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Low infectivity of *Plasmodium falciparum* gametocytes to *Anopheles gambiae* following treatment with sulfadoxine–pyrimethamine in Mali

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Abstract

Sulfadoxine–pyrimethamine (SP) treatment increases the rate of gametocyte carriage and selects SP resistance-conferring mutations in *Plasmodium falciparum* dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS), raising concerns of increased malaria transmission and spread of drug resistance. In a setting in Mali where SP was highly efficacious, we measured the prevalence of DHFR and DHPS mutations in *P. falciparum* infections with microscopy-detected gametocytes following SP treatment, and used direct feeding to assess infectivity to *Anopheles gambiae* sensu lato. Children and young adults presenting with uncomplicated malaria were treated with SP or chloroquine and followed for 28 days. Gametocyte carriage peaked at 67% 1 week after treatment with a single dose of SP. Those post-SP gametocytes carried significantly more DHFR and DHPS mutations than pre-treatment asexual parasites from the same population. Only 0.5% of 1728 mosquitoes fed on SP-treated gametocyte carriers developed oocysts, while 11% of 198 mosquitoes fed on chloroquine-treated gametocyte carriers were positive for oocysts. This study shows that in an area of high SP efficacy, although SP treatment sharply increased gametocyte carriage, the infectiousness of these gametocytes to the vector may be very low. Accurate and robust methods for measuring infectivity are needed to guide malaria control interventions that affect transmission.

Keywords

Plasmodium falciparum; Gametocytes; Sulfadoxine–pyrimethamine; Infectivity; *Anopheles gambiae*; Mali

1. Introduction

Sulfadoxine–pyrimethamine (SP) is recommended for intermittent preventive treatment of malaria in pregnant women (IPTp) (Newman et al., 2006) in sub-Saharan Africa and is contemplated for a similar use in infants (IPTi) (ter Kuile and Steketee, 2006) and children (IPTc) (Clarke et al., 2008). The efficacy of these interventions would be compromised by the development and spread of *Plasmodium falciparum* resistant to SP (O'Meara et al., 2006). For genetically-determined drug resistance to propagate, the mature gametocyte must carry the genetic information encoding resistance to the mosquito for sporogonic development. The ability of the host to infect the mosquito vector, also termed mosquito infectivity, involves several factors including the maturity, longevity, density and sex ratio of the gametocyte population, as well as host immunity (Smalley, 1977; Graves et al., 1988; Robert et al., 1996; Buckling and Read, 2001; Drakeley et al., 2006).

It is thought that reducing the duration of gametocytemia will contribute to a lower intensity of malaria transmission (Strickland et al., 1986; Lines et al., 1991; Price et al., 1996; Enosse et al., 2000; Nosten et al., 2000). In studies of chloroquine-sensitive gametocyte populations, a constant rate of population decline was noted over the 16–28 days following chloroquine treatment with no further recruitment of gametocytes occurring following peak gametocytemia (Butcher, 1997; Hogg et al., 1998), and the *P. falciparum* gametocyte population half-life was estimated to be 2.4–2.5 days (Smalley et al., 1981; Hogg et al., 1995). Gametocytes in developmental stages 0–3 are sensitive to the gametocidal effects of both chloroquine and SP, but beyond developmental day 6 of a 10 day cycle, the gametocytes become relatively insensitive (Smalley, 1977; Smalley and Sinden, 1977). Based on these observations, gametocytes recruited into the population after the peak, or 10–12 days following treatment, would be evidence of parasite resistance. Few studies have adequately addressed the issue of antimalarial drug resistance and transmission in light of these considerations.

Single nucleotide polymorphisms (SNPs) in the *P. falciparum* genes encoding dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) confer resistance to pyrimethamine and sulfadoxine, respectively (Zolg et al., 1989; Peterson et al., 1990; Triglia et al., 1997). Increasing levels of resistance to pyrimethamine are seen with the DHFR mutations S108N, N51I and/or C59R, and I164L and DHPS mutations A437G and K540E confer resistance to sulfadoxine (Triglia et al., 1997). A set of five mutations (the “quintuple mutant”) including the mutations at DHFR codons 108, 51 and 59 and DHPS codons 437 and 540 have been found to be predictive of clinical failure in Malawi (Kublin et al., 2002) and elsewhere in Africa (Dorsey et al., 2004).

The prevalence of gametocytemia increases following treatment with SP (Robert et al., 2000; Mendez et al., 2002). Although SP treatment consistently selects for infections carrying the SP resistance-conferring mutations, it has not been directly demonstrated that SP-resistant gametocytes (as opposed to remaining asexual forms and/or residual DNA from asexual forms) are selected by SP treatment (Hallett et al., 2006; Méndez et al., 2007). Previous studies of the infectivity of post-SP treatment gametocytes to the *Anopheles* vector have yielded conflicting results (Hogg et al., 1998; Targett et al., 2001; Oesterholt et al., 2009). While earlier publications showed that pyrimethamine and related drugs decreased gametocyte infectivity to the vector (Shute and Maryon, 1954; Jeffery, 1963; Chutmongkonkul et al., 1992; Hogg et al., 1998; Robert et al., 2000), some more recent studies have suggested that SP increases gametocyte infectivity (Targett et al., 2001; Hallett et al., 2006; Méndez et al., 2007). Here, we report results of a study in which we determined the genotypes of post-SP treatment gametocytes and used direct feeding, the most natural

route of malaria transmission, to measure the infectivity of these gametocytes to *Anopheles gambiae* in Mali.

2. Materials and methods

2.1. Study site

The study was conducted in two neighboring rural villages, Kolle and Bancoumana, approximately 57 and 60 km south of Bamako, respectively, in Mali, West Africa. Residents of both villages are almost exclusively farmers. *P. falciparum* malaria is endemic and seasonal with parasite prevalence rates ranging from 40% to 50% in the dry season (October through April) and 70–85% in the rainy season (May through September) (Plowe et al., 1996). The rate of parasitological resistance to chloroquine in Kolle was 30.7% and 28.3% in 1998 and 1999, respectively (Djimde et al., 2003). At the initiation of these studies no data on in vivo SP resistance were available, but the complete absence of the DHFR 108N mutation in Kolle in 1996 suggested that SP would be highly efficacious in this village (Plowe et al., 1996).

2.2. Study design

2.2.1. Prospective in vivo SP efficacy—From September to December 2000, an SP efficacy study was carried out using a modification of the World Health Organization (WHO) 1973 in vivo 28-day protocol (WHO, 1973; Plowe et al., 2001). Persons presenting to the clinic with symptoms consistent with malaria were eligible for inclusion in the study if they were aged 6 months and older with positive malaria smears and either fever or screening parasitemia >2000 parasites/ μ l and none of the following: known pregnancy, hematocrit <15%; parasitemia >10%; prostration; respiratory distress; shock; bleeding; severe vomiting; or history of allergic or other severe adverse reaction to SP. Individuals who met these criteria and consented to participate, were enrolled in the study from September to December 2000. Symptoms, prior antimalarial drug use and physical examination data were recorded on standard data forms.

Standard recommended treatment doses of SP were administered as single oral doses (1/4 tablet per 5 kg weight for age, 1 tablet = pyrimethamine 25 mg + sulfadoxine 500 mg). Participants were observed for 60 min to monitor for adverse reactions and to make sure the drug was not vomited. If vomiting occurred within 30 min, the full dose was repeated and if within 60 min one half of the dose was repeated. Malaria thick smears for microscopic diagnosis and filter paper (3MM Whatman) samples were collected at the time of enrollment. Smears were Giemsa-stained and asexual forms were counted using a light microscope at 1000 $\times$ magnification and the number of parasites per 7500 leucocytes was determined. Ten percent of slides were read independently by an expert microscopist for quality control.

With enrollment occurring on day 0, participants were followed actively on days 3, 7, 14, 21 and 28, and passively by 24 h availability of a study clinician to evaluate and treat illnesses. At all active and passive follow-up times malaria smears and filter paper samples were obtained and brief history and physical evaluations made to seek signs or symptoms of persistent or recrudescence malaria. Cases of RI, RII or RIII in vivo resistance were defined as described (Diourte et al., 1999). Fever was treated with paracetamol.

Cases of severe malaria in any subject evaluated at any time, including the first 3 days after treatment, were fully evaluated and treated with appropriate medical care as recommended by the Malian National Malaria Control Programme. The study protocol was reviewed and

approved by Institutional Review Boards of the University of Bamako Faculty of Medicine, Mali and the University of Maryland School of Medicine, USA.

2.2.2. Molecular analyses—DNA was extracted from filter paper using a methanol-heat fixation method as previously described (Plowe et al., 1995). A nested mutation-specific PCR with a parasite detection limit of approximately 250 parasites/ μ l was performed according to published methods (Plowe et al., 1995). DHFR mutations at codons 108, 51 and 59 and DHPS mutations at codons 437 and 540 were included in the analysis. Assays for all five mutations were performed on all samples collected. Mutant and wild type positive controls as well as extraction negative controls and buffer negative controls were included in each PCR. Because the finding of the DHPS 540 mutation was unexpected, these reactions were repeated by a blinded independent investigator and PCR amplicons of the *dhps* gene were sequenced using a commercial service provider (Macrogen Inc., Seoul, South Korea).

The 2002 and 2003 studies were conducted in the context of a trial to test the efficacy of chloroquine, amodiaquine and SP in Kollo and the nearby village of Bancoumana. Detailed descriptions of these studies are reported elsewhere (Tekete et al., 2009). Treatment outcomes were classified according to the 2003 WHO's protocol as early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF) and adequate clinical and parasitological response (ACPR) (WHO, 2003).

2.2.3. Gametocytemia measurements—Prevalence of gametocyte carriage and gametocyte density was measured before treatment and after treatment. During the 2000 study, gametocytes were counted against 300 leucocytes as described above, while in 2002 and 2003, the counts were made against 1000 leucocytes for increased precision. Drug-resistant genotypes of asexual parasites and gametocytes were determined by PCR as described above. Because no pure gametocyte carriers were available before treatment, the genotypes of the post-treatment gametocytes were compared to the genotype of pre-treatment infections that consisted predominantly of asexual stages by light microscopy.

2.2.4. Direct feeding experiments—Every 1–2 weeks throughout the transmission seasons of 2002 and 2003, wild-caught, naturally blood-fed *A. gambiae* collected by aspirators from houses in the village of Kollo were brought to the Bamako laboratory and allowed to oviposit. The progeny reared to the adult stage were used in direct feeding experiments. Direct feeding experiments were done on study days 0, 7, 14, 21 and 28 if gametocytemia was detected and mosquitoes were available for feeding. Gametocyte carriers aged 6–18 years were invited to participate in the direct feeding portion of the study. Up to 90 female F1 generation mosquitoes were starved for approximately 12 h and held in two small screen-covered cups containing approximately 45 mosquitoes each. They were then allowed to blood-feed for up to 15 min on the legs of each volunteer. After feeding, unfed mosquitoes were removed, and only fed mosquitoes were kept in the village under normal environmental conditions overnight. The next day, these mosquitoes were transported back to the insectary (27 °C and 80% relative humidity) in Bamako where they were provided with 3% sucrose solution changed daily until dissections were performed.

For oocyst determination and quantification, groups of at least 15 mosquitoes per carrier were dissected 8 days p.i. in 0.5% mercurochrome. Oocysts were counted under microscopy 10 times for each individual mosquito. The proportion of oocyst-positive mosquitoes that were fed on gametocyte carriers between days 7 and 28 after SP or chloroquine treatment was determined.

2.2.5. Statistical analyses—Statistical analyses were performed with SPSS version 12.0.1 and STATA 7.0. Frequencies of gametocytes carriage, the presence of resistance mutations between asexual and sexual forms and gametocyte infectivity were compared using a Chi-square test or two-tailed Fisher's exact test with the significance level set at $P=0.05$. Proportions of individual mutations, polymorphisms at DHFR codons 108, 51 and 59 (triple mutant), triple mutant + DHPS mutations at codon 437 (quadruple mutant) and quadruple + DHPS 540 (quintuple mutant) were analyzed.

3. Results

Three hundred and forty-four participants were enrolled; 10 (2.9%) were lost to follow-up by day 28; 54.5% were male, and the mean age was 5.4 years (range 6 months–32 years).

3.1. In vivo SP efficacy

Of the 334 subjects included and successfully followed in the 2000 SP efficacy study, only two (0.6%) and three (0.9%) had infections classified as resistant at the RIII and RI levels, respectively, for a 98.5% cure rate. Treatment responses of chloroquine, amodiaquine and SP have been presented in detail elsewhere (Tekete et al., 2009). Briefly, day 28 ACPR rates were 14.1%, 62.3% and 88.9% in 2002 and 18.2%, 60% and 85.2% in 2003 for chloroquine, amodiaquine and SP, respectively. After molecular correction for re-infection, ACPR rates were 63.2%, 88.5% and 98.0% in 2002 and 75.5%, 85.2% and 96.6% in 2003 for chloroquine, amodiaquine and SP, respectively (Tekete et al., 2009).

3.2. Gametocyte carriage

In the SP study conducted in 2000, the frequency of gametocytemia rose from 12.8% (44/344) on the day of treatment to 25.7% on day 3 (88/342) and peaked at 40.4% on day 7 (136/337) before declining to 36.0% on day 14 (121/336), 16.7% on day 21 (56/335) and 7.2% at day 28 ($n=24/333$) (Fig. 1). Gametocytemia in 2002 and 2003 after SP treatment similarly peaked at day 7 (Fig. 1) with prevalences of 52% and 67%, respectively. By comparison, gametocyte prevalence following chloroquine treatment reached maximum levels of 31% in 2002 and 22% in 2003. The prevalence of gametocytemia was significantly greater for SP than for chloroquine on days 7, 14 and 21 in both 2002 and 2003 (Fig. 1). Similarly, the geometric mean densities of gametocytes were higher after SP treatment than after chloroquine treatment (data not shown).

3.3. Molecular markers of drug resistance

Samples from the 2000 study were analyzed by PCR. Day 0 genotypes were obtained from amplification of infections comprised predominantly of asexual parasites according to light microscopy results. Genotypes detected on days 7–28 represented infections consisting predominantly of gametocytes, since no asexual parasite forms were detected by microscopy at these time points. PCR amplification of samples with fewer than 75 gametocytes/ μL yielded no visible product on gel electrophoresis.

Resistance-conferring DHFR mutations at codons 108, 51 and 59 and DHPS at codons 437 and 540 were significantly more frequent in the post-SP gametocytes than in the pre-treatment asexual parasites ($P<0.001$). Prevalence of the triple mutant (DHFR 51, 59 and 108) was 20% at baseline (day 0), and increased to 25.3% on days 3–28 after SP treatment. Prevalence of the quadruple mutant genotype (DHFR 108, 51, 59 and DHPS 437) was 2.4% ($n=336$) at day 0 and rose to 7.9% during the follow-up ($P<0.001$). For the quintuple mutant genotype (DHFR 108, 51, 59 and DHPS 437 and 540) the prevalence was null (0.0%) at day 0 and 2.1% (Fisher's test; $P=0.02$) during the follow-up ($n=141$) (Fig. 2).

There was a significant increase in the prevalence of each of the drug resistance-conferring mutations in the gametocyte population during the follow-up period. Notably, no DHPS 540E mutations were detected in any of the baseline samples however, that mutation was present at a frequency of 16.1% ($n = 87$), 10.3% ($n = 136$), 36.1% ($n = 119$), 48.2% ($n = 56$), and 75% ($n = 24$) on days 3, 7, 14, 21 and 28 post-SP treatment, respectively (Fig. 3).

3.4. Infectivity study

Mosquitoes were direct-fed on 14 and 30 SP-treated volunteers in 2002 and 2003, respectively. The prevalence of oocyst positivity among the mosquitoes fed on SP-treated gametocyte carriers was 7/928 (0.8%) and 1/800 (0.1%) in 2002 and 2003, respectively, for an overall oocyst positivity of 0.5% (8/1728). Each of the positive mosquitoes had only one oocyst in its midgut. To double-check the above results with a different drug, feeding experiments were performed in 2003 on four chloroquine-treated volunteers. Oocysts were present in 22/198 (11.1%) of mosquitoes fed on these chloroquine-treated gametocyte carriers. The mean number of oocyst per mosquito was 1.9 (range 6–36) in the chloroquine group.

4. Discussion

In a setting where the clinical efficacy of SP was above 95%, direct feeding assays showed that *P. falciparum* gametocytes after SP treatment had very low infectivity to *A. gambiae* mosquitoes. This reduced infectivity was seen during two consecutive transmission seasons and was demonstrated both by the numbers of mosquitoes that were oocyst-positive and the density of oocysts in the midguts of positive mosquitoes. To examine the possibility of a systematic bias in our experiment we repeated the same procedures with gametocyte carriers after chloroquine treatment. These experiments were performed contemporaneously with the second year of SP feeding experiments. We found that mosquitoes fed on chloroquine-treated gametocyte carriers had higher oocyst positivity and higher oocyst density per midgut, suggesting that the direct feeding procedures themselves did not account for the low infectivity.

Although these findings are at odds with some recent studies that suggested that SP increases gametocyte infectivity (Targett et al., 2001; Hallett et al., 2006), they are in agreement with older literature that found that pyrimethamine and related drugs decreased gametocyte infectivity to the vector (Shute and Maryon, 1954; Jeffery, 1963; Chutmongkonkul et al., 1992; Hogg et al., 1998; Robert et al., 2000). This difference is most likely because, as was done in the older studies, we used direct feeding methods, allowing mosquitoes to feed directly on the skin of gametocytemic volunteers, while the recent studies have all used membrane feeding, which involves withdrawing venous blood into anticoagulated tubes and passing it through a series of tubes to feed to mosquitoes through a membrane (Hogg et al., 1998; Drakeley et al., 1999, 2006; Robert et al., 2000; Targett et al., 2001; Govere et al., 2003; Bharti et al., 2006; Chotivanich et al., 2006; Hallett et al., 2006). Membrane feeding and direct feeding have several differences: membrane feeding is performed through an artificial membrane (not human skin) and the gametocyte-containing blood is typically washed to replace the host plasma with non-immune sera or other surrogates for human serum (Toure et al., 1998; Bonnet et al., 2000). This washing step removes the drug and any host immunity components that would be present in the patients' blood and would be taken by the mosquito together with the blood-meal. Moreover, the temperature perturbations and other manipulations and time taken to do membrane feeds may have other effects on infectivity rates. Although the membrane feeding is a cleaner experimental procedure for comparative studies as it reduces or eliminates potential effects of drugs, immunity and other host factors that could impact on transmission, direct feeding

is the natural route of transmitting malaria gametocytes to the mosquito vector, and is presumably the most predictive of infectivity under natural conditions.

Our results show that post-SP gametocytes carried significantly more DHFR and DHPS mutations than pre-treatment asexual parasites from the same population, consistent with drug selection favoring transmission of resistant gametocytes, as has been reported previously (Mendez et al., 2002; Sowunmi and Fateye, 2003; Hallett et al., 2006). Although there was no asexual parasitemia in the post-treatment samples analyzed, remaining DNA from recently killed or persistent sub-patent asexual parasites could have been amplified by PCR (Thera et al., 2005; Ladeia-Andrade et al., 2009), confounding our results. To examine this possibility, we amplified the DHFR gene using a mutation-specific PCR method that has a lower limit of detection of approximately 250 parasites/ μ l (Plowe et al., 1995), well above the threshold for detection of asexual parasites by microscopy. Indeed, during these experiments, samples with fewer than 75 gametocytes per microliter yielded no PCR product. Previous studies showed that drug-resistant parasites appear to have an advantage in differentiating into gametocytes (Mendez et al., 2002) and being infectious to the vector.

The DHPS 540 mutation, which had not previously been found in Mali and which was absent in the pre-treatment asexual population, was present in the gametocyte population. This suggests that the parasites carrying that mutation were present at a level too low to be detected by our PCR method and subsequently expanded to reach detectable levels and differentiated into gametocytes after the elimination of the more sensitive strains by the drug treatment. We have previously shown very rapid selection of asexual stage parasites in response to pyrimethamine treatment (Doumbo et al., 2000), and the same appears to occur with gametocytes.

Evidence from field observations and from experimental in vitro systems indicates that many factors can affect the rate of differentiation of asexual blood parasites into the gametogenesis pathway (Carter and Miller, 1979; Dyer and Day, 2000). Factors including genetic differences (Day et al., 1993; Alano et al., 1995), host anemia (Von Seidlein et al., 2001), immunological responses (Smalley et al., 1981) and stress (drugs) (Sokhna et al., 2001) can all induce gametogenesis. The time course for the appearance of gametocytes harboring the resistance-conferring mutations post-SP treatment was consistent with an SP treatment effect, with the greatest prevalence of the majority of mutations occurring between days 3 and 28 following SP treatment, peaking on day 7. These results are consistent with a study from Colombia that demonstrated that an increasing number of DHFR mutations was associated with longer parasite clearance time and gametogenesis (Mendez et al., 2002).

An increased duration and density of gametocyte carriage after SP treatment has been suggested to be an early indicator of drug resistance. This increased gametocytemia among patients who carry drug-resistant *P. falciparum* would fuel the spread of resistance even before treatment failure rates increase significantly (Mendez et al., 2002; Barnes et al., 2008). The true impact of treatment on transmission may vary as a function of the drugs' pharmacodynamics and intrinsic gametocidal effects. Our data suggest that increases in gametocyte carriage in the treated population may not always translate into increased malaria transmission.

Although SP is still highly efficacious in Mali when molecular correction is used to account for re-infections, this study together with our recent publication (Tekete et al., 2009) show an upward trend in SP treatment failure and resistance in the area. This trend, together with the significant increase in the prevalence of the DHFR/DHPS quadruple mutant genotype and the appearance of the quintuple mutant genotype in the gametocyte population, signal that SP resistance is spreading in the country.

This study suggests that an intervention that causes increased gametocyte carriage may not necessarily translate into increased malaria transmission. The mere presence of gametocytes, whether measured by microscopy or by detection of gametocyte-specific RNA (Schneider et al., 2006a,b), might not signal infectivity. A better understanding of the mechanisms involved in differences in infectivity of *P. falciparum* gametocytes will shed light on the spread of antimalarial drug resistance in the field. Robust and sensitive assays that can accurately predict infectivity to mosquitoes are needed to guide the assessment of interventions aimed at blocking malaria transmission, an essential component of ongoing malaria control efforts and plans for possible elimination campaigns.

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References

- Alano P, Roca L, Smith D, Read D, Carter R, Day K. *Plasmodium falciparum*: parasites defective in early stages of gametocytogenesis. *Exp. Parasitol.* 1995; 81:227–235. [PubMed: 7556565]
- Anonymous. Chemotherapy of Malaria and Resistance to Antimalarials. WHO Technical Report Series; World Health Organization, Geneva: 1973. p. 529
- Anonymous. Assessment of Therapeutic Efficacy of Antimalarial Drugs for Uncomplicated *falciparum* Malaria. Geneva: 2003. WHO/HTM/RBM/2003.50.
- Barnes KI, Little F, Mabuza A, Mngomezulu N, Govere J, Durrheim D, Roper C, Watkins B, White NJ. Increased gametocytemia after treatment: an early parasitological indicator of emerging sulfadoxine–pyrimethamine resistance in *falciparum* malaria. *Infect. Dis.* 2008; 197:1605–1613.
- Bharti AR, Chuquiyauri R, Brouwer KC, Stancil J, Lin J, Llanos-Cuentas A, Vinetz JM. Experimental infection of the neotropical malaria vector *anopheles darlingi* by human patient-derived *Plasmodium vivax* in the Peruvian Amazon. *Am. J. Trop. Med. Hyg.* 2006; 75:610–616. [PubMed: 17038681]
- Bonnet S, Gouagna C, Safeukui I, Meunier J, Boudin C. Comparison of artificial membrane feeding with direct skin feeding to estimate infectiousness of *Plasmodium falciparum* gametocyte carriers to mosquitoes. *Trans. R. Soc. Trop. Med. Hyg.* 2000; 94:103–106. [PubMed: 10748913]
- Buckling A, Read AF. The effect of partial host immunity on the transmission of malaria parasites. *Proc. R. Soc. Lond. B Biol. Sci.* 2001; 268:2325–2330.
- Butcher GA. Antimalarial drugs and the mosquito transmission of *Plasmodium*. *Int. J. Parasitol.* 1997; 27:975–987. [PubMed: 9363480]
- Carter R, Miller LH. Evidence for environmental modulation of gametocytogenesis in *Plasmodium falciparum* in continuous culture. *Bull. World Health Organ.* 1979; 57(Suppl. 1):37–52. [PubMed: 397008]
- Chotivanich K, Sattabongkot J, Udomsangpetch R, Looareesuwan S, Day NP, Coleman RE, White NJ. Transmission-blocking activities of quinine, primaquine, and artesunate. *Antimicrob. Agents Chemother.* 2006; 50:1927–1930. [PubMed: 16723547]
- Chutmongkonkul M, Maier WA, Seitz HM. *Plasmodium falciparum*: effect of chloroquine, halofantrine and pyrimethamine on the infectivity of gametocytes for *Anopheles stephensi* mosquitoes. *Ann. Trop. Med. Parasitol.* 1992; 86:103–110. [PubMed: 1417200]
- Clarke SE, Jukes MCH, Kiambo Njagi H, Khasakhala L, Cundill B, Otido J, Crudder C, Estambale BB, Brooker S. Effect of intermittent preventive treatment of malaria on health and education in schoolchildren: a cluster-randomized, double-blind, placebo-controlled trial. *Lancet.* 2008; 372:127–138. [PubMed: 18620950]

- Day KP, Karamalis F, Thompson J, Barnes DA, Peterson C, Brown H, Brown GV, Kemp DJ. Genes necessary for expression of a virulence determinant and for transmission of *Plasmodium falciparum* are located on a 0.3-megabase region of chromosome 9. *Proc. Natl. Acad. Sci. USA*. 1993; 90:8292–8296. [PubMed: 8367496]
- Diourte Y, Djimde A, Doumbo OK, Sagara I, Coulibaly Y, Dicko A, Diallo M, Diakite M, Cortese JF, Plowe CV. Pyrimethamine–sulfadoxine efficacy and selection for mutations in *Plasmodium falciparum* dihydrofolate reductase and dihydropteroate synthase in Mali. *Am. J. Trop. Med. Hyg.* 1999; 60:475–478. [PubMed: 10466980]
- Djimde A, Doumbo OK, Traore O, Guindo A, Kayentao K, Diourte Y, Doumbo SN, Coulibaly D, Kone A, Cissoko Y, Tekete M, Fofana B, Dicko A, Diallo DA, Wellem TE, Kwiatkowski D, Plowe CV. Clearance of drug-resistant parasites as a model for protective immunity in *falciparum* malaria. *Am. J. Trop. Med. Hyg.* 2003; 69:558–563. [PubMed: 14695097]
- Dorsey G, Dokomajilar C, Kiggundu M, Staedke SG, Kamya MR, Rosenthal PJ. Principal role of dihydropteroate synthase mutations in mediating resistance to sulfadoxine–pyrimethamine in single-drug and combination therapy of uncomplicated malaria in Uganda. *Am. J. Trop. Med. Hyg.* 2004; 71:758–763. [PubMed: 15642967]
- Doumbo OK, Kayentao K, Djimde A, Cortese JF, Diourte Y, Konaré A, Kublin JG, Plowe CV. Rapid selection of *P. falciparum* DHFR mutants by pyrimethamine prophylaxis. *J. Infect. Dis.* 2000; 182:993–996. [PubMed: 10950805]
- Drakeley CJ, Secka I, Correa S, Greenwood BM, Targett GA. Host haematological factors influencing the transmission of *Plasmodium falciparum* gametocytes to *Anopheles gambiae* s.s. mosquitoes. *Trop. Med. Int. Health.* 1999; 4:131–138. [PubMed: 10206267]
- Drakeley CJ, Bousema JT, Akim NI, Teelen K, Roeffen W, Lensen AH, Bolmer M, Eling W, Sauerwein RW. Transmission-reducing immunity is inversely related to age in *Plasmodium falciparum* gametocyte carriers. *Parasite Immunol.* 2006; 28:185–190. [PubMed: 16629703]
- Dyer M, Day KP. Commitment to gametocytogenesis in *Plasmodium falciparum*. *Parasitol. Today.* 2000; 16:102–107. [PubMed: 10689328]
- Enosse S, Butcher GA, Margos G, Mendoza J, Sinden RE, Hogh B. The mosquito transmission of malaria: the effects of atovaquone-proguanil (malarone) and chloroquine. *Trans. R. Soc. Trop. Med. Hyg.* 2000; 94:77–82. [PubMed: 10748906]
- Govere JM, Durrheim DN, Mngomezulu NM, Barnes K, Sharp B. Infectivity of *Plasmodium falciparum* gametocytes to *Anopheles arabiensis* after treatment with sulfadoxine–pyrimethamine. *Trans. R. Soc. Trop. Med. Hyg.* 2003; 97:707–708. [PubMed: 16117968]
- Graves PM, Burkot TR, Carter R, Cattani JA, Lagog M, Parker J, Brabin BJ, Gibson FD, Bradley DJ, Alpers MP. Measurement of malarial infectivity of human populations to mosquitoes in the Madang area, Papua, New Guinea. *Parasitology.* 1988; 96:251–263. [PubMed: 3374964]
- Hallett RL, Dunyo S, Ord R, Jawara M, Pinder M, Randall A, Allouche A, Walraven G, Targett GA, Alexander N, Sutherland CJ. Chloroquine/sulphadoxine–pyrimethamine for Gambian children with malaria: Transmission to mosquitoes of multidrug-resistant *Plasmodium falciparum*. *PLoS Clin. Trials.* 2006; 1:e15. [PubMed: 16871318]
- Hogh B, Thompson R, Hetzel C, Fleck SL, Kruse NA, Jones I, Dgedge M, Barreto J, Sinden RE. Specific and nonspecific responses to *Plasmodium falciparum* blood-stage parasites and observations on the gametocytemia in schoolchildren living in a malaria-endemic area of mozambique. *Am. J. Trop. Med. Hyg.* 1995; 52:50–59. [PubMed: 7856825]
- Hogh B, Gamage-Mendis A, Butcher GA, Thompson R, Begtrup K, Mendis C, Enosse SM, Dgedge M, Barreto J, Eling W, Sinden RE. The differing impact of chloroquine and pyrimethamine/sulfadoxine upon the infectivity of malaria species to the mosquito vector. *Am. J. Trop. Med. Hyg.* 1998; 58:176–182. [PubMed: 9502601]
- Jeffery GM. Infectivity to mosquitoes of *Plasmodium vivax* following treatment with chloroquine and other antimalarials. *Am. J. Trop. Med. Hyg.* 1963; 4:207–211.
- Kublin JG, Dzinjalimala FK, Kamwendo DD, Malkin EM, Cortese JF, Martino LM, Mukadam RA, Rogerson SJ, Lescano AG, Molyneux ME, Winstanley PA, Chimpeni P, Taylor TE, Plowe CV. Molecular markers for failure of sulfadoxine–pyrimethamine and chlorproguanil-dapsone treatment of *Plasmodium falciparum* malaria. *J. Infect. Dis.* 2002; 185:380–388. [PubMed: 11807721]

- Ladeia-Andrade S, Ferreira MU, de Carvalho ME, Curado I, Coura JR. Age-dependent acquisition of protective immunity to malaria in riverine populations of the Amazon Basin of Brazil. *Am. J. Trop. Med. Hyg.* 2009; 80:452–459. [PubMed: 19270298]
- Lines JD, Wilkes TJ, Lyimo EO. Human malaria infectiousness measured by age-specific sporozoite rates in *Anopheles gambiae* in tanzania. *Parasitology.* 1991; 102(Pt. 2):167–177. [PubMed: 1852484]
- Mendez F, Munoz A, Carrasquilla G, Jurado D, Arevalo-Herrera M, Cortese JF, Plowe CV. Determinants of treatment response to sulfadoxine–pyrimethamine and subsequent transmission potential in *falciparum* malaria. *Am. J. Epidemiol.* 2002; 156:230–238. [PubMed: 12142257]
- Méndez F, Herrera S, Murrain B, Gutiérrez A, Moreno LA, Manzano M, Muñoz A, Plowe CV. Selection of antifolate-resistant *Plasmodium falciparum* by sulfadoxine–pyrimethamine treatment and infectivity to *Anopheles* mosquitoes. *Am. J. Trop. Med. Hyg.* 2007; 77:438–443. [PubMed: 17827356]
- Newman RD, Moran AC, Kayentao K, et al. Prevention of malaria during pregnancy in West Africa: policy change and the power of subregional action. *Trop. Med. Int. Health.* 2006; 11:462–469. [PubMed: 16553929]
- Nosten F, van Vugt M, Price R, Luxemburger C, Thway KL, Brockman A, McGready R, ter Kuile F, Looareesuwan S, White NJ. Effects of artesunate–mefloquine combination on incidence of *Plasmodium falciparum* malaria and mefloquine resistance in western thailand: a prospective study. *Lancet.* 2000; 356:297–302. [PubMed: 11071185]
- Oesterholt MJ, Alifrangis M, Sutherland CJ, Omar SA, Sawa P, Howitt C, Gouagna LC, Sauerwein RW, Bousema T. Submicroscopic gametocytes and the transmission of antifolate-resistant *Plasmodium falciparum* in Western Kenya. *PLoS One.* 2009; 4(2):e4364. [PubMed: 19194499]
- O’Meara WP, Smith DL, McKenzie FE. Potential impact of intermittent preventive treatment (IPT) on spread of drug-resistant malaria. *PLoS Med.* 2006; 5:e141. [PubMed: 16573365]
- Peterson DS, Milhous WK, Wellems TE. Molecular basis of differential resistance to cycloguanil and pyrimethamine in *Plasmodium falciparum* malaria. *Proc. Natl. Acad. Sci. USA.* 1990; 87:3018–3022. [PubMed: 2183222]
- Plowe CV, Djimde A, Bouare M, Doumbo O, Wellems TE. Pyrimethamine and proguanil resistance-conferring mutations in *Plasmodium falciparum* dihydrofolate reductase: polymerase chain reaction methods for surveillance in Africa. *Am. J. Trop. Med. Hyg.* 1995; 52:565–568. [PubMed: 7611566]
- Plowe CV, Djimde A, Wellems TE, Diop S, Kouriba B, Doumbo OK. Community pyrimethamine–sulfadoxine use and prevalence of resistant *Plasmodium falciparum* genotypes in Mali: a model for deterring resistance. *Am. J. Trop. Med. Hyg.* 1996; 55:467–471. [PubMed: 8940973]
- Plowe, Christopher V, Doumbo, Ogobara K, Djimde, Abdoulaye, Kayentao, Kassoum, Diourte, Yacouba, Doumbo, Safi N, Coulibaly, Drissa, Thera, Mahamadou, Wellems, Thomas E, Diallo, Dapa A. Chloroquine treatment of uncomplicated *Plasmodium falciparum* malaria in Mali: parasitological resistance vs. therapeutic efficacy. *Am. J. Trop. Med. Hyg.* 2001; 64:242–246. [PubMed: 11463110]
- Price R, Nosten F, Luxemburger C, ter KF, Paiphun L, Chongsuphajaisiddhi T, White N. Effects of artemisinin derivatives on malaria transmissibility. *Lancet.* 1996; 347:1654–1658. [PubMed: 8642959]
- Robert V, Read AF, Essong J, Tchuinkam T, Mulder B, Verhave JP, Carnevale P. Effect of gametocyte sex ratio on infectivity of *Plasmodium falciparum* to *Anopheles gambiae*. *Trans. R. Soc. Trop. Med. Hyg.* 1996; 90:621–624. [PubMed: 9015496]
- Robert V, Awono-Ambene HP, Le Hesran JY, Trape JF. Gametocytemia and infectivity to mosquitoes of patients with uncomplicated *Plasmodium falciparum* malaria attacks treated with chloroquine or sulfadoxine plus pyrimethamine. *Am. J. Trop. Med. Hyg.* 2000; 62:210–216. [PubMed: 10813475]
- Schneider P, Bousema T, Omar S, Gouagna L, Sawa P, Schallig H, Sauerwein R. (Sub)microscopic *Plasmodium falciparum* gametocytaemia in Kenyan children after treatment with sulphadoxine–pyrimethamine monotherapy or in combination with artesunate. *Int. J. Parasitol.* 2006a; 36:403–408. [PubMed: 16500657]

- Schneider P, Bousema T, Omar S, Gouagna L, Sawa P, Schallig H, Sauerwein R. (Sub) microscopic *Plasmodium falciparum* gametocytaemia in Kenyan children after treatment with sulphadoxine–pyrimethamine monotherapy or in combination with artesunate. *Int. J. Parasitol.* 2006b; 36:403–408. [PubMed: 16500657]
- Shute PG, Maryon M. The effect of pyrimethamine (daraprim) on the gametocytes and oocysts of *Plasmodium falciparum* and *Plasmodium vivax*. *Trans. R. Soc. Trop. Med. Hyg.* 1954; 48:50–63. [PubMed: 13136412]
- Smalley ME. *Plasmodium falciparum* gametocytes: the effect of chloroquine on their development. *Trans. R. Soc. Trop. Med. Hyg.* 1977; 71:526–529. [PubMed: 343314]
- Smalley ME, Sinden RE. *Plasmodium falciparum* gametocytes: their longevity and infectivity. *Parasitology.* 1977; 74:1–8. [PubMed: 320542]
- Smalley ME, Brown J, Bassett NM. The rate of production of *Plasmodium falciparum* gametocytes during natural infections. *Trans. R. Soc. Trop. Med. Hyg.* 1981; 75:318–319. [PubMed: 7029806]
- Sokhna CS, Trape JF, Robert V. Gametocytaemia in Senegalese children with uncomplicated *falciparum* malaria treated with chloroquine, amodiaquine or sulfadoxine + pyrimethamine. *Parasite.* 2001; 8:243–250. [PubMed: 11584755]
- Sowunmi A, Fateye BA. *Plasmodium falciparum* gametocytemia in Nigerian children: before, during and after treatment with antimalarial drugs. *Trop. Med. Int. Health.* 2003; 8:783–792. [PubMed: 12950664]
- Strickland GT, Fox E, Sarwar M, Khaliq AA, Macdonald M. Effects of chloroquine, amodiaquine and pyrimethamine–sulfadoxine on *Plasmodium falciparum* gametocytemia. *Am. J. Trop. Med. Hyg.* 1986; 35:259–262. [PubMed: 3513644]
- Targett G, Drakeley C, Jawara M, von Seidlein L, Coleman R, Deen J, Pinder M, Doherty T, Sutherland C, Walraven G, Milligan P. Artesunate reduces but does not prevent posttreatment transmission of *Plasmodium falciparum* to *Anopheles gambiae*. *J. Infect. Dis.* 2001; 183:1254–1259. [PubMed: 11262208]
- Tekete M, Djimde AA, Beavogui AH, Maiga H, Sagara I, Fofana B, Ouologuem D, Dama S, Kone A, Dembele D, Wele M, Dicko A, Doumbo OK. Efficacy of chloroquine, amodiaquine and sulphadoxine–pyrimethamine for the treatment of uncomplicated *falciparum* malaria: revisiting molecular markers in an area of emerging amodiaquine and SP resistance in Mali. *Malar. J.* 2009; 26:34. [PubMed: 19245687]
- ter Kuile FO, Steketee RW. Intermittent preventive treatment in infants adjusting expectations and seeing opportunity. *J. Infect. Dis.* 2006; 194:269–272. [PubMed: 16826472]
- Thera MA, Sehdev PS, Coulibaly D, Traore K, Garba MN, Cissoko Y, Kone A, Guindo A, Dicko A, Beavogui AH, Djimde AA, Lyke KE, Diallo DA, Doumbo OK, Plowe CV. Impact of trimethoprim–sulfamethoxazole prophylaxis on *falciparum* malaria infection and disease. *J. Infect. Dis.* 2005; 192:1823–1829. [PubMed: 16235184]
- Toure YT, Doumbo O, Toure A, Bagayoko M, Diallo M, Dolo A, Vernick KD, Keister DB, Muratova O, Kaslow DC. Gametocyte infectivity by direct mosquito feeds in an area of seasonal malaria transmission: implications for Bancoumana, Mali as a transmission-blocking vaccine site. *Am. J. Trop. Med. Hyg.* 1998; 59:481–486. [PubMed: 9749648]
- Triglia T, Menting JGT, Wilson C, Cowman AF. Mutations in dihydropteroate synthase are responsible for sulfone and sulfonamide resistance in *Plasmodium falciparum*. *Proc. Natl. Acad. Sci. USA.* 1997; 94:13944–13949. [PubMed: 9391132]
- von Seidlein L, Jawara M, Coleman R, Doherty T, Walraven G, Targett G. Parasitaemia and gametocytaemia after treatment with chloroquine, pyrimethamine/sulfadoxine, and pyrimethamine/sulfadoxine combined with artesunate in young Gambians with uncomplicated malaria. *Trop. Med. Int. Health.* 2001; 6:92–98. [PubMed: 11251903]
- Zolg JW, Plitt JR, Chen GX, Palmer S. Point mutations in the dihydrofolate reductase-thymidylate synthase gene as the molecular basis for pyrimethamine resistance in *Plasmodium falciparum*. *Mol. Biochem. Parasitol.* 1989; 36:253–262. [PubMed: 2677719]

Fig. 1. Evolution of *Plasmodium falciparum* gametocyte carriage during 28 days of follow-up after treatment with chloroquine or sulfadoxine-pyrimethamine.

Fig. 2. Prevalence of mutations in the *Plasmodium falciparum* dihydrofolate reductase gene (*Pfdhfr*) and dihydropteroate synthase gene (*Pfdhps*) in trophozoites (Day 0) and post-sulfadoxine–pyrimethamine gametocytes in 2000. * indicate statistically significant differences.

Fig. 3.

Evolution of the prevalence of drug resistance-conferring mutations within the dihydrofolate reductase gene (*Pfdhfr*) and dihydropteroate synthase gene (*Pfdhps*) of a *Plasmodium falciparum* gametocyte population during 28 days of follow-up after treatment with sulfadoxine-pyrimethamine.