

Effect of intermittent treatment with amodiaquine on anaemia and malarial fevers in infants in Tanzania: a randomised placebo-controlled trial

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Abstract

Background Malaria is a major cause of infant morbidity and mortality in sub-Saharan Africa, and is often complicated by severe anaemia. Resistance of *Plasmodium falciparum* to most affordable antimalarial drugs is an impediment to intermittent chemotherapy. We investigated the effect of presumptive intermittent treatment with amodiaquine and daily iron supplementation in infants on malarial fevers and anaemia, in a holoendemic area of Tanzania where malaria is largely resistant to chloroquine and sulfadoxine/pyrimethamine.

Methods 291 infants aged 12–16 weeks who attended three clinics were randomised to receive amodiaquine, iron supplementation, amodiaquine plus iron supplementation, or placebo. Over 6 months, we gave amodiaquine three times with intervals of 60 days; oral iron supplementation was given daily. Malarial fevers and anaemia were monitored at bimonthly treatment visits and by self-reporting to health centres.

Findings The protective efficacy of intermittent amodiaquine treatment in prevention of malarial fevers and anaemia was 64.7% (95% CI, 42.4–77.2) and 67.0% (95% CI, 34.5–83.4), respectively. Protective efficacy was similar in the group receiving amodiaquine plus iron supplementation. Infants receiving iron supplementation only were partly protected against anaemia (protective efficacy 59.8%; 95% CI, 23.4–78.9), but not against malarial fevers. 4 months' follow-up did not show rebound morbidity. We noted no haematological or clinical adverse effects.

Interpretation Presumptive intermittent treatment for malaria with amodiaquine reduced malarial fevers and anaemia in infants, in an area with high resistance to other antimalarials. Intermittent treatment strategies for malaria in highly endemic areas could be of great benefit to public health.

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Introduction

Despite control efforts over the past decades, falciparum malaria remains a major cause of childhood morbidity and mortality in sub-Saharan Africa.¹ In areas with intense transmission, the rate of clinical incidents of malaria peaks in infants,^{2–4} and severe anaemia is the most common life-threatening complication.^{5,6} In these regions, children between the fourth and seventh months of life are most affected and at highest risk during and immediately after the wet season.^{7–9} Effective interventions targeted at this period could have substantial effects in reduction of malarial morbidity, including anaemia, and might avert the increasing risk of HIV transmission through blood transfusion.

Given that chemotherapy is the centrepiece for malaria control in most endemic countries, including Tanzania, chemoprophylaxis would be an ideal strategy. Several investigators have reported that weekly chemoprophylaxis has protective effects against malarial fevers as well as anaemia.^{10,11} However, this approach could compromise acquisition of natural immunity,¹¹ a side-effect that might be avoided if interventions are designed to prevent high parasite densities without interfering with exposure to the parasite.⁸ Furthermore, frequent use of antimalarial drugs in low doses can exert drug pressure and accelerate development of drug resistance,¹² as noted in an earlier chemoprophylaxis trial in north-eastern Tanzania.¹⁰ Thus, new strategies are urgently needed.

Recently, Schellenberg and colleagues¹³ reported that intermittent treatment for malaria with three therapeutic doses of sulfadoxine/pyrimethamine (S/P) during the first year of life, given as part of the Expanded Programme on Immunisation, reduced malarial morbidity in infants. However in northern Tanzania, as in many other parts of Africa where the drug has been used extensively, resistance to S/P is frequent,^{14,15} and is expected to increase after S/P has become first line treatment. Therefore, we undertook a randomised double-blind placebo-controlled clinical trial to assess the protective efficacy of intermittent treatment with amodiaquine and continuous iron supplementation singly or together on malarial fevers and anaemia in infants. Drugs were administered through Maternal and Child Health (MCH) clinic schedules.

Materials and methods

Setting and population

The study took place at Maramba, Mjesani, and Mkuzi rural health centres from June, 1999, to May, 2000, in Muheza district, north-eastern Tanzania. The climate is tropical with two rainy seasons; long rains (March–June) and short rains (October–December). The main sources of income are subsistence farming, fishing, trading, and animal husbandry. The district has a well-established health system, served by a district hospital, three health

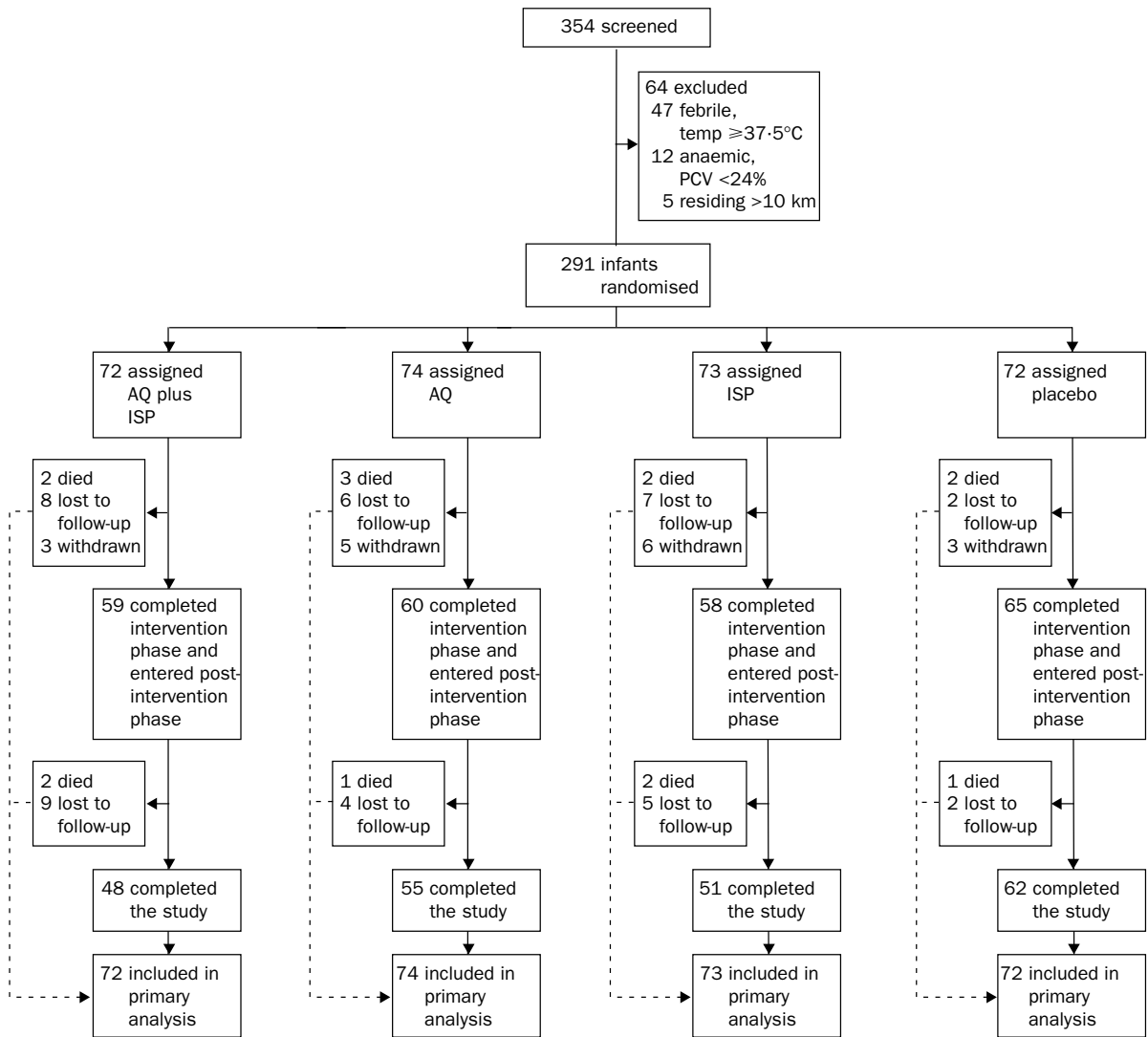


Figure 1: **Trial profile**
AQ=amodiaquine. ISP=iron supplementation.

centres, and 32 dispensaries. At each health delivery there is an effective MCH service providing immunisation, growth monitoring and antenatal services. About 93% of infants attend MCH clinics for immunisation and for growth monitoring until their fifth birthday.

Malaria is holoendemic with perennial transmission, but transmission peaks during and following the long rainy season. Annual entomological inoculation rate is 405 infective bites per person; *Plasmodium falciparum* accounts for more than 90% of all infections¹⁶ and infects about 70% of infants.¹⁷ The rate of clinical malaria episodes ranges between 3 and 3.5 per infant per year¹⁸ and prevalence of malaria-associated anaemia is about 38%.¹⁶ Infant mortality rate is estimated at 133 per 1000 livebirths.¹⁹ Chemotherapy is the main mode of malaria control in the area. Rates of resistance to chloroquine²⁰ and S/P^{14,15} are high. Amodiaquine was used in this investigation after preliminary investigations indicating that the drug was safe and efficacious (unpublished data).¹⁸

The study was a double-blinded randomised placebo-controlled clinical trial with a 2×2 factorial design.

Infants aged 12–16 weeks attending MCH clinics for growth monitoring or to receive their third diphtheria-pertussis-tetanus and oral poliovirus vaccine were recruited, if they lived within a 10 km radius of the health centre. Enrolment schedule was set to correspond with the period when children were most vulnerable to malaria. Infants with congenital malformation, severe conditions that needed treatment in hospital, a history of fever within the preceding 2 days, a packed-cell volume of less than 24, or taking chemoprophylaxis were excluded from the study. Infants were withdrawn from the study, but were monitored and treated if they reported 7 days or more after the scheduled treatment date, if they became sick from malaria or other diseases, or if they had adverse effects that were thought to be related to the test drugs.

Before and after the start of the study, we held a series of meetings with health authorities, village health committees, and villagers to explain the purpose and methods of the study. These meetings were subsequently supplemented with further sessions convened at MCH clinics. We obtained parental oral informed consent for every infant before randomisation. As part of the consent procedure, information was read aloud from a consent form to ensure consistency, and

	Placebo (n=72)	Iron supplementation (n=73)	Amodiaquine (n=74)	Amodiaquine plus iron supplementation (n=72)
Sex (female/male)	36/36	42/31	41/33	39/33
Age (weeks)*	14.4 (1.09)	14.4 (1.09)	14.5 (1.08)	14.2 (1.05)
Parasite positive	24%	34%	32%	36%
Log MPD (range)	3.2 (1.9–4.3)	3.2 (1.6–4.6)	3.5 (1.9–4.7)	3.3 (1.6–4.8)
PCV*	30.4 (4.2)	29.8 (4.7)	29.6 (4.9)	29.2 (4.9)
Temperature*	36.3 (0.4)	36.7 (0.4)	36.7 (0.4)	36.7 (0.3)
WBC $\times 10^9$ (range)	8.8 (4.6–23.2)	9.0 (5.1–15.3)	8.9 (4.8–15.3)	8.9 (5.2–14.6)
Neutrophils $\times 10^9$ (range)	2.4 (0.61–6.7)	2.6 (6.0–8.0)	2.5 (0.8–9.3)	2.6 (0.8–9.5)
Used ITN	21 (29%)	23 (32%)	24 (32%)	22 (31%)
Distance to health facility				
≤5 kms	52 (72%)	52 (71%)	44 (60%)	47 (65%)
5–10 km	20 (28%)	21 (29%)	30 (41%)	25 (35%)
Haemoglobin genotype*				
AA	67 (93%)	66 (90%)	67 (91%)	69 (96%)
AS	5 (7%)	7 (10%)	6 (8%)	3 (4%)
SS	0	0	1 (1%)	0
Height (cm)*	60.3 (3.7)	59.7 (4.9)	59.4 (3.6)	59.4 (4.0)
Weight (kg)*	5.9 (0.97)	5.9 (0.86)	5.8 (0.81)	5.9 (0.80)

*Mean (SD). Log MPD=logarithm mean parasite density, PVC=packed-cell volume. WBC=white cell blood count. ITN=insecticide treated nets.

Table 1: Demographic and clinical characteristics by treatment group at recruitment

any questions from the parent were answered. Because of the high rate of illiteracy in the study area, written consent was not obtained. Ethical approval for the study was obtained from the Medical Research Coordinating Committee of the National Institute for Medical Research, Tanzania.

After enrolment, we issued an identity card with a unique project number to every infant and obtained a clinical history. The children then underwent a thorough physical examination and blood was obtained by finger pricking. Infants were randomised to receive one of the regimens: (1) amodiaquine (Strides Pharmaceutical Ltd, Mumbai, India) and iron supplement (Amy Pharmacy, Dar es Salaam, Tanzania), (2) amodiaquine and iron supplement placebo, (3) iron supplementation and amodiaquine placebo, or (4) placebos for both amodiaquine and iron. A randomisation code list produced by a statistician not connected to the study was made in blocks of eight by means of a computer generated-permuted random numbers linked to intervention groups codes; and was concealed from the investigating team. Randomisation code lists were issued to health personnel in charge and responsible for allocating eligible infants to intervention groups. To ensure that treatment allocation was concealed from parents and the research team, and to ensure that infants received the right dose of medication, the trial drugs were coded and pre-packed. Inert and active amodiaquine were identical in shape and appearance, and the iron supplement placebo was identical in appearance and taste to active formulation. Placebos for amodiaquine and iron were specifically produced for the study by Almega, Denmark, and Amy Pharmacy, Dar es Salaam, Tanzania, respectively. The intervention drugs were all products of the same batch. All personnel involved in the study remained unaware of intervention groups until all children had completed the trial and the data had been assessed.

Procedures

Intermittent treatment with amodiaquine or placebo was given every 2 months. The drug was administered at health centres under supervision over three days in doses of 10 mg/kg, 10 mg/kg, and 5 mg/kg, respectively. Tablets were crushed, suspended in water, and given by spoon. Infants were observed for 30 min and drug administration was repeated in those who vomited.

Infants received 2.5 mL daily supplementation of iron or placebo for 6 months. Iron was given as ferric ammonium citrate mixture (3 mg elemental iron/mL). The first dose was given by the team and mothers were instructed how to administer the drug at home. A research team member made fortnightly house-to-house visits to crosscheck and encourage administration of iron supplements. Compliance with supplementation was assessed by inspection of the volume of fluid remaining in containers, when mothers brought them for replenishment. Furthermore, when asked about dose administered, all mothers indicated the correct dose. Concentration of iron in serum was not measured.

Infants were recruited between June and August, 1999, and monitored clinically, haematologically, and parasitologically for 10 months—ie, 4 months in addition to the 6 months when drugs were given. Malaria-attributable fevers and anaemia were assessed by active case detection during bimonthly treatment sessions, and between sessions by passive case detection when patients self-reported to health centres. Parents were told the importance of treatment and advised not to self-administer medication including antimalarial drugs to infants, but to return for assessment at the MCH clinic if the infant had symptoms thought to be associated with malarial infection or any other disease between treatment cycles. At MCH clinics, uncomplicated malaria cases were treated with S/P, whereas cases of severe or complicated malaria were referred to a referral hospital. Since free treatment was provided at MCH clinics, we believe that very few malarial infections were treated without our knowledge.

At every health facility attendance, mothers or guardians completed a questionnaire on clinical history including information on disease symptoms and drug use since the previous visit (chemical testing to verify use of antimalarial drugs was not attempted) after which children underwent a clinical investigation including measurement of axillary temperature and respiration rate, and inspection for signs of jaundice, pruritus, or sore throat. Blood was obtained from a finger prick; thin and thick blood films were made and stained with giemsa. Parasitaemia was microscopically assessed by counting asexual forms against 200 leukocytes. Blood films were declared negative after examination of 200 high power fields. Anaemia was assessed by

	Placebo (n=72)	Iron supplementation (n=74)	Amodiaquine (n=73)	Amodiaquine plus iron supplementation (n=72)
First or only malaria episode				
Number of malaria episodes	45	39	24	25
Infant-days at risk	7501	7954	10 940	10 601
Rate per year	2.19	1.79	0.80	0.86
PE for malaria at ≥ 40 parasite/ μ l (95% CI)	Reference group	18.3 (-25.5 to 46.8), p=0.356	63.4 (40.0 to 77.7), p<0.001	60.7 (35.9 to 75.9), p<0.001
All malaria episodes				
Number of malaria episodes	72	62	28	28
Infant-days at risk	11 421	11 299	12 584	12 294
Incidence rate per year	2.30	2.0	0.81	0.83
PE for malaria at ≥ 40 parasite/ μ l (95% CI)	Reference group	13.0 (-22.2 to 38.0), p=0.423	64.7 (42.4 to 77.2), p<0.001	63.9 (44.1 to 76.7), p<0.001
All malaria episodes for ≥ 5000 parasite/μl				
Number of malaria episodes	57	48	17	19
Infant-days at risk	11 953	11 691	12 892	12 519
Incidence rate per year	1.74	1.50	0.48	0.55
PE for malaria at ≥ 5000 parasite/ μ l (95% CI)	Reference group	13.9 (-26.4 to 41.4), p=0.445	72.4 (52.5 to 83.9), p<0.001	68.2 (46.5 to 81.1), p<0.001

PE=protective efficacy calculated on the basis of rates.

Table 2: Rate of malarial fever and protective efficacy of amodiaquine during the 6-month intervention phase by treatment group

estimation of packed-cell volume with a micro-haematocrit centrifuge machine. For practical reasons, we were unable to do liver function tests, but results from a previous hospital-based trial in adult volunteers did not suggest that concentrations of liver enzyme would be raised during treatment with amodiaquine (unpublished data). However, we did total and differential white blood cells counts with a Neubauer haematocytometer kit and by examination of thin blood films. We assessed haemoglobin genotype at recruitment by haemoglobin electrophoresis.²¹ At recruitment and 2 months later during the second administration of amodiaquine, further blood samples were taken into microtubes containing sodium ethylene-diaminetetraacetate (EDTA). Plasma was separated within 24 h by micro-centrifuge and stored at -20°C until assay.

We assessed the infants' antibody responses to vaccines given as part of the Expanded Programme on Immunisation. All infants had received BCG, and the first and second dosages of diphtheria-pertussis-tetanus and oral poliovirus vaccines before the start of the study. 174 infants received the third dose of these vaccines at enrolment, and samples were available from 140 of these infants for measurement of plasma IgG and IgM to diphtheria, tetanus toxoid, and oral poliovirus with a commercially available ELISA kit (Institut Viriorn, Serion GmbH, Germany). Of the 140 infants, 41, 36, 37, and 26 belonged to the placebo, iron supplement, amodiaquine, and amodiaquine plus iron supplement groups, respectively.

Statistical analysis

The sample size was calculated on the basis of available data on incidence of febrile malaria episodes and prevalence rate of anaemia in the trial area.^{16,18} Assuming a 20% dropout rate, the sample size of 298 infants was estimated to give 80% power to detect a 60% reduction in rate of febrile malaria at a significance level of 0.05 by the end of extended follow-up, 300 days after enrolment. Data forms were manually checked by the supervisor (JJM) for completeness before computer entry. All data were double entered and validated with Epi Info, version 6.04b. Any differences were resolved by checking against the original case record form.

The main outcome measures were cumulative rates of febrile malarial episodes and of anaemia. We did an intention-to-treat analysis, in which all infants randomised were included in the primary analysis according to the randomly assigned treatment group until completion or exit from the study due to death, loss to follow-up, or withdrawal. A febrile malarial episode was diagnosed in infants with a reported history of fever within the last 24–72 h or a measured temperature of 37.5°C or greater (or both), who had a positive blood slide with asexual forms of *P. falciparum* at any level of parasite density at time of contact with MCH clinic. An additional case definition requiring a parasite density of 5000/ μ l or greater was also used in assessment of the protective efficacy of the intervention. Anaemia was defined as packed-cell volume of less than 24%, and once an infant developed anaemia they were deemed not at risk (because anaemia is a chronic condition).

	Placebo (n=72)	Iron supplementation (n=74)	Amodiaquine (n=73)	Amodiaquine plus Iron supplementation (n=72)
Number of anaemic cases	26	12	9	8
Infant-days at risk	10 163	11 436	12 208	12 211
Incidence rate per year	0.93	0.38	0.27	0.24
PE for anaemia <24% (95% CI)	Reference group	59.0 (18.7 to 79.3), p=0.011	71.2 (38.5 to 87.0), p=0.001	74.4 (43.4 to 88.4), p=0.001
Number of outpatient attendances	115	110	81	79
Infant-days at risk	13 437	13 035	13 368	13 078
Incidence rate per year	3.12	3.08	2.21	2.20
PE for outpatient attendances	Reference group	1.4 (-28.1 to 24.1), p=0.916	29.2 (5.9 to 46.7), p=0.017	29.4 (6.0 to 47.0), p=0.017
Number of hospital admissions	30	21	13	15
Infant-days at risk	11 296	11 579	12 188	11 985
Incidence rate per year	0.97	0.66	0.39	0.46
PE for hospital admission	Reference group	31.7 (-19.2 to 60.9), p=0.180	59.9 (23.0 to 79.1), p=0.006	52.9 (12.4 to 74.7), p=0.017

PE=protective efficacy calculated on the basis of rates.

Table 3: Rates of anaemia, protective efficacy of amodiaquine, outpatient attendances, and hospital admissions during the 6-month intervention phase by treatment group

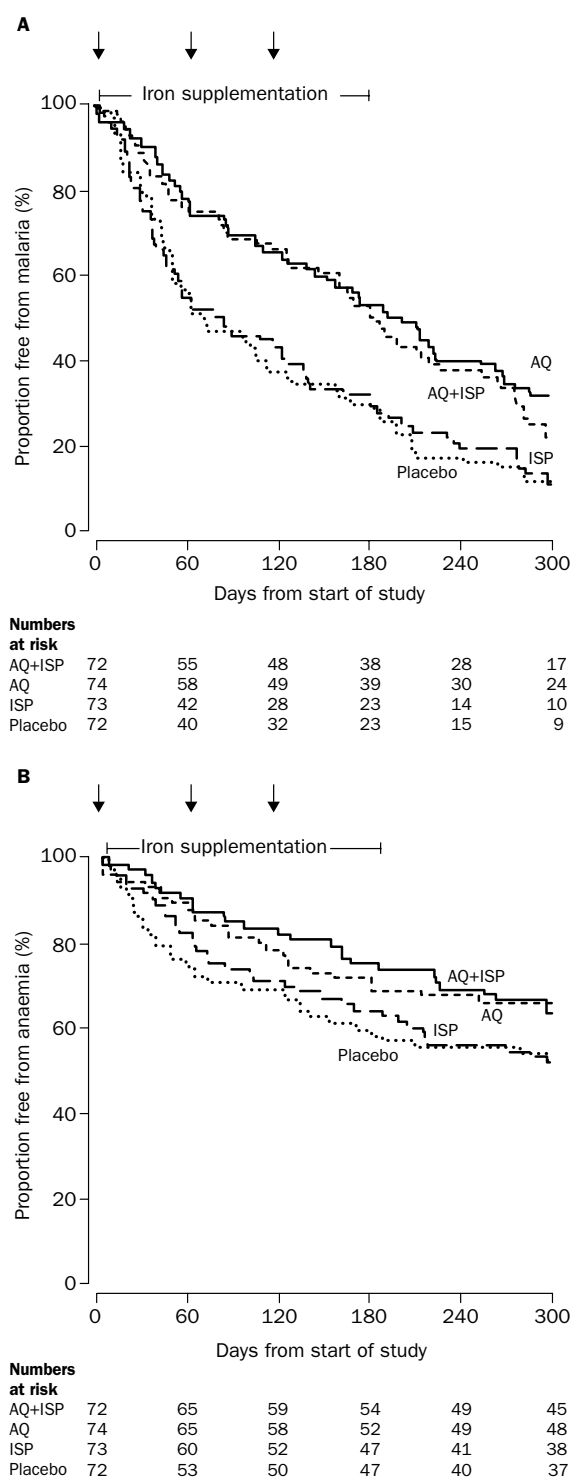


Figure 2: Proportions of infants without febrile malaria (A) and without anaemia (B) by treatment group

AQ=intermittent treatment with amodiaquine. ISP=iron supplementation. Arrows show times when drugs were given. Line shows period during which iron supplementation was given. (A) Log rank testing showed that the curves for infants in groups AQ ($p=0.0005$) and AQ+ISP ($p=0.00073$) were significantly different from the curves for placebo, whereas the ISP group curve was not ($p=0.56$). (B) Log rank testing showed that the curves for infants in groups AQ ($p=0.0011$) and AQ+ISP ($p=0.0018$) were significantly different from the curves for placebo, whereas the ISP group curve was not ($p=0.09$).

Relative risks (RR), 95% CIs, and p values were estimated and compared by means of Poisson regression. Protective efficacy was calculated on the basis of the infant's first or only malarial episode or cumulative rates of all malarial fevers. For the latter calculation, the infant was considered not at risk for 28 days after a malarial episode. Protective efficacy was calculated as $(1-RR) \times 100$. Time-to-event analyses for the first or only malarial fever or anaemia episode were done by use of Kaplan-Meier and compared by log-rank test. Secondary data analysis of adverse effects and effect of amodiaquine to diphtheria-pertussis and oral poliovirus vaccine responses were also undertaken. The effect of treatment with amodiaquine on antibody responses to diphtheria-pertussis and oral poliovirus vaccines were assessed by grouping infants receiving amodiaquine alone or amodiaquine plus iron, and comparing them with infants who had received placebo or iron alone. Plasma antibody levels (IU/mL) were compared with the Mann-Whitney rank sum test. Stata version 7 and SPSS version 10.0 were used for data analysis.

Role of the funding source

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

Results

Figure 1 shows that 242 (83%) of the 291 recruited infants completed the intervention phase and received intermittent treatment with amodiaquine or placebo on three occasions. Of these, 216 (74.2%) also completed follow-up during the 4-month period (figure 1). Those lost to follow-up included 13 infants who died, and 36 infants who had either migrated or were withdrawn because they violated the study protocol by not reporting on time to the health centres for medication and clinical surveillance. Infant mortality was equally distributed between the groups and was lower than the recorded district infant mortality. In no patient could death or withdrawal be associated with severe adverse effects previously linked to use of amodiaquine (eg, agranulocytosis, liver failure). The mean follow-up of those who did not complete the study was 155 (SD 48) days, and did not differ between the intervention groups (data not shown). Baseline demographic and clinical characteristics were similar between the four treatment groups, except for an unbalanced rate of haemoglobin genotypes (table 1).

The effects of intermittent amodiaquine treatment and iron supplementation on malarial fevers are shown in table 2. The rates of malarial fevers during the 6-month intervention phase ranged from 0.80 episodes per infant per year in the amodiaquine group to 2.19 in the placebo group. Data for the first or only episode of malaria showed that intermittent amodiaquine treatment reduced the number of malarial fevers by 63.4% (95% CI 40.0–77.7; $p<0.001$) compared with the controls; when all malaria episodes were taken into account the protective efficacy was 64.7% (95% CI, 42.4–77.2; $p<0.001$). When malarial fever was defined by a parasite density of $5000/\mu\text{L}$ or greater, the calculated protective efficacy was slightly increased (table 2). The infants who received amodiaquine plus iron also had a lower incidence of malarial fever than the controls, but there was no additional effect of iron supplementation compared with infants who received amodiaquine alone, and infants who only received iron did not have a significantly reduced rate of malarial fevers compared with controls (table 2).

	Placebo (n=72)	Iron supplementation (n=73)	Amodiaquine (n=74)	Amodiaquine plus iron supplementation (n=72)
Days 0–60 (high transmission season)				
PE* for malaria at ≥ 40 parasite/ μ l (95% CI)	Reference group	13.6 (–41.2 to 47.1), p=0.560	63.0 (32.0 to 79.8), p=0.001	61.5 (29.4 to 79.1), p=0.002
PE for anaemia <24%	Reference group	60.6 (9.9 to 82.7), p=0.027	62.2 (13.7 to 83.5), p=0.021	76.0 (35.7 to 91.0), p=0.005
Days 61–180 (low transmission season)				
PE for malaria at ≥ 40 parasite/ μ l (95% CI)	Reference group	13.9 (–37.8 to 46.2), p=0.533	68.4 (40.7 to 83.2), p<0.001	66.3 (34.2 to 82.8), p=0.001
PE for anaemia	Reference group	61.0 (–9.4 to 86.1), p=0.073	77.5 (20.9 to 93.57), p=0.020	77.1 (19.5 to 93.5), p=0.022

PE=protective efficacy calculated on the basis of incidence rates. Days 0–60 coincided with the period of highest malaria transmission; during days 61–180 malaria transmission was lower. p values refer to comparison between intervention group and placebo.

Table 4: **Protective effect of amodiaquine on malaria and anaemia episodes during the first and last parts of the intervention**

The effect of the intervention on anaemia is summarised in table 3. Compared with controls, intermittent amodiaquine treatment reduced the prevalence of anaemia by 71.2% (95% CI 38.5–87.0; p=0.001). Protective efficacy was slightly but not significantly increased in the group who received amodiaquine plus iron (table 3). Compared with controls, iron supplementation alone reduced the number of cases of anaemia by 59.0% (95% CI, 23.4–78.9; p=0.006). The protective efficacy for prevention of malarial fevers and anaemia were similar during the first 60 days (including peak transmission) and last 120 days (low transmission period) of the intervention (table 4). There was no indication that efficacy dropped as a result of drug resistance. Additionally, individuals in the amodiaquine and amodiaquine plus iron groups had a significantly lower rate of outpatient attendance and hospital admission than controls (table 3).

Figure 2 shows the effects of the treatments on first or only malaria fever (A) or anaemia (B) from recruitment (day 0) until the end of follow-up (day 300). The plots confirm the previously described effects of the treatments during the intervention period (day 0–180), and show that there was no rebound effect after the intervention in the groups who received the active compounds. At day 300—ie, 177 days after the last dose of amodiaquine—the proportion of infants who had not had malarial fever or developed anaemia was substantially greater in the groups who had received intermittent treatment with amodiaquine than in controls. Iron supplementation did not significantly affect rates of fever (rate difference 6.27×10^{-4} [95% CI -2.96×10^{-4} to 1.7×10^{-3}] and 5.44×10^{-4} [-8.2×10^{-4} to 1.9×10^{-3}] for comparisons between iron and placebo

groups, and amodiaquine and amodiaquine plus iron groups, respectively). Survival analysis calculating the proportion free of malarial fevers showed that 62% (95% CI 29–45) and 35% (27–43) of infants who had received amodiaquine had remained free of malaria on days 180 and 300, respectively. The corresponding values for infants who did not receive amodiaquine were 37% (29–45) in the placebo group, and 15% (9–22) in the iron supplementation group.

Haematological and clinical safety data were available in 242 infants (65, 58, 60, and 59 in placebo, iron, amodiaquine, and amodiaquine plus iron treatment groups, respectively). No clinical adverse effects such as sore throat or agranulocytosis were reported or observed during the study. One infant in the amodiaquine plus iron group had a neutrophil count of 1.07×10^9 , which was below the lower limit (1.5×10^9) of the normal range. This child was clinically healthy and attended the health facility to receive the second course of intermittent treatment. Because of the low neutrophil count, we postponed drug administration until 2 weeks later when the count was within the normal range. None of the other infants had low leucocyte counts at any time during the study, and we noted no significant difference in mean leucocyte counts between the groups (data not shown).

Blood samples for analysis of antibody responses to vaccines were available from 140 infants who received their third round of immunisations simultaneously with the first round of intermittent treatment. After 60 days of iron supplementation, concentrations in plasma of IgG for diphtheria, tetanus toxoid, and polio did not differ between infants who had received placebo and those who received only iron (p=0.64, p=0.96, and p=0.50 for diphtheria, tetanus toxoid, and polio, respectively). Thus, to test the effect of amodiaquine treatment on responses to vaccine, infants in the amodiaquine and amodiaquine plus iron groups (intervention group) were judged together and compared with children in the placebo and iron supplementation groups (control group). The IgG and IgM concentrations for tetanus toxoid, oral poliovirus, and diphtheria vaccines at recruitment and 60 days after the third round of vaccination and first dose of amodiaquine are shown in table 5. All children had satisfactory IgG responses to vaccination and there were no significant differences in antibody concentrations between the groups. No clinical cases of poliomyelitis, diphtheria, whooping cough, or tetanus were noted during the study.

Discussion

The use of presumptive intermittent treatment for control of malaria in infants was suggested by WHO in 1998.²² This strategy, alone or in combination with iron supplementation, was expected to decrease malarial morbidity. We have shown that malarial morbidity is

	Placebo (n=77)	Amodiaquine (n=63)	p (amodiaquine vs placebo)*
At recruitment			
Tetanus toxoid			
IgG	0.7 (0.3–1.1)	1.6 (0.8–2.1)	0.06
IgM	0.02 (0.02–0.03)	0.03 (0.02–0.04)	0.16
Poliovirus			
IgG	278 (156–489)	397 (196–598)	0.22
IgM	262 (158–432)	364 (244–629)	0.15
Diphtheria			
IgG	0.2 (0.1–0.3)	0.2 (0.1–0.5)	0.66
After 60 days			
Tetanus toxoid			
IgG	10.4 (6.1–13.7)	12.7 (7.6–18.7)	0.28
IgM	0.02 (0.01–0.03)	0.02 (0.02–0.04)	0.45
Poliovirus			
IgG	717 (599–877)	652 (511–848)	0.69
IgM	147 (99–229)	201 (127–295)	0.31
Diphtheria			
IgG	1.2 (0.9–1.5)	1.3 (1.0–1.5)	0.91

Values are median (95% CI) in IU/mL except *Mann-Whitney rank sum test.

Table 5: **Concentration of antibodies to vaccines**

substantially reduced by intermittent treatment with amodiaquine given to infants at 3, 5, and 7 months of age. During the 6-month intervention period, in which children were aged between 3 and 9 months and at the highest risk of malaria, the incidence of malarial fevers was greatly reduced. Our findings and the levels of protection are in accord with a study in which infants received intermittent treatment with S/P.¹³ Our study was undertaken in an area where resistance to S/P is high,¹⁴ and the results suggest that amodiaquine could be considered for intermittent treatment in infants either as the first-line or second-line drug in areas where such resistance is a difficulty. In accord with the previous study,¹³ we noted a significant reduction of admissions to hospital in children receiving intermittent treatment. Furthermore, in our study such treatment greatly reduced number of outpatient attendances.

We gave amodiaquine treatment during routine visits to MCH clinics, to which more than 90% of the children in the area are brought for growth monitoring and immunisation. In the study¹³ with intermittent S/P treatment, the drugs were given in connection with the Expanded Programme on Immunisation vaccinations, when the children were 2, 3, and 9 months old. The protective efficacies in the two studies were roughly similar, suggesting that the intervention is robust with regard to the exact timing schedule of the intermittent treatments. However, malarial morbidity was generally higher during our study, and the Kaplan-Meier plots show that a fairly high proportion of malarial morbidity in the groups who received amodiaquine fell in a period of 30 days before the second course of the drug (figure 2); a shorter spacing or addition of a fourth treatment cycle of amodiaquine would possibly have resulted in an even higher protective efficacy.

The efficacy of the intervention did not drop during the study; in fact it was slightly higher during the last 120 days compared with the first 60. The difference was not significant, but if it represents a real effect, it could be explained by the fact that transmission of malaria was highest during the first part of the investigation. More importantly, we could not detect any rebound effect in the 4 months after the intervention. Although we did not measure concentrations of antimalarial antibodies in the infants, findings of follow-up did not suggest that intermittent treatment impaired acquisition of clinical immunity.

Although resistance to chloroquine and S/P was high in the study area, amodiaquine was 97% efficacious before the onset of our investigation (unpublished data). Tanzanian drug policy has recently changed; S/P is now the first-line treatment for malaria in this country and amodiaquine is the second. This change will increase the use of both drugs and put additional drug pressure on the parasites. On the basis of findings from Malawi,²³ it seems probable that resistance to the drugs will increase in Tanzania. The consumption of antimalarials in Tanzania is high, and compliance with treatment is low.²⁴ Furthermore, self-medication with these drugs is common, and accelerates the spread of drug resistance. In such settings, the controlled use of an effective drug in a restricted age-group through the established health system might not, in itself, reduce the effectiveness of the drug. Thus, the use of amodiaquine for intermittent treatment of infants might not greatly accelerate development of resistance. Additionally, compared with other antimalarial drugs, amodiaquine has a short terminal elimination half-life of 7–21 days;²⁵ therefore, intermittent use might not select for *Plasmodia*

resistance, which can arise when weekly prophylaxis is used.¹² Combination treatment strategies can help to delay development and spread of resistance to the constituent drugs,²⁶ and the possibility of combining another effective antimalarial drug with amodiaquine in an intermittent treatment programme in infants should be investigated.

The use of iron supplementation was associated with protective efficacy against anaemia without increasing susceptibility to malarial infections. This finding is in agreement with results from previous studies.^{11,13} We did not find a significant added effect of iron supplementation, when amodiaquine plus iron was compared with amodiaquine alone. A minor imbalance in the percentage of sickle cell carriers between amodiaquine and amodiaquine plus iron groups could have slightly favoured the amodiaquine group in this comparison, but the results suggest that the public health benefit from daily administration of iron supplementation in infants might be limited in areas where effective intermittent antimalarial treatment is administered.

Our results show that the three intermittent courses of amodiaquine treatment were not associated with serious clinical or haematological adverse effects, and amodiaquine might prove to be safe when used in the broader population of infants. These findings confirm the safety of amodiaquine noted in previous chemotherapeutic studies.²⁷ However, although agranulocytosis or hepatic dysfunction have not been reported in malaria patients treated with amodiaquine,²⁸ careful attention should be paid to long-term safety, because prophylactic use of amodiaquine has previously been linked with haematological and hepatic toxicity in patients with low immunity,²⁹ and in a health volunteer treated in combination with artesunate.³⁰ The possibility that intermittent treatment might interfere with antibody response to some vaccines is a matter of concern. However, recent findings with intermittent S/P treatment in infants,¹³ and antibody measurements in a subgroup of individuals in our study, do not suggest that intermittent treatment impairs responses to vaccination.

We have shown that, in an area where resistance of malaria to chloroquine and S/P is common, presumptive intermittent treatment with amodiaquine protected infants against malarial fevers and anaemia. Regular general use of amodiaquine might eventually reduce the effectiveness of the intervention, as parents might fail to administer the full dose to asymptomatic infants. However, continued health education and promotion of the importance of the intervention are likely to increase compliance. Our findings, and results from a previous trial of intermittent treatment with S/P, show that the public-health benefits of intermittent treatment strategies for malaria in highly endemic areas could be great.

Contributors

J J Massaga was the principal investigator, who designed and undertook the study, analysed and interpreted data, and drafted and revised the manuscript. A Y Kitua helped with the design of the study and supervision of clinical data collection. M M Lemnge was responsible for administrative and technical logistics and overall supervision of data collection. J A Akida and L N Malle obtained and assembled data. A M Ronn helped with study design and supervised collection of clinical data. T G Theander took part in the study design, supervision of data analysis, and revision of the article. I C Bygbjerg took part in administrative and technical logistics, obtained funds, supplied the trial drugs, and supervised analysis of data. All authors planned the statistical assessment, revised the manuscript, and approved the final version.

Conflict of interest statement

None declared.

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