

Open randomized study of pyrimethamine–sulphadoxine vs. pyrimethamine–sulphadoxine plus probenecid for the treatment of uncomplicated *Plasmodium falciparum* malaria in children

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Summary

BACKGROUND Increasing drug resistance in *Plasmodium falciparum* has necessitated renewed search for cheap, effective alternatives to commonly available antimalarials, chloroquine and pyrimethamine–sulphadoxine, for the treatment of malaria in Africa. Probenecid, an inhibitor of organic anion transporters and multiresistance-associated proteins, can chemosensitize *P. falciparum* to pyrimethamine and sulphadoxine *in vitro*, but the clinical significance is unclear. We assessed the safety, treatment efficacy, and effects on gametocyte carriage of adding probenecid to pyrimethamine–sulphadoxine.

METHODS We evaluated 151 children aged 12 years or younger who had uncomplicated *P. falciparum* malaria. Patients were randomly assigned pyrimethamine–sulphadoxine (25 mg/kg of the sulphadoxine component) or pyrimethamine–sulphadoxine as above plus probenecid 20–25 mg/kg of bodyweight in two divided doses daily for 3 days. The primary endpoints were parasitological cure rates on days 14 and 28.

RESULTS Both regimens were well tolerated; no child was withdrawn because of drug intolerance. Fever (1.9 ± 1.1 vs. 2.4 ± 1.2 days, $P = 0.02$) and parasite clearance (2.3 ± 0.9 vs. 2.7 ± 1.1 days, $P = 0.04$) were significantly shorter, and the parasitological cure rate on day 14 (96.2% vs. 83.5%, $P = 0.02$) but not day 28 (79.4% vs. 72.6%, $P = 0.4$), was significantly higher in children treated with pyrimethamine–sulphadoxine–probenecid than in those treated with pyrimethamine–sulphadoxine. Gametocyte carriage was similar with both treatment regimens.

CONCLUSIONS The combination of pyrimethamine–sulphadoxine, and probenecid, at a relatively moderate dose, improved treatment efficacy but had no effect on gametocyte carriage. The pyrimethamine–sulphadoxine–probenecid combination merits further evaluation as a potential treatment for use in Nigeria.

keywords probenecid, pyrimethamine–sulphadoxine, malaria, children, Nigeria

Introduction

Drug resistance in *Plasmodium falciparum* to chloroquine is a major public health problem in much of sub-Saharan Africa, accounting for recent increases in malaria-related morbidity and mortality (Trape *et al.* 1998; Trape 2001), gametocyte carriage, and enhanced transmission of drug-resistant malaria in Africa (Robert *et al.* 1996, 2000; Sutherland *et al.* 2002; Drakeley *et al.* 2004; Happi *et al.* 2003; Sowunmi & Fateye 2003a,b).

As an alternative to chloroquine, pyrimethamine–sulphadoxine is widely used in sub-Saharan Africa, but

resistance is rapidly emerging (Sibley *et al.* 2001). It is associated with point mutations in dihydrofolate reductase and dihydropteroate synthetase genes of the parasite (Plowe *et al.* 1997; Wang *et al.* 1997; Diourté *et al.* 1999), and confers survival and propagation advantages on the parasite in the population (Sowunmi & Fateye 2003b).

These developments have led to the renewed search for effective alternatives to both chloroquine and pyrimethamine–sulphadoxine, and to the use of both drugs in combination with each other, or in combination with other antimalarials with modes of action different from those of chloroquine and pyrimethamine–sulphadoxine, with the

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aims of slowing the progression of resistance to these drugs and prolonging their lifespan (von Seidlein *et al.* 2000; Basco *et al.* 2002; Sowunmi 2002; Drakeley *et al.* 2004; Gasasira *et al.* 2003). It has also led to the use of chloroquine in combination with resistance modulators, e.g. chlorpheniramine (Sowunmi *et al.* 1997).

Experience with chloroquine plus chlorpheniramine for treating chloroquine-resistant infections comes from southwest Nigeria where the prevalence of chloroquine-resistant infection is 35–40% (Sowunmi *et al.* 1998a,b,c; Sowunmi 2003). A recent study has shown that probenecid, an inhibitor of organic anion transporters and multiresistance-associated proteins can chemosensitize *P. falciparum* to pyrimethamine, sulphadoxine or chloroquine *in vitro* (Nzila *et al.* 2003), but the clinical significance is unclear. To date no study has examined, clinically, the usefulness of probenecid in combination with pyrimethamine–sulphadoxine for the treatment of malaria in African children. Such a study is essential for a number of reasons; it is possible that the combination, given in appropriate doses, may improve treatment efficacy. Malaria transmission may be reduced if probenecid modulates the gametocytocite-releasing effect of pyrimethamine–sulphadoxine. It can help alter the management of paediatric cases of malaria.

Here we report the safety, antimalarial treatment efficacy, and effect on gametocyte carriage of pyrimethamine–sulphadoxine–probenecid and pyrimethamine–sulphadoxine alone in children aged 12 years or younger with acute, symptomatic, uncomplicated *P. falciparum* malaria.

Materials and methods

Study area

The study was carried out in Ibadan, southwest Nigeria from July to September 2003. In this area of hyperendemic malaria, transmission occurs all year round but is more intense during the rainy season, April to October. In the area, it is difficult, clinically, to distinguish recrudescence from re-infection 14 days after commencing antimalarial treatment, and usually antimalarial efficacy tests have been conducted for 14 rather than the customary 28 days (Ekanem *et al.* 1987; Salako *et al.* 1990). Chloroquine resistance was reported in the area in the 1980s (Ekanem 1985; Salako & Aderounmu 1987) and pyrimethamine–sulphadoxine resistance in the 1990s (Sowunmi *et al.* 1993, 1998a; Falade *et al.* 1997). Presently, chloroquine resistance reaches approximately 35–40% (Sowunmi 2003) and, pyrimethamine–sulphadoxine resistance approximately 25% in the under 5-year olds (A. Sowunmi & B. A. Fateye, unpublished data).

Patients, treatment and follow-up

Patients were eligible to join the study if they were aged 12 years or younger, had symptoms compatible with acute uncomplicated malaria, with pure *P. falciparum* parasitaemia >2000 asexual forms/ μ l, a temperature >37.4 °C or recent pyrexial antecedents, absence of other concomitant illness, no history of antimalarial use in the 2 weeks preceding presentation, negative urine tests for antimalarial drugs (Dill-Glazko and Lignin), and written informed consent from parents or guardians. Patients with severe malaria (WHO 2000), severe malnutrition, serious underlying diseases (renal, cardiac, or hepatic), and known allergy to study drugs were excluded from the study. Ethical clearance for the study was provided by the Ethics Committee of Oyo State Ministry of Health, Ibadan, Nigeria. The disease history was recorded by asking patients or their parents when the present symptomatic period had started, and was followed by a full physical examination.

Enrolled patients were randomly assigned pyrimethamine–sulphadoxine 25 mg/kg of bodyweight of the sulphadoxine component at presentation (days 0) or pyrimethamine–sulphadoxine as above plus probenecid (Batch 2D13, Industria Farmaceutica Nova Argentina, Milano, Italy); 20–25 mg/kg of bodyweight in two divided doses daily for 3 days (days 0, 1 and 2). The randomization was computer-generated and treatment codes were sealed in individual envelopes. Once enrolled, the study drugs were administered by a physician. Patient evaluation and follow-up after drug administration was performed by another physician blinded to the drug treatment. All drugs were given orally; except the second daily doses of probenecid, all drugs were administered in the clinic, and all patients waited for at least 3 h after drug administration to ensure that the drug was not vomited. If it was, the patient was excluded from the study. If necessary, patients were provided with antipyretics (paracetamol tablets, 10–15 mg/kg every 8 h for 24–48 h). The study nurse obtained thick and thin blood films from each child as soon as they came to the clinic. The slides were carefully labelled with the patients' codes and were air dried before being stained.

Follow-up with clinical and parasitological evaluation was performed every day for 7 days (days 1–7) and then on days 14, 21 and 28. Thick and thin blood films prepared from a finger prick were Giemsa stained and were examined by light microscopy under an oil-immersion lens, at 1000 \times magnification, by two independent assessors who were blinded to the treatment of the patient. Parasitaemia (asexual or sexual forms) in thick films was estimated by counting asexual or sexual forms relative to 1000 leucocytes, or 500 asexual or sexual forms, whichever occurred

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first. From this figure, the parasite density was calculated assuming a leucocyte count of 6000/ μ l of blood.

Routine haematological (haematocrit) and biochemical tests (concentrations of alanine aminotransferase, aspartate aminotransferase, bilirubin, and creatinine) were performed in 54 randomly selected children, pre-treatment and on day 14 after treatment. Blood was spotted on filter papers on days 0, 3, 7, 14, 21 and 28, in all patients, and at the time of treatment failures for parasite genotyping. Parasite genotyping will be reported elsewhere.

Response to drug treatment was assessed using WHO (1973) criteria, as follows: S, sensitive, clearance of parasitaemia without recurrence; RI (mild resistance), parasitaemia disappears but reappears within 7–14 days; RII (moderate resistance), decrease of parasitaemia but no complete clearance from peripheral blood; RIII (severe resistance), no pronounced decrease or increase in parasitaemia at 48 h after treatment. In those with sensitive or RI response, parasite clearance time was defined as the time elapsing between drug administration and absence of detectable parasitaemia for at least 48 h. Fever clearance time was defined as the time from drug administration until the core temperature fell to or below 37.4 °C and remained so for 48 h. Cure rates were defined as the percentages of patients whose asexual parasitaemia cleared from peripheral blood and who were free of patent asexual parasitaemia on days 14, 21 and 28 of follow-up.

Re-treatment of drug treatment failures

In patients who failed treatment (within 14 days), the codes were broken, and if the patient was initially treated with pyrimethamine–sulphadoxine, she or he was re-treated with pyrimethamine–sulphadoxine–probenecid and followed up for another 14–28 days. Those failing initial treatment with pyrimethamine–sulphadoxine–probenecid were re-treated with oral amodiaquine 30 mg/kg over 3 days and followed up for another 14–28 days. Patients were re-treated whenever they became symptomatic (usually 14–21 days after initial enrollment). Patients with profound clinical (hyperpyrexia, oral fluid intolerance) and parasitological deterioration during follow-up were treated with artemether (9.6 mg/kg, over 5 days) and were regarded as treatment failures.

Study size and statistical analysis

Sample size was calculated so that the study would be able to detect a difference of 22% in the parasitological failure rate between pyrimethamine–sulphadoxine–probenecid and pyrimethamine–sulphadoxine groups, with 95%

power at a 5% significant level (it was assumed that 75% of those given pyrimethamine–sulphadoxine, based on the current cure rate in the under 5-year olds, and 97% of those given pyrimethamine–sulphadoxine–probenecid would be cured on first treatment). At least 71 children were needed in each treatment arm. Data were analysed using version 6 of the Epi-Info software (Anonymous 1994). Variables considered in the analysis were related to the densities of *P. falciparum* gametocytes and trophozoites. Proportions were compared by calculating chi-squared value with Yates' correction or by Fisher exact or by Mantel–Haenszel tests. Normally distributed, continuous data were compared by Student's *t*-tests and analysis of variance (ANOVA). Data not conforming to a normal distribution were compared by the Mann–Whitney *U*-tests and the Kruskal–Wallis tests (or by Wilcoxon ranked-sum test). All tests of significance, except where specifically indicated, were two tailed. *P*-values of <0.05 were indicated significant differences. Data were entered serially using the patients codes and were only analysed at the end of the study.

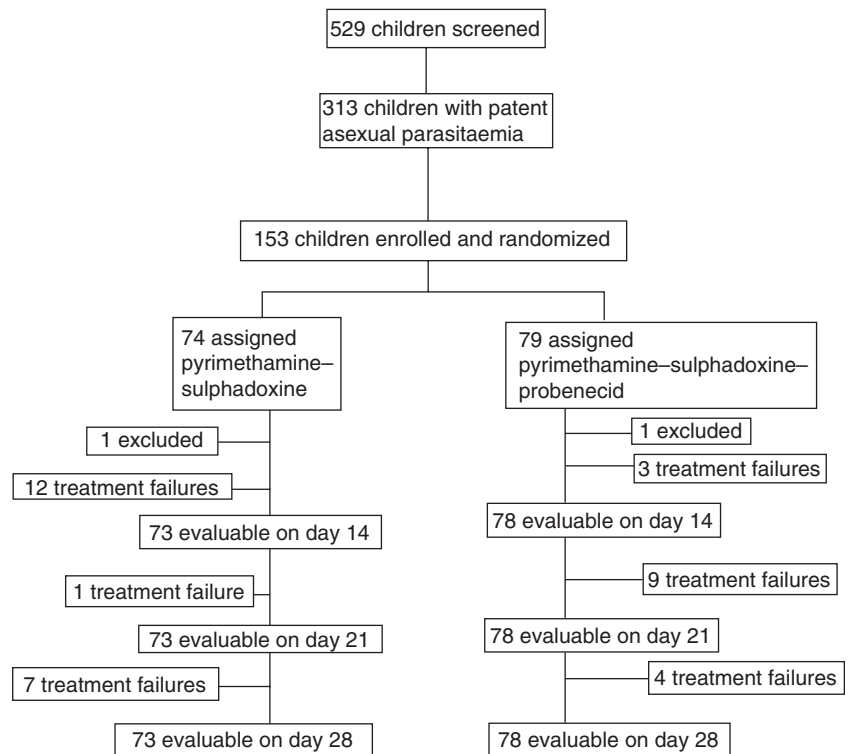
Results

Patients' characteristics

One hundred and fifty-three children were enrolled, 79 were treated with pyrimethamine–sulphadoxine–probenecid and 74 with pyrimethamine–sulphadoxine. Two children, one from each of the treatment arms, were lost to follow-up after day 7 because of parental re-location. These children were excluded from data analysis. Figure 1 shows the trial profile. Overall results are for 151 children. The demographic and clinical characteristics of patients at enrollment are shown in Table 1. These characteristics were similar in the two treatment arms, but the duration of illness at presentation was significantly longer in those treated with pyrimethamine–sulphadoxine–probenecid ($P = 0.04$).

Fever and parasite clearance, and gametocyte carriage

One hundred and seven children were febrile at enrollment, 57 in the pyrimethamine–sulphadoxine–probenecid and 50 in the pyrimethamine–sulphadoxine group. By day 2, fever cleared in 42 and 26 children, respectively. There was a significant difference in the proportion of patients whose fever cleared by day 2 [$\chi^2 = 4.5$, $P = 0.03$, odd's ratio (OR) = 0.47, 95% confidence interval (CI) = 0.23–0.96]. Overall, fever clearance was significantly quicker in those treated with pyrimethamine–sulphadoxine–probenecid (1.9 ± 1.1 vs. 2.4 ± 1.2 days, $P = 0.02$) (Table 2).

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	Pyrimethamine–sulphadoxine–probenecid	Pyrimethamine–sulphadoxine
No. of patients	78	73
Male/female	41/37	38/35
Age (years)		
Mean ± SD	6.3 ± 2.9	5.9 ± 2.9
Range	1.5–12	0.8–11.5
<i>n</i> (<5 years)	29	25
Weight (kg)		
Mean ± SD	17.8 ± 6.1	17.2 ± 5.6
Range	7–35	5–30
Duration of illness (days)		
Mean ± SD	3.5 ± 1.7	3.0 ± 1.3
Range	1–10	1–9
Temperature (°C)		
Mean ± SD	38.1 ± 1.0	38.4 ± 1.2
Range	35.9–40.3	36.1–40.5
Parasite count (/µl)		
Geometric mean	46 792	57 745
Range	2010–1 388 000	2020–1 254 000
Haematocrit (%)		
Mean ± SD	31.6 ± 5.5	33.1 ± 5.1
Range	18–43	22–46
<i>n</i> (<25%)	8	2

Compared with pyrimethamine–sulphadoxine, pyrimethamine–sulphadoxine–probenecid substantially accelerated the clearance of parasitaemia. By day 2, 53 and 37 children in the pyrimethamine–sulphadoxine–probenecid and pyrimethamine–sulphadoxine treatment arms, respectively, had their parasitaemia cleared. The difference in this proportion was significant ($\chi^2 = 3.98$, $P = 0.04$, OR = 2.06, 95% CI = 1.01–4.22). Overall, parasite clearance was significantly quicker in those treated with pyrimethamine–sulphadoxine–probenecid (2.3 ± 0.9 vs. 2.7 ± 1.1 days, $P = 0.04$) (Table 2). The cure rate on day 14 (96.2% vs. 83.5%, $\chi^2 = 5.3$, $P = 0.02$, OR = 4.92, 95% CI = 1.24–28.0) but not day 28 (79.4% vs. 72.6%, $\chi^2 = 0.6$, $P = 0.4$, OR = 1.46, 95% CI = 0.64–3.35), was significantly higher in children treated with pyrimethamine–sulphadoxine–probenecid than in those treated with pyrimethamine–sulphadoxine. Response to both treatment regimens was not related to age: one child and two children from the 29 and 49 <5- and ≥ 5 -year olds, respectively, treated with pyrimethamine–sulphadoxine–probenecid failed treatment by day 14 ($P = 1.0$, by Fisher exact test, OR = 0.84, 95% CI = 0.01–16.8). Similarly, four and eight children from the 25 and 48 <5- and ≥ 5 -year olds, respectively, treated with pyrimethamine–sulphadoxine failed treatment by

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	Pyrimethamine-sulphadoxine-probenecid	Pyrimethamine-sulphadoxine	<i>P</i> -value
No. of patients	78	73	-
Fever clearance time (days)			
Mean \pm SD	1.9 \pm 1.1 (<i>n</i> = 67)	2.4 \pm 1.2 (<i>n</i> = 60)	0.02
Range	1-5	1-7	
Parasite clearance time (days)			
Mean \pm SD	2.3 \pm 0.9 (<i>n</i> = 76)	2.7 \pm 1.1 (<i>n</i> = 71)	0.04
Range	1-5	1-6	
Day 14 responses			
No. cured	75	61	
No. RI	1	10	
No. RII	2	1	
No. RIII	0	1	
Cure rate (%)	96.2	83.5	0.02
Day 21 responses			
No. cured	66	60	
No. RI (cumulative)	10	11	
No. RII	2	1	
No. RIII	0	1	
Cure rate (%)	84.6	82.2	0.8
Day 28 responses			
No. cured	62	53	
No. RI (cumulative)	14	18	
No. RII	2	1	
No. RIII	0	1	
Cure rate (%)	79.4	72.6	0.4

Table 2 Therapeutic responses to pyrimethamine-sulphadoxine-probenecid or pyrimethamine-sulphadoxine

day 14 ($P = 1.0$, by Fisher exact test, OR = 0.98, 95% CI = 0.19-4.18).

Gametocyte carriage in those who did not have gametocytaemia at enrollment ($n = 73$ and 72 in the pyrimethamine-sulphadoxine-probenecid and pyrimethamine-sulphadoxine, respectively) was similar on days 7 [32/73 (43.8%) *vs.* 31/72 (43%), $\chi^2 = 0.01$, $P = 0.9$, OR = 1.3, 95% CI = 0.51-2.10] and 14 [16/73 (21.9%) *vs.* 21/72 (29.1%), $\chi^2 = 0.66$, $P = 0.4$, OR = 0.68, 95% CI = 0.30-1.54] with both regimens.

Response to pyrimethamine-sulphadoxine-probenecid of children with pyrimethamine-sulphadoxine-treatment failures

Seven of 12 children who had reappearance or no clearance of parasitaemia within 14 days of initial treatment with pyrimethamine-sulphadoxine were re-treated with pyrimethamine-sulphadoxine-probenecid. The therapeutic responses of these children following re-treatment with pyrimethamine-sulphadoxine-probenecid are summarized in Table 3. Parasitaemia and fever cleared within 2-4 days of treatment with pyrimethamine-sulphadoxine-probenecid. The child with RII

response to pyrimethamine-sulphadoxine during initial treatment had an RI response following re-treatment with pyrimethamine-sulphadoxine-probenecid. The cure rates on days 14 and 28 were 86% and 72%, respectively. None of the three children who failed treatment with pyrimethamine-sulphadoxine-probenecid on or before day 14 (Table 2) and who were subsequently re-treated with amodiaquine failed treatment during a 28-day follow-up period. In these children fever and parasitaemia cleared within 2-3 days of initiation of amodiaquine therapy.

Adverse events

Pyrimethamine-sulphadoxine-probenecid and pyrimethamine-sulphadoxine were well tolerated; no child was withdrawn because of drug intolerance. Symptoms reported within the first week and during follow-up were similar (Table 4). However, vomiting was more frequently reported by those treated with pyrimethamine-sulphadoxine. None of the seven children who failed initial treatment with pyrimethamine-sulphadoxine and were re-treated with pyrimethamine-sulphadoxine-probenecid reported adverse symptoms.

A. Sowunmi *et al.* **Probenecid plus pyrimethamine–sulphadoxine for uncomplicated malaria****Table 3** Clinical and parasitological parameters of the seven children with *Plasmodium falciparum* malaria who had reappearance or no clearance of parasitaemia following initial treatment with pyrimethamine–sulphadoxine and were subsequently treated with pyrimethamine–sulphadoxine–probenecid

	Pyrimethamine–sulphadoxine	Pyrimethamine–sulphadoxine–probenecid	P-value
No. of patients	7	7	–
Age (years)			
Mean ± SD	6.8 ± 2.6		
Range	3.3–11.5		
Weight (kg)			
Mean ± SD	19.9 ± 3.4	20.1 ± 3.8	0.5
Range	15–26	15–27.5	
Temperature (°C)			
Mean ± SD	38.8 ± 1.1	37.8 ± 1.4	0.1
Range	37.0–40.3	36.0–39.5	
Parasite count (/µl)			
Geometric mean	47 835	8156	0.01
Range	2020–115 500	716–27 622	
Fever clearance time (days)			
Mean ± SD	2.1 ± 1.3	1.2 ± 0.4	0.35
Range	1–4	1–2	
Parasite clearance time (days)			
Mean ± SD	3.6 ± 1.0	2.8 ± 0.9	0.26
Range	2–5	2–4	
Day 14 responses			
No. cured	0	6	0.004
No. RI	6	1	
No. RII	1	0	
No. RIII	0	0	
Cure rate (%)	0	86	

Table 4 Adverse drug reactions reported during the study

	Pyrimethamine–sulphadoxine–probenecid	Pyrimethamine–sulphadoxine
No. of children investigated	78	73
Reporting pruritus	0	0
Vomiting	2	2
Abdominal pain	5	2
Diarrhoea	0	2
Anorexia	0	4*
Drowsiness	0	0
Cough	4	7
Headache	3	3
Weight gain (≥1.0 kg)	38 (<i>n</i> = 66)	32 (<i>n</i> = 60)

* Significant statistical difference, *P* = 0.05.**Haematological and biochemical parameters**

Except for haematocrit values below 25% at enrollment in eight and two children in pyrimethamine–sulphadoxine–probenecid and pyrimethamine–sulphadoxine groups, respectively, and at day 7 in four and four children, respectively, haematological, biochemical and other parameters remained normal before and after treatment in all subjects. Thrombocytopenia was present in 10 and 12

children in pyrimethamine–sulphadoxine–probenecid and pyrimethamine–sulphadoxine groups, respectively, at enrollment, but was not seen on day 14 in any child.

Discussion

Given the increasing prevalence and intensities of resistant infections to pyrimethamine–sulphadoxine in much of sub-Saharan Africa (Falade *et al.* 1997; Sowunmi *et al.*

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1998a; van Dillen *et al.* 1999; Omar *et al.* 2001; Sibley *et al.* 2001) and the tendency for the drug to increase gametocyte carriage after its use for the treatment of malaria (Robert *et al.* 2000; von Seidlein *et al.* 2001; Sowunmi & Fateye 2003a,b), there is a need to review chemotherapeutic strategies based on the use of this drug alone. The results of the present study indicate that probenecid, an inhibitor of organic anion transporters and multidrug-resistance-associated proteins (Borst *et al.* 2000), and a chemosensitizer of *P. falciparum in vitro* to antifolate agents (Nzila *et al.* 2003), enhances the antimalarial effect of pyrimethamine-sulphadoxine *in vivo* in children with uncomplicated falciparum infections. This is the first report of the enhancement of the antimalarial activity of an antifolate agent by probenecid in humans.

The relatively accelerated clearance of fever and parasitaemia produced a cure rate of 96% by day 14 after treatment with pyrimethamine-sulphadoxine-probenecid. Interestingly, only one of the three children who failed treatment was <5 years old. Evaluation of the acetylation status of the failures would have been helpful. Longer duration of illness (>2-3 days) is associated with increased risk of gametocyte carriage in uncomplicated falciparum malaria (Price *et al.* 1999). However, despite a longer duration of illness before presentation in the pyrimethamine-sulphadoxine-probenecid-treated children, gametocyte carriage before and following treatment was similar to those of pyrimethamine-sulphadoxine-treated children. The similar gametocyte carriage following treatment with both regimens indicates that the use of pyrimethamine-sulphadoxine-probenecid may not overtly discourage the transmission of gametocytes arising from drug-sensitive or -resistant infections.

Virtually all of the pyrimethamine-sulphadoxine-treatment failures were cured of their infections when they were re-treated with pyrimethamine-sulphadoxine-probenecid. Overall, this indicates a beneficial effect of probenecid. However, this beneficial effect may also be due to additional increases in drug levels arising from repeated administration of pyrimethamine-sulphadoxine. Although no untoward effect was observed following re-treatment, caution is required with this step, as it may increase the chances of adverse drug reactions to pyrimethamine-sulphadoxine.

The drugs used were well tolerated. The most frequently reported adverse reactions were of gastrointestinal origin, and most were indistinguishable from the symptoms of malaria. Malaria, and the drugs evaluated, can cause anorexia. It is possible that the significantly reduced reporting of anorexia by those treated with pyrimethamine-sulphadoxine-probenecid was related to the accelerated clearance of fever and parasitaemia. Both

probenecid and sulphadoxine can also induce haemolysis in glucose 6 phosphate dehydrogenase (G6PD)-deficient subjects, but no child, following treatment, reported features suggestive of drug-induced haemolytic anaemia.

It remains unclear exactly how probenecid enhanced the antimalarial effect of pyrimethamine-sulphadoxine in the cohort of children studied. Probenecid can reduce folate uptake by *P. falciparum in vitro* (Nzila *et al.* 2003), in addition to increasing plasma sulphonamides concentrations by reducing renal tubular secretion of the latter. Both of these actions are independent of parasite sensitivity status to pyrimethamine-sulphadoxine. It is possible that following treatment with pyrimethamine-sulphadoxine-probenecid, sulphadoxine concentrations were significantly higher than in those treated with pyrimethamine-sulphadoxine alone, but drug levels were not measured. Probenecid can also reverse resistance in cancer cells to methotrexate (Hooijberg *et al.* 1999) and resistance in *P. falciparum* to chloroquine *in vitro* (Nzila *et al.* 2003), by inhibiting the multi-drug resistance associated proteins. Inhibition of the multi-drug resistance associated proteins is an unlikely mechanism of the enhancement of the antimalarial effect of pyrimethamine-sulphadoxine by probenecid as resistance to pyrimethamine-sulphadoxine is associated with mutations in the dihydropteroate synthase and dihydrofolate reductase, and not mutations in the *pfmdr1* gene of the parasite (Duraisingh *et al.* 1997; Wang *et al.* 1997; Diourte *et al.* 1999).

There are justifications for our dosing regimen: the relatively moderate dose was based on the dose used to retard tubular secretion of penicillin in children – a convenient starting point as the drug has not been previously co-administered with pyrimethamine-sulphadoxine for the treatment of malaria in children; the 3-day dosing regimen is practicable, and compliance is more likely than if it were for longer periods. Certainly pharmacokinetic and pharmacodynamic studies are required before optimal dosing regimens can be achieved. There are potential clinical applications of our findings. If at moderate doses probenecid enhances the antimalarial efficacy of pyrimethamine-sulphadoxine, it follows that as resistance increases to pyrimethamine-sulphadoxine, higher doses of probenecid may be effective, as it is possible that enhancement may be dose related.

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