

## Parasitological Rebound Effect and Emergence of Pyrimethamine Resistance in *Plasmodium falciparum* after Single-Dose Sulfadoxine-Pyrimethamine

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(See the editorial commentary by Bremen and O'Meara, on pages XXX–XX.)

**Intermittent preventive treatment for malaria in infants (IPTi) is a promising malaria control strategy. However, mass preventive treatment for malaria inherently bears the risk of increasing drug resistance. Here, the effect of single-dose sulfadoxine-pyrimethamine (S-P) versus placebo on *Plasmodium falciparum* infection rates was assessed in 63 selected infants who were aparasitemic at enrollment. An increase in the proportion of infants with isolates exhibiting drug resistance-associated mutations was detected 3 weeks after drug application in the treatment group. S-P, in the setting of IPTi, appears to cause a parasitological rebound effect in which there is selection of drug-resistant parasites for a short period after drug clearance.**

Since the recent revival of intermittent treatment strategies to prevent *Plasmodium falciparum* malaria in infants, various efforts with a strong focus on operational research are being pursued. The concept of intermittent preventive treatment (IPT) is to periodically deliver long-acting antimalarials to suppress parasitemia and the number of episodes of clinical malaria and to

protect from reinfections for a prolonged period. At the same time, IPT aims at allowing for the development of appropriate natural antidiarrheal and antiparasite immunity. A first clinical trial using sulfadoxine-pyrimethamine (S-P) for IPT for malaria in infants (IPTi) in Tanzania resulted in a significant reduction in the number of episodes of clinical malaria and severe anemia, and a clinical rebound did not occur during the first months after the last IPTi application [1]. During a further follow-up of the same study group, an extended positive effect of IPTi was shown, although the effect was decreased when adjustment was made for history of clinical malaria during the preceding IPTi period [2]. In addition, IPT given at least twice during pregnancy, in accordance with present guidelines [3], has been proven to decrease the severity of anemia, the degree of placental parasitemia, and the incidence of low birth weight [4, 5]. After pretreatment with S-P in a vaccine trial, the median time between treatment and the first episode of clinical malaria was delayed by ~1 month, compared with that in a control group, irrespective of the parasitological drug-resistance pattern [6].

To exert prolonged protection, drugs with long half-lives appear to be required in IPT, although they inherently bear the risk of increasing drug resistance [7, 8]. This problem has been addressed [1] but was considered to be of marginal importance because the total number of treatment courses in IPTi would be comparatively low, drug selection pressure would be reduced (because a lower number of episodes of clinical malaria would need to be treated), and subtherapeutic drug concentrations (which promote resistance) would largely be avoided.

Resistance to pyrimethamine is caused by the consecutive acquisition of mutations (Ser108Asn, Asn511Ile, and Cys59Arg) in the *P. falciparum* dihydrofolate reductase (*pf<sub>dhfr</sub>*) gene, whereby each mutation causes a successive decrease in drug sensitivity by additive steric constraints to the drug binding site. Ser108Asn is invariably the first mutation to occur. Resistance to sulfadoxine is mediated by a 2-fold mutation (Ala437Gly and Lys540Glu) of the dihydropteroate synthase (*pf<sub>dhps</sub>*) gene [8]. Both antifolates act synergistically by mimicking folates. The structural variants caused by the *pf<sub>dhfr</sub>* and *pf<sub>dhps</sub>* mutations do not completely inhibit the parasite's folate metabolism, and even multifold mutated parasites do not necessarily confer drug resistance and thereby cause treatment failure [9].

**Patients, materials, and methods.** During a randomized clinical trial of the protective efficacy of IPTi with S-P, 63 infants who were a mean  $\pm$  SD of 9 months ( $\pm 3$  weeks) old were enrolled in the present study. To assess the prophylactic efficacy of single-dose S-P and to determine the patterns of S-P resis-

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tance after drug application, only aparasitemic and afebrile infants who had not undergone treatment with S-P during the preceding 6 months were enrolled. The study took place in the Afigya Sekyere District, which is a 714-km<sup>2</sup> rural area of stable malaria transmission (hyperendemic/holoendemic) close to Kumasi in the forest belt of the Ashanti region of Ghana. Study participants were randomly and blindly allocated to receive single-dose S-P (12.5 mg of sulfadoxine and 250 mg of pyrimethamine;  $n = 28$ ) or placebo ( $n = 35$ ). Ethical clearance was granted by the Ethics Committee, School of Medical Sciences, University of Science and Technology, Kumasi, Ghana, and written or thumb-printed consent was obtained from each participant's mother.

Eight visits were performed in weekly intervals, followed by 4 monthly visits, for an observation period of 26 weeks. Heel-prick blood samples were collected at each visit, to assess parasite density and the first appearance of isolates exhibiting either 4 or <4 drug resistance-associated mutations, as described elsewhere [10]. Clinical indices, including relevant anamnestic details, were documented, and, when clinical malaria was diagnosed, patients were treated with amodiaquine or, in the case of treatment failure, additionally with artesunate. Statistical analyses ( $\chi^2$  tests, Kaplan-Meier analyses, Cox proportional hazards model, and computation of prevalence/incidence rates) were performed using Stata software (version 8.2; StataCorp), and  $P < .05$  was considered to be statistically significant. Snapshot data were converted to time-span data for longitudinal analyses.

**Results.** Thirty-three patients remained aparasitemic during the entire observation period, and 30 patients had a total of 55 *P. falciparum* infections at 579 active assessments (7231 observation days at risk), irrespective of the infecting parasite's drug resistance-associated mutations (table 1). Twelve patients had 2 infections, 2 patients had 4 infections, and 2 patients had 5 infections. The interval between enrollment and the first

*P. falciparum* infection was slightly delayed during the first weeks in the treatment group ( $P$ , not significant [NS], log-rank test for equality of survivor functions) (figure 1A). After week 9, the time-dependent proportion of parasitemic patients tended to be higher in the treatment group than in the placebo group, for a short period. The incidence rate of *P. falciparum* infections was 3.5 infections/person-year (95% confidence interval [CI], 2.4–4.9 infections/person-year) in the treatment group and 2.2 infections/person-year (95% CI, 1.5–3.3 infections/person-year) in the placebo group (incidence rate ratio [IRR], 1.6 [95% CI, 0.9–2.8];  $P$ , NS).

The period between treatment and the first detection of *P. falciparum* isolates exhibiting 4 drug resistance-associated mutations (*pfdhfr* Ser108Asn, Asn51Ile, and Cys59Arg and *pfdhps* Ala437Gly; *pfdhps* Lys540Glu was not found) was shorter in the treatment group than it was in the placebo group ( $P < .05$ ) (figure 1B). Differences became apparent after week 8, when the incidence rate for infection with isolates exhibiting 4 mutations was 2 times higher in the treatment group (2.7 infections/person-year [95% CI, 1.8–4.0 infections/person-year]) than it was in the placebo group (1.3 infections/person-year [95% CI, 0.8–2.2 infections/person-year]; IRR, 2.1 [95% CI, 1.0–4.3];  $P < .05$ ). This corresponds to a relative risk of 2.1 (95% CI, 1.1–4.0;  $P < .05$ , Cox proportional hazards model). In contrast, treatment did not significantly affect the rate of infections with isolates exhibiting <4 mutations (IRR, 0.7 [95% CI, 0.2–2.2];  $P$ , NS). Notably, the presence of isolates exhibiting 4 mutations did not exclude concomitant infections with isolates exhibiting <4 mutations. Parasite densities did not differ between groups at different time points (generalized estimating equation, within-group correlation structure).

Among the 55 events of parasitemia in 30 patients, mild malaria, as defined by a body temperature  $>38.0^\circ\text{C}$  and concomitant parasitemia, occurred in 18 patients during the ob-

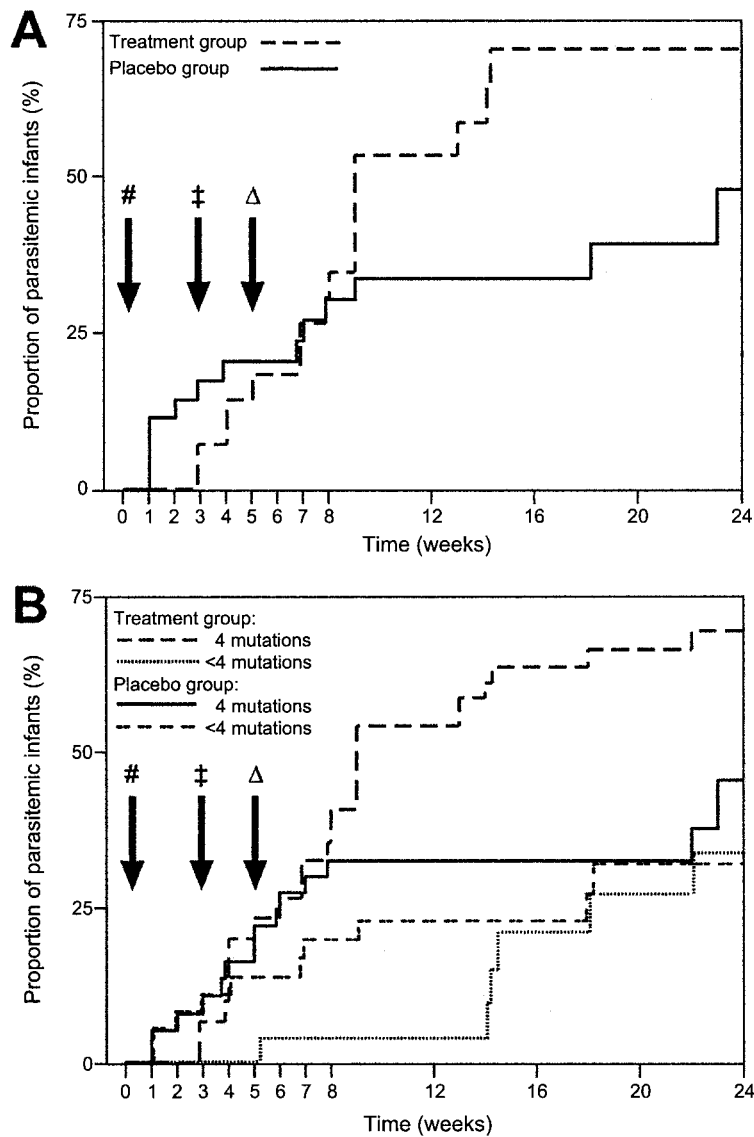
**Table 1. Parameters of *Plasmodium falciparum* infections.**

Parameter	Treatment group ( $n = 28$ )	Placebo group ( $n = 35$ )
Days at risk, no.	3955	3276
Assessments		
Total no.	259	320
Per patient, median no. (range)	10 (2–14)	10 (4–13)
Infections, total no.	31	24
Parasite density, geometric mean (95% CI), 1000 parasites/ $\mu\text{L}$	6.0 (2.5–14.4)	4.7 (1.4–15.7) <sup>a</sup>
Infections with isolates exhibiting 4 drug-resistance mutations, no.	24	14
Parasite density, geometric mean (95% CI), 1000 parasites/ $\mu\text{L}$	6.3 (2.2–17.8)	9.9 (2.5–38.7) <sup>a</sup>
Individuals never parasitemic, no.	12	21
Cases of mild malaria (temperature $>38.0^\circ\text{C}$ and parasitemia), no.	9	7
Hemoglobin, mean $\pm$ SD, g/dL	10.5 $\pm$ 1.3	10.3 $\pm$ 1.0 <sup>b</sup>

**NOTE.** CI, confidence interval.

<sup>a</sup> Not significantly different from treatment group (Wilcoxon rank-sum test).

<sup>b</sup> Not significantly different from treatment group; only 32 patients were assessed.



**Figure 1.** Parasitemia and sulfadoxine-pyrimethamine (S-P) resistance-associated mutations in *Plasmodium falciparum*. The end points of the Kaplan-Meier estimates were defined as the first polymerase chain reaction-based detection of *P. falciparum*, the first occurrence of isolates exhibiting drug resistance-associated mutations (4 *pf dhfr/pf dhps* mutations), and the first occurrence of parasites partly sensitive to S-P (<4 *pf dhfr/pf dhps* mutations). *A*, Appearance of the first parasitemia in the treatment and placebo groups. *B*, Appearance of *P. falciparum* isolates exhibiting 4 mutations and isolates exhibiting <4 mutations in the treatment and placebo groups. #, Application of either S-P or placebo; ‡, approximate elimination of pyrimethamine (5 half-life cycles); Δ, complete elimination of sulfadoxine.

ervation period. The incidence was not significantly higher in the treatment group than it was in the placebo group (IRR, 1.6 [95% CI, 0.5–5.0]; *P*, NS).

**Discussion.** In the present study, 3 consecutive phases differentially influenced infection dynamics after S-P application. First, during a period of high drug concentrations, reinfections with sensitive and partly resistant isolates were delayed. Second, during a limited phase after complete drug elimination, the parasitological rebound became apparent with a sharp increase in infections with isolates exhibiting 4 drug resistance-associated

mutations. Third, after this short rebound phase, a similar increase in the proportion of infected patients occurred in both groups.

The parasitological rebound effect observed in infants treated with single-dose S-P raises considerable concern, in particular on the background of the preexisting high levels of drug resistance-associated mutations [10]. One might assume that S-P in IPTi provides a disadvantage (development of drug-resistant strains), rather than a benefit (prevention of disease). With regard to the number, severity, and outcome of episodes of

clinical malaria, results from IPTi studies are critical if future strategies are to be designed appropriately. IPT studies with other drug regimens should be initiated, so that alternatives to an S-P regimen might be available.

In light of the high levels of preexisting drug resistance—associated mutations, accumulating drug selection pressure, and possible increased incidence of severe adverse events, is S-P a realistic option for forthcoming IPT programs? If, as is suspected for IPT during pregnancy, the efficacy of S-P in IPTi decreases with increasing drug resistance, relevant policy implications may be anticipated [11]. Our note of caution suggests that drug pressure exerted sporadically by IPT might lead to further substantial selection of drug-resistant parasites. It remains to be determined whether the benefits—in particular, the significant reduction in the number of episodes of clinical malaria—of IPT with drug combinations other than S-P outweigh the possible increase in drug resistance.

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### References

1. Schellenberg D, Menendez C, Kahigwa E, et al. Intermittent treatment for malaria and anaemia control at time of routine vaccinations in Tanzanian infants: a randomised, placebo-controlled trial. *Lancet* **2001**; 357:1471–7.
2. Schellenberg D, Menendez C, Aponte JJ, et al. Intermittent preventive antimalarial treatment for Tanzanian infants: follow-up to age 2 years of a randomised, placebo-controlled trial. *Lancet* **2005**; 365:1481–3.
3. World Health Organization (WHO). A strategic framework for malaria prevention and control during pregnancy in the African region. AFR/MAL/04/01. Brazzaville, Republic of the Congo: WHO, **2004**.
4. Shulman CE, Dorman EK, Cutts F, et al. Intermittent sulphadoxine-pyrimethamine to prevent severe anaemia secondary to malaria in pregnancy: a randomised placebo-controlled trial. *Lancet* **1999**; 353:632–6.
5. Kayentao K, Kodio M, Newman RD, et al. Comparison of intermittent preventive treatment with chemoprophylaxis for the prevention of malaria during pregnancy in Mali. *J Infect Dis* **2005**; 191:109–16.
6. Coulibaly D, Diallo DA, Thera MA, et al. Impact of pre-season treatment on incidence of falciparum malaria and parasite density at a site for testing malaria vaccines in Bandiagara, Mali. *Am J Trop Med Hyg* **2002**; 67:604–10.
7. Watkins WM, Mosobo M. Treatment of *Plasmodium falciparum* malaria with pyrimethamine-sulfadoxine: selective pressure for resistance is a function of long elimination half-life. *Trans R Soc Trop Med Hyg* **1993**; 87:75–8.
8. May J, Meyer CG. Chemoresistance in falciparum malaria. *Trends Parasitol* **2003**; 19:432–5.
9. Kublin JG, Dzinjalama FK, Kamwendo DD, et al. Molecular markers for failure of sulfadoxine-pyrimethamine and chlorproguanil-dapsone treatment of *Plasmodium falciparum* malaria. *J Infect Dis* **2002**; 185:380–8.
10. Marks F, Evans J, Meyer CG, et al. High prevalence of markers for sulfadoxine and pyrimethamine resistance of *Plasmodium falciparum* in the absence of drug pressure in the Ashanti region of Ghana. *Antimicrob Agents Chemother* **2005**; 49:1101–5.
11. White NJ. Intermittent presumptive treatment for malaria. *PLoS Med* **2005**; 2(1):e3.