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THE EFFECT OF A SINGLE DOSE OF PYRIMETHAMINE AND PRIMAQUINE IN COMBINATION UPON GAMETOCYTES AND SPOROGONY OF LAVERANIA FALCIPARA (= PLASMODIUM FALCIPARUM) IN LIBERIA[1]

by

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[1] "While the term Plasmodium falciparum is in common use the choice between the generic names Plasmodium and Laverania is left open" (International Commission on Zoological Nomenclature, Opinion No. 285). The author prefers to adjust the specific name of the parasite to the feminine generic name Laverania. (Editor's remark)

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Introduction

The arrest of sporogony of L. falcipara following the administration of pyrimethamine, was first reported by Foy and Kondi (1952). Shute and Maryon (1954) showed that following administration of a single dose of 25 mg of pyrimethamine, 74 per cent. of mosquitos still became infected, but that these had fewer oocysts than control mosquitos and that none of the oocysts reached maturity. Jeffery et al. (1956) found that pyrimethamine did not eliminate gametocytes but rendered them non-infective to mosquitos as rapidly as did primaquine. Burgess and Young (1959) showed that administration of a single dose of pyrimethamine was effective within four hours, and that mosquitos fed as late as four days after medication showed only oocysts degenerate. Bray et al. (1959) found, in Liberia, that a single dose of pyrimethamine (25 or 50 mg) prevented the development of sporogony of L. falcipara in A. gambiae, in feeds made up to 28 days after medication. Retarded oocysts, which did not reach maturity, appeared in mosquitos fed as late as 14 days following medication. At the same time, Bray et al. pointed out that the formation of such oocysts with the Liberian strain of L. falcipara in A. gambiae was the exception rather than the rule as in the earlier studies reported.

Non-infectivity and disappearance of gametocytes of L. falcipara following a single dose of pamaquine was reported by Whitmore et al. (1930), Jerace and Giovannola (1933) and Mackerras and Ercole (1949). More recently, Jeffery et al. (1956) found primaquine to act similarly. Burgess and Bray (in press) reported on single dose administration of primaquine from Liberia. In three of eight subjects sporogony proceeded to sporozoite formation in mosquitos fed one day after drug administration, and some of the mosquitos fed two to five days after medication developed degenerate oocysts but failed to show sporozoites. Gametocytes were cleared in four to eight days, with an average of 5.4 days for the series of 11 subjects.

The object of the present investigation has been to study the effect of a combination of pyrimethamine and primaquine, given as a single dose, upon gametocytes and sporogony of L. falcipara.

Materials and methods

All persons investigated were Liberian subjects who attended this Institute's clinic; they had all acquired the infection naturally. Their ages ranged from one year to adulthood (20 to 125 lb. in weight).

Only L. falcipara infections with gametocytes found in the peripheral blood were observed; no asexual parasites were seen. No other species of malaria parasites were seen in any of the subjects. The pyrimethamine and primaquine combination was in the form of tablets, each containing 25 mg and 20 mg (base) respectively, or a smaller tablet containing 12.5 mg and 10 mg (base) respectively. Dosage of the combination was proportional to the patient's weight.

20-50 lb.	12.5 mg	pyrimethamine	+	10 mg	primaquine	base
51-100 lb.	25	"	"	+ 20	"	"	"
101- lb.	50	"	"	+ 40	"	"	"

Gametocyte counts were made prior to medication and at daily intervals thereafter. In several instances, counts were made at more frequent intervals during the first 48 hours after medication. The elimination of gametocytes was confirmed by two negative examinations on consecutive days. At least 5000 leucocytes were counted in all cases before a film was declared negative for gametocytes. In several cases further checks were made periodically over the following month, to determine whether gametocytes had reappeared.

The morphology of gametocytes was studied, both before and after drug administration, in thick and thin films taken in an air-conditioned room and stained with Giemsa stain.

All mosquitos used in these studies were Anopheles gambiae from a laboratory colony maintained at this Institute. They were maintained on sugar solution at room temperature and humidity (72-85°F; 70-100%). Control feeds were made prior to the drug administration. Subsequently mosquitos were fed at daily intervals for up to three days. Mosquitos were dissected between the seventh and tenth days for sporogony studies, and between the 11th and 14th days for the presence of sporozoites.

Results

The results of the treatment of 22 subjects with the combination of pyrimethamine and primaquine are shown in Table I. Of the 22 cases, 19 were children (average age 3.5 years and average weight 28 lb.) who received the minimum dose, i.e. 12.5 mg pyrimethamine and 10 mg primaquine (base). Only one subject received the middle dose (25 mg and 20 mg respectively), and two adults received the full dosage of 50 mg pyrimethamine and 40 mg primaquine (base).

Initial gametocytaemias varied considerably, with the highest being almost 1000 times the lowest. The average initial gametocytaemia for all 22 cases (Table II) was 483. However, this table does not show the maximal gametocytaemias recorded on day 0; in six of seven cases examined gametocytaemias were, some time within the 24 hours following medication, higher than the initial number recorded. If these higher values for day 0 are taken, the initial average gametocytaemia for that day would be 514.

Clearance of the gametocytes is shown in Table II. "Clearance time" refers to the number of days elapsing from the day of medication to the first day on which no gametocytes were found. The average clearance time for the series was five days, with a range of three to eleven days, blood films being taken at 24-hour intervals. Where checked, gametocytes did not reappear in the blood once they had been cleared.

Sporogony was studied in mosquitos fed on ten gametocyte carriers over the first three days following medication. These studies are summarized in Table I. In all, 252 mosquitos fed following medication were dissected; 200 (from eight subjects) were gut dissections, and the rest were for sporozoites in the salivary glands.

The dissections (Table I) showed that the drug combination disallowed sporogony. On one occasion only was a small retarded oocyst found.

Morphological studies on thin films showed that there was no detectable change in the gametocyte morphology following medication. Microgametogony proceeded normally on the first day after medication.

The combination of pyrimethamine and primaquine in a single tablet was uniformly well-tolerated in all patients, and no clinical side-effects were noted or mentioned. Haematological studies were carried out in one case (morphology, M.C.V., M.C.H., M.C.H.C., fragility, icteric-index and Van den Bergh); all findings were normal.

Discussion

The combination of pyrimethamine and primaquine, in the dosage regimen used here, completely disallowed sporogony, and hence transmission, in mosquitos fed one, two and three days after treatment of the gametocyte carrier. At these times, gametocytes are still to be found in the circulation.

The average gametocyte clearance time for the series was five days, with blood films being taken at 24-hour intervals. Twenty-one of the 22 subjects in Table I had initial gametocytaemias lower than 1000 per mm^3. In 18 of these 21 the clearance times were 3-6 days, with an average of 4.1 days.

The 4 subjects with gametocyte clearance times extending beyond 6 days merit separate mention. In one case (S.M.) the initial gametocytaemia was 3895 per mm^3, and the extended clearance time (10 days) is assumed to have been caused by the sheer load of gametocytes present. The remaining subjects in whom clearance time was prolonged all had intercurrent infections. Thus, J.S. (gametocytes cleared by the 8th day) was diagnosed as having a mild encephalomyelitis, which may have been a sequel to vaccination, or may have followed upon a severe M.T. attack 2 days before medication with pyrimethamine and primaquine. M.B. was receiving treatment for active pulmonary tuberculosis; this patient showed no gametocytes from the 11th day after medication. Lastly, W.O. (cleared in 7 days) had a persistent leucocytosis (26 000 initially, decreasing to 14 500) with relative eosinophilia -- for which no diagnosis was established; this subject was cleared of gametocytes by the 7th day.

It would appear that very high gametocytaemias take longer to clear. It would also appear that the rate of gametocyte clearance is influenced by an intercurrent infection of the host. Thus, the rate of gametocyte clearance, provided the dosage

is adequate, is partially a function of the initial gametocytaemia as well as some mechanism within the host. The 3 subjects last cited may be interpreted as showing impairment of the host mechanism.

Bray et al. (1959) found that with a single dose of pyrimethamine alone, sporogony was arrested as of the first day following medication, though gametocytes were not eliminated. On the other hand, when primaquine alone was given, Burgess and Bray (in press) found that in some cases sporogony did proceed normally in mosquitos fed one day after primaquine administration. With primaquine, however, gametocytes were eliminated in an average of 5.4 days (11 subjects). The combination of pyrimethamine and primaquine was found to be highly effective: gametocytes were invariably eliminated and no infection occurred in mosquitos fed on the first day after treatment. The combination thus retains the virtues of each component, and these components may be regarded as having an additive effect when used in combination.

It would seem therefore that the combination of pyrimethamine and primaquine should prove useful in mass drug administration in Tropical Africa where the aim is to sterilize gametocytes and prevent the infection of mosquitos; the use of this drug combination is advisable only in areas where no resistance to pyrimethamine has developed.

Summary

A combination of pyrimethamine and primaquine was administered as a single dose, to gametocyte carriers of Laverania falcipara, in Liberia. Dosages, proportional to the subject's weight, ranged from 12.5 and 10 mg to 50 and 40 mg of pyrimethamine and primaquine (base) respectively. The time of gametocyte clearance from the blood was studied in 22 subjects and sporogony studies were made in 10.

Sporogony was arrested in all mosquitos fed from 1-3 days after medication and on one occasion only was a retarded oocyst found. Gametocytes were cleared in an average of 5.0 days for the series, with a range of 3-11 days.

The results obtained show that the two drugs when given in combination, do not interfere with each other, and may be regarded as having an additive effect.

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TABLE I. THE EFFECT OF PYRIMETHAMINE AND PRIMAQUINE IN COMBINATION UPON THE GAMETOCYTES AND SPOROGONY OF L. FALCIPARA

Subject	Age (in yrs) Sex	Wt in lb.	mg of pyrimethamine and primaquine base	Gametocytes per cu. mm Day 0 [a] (prior to drug admin)	1st day gametocytes absent	Sporogony studies					
						Day of feed [g]	Gametocytes per cu. mm	No. dissected	No. Pos.	% Pos.	No. oocysts average (range)
1. Mw.	7 F	40	12.5 : 10	4 [b]	4 [c]						
2. M.J.	5 F	30	12.5 : 10	23	3						
3. S.D.	2½ F	26	12.5 : 10	79	3						
4. H.Ti.	2½ F	30	12.5 : 10	149 [d]	4						
5. F.G.	Ad. M	125	50 : 40	32	3						
6. C.D.	9 F	50	25 : 20	360 [e]	3						
7. J.S.	3½ M	25	12.5 : 10	689	8						
8. M.D.	3½ F	27	12.5 : 10	82	4						
9. B.G.	2 M	25	12.5 : 10	64	3						
10. H.T.	4 F	30	12.5 : 10	31	5						
11. N.J.	1½ M	25	12.5 : 10	903	5						
12. I.C.	4 M	27	12.5 : 10	256	5						
13 S.B.	2½ F	25	12.5 : 10	700	5	0	700	22	17	77	29 (14-54)
						1	525	10	0	0	
						2	126	10	0	0	
14. M.G.	Ad. F	100	50 : 40	193 [f]	4	0	193	10	8	80	
						1	228	5	0	0	
15. M.M.	9 F	40	12.5 : 10	162	5	0	162	40	36	90	26 (18-44)
						1	81	25	0	0	
						2	18	22	0	0	
						3	18	5	0	0	

TABLE I. THE EFFECT OF PYRIMETHAMINE AND PRIMAQUINE IN COMBINATION UPON THE GAMETOCYTES AND SPOROGONY OF L. FALCIPARA (continued)

Subject	Age (in yrs) Sex	Wt in lb.	mg of pyrimethamine and primaquine base	Gametocytes per cu. mm Day 0 [a] (prior to drug admin)	1st day gametocytes absent	Sporogony studies					
						Day of feed [g]	Gametocytes per cu. mm	No. dissected	No. Pos.	% Pos.	No. oocysts average (range)
16. H.Y.	2¼ M	27	12.5 : 10	631	5	0	631	4	4 [g]	100	63(16-146)
						1	410	9	1*	11	1* [g] ls +ve.
17. P.L.	1½ M	22	12.5 : 10	763	4	0	763	14	10	71	88(32-170)
						1	171	18	0	0	
						2	124	4	0	0	
18. S.M.	6 F	42	12.5 : 10	3895	10	0	3895	22	2	9	10 (8-12)
						1	2720	19	0	0	
						2	1470	8	0	0	
19. M.B.	2½ F	25	12.5 : 10	772	11	0	772	13	13	100	85(24-180)
						1	451	38	0	0	
20. W.O.	1 M	21	12.5 : 10	364	7	0	364	13	12	92	31 (7-87)
						1	154	3	0	0	
21. B.J.	1½ F	20	12.5 : 10	295	6	0	295	8	6	75	3 (1-7)
						1	246	8	0	0	
22. T.P.	4½ F	32	12.5 : 10	185	3	0	185	25	24	96	32 (1-103)
						1	132	68	0	0	
Average				483	5.0						

[a] The day on which drug was administered was day 0

[b] 30 gametocytes, 6 hours after medication

[c] Less than 1 gametocyte to 4000 Lc. on the 3rd day

[d] 417 gametocytes, 6 hours after medication

[e] 707 gametocytes, 6 hours after medication

[f] 246 gametocytes, 30 hours after medication

[g] Mosquitos were fed prior to drug administration on Day 0

* Retarded oocysts

TABLE II. CLEARANCE OF GAMETOCYTAEMIA FOLLOWING ADMINISTRATION OF A SINGLE COMBINED DOSE OF PYRIMETHAMINE AND PRIMAQUINE

Day	No. of Subjects	Gametocytes per mm^3		
		Average	Maximum	Minimum
0	22	483*	3895	4
1	22	326	2720	13
2	22	145	1470	4
3	16	53	375	3
4	10**	43	260	1
5	5	52	150	7
6	4	43	115	3
7	3	14	18	10
8	2	10	10	9
9	2	10	11	9
10	1	6	6	6

* When the maximum recorded gametocytaemia is taken, the average for Day 0 is 514

** 11 subjects were positive, 10 only were examined

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