Am. J. Trop. Med. Hyg., 73(1), 2005, pp. 166–170

REDUCTION OF THE EFFICACY OF ANTIFOLATE ANTIMALARIAL THERAPY BY FOLIC ACID SUPPLEMENTATION

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Abstract. Malaria and anemia are common conditions in patients presenting to outpatient clinics in Kenya. Anemia is usually due to malaria infection with underlying micronutrient deficiency. Iron therapy has been shown to enhance recovery from anemia in children with malaria, without affecting malaria treatment. Iron and folic acid are often prescribed together for anemic individuals. Until recently in Kenya, the drug of first choice for non-severe malaria was sulfadoxine-pyrimethamine (SP), an antifolate antimalarial drug. In this study, 303 patients of all ages with anemia and uncomplicated *Plasmodium falciparum* malaria attending an outpatient clinic in an area of seasonal malaria were treated with SP and iron, and were randomized to receive folic acid. Parasite clearance rates were measured using a survival analysis plot for both parasitologic and clinical failure. There was a significant reduction in the efficacy of SP in patients taking standard therapeutic doses of folic acid using the survival curve for parasitologic failure ($P < 0.0001$), but no difference for clinical failure ($P = 0.7008$). Folic acid supplementation did not enhance recovery from anemia.

INTRODUCTION

Anemia is a common cause of morbidity in eastern Africa, affecting mainly children less than five years old and women of child-bearing age. In a population-based survey carried out in coastal Kenya in 1986, the mean hemoglobin for the whole population was 10.8 g/L (Oppenheimer SJ, unpublished data). The main determinants of anemia were hookworm infestation (in those ≥ 5 years old) and malaria (in those < 5 years old). Five percent of the population had red blood cell folic acid levels below normal. In another study carried out in Kenya, the overall prevalence of anemia in primary school children in coastal Kenya was 75.6%, using a hemoglobin cutoff of 12 g/dL.[1] In a facility-based survey carried out in the southern Rift Valley of Kenya during the low malaria season, more than half the patients attending the outpatient clinic had hemoglobin levels below the lower reference limits, suggesting underlying nutritional deficiency contributing to anemia.[2] A study carried out in western Kenya suggested that hookworm infection may contribute to anemia through deficiencies of other nutritional factors, in addition to iron.[3] Since both iron and folic acid are provided in the Kenya national drug supply system, they are often prescribed together for anemic individuals.

Plasmodium falciparum malaria is a major cause of anemia of infectious origin. The etiology of anemia associated with malaria includes shortened red blood cell survival (hemolysis) of both parasitized and non-parasitized red blood cells, and reduced bone marrow production.[4,5] While some studies have suggested that iron supplementation may stimulate malarial parasitemia,[6–9] more recent studies have shown an improved hematologic response with no increase in malarial parasitemia if iron is administered together with an antimalarial drug.[1,10–12]

Antifolate antimalarial drugs, which act by inhibition of the parasite enzyme dihydrofolate reductase,[13] have been until recently the recommended first-line drugs for treatment of uncomplicated malaria in Kenya. Antifolate drugs may cause hematologic effects if administered for a long enough period[14] or in the face of existing folic acid deficiency.[15] Malaria hemolysis increases folic acid requirements and may lead to folic acid deficiency and megaloblastic anemia in the face of inadequate dietary folic acid.[16,17] In a study carried out in the Gambia, supplementation of folic acid did not improve the hematologic response but increased the failure rate of treating malaria using sulfadoxine-pyrimethamine (SP).[18]

We report the results of a prospective, randomized study of anemic patients with uncomplicated *P. falciparum* malaria treated with SP and oral iron, with an evaluation of the effect of daily folic acid supplementation on antimalarial drug efficacy and recovery from anemia.

MATERIALS AND METHODS

Study area and population. The study was conducted at the Entasopia Health Center in the Magadi Division of the Kajiado District in the southern Rift Valley of Kenya. The Entasopia Health Center is located approximately 2,500 feet above sea level and is in a low-to-moderate malarial transmission area. The study was conducted on patients of all ages attending the health facility who met the following entry criteria: 1) hemoglobin level < 11 g/dL and ≥ 5 g/dL; 2) pure *P. falciparum* malaria with a parasite concentration > 20 parasites/200 white blood cells and < 10,000 parasites/200 white blood cells on thick blood film examination; 3) no criteria of severe and complicated malaria; 4) no use of sulfa drugs in the previous month; 5) no previous reaction to a sulfa drug; and 6) informed consent obtained from the patient, parent, or guardian. Pregnant patients were excluded from the study because folic acid supplementation was withheld from half of the study patients.

Study design. The study was reviewed and approved by the Standards and Ethical Committees of the Kenya Medical Research Institute. Patients were seen during routine daily clinics and recruited following initial screening in the health center laboratory by a finger prick sample (examination of a thick blood film stained with Field's stain and hemoglobin estimation using the hemiglobincyanide method in a colorimeter (Jenway Ltd., Dunmow, United Kingdom). Informed consent was obtained from all human adult participants and

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from parents or legal guardians of minors. A clinical history and physical examination were performed by one of two study clinicians. Patients returned to the laboratory for venipuncture for baseline full blood counts and red blood cell indices (model CBC 5 analyzer; Coulter, Hialeah, FL) and for thick and thin blood films (Field's stain). These values were taken as the initial baseline study data. Patients received SP as a single dose by body weight according to a scale calculated at 10-kg intervals as follows: those 5–9.9 kg received 0.5 tablets; those 10–19.9 kg received 1 tablet; those 20–29.9 kg received 1.5 tablets; those 30–39.9 kg received 2 tablets; those 40–49.9 kg received 2.5 tablets; those 50–59.9 kg received 3 tablets; those 60–69.9 kg received 3.5 tablets; and those ≥ 70 kg received 4 tablets. All patients also received ferrous sulfate, 3–6 mg of elemental iron/kg/day, in divided doses for 30 days according to the following scale: those 5–9.9 kg received 0.5 tablets a day; those 10–19.9 kg received 0.5 tablets twice a day; those 20–39.9 kg received 1 tablet twice a day; those ≥ 40 kg received 1 tablet 3 times a day. Patients were randomly allocated to receive 2.5 mg of folic acid a day if < 2 years of age and 5 mg a day if ≥ 2 years of age (Kenya Ministry of Health recommendation) for 30 days. The brand of SP used was Falcidin® (Cosmos Ltd., Nairobi, Kenya) containing 25 mg of pyrimethamine and 500 mg of sulfadoxine; ferrous sulfate (200 mg tablets containing 60 mg of elemental iron) and folic acid (5-mg tablets) were obtained from Elys Chemical Industries (Nairobi, Kenya). Patients were given a 14-day supply of ferrous sulfate and folic acid in case they were unable to attend for follow-up. Patients also received paracetamol (10 mg/kg every six hours) for the duration of fever. Tablets were crushed and given with water to infants and small children. The initial doses of all drugs were given under supervision and patients were observed for one hour after administration; doses were repeated if vomiting occurred within one hour. Doses of half tablets were pre-cut before they were dispensed, and mothers were shown how to crush tablets and administer medications to small children.

Patients were reviewed on the third day and at weekly intervals for 28 days. On the third day, a clinical assessment was performed and a repeat hemoglobin examination and thick blood film examination were performed. On days 7, 14, 21, and 28, a clinical assessment including temperature measurement, full blood count, and thick and thin blood films was performed. Patients were advised to return at any time if they did not feel well. At each visit, patients visited the drug dispenser to review their hematinic consumption and to receive an additional seven-day supply. A medical officer and laboratory technologist were assigned to the study and performed clinical assessments and laboratory investigations without knowledge of the treatment group.

Patients with parasitologic failure up to day 14 were treated with halofantrine tablets or syrup (8 mg/kg every six hours in three doses). Parasitologic failure after day 14 was treated with a repeat single dose of SP; subsequent parasitologic failure was treated with halofantrine. If the hemoglobin level decreased below 5 g/dL during the study and patients remained stable, the code was broken and patients were treated with folic acid if they were in the non-folate study group.

Statistical methods. The sample size was calculated to be able to detect a difference of hemoglobin > 0.5 g/L in the two study groups with a 5% significance and 80% power. The data were analyzed using SPSS software (SPSS Inc., Chicago, IL) and chi-square and *t*-tests were used to compare differences in the outcomes of the two groups. Parasitologic data was presented as survival based on parasitologic failure (slide positive) and clinical failure (slide positive and a temperature ≥ 37.5°C).

RESULTS

Patients. Between March 1998 and March 1999, 382 patients were screened, of whom 303 met the entry criteria for the study. Four patients developed signs of severe malaria by day 3 and were withdrawn from further follow-up. Several patients did not attend for regular follow-up, mainly due to difficulties in traveling to the health facility during a period of heavy rain (El Niño); these patients were removed from further analysis. All drug regimens were well tolerated with no reported side effects.

Admission characteristics. The admission characteristics are shown in Table 1. Group A patients received no folic acid supplementation and group B patients received folic acid supplementation. The two groups were comparable with no significant differences in age, sex, clinical features, or laboratory indices at the time of entry into the study. The admission hemoglobin level was ≥ 11 g/dL in 29 (19%) patients in group A and 36 (24%) patients in group B. This was due to differences in hemoglobin measurement between the initial finger prick screen and the subsequent analysis. The admission mean corpuscular volume (MCV) was < 77 fL in 45 (29%) patients in group A and 58 (39%) patients in group B; the mean corpuscular hemoglobin concentration (MCHC) was < 32 g/dL in 80 (52%) patients in group A and in 73 (49%) patients in group B.

Parasitologic and clinical failure. Figures 1 and 2 compare the cumulative survival rates for patients in each study group on days 3, 7, 14, 21, and 28. Figure 1 compares cumulative survival based on parasitologic criteria, with survival equating to a negative blood slide on all study days except day 3 (> 25% reduction in parasitemia). Figure 2 compares cumulative survival based on clinical criteria defined as both parasitologic failure and a body temperature ≥ 37.5°C. The weekly data include patients reviewed during the week before

TABLE 1
Patients characteristics at the start of the study (n = 303)*

	Group A	Group B
Sex		
Male	77	87
Female	76	63
Age, months	78.84 (106.7)	81.96 (97.2)
Weight (kg)	18.72 (14.4)	19.38 (14.9)
Temperature (axillary), °C	37.2 (5.4)	36.3 (7.5)
Respiration rate (min)	23.5 (9.4)	23.6 (6.8)
Heart rate (min)	98.4 (28.9)	98.7 (27.7)
Splenomegaly	22	23
Parasites/μL	2,147 (36.4)	1,848 (27.7)
Hemoglobin (g/dL)	9.6 (1.7)	9.6 (1.8)
Red blood cell count ($\times 10^{12}$/L)	3.7 (0.7)	3.8 (0.7)
Mean corpuscular volume (fL)	81.4 (8.4)	78.8 (8.4)
Hematocrit (%)	30.3 (5.2)	30.0 (5.5)
Mean corpuscular hemoglobin concentration (g/dL)	31.7 (2.7)	32.1 (2.6)
Total white blood cell count ($\times 10^{9}$/L)	8.7 (3.8)	9.0 (4.4)

* Values are the mean (SD) unless otherwise indicated.

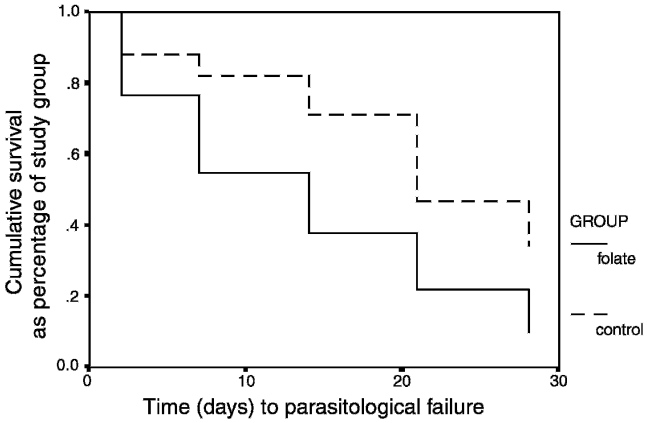

FIGURE 1. Cumulative survival rates as a percentage of each study group by parasitologic failure.

each assessment date. Folic acid supplementation made a significant difference to survival using parasitologic criteria on all study days ($P < 0.0001$), with a more pronounced difference on day 7, indicating that folic acid supplementation results in greater early, rather than late, treatment failure. There was no significant difference between the study groups on any study day using the criteria for clinical failure ($P = 0.7008$).

Gametocytes were noted in the peripheral blood films of patients following treatment in 32 (10.6%) patients on day 2, 110 (37.6%) on day 7, 68 (24.2%) on day 14, 21 (8.2%) on day 21, and 22 (8.9%) on day 28.

Hematology. The hematologic recovery of patients in each study group was compared, taking all patients and patients with hemoglobin levels < 8 g/dL. There was no significant difference in the recovery rate from anemia in patients taking folic acid supplementation in either group.

DISCUSSION

In this study, SP was given according to body weight because SP under-dosage is an important determinant of treatment failure in children and may occur when the drug is given according to age.[19] According to our dosage scale, the heavier patients received the correct dose of sulfadoxine (25 mg/kg) and pyrimethamine (1.25 mg/kg), and the lighter patients received a maximum dose of pyrimethamine (2.5 mg/kg) and sulfadoxine (50 mg/kg). Dosage according to body weight should be possible for all malaria managed at the facility level. The study dispenser had little difficulty in administering tablets to small children, which has also been the experience of others.[19,20] Mothers were instructed on how to administer tablets to children before leaving the health facility. Drug formulations used were those commonly available in health facilities in Kenya; as a result, both iron and folic acid tablets had to be cut in half before dispensing. For iron, which is an unscored tablet, this results in unequal amounts of drug in the two parts of the tablet. The production of appropriate tablet formulations of hematinics for the pediatric age group would be helpful.

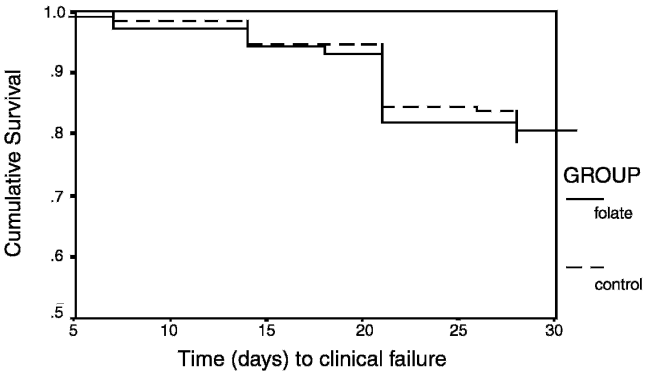

FIGURE 2. Cumulative survival rates as a percentage of each study group by clinical failure.

For low-to-moderate malaria transmission areas, parasitologic cure is important due to the lack of immunity in the patient population. In the current classification of response to treatment, follow-up is limited to 28 days, although the time to recrudescence when malaria is treated with slowly eliminated drugs may be considerably longer.[21] As resistance to the drug increases, this time becomes shortened. In the absence of molecular genotyping, reinfection and recrudescence cannot be distinguished; however, a recent study from Tanzania showed that malaria recurred within 28 days in most patients treated with SP due to parasite recrudescence.[22] The efficacy of SP treatment decreased with re-treatment and selected for parasites with point mutations for resistance.[22] In the study of Terlouw and others,[19] children who received SP within the previous 15–35 days had a 1.7 times higher failure risk by day 7, suggesting that these cases represented re-treatment of recrudescent infections with a selected parasite population. In our study, several of the patients who received repeat doses of SP after day 14 required re-treatment with halofantrine. Our study has demonstrated that the daily administration of folic acid in currently accepted therapeutic doses reduces the efficacy of SP. Using parasitologic criteria to determine weekly cumulative survival rates, there was a significant difference between the two study groups up to day 28 ($P = 0.0001$), indicating the inhibitory effect of folic acid on the antiparasitic effect of SP. This confirms the findings of a previous study,[18] although the doses of folic acid administered in that study were considerably higher than the recommended therapeutic doses. Clinical criteria showed no difference in survival rates between the two groups, indicating that normal body temperature is a poor guide to parasite clearance. The response to SP in the non-folic acid group represents the baseline sensitivity of SP in this area of Kenya, with failure rates of 17.2% on day 7 and 28.5% on day 14. In a previous study carried out on children in the same location in 1986, there were no failures up to day 7, only 1 of 17 failures on day 14, and 2 of 14 failures on day 28,[23] indicating the deteriorating efficacy of SP in the last 10 years.

No measurements of iron or folic acid levels, or the extent of hookworm burden, were performed in this study; however, the existence of background iron deficiency in the community is suggested by the low MCV and MCHC at the study start. Iron deficiency is known to affect psychomotor development in children[24] and iron supplements improve cognition and growth in deficient children.[25,26] Iron supplementation has been shown to enhance recovery from anemia in children with malaria.[18] In our study, all the patients recovered rapidly from anemia after a combination of effective antimalarial treatment and iron therapy. A recent systematic review has

shown no apparent harmful effect of iron on the incidence of infectious diseases in children, including malaria,[27] suggesting that there are no longer grounds for withholding iron supplementation from anemic individuals, especially children, in malarial areas. In children, folic acid deficiency may occur as a result of poor diet and hookworm infestation,[4] and it is therefore regarded as useful to administer folic acid together with iron when treating deficiency anemia. Folic acid supplements are also important in antenatal care and are used to reduce the risk of fetal neural tube defects in early pregnancy and to prevent anemia in late pregnancy.

This study has shown that generally accepted therapeutic doses of folic acid interfere with parasite clearance using antifolate antimalarial drugs and that the addition of folic acid has no impact on recovery from anemia in patients treated effectively for malaria. These findings have been supported by others.[18] The findings suggest that folic acid in commonly used therapeutic doses should not be co-administered with antifolate antimalarial drugs. This finding has major practical implications for intermittent preventive treatment (IPT) programs for both children and pregnant women, for which SP is currently the drug of first choice. In children, the benefits of IPT are currently being evaluated;[12,28] however, in pregnant women, IPT is one of the core strategies of malaria control programs in several African countries to prevent severe anemia and reduce low birth weight, especially in primigravidae and secundigravidae. In studies showing the benefits of intermittent treatment with SP, pregnant women were given therapeutic doses of folic acid (5 mg),[29] combined hematinic formulations containing low doses of folic acid (250–500 μg),[30] or no folic acid.[31] Low-dose folic acid formulations are not consistently provided to primary health care clinics, and anemic women are likely to be given 5-mg tablets. Our study suggests that folic acid supplementation should be withheld for at least one week after antifolate drug administration. The adverse effects of concurrent folic acid administration on the efficacy of SP administered for IPT and the correct timing for reintroducing folate supplementation require further evaluation.

Our results have operational implications for the new antifolate antimalarial drug Lapdap™ (chlorproguanil-dapsone), which has recently become commercially available. Lapdap™ has been developed in response to the need for an effective, safe and affordable malaria treatment[32] for Africa, but it is a synergistic combination of antifolate drugs, with the same mechanism of action as SP. If concomitant folate inhibits the antiparasitic activity of SP, then the same effect will occur with Lapdap™ treatments. Urgent steps are required to define the effect of folate supplementation on the activity of this new antimalarial drug as soon as possible.

The high rate of gametocytemia after SP use has been found in other studies[33] and is a further consideration for the selection of SP as an antimalarial drug, since prevention of malaria transmission is another important objective of treatment.

Received June 24, 2004. Accepted for publication August 6, 2004.

Acknowledgments: We thank the staff of the Entasopia Health Centre for the use of their facilities and support in carrying out this study. We also thank Professor Bob Snow and the Kenya Medical Research Institute/Wellcome Trust Programme for their assistance with data entry and analysis.

Financial support: This work was supported by a grant from the European Union Cofinancing Scheme through the African Medical and Research Foundation (Germany). W. M. Watkins is a Wellcome Trust resettlement fellow (grant #056305).

Disclosure: William M. Watkins is a member of the Lapdap™ product development team, but he receives no renumeration other than travel costs and per diems for this work. This statement is made in the interest of full disclosure, and not because the author considers this to be a conflict of interest.

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