Mathematical modelling of malaria chemotherapy: combining artesunate and mefloquine

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SUMMARY

Clinical data on the use of artesunate combined with mefloquine in a variety of treatment regimens and parasite loads in Thailand were modelled on the basis of experimentally determined pharmacokinetic data. The model assumed no pharmacodynamic interaction between artesunate and mefloquine, but that the parasites were already resistant to mefloquine. Predictions of the model accorded well with the data. In particular, in accordance with clinical observations, the model showed that monotherapy with either drug failed to cure at moderate parasitaemia, yet such patients could be treated effectively with the combination of 3 days of artesunate + mefloquine. For high levels of parasitaemia, 5 days of artesunate+mefloquine were needed. Simulations were also performed for situations of lower resistance to mefloquine and for the immune human populations found in Africa. The importance of mathematical modelling of combination therapy is borne out by this study and suggests its wider application for other drug combinations.

Key words: artesunate, mefloquine, combination therapy, PK–PD model, falciparum, resistance, immunity.

INTRODUCTION

The development of resistance to anti-malarial drugs is causing massive pressure on the world's health budgets. The gradual loss of relatively cheap chemotherapeutic agents, such as chloroquine (CQ) (Sowunmi & Oduola, 1995) and pyrimethamine-sulfadoxine (Fansidar®), to resistance (Hurwitz, Johnson & Campbell, 1981; Mengesha & Makonnen, 1999) necessitates the use of more expensive drugs such as mefloquine (MQ) (Nosten *et al*. 1991; ter Kuile *et al*. 1992; Looareesuwan *et al*. 1997, 1999) and, recently, the qinghaosu derivatives (van Agtmael, Eggelte & van Boxtel, 1999). Qinghaosu is the anti-malarial component of *Artemesia annua* (Linnaeus) used for centuries by traditional Chinese medicine (Jiang *et al*. 1982; Barradell & Fitton, 1995) to cure malaria-like symptoms. The current emphasis of the WHO is on the exclusive use of combinations of anti-malarial drugs in order to minimize the emergence of drug resistance. Not every combination succeeds to meet this goal.

MQ was developed in the early 1970s and clinical trials began in Thailand in 1974. From 1984, the drug was employed there as Fansimef® (a combination with pyrimethamine and sulfadoxine). By 1990 the efficacy of this combination declined to such low levels that it had to be discontinued. MQ monotherapy by a single dose of 15 mg/kg and later, due to increased resistance, 25 mg/kg became standard (ter Kuile *et al*. 1992; White, 1992). From 1994 onwards, administration of the drug, even at these doses was halted in Thailand, and MQ was applied only in addition to qinghaosu derivatives (Nosten *et al*. 2000).

Meticulously run clinical trials on MQ and artesunate (Ar) separately and together, have produced a wealth of data in terms of pharmacokinetics and dose response. These data have provided the basis for treatment protocols. With the advent of mathematical modelling for the chemotherapy of malaria, it seems desirable to extend the models for monotherapy to drug combination. In previous papers the pharmacokinetic–pharmacodynamic (PK–PD) effects of Ar (Hoshen *et al*. 2000) and MQ (Simpson *et al*. 2000; Hoshen, Stein & Ginsburg, 2001) have been analysed. In this paper a mathematical model is formed to account for the action of MQ in combination with Ar, and to compare the simulated results to published clinical data in Thailand. This validation may allow the expansion of modelling to other drug combinations and other geographical areas.

METHODS

The model is based on the assumption that the PD effects of MQ and Ar on the parasite population *in vivo* are independent of one another. It is assumed

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that the 'dormancy' effect of Ar† does not influence MQ efficacy. The small reported *in vitro* PD synergy between Ar and MQ (Fivelman *et al*. 1999) will not be of great importance *in vivo* due to the short time during which the parasites are exposed to therapeutic concentrations of Ar, and hence to the combination.

The PK–PD parameters used for Ar have been derived in a previous paper (Hoshen *et al*. 2000) where they were validated by comparison with clinical studies. In brief, it is assumed that the parasite population is reduced 10^4-fold every 48 h or 99·99 %/cycle (White, 1997) and that the half-time of drug elimination is so rapid (Sidhu *et al*. 1998) in comparison to that of MQ, that it can be neglected. The decrease in bioavailability of Ar due to repetitive dosing was not taken into consideration since it has been shown that Ar doses are anyway higher than necessary (Ashton *et al*. 1998).

MQ, on the other hand, is a slowly eliminated drug, with a half-time of days and weeks (Karbwang & Na-Bangchang, 1994; Simpson *et al*. 1999). Thus, usually, only a single dose of MQ is administered, usually divided in 2 treatments to assure bioavailability. The parasite population drops 10^2-fold in 48 h or roughly 99 %/cycle (White, 1992, 1997). In a previous paper (Hoshen *et al*. 2001) it was established that the maximum kill rate constant k_1 for MQ (where the fractional reduction in parasitaemia during time t is exp $(-k_1 t)$) is 3·3/day. The MQ PK model is based on the population PK model of Simpson *et al*. (1999), studying whole-blood PK for various groups, among which a group of 31 patients (group C) received 25 mg/kg of MQ in a divided dose as well as 3 days of Ar. We used this population since most clinical studies that we are attempting to model are based on a split dose protocol. In fact, there is very little difference in the calculated PK parameters for those given single or divided dose. For this group of patients the apparent clearance (Cl/F) was established as $1{\cdot}027 \pm 0{\cdot}083$ L/kg/day and the apparent volume of distribution (V/F) was given as $13{\cdot}99 \pm 0{\cdot}70$ L/kg. On an MS Excel 2000© worksheet we formed a random distribution of 20 pairs of Cl/F and V/F assuming normal distribution of each, based on the above results. Division of Cl/F by V/F will supply the drug elimination rate constant $\boldsymbol{k}$. The value of $C_{\max}$ (the maximal drug concentration in the blood) is obtained by dividing the dose by apparent volume of distribution. By this method 20 random sets of pairs of $C_{\max}$ and $\boldsymbol{k}$ were obtained and served as the basis for single exponential PK simulations. The MQ whole blood concentration was now calculated for each set at 0·1 day intervals (neglecting absorption kinetics).

† Dormancy is the term we have used to describe the temporal suspension of the parasite's life-cycle that is induced by Ar treatment (Hoshen *et al*. 2000).

The initial parasitaemia was assumed to be identical for each random sample. The PD was based on the Michaelis-Menten (M-M) dynamics in which the population increased with a rate constant $\boldsymbol{a} =$ 1/day and decreased with the MQ M-M dynamics. The kill rate constant k_C at an MQ concentration C is given by $k_C = k_1 C/(C+K)$, with k_1 the maximal kill rate (at saturation), C the instantaneous plasma drug concentration and K the concentration at which 50 % of effect is attained. The Ar kill rate constant (99 % kill/day) is added (in the exponent) to k_C. The instantaneous PD of MQ for the parasitaemia, P, as a function of time, t, is governed by the differential equation $dP/dt = \boldsymbol{a}P - k_C P$. The parasitaemia at any given moment is established by numerical integration of this differential equation at 0·1 day time-steps. In all the figures in this report, time zero on the X-axis is the time at which the maximum plasma concentration of the drug is reached. The clinical parasite detection limit is usually 50/μl or $2{\cdot}5 \times 10^8$ parasites/patient. The full clearance, or radical cure, is evaluated as 2×10^{-7}/μl, that is less than 1 parasite in a body blood volume of 5 l. Since the notion of MIC (minimal inhibitory concentration) is used extensively in the literature, it is worth relating it to the other parameters of the model mentioned above. This is an unobservable parameter and in the present context it is defined as the whole blood concentration at which the kill rate is equal to the growth rate. Mathematically, $\text{MIC} = K/(k_1/\boldsymbol{a} - 1)$ (Hoshen, Stein & Ginsburg, 1998).

In the simulations, the probability of recrudescence was evaluated by verifying if the parasitaemia remains above 1 parasite per body, leading to an observable clinical recrudescence. Obviously, the higher the parasitaemia at the minimum, the shorter is the time for recrudescence.

RESULTS AND DISCUSSION

The results of a survey of clinical data reported in the literature of the effect of MQ co-administered with Ar appear in Table 1. It is clear that high values of recrudescence are found when the initial parasitaemia is high and the number of cycles of Ar treatments is low. Modelling of the effect of combined MQ-Ar chemotherapy must be able to account for these data.

Fig. 1 depicts a simulation of the progress of parasitaemia after a 3 day treatment with 4 mg/kg/day of Ar and 25 mg/kg MQ given at time zero. The initial parasite load was set at 6×10^{11} parasites/patient (~ 3 % parasitaemia). The various lines represent the computation based on normal distribution of the two PK parameters, apparent clearance rate and volume of distribution, as described in the Methods section. The rapid fall in parasitaemia depicted in Fig. 1 would indeed show

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Table 1. Summary of clinical trials of artesunate + mefloquine treatment

Source*	Year†	Parasite load‡	Recrudescence§	Notes
Luxemburger 94	1991	Low 1600–4300	19 (28)	1 day Ar 10 mg/kg
Karbwang 94	?	Medium 31000	34 (33)	1 day Ar 3 mg/kg
Luxemburger 95	< 1995	High 350000	30 (28), 33 (42)	3 days Ar 4 mg/kg
Luxemburger 95	< 1995	Low	4 (28), 6 (42)	3 days Ar 4 mg/kg
Nosten 95		High	2 (28)	3 days Ar 4 mg/kg
Price 95	1994	3750–6800	4 (28), 6 (42), 14 (63)	3 days Ar 4 mg/kg
Bunnag 96	1992–1994	180–46 800	2 (42)	1 day Ar 6 mg/kg
Price 97	1992–1995	Low 6000	8 (28), 14 (42)	3 days Ar 4 mg/kg
Price 97	1992–1995	High > 175 000	37 (42)	3 days Ar 4 mg/kg
Price 98	1993–1995	High ~ 350000	2 (28), 6 (42)	5 and 7 days Ar 4 mg/kg No difference in outcome between 5 and 7 days
Van Vugt 98		Low 4500	6 (63)	3 days Ar 4 mg/kg
Sabchareon 98	1995–1996	1000–20000	0 (28)	3 days Ar 6 mg/kg
Price 99	1994–1995	Low 5000–10000	3 (42)	3 days Ar 4 mg/kg
Na-Bangchang 2000	?	Low 12000	0 (42)	DHA + Lariam
Simpson 99	1994–1995	Low 3000	2 (28)	3 days Ar 4 mg/kg

* Only first author and abridged year of publication is shown.
† Years during which trials were conducted.
‡ Parasite load is given as infected cells/μl of whole blood.
§ Recrudescence is shown as % treatment failures monitored at (day) of follow-up.

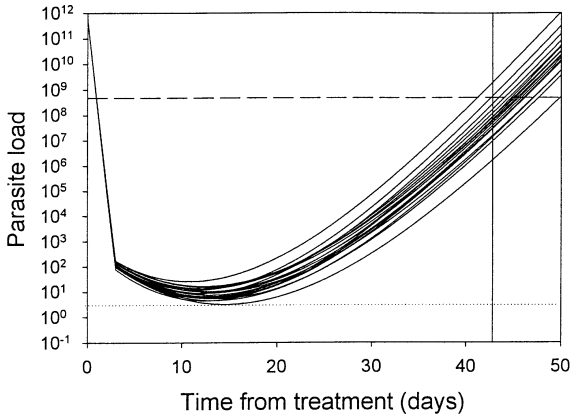

Fig. 1. Simulated parasite load after treatment with 25 mg/kg MQ and 3 days Ar at 4 mg/kg. Initial parasite load 6×10^{11}/body, MIC = 700 ng/ml, $k_1 = 3{\cdot}3$/day and $\boldsymbol{a} = 1$/day. Each line represents the response to a randomly generated pair of PK parameters, as described in the Methods section. Horizontal dashed line represents the detection limit for parasitaemia (50 parasites/μl), and dotted line marks the 1 parasite/body level. The vertical line marks the 42nd day after initiation of treatment.

some variation if we had made the assumption that the Ar kill rate constant varied. We could have done this but it would have shown up as a pencil of lines radiating from time zero, yielding a small fuzziness of the initial drop without significance for the overall shape of the curve nor for the time of recrudescence between 30 and 40 days later. For the PD, a MIC = 700 ng/ml whole blood was chosen. When the calculated parasitaemia lines rise above the detectable parasitaemia level, a recrudescence is observed. When the calculated parasitaemia falls below the 1/body level, a radical cure is achieved. (In the simulations, 'growth curves' beyond this point are not shown). The prediction suggests that no patient was cured of parasites by this treatment (no patient parasitaemia fell below 1/body) and a substantial fraction of them were observed to be recrudescent by 42 days post-treatment. This accords well with the report of Price *et al.* (1997) where with this regime and an initial parasite load of $7{\cdot}5 \times 10^{11}$ parasites/patient, some 37% recrudescence was reported at 42 days. Indeed the value of MIC was chosen so as to accord with this clinical picture. The simulation accords also with Nosten *et al.* (1995) who reported a 2% recrudescence from high parasitaemia at 28 days. From Fig. 1 no recrudescence is expected at this time of follow up. It should be noticed that no PD synergism between the two drugs is assumed, and the only independent variable is the MIC value.

This prediction does not, however, fit the clinical data of Luxemburger *et al.* (1995), although collected in the same location, where 30% recrudescence was reported by day 28 and 33% by day 42. In other words, between days 28 and 42 only 3% additional patients recrudesced, suggesting that the most MQ-sensitive parasites were already eliminated by day 28. In order to account for these results, an attempt was made to study the effect of variation in the MIC values over a wide range. Fig. 2 depicts the results of these simulations, where, for simplicity, only the average values of the PK parameters of the Simpson *et al.* (1999) paper were used. The values of MIC used are indicated on the graph. The predictions are of interest in themselves: it would appear (looking at this figure and Fig. 1 together) that for all values of

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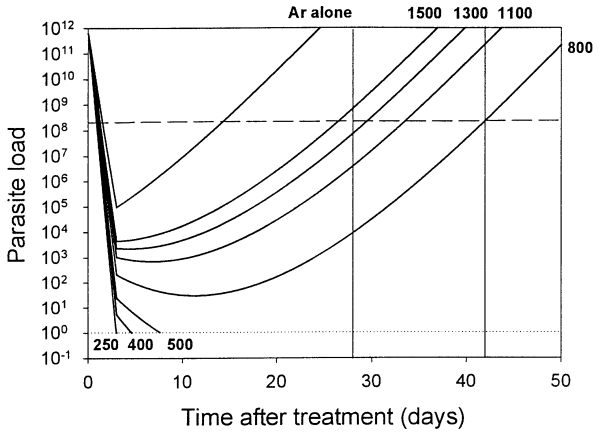

Fig. 2. Effect of parasite resistance to MQ. Curves represent the simulated parasite load after treatment with 25 mg/kg MQ and 3 days Ar at 4 mg/kg. Initial parasite load 6×10^{11}/body, $k_1 = 3{\cdot}3$/day and $\boldsymbol{a} = 1$/day and different values of MIC were used: 250, 400, 500, 800, 1100, 1300 and 1500 ng/ml. Monotherapy with Ar alone is also shown. For the sake of clarity the curves were generated using only mean PK values. Horizontal dashed line represents the detection limit for parasitaemia (50 parasites/μl), and dotted line marks the 1 parasite/body level. The vertical lines mark the 28th and the 42nd day after initiation of treatment.

MIC > 700 ng/ml recrudescence is expected sooner or later. Whenever MIC $\leqslant$ 500 ng/ml the combination treatment results in radical cure. Notice that even with the highest MIC value used, the combination is advantageous over Ar monotherapy ('Ar alone' in the figure). No simulation using a single MIC predicts, however, the Luxemburger *et al*. (1995) results of only a small increase in recrudescence between days 28 and 42. The results can be explained by assuming the presence of at least 2 populations of parasites, distinguished by high or low MIC values.

In recent years, in the refugee camps of Thailand, low transmission in conjunction with early diagnosis and combination therapy has led to the partial restoration of sensitivity to MQ (Nosten *et al*. 2000). This is reflected in a measured decrease in the IC_{50} from $\sim$ 50 ng/ml in 1995 to $\sim$25 ng/ml in 1999 (Brockman *et al*. 2000). In the present simulations, this must be paralleled with a decrease of 50 % in the value of K, and hence MIC. If, previously, Price *et al*. (1995) reported substantial (14 %) recrudescence with 3 cycles of Ar combined with MQ, recrudescence is now reported to be close to zero with the same protocol (Nosten *et al*. 2000). This is reflected in Fig. 2 where a decrease of MIC from 800 ng/ml, where late recrudescence is predicted, to 400 ng/ml, where radical cure is expected and found.

Another feature conspicuous in the data of Table 1, is the importance of the number of cycles of Ar treatment. Compare for example Karbwang *et al*. (1994) and Luxemburger *et al*. (1994) (1 cycle), Price *et al*. (1997) (3 cycles) and Price *et al*. (1998) (5 cycles). It is clear that the recrudescence rate decreases with the number of Ar treatment cycles. The additional simulations depicted in Fig. 3, where the number of cycles of Ar treatment is the variable, show a good agreement with the clinical outcome. We see that 1 day of combined Ar-MQ treatment is quite insufficient for cure of MQ resistant parasites. Three cycles of Ar may still result in a late recrudescence (beyond the 42 day follow-up period). Only 5 cycles of Ar provide radical cure. The failure of 5 cycles of Ar alone to reach this result (dashed falling and rising lines in Fig. 3), underscores the importance of MQ as an adjunct drug, even in areas of drug resistance.

The last important factor in the outcome of this particular drug combination, is the initial parasite burden. A high parasite burden can be considered to be above 200000 infected cells/μl blood which is a parasite load of some 6×10^{11} parasites/patient. Low parasitaemia will be around 3000 infected cells/μl blood, or a parasite load of 10^{10}, while a very low parasitaemia will be 200 parasites/μl blood or a parasite load of 6×10^{8}. From Table 1, it can be seen that high parasitaemia is associated with high recrudescence rates. To model this phenomenon, the initial parasite burden was set at various levels, and the outcome of treatment of 3 days 4 mg/kg Ar in combination with 25 mg/kg MQ simulated. Results shown in Fig. 4, where the initial parasite burden is shown for each simulation, indicate that indeed this treatment very effectively eradicates infections of low or even medium parasitaemias, but that recrudescences are predicted for heavier infections, as observed in the field (see Table 1 and Luxemburger *et al*. (1995); Price *et al*. (1995), (1997), (1999); van Vugt *et al*. (1998), Simpson *et al*. (1999), Na-Bangchang *et al*. (2000)), Obviously, the protocol of Sabchareon (1998),where 6 mg/kg were given for 3 days and followed up at 28 days, also accords with the simulations. Referring to Figs 2 and 4 it can be seen that the predictions accord also in a general fashion with the data of Luxemburger *et al*. (1994), Karbwang *et al*. (1994) and Bunnag *et al*. (1996), where low or medium parasitaemias were treated for 1 day at levels around those that were chosen for the depicted simulations, but where we have not performed the precise simulations needed. In addition, in Fig. 4 it is observed that in accord with clinical observations, monotherapy for 3 days with Ar fails even for low parasitaemia. Hence, the combination with MQ is essential for successful treatment.

Since the model predicts well the clinical outcome of combination therapy in the South-East Asian context, it may be worth using it in the setting of holoendemic malaria, such as can be found in Africa. In such a situation the adult population is often immune, i.e. parasite growth is inhibited by the host's immune system, and infection is less danger-

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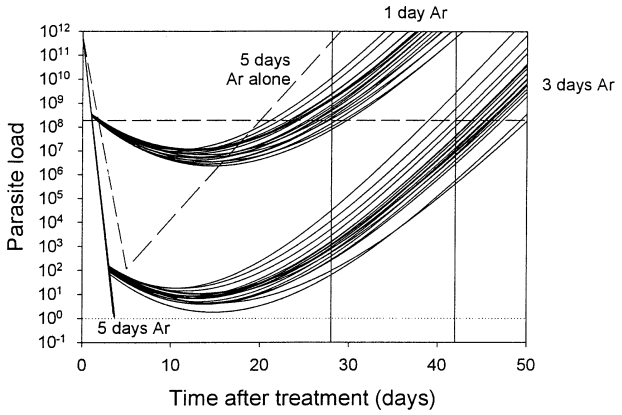

Fig. 3. Effect of number of Ar treatment cycles. Curves represent the simulated parasite load after treatment with 25 mg/kg MQ and 1, 3 or 5 days of Ar treatment at 4 mg/kg. Initial parasite load 6×10^{11}/body, $k_1 =$ 3·3/day and $\boldsymbol{a} = 1$/day and MIC = 700 ng/ml. Monotherapy with Ar alone for 5 days is also shown. Each curve represents the response to a randomly generated PK, as described in the Methods section. Horizontal dashed line represents the detection limit for parasitaemia (50 parasites/μl), and dotted line marks the 1 parasite/body level. The vertical lines mark the 28th and the 42nd day after initiation of treatment.

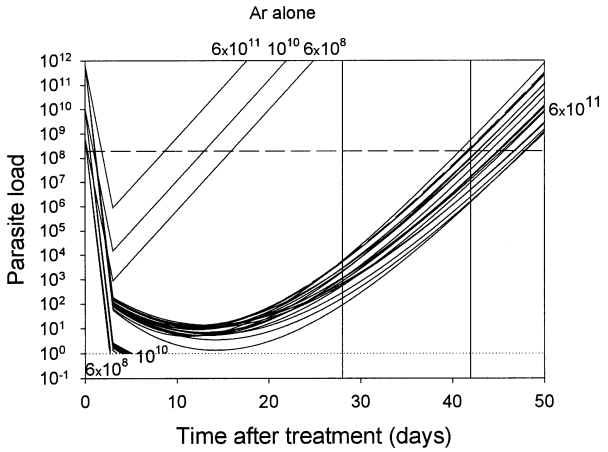

Fig. 4. Effect of initial parasite load. Curves represent the simulated parasite load after treatment with 25 mg/kg MQ and 3 days of Ar treatment at 4 mg/kg. Initial parasite load was set at 6×10^{11}/body, 10^{10} or 6×10^{8} for hyper-, high or low parasitaemia $k_1 = 3{\cdot}3$/day and $\boldsymbol{a} = 1$/day and MIC = 700 ng/ml. Monotherapy with Ar alone at each level of parasitaemia for 3 days is also shown. Each curve for the combination represents the response to a randomly generated PK, as described in the Methods section. Horizontal dashed line represents the detection limit for parasitaemia (50 parasites/μl), and dotted line marks the 1 parasite/body level. The vertical lines mark the 28th and the 42nd day after initiation of treatment.

ous to the host. The relevant parameter that should be changed in order to simulate this situation, is the parasite growth rate $\boldsymbol{a}$ which, in the following simulation, is equated to 0·1. Although the value of

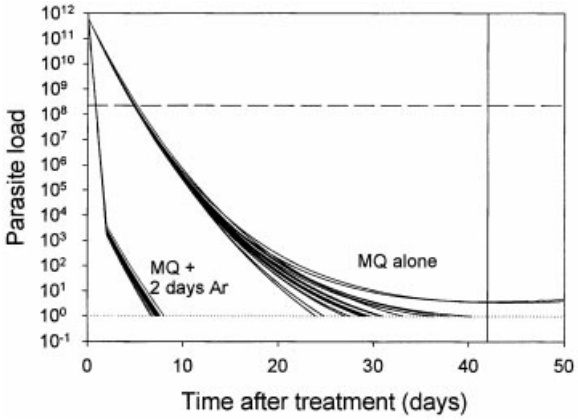

Fig. 5. Predictions for the African setting. Curves represent the simulated parasite load after treatment with 25 mg/kg MQ with 2 days of Ar treatment or without Ar. Initial parasite load was set at 6×10^{11}/body, $k_1 = 3{\cdot}3$/day and $\boldsymbol{a} = 0{\cdot}1$/day MIC = 50 ng/ml. Each curve represents the response to a randomly generated PK, as described in the Methods section. Horizontal dashed line represents the detection limit for parasitaemia (50 parasites/μl), and dotted line marks the 1 parasite/body level. The vertical line marks the 42nd day after initiation of treatment.

K (which is intrinsic to the parasite itself) may be as high as in the South-East Asian setting, the MIC will be much lower, since $\text{MIC} = K/(k_1/\boldsymbol{a} - 1)$. Results depicted in Fig. 5 simulate an extreme case of high resistance to MQ ($K =$ 1600 ng/ml, which is parallel to a MIC of 700 ng/ml in South-East Asia) with hyperparasitaemia. The corresponding MIC, with $\boldsymbol{a} = 0{\cdot}1$ is 50 ng/ml. Monotherapy with MQ with 25 mg/kg is borderline curative. In the treatment failure cases, recrudescence will be very late (months, not shown). In combination with 2 cycles of 4 mg/kg Ar, however, full cure is demonstrably achieved. Obviously, with the lower prevalent parasitaemia and lower resistance existing in this population in Africa, monotherapy with MQ will still be highly effective. However, when most parasites become resistant to MQ, combination therapy will have to be used in the case of hyperparasitaemia. For the treatment of young children (non-immune) the simulations presented for the South-East Asian setting should apply.

It is clear that the general strategy employed in the present investigation could be used to predict the effect on the cure rate of changing the MQ dosing strategy. One could evaluate the effect of a delayed second dose of MQ (Hoshen *et al*. 2001), both with long and short treatments of Ar. We have not taken into account in our simulations the effect of reinfection. In Thailand, from where our data are taken, the bite rate is low so this may be appropriate. For Africa, however, where bite rates are high (Bloland Ettling & Meek, 2000), it will be certainly worthwhile to include this factor in future simulations.

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