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MALARIA SUPPRESSION WITH FORTNIGHTLY DOSES OF PYRIMETHAMINE
ALONE OR COMBINED WITH SULFADOXINE¹ IN THE GAMBIA

by

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INDEXED



1. Introduction

Clinical trials among hospital patients in The Gambia have shown that potentiating combinations of pyrimethamine and sulfadoxine or diaphenylsulfone (Dapsone) are effective in eliminating high density falciparum parasitaemia within two or three days (Laing, 1969). The actual quantity of drugs required to achieve this was found to be extremely small, doses of pyrimethamine as low as 0.01 mg/kg body weight, combined with sulfonamide or sulfone, sufficing to clear asexual parasitaemia. Doses of pyrimethamine at a level of 0.1 mg/kg in combination with sulfadoxine were completely effective even in very heavy infections with parasite counts of 500 000 per mm³. These trials were carried out in "semi-immune" Gambian patients but most of them were infants over six months or young children under four years of age in which susceptibility to heavy infection was high and death due to malaria not uncommon.

The extraordinary efficacy of these low dosage combinations in treatment suggested that they might also be used for suppression: the dosage of sulfonamide or sulfone would probably be well below a level producing toxic effects and the possibility that parasites would become resistant has been shown by experiments with non-human Plasmodia to be unlikely (Richards, 1968). It was decided, therefore, to carry out a small-scale field trial to compare the effects of fortnightly doses of a combination of pyrimethamine and sulfadoxine with weekly doses of pyrimethamine alone over a six-month period during and just after the rainy season when malaria was hyperendemic.

2. Materials and methods

The subjects of the trial were 86 schoolchildren between the ages of six and ten years attending the nearby Government school at Bakau, Cape St Mary and 36 infants or toddlers between the ages of six months and three-and-a-half years from Bakau town. A preliminary blood survey was carried out among the schoolchildren in June 1968. Those found with patent parasitaemias were divided into two groups of 26 each, one group to receive fortnightly pyrimethamine 2 mg + sulfadoxine 40 mg and the other pyrimethamine 25 mg weekly; the remaining 34 children with negative blood films were allocated to a control group who were to receive no antimalarial regimen. The groups of younger children, 12 in each, were made up from those suffering from acute attacks of falciparum malaria attending as out-patients in June, July and early August. One group received pyrimethamine 1 mg + sulfadoxine 20 mg for the treatment of the first attack observed and then fortnightly thereafter and another group pyrimethamine 12.5 mg initially and weekly thereafter; some of those in the control group received treatment for overt malaria, not necessarily with pyrimethamine, for attacks occurring during the trial period.

Thick blood films stained by Field's rapid method were used to detect or assess parasitaemias every two weeks in those groups given suppressive chemotherapy; in the school control group, blood surveys were only carried out at the beginning of the trial, after three months and after six months. Parasites were counted against leucocytes and densities per mm³ estimated accordingly; a thick blood film was reported as negative if no parasites were seen in 100 microscopic fields (magnification x 600).

¹ Sulformethoxine (Fanasil).

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The MRC Grey Wedge photometer was used to estimate haemoglobin concentrations from finger-prick blood samples.

All antimalarials were administered personally by the writer. Sulfadoxine was given in the form of "Fanasil" suspension from drop bottles containing 5 mg sulfadoxine per drop and pyrimethamine as Daraprim tablets of 25 mg "Daraprim" and of "Elixir" containing 1.25 mg "Daraprim" per ml. During the school holidays of 10 weeks from the end of July to the end of September, the subjects were examined and treated in their homes; a few during this time were away from the district for varying periods.

For the detection of pyrimethamine in urine, 25 ml samples, preserved by the addition of two drops of toluene to each, were sent to the Wellcome Research Laboratories where extracts were examined with thin-layer chromatography.

3. Results

3.1 Schoolchildren

Pyrimethamine 2 mg with sulfadoxine 40 mg fortnightly. Twenty-six schoolchildren were given fortnightly doses of this combination. All were symptomless carriers of malaria, the majority infected with Plasmodium falciparum at the time of the first dose; two weeks later all blood films were negative except for seven with scanty falciparum gametocytes. Thereafter no patent parasitaemia was found in eight children taking regular doses or eight children who had missed one dose; falciparum trophozoites were seen in a blood film from one child who had missed three consecutive doses and gametocytes in another from a child who had missed five doses during the school holidays; otherwise blood films were negative.

White cell counts were done on each subject monthly for the last three months of the trial. All counts were within normal limits and although three had relatively low counts of 3750, 4000 and 5000 per mm^3 at one or other examination; however these counts increased at subsequent checks and there was no suggestion of leucopenia occurring.

Haemoglobin values (see Table 1) increased within the first three months from an average of 12.3 to 14.0 g% and were 12.5 g% at the end of the trial, similar values to those given weekly pyrimethamine.

Splenic enlargement was found in 19 children to begin with, three after three months and in two at the conclusion of the trial. The average dose of pyrimethamine was 0.1 mg/kg body-weight and sulfadoxine 2.1 mg/kg.

Pyrimethamine 25 mg weekly. Twenty-six school children, all symptomless carriers, were given weekly doses and surveyed fortnightly on the same occasions as those given the fortnightly doses of the combination. In three children asexual falciparum parasitaemia remained patent in spite of the repeated pyrimethamine dosage and in these three cases, weekly instead of fortnightly blood films were taken while parasitaemia was patent; details of these infections are given in Table 2. In one child (D3) falciparum trophozoites persisted up to the fifth week and although the next five doses were missed, the infection persisted subsequently in spite of a resumption of regular dosage. Another child (D17) missed the third dose but asexual parasitaemia cleared after the fourth dose to reappear about a month later, despite regular weekly dosage. In the third child (D18) asexual parasitaemia persisted until after the sixth week when it disappeared; but further parasitaemia was apparent about two months later. The presence of pyrimethamine in the urine of all three children was confirmed from urine samples taken in the sixteenth week, prior to dosage with the combination of pyrimethamine 2 mg + sulfadoxine 40 mg instead of pyrimethamine 25 mg. This combination of drugs apparently eliminated parasitaemia in all three, as all blood examinations were negative thereafter.

Nineteen children had enlarged spleens at the start of the trial, six after three months and four at the end of the trial. The average dose of pyrimethamine was 1.2 mg/kg.

3.2 Control group

Thirty-four children who had negative blood films at the time of the first survey were examined again after three months and again at the end of the trial period. Twelve out of 30 examined at three months had positive films (11 P. falciparum, one P. ovale) and at the end of the trial period 11 out of 29 examined were positive (all P. falciparum, three being previously negative). Spleen rates at the time of these surveys were 32, 42 and 41 per cent. respectively. Two children had antimalarial treatment for acute attacks of falciparum malaria.

3.3 Infants and young children

Pyrimethamine 1 mg with sulfadoxine 20 mg fortnightly. Twelve infants or young children were treated for acute attacks of falciparum malaria during the early part of the rains with a single dose of pyrimethamine 1 mg + sulfadoxine 20 mg. Thereafter for five to six months, they received approximately fortnightly the same dose of both drugs, except for two subjects who left the district after one and two months. No detailed follow-ups of the immediate response to the first treatment were made, but in all cases the blood films were negative for asexual parasites although seven showed falciparum gametocytes at the time of the next dose of the combination about two weeks later; subsequently except for scanty gametocytes in one blood film at the third examination, no parasites were seen throughout the period of observation. Leucocyte counts done in the last week of the trial were all within normal limits. Haemoglobin values increased from an average of 8.0 to 11.4 g% over the trial period. The average dose of pyrimethamine was 0.1 mg/kg and sulfadoxine 2.1 mg/kg.

Pyrimethamine 12.5 mg weekly and fortnightly. Twelve infants or young children were treated for acute attacks of falciparum malaria with pyrimethamine 12.5 mg and subsequently given the same dose weekly. After six to eight doses the interval between dosage was increased to fortnightly because it was becoming increasingly difficult to continue with weekly dosage as none of the parents could be relied on to bring their children regularly, and much time and travelling was required to visit them or bring them from their homes. One child under study for immunological purposes was treated with pyrimethamine 12.5 mg for an acute attack of falciparum malaria a week before being registered for inclusion in this series and, when still found to have asexual parasitaemia a week later, was referred to the writer; the child had within the last nine months been treated with pyrimethamine on two occasions, apparently successfully. However, on this occasion the infection persisted with continuing schizogony despite an extra dose of pyrimethamine 25 mg in the fifth week. In the seventh week with asexual parasitaemia still present a dose of the combination of pyrimethamine 1 mg with sulfadoxine 20 mg was given; this appeared to eliminate the infection as no further asexual parasitaemia was found over the subsequent observation period of three months. On the fortnightly regimen one child developed asexual falciparum parasitaemia which, however, disappeared on increasing the dose to 25 mg. Four of the 12 subjects in this series were away or otherwise not available for two months or longer. The average dose of pyrimethamine was 1.1 mg/kg.

3.4 Control group

Twelve infants or young children were treated for acute attacks of falciparum malaria, mostly with pyrimethamine 12.5 mg and their parents asked to bring them for further treatment should they again have fever. In this way all but one, whose parents were unco-operative, were seen again with acute attacks of malaria during the trial period - six with one further attack, four with two and one with three further attacks. Detailed follow-ups were not undertaken so that any development of pyrimethamine-resistant infections was undetected.

4. Discussion

Compared to the many recent investigations on the effect of combinations of sulfonamide with pyrimethamine or other folic acid antagonists in the treatment of overt malaria, particularly their effectiveness in multiple-drug resistant falciparum malaria (Chin et al., 1966; Bartelloni et al., 1967; Herrero, 1967; Sheehy & Reba, 1967), there have been few reports on the effect of such combinations in chemoprophylaxis. The original antimalarial trials of sulfadoxine in the United Republic of Tanzania included a six-week trial of weekly 500 mg doses in schoolchildren with and without pyrimethamine 12.5 mg or 25 mg and another of sulfadoxine 250 mg with and without pyrimethamine 12.5 mg for eight weeks (Laing, 1968a). These trials showed that this combination was effective against pyrimethamine-resistant falciparum malaria and a further trial in West Malaysia showed that similar doses given weekly eliminated chloroquine-resistant P. falciparum and were also effective against P. vivax (Laing, 1968b). The only other published report to date is that of the trial of weekly sulfadoxine or diaphenylsulfone with pyrimethamine in 280 Western Nigerian schoolchildren (Lucas et al., 1969). These regimens were successful in suppressing malaria parasitaemias over a year-long period without serious side-effects and without the emergence of resistant parasites; the doses of sulfadoxine used were 250 and 125 mg in combination with pyrimethamine.

In the Gambian trial the dose of sulfadoxine was only 40 mg in combination with pyrimethamine 2 mg in schoolchildren and half these quantities in the younger children. These doses, by comparison extremely small, had been shown to be more than sufficient for the treatment of heavy falciparum infections in the Gambian indigenes (Laing, 1969, loc. cit.). In view of the smallness of the doses, the emergence of resistant parasites was thought possible and blood films were taken routinely every two weeks from every subject on suppressive regimens. However, no sign of resistance to sulfadoxine with pyrimethamine was found even with fortnightly doses, complete suppression being achieved over the whole trial period of six months. This was not so with weekly pyrimethamine in doses of 25 mg which failed to suppress asexual falciparum parasitaemias in three schoolchildren and in one infant; however, these pyrimethamine-resistant parasitaemias were sensitive to combined sulfadoxine and pyrimethamine.

In the control group of 34 schoolchildren the parasite rate rose from 0 to 40 per cent in three months and was at about the same level at the end of the trial, a low rate for the time of year when it would be more often than not over 60 per cent for this age-group. However, over this wet season there was barely half the usual rainfall so that transmission was almost certainly not as high as usual; nonetheless, as shown in the younger of the two control groups, the possibility of becoming infected was still high as all but the one child who did not co-operate in the trial, after the first treatment, returned with one or more acute attacks of falciparum malaria.

The over-all fall in haemoglobin levels in the schoolchildren over the last three months of malaria suppression, both in those given the combination and those given pyrimethamine alone, is interesting. The removal of patent asexual parasitaemia with consequent haemolysis would stimulate reticulocytosis and red cell regeneration so that an increased haemoglobin level could be expected and this is reflected in the raised levels after three months of suppression. Thereafter, instead of persisting at a high level, a reversion to the same level as that found in the control group appears to have taken place. The reason for such a reversion on the data available is not clear but the finding of the same levels of haemoglobin in both parasitized and non-parasitized groups of children suggests that in the former the rate of destruction of red cells is fully compensated and does not exceed the ability of the host to replace them.

The successful outcome of this trial together with that of the more comprehensive Nigerian trial show that combinations of sulfonamide and pyrimethamine are highly effective in suppressing falciparum malaria in indigenous African populations, more so in fact than by pyrimethamine alone. In neither trial did any side-effects occur, although in the Nigerian trial sulfadoxine may have been responsible for transient anaemia associated with glucose-6-phosphate dehydrogenase deficiency in two children and leucopenia in three children; nor was

there any suggestion that parasites became resistant to drugs in combination. However, bacteriological examination of stool specimens in the Nigerian trial suggested that sulfonamide-resistant strains of Escherichia coli were detected in the first three months of the trial but these did not become more prevalent later. Further investigations on this aspect of the use of sulfonamides in malaria chemoprophylaxis are required and if these show negative results, there would appear to be no objection to the inclusion of sulfadoxine in combination with pyrimethamine among the recommended agents for the prevention of malaria.

TABLE 1. HAEMOGLOBIN VALUES (g/100 ml) BEFORE, DURING AND AT THE END OF THE TRIAL

Group	On 1st treatment			After 3 months			After 5-6 months		
	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.
Pyrimethamine 2 mg sulfadoxine 40 mg fortnightly (6-10 years)	11.0	<u>12.3</u>	13.6	12.4	<u>14.0</u>	15.9	11.1	<u>12.5</u>	14.2
Pyrimethamine 25 mg weekly (6-10 years)	7.5	<u>11.9</u>	13.8	11.6	<u>13.4</u>	15.3	10.8	<u>12.0</u>	14.4
Control (6-10 years)	11.1	<u>12.5</u>	13.9	9.3	<u>12.7</u>	14.5	9.9	<u>12.3</u>	14.8
Pyrimethamine 1 mg sulfadoxine 20 mg fortnightly (1/2 - 3-1/2 years)	5.0	<u>8.0</u>	11.3				10.1	<u>11.4</u>	13.2

TABLE 2. ASEXUAL FALCIPARUM PARASITAEMIAS (per mm³) IN THREE CHILDREN TAKING
PYRIMETHAMINE 25 mg WEEKLY

Subject number	1	5	10	15	20									
	WEEKS													
<u>D3</u>	p 200	p 300	p 100	p 900	p 12 000	p 3 000	p 1 500	p* 2 100	ps* 4 800	p 0	p 0	p 0	p 0	p 0
<u>D17</u>	p 200	p 100	p 1 200	p 16 000	p 0	p 900	p 100	p 400	ps 4 000	p 0	p 0	p 0	p 0	p 0
<u>D18</u>	p 1 600	p 100	p 100	p 300	p 0	p 0	p 0	p 100	ps 300	p 0	p 0	p 0	p 0	p 0

* falciparum gametocytes present

p = pyrimethamine 25 mg ps = pyrimethamine 2 mg + sulfadoxine 40 mg

SUMMARY

The suppressive effect of fortnightly doses of pyrimethamine 2 mg + sulfadoxine 40 mg for six months was compared with that of pyrimethamine 25 mg weekly over the same period in Gambian children aged six to 10 years, all of whom had asymptomatic falciparum parasitaemias. The combination of drugs achieved complete suppression after the first dose whereas three children on weekly pyrimethamine alone had persistent asexual falciparum parasitaemias which were cleared eventually by a dose of the combination of drugs.

Similarly the suppressive effect of pyrimethamine 1 mg with sulfadoxine 20 mg fortnightly for five to six months over the same period was compared with that of weekly, and latterly fortnightly, doses of pyrimethamine 12.5 mg alone in young Gambian children aged six months to three-and-a-half years. Again complete suppression was achieved with the combination but one child on weekly pyrimethamine had persisting falciparum asexual parasitaemia which was eventually cleared by a dose of the combination.

The periodic examination of control groups showed that malaria transmission continued at a high level during the period of the trial.

RESUME

Des essais cliniques effectués en Gambie sur des paludéens "semi-immuns" hospitalisés ont montré que l'administration de pyriméthamine associée à la sulfadoxine ou à la diaphénylsulfone, qui la potentialisent, permet d'éliminer en 2 ou 3 jours une forte parasitémie à falciparum. On a constaté que la quantité de médicaments nécessaire était extrêmement faible : associées à un sulfamide ou à une sulfone, des doses de pyriméthamine n'excédant même pas 0,01 mg/kg de poids corporel venaient à bout d'une parasitémie à formes asexuées. L'extraordinaire efficacité de faibles doses de ces associations de médicaments a conduit à penser qu'elles seraient peut-être utilisables également dans le traitement suppressif.

On a donc comparé l'effet suppressif de doses de 2 mg de pyriméthamine + 40 mg de sulfadoxine, administrées tous les 15 jours pendant 6 mois, à celui de 25 mg de pyriméthamine seule donnée une fois par semaine, pendant le même laps de temps, chez des enfants gambiens âgés de 6 à 10 ans, tous atteints de parasitémie asymptomatique à falciparum. Avec l'association médicamenteuse, on a pratiquement obtenu une suppression totale de la parasitémie après la première dose, alors que chez 3 des enfants traités chaque semaine par la pyriméthamine seule persistait une parasitémie à formes asexuées de falciparum, qu'une dose de l'association médicamenteuse fit, par la suite, disparaître.

De même, on a comparé l'effet suppressif de doses de 1 mg de pyriméthamine + 20 mg de sulfadoxine, administrées tous les 15 jours pendant 5 ou 6 mois, à celui de 12,5 mg de pyriméthamine seule donnée une fois par semaine, puis tous les 15 jours, chez des enfants gambiens âgés de 6 mois à 3 1/2 ans. A nouveau, l'effet suppressif de l'association médicamenteuse a été total, alors que chez un des enfants traités chaque semaine par la pyriméthamine seule persistait une parasitémie à formes asexuées de falciparum, qui disparut après l'administration d'une dose de l'association médicamenteuse.

L'examen périodique de groupes témoins a mis en évidence que le niveau de transmission du paludisme était resté élevé pendant toute la période des essais.

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