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MINIREVIEW

Assessment of the Pharmacodynamic Properties of Antimalarial Drugs In Vivo

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Malaria is unusual among the systemic infections of humans in that the number of organisms causing the disease may be quantitated with reasonable precision. This applies particularly to those causing benign human malarias, i.e., *Plasmodium vivax*, *P. malariae*, and *P. ovale*, as these parasites are not sequestered in the microcirculation (13). The assessment of parasite burden for the sequestering, potentially lethal parasite *P. falciparum* is more difficult, but there are some clues which allow a rough estimation of the proportion of parasites circulating in the bloodstream (65). If the number of organisms causing an infection is known, then the pharmacodynamic properties required of an anti-infective drug to produce a cure can be defined. The assessment of the treatment response in malaria rests on the clinical outcome (mortality, speed of recovery from coma, fever clearance, etc.) and the parasitological outcome—the subject of this discussion. Although a great deal remains to be learned about the pharmacodynamic properties of antimalarial drugs in vivo, sufficient information is already available to construct simple models which predict for how long antimalarial treatment should be given and the chances of treatment failure (i.e., recrudescence of the infection). In the management of individual patients, the ratio of the parasitemia at the time of treatment to the count 48 h later (the parasite reduction ratio [PRR]), representing the fractional reduction per asexual life cycle, may be a simple but useful predictive index.

General principles. Parasitological recovery from malaria is assessed conventionally by the clearance of parasites from peripheral blood smears (68). In highly drug-resistant infections, parasites do not disappear from the peripheral blood or may increase following the administration of antimalarial drugs. Parasites with lower grades of resistance disappear from the peripheral blood (in fact, the concentration falls below the level of microscopic detection) but recur at a later time, usually in association with a return of symptoms. The efficacy of antimalarial drug treatment is assessed in terms of the speed at which symptoms and signs resolve and parasitemia declines (usually recorded as the parasite clearance time [PCT]) and the proportion of patients in whom infections recur within a defined period (71).

Relationship between parasitemia and disease. Assessment of the therapeutic response in falciparum malaria is complicated by the loose relationship between parasitemia (number of parasites per unit volume of blood) and disease severity (10). A patient may be admitted in a deep coma with evidence of liver and renal dysfunction and severe metabolic acidosis, yet parasites are visible only on the thick blood film (<0.02%), whereas in areas where the disease is endemic a child may be able to walk and continues to eat when nearly half of the erythrocytes are parasitized. Several factors explain this discrepancy. As immunity to malaria develops with repeated infections in areas where the disease is endemic, the parasitemia threshold at which symptoms develop rises. This is often termed antitoxic immunity or premunition (2). It reflects a change in the relationship between parasite burden and both the release of and the response to illness-inducing cytokines (25). Interestingly, this relationship differs among the parasites causing human malarias; for example, *P. vivax* has a lower pyrogenic threshold (ca. 200/μl) than *P. falciparum* (ca. 10,000/μl) (23). As a consequence, adults living in areas where malaria is endemic who have been exposed repeatedly to malaria during their lives tolerate detectable parasitemias (total parasite burden, $>10^8$) without symptoms, whereas nonimmune persons with such burdens are ill. The second important factor explaining the discrepancy between parasitemia and disease severity relates to the stage and synchronicity of infection (53, 65). In *P. falciparum* malaria, only the first half of the 48-h life cycle is visible to the microscopist. At approximately 16 to 24 h of asexual development, intraerythrocytic parasites start to induce the expression of adhesins on the infected erythrocyte surface (20). Parasitized erythrocytes aggregate with uninfected erythrocytes (rosetting) and also begin to adhere to vascular endothelium, particularly in the venules. This process is termed cytoadherence, and it leads to sequestration of the mature parasites in the deep vasculature (67). Thus, in *P. falciparum* malaria, depending on stage and synchronicity, the bulk of the infecting asexual stage parasites may be either circulating (when the mean age of development is in the first half of the cycle) and measurable or sequestered (when the mean age of development is in the second half of the cycle) and not measurable (53). In the expanding phase of the infection, there are usually more circulating than sequestered parasites and there can be considerable differences in synchronous infections (65). As pathophysiological processes in falciparum malaria are thought to relate to the sequestered forms of the parasite and subsequent merogony (67), and not to the younger circulating asexual stages, patients tend to be more ill when the majority of their parasites are sequestered (i.e., not visible to the microscopist) or just after schizont rupture (merogony). In benign human malarias, which synchronize more readily than falciparum malaria, fever and rigors are associated with synchronous merogony (22, 23, 28). These symptoms result from the simultaneous release of malaria-related pyrogens

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(often termed toxins) and subsequent production of pyrogenic cytokines. In very synchronous infections with these parasites, the patient may be remarkably well between fever spikes (paroxysms). This is comparatively rare nowadays, as infections are usually treated promptly.

Thirdly, like all pathogenic microorganisms, malaria parasites vary in terms of virulence or pathogenicity (52, 67). Some parasites multiply faster than others, and they differ in resistance to antimalarial drugs, adhesion characteristics, and the amounts of toxin liberated at schizont rupture. The fourth factor affecting the relationship between parasitemia and disease severity is the use of antimalarial drugs before admission to a hospital or treatment facility. In the tropics, unregulated use of antimalarial drugs is widespread and it is often difficult or impossible to ascertain whether the patient has taken antimalarial treatment previously or not. Peripheral blood parasite counts may be relatively low or may have fallen below the level of detection (about 50 parasites/μl, which corresponds to approximately 500×10^6 parasites in total in an adult) while the patient remains clinically ill. This is more likely following treatment with artemisinin or one of its derivatives, as these compounds accelerate the clearance of ring form-infected erythrocytes (64). All of these different factors may confound interpretation of the initial antimalarial treatment response.

Parasitemia during the course of untreated infections. To interpret changes in parasitemia following antimalarial drug treatment, the changes that occur during natural (untreated) infections need to be considered (i.e., the changes that would have happened anyway if antimalarial drug treatment had not been given). Much of this information derives from studies conducted during the first half of this century with volunteers or in the malaria therapy of syphilis. During the exponential growth phase of *P. falciparum*, the principal parasite brood expands in a rising sine wave pattern with a 2-day cycle (tertian) (9, 65, 67). Smaller broods may be out of phase, and some infections consist of two broods, approximately equal in size, oscillating 24 h out of phase with each other (quotidian) (28). The more synchronous the infection, the more marked are the rises and falls in peripheral parasitemia, which correspond to merogony and sequestration, respectively. Parasitemia in benign (nonsequestering) malarias rises like a step ladder if the infection is highly synchronous, whereas the rise is log linear if the infection is completely asynchronous (65). Although it is generally thought that hepatic merogony (schizogony) is relatively synchronous, there is evidence that this process is discontinuous and continued merozoite release from the liver may also contribute to changes in parasitemia (37). In most untreated falciparum malaria infections and all untreated benign human malaria infections, this exponential rise in parasitemia is abruptly checked. There follows a period of numerical instability during which the parasitemia usually subsides to a rough plateau in the untreated infection. This is followed by a gradual decline in parasite counts over weeks or months during which the parasitemia shows peaks or waves approximately every 9 cycles (18 days), which probably represent the emergence of a new subpopulation of antigenic variants (3a). Eventually, the parasitemia sinks below the level of detection, although gametocytes may still be present in peripheral blood smears. The duration of the untreated infection averages 7 months but in many cases exceeds 1 year (8). During the plateau phase, parasite production is equal to parasite clearance (an approximation of a steady state); the effective multiplication rate is therefore 1. Background immunity reduces parasite multiplication rates and lowers the threshold at which parasite expansion is checked. In areas where the disease is endemic, a steady state below the level of parasite detection ($<10^8$ parasites in total for an adult) is often reached.

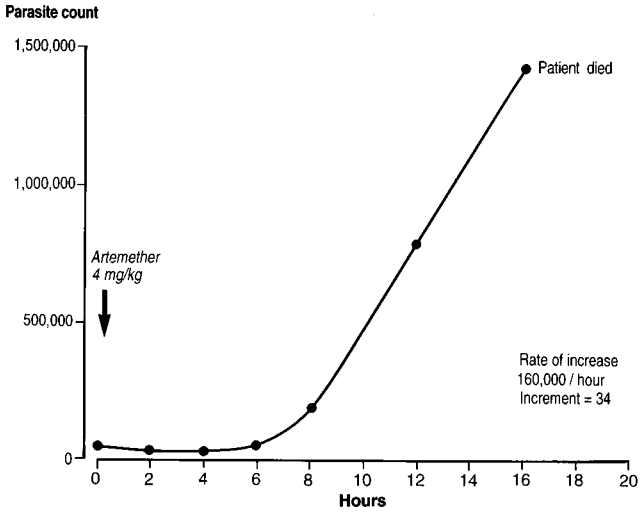

FIG. 1. Abrupt rise in parasite count (per microliter) in a child with severe falciparum malaria beginning 6 h after intramuscular administration of artemether. This does not reflect drug resistance, although it may reflect poor absorption. Antimalarial drugs have relatively little activity against mature schizonts and do not prevent imminent merogony.

Changes in parasitemia immediately following the start of antimalarial treatment. As none of the antimalarial drugs acts instantaneously, even if given intravenously, the pattern of parasitemia in the first few hours following the start of treatment will be the same as that which would have occurred without treatment. If the patient happens to be admitted at a time when the majority of the parasites are sequestered mature meronts (schizonts), then parasitemia may rise alarmingly in the hours following treatment as these meronts rupture, liberating merozoites which invade circulating uninfected erythrocytes (Fig. 1). Mature *P. falciparum* meronts may contain up to 34 (usually 18 to 24) merozoites (4, 13) and so, in a highly efficient expanding infection, the parasite burden could increase up to 20-fold following merogony (9, 23, 65). But as the parasite burden is not linearly related to peripheral blood parasite counts in falciparum malaria, parasitemia can increase more (65). This is because the majority of parasites in the body can emerge from sequestration during merogony over a period of several hours (i.e., a few hours previously, most of the parasites in the body were stuck in the microcirculation and invisible, whereas all of the newly infected erythrocytes following merogony circulate and their representatives are visible to the microscopist). Rises in parasitemia immediately following treatment are worrying if they are recognized, but they should not be misinterpreted as reflecting drug resistance. They are a natural consequence of infection. The parasites will be young; nearly all of the forms in a peripheral blood smear will be tiny rings (53).

Although abrupt rises in parasitemia may occur with any of the human malarias, they are observed most commonly in falciparum malaria, where parasites are sequestered for half of the life cycle and parasite burdens are generally heavier (Fig. 1). Close monitoring of parasitemia is also more likely in severe *P. falciparum* infections with heavier parasite burdens. Abrupt rises in parasitemia are not usually noticed in other human malarias, where peripheral parasitemias are seldom more than 2% at their maximum. Falciparum malaria may also

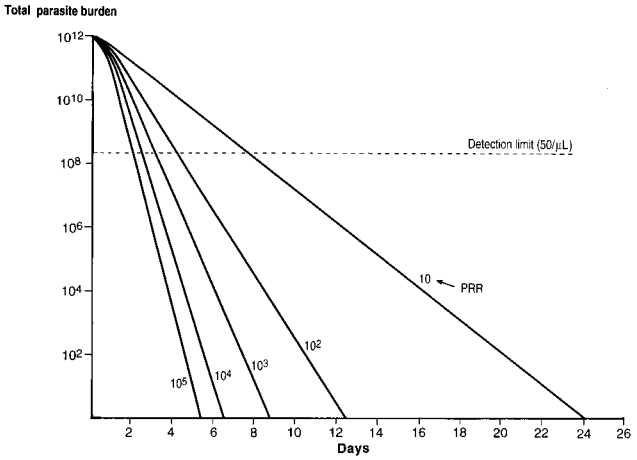

FIG. 2. Parasite clearance following different antimalarial treatments. The initial parasite burden corresponds to a parasite count of approximately 100,000/μl, or 2% parasitemia, in an adult with falciparum malaria. The times taken to eradicate all parasites from the body are shown at different killing rates (PRRs), assuming constant fractional reduction and no contribution from induced immunity. It is evident that rapidly eliminated drugs with PRRs of $\leq 10^3$ need to be given for >1 week.

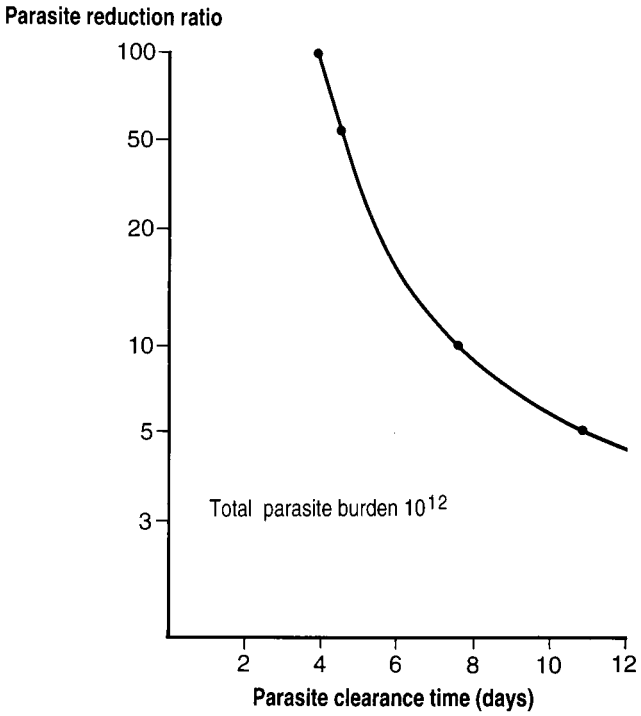

FIG. 3. Relationship between PRR and PCT for drugs with low efficacy (PRR, ≤100) illustrated with an initial total burden of 10^{12} parasites.

exhibit a rapid decline in parasitemia immediately following treatment as a result of sequestration of a synchronous infection (65). In this case, before parasite numbers fall rapidly the majority of peripheral blood parasites are large rings or early trophozoites (in which some parasite-associated pigment is visible) (53). In this situation, the clinician should not be misled into thinking that the drugs are being rapidly effective. Indeed, the patient may deteriorate coincident with sequestration in heavy parasite burdens. However, the effects of antimalarial drugs on later changes in parasitemia provide the basis for in vivo pharmacodynamic assessment.

Parasite clearance times. The PCT is the time from the beginning of antimalarial treatment until parasites are no longer detectable in the peripheral blood film (Fig. 2). This depends very much on the admission parasitemia and the frequency with which blood films are taken (68) (Fig. 3). PCTs will obviously be short if admission parasitemias are very low and will vary by ±24 h if parasite counts are taken only once each day. For these reasons, in small comparative clinical trials, parasitemias should be normalized and parasite counts should be taken at least every 12 h (preferably every 4 to 6 h) (68). As the sensitivity of microscopy depends on the quality of the microscope, the staining procedure, the slide, the duration of the examination, and the experience of the microscopist, the parasite clearance curve should be defined by more than one parameter. We use PC_{50} (time until reduction by 50%), PC_{90} (time until reduction by 90%), and PCT, and later in this discussion I will describe the use of the ratio between the admission parasite count and the count 48 h later (the parasite reduction ratio). Others have used PC_{95} and PC_{99}. These measures are all misleading at low parasitemias because the intrinsic variability of the estimates of the parasite count increases disproportionally near the limits of detection. For drug comparisons, it may be better to exclude patients with admission parasite counts under 10,000/μl.

IN VIVO PHARMACODYNAMICS

Antimalarial drug effects on circulating parasites. The dihydrofolate reductase (DHFR) inhibitors (cycloguanil and pyrimethamine) and the quinolines quinine, quinidine, and mefloquine appear to have relatively little effect on asexual malaria parasites in the first half (24 h) of the life cycle (6, 14, 47, 58). As a result, most circulating *P. falciparum* parasites continue to mature, express adhesins on the infected erythrocyte surface, and begin to cytoadhere and form rosettes despite treatment (60). Thus, exposure of ring form parasites to these drugs does not appear to prevent sequestration significantly (60, 65). As a consequence, the early decline in parasitemia following treatment with these antimalarial agents is largely that which would have occurred in their absence. In contrast, chloroquine, halofantrine, and, to a greater extent, artemisinin and its derivatives do attenuate the growth of young parasites in vitro and increase the clearance of ring forms in vivo (6, 14, 18, 47, 58, 60, 72, 74). As a result, the early decline in parasitemia following treatment with these drugs is usually more rapid than that seen with treatment with those in the first category (69). These differences may not be appreciated from an individual patient's records but become evident when a series of patients with similar admission parasitemias and degrees of background immunity and treated with the different drugs are compared (64). A population of patients with malaria will have infections different in magnitude (parasite numbers), stage, and synchronicity and therefore parasitemia-time profiles. The population parasite clearance curve represents the average of the individual parasitemia-time profiles normalized to a baseline parasitemia of 100%. Following treatment with quinine or mefloquine, the average parasitemia-time profile is that of a lag phase, during which the population mean parasitemia remains unchanged, and then a decline. The population parasite clearance curves following treatment with chloroquine (69), halofantrine, and particularly the artemisinin derivatives (1, 19) show a shorter lag phase and thus shorter PC_{50}, PC_{90}, and PCT, provided the infecting organisms are

fully sensitive. These changes are too rapid to be accounted for by reduced merogony and can only represent accelerated ring form clearance (64). Accelerated cytoadherence (leading to enhanced sequestration) is unlikely as an alternative explanation, as the rapid decline in parasitemia coincides with morphological changes in the circulating parasites and a reduced ability to cytoadhere in vitro (60). The number of additional ring form parasites cleared as a result of treatment can be estimated by subtracting (i) the area under the population-normalized parasitemia-time curve following administration of the drug from (ii) that of the corresponding population following quinine or mefloquine antimalarial treatment (64). Thus, the stage specificity of drug action and the magnitude of the antimalarial effect can be deduced from population parasite clearance curves.

Antimalarial effects on sequestered parasites. Antimalarial drugs are maximally active on the mature trophozoite stage of parasite development (roughly the middle third of the life cycle) (6, 14, 18, 47, 58, 60, 72, 74). This is assessed in vitro in terms of inhibition of glucose, amino acid, and purine (hypoxanthine) uptake and also inhibition of morphological development and subsequent multiplication. Stage specificity varies between compounds and has not been defined precisely. Available evidence suggests that DHFR inhibitors have a relatively narrow time window of activity in the second half of the life cycle; quinine and mefloquine have a broader time window but still act mainly in the second half of the cycle. Chloroquine and halofantrine have a still wider time window of activity (hence their action on circulating forms), but artemisinin derivatives have the broadest range of all, with considerable effects on ring stage parasites. Population studies may also be used to deduce the stage specificity of antimalarial action on meront development. If the stages of parasite development are assumed to be distributed randomly in the patient population (i.e., there is no reason why patients should present to medical attention at a particular stage of parasite development), then some of the patients, by chance, will have a predominance of mature meronts when drug treatment is given. If the drug has no action on these mature-stage parasites, then parasitemia will be seen to rise shortly after treatment in this patient group (61). If the drug does exert an effect, then merogony will be reduced or stopped. There will be fewer patients with sudden rises in ring form parasitemias shortly after treatment. Thus, the relative effects of two antimalarial drugs on meront development may be assessed by comparing the proportion of patients whose parasitemia shows an early rise (e.g., within 6 to 12 h) after the start of antimalarial treatment. Following treatment with oral artesunate, early rises in parasitemia are significantly less than those that occur following quinine or mefloquine treatment. This suggests that artesunate affects meront development at a later stage than these other antimalarial drugs (unpublished observations).

Timing and frequency of antimalarial administration. Mature schizonts and tiny rings are the most drug-resistant stages of the blood infection (58). Most of the available antimalarial drugs are active predominantly on the sequestered parasites that are not visible to the microscopist. The consequences of this are inhibition of trophozoite development and a considerable reduction in the parasite multiplication rate. As susceptibility is stage dependent, the timing of drug administration with respect to the stage and synchronicity of parasite development may determine the therapeutic response (chronotherapy). Antimalarial treatment with chloroquine and, possibly, some other compounds can be optimized if peak drug levels coincide with the most sensitive stage of parasite development (27). This is rather difficult to arrange in practice.

Qinghaosu (artemisinin) and its derivatives are peroxidic antimalarial agents which are particularly active against ring stage parasites. In the early Chinese studies with these drugs, treatment was started only when most of the infecting parasites were at the tiny-ring stage of development (21). The advantages of chronotherapy remain to be established, although this is an important area for further research. The frequency of drug administration is usually determined by pharmacokinetic considerations. Conventionally, a drug should be given at intervals roughly corresponding to its biological half-life (estimated in patients with the disease being treated, not extrapolated from studies with healthy volunteers). If the drug exerts its effects only while concentrations remain above a therapeutic level, then the biological half-life corresponds to the drug half-life. However, if there is concentration-dependent killing and biological effects persist well after the drug has declined below the therapeutic level, then the drug can be given less frequently than predicted from the drug half-life determined in pharmacokinetic studies (5, 7, 66). We know relatively little about antimalarial dose-response or concentration-effect relationships in vivo. In vitro, the relationships for antimalarial drug effects on hypoxanthine uptake or on schizont maturation display a sigmoid relationship (3, 58). These concentration-effect relationships are probably different at different stages of parasite development. Although therapeutic concentrations are assumed to reflect drug activity at the flat (top) part of the sigmoid dose-response (concentration-effect) curve i.e., E_{max}, there are few data to support this for any of the antimalarial drugs. Recent studies on the in vitro pharmacodynamics of quinine have shown that for the Tanzanian isolate tested (F32), at concentrations below 0.65 to 2.6 μmol/liter (0.21 to 0.83 mg/liter), parasite killing was concentration dependent, but above this level the reduction in parasites was proportional only to the duration of exposure (34). These considerations may also be important for other short-half-life antimalarial agents, in particular, the artemisinin derivatives. The available studies suggest that despite rapid absorption and elimination half-lives for both the parent compound and the biologically active metabolite dihydroartemisinin that are less than 1 h, oral artesunate is equally effective when given once or twice daily (29, 39, 64). This suggests that the biological effects of the artemisinin compounds extend beyond their presence at therapeutic concentrations in plasma. The broad stage specificity of action of these drugs ensures that only two exposures of a few hours in each asexual life cycle (i.e., once-daily administration) is sufficient for a maximal antimalarial effect. This challenges the concept that antimalarial drugs need to remain above parasiticidal levels throughout the dosing interval.

Parasite reduction ratio. The number of new young parasites which enter the circulation and invade erythrocytes depends on a number of factors, i.e., the sequestered biomass, its stage and synchronicity of parasite development, the number of viable merozoites released per schizont, the presence of merozoite agglutinating antibodies, the susceptibility of the remaining erythrocytes to invasion, the timing of antimalarial drug administration, the drug levels in blood achieved, and the drug's activity (68). Antimalarial drugs differ in their concentration-effect relationships and also in their intrinsic activity (Fig. 2). Thus, the E_{max} for one drug will be different from that of another, and so will the minimum concentration in blood or plasma required for a maximal effect (minimum parasiticidal concentration [MPC]). These concentrations will obviously be higher than those required to prevent parasite multiplication (i.e., to reduce parasite multiplication to ≤ 1 [the MIC]). In the presence of effective antimalarial drug concentrations (i.e., concentrations greater than the MIC), parasitemia falls as a

TABLE 1. In vivo pharmacodynamics

Antimalarial drugs	Estimated PRR in vivo[a]
Artemisinin, artesunate, artemether	10^3–10^5
4-Aminoquinolines, halofantrine	10^2–10^4
Quinine, mefloquine, pyrimethamine-sulfadoxine	10–10^3
Antimalarial antibiotics, desferrioxamine	5–10

[a] PRR = baseline parasite count/parasite count 48 h later; this rises if there is background immunity and falls with resistance. The data shown were derived from references 15, 19, 24, 36, 45, 47, 55, 57, and 59.

consequence of a reduced input of new young parasites, clearance of circulating rings, and sequestration. In the nonsequestering benign human malarias (those caused by *P. vivax*, *P. ovale*, and *P. malariae*), the reduction in the total-body parasite burden following antimalarial treatment can be estimated by comparing the baseline and 48-h parasite counts and multiplying by the blood volume. Although the precise total burden of *P. falciparum* cannot be estimated, this baseline-to-48-h parasitemia ratio is still informative. The PRR or fractional reduction in parasitemia per asexual life cycle is analagous to the killing rate. The considerable differences in PRR between antimalarial agents reflect the differences in intrinsic activity (E_{max}) between the drugs (Table 1). Sir Ronald Ross used a similar concept over 70 years ago to estimate the number of quinine doses that should be required to cure malaria. He described the single-dose reduction rate (SDRR) as the fractional reduction in parasitemia for each dose of quinine (50). Based on careful enumerative studies with David Thomson, he estimated that the SDRR for quinine is approximately 0.75 (51).

The duration of antimalarial treatment. Estimation of the SDRR or PRR in vivo allows an assessment of the duration for which therapeutic concentrations of an antimalarial drug must be present (Fig. 2), although this does make the considerable assumption that the reduction rate or killing rate remains constant with succeeding cycles (i.e., it is a first-order process). Although both in vitro and in vivo data support this, there are several reasons why the decline in parasitemia may not remain log linear until the last parasite has been removed from the body. First, the concentrations of the antimalarial drug in blood will fall and the antimalarial effect will decline (as levels fall below the MPC determined by the intrinsic susceptibility of the infecting parasites). Second, there may be selection of more resistant parasites with each generation under the drug pressure. Third, parasites may be prevented from multiplying but not killed. Very young rings can be put into a state of temporary "suspended animation" in which there is no further development and cell cycle activity shuts down by concentrations of antimalarial drugs that kill parasites in other stages of development (26a). The selection of resistant parasites is not usually measurable over a few asexual cycles, although it has been possible to demonstrate selection over 8 to 20 cycles (16 to 40 days) in recrudescent infections following treatment with atovaquone and mefloquine (30, 31). Precise estimation of the PRR also depends on exact timing of blood smears (this is critical in very synchronous infections) and assumes that the asexual life cycle lasts exactly 48 h. It is also likely to be inaccurate at low-level parasitemias because of counting errors, particularly in the transition from thin to thick smear counts, as the latter are usually underestimates.

We do not know how important these potential sources of error are. Nevertheless, the concept of a first-order decline in viable parasitized erythrocyte numbers (a fixed fractional reduction) provides a simple basis for assessing and comparing drug activity and one that fits best the decline in parasitemia following antimalarial treatment (41). The factors described above all reduce the rate of parasitized erythrocyte decline, so predictions based on PRR estimates give an estimate of the optimum drug effect. From available data, it is apparent that although the artemisinin derivatives have the highest PRRs of all available antimalarial drugs ($>10^4$ or $>99.99\%$ per cycle [52]), they still need to be present at parasiticidal concentrations during at least three parasite life cycles (i.e., >6 days) to remove all of the parasites from the blood (Fig. 2). This explains why 5-day regimens with these drugs are not very effective, as they can cover only three parasite life cycles and would require sustained PRRs of over 10^4 to eradicate infections with a total parasite biomass of 10^{12} (29). If the PRRs are reduced slightly, then four cycles need to be covered by therapeutic antimalarial drug concentrations, i.e., there will be failures with 7-day drug regimens (Fig. 4). Short-course regimens of quinine (i.e., less than 7 days) are unlikely to be effective for the same reasons. Mefloquine has considerably less intrinsic antimalarial activity (E_{max} values) than the artemisinin derivatives and gives lower PRRs ($\approx 10^2$/cycle), but its long half-life allows therapeutic (i.e., effective) concentrations to remain until all parasites have been eliminated in drug-sensitive infections (Fig. 5). It can be seen from Fig. 2 that PCTs of less than 48 h are associated with PRRs of over 10^4, whereas PRRs of less than 10 will result in PCTs of more than 1 week (R_2 resistance) and either do not clear parasitemia at all or presage later recrudescence (38, 39, 55–57). Therefore, if the pharmacokinetic properties of the antimalarial drug are known, there is some estimate of the therapeutic level (MPC), and if the PRR is known (to the nearest order of magnitude), then the dose and duration of antimalarial treatment required to eliminate all of the parasites from the body can be estimated.

The therapeutic level of an antimalarial drug in plasma or blood in vivo and the parasite killing rate reflected by the PRR are clearly related. Thus, the MPC (18a) can be defined as the lowest concentration of the antimalarial agent in blood or plasma that results in a maximal effect, i.e., a maximum PRR. The MIC is the level required to maintain the PRRs at unity. When the interval from primary treatment to recrudescence and the concentration in blood at that time are known (for example, with chloroquine or mefloquine resistance), it may be possible to estimate the MIC (Fig. 5 and 6). Some parasites may be associated with low PRRs (low E_{max}) and yet remain

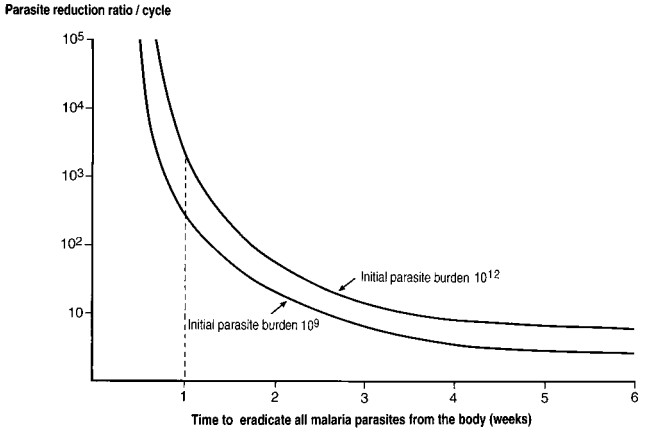

FIG. 4. Effects of parasite burden on the relationship between PRR and the PCT (assuming a constant PRR).

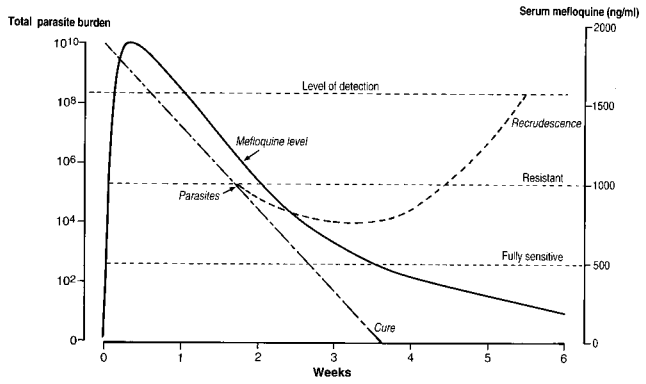

FIG. 5. Development of mefloquine resistance. Shown are mefloquine concentrations in serum and parasitemias in a sensitive *P. falciparum* infection with an in vivo MPC of 500 ng/ml and in a resistant infection with an in vivo MPC of 1,000 ng/ml which recrudesced 38 days after the initial treatment. The PRR was low (<100) in both cases. This illustrates the need to interpret PRRs against the background context of prevailing antimalarial resistance.

susceptible to the treatment drugs (i.e., the MPC is low). For example, in 1986 on the Thai-Burmese border, occasional patients with very slow parasite clearance following mefloquine treatment were seen, but the infections were usually cleared ultimately (38). This was because the parasites were relatively drug sensitive and so the estimated in vivo MPCs were low (less than 500 ng/ml) and therefore maximum killing rates (PRRs) were maintained long enough to eradicate the infection. However, as the level of resistance (therapeutic level) rose these maximum PRRs could not be sustained as the levels of mefloquine in blood fell. All infections with long PCTs (reflecting low PRRs) recrudesced subsequently (39, 55–57). Thus, the value of the PRR or PCT in predicting recrudescence depends on the prevailing level of antimalarial drug resistance (Fig. 5). In the case of mefloquine, increasing the dose ensures that concentrations remain above the MPC longer and therefore gives a greater chance of eradicating all of the parasites (54, 57). As resistance worsens, the MPC rises and even higher doses cannot maintain effective concentrations in blood long enough to achieve total-body parasite clearance (63).

The simple PRR concept does not explain all aspects of the therapeutic response in malaria. The combination of quinine with tetracycline is consistently more effective than quinine alone, particularly in areas with quinine-resistant falciparum malaria, but mean PRRs are not significantly different whether or not tetracycline is added. However, the distribution of PRRs is usually smaller with tetracycline; i.e., addition of the antibiotic may augment killing of the more resistant parasites and thereby prevent very long PCTs (low PRRs).

Treatment failure: the time to recrudescence. From these observations, the time to recrudescence of malaria can be estimated roughly in situations in which the prevailing level of drug resistance in vivo is known. It can be seen from Fig. 5 and 6 that for relatively weak drugs (low PRR) which are eliminated slowly (e.g., mefloquine), any recrudescences that occur will tend to be late (if the population of parasites is relatively sensitive). This explains why recrudescences of *P. falciparum*, or the appearance of *P. vivax* in patients with mixed infections, may occur for up to 2 months after mefloquine treatment (16, 32, 39, 54, 55, 63). Parasite reduction rates are relatively low to start with, and the continuously declining concentrations of mefloquine in blood (elimination half-life of 2 to 3 weeks) exert a progressively diminishing effect. As the parasiticidal effect declines below the E_{max} value, the PRR falls slowly to unity (Fig. 5) and then below it (i.e., the infection begins to re-expand). On the Thai-Burmese border, mefloquine resistance in *P. falciparum* has increased rapidly. Now nearly all patients with parasitemia persisting beyond 3 days (i.e., a PRR of $<10^2$) will have a recrudescent infection subsequently (55–57). This reflects an increase in resistance such that the low levels of mefloquine that occur weeks after treatment are below the MIC; i.e., they are unable to maintain the PRR above unity. This is the drug's Achilles heel; selection of more resistant parasites occurs under drug pressure. Initially, when the drug is highly effective, the primary infection is always cleared. It is only new infections acquired weeks or months later, when mefloquine concentrations have fallen below the low inhibitory level, which survive. Obviously, the addition of drugs with shorter elimination half-lives than mefloquine, such as pyrimethamine and sulfadoxine, cannot protect mefloquine at this stage even if the malaria parasites are sensitive to all three drugs. But eventually treatment failures of the primary infection occur, and as resistance worsens the mean time to recrudescence of the disease shortens (39, 63). In contrast, the artemisinin derivatives and quinine are eliminated relatively rapidly (half-life, <16 h), so within hours or days of stopping treatment, drug concentrations in blood have fallen to subinhibitory levels. After quinine treatment is stopped, residual drug levels may still have some direct inhibitory effect on parasite multiplication for three to four half-lives (≈48 h), whereas artemisinin and its biologically active metabolites are eliminated in hours. If some viable parasites still survive when the concentrations of these drugs in blood are subtherapeutic, their multiplication is then constrained only by any residual second-cycle or postantimalarial effect or by immune defenses (see Appendix) which may retard the initial phase of re-expansion of the parasite population. Recrudescences tend to occur earlier than those following mefloquine treatment, often occurring around 2 weeks after treatment is stopped. Thus, the selective pressure to drug resistance with artemisinin derivatives or quinine is minimal (43, 63). Resistance therefore develops slowly or not at all. It is also evident that recrudescences that occur more than 4 weeks after antimalarial treatment with a drug that is eliminated rapidly is stopped are most unlikely. This accords with clinical observations.

Treatment failure and the PRR. To eradicate all of the parasites in the body, an effective antimalarial drug concentra-

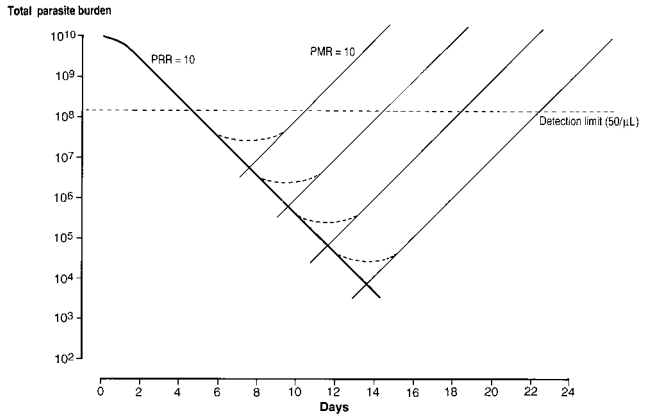

FIG. 6. Relationship between duration of treatment with a weak antimalarial agent (PRR, 10) and the time to subsequent recrudescence. PMR, parasite multiplication rate.

tion (at least the MIC but preferably the MPC) needs to be present in the blood until either the last parasite has been removed or immune defenses are able to deal with the residuum. Figure 2 shows that courses of short-acting antimalarial agents of less than 7 days in nonimmune patients will not eradicate all of the malaria parasites in all patients (i.e., there will be significant recrudescence rates). Failure rates will be higher in patients with greater initial parasite burdens because they have more parasites to remove. Recent studies have shown that high presenting parasitemia is a risk factor for subsequent recrudescence in multidrug-resistant falciparum malaria (12, 23a, 33, 56) (Fig. 4).

Antibacterial drugs with antimalarial properties, such as the sulfonamides, tetracycline, clindamycin, or rifampin, or other compounds such as desferrioxamine are much less active than the antifols, quinoline, or peroxidic antimalarial agents. They have estimated PRRs of only about 6 to 10 per cycle (15, 17, 24, 45, 48). Therapeutic concentrations of these drugs alone need to be present for approximately 2 weeks in nonimmune patients; otherwise, the infection will recrudesce (this accords with the results of clinical trials) (Fig. 2). Chloroquine is more complex. Although it has an extremely long terminal elimination half-life (1 to 2 months), the blood concentration profile reflects a multiexponential decline after treatment. Concentrations in blood fall rapidly after absorption, and the levels that persist in the blood for weeks after treatment are relatively low compared to those during the treatment phase (62). If killing rates are low initially, then the infection cannot be eradicated. Prasad et al. evaluated 48-h parasite counts as predictors of treatment failure (assessed at 7 days) in Indian patients with chloroquine-treated falciparum malaria (44). The minimum geometric mean PRR (95% confidence interval) was 43 (20 to 90) in treatment successes compared to 2.4 (0.8 to 6.7) in failures. Turaman et al. (59) provided parasitological data from northern Guinea during a period of increasing chloroquine resistance. The minimum geometric mean PRR (95% confidence interval) following chloroquine treatment derived from these data was 183 (78 to 432) for infections which cleared within 1 week and 19 (7 to 48) for infections which did not ($P < 0.001$). Although high PRRs were excellent predictors of clearance by 7 days in this study, low values did not necessarily predict failure. In hospital studies of chloroquine treatment of severe malaria in childhood in The Gambia, PRRs were approximately 10^3 in 1988 and 1989 (69, 70) before the advent of chloroquine resistance, but by the following year they had fallen to 10^2 with subsequent recrudescences (R_1) in 41% of children (26). Watt et al. reviewed several studies involving 230 patients treated with quinine, mefloquine, and sulfadoxine-pyrimethamine for uncomplicated falciparum malaria on the Thai-Cambodian border (61). Rising parasite counts >12 h after the start of treatment were associated with an increased risk of treatment failure. In successfully treated patients, the median PRR was >100 compared to 25 in patients whose infections recrudesced ($P < 0.05$). Fontanet and Walker (12) recently reported on predictors of mefloquine treatment failure on the Thai-Cambodian border; failure rates were 73% in patients with positive blood smears on day 2 (PRR, $<10^3$) compared to 43% in those with negative smears (PRR, $>10^3$). On the western border of Thailand, persistence of parasitemia to day 4 after mefloquine treatment (25 mg/kg), which corresponds to a PRR of <50, now presages a subsequent recrudescence of *P. falciparum* infection in 100% of cases (39, 56).

Resistance to the antifols (11, 42, 63) usually develops more rapidly than resistance to quinoline antimalarial agents. Resistance results from reductions in parasite DHFR affinity for these antimalarial agents and is usually caused by single or double base pair mutations in the DHFR gene. As only one or two steps are required to reduce affinity by several orders of magnitude, high-grade resistance develops rapidly. When proguanil (chloroguanide) was first introduced in peninsular Malaya, both *P. falciparum* and *P. vivax* were very sensitive to the drug, but over a 3-year period the mean PRR following administration of a single dose of 250 to 300 mg in uncomplicated falciparum malaria fell from 500 in 1947 to 100 in 1948 to 25 in 1949. By mid-1949, the early failure rate had risen to 25% (11, 42).

The PCT and the PRR are obviously related closely, although the latter is much easier to measure (requiring only two counts) and is more precise. Parasite counts taken at 2 days are already part of the standard assessment protocol for determining the level of 4-aminoquinoline drug resistance. PRRs of <4 are classified as R_3 resistance. However, these observations suggest that the measurement may have a broader application. PRRs of <10 tend to be associated with R_2 resistance (i.e., parasite PCTs of over 7 days). The prognostic sensitivity and specificity of the PRR as a predictor of therapeutic failure should be defined for different drugs at different levels of antimalarial drug resistance.

Effects of immunity on the therapeutic response. Yorke and Macfie (73) were the first to note that antimalarial treatment responses in semi-immune or immune patients were always better than those in nonimmune patients. Even nonimmune patients eventually develop a strain-specific immunity to their malaria infection which at first limits and finally eradicates it. This is a lengthy process (several months) which approximates to the natural history of untreated falciparum malaria. Nonimmune patients with highly (multiple drug) resistant parasites may require three or more courses of antimalarial treatment before they are finally cured. In areas where malaria is endemic, the well-developed immune response acts together with the antimalarial drugs, allowing shorter treatment courses to be given and giving good therapeutic responses even when there is some degree of drug resistance. In these areas, the treatment response improves with age, coincident with the acquisition of immunity. Thus, age is an important factor determining the therapeutic response in such areas and must be taken into account in drug trials. Immunity is temporarily disrupted in pregnancy, and pregnant women are a particularly difficult group of patients to treat, as the choice of drugs is also limited.

Implications for the prevention of antimalarial resistance. The initial parasite burden in malaria is an important factor in determining not only the therapeutic response (23a, 33, 56) but also the propensity to develop antimalarial drug resistance. This was shown initially in animal studies (46, 49) and then in human volunteer studies with pyrimethamine (35). These volunteer studies were particularly illuminating, as they showed clearly, by further challenge studies, that the recrudescent parasite isolates had become highly drug resistant. To counter this phenomenon and prevent the emergence of resistant mutants, combinations of antimalarial drugs with different loci of action have been employed (42). A similar strategy is employed in antituberculous chemotherapy, where spontaneous mutation rates for individual antibiotics are on the order of 1 in 10^{10} organisms, and also in antiretroviral and cancer chemotherapy. This works best if the drugs have well-matched pharmacokinetic properties and all have significant antimalarial activity against prevalent parasite strains. In a rodent model, the combination of sulfadoxine-pyrimethamine-mefloquine delayed the onset of mefloquine resistance in a strain of *P. berghei* (which was sensitive to all three drugs) (43), but this did not work in falciparum malaria in Thailand, where the combina-

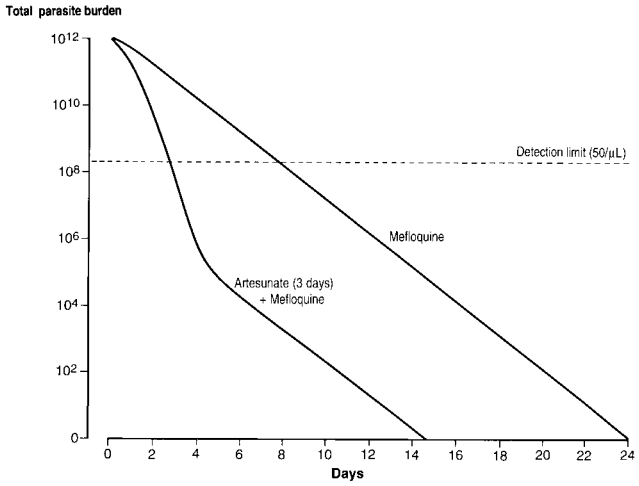

FIG. 7. Addition of artesunate to mefloquine accelerates parasite clearance, shortens the PCT, reduces the number of parasites exposed to mefloquine, and thus reduces the chances of an "escape" mefloquine-resistant mutant arising.

tion was introduced in 1984, because the drugs were not well matched in terms of pharmacokinetic properties (mefloquine had a much longer terminal elimination half-life than the other two) and *P. falciparum* was already highly resistant to sulfadoxine and pyrimethamine (40).

The pharmacodynamic properties of antimalarial drugs are also relevant to the prevention of resistance. If resistance potential is a function of viable parasite biomass (i.e., the more parasites there are, the more likely it is that a resistant mutant will emerge), then it follows that the risk of resistant isolates emerging from a primary infection will be greatly reduced if the parasite biomass is reduced. For example, artesunate is now increasingly used in combination with mefloquine for multidrug-resistant falciparum malaria. This is associated with a significant increase in cure rates, even in infections with highly mefloquine-resistant parasites (29, 30, 39, 63). Artesunate given for only 3 days will reduce the parasite biomass by a factor of between 10^6 and 10^8. Thus, relatively few parasites (up to 10^4) remain for mefloquine to remove and the chances of a more mefloquine-resistant mutant emerging from this primary infection are reduced correspondingly (Fig. 7). Of course, the use of artesunate cannot protect mefloquine from any subsequent (new) infection which is acquired later, when mefloquine levels have declined to subtherapeutic values. In successfully treated patients, mefloquine also protects artesunate by removing all residual parasites originally exposed to the drug; thus, there is no pressure to artesunate resistance. The same principles should also apply to other combinations of artemisinin derivatives with more slowly eliminated antimalarial agents. This would argue in favor of deploying, de novo, an artemisinin derivative in combination with any new, slowly eliminated antimalarial drug.

CONCLUSION

Much of the above hypothesis, although consistent with clinical observations, has not been tested formally. Our understanding of the pharmacodynamic properties of antimalarial drugs is still incomplete. For example, the precise relationship between killing, drug concentrations, and stage of development at concentrations well in excess of the 50% inhibitory concentration has not been defined. The PRR is a simple and easily understood measurement. Its utility should be established prospectively. The advantages of defining the pharmacodynamic properties of antimalarial drugs more precisely would be better methods of assessing the therapeutic response. This would also provide a firm basis for designing antimalarial drug regimens (dose, frequency, and duration), predicting treatment failure, and slowing the development of antimalarial drug resistance.

APPENDIX

Parasite clearance rates. The PRR (τ) is defined as the ratio of the parasite count before treatment (P_0) to the count at 48 h or 2 days (P_2).

$$\tau = P_0/P_2 \quad (1)$$

If it is assumed that the decline in parasitemia is first order if assessed at 2-day intervals, then the parasite count (P_t) at any time (t) is given by

$$P_t = P_0\, e^{-kt} \quad (2)$$

where k is the first-order elimination rate constant. So, for $t = 2$ days, $P_2 = P_0 e^{-k(2)}$ or $P_0/P_2 = 1/e^{-k(2)}$ but $\tau = P_0/P_2$ from equation 1; therefore, $\tau = 1/e^{k(2)}$. So, the relationship between PRR and the parasite clearance rate is given by $k = 0.5 \log_e 1/\tau$.

Time required to clear parasites from the body. If the blood volume of an average adult human is assumed to be 5,000 ml and P is the parasite count per microliter, then the total parasite burden (B) is given by $B = 5{,}000 \times 10^3 \times P = 5P \times 10^6$; therefore,

$$\log_e B = \log_e P + 15.4 \quad (3)$$

If $\tau = P_0/P_{48}$ (where P_0 and P_{48} are the parasite counts per microliter before treatment and at 48 h, respectively, then the time (T) in days for which parasites are present in the body (with an asexual cycle of 2 days) is given by

$$T = \frac{2(\log_e P + 15.4)}{\log_e \tau} \quad (4)$$

This is the time for which therapeutic concentrations of antimalarial drugs must be present, assuming that τ is constant and that there is no induction of immune mechanisms.

Worked example. On admission, a 22-year-old male with acute, uncomplicated falciparum malaria has a parasitemia of 2.3% and a hematocrit of 35%, equivalent to a parasite count of 101,110/μl. Exactly 48 h after mefloquine (25 mg/kg) administration, the parasitemia declines to 86/200 leukocytes of 3,440/μl. τ = 101,110/3,440 = 29.4. The time to eradication of all of the parasites in the body, assuming a constant PRR estimated from equation 4, is calculated as follows: $2(\log_e 101{,}110 + 15.4)/\log_e 29.4 = 16$ days. This approximates to the half-life of mefloquine. It can also be seen that at 4 days (96 h) the parasite count will fall to approximately 117/liter, i.e., a detectable count. The PCT is therefore >4 days. In areas with mefloquine-resistant parasites, a PCT of ≥4 days predicts treatment failure. This is because by 16 days, mefloquine levels will have fallen below the concentrations required to inhibit parasite development (i.e., τ falls below unity).

It is also evident from this example that counting errors in the denominator have a significant effect on the calculation. If the true parasite count was underestimated by 50% in the thick-film count of the above example, then the time to eradication of all parasites from the body becomes 18 days.

Prediction of time to recrudescence. During the acute phase of infection, parasitemia can rise as much as 20-fold per cycle. This represents highly efficient invasion (>90%) and no loss of parasites during intraerythrocytic development; i.e., nearly all schizonts survive, and nearly all liberated merozoites invade new erythrocytes. Although this may occur, multiplication is usually less efficient. Approximately 50% efficiency or survival, i.e., a multiplication rate of 10-fold per cycle (equivalent to $\tau = 0.1$), is a reasonable average from the well-documented clinical studies of the malaria therapy and artificial infection era. The time to recrudescence for short-acting drugs can be estimated

by calculating the number of parasites likely to be present at the end of treatment (from equation 2 or 4) and then assuming unrestrained multiplication in the cycle after drug levels have fallen below the inhibitory concentrations (Fig. 6).

For example, at the end of a usual treatment course, e.g., 7 days with a short-acting drug such as quinine, which did not prove effective (i.e., treatment τ of <100), some parasites persist. If 100 viable parasites remain in the body after four cycles (8 days), then with an unrestrained multiplication rate of 10-fold and a pyrogenic density of 10,000/μl, symptomatic recrudescence would be expected in 21 days (14 days after treatment is stopped).

A more complex equation is necessary for antimalarial agents which are eliminated slowly. Their antimalarial effect wanes (τ falls) as concentrations in blood decline. Recrudescences are therefore considerably delayed in relatively drug-sensitive infections.

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