabolically active and cannot mature to the point where they sequester. Thus, drugs which induce rapid peripheral blood parasite clearance (such as the artemisinin derivatives) may improve outcome through this property alone [20].

Because of the presence of an unquantifiable number of sequestered forms, changes in the peripheral parasitaemia with time in a patient with falciparum malaria can be complex, even after treatment has started. However, the models assessed in this report provide a mathematical description of the parasitaemia-time curve in a uniquely simple clinical situation, that in which only the clearance of a fixed density of young ring forms occurs. The comparison of plausible models applied in a descriptive sense has direct parallels in other areas of medicine [6, 21]. Given the fate of intraerythrocytic parasites in the second half of their life-cycle, more complex approaches would be needed to characterize clearance in other clinical contexts. Incorporation of drug pharmacokinetic parameters would further increase model complexity. Nevertheless, pharmacoepidemiological studies which examine the efficacy of different antimalarial drug regimens in different patient populations could benefit from the type of approach used in the present study.

The modified MM equation provides a simple but adequate model of the post-treatment clearance of young parasite forms in our patient groups, proving preferable to both a linear decline and a log-linear model. The latter, like the MM model itself, could be applied to a capacity-limited system. Although mechanisms governing parasite removal from the circulation remain incompletely understood, host immune and reticuloendothelial function (especially that of the spleen) are probably key factors. These mechanisms, as with enzyme kinetics and other biological processes [10], would be expected to display nonlinearity and saturation in patients with malaria especially at parasite counts higher than those in our uncomplicated patients.

The lag time between drug administration and the start of parasite clearance reflects both pharmacokinetic (absorption and distribution) and pharmacodynamic (rapidity of action and stage specificity) properties of the drugs and combinations used. Artemisinin derivatives are absorbed promptly and are highly active against young parasite forms [20, 22, 23], consistent with the short lag times seen in patients who received artemisinin alone or as part of combination therapy. Mefloquine, which is available only as an oral preparation, is more slowly absorbed, reaching peak concentrations 14 h or more after dosing [24]. Like the chemically-related drug quinine [22], mefloquine is primarily active against parasite stages later than rings. Fansidar acts on the most mature parasite forms [25]. These considerations account for the relatively long lag times after mefloquine and Fansidar given alone or in combination.

The MM parameter k_2 represents a more specific index of parasiticidal action than lag time. Nevertheless, as mentioned previously, the ability to clear parasites effectively will depend on most immunological and reticuloendothelial function as well as drug effects. Our patients were a relatively homogeneous group in terms of general health and previous malaria exposure. The similarity of k_2 values in most treatment groups could, therefore, indicate that clearance mechanisms in our patients were similar once parasite death has occurred from any drug regimen. The significant lower values of k_2 and their narrow range in Group 3 patients are in accord with previous data on the action of Fansidar. Because this combination therapy has little activity against circulating parasite forms [25], the pattern of a long lag time followed by relatively fast clearance may thus reflect continued maturation leading to sequestration rather than relatively late parasite death and removal from the circulation.

Support for this hypothesis comes from the more detailed analysis of parasite clearance in the patients who received intravenous therapy. Artesunate induced prompt clearance of R1 and R2 forms with minimal continued maturation of either to R3, consistent with *in vitro* data relating to drug stage specificity [20, 22, 23]. Rapid quinine loading did not prevent the majority of R1 rings present initially developing through the R2 stage into R3 forms, as might also be

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expected for mefloquine and Fansidar. The R3 forms cleared with a value of k_2 which was approximately half those of R1 and R2 in the patient treated with artesunate. This suggests that the mechanisms governing parasite disappearance were different during treatment with each drug, and that sequestration was a significant contributing factor in the case of quinine.

Persistence of small numbers of microscopically drug-affected young ring forms to 32 h and beyond (see Figs 2–5) suggests that all regimens interfered to some extent with normal parasite maturation to trophozoite stage and prevented cytoadherence. In the two patients treated intravenously, a proportion of R1, R2 and R3 forms persisted well beyond the point at which all should have matured to the next stage or sequestered. In some groups, parasites were still being cleared at a time ($>$ 36–40 h after initial drug administration) when a new generation of tiny ring forms would be entering the circulation in an untreated patient. The modified MM model accommodates this non-linearity in the terminal phase of the log parasitaemia-time curve whereas the other two models do not. It is interesting to speculate whether these persistent forms may still be viable and contribute to recrudescence, a well recognized phenomenon in the case of the artemisinin derivatives [20].

The times to 50% or 95% parasite clearance in the present patients would be functions of both lag time and k_2, and thus reflect a combination of drug, parasite and host effects. The time to 100% clearance is a function of the sensitivity of microscopy *per se* as well as the skill of the microscopist, and ignores completely the preceding changes in parasitaemia. These considerations indicate that conventional measures are inappropriate where detailed comparisons of antimalarial drugs, or of different groups of patients receiving the same treatment, are proposed. The modified MM model adequately fitted clearance curves with a long lag time and could, therefore, be considered in any patient, whether with a synchronous infection or not, to describe the terminal phase of parasite clearance when fluxes to and from the sequestered biomass are likely to have stopped.

Although the frequency at which parasite counts can be taken is limited by considerations of patient comfort and convenience, the modified MM model was easily applicable to individual and pooled data from 4-hourly sampling. Absolute errors in parasite counts are greatest at higher parasitaemias but the effect of this is minimized by the use of a logarithmic distribution. Consistent with these considerations, the 95% confidence intervals for pooled data in Table 1 for MM parameter estimates were relatively narrow. Techniques from time series analysis could have been applied to the parasite count data. However, such methods are most successful when there are relatively many sampling points. There are on average less than 10 sampling points per group of patients in the present data, which casts doubt upon the validity of a time series approach. In any case, a major advantage of the current analysis is that it uses standard numerical techniques (including nonlinear optimization and Runge–Kutta solution of differential equations) and readily available, nonproprietary numerical software such as FSQP.

Notwithstanding its possible use in the characterization of the terminal phase of any parasitaemia-time curve, the modified MM model could form part of a more complex integrated system including fluxes to and from the sequestered biomass in patients with asynchronous infections. As discussed in recent reports [4, 5], an expanded model would need to include an estimate of the parasite multiplication rate (the number of merozoites produced from a single mature schizont which successfully invade uninfected red cells) as well as a mathematical description of the effect of sequestration on parasite numbers in the peripheral blood. Such a systematic evaluation of parasite kinetics in patients with falciparum malaria should allow a better understanding of disease pathophysiology and may have direct application to clinical management.

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