

Sulfadoxine/pyrimethamine alone or with amodiaquine or artesunate for treatment of uncomplicated malaria: a longitudinal randomised trial

Grant Dorsey, Denise Njama, Moses R Kamya, Adithya Cattamanchi, Daniel Kyabayinze, Sarah G Staedke, Anne Gasasira, Philip J Rosenthal

Summary

Background New antimalarial treatments are urgently needed in sub-Saharan Africa. Improved therapies should decrease failure rates in the short term, but their effect on incidence of subsequent episodes of malaria is little studied. We aimed to compare the short-term and long-term effectiveness of three antimalarial regimens in children from Kampala, Uganda.

Methods We randomly allocated healthy children aged 6 months to 5 years to receive 25 mg/kg sulfadoxine and 1.25 mg/kg pyrimethamine plus either placebo, 25 mg/kg amodiaquine, or 12 mg/kg artesunate. Participants were followed up for 1 year and received the same preassigned treatment for every new episode of uncomplicated malaria diagnosed during follow-up. Recrudescence and new infections were distinguished by comparison of polymorphisms in merozoite surface protein 2 (MSP2). Our primary endpoint was the total number of treatments for malaria per time at risk. Analyses were done per protocol.

Findings 183 (61%) of 316 participants were diagnosed with at least one episode of uncomplicated malaria. A total of 577 episodes of uncomplicated *Plasmodium falciparum* malaria were treated with study drugs; all regimens were safe and well tolerated. Clinical treatment failure after 14 days was significantly more frequent in the sulfadoxine/pyrimethamine group (38 of 215, 18%) compared with either the sulfadoxine/pyrimethamine plus amodiaquine group (two of 164, 1%; $p < 0.0001$) or sulfadoxine/pyrimethamine plus artesunate group (one of 198, 1%; $p < 0.0001$). After 28 and 42 days, patients in the sulfadoxine/pyrimethamine plus amodiaquine group were significantly less likely to develop malaria than were those in the other groups. Overall, sulfadoxine/pyrimethamine plus amodiaquine reduced the rate of subsequent treatments for malaria by 54% (95% CI 36–66, $p < 0.0001$) compared with sulfadoxine/pyrimethamine alone and by 37% (12–54, $p = 0.007$) compared with sulfadoxine/pyrimethamine plus artesunate.

Interpretation Sulfadoxine/pyrimethamine plus amodiaquine could be used as an inexpensive regimen to decrease the rate of subsequent episodes of malaria.

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Department of Medicine, San Francisco General Hospital, University of California, San Francisco, CA, USA (G Dorsey MD, A Cattamanchi BA, S G Staedke MD, P J Rosenthal MD); Makerere University Medical School, Kampala, Uganda (D Njama MBChB, M R Kamya MMed, D Kyabayinze MBChB, A Gasasira MBChB)

Correspondence to: Dr Grant Dorsey, Department of Medicine, San Francisco General Hospital, 1001 Potrero Avenue, Building 30, Room 421, San Francisco, CA 94110, USA (e-mail: grantd@itsa.ucsf.edu)

Introduction

Malaria remains a leading cause of morbidity and mortality in African children.¹ The basis for malaria control throughout sub-Saharan Africa is appropriate case management, focusing on prompt treatment with effective antimalarial drugs.² Chloroquine has been the mainstay of antimalarial treatment, but the emergence of *Plasmodium falciparum* resistance to this drug has challenged control efforts and been linked to an increase in childhood mortality.³ As African countries are reassessing their antimalarial drug policies, no clear alternative to chloroquine for treatment of uncomplicated malaria has emerged.

The fixed combination of sulfadoxine and pyrimethamine has replaced chloroquine as the first-line therapy for uncomplicated falciparum malaria in several African countries.⁴ Advantages of this combination include low cost, simple dosing, and relative safety. However, the therapeutic lifespan of sulfadoxine/pyrimethamine will be limited by the rapid emergence of parasites resistant to this combination.⁵ Amodiaquine, a 4-aminoquinoline related to chloroquine, has retained efficacy even in regions of substantial resistance to chloroquine, with a side-effect profile similar to that of chloroquine and sulfadoxine/pyrimethamine.⁶ However, concerns about safety and the potential for cross-resistance between the 4-aminoquinolines has led to arguments against replacement of chloroquine with amodiaquine.⁷ The artemisinin derivative artesunate has potent antimalarial activity and an attractive safety profile, and has yet to be associated with resistance.⁸ However, when a short course of artesunate is used on its own, late recrudescence frequently occurs,⁹ and the drug is still too expensive for widespread use in most African countries. Recently, attention has focused on the strategy of combining drugs with different modes of action to improve antimalarial therapeutic efficacy and delay emergence of drug resistance.⁸ In studies in Africa, the combinations of sulfadoxine/pyrimethamine plus amodiaquine¹⁰ and sulfadoxine/pyrimethamine plus artesunate¹¹ improved effectiveness when compared with sulfadoxine/pyrimethamine alone.

In sub-Saharan Africa, transmission levels are generally high, and children who are semi-immune generally need treatment for malaria several times a year.^{12,13} After antimalarial treatment, patients are at risk of short-term (14-day) treatment failure; late recrudescence, which may take place weeks to months after treatment;¹⁴ and illness due to reinfection with new parasite strains. Most studies of drug efficacy in Africa have limited follow-up to 14 days, in accordance with WHO recommendations, and thus only the short-term effectiveness of the drugs has been compared.¹⁵ Therefore, such studies may not identify important differences between treatment regimens. Rather, differences in effectiveness can be fully appreciated only with longer-term follow-up, especially for treatments with varied pharmacokinetics.

In Kampala, Uganda, failure rates with chloroquine have reached unacceptable levels, and resistance to sulfadoxine/pyrimethamine is common.^{16,17} We aimed to compare the efficacy of sulfadoxine/pyrimethamine alone, sulfadoxine/pyrimethamine plus amodiaquine, and sulfadoxine/pyrimethamine plus artesunate for treatment of uncomplicated malaria in children using a longitudinal design. This approach enabled us to compare the effects of different regimens on short-term effectiveness and on incidence of recurrent disease over an extended period of risk.

Methods

Study area and recruitment

The study took place between July, 2000, and August, 2001, at the outpatient department of Mulago Hospital in Kampala, Uganda. Kampala is an urban centre where malaria is mesoendemic, occurring perennially with peaks during the two rainy seasons (Nathan Bakyaata, Ugandan Ministry of Health, unpublished data). We recruited healthy children between the ages of 6 months and 5 years over a 6-week period from five distinct zones within one geopolitical division (Kawempe) around Mulago Hospital. We used community-based, convenience sampling of interested parents or guardians and their children to bring potential participants to our clinic, where study physicians did a screening interview. Children were enrolled if they fulfilled all of the following eligibility criteria: age 6–59 months; no history of treatment for malaria in the previous 2 weeks, or fever in the previous 48 h; no history of adverse reactions to any of the study drugs; no history of sickle-cell disease; haemoglobin of 50 g/L or more; willingness to remain in the city of Kampala and follow the study protocol for the next 12 months; and willingness of parent or guardian to provide informed consent. All eligible children living in a single household were enrolled. The study was approved by the institutional review boards of the University of California, San Francisco and Makerere University, Kampala.

Treatment allocation and case management

Immediately after enrolment, we randomly allocated children to one of three regimens to be given for all future episodes of uncomplicated malaria diagnosed: 25 mg/kg sulfadoxine and 1.25 mg/kg pyrimethamine (Fansidar, Roche, Basel, Switzerland) given as a single dose on the first day plus vitamin C placebo given once a day for 3 days; sulfadoxine/pyrimethamine plus amodiaquine (Camoquin, Parke-Davis, Senegal, 10 mg/kg for the first 2 days and 5 mg/kg on the third day) given once a day for 3 days; or sulfadoxine/pyrimethamine plus 4 mg/kg artesunate (Arsumax, Sanofi, France) given once a day for 3 days. Children were allocated to treatment by a study investigator who was not directly involved in patients' enrolment with a predetermined, computer-generated randomisation list stratified into 18-month age groups in blocks of six. Study participants and their parents or guardians were unaware of their treatment group, but study investigators were not masked.

We maintained a daily clinic at the outpatient department of Mulago Hospital. Parents and guardians were instructed to bring their child to clinic whenever they needed medical attention and to avoid using any drugs not administered or approved by a study physician. Participants were followed up for 12 months. If more than 30 days passed without a child being seen in the clinic, participants and their parents or guardians were visited at home to remind them of the study protocol and to assess the children for use of other drugs. Follow-up was

terminated early if the child permanently moved from the city of Kampala; did not have contact with the clinic for more than 60 consecutive days; did not attend more than three scheduled follow-up visits for malaria; withdrew informed consent; had an adverse reaction to study drugs; or died.

Every time a child presented to the clinic with a new history of fever (previous 48 h) or a tympanic temperature of 38.0°C or greater, we obtained a thick blood smear. Patients were diagnosed with malaria if they fulfilled any of the following criteria: complicated malaria (defined as presence of severe malaria¹⁸ or danger signs¹⁵) and any parasitaemia; tympanic temperature of 38.0°C or greater and any parasitaemia; history of fever (not documented) and 500 or more asexual parasites/ μ L. On a diagnosis of malaria, patients received a second finger prick for thin smear and measurement of haemoglobin. All patients with complicated malaria were referred to the hospital's inpatient service for treatment with quinine (10 mg/kg every 8 h for 7 days) and after discharge completed standard follow-up in our clinic. Patients who developed criteria for complicated malaria within 24 h of the initial diagnosis were managed as above and quinine was judged their first-line regimen. Patients diagnosed with uncomplicated malaria began treatment with their preassigned regimen. Children in each treatment group were given the same treatment for every new episode of uncomplicated malaria diagnosed. All treatment with study drugs was directly observed. After administration of the drugs, patients were observed for 30 min, with the dose re-administered if the child vomited. All patients were provided with a 3-day supply of acetaminophen for treatment of febrile symptoms. Patients with a haemoglobin of less than 100 g/L on day 0 were given 100 mg ferrous sulphate a day for 14 days and given antihelmintic treatment if they were older than 1 year and had not been treated in the previous 6 months.

Patients diagnosed with non-malarial illnesses were managed at the discretion of the study physician. Patients diagnosed with malaria and concomitant illnesses were treated for both conditions and followed up according to the same protocol as if malaria were diagnosed alone. Use of non-study drugs with antimalarial activity was avoided if suitable alternatives were available.

Malaria follow-up and outcome classification

Malaria follow-up appointments were scheduled for days 1, 2, 3, 7, and 14 and consisted of a focused physical examination and completion of a standard history form. We obtained blood by fingerprick on days 3, 7, and 14 (or any additional days on which patients presented with a history of fever) for repeat thick blood smears. Haemoglobin measurements were repeated on day 14. We encouraged patients to return to the clinic at any time if they felt ill. If patients did not return for a scheduled appointment, they were visited at home.

For every episode of malaria, patient outcomes were determined using the WHO 14-day parasitological and clinical classification systems.^{15,19} Parasitological response was classified as S, RI, RII, or RIII and dichotomised for analysis into sensitive (S) or resistant (RI/RII/RIII) outcomes. Clinical response was classified as adequate clinical response, early treatment failure, or late treatment failure, and was dichotomised into success or failure (early or late). The WHO clinical classification system was slightly modified by adding a criterion for late treatment failure: reported fever in the past 48 h on days 4–14 in the setting of parasitaemia. Early treatment failures that did not fulfil criteria for an RIII response were classified as

RII. All patients classified as clinical failures were treated with standard doses of quinine, and at that time started a new 14-day follow-up schedule. Any case of malaria diagnosed more than 14 days after a previous episode was considered a new event (for treatment purposes) and managed according to the protocol above.

Laboratory methods

We stained thick and thin blood smears with 2% Giemsa for 30 min. All thick smears from symptomatic patients were initially read in the clinic to determine whether treatment should be started or a clinical failure had occurred. Readings used for data analyses were done within 48 h by skilled microscopists who were unaware of the patient's clinical status and treatment group. We measured the density of parasites from the thick blood smear by counting the number of asexual parasites per 200 white blood cells and calculating parasites/ μL assuming a white blood cell count of 8000/ μL .¹⁵ A smear was judged negative if no parasites were seen after review of 100 fields at $\times 1000$ magnification. We assessed the density of gametocytes from every thick blood smear with the same method, and used thin smears to determine the species of parasite. All thick smears were read by a second skilled microscopist who was also unaware of the patient's clinical status and treatment. Any discrepancies (positive *vs* negative, results that would change outcome classification, more than 25% difference in parasite density) were resolved by a third microscopist. We measured haemoglobin in the study clinic with a portable spectrophotometer (HemoCue, Anglholm, Sweden).

Blood was collected on filter paper (Whatman No 3) every time a new episode of malaria was diagnosed and when treatment failed. We did molecular genotyping for all successive episodes of uncomplicated *P falciparum* malaria treated with study drugs, except that failures within 6 days of treatment were judged as recrudescence, episodes due to *P falciparum* followed by non-*falciparum* malaria were judged as reinfections, and episodes after quinine therapy (for severe malaria or clinical failure within 14 days) were considered new infections. Parasite DNA was extracted from filter paper with chelex. The block 3 polymorphic region of MSP2 was amplified by nested PCR. Initial PCR primers corresponded to conserved sequences flanking this region.²⁰ Nested PCR primers were then used to amplify the IC3D7 and FC27 allelic families of MSP2.²¹ For controls, genomic DNA from HB3 and 3D7 laboratory strains was isolated by standard techniques. PCR products were analysed by electrophoresis with 2% agarose. Samples from an individual patient were run in adjacent lanes. Gel images were digitised and molecular weights assigned to bands by GelCompar II software (Applied Maths, Sint-Martens-Latem, Belgium). We calculated densitometric curves for every gel lane, and assigned molecular weights to bands with greater than 2% of the density of the dominant band in each lane. An outcome was defined as recrudescence if a subsequent episode contained identical alleles or a subset of the alleles present in the previous episode and reinfection if a subsequent episode contained only new alleles. If a subsequent episode contained both alleles present in the previous episode and new alleles (10% of episodes), the outcome was judged a reinfection if half or more of the bands were new, and recrudescence if more than half of the bands were present in the previous episode. A recent detailed analysis has shown this system to provide an accurate means of distinguishing recrudescence from new infections (unpublished).

Statistical analysis

Our primary aim was to test the hypothesis that combination therapy would reduce the number of treatments (both first and second-line) for malaria compared with sulfadoxine/pyrimethamine alone. Using information from previous studies in Kampala, we estimated that 70% of children in our control group would develop at least one episode of malaria, with an average of four episodes per year, and an anticipated 20% loss of follow-up time due to early study termination. Based on these estimates, a study with 100 participants in each group would have 80% power to detect at least a 25% decrease in the total number of treatments for malaria per time at risk for either combination therapy group compared with sulfadoxine/pyrimethamine monotherapy (with a two-sided significance of 5%).

For our primary outcome, we assessed only participants who had at least one treatment for malaria with a study drug. To eliminate observation time before study drugs were first given we calculated the total number of treatments for malaria per time at risk using only treatments and time at risk occurring after the first treatment with a study drug was started. Observation time 14 days after starting quinine and 1 day after starting study drugs was not included, since patients were not considered at risk for additional treatment during these intervals. Data were censored for participants if follow-up was for less than 1 year. Comparisons of the primary outcome were made with a negative binomial regression model with assigned treatment groups as covariates and exposure reflected by the time at risk after the first treatment with a study drug.

Secondary analysis included outcomes after treatments of individual episodes of malaria, restricted to *P falciparum* infections treated with study drugs and having complete follow-up. Clinical outcomes, parasitological outcomes, fever clearance, parasite clearance, and gametocyte prevalence were compared after 14 days of follow-up. Day 0 parasite densities were normalised by log transformation. We compared proportions with χ^2 or Fisher's exact tests, and used analysis of variance for comparison of continuous variables. Risks of recrudescence and reinfection after extended follow-up and correction by genotyping were estimated with Kaplan-Meier survival analysis techniques. Survival curves were compared with the log-rank test. We estimated the relative risk of PCR-corrected recrudescence after each episode of malaria at 2, 4, and 6-week time intervals using the odds ratio for each pairwise comparison. To control for repeated measures of malaria treatment response in the same patients and day 0 parasite densities, we calculated odds ratios with generalised estimating equations with exchangeable correlation and robust SEs.²²

All data were entered and verified with Epi-info version 6.04. Analysis was done on STATA statistical software version 7.0. Pairwise comparisons between treatment groups were planned a priori. A p value of 0.05 or less was judged significant.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or in the writing of the report.

Results

We enrolled 316 children from 211 different households in the study and randomised them into three groups for future treatment of uncomplicated malaria (figure 1). The cumulative period of observation covered 93% of potential follow-up time, and 282 participants (89%) completed the

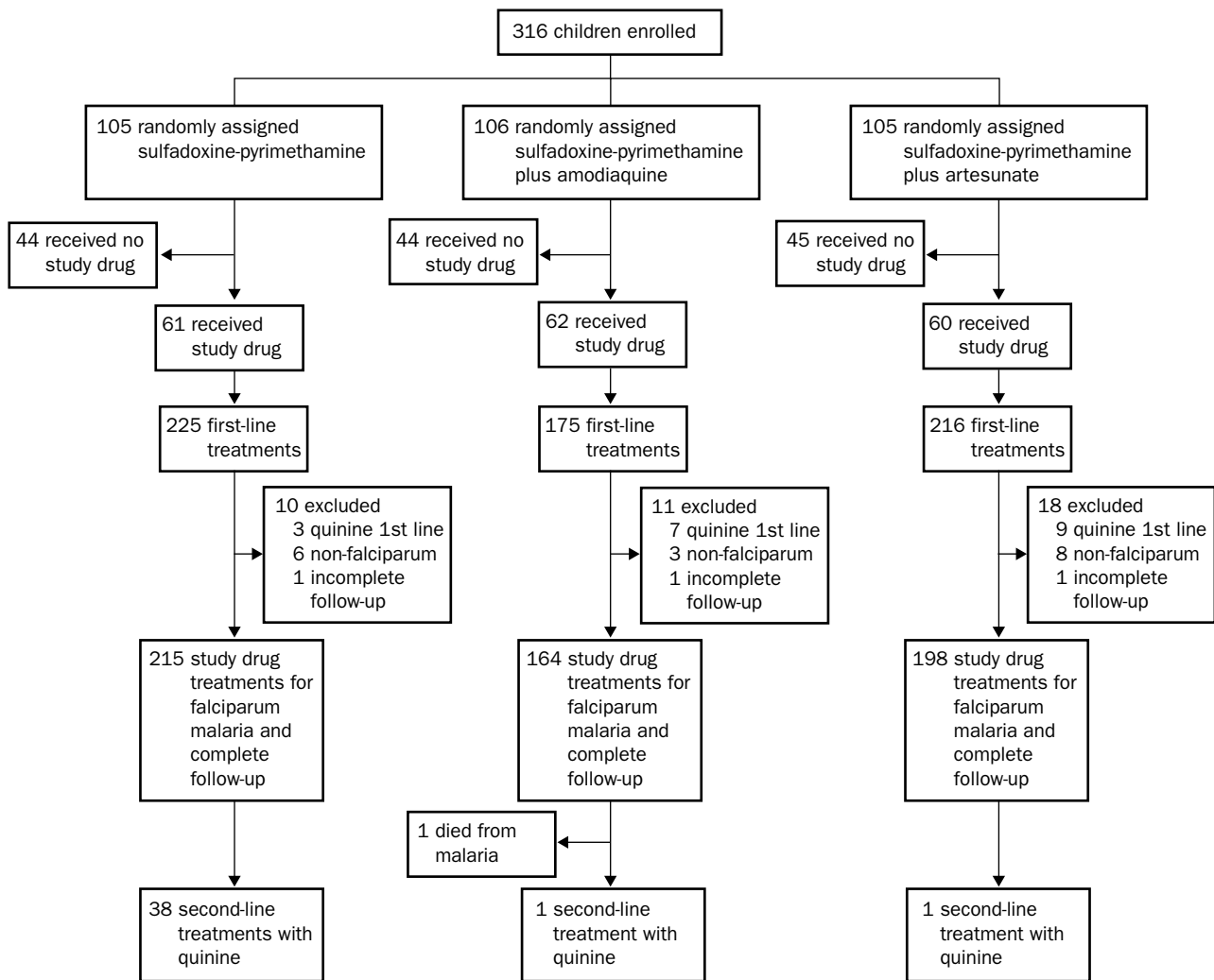


Figure 1: Trial profile and treatment episodes

full 1-year of follow-up. Reasons for early study termination included permanent movement from the study area ($n=23$), withdrawal of informed consent ($n=7$), death ($n=3$, one death due to malaria, one to aspiration pneumonia, and one to chronic diarrhoea, wasting, and probable AIDS), and failure to show up for more than three scheduled malaria follow-up visits ($n=1$). Four patients treated for malaria with study drugs did not fulfil our original criteria (all had positive smears, but had parasite densities of less than $500/\mu\text{L}$, with reported but undocumented fever). Excluding follow-up visits for malaria and non-malaria illnesses, a total of 5462 encounters with participants occurred for new illnesses or routine assessments (mean 17 per participant, SD 6.66). A history of drug use not started by study physicians was obtained in 75 (1%) of these encounters in 62 participants. This drug was reported to be an antimalarial (chloroquine on all but one occasion) or unknown drug on 38 occasions (in 34 participants) and a single dose was given in most cases. A history of malaria

reportedly confirmed by blood smear outside of our study clinic was obtained on three occasions. In view of the infrequent occurrence of protocol violations, outside antimalarial drug use is unlikely to have affected our results greatly.

We gave 616 treatments for malaria to the 183 participants who received at least one study drug treatment. Quinine was used as first-line treatment for complicated malaria in 19 episodes (3%), 17 episodes (3%) were due to non-falciparum infections, and three episodes (0.5%) had incomplete follow-up (figure 1). Table 1 shows the baseline characteristics averaged over every episode of *P. falciparum* malaria treated with study drugs and having complete follow-up.

We assessed short-term patient outcomes using clinical and parasitological criteria.^{15,19} After 14 days, we recorded significantly fewer clinical treatment failures and less parasitological resistance in the combination treatment groups than in the sulfadoxine/pyrimethamine alone groups

Characteristic	Sulfadoxine/pyrimethamine plus placebo (n=215)	Sulfadoxine/pyrimethamine plus artesunate (n=198)	Sulfadoxine/pyrimethamine plus amodiaquine (n=164)
Temperature (mean [SD], °C)	38.1 (1.2)	38.3 (1.1)	38.2 (1.2)
Temperature $\geq 38.0^\circ\text{C}$ (95% CI)	59% (52–65)	66% (59–72)	63% (55–70)
Parasite density (geometric mean [range], per μL)	27 300 (48–987 840)	44 700 (120–968 000)	29 000 (120–611 520)
Parasite density $>100\ 000/\text{mL}$ (95% CI)	30% (24–36)	37% (31–44)	27% (21–34)
Haemoglobin (mean [SD], g/L)	106 (17)	106 (17)	106 (18)

Table 1: Characteristics of episodes due to *P. falciparum* at day 0 treated with study drugs and with complete follow-up

	Sulfadoxine/ pyrimethamine plus placebo (n=215)	Sulfadoxine/ pyrimethamine plus artesunate (n=198)	Sulfadoxine/ pyrimethamine plus amodiaquine (n=164)
Clinical outcomes			
Adequate clinical response	177 (82%)	197 (99%)	162 (99%)
Early treatment failure	22 (10%)	0	2 (1%)
Late treatment failure	16 (7%)	1 (1%)	0
Parasitological outcomes			
S	147 (68%)	189 (95%)	161 (98%)
RI	33 (15%)	9 (5%)	1 (1%)
RII	19 (9%)	0	2 (1%)
RIII	16 (7%)	0	0

Table 2: Clinical and parasitological outcomes after 14 days of follow-up

($p < 0.0001$ for all comparisons, table 2). 38 (18%) of 215 episodes treated with sulfadoxine/pyrimethamine alone were clinical treatment failures, compared with only two (1%) of 164 episodes treated with sulfadoxine/pyrimethamine plus amodiaquine. One patient presented to the emergency room after a convulsion about 60 h after treatment began and died immediately after a blood transfusion. The density of parasites had decreased from 114 240/ μL on day 0 to 3360/ μL on day 2, and a repeat smear was negative in the emergency room. The other patient was classified as an early treatment failure after having a convulsion about 36 h after treatment began, despite a decrease in parasite density from 578 000/ μL on day 0 to 960/ μL on day 2. Only one (1%) of 198 episodes treated with sulfadoxine/pyrimethamine plus artesunate was a clinical treatment failure. This patient recovered fully and had a negative smear on day 7, but had documented fever and a positive smear on day 14.

Use of sulfadoxine/pyrimethamine with amodiaquine and sulfadoxine/pyrimethamine with artesunate, respectively, significantly reduced the proportion of patients with documented fever on days 1 ($p = 0.006$ and $p < 0.0001$), 2 ($p < 0.0001$ for both comparisons), and 3 ($p = 0.004$ and $p = 0.003$, figure 2), and the proportion of patients with positive smears on day 3 (sulfadoxine/pyrimethamine group 74 [34%], sulfadoxine/pyrimethamine plus artesunate group 0, sulfadoxine/pyrimethamine plus amodiaquine group 4 [2%]; $p < 0.0001$ for both comparisons). Clearance of fever between days 1 and 3 did not differ between the two combination groups (day 1 $p = 0.27$, day 2 $p = 0.23$, and day 3 $p = 0.90$). Gametocytes first appearing on days 1–14 were much more common in the sulfadoxine/pyrimethamine group (84, 39%) than in the sulfadoxine/pyrimethamine group plus amodiaquine group

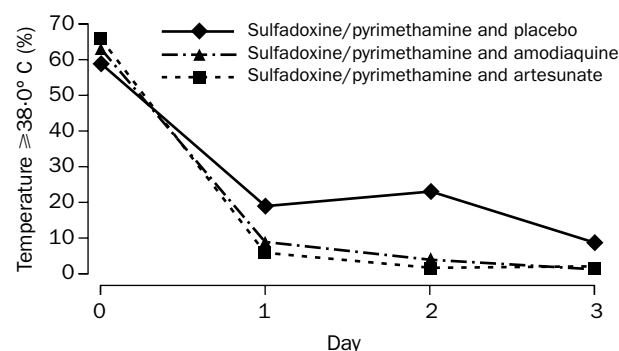
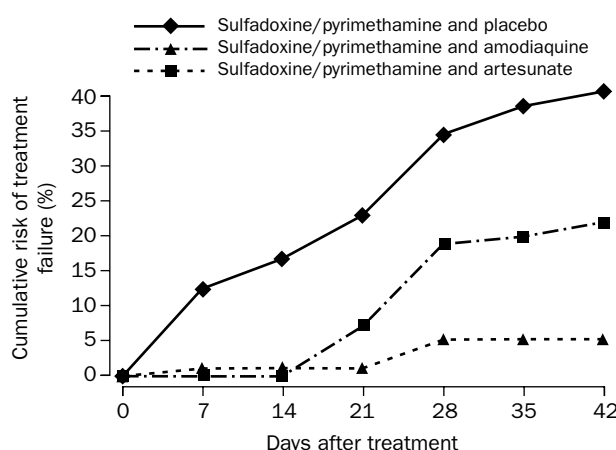


Figure 2: Proportion of patients with documented fever ($\geq 38.0^\circ\text{C}$ tympanic)

Early treatment failures occurring on day 2 counted as febrile on day 3.



Patients at risk

SP	203	181	173	142	109	84	70
AQ	158	156	156	147	130	111	98
AS	193	193	193	160	129	112	94

Figure 3: Cumulative risk of treatment failure

Data are based on survival analysis after correction by genotyping with event defined as recrudescence and survival time censored for episodes followed by reinfection or the end of the study.

(16, 10%; $p < 0.0001$) or the sulfadoxine/pyrimethamine group plus artesunate group (two, 1%; $p < 0.001$).

No participant developed severe adverse reactions to study drugs, and fewer than 1% of patients vomited. No patient developed jaundice after day 0, and no serious dermatological conditions were recorded in any of the study patients. Potential mild adverse reactions (generally indistinguishable from malaria symptoms), with onset 1–14 days after therapy was started, did not differ between the three treatment groups, with the exception of malaise, which was more common in those receiving sulfadoxine/pyrimethamine plus amodiaquine than in those receiving either sulfadoxine/pyrimethamine alone or sulfadoxine/pyrimethamine plus artesunate (89 [54%] vs 157 [38%], $p = 0.01$).

Patients judged as clinical treatment failures within 14 days of diagnosis were given quinine. Every patient with an adequate clinical response was followed up for any subsequent episodes of malaria until study termination. This design enabled us to estimate the long-term risk of repeat treatment. Comparison of MSP2 alleles allowed us to distinguish recrudescence from new infections, and thus determine the long-term risk of recrudescence in children given each regimen. Both combination treatments significantly reduced the risk of treatment failure compared with sulfadoxine/pyrimethamine monotherapy at 2, 4, and 6 weeks of follow-up (figure 3, table 3). After the first 2 weeks of follow-up, the risk of treatment failure did not differ between the two combination groups ($p = 0.4$). However, 4 and 6 weeks after therapy, the sulfadoxine/pyrimethamine plus amodiaquine regimen was more effective than the sulfadoxine/pyrimethamine plus artesunate group (4 weeks $p = 0.002$, 6 weeks $p = 0.001$). Compared with sulfadoxine/pyrimethamine plus amodiaquine, patients given sulfadoxine/pyrimethamine plus artesunate were more than three times as likely to have their treatment fail after 4 and 6 weeks (table 3). Higher parasite density at the time of diagnosis was a significant independent predictor ($p = 0.002$) of treatment failure at 4 and 6 weeks, and was controlled for in the analysis. Stratification for hyperparasitaemia ($> 100\,000/\mu\text{L}$) did not change the results; children with parasite densities of

Comparison groups	Day 14*		Day 28†		Day 42†	
	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p
SP vs SP+amodiaquine	14.9 (3.7–60.0)	<0.0001	6.8 (3.3–14.1)	<0.0001	5.9 (3.0–11.6)	<0.0001
SP vs SP+artesunate	43.8 (6.0–318.0)	<0.0001	2.1 (1.4–3.2)	0.001	1.8 (1.3–2.6)	0.001
SP+artesunate vs SP+amodiaquine	0.34 (0.03–37.0)	0.4	3.3 (1.5–7.0)	0.002	3.2 (1.6–6.4)	0.001

SP=sulfadoxine/pyrimethamine. Odds ratios are adjusted for repeated measures and day 0 parasite density. *WHO clinical treatment failure (early or late). †Treatment failure after correction for genotyping.

Table 3: Odds ratios of treatment failure at 2 week intervals after each episode of malaria

100 000 parasites/ μ L or less were 1.8 (95% CI 1.4–2.4) times more likely to have recrudescence with 42 days than were those with a parasite density of less than 100 000 parasites/ μ L, irrespective of the treatment given. By contrast with treatment failure, the risk of reinfection over the extended period of follow-up (as estimated by survival analysis techniques) did not differ significantly between the three treatment groups ($p=0.06$).

To compare the long-term effectiveness of these treatments, we measured the total number of treatments for malaria per time at risk over an extended period of follow-up. Table 4 shows the baseline characteristics and subsequent treatment data for patients with at least one study drug treatment. Children were similar with respect to age, sex, haemoglobin concentration, height, weight, and duration to first study drug treatment. Figure 4 shows the total number of malaria treatments at time of risk. Use of sulfadoxine/pyrimethamine plus amodiaquine reduced the subsequent rate of treatments by 54% (95% CI 36–66, $p<0.0001$) compared with sulfadoxine/pyrimethamine alone and by 37% (12–54, $p=0.007$) compared with sulfadoxine/pyrimethamine plus artesunate. Use of sulfadoxine/pyrimethamine plus artesunate reduced the subsequent rate of treatments by 27% (1–46, $p=0.05$) compared with sulfadoxine/pyrimethamine alone. Increases in haemoglobin concentrations from the first episode of malaria to the end of follow-up were higher in the sulfadoxine/pyrimethamine plus amodiaquine group (mean increase 17 g/L, SD 18) than in the sulfadoxine/pyrimethamine plus artesunate group or the (mean increase 14 g/L, 19) and sulfadoxine/pyrimethamine monotherapy group (10 g/L, 20), although only the comparison of sulfadoxine/pyrimethamine plus amodiaquine versus sulfadoxine/pyrimethamine alone was of borderline significance ($p=0.05$).

Discussion

Our results show that combinations of sulfadoxine/pyrimethamine plus amodiaquine and sulfadoxine/pyrimethamine plus artesunate were very effective after 14 days. By contrast, high rates of clinical (18%) and parasitological (32%) failure were recorded when sulfadoxine/pyrimethamine was used alone, consistent with results of previous studies from Kampala.^{10,17} Compared with sulfadoxine/pyrimethamine monotherapy,

the combination regimens also improved clearance of fever and parasites, and reduced incidence of gametocytaemia. These findings lend support to use of combination therapy for treatment of uncomplicated malaria. The combination of sulfadoxine/pyrimethamine and artesunate was the most potent treatment, with no parasites detected in any patients 3 days after the start of treatment, and only a 1% incidence of gametocytaemia within 14 days of treatment. All three regimens were tolerated well, with no severe adverse events reported, and an equal number of minor side-effects, all of which were difficult to distinguish from the symptoms of uncomplicated malaria. When follow-up was extended beyond 14 days, the combination of sulfadoxine/pyrimethamine plus amodiaquine significantly reduced the risk of recrudescence compared with sulfadoxine/pyrimethamine plus artesunate. Over the entire at-risk observational period, use of sulfadoxine/pyrimethamine plus amodiaquine reduced the subsequent rate of treatments by more than a half compared with sulfadoxine/pyrimethamine alone and by more than a third compared with sulfadoxine/pyrimethamine plus artesunate.

Drug-resistant parasites can be selected both when infecting parasites survive beyond the initial treatment period and when new populations of parasites are exposed to suboptimum drug concentrations.⁸ Combining short-acting artemisinin compounds with long-acting agents could provide the most useful combination regimens.⁸ Use of artesunate combined with mefloquine in southeast Asia has been highly effective, and this combination could reduce transmission and delay the spread of resistance.^{23,24} However, care must be taken in extrapolation of these results to Africa, where transmission, acquired immunity, risk of infection leading to clinical disease, and treatment practices are generally quite different.²⁵

The ability of antimalarials to clear parasites could be affected by the levels of parasitaemia at presentation.⁸ Young patients and those from regions with high transmission rates could have higher levels of parasitaemia on presentation than older patients and those living in regions with low rates of transmission.¹² Thus, parasites in African children, who frequently present with high densities of parasites, are more likely to survive initial exposure to a short-acting drug than would those in children from other countries, subsequently increasing the chance of

Variable	Sulfadoxine/pyrimethamine plus placebo (n=61)	Sulfadoxine/pyrimethamine plus amodiaquine (n=62)	Sulfadoxine/pyrimethamine plus artesunate (n=60)
Age at enrolment (mean [SD], months)	32.6 (16.1)	30.7 (15.6)	32.1 (16.7)
Female sex	32 (52%)	35 (56%)	32 (53%)
Weight at enrolment (mean [SD], kg)	11.8 (3.0)	12.0 (2.6)	12.2 (3.5)
Height at enrolment (mean [SD], cm)	96.0 (12.9)	96.0 (11.8)	89.0 (13.7)
Haemoglobin at time of first treatment with study drug (mean [SD], g/L)	104 (19)	100 (21)	104 (18)
Time from enrolment to first treatment with a study drug (mean [SD], days)	129 (102)	108 (101)	130 (101)
Number of malaria treatments excluding first episodes	202	110	154
Total time at risk after first episodes (days)	12 797	14 653	13 065

Table 4: Baseline characteristics for participants with at least one treatment with study drugs and subsequent numbers of treatments and time at risk

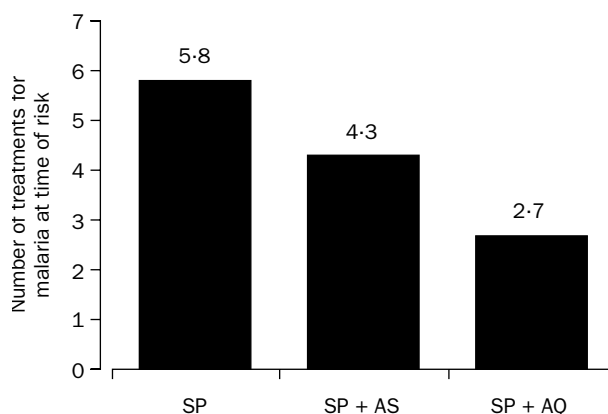


Figure 4: **Malaria treatment incidence density**

Data are the total number of first-line and second-line treatments for malaria (after the first treatment with a study drug is given) per person year of time at risk. SP=sulfadoxine/pyrimethamine. AS=artesunate. AQ=amodiaquine.

recrudescence during the elimination phase of the long-acting companion drug. In our study, children with initial parasite densities of greater than 100 000 parasites/ μ L, were 1.8 times more likely than those with parasitic densities of 100 000 parasites/ μ L to have recrudescence within 42 days, irrespective of the treatment given.

Recrudescence in the sulfadoxine/pyrimethamine plus artesunate group occurred almost exclusively more than 14 days after treatment, when concentrations of artesunate are expected to be negligible and concentrations of sulfadoxine/pyrimethamine are reduced to those that likely select for parasites resistant to this combination.²⁶ Thus, our results suggest that sulfadoxine/pyrimethamine plus artesunate is not an appropriate new regimen for treatment of uncomplicated malaria in regions with substantial resistance to sulfadoxine/pyrimethamine. As potential alternatives, combinations of artesunate and amodiaquine are very effective in regions where resistance to amodiaquine is low²⁷ and combinations with the new antifolate chlorproguanil-dapsone are promising.²⁸ Alternatively, combining two long-acting drugs, amodiaquine and sulfadoxine/pyrimethamine, could be acceptable, even in regions with moderate resistance to either drug.¹⁰ In our study, this combination was highly effective, with a low risk of late recrudescence.

The Ugandan Ministry of Health recently replaced chloroquine with a combination of chloroquine and sulfadoxine/pyrimethamine as the recommended first-line agent for uncomplicated malaria.²⁹ In Kampala, where resistance to chloroquine is very high,^{16,17} combining chloroquine with sulfadoxine/pyrimethamine would be expected to offer little benefit over sulfadoxine/pyrimethamine monotherapy. Our results indicate that use of sulfadoxine/pyrimethamine is associated with frequent treatment failures after 14 days, and that the true risk of failure of this regimen is very high (approaching 40% in our study population). This finding is especially concerning since resistance to sulfadoxine/pyrimethamine has developed without widespread use of this drug in Uganda, and because there are few alternatives to chloroquine that are affordable for many countries. However, combining sulfadoxine/pyrimethamine with drugs with different mechanisms of action offered short-term and long-term benefits for treatment of uncomplicated malaria. Most notably, the inexpensive regimen of sulfadoxine/pyrimethamine and amodiaquine was most effective at reducing subsequent malarial illnesses, and could be the ideal immediate solution for regions of Africa where

chloroquine is no longer effective and resistance to sulfadoxine/pyrimethamine is emerging.

Our results suggest that 14 days of follow-up is inadequate for assessment of the effectiveness of antimalarial drugs. We suggest that standard practice should be changed so that clinical treatment outcomes are followed up for at least 4 weeks after treatment. This practice should also be linked to simple genotyping methods to distinguish between recrudescence and reinfection, and thereby estimate the true risk of resistance more accurately.

Contributors

G Dorsey, M Kanya, and P Rosenthal designed and coordinated the study. G Dorsey, D Njama, A Gasasira, S Staedke, and M Kanya supervised enrolment and follow-up of patients. A Cattamanchi and D Kyabayinze completed all of the genotyping studies. G Dorsey analysed and interpreted the data, with assistance from P Rosenthal, M Kanya, and S Staedke. All investigators contributed to the preparation of the report.

Conflict of interest statement

None declared.

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References

- Breman JG. The ears of the hippopotamus: manifestations, determinants, and estimates of the malaria burden. *Am J Trop Med Hyg* 2001; **64** (suppl): 1–11.
- Remme JHF, Binka F, Nabarro D. Toward a framework and indicators for monitoring roll back malaria. *Am J Trop Med Hyg* 2001; **64** (suppl): 76–84.
- Trape JF. The public health impact of chloroquine resistance in Africa. *Am J Trop Med Hyg* 2001; **64** (suppl): 12–17.
- White NJ, Nosten F, Looareesuwan S, et al. Averting a malaria disaster. *Lancet* 1999; **353**: 1965–67.
- Mberu EK, Mosobo MK, Nzila AM, Kokwaro GO, Sibley CH, Watkins WM. The changing in vitro susceptibility pattern to pyrimethamine/sulfadoxine in *Plasmodium falciparum* field isolates from Kilifi, Kenya. *Am J Trop Med Hyg* 2000; **62**: 396–401.
- Olliaro P, Nevill C, LeBras J, et al. Systematic review of amodiaquine treatment in uncomplicated malaria. *Lancet* 1996; **348**: 1196–201.
- White NJ. Can amodiaquine be resurrected? *Lancet* 1996; **348**: 1184–85.
- White N. Antimalarial drug resistance and combination chemotherapy. *Philos Trans R Soc Lond B Biol Sci* 1999; **354**: 739–49.
- de Vries PJ, Dien TK. Clinical pharmacology and therapeutic potential of artemisinin and its derivatives in the treatment of malaria. *Drugs* 1996; **52**: 818–36.
- Staedke SG, Kanya MR, Dorsey G, et al. Amodiaquine, sulfadoxine/pyrimethamine, and combination therapy for treatment of uncomplicated falciparum malaria in Kampala, Uganda: a randomised trial. *Lancet* 2001; **358**: 368–74.
- von Seidlein L, Milligan P, Pinder M, et al. Efficacy of artesunate plus pyrimethamine-sulphadoxine for uncomplicated malaria in Gambian children: a double-blind, randomised, controlled trial. *Lancet* 2000; **355**: 352–57.
- Rogier C, Tall A, Diagne N, Fontenille D, Spiegel A, Trape JF. *Plasmodium falciparum* clinical malaria: lessons from longitudinal studies in Senegal. *Parasitologia* 1999; **41**: 255–59.
- Marsh K, Snow RW. Malaria transmission and morbidity. *Parasitologia* 1999; **41**: 241–46.
- Basco LK, Ringwald P. Molecular epidemiology of malaria in Yaounde, Cameroon. VII. Analysis of recrudescence and reinfection in patients with uncomplicated falciparum malaria. *Am J Trop Med Hyg* 2000; **63**: 215–21.
- WHO. Assessment of therapeutic efficacy of antimalarial drugs for uncomplicated falciparum malaria in areas with intense transmission. Geneva: World Health Organization, 1996.

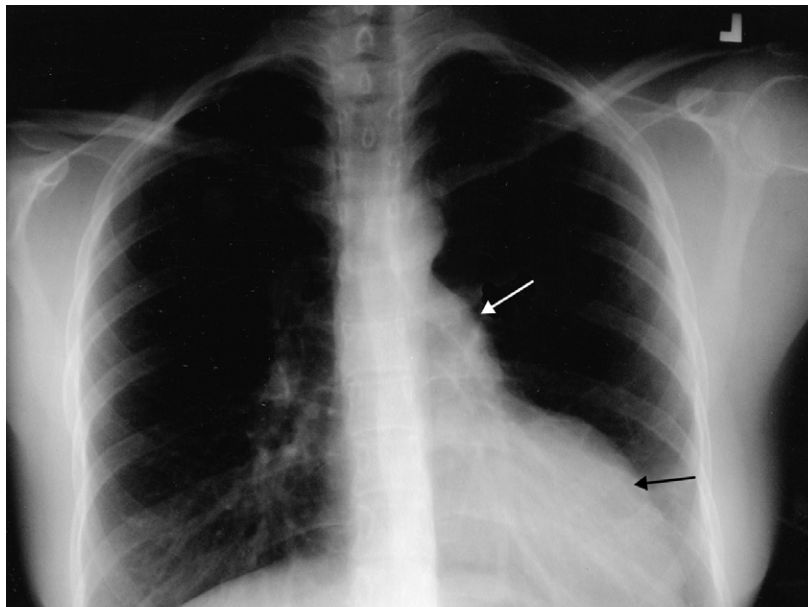
- 16 Dorsey G, Kamya MR, Ndeezi G, et al. Predictors of chloroquine treatment failure in children and adults with falciparum malaria in Kampala, Uganda. *Am J Trop Med Hyg* 2000; **62**: 686–92.
- 17 Kamya MR, Dorsey G, Gasasira A, et al. The comparative efficacy of chloroquine and sulfadoxine-pyrimethamine for the treatment of uncomplicated falciparum malaria in Kampala, Uganda. *Trans R Soc Trop Med Hyg* 2001; **95**: 50–55.
- 18 Warrell DA, Molyneux ME, Beales PF. Severe and complicated malaria. *Trans R Soc Trop Med Hyg* 1990; **84** (suppl): 1–65.
- 19 WHO. Antimalarial drug policies: data requirements, treatment of uncomplicated malaria and management of malaria in pregnancy. Geneva: World Health Organization, 1994.
- 20 Zwetyenga J, Rogier C, Tall A, et al. No influence of age on infection complexity and allelic distribution in *Plasmodium falciparum* infections in Ndiop, a Senegalese village with seasonal, mesoendemic malaria. *Am J Trop Med Hyg* 1998; **59**: 726–35.
- 21 Fenton B, Clark JT, Khan CM, et al. Structural and antigenic polymorphism of the 35- to 48-kilodalton merozoite surface antigen (MSA-2) of the malaria parasite *Plasmodium falciparum*. *Mol Cell Biol* 1991; **11**: 963–74.
- 22 Liang KY, Zeger SL. Longitudinal data analysis using generalized linear models. *Biometrika* 1986; **73**: 13–22.
- 23 Nosten F, Luxemburger C, ter Kuile F, et al. Treatment of multidrug-resistant *Plasmodium falciparum* malaria with 3-day artesunate-mefloquine combinations. *J Infect Dis* 1994; **170**: 971–77.
- 24 Nosten F, van Vugt M, Price R, et al. 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.
- 25 Bloland PB, Ettling M, Meek S. Combination therapy for malaria in Africa: hype or hope? *Bull World Health Organ* 2000; **78**: 1378–88.
- 26 Watkins WM, Mberu EK, Winstanley P, Plowe CV. The efficacy of antifolate antimalarial combinations in Africa: a predictive model based on pharmacodynamic and pharmacokinetic analyses. *Parasitology Today* 1997; **13**: 459–64.
- 27 Adjuik M, Agnamey P, Babiker A, et al. Amodiaquine-artesunate versus amodiaquine for uncomplicated *Plasmodium falciparum* malaria in African children: a randomised, multicentre trial. *Lancet* 2002; **359**: 1365–72.
- 28 Winstanley P. Chlorproguanil-dapsone (LAPDAP) for uncomplicated falciparum malaria. *Trop Med Int Health* 2001; **6**: 952–54.
- 29 Wendo C. African scientists discuss drug-resistant malaria. *Lancet* 2002; **359**: 770.

Clinical picture

Congenital absence of the left pericardium

Atul Aggarwal, Robert W Battle

A 35-year-old woman with diabetes had chest pain at rest, particularly when lying on her left side. A chest radiograph showed a prominent pulmonary conus (figure, white arrow), and abnormal leftward displacement of the heart (black arrow). Stress myocardial perfusion imaging did not show any abnormalities. A transthoracic echocardiogram showed herniation of the left atrial appendage and levoposition of the left ventricle due to congenital absence of the left pericardium. Computed tomography of the chest confirmed that the left ventricle was located superolaterally, without a surrounding pericardium. We did a partial pericardiectomy, found a large defect in the left pericardium, and removed the remainder of the left pericardium and that of the diaphragmatic surface to minimise any chance of entrapment of the left heart structures. The patient did well postoperatively.



Cardiology Unit, Burlington, Vermont 05401, USA (A Aggarwal MD, R W Battle MD)