Effects of pyrimethamine-sulphadoxine, chloroquine plus chlorpheniramine, and amodiaquine plus pyrimethamine-sulphadoxine on gametocytes during and after treatment of acute, uncomplicated malaria in children

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The effects of pyrimethamine-sulphadoxine (PS), chloroquine plus chlorpheniramine, a H1 receptor antagonist that reverses chloroquine resistance in Plasmodium falciparum *in vitro and in vivo (CQCP), and amodiaquine plus pyrimethamine-sulphadoxine (AQPS) on gametocyte production were evaluated in 157 children with acute, symptomatic, uncomplicated falciparum malaria who were treated with these drugs. PS was significantly less effective than CQCP or AQPS at clearing asexual parasitaemia or other symptoms of malaria. Gametocyte carriage on days 3, 7, and 14 were significantly higher in those treated with PS. The ratio of the density (per µl blood) of peripheral young gametocyte (PYG), that is, ≤ stage III to peripheral mature gametocyte (PMG), that is, stage IV and V, an index of continuing generation of gametocytes, rose to 1 by day 7 of treatment in those treated with PS, but remained consistently below 1 in the other treatment groups. PYG-PMG density ratio increased significantly from day 0-14 in those treated with PS and CQCP ($\chi 2 = 76$, $P = 0.000001$ and $\chi 2 = 42.2$, $P = 0.00001$, respectively) but decreased significantly in those treated with AQPS ($\chi 2 = 53.2$, $P = 0.000001$). Both PS-sensitive and -resistant infections generated PYG (18 of 29 vs 13 of 20, $\chi 2 = 0.04$, $P = 0.93$) but PYG was present only in those with resistant response to CQCP. Combination of PS with amodiaquine (AQ), that is, (AQPS) resulted in less production of PYG, but in this setting, PYG was not indicative of response to AQPS. These data indicate that PS enhanced production or release of young gametocytes when used alone, but generated less young gametocytes when used in combination with AQ. PYG may be used as an indicator of response to CQCP but not PS or PS-based combination drugs.*

Key words: malaria - gametocytaemia - pyrimethamine - sulphadoxine - chloroquine - chlorpheniramine - amodiaquine - children - Nigeria

As resistance to chloroquine (CQ) increases in extent and severity, alternative regimens available to control programmes in developing endemic countries including pyrimethamine-sulphadoxine (PS), amodiaquine (AQ) (Olliaro et al. 1996, Brasseur et al. 1999, Sowunmi et al. 2001) or combination of AQ with PS (AQPS) (Sowunmi 2002) or other suitable combinations have become increasingly used in the treatment of CQ-resistant falciparum infections. These alternatives have varying effects on clearance of asexual parasitaemias or sexual forms of *Plasmodium falciparum*. For example, PS may (Puta & Manyando 1997) or may not (Hogh et al. 1995) enhance gametocyte carriage during treatment of acute falciparum infections. Although the presence of gametocytes in peripheral blood after antimalarial treatment is no proof of viability, their generation is required for the transmission of the infection from the vertebrate to the anopheline host. In order to improve the management of paediatric cases of malaria and reduce transmission in our area of study, the effects of these drugs on gametocyte production needs urgent assessment. In addition, it is not clear whether the enhancement or non-enhancement of gametocyte production by PS will be influenced by its use in combination with other antimalarial drugs. It is noteworthy that antifolates are ineffective in the treatment of uncomplicated falciparum malaria in South America, for example, in Brazil (Fontes et al. 2002).

Resistance to CQ in *P. falciparum* can be reversed by chlorpheniramine (CP) in vitro and in vivo (Sowunmi et al. 1997, 1998a, b, c). We have recently shown that, the presence in peripheral blood of very young gametocytes (PYG) 72 h after commencing CQ may be used as indicator of response to CQ (Sowunmi et al. 2003). However, it is unclear whether the addition of CP to CQ will alter the use of PYG as an indicator of response to CQ or indeed as an indicator of failure of reversal of CQ resistance in vivo by CP. Although the combination of CQ with CP will not be employed by control programmes in Africa in the very near future, it is still essential to study PYG and peripheral mature gametocyte (PMG) generation during treatment with CQCP in the event that this or other similar combination become available.

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In order to address these issues, we have evaluated gametocyte generation during treatment of falciparum malaria in children with PS, CQCP and AQPS. The main aims of our study were (i) to evaluate the effects of PS, CQCP and AQPS on gametocyte generation during treatment with these drugs, (ii) to determine whether or not the addition of PS to AQ will influence the generation of gametocytaemia by PS, and (iii) to evaluate PYG as an indicator of response to PS, CQCP or AQPS.

PATIENTS AND METHODS

Study site - The study site, Ibadan, is a hyperendemic area for malaria in Southwestern Nigeria (Salako et al. 1990). In the area, it is difficult to distinguish, clinically, re-infection from recrudescence after day-14 of treatment because of intense transmission. Antimalarial drugs have therefore generally, until recently, been evaluated on the basis of data recorded up to day 14, rather than the customary day 28 (Ekanem et al. 1990, Sowunmi & Salako 1992).

Patients - The study took place at the University College Hospital in Ibadan, Nigeria. Overall, 166 children who presented with acute, symptomatic, uncomplicated *P. falciparum* malaria were enrolled in the study between September 1999 and September 2001.

The study was designed to elicit a 20% difference in cure rates between AQPS/CQCP on one hand and PS on the other hand with 80% power and at 95% level of confidence. The minimum number of patients required for each treatment arm is 45. In general, to be enrolled, the children had to be aged 0.5-10 years, and have symptoms compatible with acute, falciparum malaria (with fever or history of fever in the 24-48 h preceding presentation) and a pure *P. falciparum* parasitaemia of > 2000 asexual forms/µl blood. Those who had taken antimalarial drugs in the 2 weeks preceding presentation, provided a urine sample found positive for 4 aminoquinolines or sulphonamides (by the Dill-Glazko and lignin tests, respectively), or who had a concomitant illness, such as sickle-cell anaemia, or severe or complicated malaria (Warrell et al. 1990, WHO 2000) were excluded. The informed consent of a parent or guardian was obtained for each child included in the study. A child was withdrawn from the study if she/he developed concomitant illness during the follow-up period, or if his/her parent or guardian requested it. The study received ethical approval from the local ethics committee.

Before enrolment in the study, a medical history of each child was obtained from an accompanying parent/guardian and each was physically examined. Body weight and oral or rectal temperature were recorded, and thick and thin films were prepared from finger-prick blood samples. These smears were Giemsa-stained for parasite identification and quantification of any peripheral parasitaemias.

Drug treatment - Children were randomly allotted to one of 3 treatment groups. One group received PS at presentation (day 0) at a dose 25 mg/kg of the sulphonamide component. Each tablet of PS contained 500 mg of sulphadoxine and 25 mg pyrimethamine. The other groups received chloroquine base, 30 mg/kg of body weight over 3 days (days 0-2) plus chlorpheniramine maleate, 6 mg at presentation followed by 4 mg every 8 h for 7 days (days 0-6) if the child was aged < 5 years, or 8 mg at presentation followed by 6 mg every 8 h if the child was ≥ 5 years; or a single dose of PS at presentation plus AQ 30 mg/kg over 3 days (days 0-2). All drugs were given by a physician orally and each child was observed for at least 3 h after each such supervised drug treatment, in order to ensure that the drug was not vomited. If it was, the child was excluded from the study. Additional management of some children included the administration of an antipyretic (e.g. 10-15 mg paracetamol/kg, every 8 h for 24 h) and fanning and tepid sponging when necessary.

Evaluation of response - Clinical observations were recorded daily for 8 days (days 0-7) and then on day 14. Thick and thin blood films, for quantification of parasitaemia, were prepared at the same times. At each follow-up, the guardians or parents (and, when possible, the children) were actively questioned, using a standard questionnaire, and the children were examined for the presence of adverse reactions to drugs.

Giemsa-stained blood films were examined by light microscopy under an oil-immersion objective, at × 1000 magnification, by two independent assessors who did not know the drug treatment of the patients. Parasitaemia in thick films was estimated by counting asexual parasites relative to 1000 leukocytes, or 500 asexual forms, whichever occurred first. From this figure, the parasite density was calculated assuming a leukocyte count of 6000/µl blood. Young gametocytes (stage I-III) and mature gametocytes (stage IV and V) (Sinden 1998) were also counted in thick blood films against 1000 leukocytes on days 0, 3, 4, 5, 6, 7, and 14. The responses to drug treatment were classified according to World Health Organization (1973) criteria. Treatment was considered a failure if the day-3 parasitaemia was > 25% of the day 0 value, if parasitaemia did not clear by day 7, or if parasitaemia cleared before day 7 but re-appeared before day 28. The parasite clearance time (PCT) was defined as the time elapsing from drug administration until there was no patent parasitaemia. The fever clearance time (FCT) was defined as the time from drug administration until the oral or rectal temperature fell to ≤ 37.4°C and remained so for at least 72 h. (This definition was necessary because of the routine use of paracetamol during the first 36 h of treatment in some children).

Cure rates were defined as the proportions of patients who remained free of parasitaemia on day 14 of follow-up.

Re-treatment of drug treatment failures - All treatment failures were re-treated with AQPS on day 14 provided they were not symptomatic before this time. Patients with profound clinical (hyperpyrexia, oral fluid intolerance) and parasitological deterioration during follow-up were treated with artemether, 9.6 mg/kg of body weight over five days and were regarded as treatment failures.

Statistical analysis - Data were analyzed using version 6 of the Epi-Info software (Anon 1994). Proportions were compared by calculating χ^2 with Yates' correction or by Fisher exact 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 rank sum test). The values presented below are generally means and standard deviations (sd). P-values of < 0.05 were taken to indicate significant differences.

RESULTS

Clinical and parasitological characteristics at enrolment and therapeutic responses - A total of 166 children was enrolled in the study; 51, 52, and 63 children were enrolled in the PS, CQCP, and AQPS groups, respectively. Of these, 49, 48, and 60 children in the PS, CQCP, and AQPS groups, respectively completed the mandatory 14-day follow-up period and were analysed. The clinical and parasitological characteristics at enrolment were similar in all groups (Table I). The therapeutic responses to drug treatment are also summarized in Table I. AQPS was significantly more effective than CQCP or PS in clearing fever and parasitaemia and with a significantly higher cure rate on day 14. Direct comparison of PS and CQCP showed that fever (2.2 ± 1.1 *vs* 1.6 ± 0.8 day, P = 0.008) but not parasite clearance in those with sensitive response (2.7 ± 1.1 *vs* 2.5 ± 0.8 day, P = 0.33) was significantly faster with CQCP than with PS. The cure rate on day 14 was also significantly higher with CQCP than with PS (80.8 vs 59.2%, P = 0.03).

Gametocytaemia during follow-up - The prevalence and intensities of gametocytaemia before, during and after treatment are summarized in Table II. Gametocyte carriage on days 3, 7, and 14 or days 3, 7, and 14 combined were significantly higher in the PS group than in the other treatment groups. However, the geometric mean gametocyte densities (GMGD) were similar in all the treatment groups.

The median survival time for peripheral young gametocytes (PYG) in PS, CQCP, and AQPS treatment groups were 3.5, 1.5, and 1.5, respectively. There was a significant difference in the overall comparison of the survival experience using Wilcoxon (Gehan) statistics ($\chi^2 = 14.7$, P = 0.0006). The ratios of the densities (per μl blood) of peripheral young gametocytes (PYG) to peripheral mature gametocytes are summarized in Table

TABLE I

Clinical and parasitological parameters of the children enrolled in the study

	PS (n =49)	CQCP (n = 48)	AQPS(n = 60)	P value
Age (years)				
mean + s.d.	5.1 ± 2.7	6.0 ± 2.3	5.5 ± 2.5	0.52
range	0.6-10	2.0-10	1.2-10	
Weight (kg)				
mean ± s.d.	15.4 ± 5.6	16.8 ± 5.2	15.5 ± 4.7	0.32
range	6.5-26	8.1-35	6-26	
Duration of illness (d)				
mean ± s.d.	3.1 ± 1.4	3.6 ± 2.4	2.8 ± 1.3	0.06
range	1-7	2-14	1-8	
Presenting body temp. (°C)				
mean ± s.d.	38.5 ± 1.2	38.6 ± 1.2	38.1 ± 1.0	0.05
range	35.8-40.5	36.2-40.5	36-40.2	
Parasitaemia (per ml)				
geometric mean	37858	29248	30482	0.56
range	3310-375476	2511-219600	878-716000	
Fever clearance times (d)				
mean ± s.d.	2.2 ± 1.1	1.6 ± 0.8	1.2 ± 0.9	0.000001
range	1-5	1-4	1-3	
Parasite clearance time (d)				
mean ± s.d.	2.7 ± 1.1	2.5 ± 0.8	2.2 ± 0.7	0.012
range	1-6	1-4	1-4	
No. of children				
cured	29	38	60	0.000000
RI	10	10	-	
RII	7	-	-	
RIII	3	-	-	

95%CI: 95% confidence interval; PS: pyrimethamine-sulphadoxine; CQCP: chloroquine plus chlorpheniramine; AQPS: amodiaquine plus pyrimethamine-sulphadoxine.

III. The ratios were consistently below 1 in the CQCP and AQPS groups up till day 7. However, in the PS group, this ratio rose progressively to 1 on day 7 indicating continuing production (or generation or mobilization) of young gametocytes. PYG-PMG density ratio increased significantly from day 0-14 in those treated with PS and CQCP (χ^2 = 76, P = 0.000001and χ^2 = 42.2, P = 0.00001, respectively) but decreased significantly in those treated with AQPS (χ^2 = 53.2, P = 0.000001).

Relationship between PYG and responses to drug treatment - None of the children successfully treated with CQCP had PYG during the follow-up. In children who had sensitive response to PS treatment (n = 29), PYG was present on days 0, 3, 5, 7, and 14 in 5, 12, 13, 13, and 7 children, respectively. Similarly in those successfully treated with AQPS (n = 60), PYG was present on days 0, 3, 5, 7, and 14 in 2, 2, 3, 3, and 0 patient, respectively. The PYG rates were significantly higher in those treated with PS than in those treated with AQPS at all times during follow up (P ≤ 0.006 in all comparisons).

Post Hoc Turkey HSD test for repeated measure of the effect of PYG generation over the 14 day follow up showed significant differences in the comparisons of PS vs AQPS and PS *vs* CQCP (P = 0.0001 and 0.0001 respectively). There was no significant difference in the comparison of PYG generated by those treated with AQPS and CQPS (P = 0.08)

Relationship between PYG and outcomes of treatment in the children treated with PS and CQCP - The relationship between treatment outcomes and presence of PYG in children treated with PS and CQCP are shown in Tables IV and V. PYG rates were similar in those with sensitive or resistant responses to PS (18 of 29 *vs* 13 of 20, χ^2 = 0.04, P = 0.93) and the rates were similar from days 0-14. In contrast, PYG was seen only in those with resistant response to CQCP. In those without gametocytaemia at presentation, but who subsequently developed PYG 72 h after commencement of CQCP, the presence of PYG was associated with treatment failure on or before day 14 (Table V).

TABLE II

Gametocytaemias before, during and after the treatment, with pyrimethamine-sulphadoxine (PS), chloroquine plus chlorpheniramine (CQCP) or amodiaquine plus pyrimethamine-sulphadoxine (AQPS), of *Plasmodium falciparum* infections in children

	PS (n = 49)	CQCP (n = 48)	AQPS (n = 60)	P value
Day 0 gametocytaemia				
Geometric mean (/µl)	36	22	40	0.49
Mean + S.E.	87 ± 52.1	26 ± 5.3	74 ± 43.3	
Range	12-444	12-36	12-288	
Day 3 gametocytaemia				
Geometric mean (/µl)	36	44	40	0.92
Mean + S.E.	100 ± 43.5	127 ± 83.5	86 ± 54.1	
Range	12-876	12-612	12-408	
Day 5 gametocytaemia				
Geometric mean (/µl)	70	61	43	0.74
Mean + S.E.	243 ± 112.9	118 ± 64.3	141 ± 121.0	
Range	12-468	12-518	12-504	
Day 7 gametocytaemia				
Geometric mean (/µl)	135	39	36	0.38
Mean + S.E.	399 ± 141.2	136 ± 73.6	98 ± 74.1	
Range	12-3520	12-696	12-468	
Day 14 gametocytaemia				
Geometric mean (/µl)	83	34	19	0.21
Mean + S.E.	139 ± 29	60 ± 30.1	24 ± 12	
Range	12-480	12-168	12-48	
No. of patients with gametocytaemia on				
Day 0	9	8	6	0.42
Day 3	25	8	7	0.000004
Day 7	26	8	6	0.000002
Day 14	20	4	3	0.000001
Day 3, 7, 14	32	11	3	0.000000
Day 7, 14	27	11	3	0.000000

Wilcoxon (Gehan) for survival analysis (χ2 = 14.7, P = 0.0006).

TABLE III

Prevalence and intensities of peripheral young gametocytes and peripheral mature gametocytes in children treated with pyrimethamine-sulphadoxine (PS), chloroquine plus chlorpheniramine (CQCP) or amodiaquine plus pyrimethamine-sulphadoxine (AQPS)

	PS		CQCP		AQPS	
Day	GMPYGD (/µl)	GMPMGD (/µl)	GMPYGD(/µl)	GMPMGD(/µl)	GMPYGD (/µl)	GMPMGD (/µl)
0	21 (5)[a] 12-36[b] 1[c]	65 (4) 12-408 3.1[c]	12 (4) 12-12 1	36 (1) - 3	42 (2) 24-72 1	51 (2) 12-216 1.2
3	28 (20) 12-372 1	57 (9) 12-552 2.0	55 (4) 12-144 1	106 (2) 24-468 1.9	27 (2) 12-60 -[d]	348 (1) -[d]
5	37 (21) 12-640 1	54 (17) 12-1840 1.5	46 (6) 12-348 1	58 (4) 12-180 1.3	23 (3) 12-84 1	39 (3) 12-420 1.7
7	79 (24) 12-1320 1	85 (21) 12-2210 1.1	42 (7) 12-216 1	97 (4) 12-600 2.3	28 (3) 12-156 1	87 (2) 24-312 3.1
14	50 (14) 12-240 1	78 (12) 12-444 1.6	21 (4) 12-120 1	35 (3) 12-72 1.7	-[e] -[e] -	19 (3) 12-48

GMPYGD: geometric mean peripheral young gametocyte density; GMPMGD: geometric mean peripheral mature gametocyte density; *a*: values in parentheses represent number of children with gametocytaemia; *b*: range; *c*: GMPYGD:GMPMGD ratio; *d*: not calculated; *e*: no peripheral young gametocytes observed

TABLE IV

Prevalence of peripheral young gametocytaemia (PYG) in the children with sensitive or resistant response following treatment with oral pyrimethamine-sulphadoxine (PS) or chloroquine plus chlorpheniramine (CQCP)

No. of children with PYG on day	PS-sensitive (n = 29)	PS-resistant (n = 20)	P value	CQCP-sensitive (n = 38)	CQCP-resistant (n = 10)	P value
0	5	4	0.81	-	3	-
3	12	8	0.96	-	4	0.001
5	13	8	0.85	-	6	0.00002
7	13	11	0.38	-	7	0.000002
14	7	7	0.34	-	4	0.001
3, 7, and/or 14	18	13	0.65	-	8	0.000002
7 and/or 14	13	13	0.11	-	7	0.0000001

TABLE V

Peripheral young gametocyte (PYG) carriage at or after day 3 in children treated with oral pyrimethamine-sulphadoxine (PS) or chloroquine plus chlorpheniramine (CQCP) and who did not have gametocytaemia on presentation

No. of children with PYG on day	PS-sensitive (n = 29)	PS-resistant (n = 20)	P value	CQCP-sensitive (n = 38)	CQCP-resistant (n = 10)	P value
3	5	6	0.46	-	-	-
4	6	6	0.72	-	2	0.008
5	9	7	0.91	-	1	0.08
6	10	9	0.69	-	1	0.08
7	11	10	0.61	-	2	0.008
14	7	7	0.64	-	2	0.008

DISCUSSION

The ideal antimalarial drugs or drug combinations for the treatment of falciparum malaria should not only promptly clear parasitaemia, fever or other symptoms of malaria, but should also prevent the generation of gametocytes from asexual forms during treatment. In the present study, PS was significantly less effective than CQCP or AQPS in clearing parasitaemia or fever in children with acute falciparum infections. This is not surprising since progressive decline in sensitivity of *P. falciparum* to PS has been reported from the area of study from the late 1990s (Falade et al. 1997, Sowunmi et al. 1998a). The decline in sensitivity of the parasite to PS has also occurred in many areas of Africa (Sibley et al. 2001).

In addition to their effects on the sexual forms, gametocyte carriage may be influenced to a considerable extent by the sensitivity of the asexual parasites to the drugs used for the treatment of infections. For example, as resistance of the asexual parasites to the 4-aminoquinolines, CQ, and AQ, increases, gametocyte carriage also increases (Strickland et al. 1986, Hogh et al. 1995). In these studies, gametocyte carriage rates 28 days after PS treatment were significantly less than those of CQ and AQ since PS was more effective than the 4-minoquinolines on asexual parasites in the settings of these studies. However, increased carriage may also be related to decreased sensitivity to PS in certain circumstances (Sowunmi et al. 1998a, Tjitra et al. 2002). In our cohort of children, gametocyte carriage was significantly higher at all times after treatment with PS than in the other treatment groups. In addition, PYG rates were similar in both PS- sensitive and -resistant infections supporting a known fact that PS enhanced generation or release of gametocytes during treatment of acute falciparum infections (Puta & Mayando 1997). However, GMGD were similar in all the treatment groups.

Many antimalarial drugs appear to reduce gametocytaemia by clearing the asexual stage infections. This clearance, if exceptionally rapid, may reduce transmissibility particularly in areas of low transmission. For example, the artemisinin derivatives have reduced transmissibility in some parts of Thailand by this process (Price et al. 1996).

In order to determine the influence of treatment with antimalarial drugs on gametocyte production and densities, we have quantified both young and mature gametocytes and expressed these as ratios. The ratios of PYG to PMG were consistently below 1 up to day 7 in those treated with CQCP and AQPS, but rose to 1 by day 7 in those treated with PS irrespective of the sensitivity of the asexual parasite to PS. This showed continuing and enhanced production or, preferential mobilization of gametocytes by PS irrespective of the sensitivity of the asexual parasites to PS. This process of continuing or preferential mobilization of young gametocytes by PS may explain why gametocytes persist longer in some patients treated with PS. This is plausible because the young gametocytes must grow and run the normal time-course of survival of the normal mature gametocytes.

Given that gametocyte density may correlate with mosquito infectivity and therefore transmission success (Tchuinkam et al. 1993, Drakeley et al. 1999), the effects of PS on gametocytes carriage and mobilization have implications for malaria control programmes with respect to the use of this drug. Recent WHO recommendations (WHO 2001a, b) have focused on the use of combination antimalarial therapy (CT), particularly artemisinin-based combination therapy (ACT). Although several control programmes in Africa have switched to CT, some programmes use PS-based combination, for example, AQPS (Sowunmi 2002). The modulating effect of AQ on enhanced production of PYG by PS may provide supporting argument for the use of combination therapy. However, the reduced generation of PYG by PS despite its combination with other drugs suggests that generation of gametocyte is an inherent property of antifolate antimalarials (Sowunmi et al. 2005, Hamel et al. 2005).

In a recent study, we have shown that the detection of PYG 72 h after the start of CQ therapy may be used as an indicator of response to this drug (Sowunmi & Fateye 2002). Our results show that PYG may also be used as an indicator of response to CQCP. Failure of the enhancement of the antimalarial efficacy of CQ by CP *in vivo* was associated with the presence in peripheral blood of young gametocytes. However, PYG was not an indicator of response to PS, since both PS-sensitive and -resistant infections generated PYG. In addition, the presence of PS in combination with AQ also generated PYG and was clearly not an indicator of response to AQPS since the cure rate in this group was 100%.

The limitation of the present study is the fewer number of gametocyte carriers in the AQPS and CQCP groups following treatment. Therefore caution is required with the interpretation of the data from these two groups.

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