

Prevention of Increasing Rates of Treatment Failure by Combining Sulfadoxine-Pyrimethamine with Artesunate or Amodiaquine for the Sequential Treatment of Malaria

Grant Dorsey,¹ Jonathan Vlahos,¹ Moses R. Kamya,² Sarah G. Staedke,¹ and Philip J. Rosenthal¹

¹Department of Medicine, San Francisco General Hospital, University of California, San Francisco; ²Makerere University Medical School, Kampala, Uganda

Combination antimalarial therapy may delay the spread of drug resistance, but clinical data supporting this notion are limited. For 1 year, we studied Ugandan children who were treated for uncomplicated malaria with sulfadoxine-pyrimethamine (SP), SP + amodiaquine (AQ), or SP + artesunate (AS). We compared treatment responses and the prevalence of resistance-conferring mutations of new infections with those of recrudescing infections due to parasites that survived prior treatment. Recrudescing infections were associated with the selection of SP resistance-conferring mutations in all treatment groups, but responses to repeat therapy differed. Compared with initial treatments, treatment of recrudescing infections was associated with a higher rate of treatment failure (hazard ratio [HR], 2.44; $P = .01$), for the SP group, but with a lower rate of treatment failure (HR, 0.40; $P = .08$), for the SP + AS group. Treatment failure in the SP + AQ group was uncommon, limiting the analysis of recrudescing parasites. Our results suggest that the use of combination antimalarial therapy in Africa may slow the spread of drug-resistant malaria and prolong the therapeutic life span of available treatment regimens.

Resistance to antimalarial drugs is a major problem in Africa. Chloroquine (CQ) has been the mainstay of treatment for >50 years, but resistance to this drug is now widespread. The optimal replacement for CQ remains unclear, because effective, affordable treatment options are currently limited. The fixed combination of sulfadoxine-pyrimethamine (SP) has replaced CQ in several African countries and is the second-line agent in most countries where CQ is still recommended [1]. However, substantial resistance to SP already has been reported

from Eastern and Southern Africa and is likely to spread rapidly, as already seen in Southeast Asia [2, 3].

Combining antimalarial drugs that have different modes of action has been shown to improve the treatment efficacy of individual drugs. The use of artesunate (AS) in combination with mefloquine (MQ) was highly effective in parts of Thailand, where multidrug-resistant malaria is common [4]. In Africa, the efficacies of combination regimens—including SP + AS, SP + amodiaquine (AQ), and AS + AQ—were superior to those of SP or AQ monotherapy [5–8]. The use of combination antimalarial therapy also has been proposed as a means of delaying the spread of drug resistance and prolonging the therapeutic life span of antimalarial drugs. However, there are limited clinical data to support this strategy. The introduction of AS + MQ in parts of Thailand has been temporally associated with stabilization of failure rates and decrease in malaria transmission [9, 10]. However, a clear cause-and-effect relationship between the introduction of AS + MQ and these results is difficult to establish [11]. In Africa, where the epidemi-

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Reprints or correspondence: Dr. Grant Dorsey, University of California, San Francisco, PO Box 0811, San Francisco, CA 94143 (grantd@itsa.ucsf.edu).

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ology of malaria is markedly different from that in Southeast Asia, it is difficult to predict how combination therapy may affect the spread of drug resistance. The added cost and logistical challenges of implementing a policy of first-line combination therapy have discouraged public health officials from adopting this approach on a wide scale in Africa [12].

Previously, Dorsey et al. published the results of a longitudinal clinical trial comparing SP monotherapy to combinations of SP + AQ and of SP + AS, in a cohort of Ugandan children [6]. The longitudinal study design and the inclusion of genotyping to distinguish recrudescence infections from new infections provided the unique opportunity to evaluate the effects of repeated therapy on drug-resistant parasites among the 3 treatment groups. We now present clinical data supporting the theory that combination therapy can delay the spread of drug resistance and discuss possible mechanisms behind this observation.

SUBJECTS, MATERIALS, AND METHODS

Clinical study. The clinical study took place between July 2000 and August 2001 at the outpatient department of Mulago Hospital in Kampala, Uganda. The full details of the clinical trial have been published elsewhere [6]. The study was approved by the institutional review boards of the University of California, San Francisco, and Makerere University, Kampala. In brief, 316 healthy children between the ages of 6 months and 5 years were enrolled from the community and randomly assigned to 1 of 3 regimens: SP (25 mg/kg sulfadoxine component given as a single dose) plus placebo, SP + AQ (10 mg/kg given on days 0 and 1, then 5 mg/kg given on day 2), and SP + AS (4 mg/kg given daily for 3 days). Participants were monitored for 1 year and diagnosed with uncomplicated falciparum malaria if they presented with a tympanic temperature of $\geq 38.0^{\circ}\text{C}$ and *Plasmodium falciparum* parasitemia or a history of fever (not documented) and ≥ 500 asexual parasites/ μL (without meeting criteria for severe malaria [13] or displaying danger signs [14]). For each episode of uncomplicated malaria diagnosed, participants received the same preassigned treatment. Clinical outcomes were assessed using a standard World Health Organization (WHO) 14-day follow-up protocol [14]. All episodes classified as treatment failures within 14 days were treated with standard dosage of quinine (10 mg/kg every 8 h for 7 days). Any case of uncomplicated malaria diagnosed >14 days after a previous episode was considered to be a new event (for treatment purposes) and retreated with the same preassigned treatment regimen.

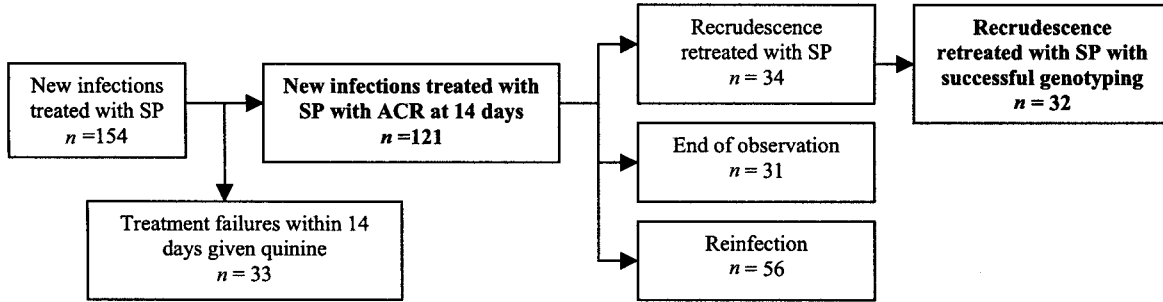
Molecular genotyping. After the clinical trial, malaria parasites were genotyped, to distinguish new from recrudescence infections. The details of the genotyping methods used in this study have been published elsewhere [6]. In brief, polymor-

phisms in the merozoite surface protein-2 gene were characterized for isolates from consecutive episodes of uncomplicated malaria. When comparing consecutive episodes of malaria, a recrudescence infection was defined as an episode containing identical alleles or a subset of the alleles present in the previous episode, and a new infection was defined as an episode containing different alleles from those present in the previous episode. An episode that contained both alleles present in the previous episode and new alleles (10% of total) was classified as a new infection if half or more than half of the alleles were new and, otherwise, was classified as a recrudescence infection. A recent detailed sensitivity analysis showed that this method provided an accurate means of distinguishing recrudescence from new infections [15].

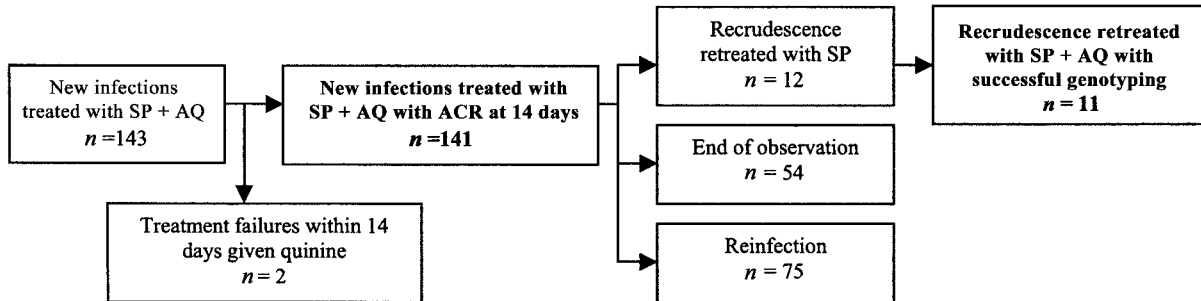
Sample selection. In the present study, we were interested in comparing the treatment outcome of new infections with the outcome of recrudescence infections retreated with the same therapy. New infections were defined as all first episodes of uncomplicated malaria and any episode of uncomplicated malaria due to parasite strains not present at the time that a prior treatment was initiated. For this analysis, in the new infection group, we excluded all infections that were treatment failures in the first 14 days, because these were treated with quinine and, thus, were not retreated with the same regimen. Recrudescence infections were defined as all episodes of uncomplicated malaria due to parasites that survived prior treatment with the same regimen. For analysis of the recrudescence infection group, we included only first-time recrudescence infections (those following a new infection), because the number of recrudescence infections that followed a previous recrudescence infection was too small for meaningful comparisons.

Molecular markers of drug resistance. Kublin et al. [16] and Kyabayinze et al. [17] previously found a strong association between key mutations in the parasite dihydrofolate reductase (DHFR; Arg-59) and dihydropteroate synthetase (DHPS; Glu-437 and Gly-540) genes and clinical treatment outcomes in children treated with SP [17]. The prevalences of 2 other relevant mutations (DHFR 108 and 51) were too high ($\geq 95\%$) to be useful independent predictors of treatment outcome and were, therefore, not evaluated in the present study. The prevalences of the DHFR Arg-59, DHPS Glu-437, and DHPS Gly-540 mutations in new and recrudescence infections were compared among the 3 treatment groups. Molecular methods used to identify these mutations have been published elsewhere [17, 18]. In brief, parasite DNA was extracted with chelex, from filter-paper samples obtained each time a patient was diagnosed with uncomplicated malaria [19]. Parasite DNA was amplified using nested polymerase chain reaction, and the presence of mutations at each codon of interest was determined using restriction-enzyme digestion [18]. Digestion products were visualized by electrophoresis, and results were classified as wild-

SP monotherapy



SP/AQ combination therapy



SP/AS combination therapy

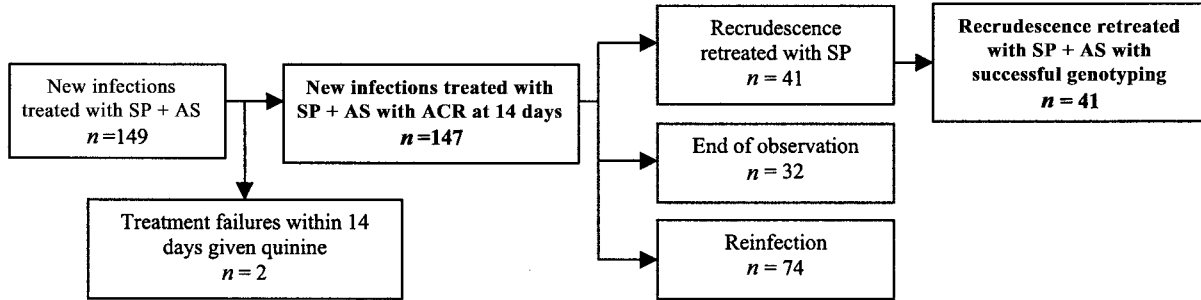


Figure 1. Sample selection for each of the treatment groups. Bold type indicates groups for comparison (new vs. recrudescence infections). ACR, adequate clinical response (as defined elsewhere [14]); AQ, amodiaquine; AS, artesunate; SP, sulfadoxine-pyrimethamine.

type, pure mutant, or mixed (both wild-type and mutant genotypes present in the same infection).

Statistical analysis. Our primary outcome of interest was time to treatment failure. Treatment failure was defined as an episode of symptomatic malaria due to recrudescence parasites. Treatment-failure rates were estimated using survival analysis, with an event defined as recrudescence and data censored for new infections or the end of observation [6]. Associations between the type of infection (recrudescence vs. new) and treatment outcomes were estimated using a Cox proportional hazards model, controlling for day-0 parasite density (normalized by log transformation) and repeated measures, in the same

patient. Hypothesis testing for differences in HRs for treatment failure among the 3 treatment groups was done by modeling for interaction between the treatment group and type of infection (recrudescence vs. new) in our Cox proportional hazards model. Hypothesis testing for differences in the prevalence of DHFR and DHPS mutations in recrudescence versus new infections used generalized estimating equations with exchangeable correlation and robust SEs [20], controlling for day-0 parasite density and repeated measures, in the same patient. All data were entered and verified using Epi-Info 6.04 (Centers for Disease Control and Prevention) and SPSS (SPSS) software. Analysis was performed using STATA statistical software

Table 1. Comparison of treatment outcomes for recrudescing versus new infections, by treatment group.

| Treatment group, type of infection ^a | Treatment outcome, no. of patients | | HR (95% CI) ^d | P |
|---|------------------------------------|----------------------|--------------------------|-----|
| | Failure ^b | Success ^c | | |
| SP | | | | |
| Recrudescing | 15 | 17 | 2.44 (1.32–4.50) | .01 |
| New | 34 | 87 | | |
| SP + AQ | | | | |
| Recrudescing | 1 | 10 | 1.05 (0.21–5.29) | .96 |
| New | 12 | 129 | | |
| SP + AS | | | | |
| Recrudescing | 4 | 37 | 0.40 (0.14–1.12) | .08 |
| New | 41 | 106 | | |

NOTE. AQ, amodiaquine; AS, artesunate; CI, confidence interval; HR, hazard ratio; SP, sulfadoxine-pyrimethamine.

^a Recrudescing infection is defined as an episode of malaria due to parasites that survived prior treatment with the same regimen. New infection is defined as all first episodes of malaria and episodes due to parasites not present at the time of prior treatment.

^b Treatment followed by another episode of malaria due to the same parasite strain.

^c Treatment followed by an episode of malaria due to a new parasite strain or the end of the study.

^d HR controlling for day-0 parasite density and repeated measures.

(StataCorp). $P < .05$ was considered to be statistically significant. All confidence levels were set at 95%.

RESULTS

Clinical treatment outcomes for recrudescing versus new infections. For 1 year, we evaluated outcomes after treatment for episodes of uncomplicated malaria that occurred in the 3 treatment groups of our cohort. New infections that resulted in treatment failure (primary treatment failure) >14 days after initial diagnosis were relatively common (figure 1). In the SP treatment group, 67 (44%) of 154 new infections resulted in treatment failure, 34 (51%) of which occurred >14 days after the onset of therapy. Primary treatment failure was less common in the SP + AQ (14/143 [10%]) and SP + AS (43/149 [29%]) treatment groups and occurred almost exclusively >14 days after the onset of therapy (12/14 [86%] for SP + AQ and 41/43 [95%] for SP + AS).

In the longitudinal trial, episodes of uncomplicated malaria that occurred >14 days after a previous episode were retreated with the same preassigned regimen. Molecular genotyping allowed recurrent episodes to be classified as due to either recrudescing or new infections. Treatment outcomes for recrudescing and new infections were compared among the 3 treatment groups (table 1). In the SP group, recrudescing infections had more than twice the rate of treatment failure, compared with new infections ($P = .01$). In the SP + AQ group,

power was limited because of the low numbers of recrudescing infections, and there was no significant difference in the hazard of treatment failure between new and recrudescing infections ($P = .96$). Of note, only 1 of 11 recrudescing infections retreated with SP + AQ resulted in treatment failure, and this recurrent episode occurred 309 days after therapy, suggesting that this case did not represent a true failure but that a new infection with the same genotype as the original infection occurred. Interestingly, in the SP + AS group, recrudescing infections had less than half the rate of treatment failure, compared with new infections, although this comparison did not achieve statistical significance ($P = .08$). Most importantly, in a pair-wise comparison of HRs among the different treatment groups, associations between recrudescing infections and treatment failure were significantly different in the SP group, compared with those in the SP + AS group (2.44 vs. 0.40; $P = .004$). Pair-wise comparisons of HRs for SP versus SP + AQ (2.44 vs. 1.05; $P = .46$) and SP + AQ versus SP + AS (1.05 vs. 0.40; $P = .37$) were not significant.

Prevalence of mutations. A total of 493 samples were tested for polymorphisms in the 3 codons that best predicted SP treatment failure in prior studies (DHFR Arg-59 and DHPS Glu-437 and Gly-540) [17], resulting in 1463 (99%) of 1479 successful reactions. The pretreatment prevalence of these 3 mutations was strongly associated with treatment failure (table 2). Samples with any 2 mutations had 3 times the rate of treatment failure, and samples with all 3 mutations had 4 times the hazard of treatment failure, compared with pretreatment samples with only wild-type sequences or only 1 mutation.

To determine whether the differences in treatment failure rates for recrudescing and new infections among treatment groups were due to variation in the selection of resistance-mediating mutations in SP target enzymes, we compared the prevalence of key DHFR and DHPS mutations in recrudescing and new infections. Among new infections, the baseline prevalences of wild-type, mixed, and mutant genotypes were not significantly different between the treatment groups (figure 2).

Table 2. Association between dihydrofolate reductase (DHFR) and dihydropteroate synthetase (DHPS) mutations and treatment failure.

| Pretreatment mutation analysis ^a | No. of samples ^b | HR (95% CI) ^c | P |
|---|-----------------------------|--------------------------|-------|
| All wild-type or any 1 mutation | 90 | 1.0 (referent) | ... |
| Any 2 mutations | 131 | 2.93 (1.48–5.80) | .002 |
| All 3 mutations | 262 | 3.74 (1.93–7.26) | <.001 |

NOTE. CI, confidence interval; HR, hazard ratio.

^a Considering DHFR Arg-59 and DHPS Glu-437 and Gly-540 mutations. Presence of mutation is defined as mixed or pure mutant genotype.

^b The samples shown are only those which gave successful results for all 3 mutations tested.

^c HR controlling for day-0 parasite density and repeated measures.

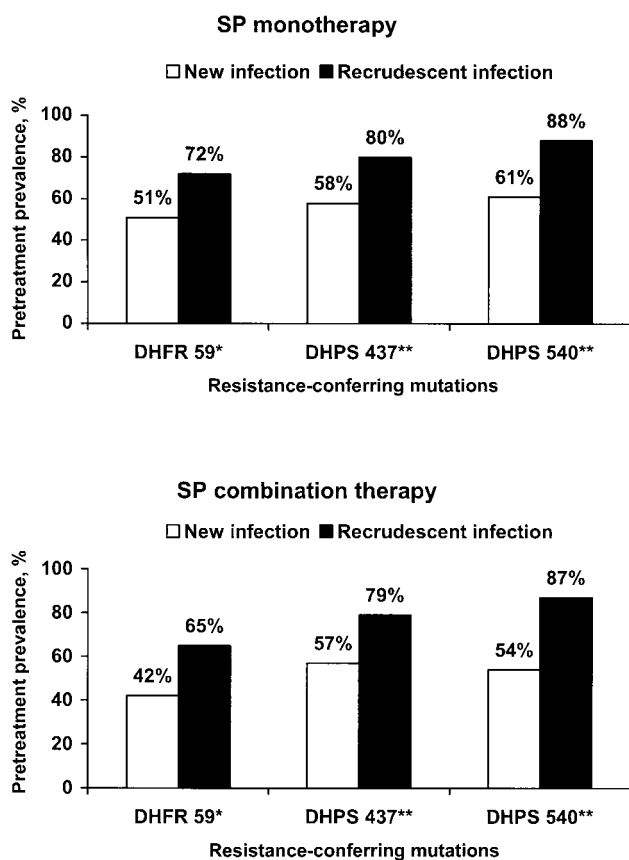


Figure 2. Comparison of pretreatment prevalence of resistance-conferring mutations for new versus recrudescence infections. * $P < .01$ and ** $P < .001$, pair-wise comparisons of prevalences (controlling for day-0 parasite density and repeated measures in the same patient). Prevalence of mutations is defined as the presence of the pure mutant genotype. DHFR 59, dihydrofolate reductase Arg-59; DHPS 437, dihydropteroate synthetase Glu-437; DHPS 540, dihydropteroate synthetase Gly-540; SP, sulfadoxine-pyrimethamine.

In the SP monotherapy group and the combination therapy groups, the prevalences of all 3 mutations were significantly higher in recrudescence infections, compared with those in new infections (figure 2), which is consistent with the selection of resistant parasites, regardless of the treatment regimen.

An increased prevalence of resistance-conferring mutations after treatment failure could have occurred either through selection of mutant parasites that were present before initiation of therapy or through the appearance of mutant parasites after therapy was initiated. We compared the pretreatment distribution of DHFR and DHPS mutations in recrudescence infections with those in new infections that resulted in treatment success and new infections that resulted in recrudescence (table 3). The distribution of mutations in recrudescence infections was very similar to that in new infections that resulted in recrudescence, suggesting that recrudescence was due to the selection of parasites that were already present before therapy.

DISCUSSION

Combination antimalarial therapy may improve treatment efficacy, but will it affect the spread of drug resistance in Africa? In our original longitudinal clinical trial, patients diagnosed with malaria ≥ 14 days after a previous episode were retreated with their preassigned regimen, a method that is consistent with standard clinical practice. However, in all the treatment groups, the majority of clinical failures occurred after 14 days, resulting in repeat therapy of recrudescence parasites with the same regimen. When recrudescence parasites were retreated with SP, the rate of clinical treatment failure was significantly higher than that for new infections. In contrast, retreatment of recrudescence parasites with SP + AS combination therapy led to a lower rate of clinical treatment failure. These differences in treatment-failure rates could not be explained by differences in day-0 parasite densities, because this was controlled for in our analyses. There were too few treatment failures in the SP + AQ combination group to make meaningful comparisons, but clinical response in the limited number of retreatments was excellent. In all treatment groups, clinical treatment failure was associated with an increased prevalence of SP resistance-conferring mutations, suggesting that the improvement in clinical outcome observed with combination treatment of recrudescence infections was not due simply to a decrease in the selection of SP-resistant parasites.

Since 1996, most studies of drug efficacy in Africa have followed WHO guidelines [14] for areas of intense transmission. The WHO protocol relies on clinical response to therapy and limits follow-up to 14 days because of the operational difficulties of longer follow-up and the inability to distinguish re-

Table 3. Distribution of dihydrofolate reductase (DHFR) and dihydropteroate synthetase (DHPS) mutations for different types of infections.

| Treatment group, type of infection | No. of mutations ^a | | | |
|--|-------------------------------|----|----|-----|
| | 0 | 1 | 2 | 3 |
| SP monotherapy | | | | |
| New infections resulting in treatment success ^b | 8 | 10 | 23 | 42 |
| New infections resulting in recrudescence ^c | 0 | 1 | 10 | 23 |
| Recrudescence infections | 0 | 1 | 6 | 23 |
| SP combination therapy | | | | |
| New infections resulting in treatment success ^b | 17 | 42 | 61 | 111 |
| New infections resulting in recrudescence ^c | 2 | 5 | 16 | 30 |
| Recrudescence infections | 0 | 4 | 15 | 33 |

NOTE. SP, sulfadoxine-pyrimethamine.

^a Considering mutations DHFR Arg-59 and DHPS Glu-437 and Gly-540 at the time of diagnosis. Data are no. of samples; the samples shown are only those which gave successful results for all 3 mutations tested.

^b Treatment followed by episode due to different parasite strain or the end of the observation period.

^c Treatment followed by episode due to parasites surviving prior treatment with the same regimen.

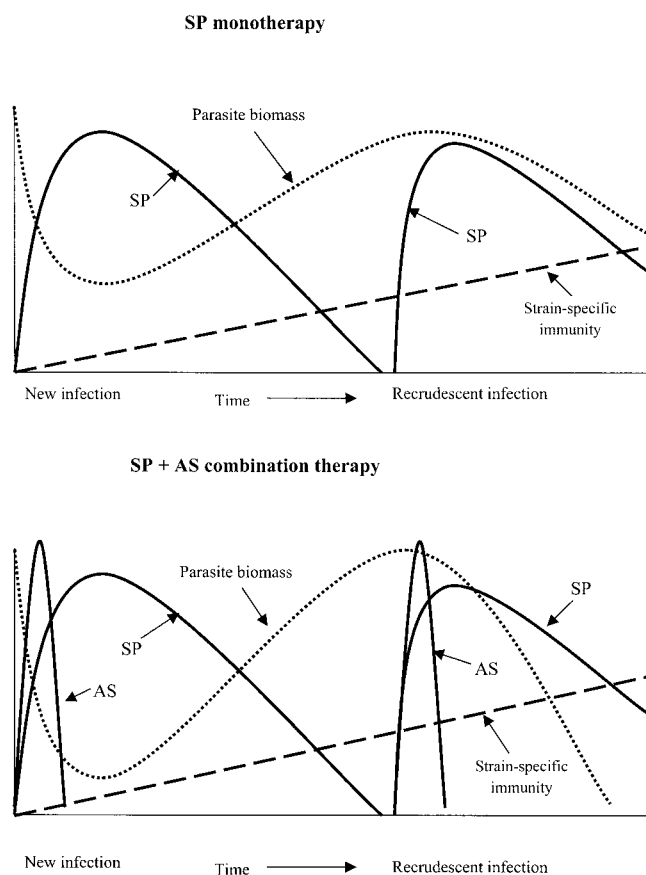


Figure 3. Hypothetical relationship between drug levels, parasite density, and strain-specific immunity for a new infection followed by retreatment of recrudescent parasites. AS, artesunate; SP, sulfadoxine/pyrimethamine.

crudescent from new infections without molecular analysis. However, increasing amounts of evidence suggest that 14-day follow-up significantly underestimates the level of antimalarial drug resistance [21]. Several recent studies in which longer follow-up and molecular genotyping were used have documented that most treatment failures occur after 14 days [5, 6, 22]. Indeed, 14 days of follow-up is sufficient only to detect high levels of resistance [21], when a drug is no longer useful and is likely having an adverse effect on morbidity and mortality [23]. In practice, treatment failures that occur >14 days after therapy are assumed to be new infections, leading to repeat therapy with the same first-line drug. Few studies have looked at the impact of repeat therapy in Africa. In Tanzania, patients treated with SP who experienced clinical failure 7–28 days after therapy were retreated with SP [24]. The risk of parasitological failure at day 7 increased from 45% after the first treatment to 61% after retreatment, although clinical outcomes were not assessed, and genotyping was not used to distinguish recrudescence from new infections. Similarly, in the present study, patients commonly had an initial adequate clinical response to

SP but later experienced treatment failure due to the emergence of recrudescence parasites. Not surprisingly, these treatment failures led to the selection of resistance-conferring mutations and an increased rate of subsequent treatment failure. At the individual level, increased treatment failure rates will lead to more clinical attacks of malaria and, most likely, increased incidences of anemia and severe disease [25]. At the population level, increased treatment-failure rates will lead to prolonged carriage of drug-resistant parasites and likely will accelerate the spread of drug resistance. The fact that we were able to observe the ready selection of clinically relevant drug resistance in a small cohort of children raises serious concerns about the useful therapeutic life span of SP monotherapy in similar settings.

Clinical failure after initial treatment with an SP-containing combination regimen also was associated with selection of parasite mutations that confer resistance to SP. Of interest, in patients retreated with a combination regimen, this selection did not translate into an increased rate of clinical failure. How can we explain the improved outcome after retreatment with combination therapy despite the selection of resistance-mediating mutations? It is helpful to model the interactions between pharmacodynamic properties of drugs and host immunity (figure 3). In our model, a new infection is characterized by a multiclonal population of parasites, as is common in areas of Africa where malaria is endemic [26]. As the population of parasites is exposed to SP monotherapy, the parasite biomass is reduced, with the elimination of drug-sensitive parasites. Surviving parasites multiply as drug levels decrease, leading to a recrudescent episode of malaria that requires repeat therapy. Strain-specific immunity increases over time, but immunity is counterbalanced by the selection of parasites that are less susceptible to SP, resulting in an increase in the probability of subsequent treatment failure. With combination therapy, a more complex situation exists (figure 3). AS is a potent, short-acting antimalarial with no reported resistance and rapid reduction in parasite biomass [27]. In the study by Dorsey et al. [6], no patients treated with AS had detectable parasitemia 3 days after the initiation of therapy. However, late recrudescence is common after AS monotherapy [27], especially with high pretreatment parasite density [28]. With SP + AS combination therapy, parasites that survive the initial short action of AS are then subjected to the selective pressure of SP, which has a much longer half-life. Given the high baseline level of resistance to SP in our population, it was not uncommon for recrudescence to occur after therapy with SP + AS, albeit almost exclusively after 14 days. At the time of retreatment, parasites are again exposed to AS, leading to a dramatic reduction of parasite biomass, independent of susceptibility to SP. At this point, when parasite biomass is much lower than after SP monotherapy, increased strain-specific host immunity would be expected to be more likely to clear the few remaining parasites, resulting

in a greater chance of cure after repeat therapy. In contrast to the AS-containing regimen, the outstanding efficacy of SP + AQ is explained by the long half-lives of both drugs, leading to an increased chance of elimination of all parasites before resistance to both agents has been selected. In the limited number of recrudescence infections, retreatment did not appear to lead to an increase in treatment-failure rates, despite the selection of SP-resistant parasites.

The emergence of resistance to antimalarial drugs has become a critical problem in Africa. CQ has already been abandoned by several countries [29], and these countries are now struggling to select a suitable replacement. Evidence from the present study suggests that a change to SP monotherapy, as has occurred in a number of countries, will lead to the rapid selection of resistance to SP. This selection may not only compromise the future utility of SP as a component in combination regimens but may also negatively affect the efficacy of chlorproguanil-dapsone, a newly developed, affordable antimalarial with a mechanism of action similar to that of SP [30]. Combination therapy may now be the appropriate strategy for the treatment of uncomplicated malaria in Africa, but the ideal regimen is not clear and may be not be the same for every location. In particular, the short half-lives of artemisinins may lead to higher rates of treatment failure in areas of high endemicity (and, typically, high pretreatment parasite densities) than are predicted on the basis of studies in Southeast Asia. Alternatively, combinations of longer-acting drugs (e.g., SP + AQ) have shown excellent efficacy in some areas [6, 7], but high rates of resistance to these drugs in some parts of Africa may limit efficacy. Clearly, no perfect antimalarial regimen for Africa is available. Considering this situation, it is reassuring that our study showed that 2 different combination regimens prevented the selection of drug-resistant parasites after therapy, either by nearly eliminating treatment failure (SP + AQ) or by preventing increased rates of treatment failure after initial therapy (SP + AS). These results highlight the promise of combination antimalarial therapy for Africa but suggest that additional study is required to identify the specific combination regimens that are appropriate for different areas.

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