Iron, but not folic acid, combined with effective antimalarial therapy promotes haematological recovery in African children after acute falciparum malaria

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Abstract

Whether children with malarial anaemia should receive supplementation with iron or folic acid is uncertain. Therefore, the effects of supplementary treatment with iron or folic acid, given together with chloroquine or pyrimethamine–sulfadoxine (Fansidar®), has been assessed in 600 Gambian children with uncomplicated falciparum malaria. After one month, haematological recovery was significantly better in the group treated with Fansidar® than in the chloroquine-treated group (difference in mean haemoglobin level=0·54 g/dL, \( P=0·01 \)). Children who received iron had a significantly better response than those given placebo (differences in mean haemoglobin level after one month and at dry season follow-up were 0·70 g/dL, \( P=0·006 \), and 0·81 g/dL, \( P=0·001 \), respectively). Iron supplementation was not associated with increased prevalence of malaria. Supplementation with folic acid did not improve the haematological response but, among children who received Fansidar®, the treatment failure rate was significantly higher among those given folic acid than among those given placebo. Thus, supplementation with iron, but not folic acid, improves haematological recovery without increasing susceptibility to malaria.

Keywords: malaria, Plasmodium falciparum, chloroquine, pyrimethamine–sulfadoxine, iron, folic acid, children, The Gambia

Introduction

In Africa, the anaemia associated with Plasmodium falci- parum infection is a major cause of childhood morbidity and mortality and the problem of severe malarial anaemia is increasing as chloroquine resistant parasites become widespread throughout Africa (BLOLAND et al., 1993). Improved methods of treating and preventing this condition are needed.

Although malarial anaemia has a complex, multifactorial pathogenesis, 2 presentations predominate in African children: a rapid drop in haemoglobin level caused by both immune and non-immune haemolysis associated with an acute infection and dyserythropoiesis, sometimes with chronic bone marrow suppression, secondary to persistent and often low-grade parasitaemia (ABDALLA et al., 1980; WEATHERALL et al., 1983; PHILLIPS & PAS-VOL, 1992). Thus, prevention of malarial anaemia relies on both effective parasite elimination and enhancement of the bone marrow response. Optimally effective antimalarial drugs should reduce parasite persistence and hasten prompt haematological recovery. This has been demonstrated in areas of East Africa where chloroquine resistance is well established and where parasitological clearance, rather than apparent clinical recovery, was found to be necessary for an effective bone marrow response (KEUTER et al., 1992; BLOLAND et al., 1993).

Many young children in malaria-endemic areas are deficient in iron, but there has been reluctance to treat them with iron because of reports that iron therapy can predispose to malaria infection or enhance its clinical severity (MURRAY et al., 1978; OPPENHEIMER et al., 1968; SMITH et al., 1989). However, this effect has not been found in all studies (BATES et al., 1987; HARVEY et al., 1989). Prescription of folic acid supplementation after malaria infection is common in Africa. Megaloblastic changes in bone marrow aspirates combined with low serum folate levels have been reported in adults with acute malaria (STRIKLAND & KOSTINAS, 1970). However, detailed analyses of Gambian children with malarial anaemia demonstrated that the dyserythropoietic changes were not a result of folic acid deficiency (ABDALLA, 1990; ABDALLA et al., 1984), and red cell folate levels have been found to be higher in malaria patients than in control subjects (BRADLEY-MOORE et al., 1985; OPPENHEIMER & CASHIN, 1986).

We report the results of a two-sided, prospective, randomised trial evaluating the effect of iron and folic acid as supplementation treatments and chloroquine and pyrimethamine–sulfadoxine (Fansidar®) as antimalarial treatments upon haematological recovery following acute P. falciparum infection.

Patients and Methods

Study area and population

The trial was conducted with patients who attended the clinic of the Medical Research Council (MRC) Laboratories, Fajara, The Gambia, between July and December 1992. Fajara is situated on the Atlantic coast, 16 km from the capital, Banjul. The climate is typical of West African sub-Sahelian savannah with an annual rainfall of 500–1000 mm which falls almost exclusively during the months of June to October; most transmission of P. falciparum occurs from August to November.

Children who attended the MRC clinic were enrolled in the study if they fulfilled the following inclusion criteria: (i) clinical illness compatible with malaria; (ii) peripheral P. falciparum parasitaemia of at least 5 parasites per high power field (HPF) (approximately 2500/mL); (iii) age between 6 months and 9 years; and (iv) informed consent given by parents or guardians. Any child with either severe underlying disease or complicated malaria that required hospital admission was excluded.

Study design

On recruitment, a clinical history was taken and children were examined by one of the study doctors. A blood sample was taken for baseline parasitological and haematological tests. Children were then allocated at random to receive either chloroquine or Fansidar® as antimalarial treatment and iron, folic acid or placebo as supplementation. Chloroquine was given in a total dose of 25 mg/kg (initial dose 10 mg/kg then 5 mg/kg every 12 h) and Fansidar® as a single dose as close as possible to 1·25 mg/kg pyrimethamine and 25 mg/kg sulfadoxine. The first antimalarial dose was supervised and repeated if vomiting occurred within one hour of administration. Iron was given as sodium iron edetate syrup containing 5·5 mg/mL elemental iron. The dose was 5 mL thrice daily for children weighing <20 kg and 7·5 ml thrice daily for those over this weight. Folic acid (5 mg tablets) was given in daily doses of 5 mg for children weighing <15 kg, 7·5 mg for those weighing 15–20 kg, and 10 mg for children >20 kg. A single daily sugar tablet was given as placebo.

Supplementation was provided for a total of 28 d (for 7 d initially; on day 7 an additional 21 d supply was given).
Patients were asked to return for review 7 and 28 d after recruitment, when another clinical history was taken, physical signs were again recorded, and haematological and parasitological measurements repeated. Any patients with positive blood films on or after day 7 were defined as parasitological failures. Patients with any parasitaemia associated with either the presence of fever or a history of fever within the previous 48 h, or those without symptoms but with parasitaemia >10/HPF (approximately 5000/mL), were defined for management purposes as clinical failures. All clinical failures were re-treated with the other antimalarial drug (chloroquine failures received Fansidar® and vice versa). Any clinical failures that occurred after a first re-treatment were given a 5 d course of oral quinine sulphate at a dose of 10 mg/kg thrice daily. A dry season follow-up was conducted 4 months after the end of the rainy season. Deaths, hospital admissions and episodes of severe illness were recorded and a finger-prick blood sample taken for haemoglobin estimation.

I. Laboratory investigations

All laboratory investigations were performed without knowledge of the treatment group. Thick blood films were stained with Giemsa's stain and 100 HPF were examined by an experienced microscopist. Parasite densities were recorded as the number of asexual parasites per HPF; one parasite per HPF indicates a parasitaemia of approximately 500/mL (Greenwood & Armstrong, 1991).

Full blood counts (including haemoglobin concentration (Hb), haematocrit (PCV), red blood cells (RBC), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were measured with a Coulter counter. Reticulocyte counts, sickling and haemoglobin electrophoresis were all done by standard methods. Blood samples were taken on days 0 and 28 from a randomly selected sub-group of patients (the first 25 of each 100 consecutively enrolled patients with a sufficiently large sample of serum) for estimation of iron and folate status. Serum samples were collected and stored at -20°C until used. Serum iron was assayed on a Cobas MIRA® autoanalyzer using commercial kits (Biomerieux, France and Roche Diagnostics, UK); serum ferritin levels were determined using a previously described enzyme-linked immunosorbent assay (ELISA) (Snow et al., 1991). Samples for red cell folate estimation were collected, stored and measured by radioimmunoassay according to the manufacturer's instructions (Becton-Dickinson, UK). Chloroquine and pyrimethamine drug levels were measured by ELISA inhibition test on admission and at 7 d follow-up (Eggelte, 1990).

Study size and statistical analysis

The sample size was calculated so that the study would be able to detect with 95% confidence and 90% power a difference of 0.5 g/dL in the increase in Hb between children receiving iron or folate supplementation and those given placebo. The data were analysed using EpInfo and SAS System for Windows® (SAS, 1988). Groups were compared using analysis of variance if the data were normally distributed; when necessary, the data were normalized using logarithmic transformation. Discrete data were analysed using Pearson's χ² or Fisher's exact test if the frequencies were small. The trial was analysed as a factorial design, the factors being antimalarial and supplementation treatments. On the antimalarial side, patients were started on chloroquine vs Fansidar® treatment but some failed and were transferred to the other treatment; subsequent failures were then transferred to quinine. In the analysis we treated these 6 groups as separate entities; thus the trial was analysed as a 6 × 3 factorial (Mead, 1986). The supplementation treatments, folic acid and iron, were compared with the placebo, after adjusting for the antimalarial treatment. An interaction term, testing whether the supplementation treatment effect was the same in each antimalarial group, was examined but in every case was found to be not significant. In the case of Hb, baseline values were subtracted from the day 7, day 28 and dry season measurements and the differences, referred to as haematological recovery, were analysed. Regression models were fitted to explore the relationship between haematological recovery and baseline Hb for each of the supplementation groups using analysis of variance techniques. Models were compared with one another using changes in sums of squares with their corresponding changes in degree of freedom, which follow an F distribution.

Results

Patients

Between July and December 1992, 600 children were recruited into the study of whom 530 (88.3%), 445 (74.2%), and 480 (80.0%) were followed up at 7 and 28 d after presentation and during the following dry season, respectively. Most frequent reasons for failure to attend for follow-up examination were parental refusal, migration out of the study area, or the reporting of an incorrect address. Defaulters were divided equally among the study groups.

Admission characteristics

Admission characteristics are given in Table 1. The 2 antimalarial and 3 supplementation groups were comparable in terms of clinical features and laboratory measurements at the time of entry into the study (details available from the authors). Chloroquine concentrations in

<table>
<thead>
<tr>
<th>Table 2. Parasitological failure rates at day 7 and cumulative failure rate by day 28 of follow-up</th>
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<tbody>
<tr>
<td>Chloroquine</td>
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<tr>
<td>------------</td>
</tr>
<tr>
<td>Iron</td>
</tr>
<tr>
<td>Folic acid</td>
</tr>
<tr>
<td>Placebo</td>
</tr>
<tr>
<td>Total</td>
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<tr>
<td>Mean (SD)</td>
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whole blood samples obtained on entry into the study were above 300 ng/mL (equal to 60-100 ng/mL in plasma) in 18-2% of the chloroquine group (median 16 ng/mL, interquartile range [IQR] 14-159 ng/mL) and in 23-7% of the Fansidar® group (median 21 ng/mL, IQR 14-311 ng/mL). This difference is not statistically significant. Admission Hb for 74% of the children was below 11·0 g/dL, the World Health Organization definition of anaemia in children (WHO, 1968). Admission MCV and MCHC values were below the normal range in 36% and 66% of the children, and above the range in 1·4% and 0·4% respectively. Eight percent of children were folate deficient, with red cell folate values below 160 mg/L. Admission serum iron levels were below the normal range in 16-1% and all children had raised serum ferritin levels on presentation (median 2225 ng/mL), presumably reflecting the acute phase response to their infection.

Parasitology

Results of parasitological examinations are given in Table 2. Children treated with chloroquine were more likely to have persistent parasitaemia on day 7 compared to those given Fansidar® and to require re-treatment as a result (17.7% vs. 3.2%, P<0.001). At 28 d 38.9% of all patients receiving chloroquine and 14.0% of those receiving Fansidar® needed re-treatment.

There were fewer parasitological failures in the children who received iron supplementation than in those receiving placebo at both 7 and 28 d; this was most marked in the chloroquine group at 28 d (Table 2). The rate of failure to clear parasites did not differ significantly between children receiving folic acid and placebo. However, persistent parasitaemia occurred more frequently in the children given the Fansidar®-folic acid combination than in those given the Fansidar®-placebo combination at 7 and 28 d follow-up (Table 2); the difference at 7 d was not significant; P=0.75. Median parasite counts in children who failed to clear their parasites were not significantly different between the 3 supplementation groups at 7 or 28 d.

Table 3. Difference in haematological recovery (assessed by haemoglobin level) between treatment groups

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Day 7</th>
<th>Day 28</th>
<th>Dry season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron vs. placebo</td>
<td>-0.08 (0.46, 0.30)</td>
<td>0.12 (0.38, 0.62)</td>
<td>0.81 (0.56, 1.00)</td>
</tr>
<tr>
<td>Folic acid vs. placebo</td>
<td>-0.38 (0.00, 0.76)</td>
<td>0.54 (0.09, 0.97)</td>
<td>0.11 (0.01, 0.10)</td>
</tr>
<tr>
<td>Fansidar® vs. chloroquine</td>
<td>-0.15 (0.04, 0.16)</td>
<td>0.09 (0.01, 0.17)</td>
<td>0.44 (0.03, 0.18)</td>
</tr>
<tr>
<td>No. of patients</td>
<td>154</td>
<td>115</td>
<td>90</td>
</tr>
<tr>
<td>Iron + fansidar® vs. placebo + chloroquine</td>
<td>-0.30 (-1.00, 0.40)</td>
<td>0.90 (0.11, 1.68)</td>
<td>0.44 (0.03, 0.18)</td>
</tr>
</tbody>
</table>

*95% confidence interval in parentheses. Significance of results (analysis of variance) indicated thus: *P=0.006, **P=0.001, ***P=0.001, ****P=0.03.

Haematological indices

No difference in red cell indices, iron and folate status were found between the chloroquine and Fansidar®-treated groups at follow-up. Similarly, MCV and MCHC values at follow-up did not differ significantly between the folic acid and placebo groups at 28 d or the dry season follow-up (Table 3; P=0.65 and P=0.56, respectively). The reticulocyte count did not differ significantly between the 3 supplementary groups at either 7 or 28 d.

Table 4. Morbidity and mortality in treatment groups between enrolment and dry season follow-up

<table>
<thead>
<tr>
<th>Hospital admissions</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>Chloroquine plus</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*One child in this group died at home.
Morbidity and mortality

Morbidity and mortality did not differ significantly between the study groups (Table 4). During the study period 37 children (6.2%) were admitted to hospital and 9 (1.5%) died (8 in hospital and 1 at home). Of the 29 fatal admissions 12 had severe anaemia (Hb<5 g/dL), 12 moderately severe anaemia, and 5 had other illnesses; 86% of admissions occurred during 28 days after initial enrolment. Three of the 8 children who died in hospital were born on arrival, 3 had cerebral malaria, 1 severe anaemia and 1 septicaemia.

Discussion

Anaemia of sufficient severity to threaten life is an uncommon feature of acute falciparum malaria; it occurs in perhaps 1% of clinical malaria infections in African children, with a case fatality rate of around 8% (GREENWOOD et al., 1991). However, in most parts of tropical Africa children under the age of 5 years experience at least one clinical attack of malaria a year, so anaemia associated with malaria infection is a major cause of death in African children and a frequent cause of admission to paediatric clinical attack of malaria a year, so anaemia associated with malaria would be expected to have an important public health impact, even if the effect was only modest.

Animalaral treatment

Our study has demonstrated that, even in an area where high-grade chloroquine resistance is still uncommon, children given Fansidar® gained a clear advantage in terms of haematological recovery over those treated with chloroquine. Although there was no significant difference in the number of subsequent hospital admissions or deaths between the 2 groups, it is likely that this advantage has been underestimated, for 2 reasons. Firstly, due to the interaction between Fansidar® and folic acid, the Fansidar® failure rate may have been artificially high. Secondly, due to an active follow-up policy, chloroquine failures were traced and re-treated at an early stage; this resulted in 39% of children initially given chloroquine requiring re-treatment by day 28. The high re-treatment rate could also explain the reduction in the effect of the Fansidar®-iron combination on haematological recovery found at the dry season follow-up.

Iron supplementation

We have shown that iron supplementation promoted haematological recovery after an attack of acute malaria in Gambian children. Although the additional increase in haemoglobin among iron supplemented children was only modest (average just under 1 g%), this benefit was retained for several months after the malaria attack. It is unlikely that children with moderate anaemia would gain any substantial clinical benefit from an additional increase in Hb of this magnitude. However, for the small number of children with an Hb at the left of the Hb distribution curve, an additional increase in Hb of this magnitude could make the difference between potentially fatal or non-fatal infection. The present study was not large enough to demonstrate this directly, for only 12 children developed severe anaemia requiring urgent transfusion (3 of whom had received oral iron). Nevertheless, we believe that administration of oral iron to children with acute malaria will help to prevent the development of severe anaemia in a proportion of children and that further, large-scale, studies of this intervention are warranted.

Iron supplementation has not previously been recommended for the management of children with malaria anaemia, even though iron deficiency is known to be a prevalent, mainly because of concerns that administration of iron could exacerbate the malaria infection. This effect has been demonstrated in several studies in which treatment with iron has been given to iron deficient subjects (MURRAY et al., 1978; OEPPEINHEIMER et al., 1986; SMITH et al., 1989), although it has not been found in all trials (BATES et al., 1987; HARVEY et al., 1989). In our study we found no evidence of increased treatment failure rate among children supplemented with iron. Indeed, the parasitological and haematological response was similar in the iron treatment group than in the placebo group, and this effect was statistically significant in the subgroup treated with chloroquine. Parasite counts among treatment failures were no higher in those given iron than among those given other supplements, nor was the number of visits to health services increased among iron supplemented children (data not shown).

Thus, we found no evidence of any harmful parasitological consequence of giving iron supplementation to children with malaria.

Folic acid supplementation

Although iron is rarely given to children with malaria, folic acid is frequently prescribed on the grounds that deficiency of this micronutrient may compromise the enhanced erythropoiesis required to restore Hb after an episode of haemolytic anaemia. The failure of folate supplementation to produce any clinical benefit in this study cannot be explained by poor compliance, since RBC folate levels rose significantly in the folate-treated group. Only 8% of children in this study were folate deficient, in accordance with other studies showing that folate deficiency is an unusual problem in West African children (BRADLEY-MOORE et al., 1985; ABDALLA, 1990). The widely held belief that malaria parasites are obligatorily dependent upon p-aminobenzoic acid (PABA) and are unable to utilize exogenous folate for tetrahydrofolate formation during purine synthesis may well be an over-simplification (WERNSDORFER & TREUGG, 1988), as folic acid can antagonize the anti-malaria parasite activity of sulphonamide drugs in vitro (WATKINS et al., 1985). One can postulate that, in our study, the parasites that failed to respond to Fansidar® in the presence of folic acid were intrinsically pyrimethamine resistant and that this property became apparent only after the folic acid had blocked the sulfadoxine (W. M. Watkins, personal communication). The clinical relevance of this interaction has not previously been established; our results suggest that supplementary folate can compromise the antimalarial activity of Fansidar® in vitro.

Our data showed that iron supplementation, when combined with an effective antimalarial drug, promotes haematological recovery in African children with uncomplicated falciparum malaria without increasing their susceptibility to malaria. Supplementation with folic acid had no beneficial effect and may be harmful in patients treated with an antifolate drug.

Acknowledgements

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