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Acta Tropica 94 (2005) 230–240
ACTA TROPICA
www.elsevier.com/locate/actatropica

Population biology and antimalarial resistance: The transmission of antimalarial drug resistance in *Plasmodium falciparum*

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Abstract

Malaria morbidity and mortality continue to increase across sub-Saharan Africa. This is largely as a result of the continued use of chloroquine and sulfadoxine-pyrimethamine, despite widespread resistance. Although eliminating the asexual stages of *Plasmodium falciparum* is the focus of treatment of individual symptomatic patients, at a population level, reducing the carriage of gametocytes – the sexual stage responsible for infection of the mosquito vector – is necessary to limit the transmission of malaria parasites and the spread of antimalarial resistance. The probability of a mosquito being infected depends on the prevalence, duration and density of viable gametocyte carriage in the human host, although additional humoral and leukocyte factors also affect transmissibility. There is a log–sigmoid relationship between gametocyte density in the patients' blood and infectivity to the mosquito. The infectivity and thus transmission potential associated with a particular antimalarial treatment can be characterised as a function of blood gametocyte density and time, summing these over the acute and all subsequent recrudescences of that infection. Gametocyte carriage and infectivity to mosquitoes is consistently higher in patients infected with drug resistant compared with drug sensitive malaria parasites. It is the ratio of transmission potential in drug resistant versus sensitive infections that drives the spread of resistance.

Early access to highly effective antimalarial treatment reduces the risk of disease progression and limits gametocyte carriage. The remarkable spread of sulfadoxine-pyrimethamine (SP) resistance across vast regions results from the very high post-treatment prevalence and density of gametocyte carriage following SP treatment. In areas of low intensity malaria transmission, the gametocyte-reducing effect of widespread use of artemisinin-based combination therapy has resulted in a sustained decrease in malaria transmission and a decrease in the spread of resistance. Malaria treatment policy should be based primarily on therapeutic efficacy against asexual stages, but should also consider transmission reduction potential. Artemisinin-based combination therapies are the only antimalarials currently available which rapidly reduce both asexual and gametocyte stages of the *P. falciparum* lifecycle.

Keywords: Malaria; *Plasmodium falciparum*; Transmission; Resistance; Artemisinin-based combination therapy; Sulfadoxine-pyrimethamine; Chloroquine

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doi:10.1016/j.actatropica.2005.04.014

1. Introduction

Malaria is the most important parasitic disease of man. It causes over 1 million deaths, 300 million cases, and an economic loss of US$ 12 billion annually (World Health Organisation, 2002). This burden is borne by some of the world's poorest countries. Although four *Plasmodium* species infect humans, severe malaria and malaria-related deaths are almost entirely attributable to *falciparum* malaria (World Health Organization, 2000). After a steady decline in malaria deaths, the malaria mortality rate in eastern and southern Africa has increased dramatically in the last two decades, despite a drop in all-cause deaths among children (Snow et al., 2001; Korenromp et al., 2003). This increase is largely accounted for by the continued use of cheap and widely available drugs, chloroquine and sulfadoxine-pyrimethamine, which have become progressively ineffective (Attaran et al., 2004). Antimalarial resistance in *Plasmodium falciparum* parasites results in an enormous public health burden because of prolonged or recurrent illness, and progression to severe malaria, which is associated with increased hospitalisation and death. As antimalarial resistance has risen, there has been a coincident rise in malaria-related hospital admissions and malaria mortality across west, east and southern Africa (Trape, 2001; Trape et al., 2002). For example in Siaya, Western Kenya, 69% of malaria deaths are attributed to resistance to chloroquine treatment (Zucker et al., 2003). Even lower levels of resistance, which cause recrudescence of infection, are associated with return of illness, prolonged or worsening anaemia, and increased gametocyte carriage which fuels malaria transmission, particularly of the resistant parasites, and a higher risk of treatment failure in subsequent infections (Price et al., 1999, 2001). Levels of antimalarial resistance, and the subsequent public health burden and economic consequences, are frequently underestimated, particularly in areas of high intensity malaria transmission (Fevre and Barnish, 1999). This is because drug assessments are often conducted in patients with significant levels of immunity which may itself resolve the infection, and, until resistance reaches very high levels (such that parasitaemia fails to clear following treatment), the widely used day 14 in vivo therapeutic efficacy study misses the majority of treatment failures (Stepniewska et al., 2004).

2. Transmission of malaria

Malaria is a haematoprotozoan parasitic infection transmitted to humans by certain species of *Anopheline* mosquitoes. The biting insects inoculate *Plasmodium* sporozoites into the human host at the time of blood feeding. After asymptomatic pre-erythrocytic maturation in the liver, each infected hepatic schizont ruptures releasing merozoites which then invade red blood cells. These asexual blood stages cause the illness malaria, and they have historically been the focus of antimalarial treatment. Maturation of asexual stages of *P. falciparum* in the erythrocytes results in cytoadherence and sequestration of the trophozoite infected red blood cells, providing the pathophysiological basis for the development of the complications in severe malaria. Each 48 h asexual reproduction cycle ends with the rupture of schizonts and the release of merozoites, which infect further red blood cells. The parasite burden expands logarithmically by approximately ten-fold per cycle (Simpson et al., 2002; White, 2004).

In *falciparum* malaria (but not the three other human malarias) the sexual cycle is delayed with respect to the asexual cycle. Eventually after several asexual cycles, some merozoites invade red cells and there develop into either male or female gametocytes. Initially these resemble trophozoite forms, and they are sequestered. They mature in the small capillaries and venules and they are then released into the circulation. This sexual stage is responsible for infecting the *Anopholine* mosquito and thus transmission of the infection. As gamete fusion and thus meiosis takes place in the mosquito's mid gut, the Anopheline's blood meals (average 3 μL) must contain both viable female and male gametocytes, and the mosquito must survive long enough for sporozoite development and subsequent inoculation into another human host.

Gametocytes are conventionally divided into five stages of development. In the first three stages the sexual parasites are sequestered, and they are potentially susceptible to the drugs used to treat the asexual stage infection. In stage 4 they reenter the circulation and by stage 5 the gametocytes circulate and are resistant to all drugs except the 8-aminoquinolines. After an acute attack of *P. falciparum* malaria, a wave of gametocytes generally appears in the peripheral blood after 6–10 days, even when treated. Gametocytes display a diurnal subperiodic pattern, with peripheral

gametocyte densities peaking between 11:30 and 23:30 h, which may be a strategy to achieve a higher degree of transmission from human hosts to mosquito vectors (Magesa et al., 2000). The precise mechanisms that trigger the switch from asexual to sexual stage development remain unclear. Many factors can induce gametocytogenesis, particularly those which slow or inhibit asexual parasite proliferation, such as immunological stress and partially effective chemotherapy (Paul et al., 2002). With increased density and prolonged duration of the *falciparum* infection, an increasing proportion of the asexual parasites switch to sexual stage development (Sowumni and Fateye, 2003). In epidemiological studies of symptomatic patients, pre-treatment gametocyte carriage is increased in afebrile patients and those with pure *P. falciparum* asexual parasitaemia, either low density asexual parasitaemia or hyperparasitaemia, severe malaria, and anaemia (Price et al., 1999; von Seidlein et al., 2001; Nacher et al., 2002). The apparently contradictory effects of parasite density reflect different influences; high parasite numbers provide greater numbers of sexual stages at basal switch rates whereas low parasitaemias in clinical epidemiological surveys are more likely to reflect longer durations of infection, partially effective treatment or greater background immunity – all of which increase switching rates from asexual to sexual stages.

The probability of a mosquito being infected depends on the prevalence, duration and density of gametocyte carriage in the human host (Hogh et al., 1998; Drakeley et al., 1999; Targett et al., 2001). There are additional humoral (mainly specific gametocyte antibodies) and leukocyte factors which affect transmissibility but their effects have been difficult to quantify accurately. Obviously as gametocyte densities fall much below 2/μL transmission becomes impossible. The lowest density at which transmission may occur is therefore close to the limit of detection at routine microscopy, which explains reports of mosquito infection from blood which was apparently gametocyte negative. There is a predictably lower prevalence of *P. falciparum* infection and lower intensity of oocyst infection in mosquitoes fed on blood from patients with lower gametocyte density (Drakeley et al., 1999). In malariatherapy studies, there is a log sigmoid relationship between gametocyte densities in the patient's blood and infectivity to the mosquito. Individual infectiousness to mosquitoes was shown to saturate at gametocyte densities above 1000 per μL (Fig. 1) (Jeffery and Eyles, 1955). However, the infectivity described

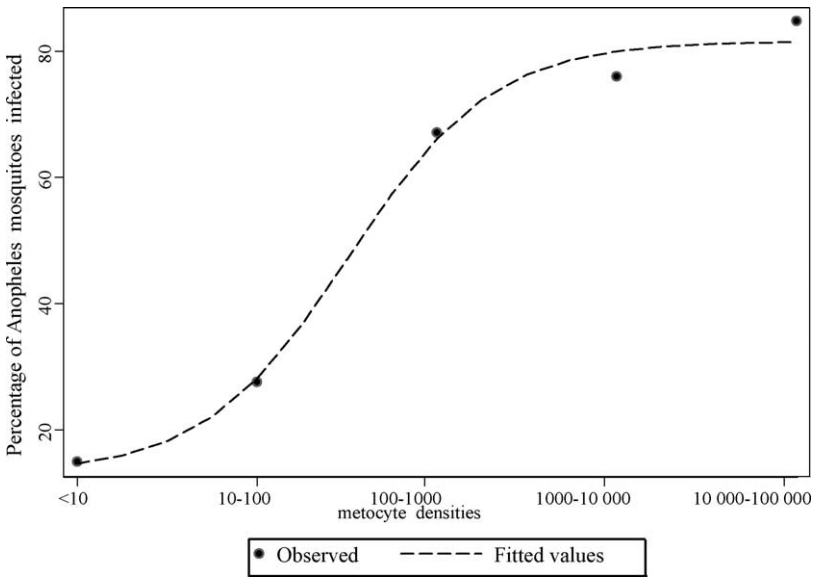

Fig. 1. The sigmoid relationship between gametocyte density (per μL) of two different strains of *P. falciparum* and infectivity, expressed as a weighted average of the proportion of *Anopheles* mosquitoes infected, in malariatherapy patients (Jeffery and Eyles, 1955). A logistic curve was fitted to observed values ($R^2 = 0.99$).

at different gametocyte densities in the malariatherapy studies of non-immune adults may overestimate that which is likely to occur in areas of high intensity malaria transmission, where humoral and cellular factors affect transmissibility, both by limiting the density of the asexual parasitaemia and by developing a sexual stage specific immunity (Buckling and Read, 2001; Akim et al., 2000). In western Kenya, for example, only 1% of mosquitoes were infected by gametocyte carriers presenting with suspected malaria. Among these patients, gametocyte densities were generally very low (geometric mean of 39 gametocytes per microlitre) and 67% of all gametocyte carriers were older than 5 years of age (Gouagna et al., 2003).

3. Measuring transmissibility

A wide variety of measurements of transmissibility of *P. falciparum* parasites have been reported. The least informative are those that simply report the proportion of individuals that have detectable gametocytaemia. Most studies report gametocyte carriage only in the initial 7–14 days following therapy. A longer duration of follow up is necessary to quantify duration of gametocyte carriage, particularly for comparing gametocyte carriage of antimalarial resistant and sensitive infections (Fig. 2A and B). The withdrawal of treatment failures from studies generally results in underestimation of the duration of gametocyte carriage in treatment failures relative to those with an adequate clinical and parasitological response who usually complete follow-up. Gametocyte carriage may be presented as the duration for which there is patent gametocytaemia and summed for all studied patients (i.e. gametocyte person weeks per unit number of studied patients).

Quantifying gametocyte density accurately (on the basis of gametocytes per 1000 leukocytes) and reporting the area under the gametocyte time curve is preferable to gametocyte person weeks as a measure of gametocyte carriage. However, this does not incorporate the non-linear relationship between gametocyte densities in the patient's blood and infectivity to the mosquito. A logistic curve ($R^2 = 0.99$) can be fitted to the association of the probability of mosquito infection and gametocyte densities in malariatherapy patients (Jeffery and Eyles, 1955). This relationship can

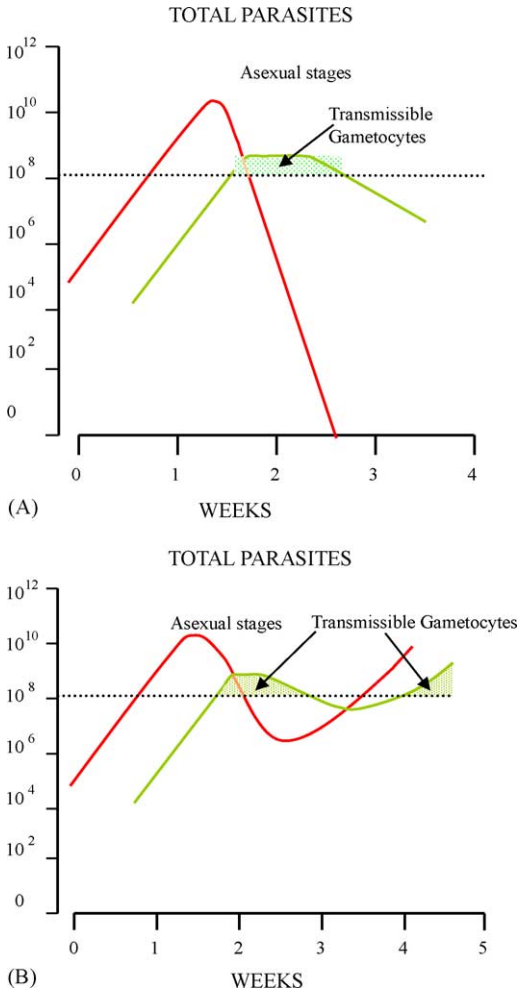

Fig. 2. An example of the density of asexual parasites and gametocytes over 28 days following treatment in a patient with an adequate clinical and parasitological response (A) and with parasitological failure (B). Routine microscopy detects asexual parasite densities down to approximately 50/μL, which corresponds to a total burden of approximately 10^8 parasites in an adult.

be used to predict the infectivity to mosquitoes from observed gametocyte densities – assuming that the effects of humoral and cellular factors are not significant (Fig. 1). The individual infectivity per unit time can be derived from this relationship and the normalized value summed for each individual patient (Fig. 3). The sum of these individual values is probably the best measure of infectivity in a group of patients. The number of oocysts in the midguts of *Anopheles* mosquitoes fed on blood samples collected from parasitaemic

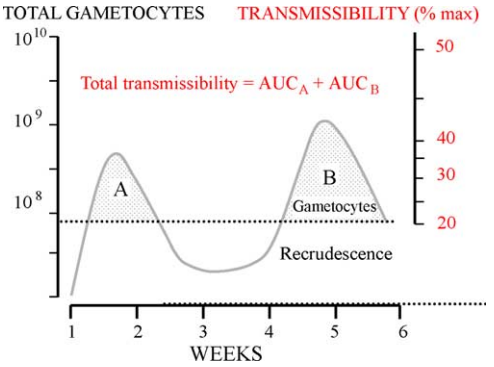

Fig. 3. Gametocytaemia in an infection which recrudesces once. Transmissibility at each measured time point (right side *y*-axis expressed as a percentage of maximum) can be extrapolated from Fig. 1 and summed over the total period of gametocyte carriage.

patients following treatment via an artificial membrane can be used as a direct measure of infectivity to the mosquito.

4. Antimalarial drug resistance

P. falciparum has developed clinically significant resistance to all classes of antimalarial drugs, with the possible exception of artemisinin derivatives. Resistance is a shift to the right of the dose–response curve, thus requiring higher drug concentrations to achieve the same parasite clearance (White, 2004). Resistance emerges de-novo through spontaneous mutations or gene duplications, which are thought to be independent of drug selection pressure, but these mutants are then selected for and spread as a result of the drug pressure which provides a selective advantage to resistant parasites. Thus there are two discrete phases to the development of resistance; de-novo emergence and subsequent spread. Resistance arises mainly during asexual reproduction, and may require only a single genetic event (e.g. antifol or atovaquone resistance) or multiple events (epistasis). But meiosis does take place in the Anopheline mosquito, and many infections are multiclonal. In addition sometimes mosquitoes bite two gametocyte carrying humans. These offer the possibility of recombination with the formation or breakdown of multigenic resistance.

The intrinsic frequency of the genetic events that confer antimalarial resistance (while retaining parasite viability) varies between antimalarials, but this is generally a rare event. The most readily occurring mechanisms of resistance to current antimalarials are the single point mutations in Cytochrome b which confer high level atovaquone resistance; the per-parasite probability of arising de novo has been estimated at approximately 1 in 10^{12} parasite multiplications in vivo (White, 1999). Viable mutations in the gene encoding dihydrofolate reductase (*Pfdhfr*), which confer pyrimethamine resistance, were also estimated to develop with a similar frequency. An adult with approximately 2% parasitaemia carries a burden of 10^{12} parasites so these arise readily. Pyrimethamine is no longer used alone; it is combined with slowly eliminated sulphonamides (usually sulfadoxine), which may well confer some independent protection against the emergence of resistance despite acting on the same pathway. The mutations associated with resistance to sulfadoxine (in *Pfdhps*) and pyrimethamine (*Pfdhfr*) are widespread, and it was thought that these arose de-novo very frequently, and that this accounted for the rapid development of sulfadoxine-pyrimethamine resistance wherever it has been deployed. However recent molecular epidemiological studies from Southeast Asia, Southeast Africa and South America illustrate the remarkable capacity for spread of these antifol resistant parasites. A single parasite (with mutations in *Pfdhfr*, at positions 108, 51 and 59) has, in recent years, spread across these vast regions (Cortese et al., 2002; Roper et al., 2003; Nair et al., 2003). The resistant haplotypes observed in both Africa and Asia share a common ancestor, as the neutral polymorphic sequences in the flanking regions (the genetic material adjacent to the *dhfr* mutations) are identical (Roper et al., 2004). The studies in southeast Africa and South America presented similar findings indicative of a single origin of mutations in dihydropteroate synthase (*dhps*) that confer sulfadoxine resistance. There are two properties of sulfadoxine-pyrimethamine that may have contributed to this remarkable spread of resistance; the apparent stimulation of gametocytogenesis associated with poor therapeutic responses to sulfadoxine-pyrimethamine and the widespread exposure of *P. falciparum* parasites in these regions to sub-therapeutic sulfadoxine-pyrimethamine concentrations either during its long elimination phase from the body or following sub-therapeutic dosing (Watkins and Mosobo, 1993; Bousema et al., 2003; Terlouw et al., 2003).

5. Transmission and resistance

Although eliminating the asexual stages of *P. falciparum* is the focus of treatment of individual symptomatic patients, at a population level, reducing carriage of the gametocytes is necessary to limit the transmission of malaria parasites, and in particular, the transmission of resistant parasites. Antimalarial drugs are important in controlling the passage of *falciparum* parasites from humans to the mosquito vector, although this relationship is complex as different antimalarials have different effects on the various stages of gametocytogenesis, on gametocyte infectivity to the mosquito, and on the development of the parasites in the mosquito vector.

Antimalarial drugs which kill the asexual stages of *Plasmodium vivax, P. malariae* and *P. ovale* also act against the sexual stages of these parasites, but the antimalarial drugs generally only affect stages 1–3 of *P. falciparum* gametocyte development. The artemisinin derivatives may affect stage 4 gametocytes, but only the 8-aminoquinolines (such as primaquine) are active against mature (stage 5) *P. falciparum* gametocytes (Bunnag et al., 1980; ter Kuile et al., 1993).

Sulfadoxine-pyrimethamine is associated with the highest post-treatment prevalence and density of gametocyte carriage of all antimalarial drugs. This may reflect a drug induced release or redistribution of gametocytes as this increase is seen as early as 4 days after treatment (Targett et al., 2001). After adjusting for gametocyte density, there is a lower probability of infecting mosquitoes in gametocyte carrying children treated with sulfadoxine-pyrimethamine compared with chloroquine (Hogh et al., 1998; Robert et al., 2000). This relatively lower infectivity per gametocyte may be due to a sulfadoxine-pyrimethamine-triggered release of immature forms that are not yet infectious (Targett et al., 2001). However, the higher prevalence and density of gametocytes following sulfadoxine-pyrimethamine treatment more than offsets this apparent lower per patent gametocyte transmissibilty. In The Gambia, where sulfadoxine-pyrimethamine resistance is uncommon, 25.6% of children treated with sulfadoxine-pyrimethamine compared with 12.2% of children treated with chloroquine were found to be infectious 7 days after treatment (Targett et al., 2001). Treatment with artemisinins result in the lowest levels of gametocyte carriage, even when administered together with sulfadoxine-pyrimethamine (International Artemisinin Study Group, 2004; Targett et al., 2001). This is as a result of both the lower production of gametocytes, due to the rapid reduction in asexual parasite biomass, and their direct gametocytocidal effect against immature stages (ter Kuile et al., 1993; White, 2004).

Gametocyte carriage is significantly higher in patients infected with resistant parasites than sensitive ones, fuelling the spread of antimalarial resistance. This is expected as the slower clearance and prolonged presence of asexual parasites associated with resistance increases gametocytogenesis (Fig. 2A and B). Furthermore, the apparent half life of gametocytes was longer and their apparent clearance slower in children with chloroquine-resistant infections than those with chloroquine-sensitive infections (Drakeley et al., 2004; Sowumni and Fateye, 2003). It should be noted that these kinetic terms are in fact hybrids of formation in and removal from the circulation. The ratios of post treatment gametocyte prevalence (i.e. the proportion of patients carrying any gametocytes) of treatment failures to sensitive *falciparum* infections was 4.0 for mefloquine (Price et al., 1996), 4.1 for sulfadoxine-pyrimethamine (Bousema et al., 2003) and for chloroquine ranged between 2.9 and 12 (Drakeley et al., 2004; von Seidlein et al., 2001; Hogh et al., 1998; Sowumni and Fateye, 2003). However, these studies differ in their baseline prevalence of gametocytaemia, age distribution, levels and definitions of antimalarial drug resistance and duration of follow up (7–28 days). Variations in the intensity of malaria transmission will result in differences in the effects of acquired immunity on gametocyte carriage. Not all studies adjusted their comparisons for these other determinants of gametocyte carriage. As mentioned previously, gametocyte prevalence ratios do not capture differences in duration of gametocyte carriage or in gametocyte density, both important determinants of the probability of mosquito infection.

In The Gambia at a time of worsening chloroquine resistance gametocytes that emerged after successful treatment with CQ were significantly more likely to be of the resistant *pfmdr1-86Tyr* and *pfcrt-76Thr* genotypes and to be infective to *Anopheles* mosquitoes. Apparent therapeutic success may thus be accompanied by public health failure as cured children pass resistance genes on to mosquitoes at an enhanced rate (Sutherland et al., 2002). This presumably reflects

differential parasite killing in multiclonal infections and in vivo selection. Similarly, *Pfdhfr* mutations insufficient to cause treatment failure, were associated with a significant increase in gametocyte carriage and thus may increase malaria transmission and promote the spread of sulfadoxine-pyrimethamine resistance (unpublished observations and Mendez et al., 2002). Furthermore, antifolates such as sulfadoxine-pyrimethamine, when ingested in a blood meal taken by a biting *Anopheline* mosquito, are sporonticidal by inhibiting further development in sensitive *P. falciparum*, damaging ookinetes and reducing oocyst numbers (Hogh et al., 1998). Thus selection also takes place in the mosquito and sulfadoxine-pyrimethamine resistant parasites are likely to produce more oocysts than sensitive parasites, fuelling the more rapid spread of resistance.

6. Artemisinin-based combination therapy

One of the most important benefits of artemisinin-based combination therapy is the potential to delay the spread of antimalarial resistance. Wide-scale use of artesunate plus mefloquine decreased mefloquine resistance in northwest Thailand (Nosten et al., 2000). This is explained both by the advantage of using two drugs with different mechanisms of action, preventing further selection of resistance and resulting in higher cure rates, and by the effect of artesunate in reducing gametocyte carriage, even in mefloquine resistant infections. ACTs decrease the transmission advantage of the resistant parasites over sensitive parasites, from a gametocyte carriage ratio of 4:1 (monotherapy resistant: sensitive) to a ratio of 1:1 (ACT resistant: sensitive) (Price et al., 1996). In patients with no detectable gametocytaemia at baseline included in a meta-analysis of randomized controlled trials comparing artemisinin-based combination therapy with monotherapy, the addition of 3 days artesunate dramatically reduced gametocyte carriage on day 7 (OR 0.11, 95% CI 0.09–0.15, $n = 2734$), with larger effects at days 14 and 28 (International Artemisinin Study Group, 2004). Decreased gametocyte carriage following artemether-lumefantrine treatment has been shown to limit post-treatment transmission of *P. falciparum* to *Anopheles* mosquitoes (Sutherland et al., 2005). Although artesunate consistently reduces post-treatment infectivity to mosquitoes, primarily by decreasing gametocytes in peripheral blood and preventing recrudescence, it does not abolish infectivity completely. The probability of malaria transmission was 8-fold lower in children treated with 3 day artesunate plus sulfadoxine-pyrimethamine regimen (0.3% probability of transmission), when compared with sulfadoxine-pyrimethamine monotherapy (Targett et al., 2001). The addition of 3-day artesunate to chloroquine significantly reduced post-treatment prevalence and mean density of gametocytes in the first 14 days (Drakeley et al., 2004). In this randomised controlled trial in The Gambia, treatment failure was associated with 2.3-fold ($p = 0.30$) higher intensity of mosquito infection at day 7 in artesunate-chloroquine treated children, compared with a 26-fold ($p = 0.002$) increase in chloroquine treated children. However cure rates were low because of the poor efficacy of chloroquine, and thus later gametocyte carriage could not be prevented. This emphasises that the overall transmission reduction benefit of artemisinin derivatives is closely related to asexual stage therapeutic efficacy of the combination.

The gametocyte-reducing effect of widespread use of a highly effective artemisinin based combination therapy (artesunate plus mefloquine), has been shown to translate into a sustained 6-fold decrease in *Plasmodium falciparum* transmission in an area of low intensity transmission in north-western Thailand (Nosten et al., 2000). Similar decreases in malaria transmission following the widespread use of other artemisinin-based combination therapies have been documented in Vietnam and South Africa (Muheki et al., 2004).

However, a double-blind, community-randomized, placebo-controlled trial conducted in a rural area of The Gambia to test whether a reduction in the infectious reservoir through mass drug administration (MDA) of a single dose of sulfadoxine-pyrimethamine combined with artesunate could reduce malaria transmission, found no benefit after two months. During the first two months of surveillance, however, the malaria incidence was lower in treated villages. The authors explained the absence of a sustained impact on malaria transmission by the very high basic reproductive rate of malaria in this area, and the persistence of mature gametocytes, which are not affected by artesunate treatment (von Seidlein et al., 2003). The use of only a single dose of artesunate may have contributed to the low impact as randomised controlled trials comparing the addition

of 1-day versus 3-day artesunate to longer acting antimalarials found that the 3-day regimen resulted in significantly lower gametocyte carriage (International Artemisinin Study Group, 2004).

7. Intensity of malaria transmission

In areas of unstable or low endemicity, most malaria transmission is likely to come from patients with symptomatic malaria, as transmission intensity is generally too low for acquiring partial immunity. Thus asymptomatic infection is relatively rare. In these settings, antimalarial drug use patterns and antimalarial pharmacokinetics and pharmacodynamics would be the major determinants of the spread of antimalarial drug resistance. In areas of high intensity malaria transmission the situation is more complex, as disease controlling immunity results in frequent asymptomatic parasitaemia and transmission from untreated healthy individuals – and thus lower drug pressure (the proportion of *falciparum* parasites exposed to sub-therapeutic antimalarial concentrations). In addition, sexual stage specific immunity gradually develops in those living in areas of high intensity malaria transmission. The probability of cure of drug resistant parasites is greater, and the selective advantage of resistant parasites is less. These factors reduce both de-novo emergence and spread of resistance (White and Pongtavornpinyo, 2003; White, 2004). These differences may explain why historically multi-drug resistance has generally developed more rapidly in areas with low intensity and unstable malaria transmission.

The majority of studies of the effect of antimalarial drugs on gametocyte carriage have been conducted in acutely symptomatic malaria patients. It is not clear the extent to which these findings can be extrapolated to those who are asymptomatic. These asymptomatic individuals make up a considerable proportion of the gametocyte pool. Asymptomatic carriers in Western Kenya were significantly more likely than symptomatic patients to infect mosquitoes. This difference was due to a higher level of infectivity of their gametocytes, as well as their higher gametocyte densities (Gouagna et al., 2004). These asymptomatic gametocyte carriers had lower asexual parasite densities than symptomatic patients. This could be explained by the parasite-mediated release of pro-inflammatory cytokines, commonly associated with high asexual parasite densities, which increases gametocyte mortality (Naotunne et al., 1993). Asymptomatic and chronic *falciparum* infections in semi-immunes are associated with recurrent, low density, intermittent gametocytaemia (Babiker et al., 1999). The proportion of malaria transmission and the contribution these individuals make to the delayed spread of resistance is currently poorly defined.

8. Conclusions

The selection and spread of resistant parasites can be limited by the widespread use of effective antimalarial combination therapies. Consensus has recently be achieved that the principle guiding the use of tuberculosis, HIV/AIDs and leprosy treatments in combination, applies equally well to malaria (Roll Back Malaria Partnership Secretariat, 2004). With the use of two antimalarials drugs with different mechanisms of action, and therefore different resistance mechanisms, the probability of any one *P. falciparum* parasite developing simultaneously the mutations required to resist both drugs is then dramatically reduced. Artemisinin-based combination therapies (ACTs) have three particular additional advantages. Therapeutically significant resistance to the artemisinin derivatives has not been identified; neither can stable significant resistance be induced in the laboratory. Artemisinins, with parasite reduction ratios of 10 000 per cycle, reduce the asexual parasite load more rapidly than any other antimalarials (White, 2004). Lastly, artemisinins are associated with marked decrease in gametocyte carriage (Price et al., 1996). The resistance prevention benefits of ACTs have been well described in northwest Thailand where the widespread use of artesunate plus mefloquine has resulted in a reversal of mefloquine resistance (Nosten et al., 2000; Brockman et al., 2000). Although improvements in cure rates, and decreased gametocyte carriage have been documented in Africa and South America, widespread use of ACTs has not been in place for long enough to determine their effects on resistance in areas of high intensity transmission (International Artemisinin Study Group, 2004).

Antimalarial drug resistance is the predominant factor increasing the global burden of malaria, particularly in sub-Saharan Africa. Antimalarial drug resistance spreads because drug resistant infections

have greater transmission potential than sensitive ones. This derives largely from their greater gametocyte carriage. Early access to highly effective treatment is essential, as in addition to reducing the risk of disease progression, this would limit gametocyte carriage which is increased by prolonged duration of symptoms and ineffective treatment. Malaria treatment policy should be based primarily on drug efficacy against asexual stages, but should also consider the antimalarial effect on gametocytes, which are pivotal in malaria transmission and the spread of antimalarial resistance. In this way malaria control interventions to reduce malaria transmission, traditionally vector control measures, could be complemented with the synergistic effects of antimalarial treatment policies which decrease gametocyte carriage. In marked contrast to the antifolate antimalarials such as sulfadoxine-pyrimethamine which actually stimulate gametocytogenesis, artemisinin derivatives reduce gametocyte carriage markedly. Artemisinin-based combination therapies (ACTs) are the only antimalarials currently available which rapidly reduce both asexual and gametocyte stages of the *P. falciparum* lifecycle, and their widespread implementation offers our best chance to reverse the increase in morbidity and mortality resulting from the continued use of ineffective antimalarials. This is one of few effective measures that will enable malaria-endemic countries to progress towards achieving the ambitious goals set in Abuja to "Roll Back Malaria", particularly the halving of malaria morbidity and mortality by 2010. The availability of international financial support, particularly the global fund for fighting AIDs, tuberculosis and malaria, has made access to ACTs through strengthened healthcare infrastructures a tangible option, even in the most resource limited malaria endemic countries, where the social and economic burden of malaria is greatest.

Acknowledgements

The authors thank Francesca Little (Department of Statistical Sciences and Division of Clinical Pharmacology, University of Cape Town) for assistance with fitting the logistic curve in Fig. 1. Karen I Barnes is employed by the University of Cape Town Division of Clinical Pharmacology. Nicholas J White is a Wellcome Trust Principal Fellow.

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