Mathematical modelling of the chemotherapy of *Plasmodium falciparum* malaria with artesunate: postulation of 'dormancy', a partial cytostatic effect of the drug, and its implication for treatment regimens

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(*Received 3 December 1999; revised 16 March 2000; accepted 16 March 2000*)

SUMMARY

Although artesunate, one of the potent derivatives of the qinghaosu family of drugs for treating falciparum malaria, is already in use in the field, its therapeutic protocol has only been developed empirically by hit-or-miss. A pharmacokinetic–pharmacodynamic (PK–PD) model, required for creating such a protocol, is not straightforward. Artesunate presents extremely fast pharmacokinetics. As a result the stage specificity of its action must be treated explicitly. Also, use of standard PK–PD modelling fails to explain the clinical results. Our PK–PD modelling of its activity leads us to the postulation of the existence of a novel effect: a small fraction of the parasites, as a result of chemotherapeutic pressure, become cytostatic, or 'dormant'. At this stage, the parasite cycle is halted, making them unsusceptible to further dosing until wakening. This slows down the antimalarial activity of the drug, entailing either many frequent doses or an extended period of treatment and surveillance. Based on our modelling, we suggest a method for deciding on rational models of chemotherapy against falciparum malaria.

Key words: *Plasmodium falciparum* malaria, artesunate, mathematical model, pharmacokinetics, pharmacodynamics, dormancy.

INTRODUCTION

A main target of modern malaria research is to plan more effective chemotherapeutic regimens which will enable radical cure, thus treating the individual patient and preventing selection of drug-resistant strains from re-emerging parasites (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 (Ronn *et al*. 1996; Price *et al*. 1997).

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 strives to give a maximal permitted dose, and follows it up by additional doses when the drug concentration has dropped considerably. The number and spacing of doses are empirical, based on failure rates. A problem is that a patient with subdetection parasitaemia will be released as 'cured', although he still bears viable parasites that 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). With these data, a more effective procedure to decide upon drug use could be developed, if one possessed 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 PK in the body and of the pharmacodynamics (PD), that is, the concentration dependence and stage dependence of the drug's effect on the parasite number. The antimalarials that were derived from the Chinese drug qinghaosu are especially important because of two of their characteristics: (1) they have very fast parasiticidal action; (2) there is no proven resistance to chemotherapy with these drugs. As a result, there is increasing use of Artemisinin or artesunate (Ar) in clinical situations, and there is a tendency to introduce Ar also into geographical regions in which resistance to traditional drugs is a growing problem (but see Oduola *et al*. 1992).

Clinical data have demonstrated that artesunate

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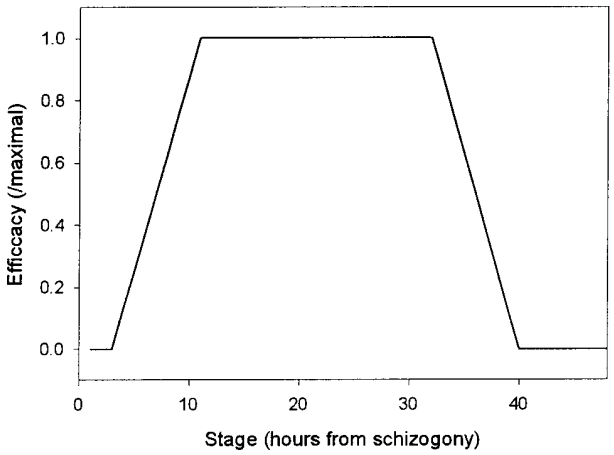

Fig. 1. Stage-dependent action of artesunate. The simulation of the maximal efficacy of artesunate for different stages of parasite development. Y-axis: the efficacy, that is the fraction of parasites at a given stage killed per h, under heavy drug concentration. X-axis: the stage, in hours after penetration of parasites into fresh erythrocytes.

has extremely fast PK, with an elimination half-time of about 0·7 h and the principal active metabolite dihydro-artesunate (DHA) too is also rapidly eliminated (Bunnag *et al*. 1991 *a–c*; White, 1994; Barradell & Fitton, 1995; Bethell *et al*. 1997; Batty *et al*. 1998). As a result, either drug is active for only a couple of hours following each dose. Classical PK suggests that the ideal dosage would be a set of very frequent doses, every few hours, keeping the drug concentration at steady-state (Rowland & Tozer, 1989). However, for Ar, treating patients at 12 h intervals is inferior to treating them at 24 h intervals, with the same total dose (Bunnag *et al*. 1991 *a*, *b*). We shall demonstrate how mathematical modelling can resolve this disagreement between a naïve theory and clinical practice.

METHODS

Mathematical model

The simple analytical PK–PD model that we used before for modelling the chemotherapy of chloroquine (Hoshen, Stein & Ginsburg, 1998) is not suitable for simulating the clinical data of Ar treatment. As opposed to its use for the long-acting chloroquine, for fast-eliminated drugs such as the qinghaosu derivatives we now have to consider the stage-dependence of drug action. Only parasites at the drug-sensitive stage will be killed during each dose period. Therefore, treatment will result in synchronization of the population by selecting certain subpopulations. One can utilize the synchronization induced by an initial stage-specific dose to time accurately the succeeding doses. This approach is the basis for our modelling of the chemotherapy of malaria with artesunate.

The PK–PD model for Ar chemotherapy is based on the following assumptions. (1) The parasite cycle lasts ordinarily roughly 48 h. Accordingly, it is convenient to divide the population into 48 boxes, each representing an age group of 1 h. All parasites are considered to be identical genetically and physiologically, except as to their age. The original population stage distribution may be set at will. We have in the simulations presented used a uniform initial distribution throughout. This conforms to the means of a random patient population. Each patient may have an asynchronous (Kwiatkowski, 1989) or a synchronous infection (Cheng *et al*. 1997). (2) We assume that the activity of the drug is stage-dependent, acting primarily on boxes 10–35 i.e. from the 10th to the 35th hour of the cycle from invasion of the erythrocyte. The stage-dependence of drug sensitivity, $f(s)$, is derived from the results obtained in cultures of *Plasmodium falciparum* (Geary, Divo & Jensen, 1989; ter Kuile *et al*. 1993). Using the opposing results of Skinner and coworkers (Skinner *et al*. 1996) does not cause great changes in the results of the model's simulations. Fig. 1 depicts the efficacy curve that we have used, assuming that the drug is always at maximal concentration. The actual kill rate at each hour, for each stage, is proportional to the drug concentration, which we derive from a simulated PK curve. Every hour, the fraction of parasites killed by the drug is subtracted from the content of the relevant box and the results in boxes 1–47 are moved to the following box. (3) The parasite population that reaches the end of its cycle (box 48 or merogony), is multiplied by a constant representing the multiplication rate, and this value is placed in box 1, to give the start of the next cycle. The rate can be set at will in cases where there is evidence that the drug affects the rupture of the schizonts or the invasion. (4) The maximal kill rate, k_{max}, is set to 1 for fully sensitive stages, that means that for saturating drug doses the whole sensitive parasite population will be killed in that hour. Indeed, inspection of clinical data from individual patients (Fig. 2) reveals that parasite numbers can decline by 10^2 to 10^3-fold every 6 h. The different times of onset of parasite clearance for individual patients implies that patients present bearing parasites at different stages in the cell cycle. Thus, for less sensitive stages k_{max} will be smaller than 1. (5) The PK data that we use are taken from clinical trials. A typical PK curve, from 11 patients, of the plasma concentration of artesunate and of DHA is shown in Fig. 3. Our model uses these PK data to take account of the time-dependent reduction of the drug concentration. (6) The actual kill rate for each box is equal to $(k_{max} \times C)/(C + K_m)$ where C is the drug concentration as found above, and K_m is the drug concentration that cause 50 % of the maximal effect. The details of the actual simulations used are presented in the Appendix.

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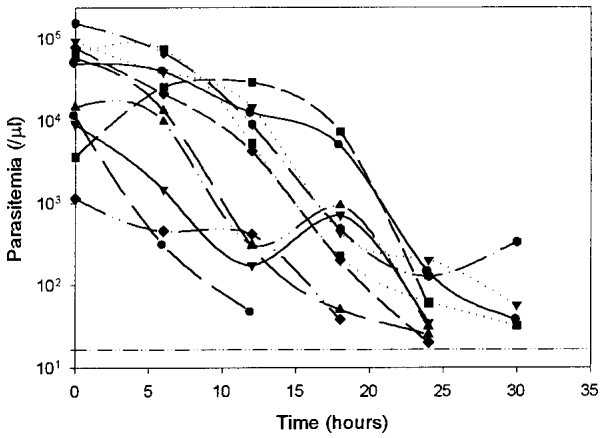

Fig. 2. Clinical parasitaemia (parasite/μl) for 11 individual adult Thai patients as a function of time (h). Treatment protocol: 300 mg/kg on day 0 and then 100 mg/kg on day 1.

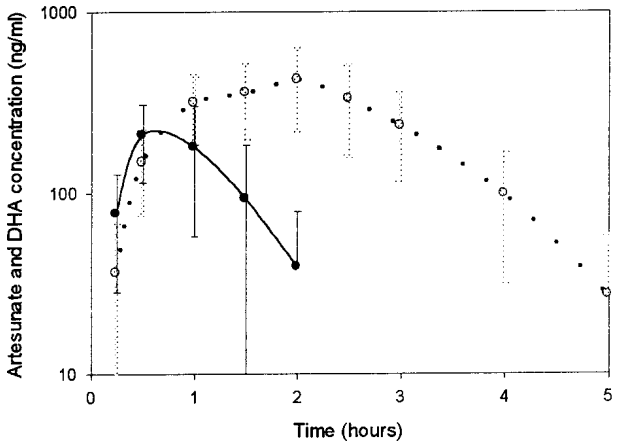

Fig. 3. Pharmacokinetics of artesunate. Plasma concentration (in ng/ml) of artesunate (———) and DHA (·····) after oral administration of 300 mg artesunate to 11 patients. Y-axis: the drug concentration. X-axis: the time after administration of the drug (h).

RESULTS

Clinical results do not fit classical assumptions of PK–PD models

With the simple model, we attempted to simulate the clinical results of drug treatment. As a data-base, we used clinical data on the fall in parasitaemia after drug treatment that were published previously (Karbwang *et al*. 1998; Na-Bangchang *et al*. 1998). Figure 2 depicts such data on 11 patients. The data show the variation that one might expect from a set of patients that come into the clinic with their disease at different stages of the parasite cycle. For most of the cases, the parasitaemia decreases from the time the drug is given, but in 2 cases it rises before it falls. In these cases, the parasite population was probably predominantly at the schizont stage, and thus mostly unobserved (due to sequestration in tissues) and unaffected, at the time of treatment. Only when some or all of the parasites are in the circulating

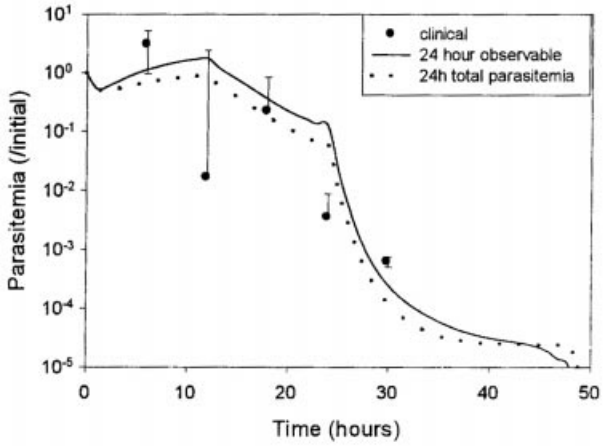

Fig. 4. Comparison of model with clinical data. Dots depict clinical data as means of the individual data points of Fig. 2 (with standard error bars), with protocol as above. (———) Simulated observable (circulating) ring-forms as fraction of initial measured parasitemia. (·····) Simulated total parasitaemia as a fraction of initial total parasitaemia. Y-axis: the parasitaemia at given time (as fraction of initial parasitaemia, for each category respectively). X-axis: the time of measurement, in hours after commencing chemotherapy. Each dose corresponds to an increase of blood concentration of 5 times K_m.

susceptible stages will we find immediate results. Inspection of these data reveals that in most cases the maximal clearance rate is the same. The value of this rate is so high, that it suggests that practically all parasites in sensitive stages are being killed. This suggests that most infections are synchronous and that the greater the synchrony the faster can be the maximal clearance rate. The precise time at which the maximal kill rate occurs reflects the stage at which the patient enters treatment. Averaging the data from all patients reduces the synchronicity of the population, approaching the asynchrony which we model. The appropriate simulation is then where we take equal initial populations in each box.

For our modelling, therefore, we used the mean value of the parasitaemia at each time interval of the data set of Fig. 2. The mean values $\pm$ S.D. are presented in Fig. 4. These mean values are used with the following reservation. When interpreting clinical results we must, clearly, distinguish between individual patients and mean values. In particular, we must pay attention to differences which represent only the initial admission condition, which may be purely incidental. For example, if the infection is synchronous, the initial shape of the parasitaemia versus time curve might reflect, to a large extent, the progress of the infection from observable to unobservable stage or *vice versa*, rather than the influence of the drug (White, Chapman & Watt, 1992). Only after a cycle or more would we be able to receive clear information about the efficacy of the treatment.

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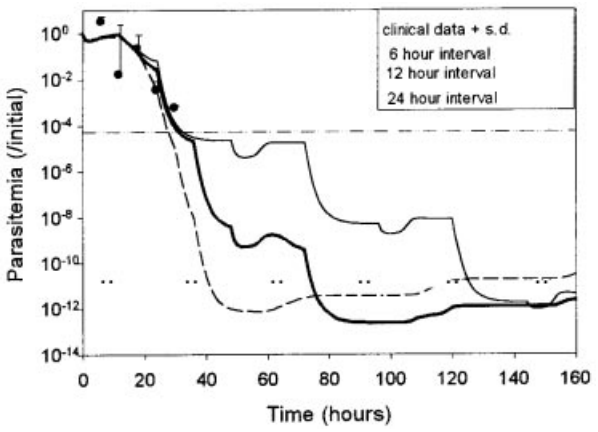

Fig. 5. Progress of parasitaemia under artesunate chemotherapy without dormancy. Different protocols were used for simulation: 7 doses given either every 6 h (– – – –), twice daily (———) or daily (———). Y-axis: the total parasitaemia as fraction of initial parasitaemia. X-axis: the time (h) after commencing treatment. Assuming no dormancy. Each dose corresponds to an increase of blood concentration of 5 times K_m. Horizontal dotted line: 1 parasite per body (10^{-11} of 1 % parasitaemia). Horizontal dash-dotted line: detection limit (10^{-5} of 1 % parasitaemia).

Figure 4 depicts the simulation of various drug dosing intervals and the mean clinical data. It is important to underscore the fact that sequestered infected erythrocytes are not seen. Hence, the disappearance of circulating parasites due to stage-specific killing that synchronizes the infection, will not accurately reflect the total parasite burden. Clearly, the fitting is quite good for these short times, irrespective of the treatment protocol, and thus confirms the choice of the numerical parameters used in the simulation.

In Fig. 5 we proceeded to extrapolate the predictions of the model to longer times, at subsequent figures. We could then determine whether radical cure is achieved, that is, whether the minimal parasitaemia attained by a given chemotherapeutic protocol would cross the single parasite per body value, shown as the dotted line on this and subsequent figures. If that value is passed, cure is ensured, otherwise recrudescence is to be expected. We show the curves appropriate for 3 protocols, treating with 7 equal doses every 6, 12 or 24 h (Fig. 5).

It is clear from the figure that all 3 dosing intervals are predicted to give radical cure, and the shorter intervals give this cure more rapidly. Thus, our model, at this stage of its development, predicted superior cure for shorter intervals. This is, however, in contradiction to clinical data (Bunnag *et al*. 1991 *c*), which showed improved outcome for longer (24 h) intervals. In these clinical studies, treatment with an equal number of doses at 6 and 12 h intervals resulted in higher rates of recrudescence than the 24 h intervals between consecutive treatments. Thus a model based on classical assumptions is incapable of predicting the observed clinical outcome.

Why should less frequent treatment be more effective? We suggest a modification of the simple model. We assume that the drug has an additional effect, in that it is cytostatic, in tandem with the killing; a certain fraction of the affected parasites, instead of dying, become 'dormant'. This is the 'dormancy fraction'. We define 'dormancy' as an arrest of the parasite cycle for a certain period, during which the parasites become unobservable and invulnerable to the drug. After this period, which we define as the 'dormancy period', they reappear in the population, at their previous stage. This emendation is justified, in general, by the observation that the parasite-cycle may be extended, as a result of Ar chemotherapy, from 48 h to 72 h or more (J. Karbwang, personal communication). The notion of 'dormancy' can also be derived from the simulation of PK in parasite cultures (Bwijo *et al*. 1997 *a*, *b*). These authors demonstrated that artesunate added to cultures of *P. falciparum* will cause radical clearance only when the treatment is extended in duration, even though not continuous. Thus treating 3 h daily for a week is more effective than treating continuously for 72 h.

The resulting curve of parasitaemia as a function of time, with this 'dormancy' effect taken into account, is depicted in Fig. 6. Here we find indeed that the 24 h interval between doses (thin solid line) gives a radical cure, while an interval of 6 h (dashed line) does not improve the ability of the model to predict recrudescence. Notice that the fraction of dormant parasites needed to accommodate the clinical data is rather small, 0·2 %.

Using mathematical modelling to improve therapeutic protocols

The advantage of mathematical modelling is the ability to suggest improved chemotherapeutic protocols. In therapy with Ar, the most important factor is the interval between doses. The best choice of interval will be highly dependent on the dormancy period and on the fraction of dormant parasites. Neither of these is, of course, known. Our approach here is to calculate, from the model, the minimal parasitaemia that is achievable using a given set of these two parameters. From these minima, we can choose the most valuable clinical protocols.

In Fig. 7 we display the simulated minimal parasitaemia, as a function of the dormancy period, at a fixed dormancy fraction of 0·2 %, achieved by 7 equal drug doses with either 12 or 24 h intervals between doses. We see that minimal parasitaemia depends on the dormancy period. If the interval between two consecutive drug applications is shorter than the dormancy period, we may expect treatment

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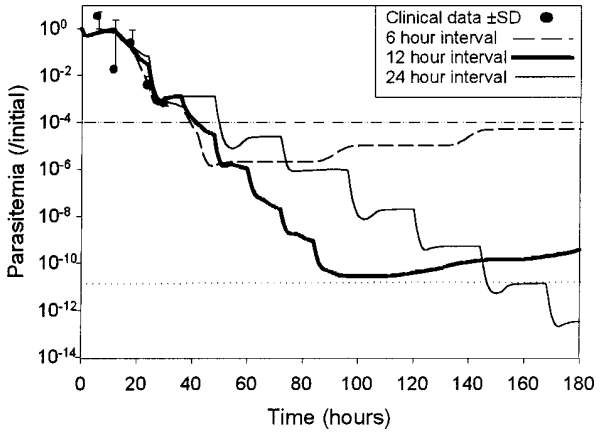

Fig. 6. Progress of parasitaemia under artesunate chemotherapy with dormancy. Total parasitaemia using artesunate chemotherapy assuming 0·2 % of parasites becoming dormant for 18 h. Comparing 3 protocols: 6 h interval (– – –), 12 h (———) and 24 h (———) interval. Each dose corresponds to an increase of blood concentration of 5 times K_m. Y-axis: the parasitaemia as a fraction of initial parasitaemia. X-axis: the time in hours from first dose. Horizontal dotted line: 1 parasite per body (10^{-11} of 1 % parasitaemia). Horizontal dash-dotted line: detection limit (10^{-5} of 1 % parasitaemia).

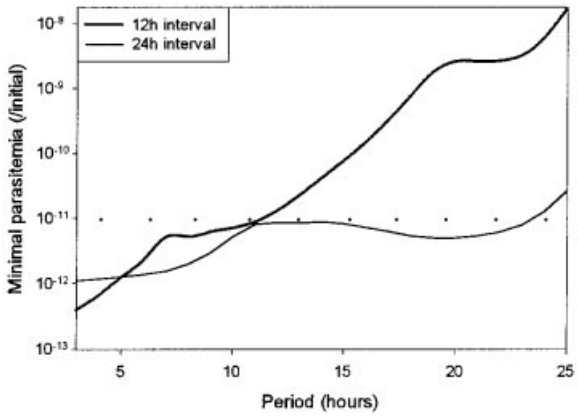

Fig. 7. Effect of dormancy period on outcome of treatment. Minimal parasitaemia (as fraction of initial parasitaemia) attained for 2 different regimens: 7 doses at 12 h (———) or 24 h (———) intervals, as function of dormancy period (X-axis, in hours). The dormant fraction was set to 0·2 %. Each dose corresponds to an increase of blood concentration of 5 times K_m. Horizontal dotted line: 1 parasite per body (10^{-11} of 1 % parasitaemia).

failure (as shown by the increase in parasitaemia after 12 h, when using the 12 h interval), while if the interval is longer we may expect cure. This result is to be expected. If the dormancy period is long, dormant parasites will be missed on the second dose. On the third dose a large fraction will still be at insensitive stages. Thus parasites will be hit once in 3 times at best, and the decrease in parasitaemia is limited.

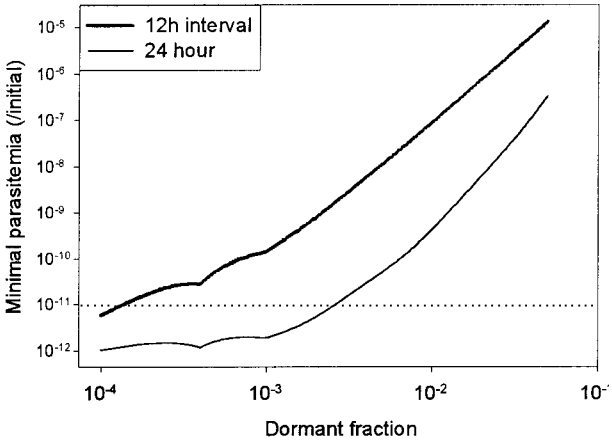

Fig. 8. Effect of dormant fraction on outcome of treatment. Minimal parasitaemia (as fraction of initial parasitaemia) as a function of the dormant fraction, assuming 7 doses, 18 h dormancy period and 12 h (———) or 24 h (———) interval. Each dose corresponds to an increase of blood concentration of 5 times K_m. Horizontal dotted line: 1 parasite per body (10^{-11} of 1 % parasitaemia).

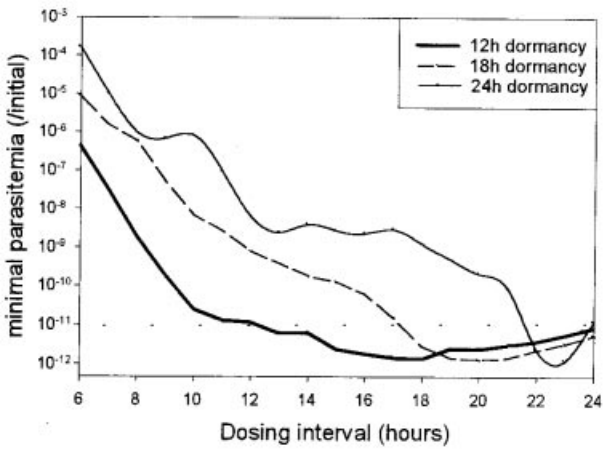

Fig. 9. The interplay between dormancy period and dosing intervals. Minimal parasitaemia achieved by 7 doses (as fraction of initial) as a function of the dosing interval (in hours). The dormancy period is 12 (———), 18 (– – –), or 24 h (———). Dormant fraction is set at 0·2 %. Each dose corresponds to an increase of blood concentration of 5 times K_m. Horizontal dotted line: 1 parasite per body level (10^{-11} of 1 % parasitaemia).

In the simulation shown in Fig. 7 we set the dormancy fraction at 0·2 %. If, now, we vary this fraction, at constant dormancy period of 18 h, as in Fig. 8, the minimal parasitaemia that can be achieved will change too. We find that the minimal parasitaemia is almost log-log-linear with the dormancy fraction. This strong correlation means that the dormancy fraction is the principal limiting factor in the kill-rate. In other words, the dormancy fraction is a limit on any form of Ar treatment of malaria. No such correlation is found between the minimal parasitaemia and dormancy period (Fig. 7).

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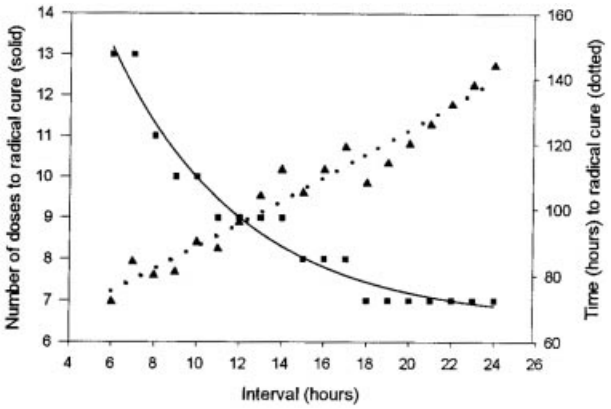

Fig. 10. The interplay between the number of doses and total time of treatment. Number of doses (left Y-axis in hours, ■, ———) and total time of treatment (right Y-axis in hours, ▲, ·····) required for radical cure, for various intervals (X-axis in hours). Assuming 18 h dormancy period and 0·2 % dormant fraction. Each dose corresponds to an increase of blood concentration of 5 times K_m.

At the present time we have no way of knowing the length of the dormancy period. However, the model allows us to simulate the effect on the final outcome of treatment of choosing different dosing intervals while holding the dormancy period fixed at various values (Fig. 9). The general conclusion that can be drawn from this simulation is that the dosing intervals should be somewhat longer than the dormancy period in order to achieve maximal effect. Consider the curve for 24 h dormancy period and the dosing interval of 10 h. The minimal parasitaemia achieved here is rather high, because after the first treatment, the 2 subsequent ones were given when parasites were dormant and insensitive to the drug. On the basis of the clinical results we can argue that the success of the 24 h interval dosing indicates that the dormancy period is shorter than 24 h, while the failure of the 12 h interval regimen demonstrates that the dormancy period is longer than 12 h.

If we manage to ascertain the dormancy fraction and period, we will want to find the shortest and/or cheapest possible chemotherapeutic protocols which will enable radical cure. The result of such an exercise is depicted in Fig. 10. Here we display the number of doses (left axis, solid points and curve) and total time to cure (in hours, right axis triangles and dotted line) needed when using a protocol of treating with a chosen interval length (hours, X-axis). The time to cure is the product of the interval and (the number of doses -1).

We find that the number of doses decreases with interval length. But the reduction in the number of doses in conjunction with the necessary increase in interval, would entail longer cure times, an unwanted consequence because of hospitalization costs or obvious difficulties in observed compliance in the field clinic. In general, every combination of number of doses and dosing interval that falls below the solid line will not result in total cure, whereas any such combination above the line will be superfluous in terms of costs of drug and hospitalization. Results of clinical trails support the connection between the number of doses and the treatment intervals as predicted by the model (Karbwang *et al*. 1994). If, however, our major objective is minimizing the total cure time, the best solution, as illustrated by the dotted line, is to give frequent treatment, which if administered every 6 h will entail full cure after 72 h.

As such a protocol is undesirable as to compliance, it might be preferable to administer 11 doses every 8 h, completing all treatment by 80 h. On the other hand, the use of 12 h intervals would require 96 h of treatment, as found by Bunnag *et al*. (1991*a*, *b*). Choosing the correct protocol would greatly economize on medical budgets, and thus allow full cure of patients, reducing the problem of increasing resistance. A more accurate knowledge of the dormancy period and the size of the dormant fraction should allow a further refinement of the model. Improvement in knowledge of the stage-dependence of drug action would also considerably assist in such refinement.

DISCUSSION

We have shown here that mathematical modelling can be used for the optimization of chemotherapeutic protocols. We show that in the particular case of artesunate (Ar), use of the classical approach that utilizes PK–PD data only, does not conform with clinical data. It is only through the judicious combination of empirical trial and error and eventual modelling, that the dormancy phenomenon has been conceived and can now be used for further improvement of chemotherapeutic regimens. To do so, we have to determine the controllable parameters.

Using the novel concept of dormancy in the pharmacology of Ar, we can explain the failure of high frequency dosage and the prolongation of the parasite cell cycle. We do not know if the postulated dormancy is equally effective at all stages of the parasite cycle, as we assumed here for simplicity. Nor do we know the length of the dormancy period or the size of the dormant fraction. Knowledge of these parameters could be obtained by carefully designed experiments in synchronized parasite cultures and could then be used for further refinement of protocols. It would also be very useful to investigate the biological mechanism of parasite growth arrest and desensitization to drug, as its deciphering may suggest means to prevent this phenomenon, and thus render Ar even more effective.

The phenomenon of dormancy should have a profound impact on the design of chemotherapeutic protocols as we have demonstrated in the simulations

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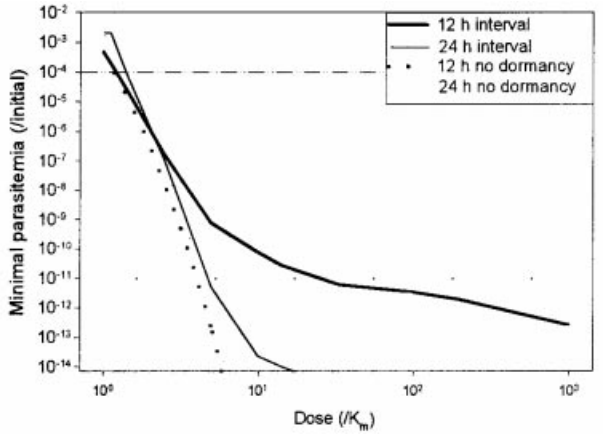

Fig. 11. Effect of dose on parasitaemia. Minimal parasitaemia attained by 7 doses as a function of the dose. (———), Treatment every 12 h. (———) Once daily treatment. Y-axis: minimal parasitaemia/initial parasitaemia. X-axis: drug concentration in units of K_m. Assuming 18 h dormancy period and 0·2 % dormant fraction or no dormancy. (·····) 1 parasite per body (10^{-11} of 1 % parasitaemia). Horizontal dash-dotted line: detection limit (10^{-5} of 1 % parasitaemia).

shown above. It bears on the choice of dosage intervals and on the total amount of drug that has to be administered for radical cure. The two last factors would have an impact in clinical pharmacology as to compliance and expense.

Although, in our model, the size of the dormant fraction is the most important parameter that determines the efficacy of drug treatment, it may well be an innate property of the parasite, which cannot be modulated at the present time. Whatever might be the cause of the dormancy, it could be a limitation on the kill rate per-cycle. If, as we found using our model, the dormancy effect is consistent with 0·2–0·5 % of the total kill, this would be the minimal survival per dormancy period. This means that, for total cure, the reduction of parasitaemia from 1 % erythrocytes infected to some 10^{-11} % (less than 1 parasite per body) would require at least 4 hits on the fully awakened parasite population at the sensitive stage. From the simulations depicted in Fig. 10, we see that the minimal number of doses needed for full cure is 7. This reflects the clinical result, which achieves radical cure by 7 daily doses of Ar (Bunnag *et al*. 1991*b*). This is a treatment requirement quite hard to meet, both because of compliance problems and because of hospital expenses. The minimal time for full cure is 72 h if 13 doses are given at 6 h intervals. This protocol has not been tested before. This might be because it too may seem prohibitive in terms of compliance and cost and the continuous presence of the drug may be toxic. The clinician must choose the protocol based on the trade-off between costs of hospitalization, and drug and compliance. The simulation shown in Fig. 10 could serve as a guideline for making such a choice.

A priori the dormancy fraction might be affected by the drug concentration. But from the fact that doubling of the dose from 600 to 1200 mg did not improve the final outcome (Bunnag *et al*. 1991*a*), we can surmise that the dose does not determine the size of the dormant fraction nor the dormancy period. This means that the dose currently used is saturating. Utilizing a Michaelis–Menten form equation, effect $= E_0C/(C+K_m)$, (where E_0 is the maximal drug effect; C is drug concentration and K_m is drug concentration producing half-maximal effect) suggests that the effect of increasing drug concentration will be diminished when $C \gg K_m$. This we depict in Fig. 11. We see that increase of dosage will be less effective than an increase in interval. Thus, we have left at our discretion mainly the number of doses and the timing of their application. For best results, the interval of dosage should be equal to, or just longer than, the dormancy period. The first treatment will synchronize the infection and it is, therefore, unimportant if the infection was initially synchronous or not. With the correct timing, the second and subsequent doses of drug will always meet a population of parasites at a drug-sensitive stage, and thus achieve a maximal effect. We have also shown how the necessary number of doses is determined by the dormancy period and dormancy fraction. This number obviously depends also on the frequency of treatments. The two parameters of dormancy period and dormancy fraction will determine the strategy of treatment, taking into account the relative costs of drug (low) and hospitalization (high), and the risk of non-compliance with frequent treatments or with long protocols.

In recent years, combination therapy of Ar with another drug, mostly mefloquine, has become more common (Looaresuwan *et al*. 1992; Hien *et al*. 1994; Karbwang *et al*. 1994; Nosten *et al*. 1994; Win, Than & Thwe, 1994; Alin *et al*. 1996; Bunnag *et al*. 1996; Na-Bengchang *et al*. 1996; Hung *et al*. 1997; Looaresuwan *et al*. 1995; Price *et al*. 1998*a*, *b*; Sabchaeron *et al*. 1998; Vanvugt *et al*. 1998), since it is more efficacious, in terms of compliance and time of hospitalization and of cure rate, as compared with monotherapy. But even then, cure is not absolute. It could well be that the efficacy of the combination could be due to sensitivity of the dormant parasites to mefloquine. The pharmacological basis of this successful combination is unknown. The model suggested by the present work expanded to include treatment with mefloquine (with its own PK–PD properties) may resolve the nature of the interaction and suggest improved treatment protocols. The theoretical basis of such combination therapy is currently being investigated in our laboratory.

This investigation received financial support from the Director's Initiative Fund of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR).

APPENDIX

The simulations in this paper were performed on a MS-Excel 97 worksheet. Forty eight boxes in column A were assigned, each corresponding to 1 of the 48 h intervals in the parasite life-cycle of 48 h. Each box was given an initial value, all being equal to 1 when representing asynchronous broods of parasites, but varying for synchronous broods. The net growth rate per cycle, r, in the absence of drug was set at a given value, usually at $r = 5$. In the absence of drug, the simulation procedure is, for every simulated hour, to move the contents of each box unchanged into the next column, say B, one row below, except for stage 48, which is multiplied by r and placed in the first box of column B. This procedure is repeated, simulated hour after simulated hour, to create columns C, D etc until the end of the time-period being simulated.

When drug is present, some parasites are killed. To simulate this, at the end of every simulated hour we subtract from each box a number equal to the fraction of parasites killed. This fraction is calculated from the efficacy of the drug at that stage of the parasite cycle and the concentration of drug in the host by that time. The efficacy of the drug at each hour of the cycle is given by Fig. 1. The efficacy increases linearly between stage-boxes 3 and 11 from 0 to 1, stays constant at 1 from box 11 until box 32, and then decreases linearly to value 0 during boxes 40–48 and boxes 1–2. The concentration of drug in the host at any time is calculated using a simplification of the pharmacokinetics that were depicted in Fig. 3. The simplification is to treat the PK as a simple 1-compartment model, with the drug being administered instantaneously and eliminated exponentially. For DHA, the principle active metabolite, the half-time of elimination is roughly $t_{1/2} = 1$ h. Thus, we allow the concentration to decrease by 50% between the consecutive 1-h time-steps. At the dosage times, the concentration is increased instantaneously by a constant value (in our case by a single unit). This concentration (C) we used in the simulations.

To evaluate pharmacodynamic efficacy, we use the Michaelis-Menten equation. We divide the concentration C by $C+K_m$ (with K_m, equivalent to IC_{50}, being a constant given by the concentration that causes 50% of the drug's maximal effect), to obtain a 'reduced' (unit-less) concentration. The result of multiplying the reduced drug concentration by the stage-specific efficacy will give us the stage-specific time-dependent kill-rate. We multiply by the box's population to receive the number of parasites in this box killed during 1 h. We subtract this value from the box's population before transferring to the next column and next row.

Where we assume dormancy, we need to add an appropriate term for the number of wakening dormants. This number is calculated as follows: a certain fraction (the dormancy fraction) of the parasites 'killed' do not vanish eternally from the calculation. They are re-added into the worksheet at the same stage as when they were removed from it, awakening after a time equal to the dormant period. We assume that this dormant period is not a sharply defined time but is best described by a Gaussian spread $r \sim N(p,2)$, with r being the quantity awakening and p the dormancy period in hours, with the sum of r equal to the total number of dormant parasites.

We display the total population of parasites as the sum of boxes 1–48, and the observable or circulating population as the sum of boxes 1–24. We find the minimum of these numbers, under any particular simulation regime, as a function of time, to compute the minimal parasitaemia attained.

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https://doi.org/10.1017/S0031182099006332 Published online by Cambridge University Press

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