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THE DEVELOPMENT OF PYRIMETHAMINE RESISTANCE BY *PLASMODIUM FALCIPARUM*

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SYNOPSIS

Sixteen patent *P. falciparum* infections (McLendon and Panama strains) in non-immunes were treated with single doses of pyrimethamine. The schizontocidal and sporontocidal response to the initial dose was rapid.

Seven blood-induced infections, of which three were treated with 100 mg and four with 50 mg, did not relapse. Of seven cases observed after 25-mg treatment, five relapsed.

Delayed treatment of the relapsing infections (Panama strain) with single doses of 25 mg or 50 mg, and subsequently 100 mg, had virtually no schizontocidal or sporontocidal effect. In one case the resistant infection was transmitted by mosquitos to another patient; the subsequent infection was also highly resistant to the drug.

It is concluded that under the experimental conditions of this study resistance to pyrimethamine by *P. falciparum* may occur rapidly after a single dose of 25 mg, being manifested during relapses on the second challenge with the drug. Increasing the drug dosage does not overcome the resistance. The resistant character is readily transmitted by mosquitos.

The development of resistance to pyrimethamine by *Plasmodium malariae* (Young, 1957) and *P. vivax* has been reported (Hernandez et al., 1953; Young & Burgess [1]). These findings led to studies to determine under what conditions such resistance might develop in *P. falciparum*. The results are given in this report.

The McLendon (USA) and Panama strains of *P. falciparum* were induced in 16 Negro neurosyphilitic patients, 15 by the injection of infected blood and one by infected mosquito bites. During the resulting primary attack, a single dose of 25, 50 or 100 mg of pyrimethamine was given. If the infection relapsed, the same dose or twice the previous dose was given.

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[1] See page 27 of this issue.

Blood smears were made daily, the density of parasites being determined by the Earle-Perez method with at least 0.1 ml of blood examined.

Mosquitos, principally *Anopheles freeborni* but some *A. quadrimaculatus*, were allowed to bite the malarious patient before and for several days following the drug administration. In some cases, to judge the rapidity of sporontocidal action mosquitos were fed upon the patient at four-hour intervals for the first day after giving the drug. The details of the procedures are given in the companion paper appearing on page 27 of this issue.

Observations

The linear passages of the two strains are shown in Fig. 1.

FIG. 1. LINEAR PASSAGES OF *PLASMODIUM FALCIPARUM*

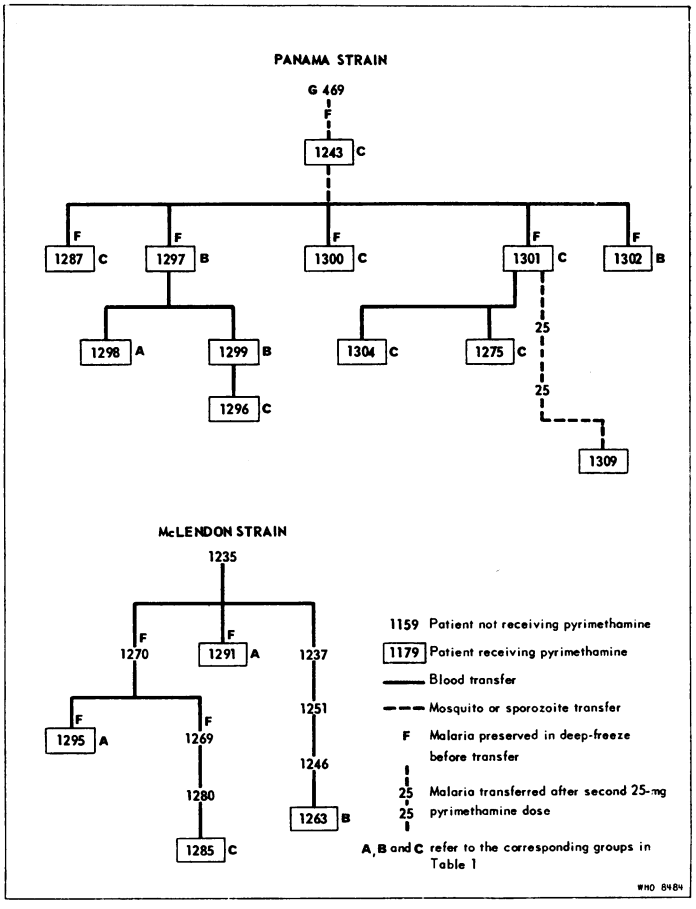


TABLE 1. RESPONSE OF *P. FALCIPARUM* TO INITIAL AND SUBSEQUENT TREATMENT WITH PYRIMETHAMINE

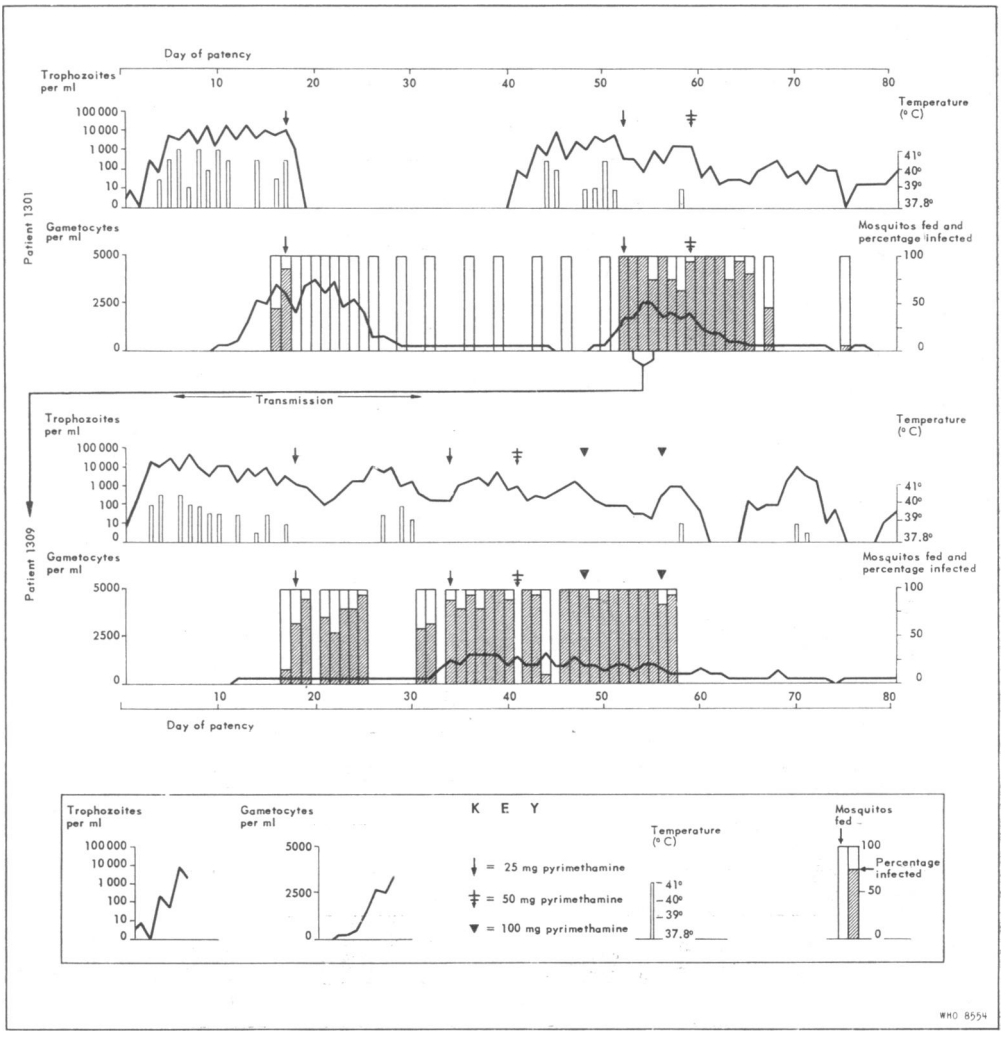
Patients	Contact with drug [a]	Quantity of drug (mg)	Average number of parasites per ml	Percentage of asexual parasites remaining: days after treatment							Days to clear		Number relapsed/ total followed	Days to relapse [c]	Remarks
				1	2	3	4	5	6	7	range [b]	average			
Group A	1	100	5 036	12	<0.5	0	0	0	0	0	2-3	2.7	0/3	—	3 patients (2 McLendon strain, 1 Panama strain)
Group B	1	50	56 539	61	15	<0.5	0	0	0	0	2-4	3.3	0/4	—	4 patients (1 McLendon strain, 3 Panama strain)
Group C	1	25	43 309	9	<0.5	<0.5	0	0	0	0	1-4	2.4	5/7	18-23	8 patients (1 McLendon strain, 7 Panama strain)
1243	2	50	150	1 280	2 400	3 300	4 493	2 040	453	460	c.p.				Group C relapse
1300	2	25	23 280	30	29	45	18	56	6	22	c.p.				Group C relapse
1300	3	50	5 130	1	11	3	1	1	3	2	c.p.				
1300	4	50	2 430	17	3	1	1	0	1	1	c.p.				
1300 [d]	5	50	1 620	7	18	1	3	0	7	7[d]					
1300	6	100	110	40	10	0	0	0	0	0			1/1	18	
1301 [d]	2	25	480	121	17	194	44	440	606	550[d]					Group C relapse
1301	3	50	2 640	2	10	<0.5	2	2	1	5	c.p.				
1309 [e]	3	25	2 680	41	19	7	10	31	107	100	c.p.				Inoculated from patient 1301 after second drug dose
1309 [d, e]	4	25	270	602	835	2 074	559	2 407	322	456[d]					
1309 [d, e]	5	50	1 230	28	46	27	56	94	145	51[d]					
1309 [d, e]	6	100	630	43	17	19	25	6	9	3[d]					
1309 [e]	7	100	470	287	249	66	15	0	0	0	5	5	1/1	9	
				Percentage of gametocytes remaining											
Group A	1	100	793	85	82	83	74	24	79	98					
Group B	1	50	1 033	182	152	158	240	290	378	665					
Group C	1	25	735	92	136	144	172	214	342	435					

[a] Number of times the particular line of parasites had been treated
[b] c.p. = continuous parasitaemia
[c] — = no data
[d] Treated on day 7
[e] Sporozoite-inoculated

The cases initially treated with 100 mg or 50 mg of pyrimethamine responded rapidly, the clinical symptoms and the asexual parasites being eliminated within one to three days. None of the seven cases relapsed (Table 1: Groups A and B).

The eight cases receiving 25 mg initially also showed the same rapid disappearance of symptoms and asexual parasites. However, of the seven cases followed, five relapsed within 18 to 23 days (Table 1: Group C).

FIG. 2. DEVELOPMENT AND TRANSMISSION OF PYRIMETHAMINE RESISTANCE IN *PLASMODIUM FALCIPARUM* (PANAMA STRAIN)



The drug did not prevent either the appearance of gametocytes, if they were absent on the day of treatment, or their increase if present. (Tables 1 and 3; Fig. 2). Frequent application of mosquitos following the various treatments showed that the sporogonous cycle was interrupted, i.e., failure to produce sporozoites, within four hours after the drug was given (Table 2). Some mosquitos fed as late as four days after the drugging had very small oocysts on the gut wall. These were obviously retarded, being about the size of normal four-day-old cysts although the mosquitos had been incubated for eight to ten days. The cell contents were unorganized, indicating degeneration. Only a few were able to develop to a slightly larger size. None ever approached mature stages nor produced sporozoites. The evidence indicates that the oocysts died in the early stages but remained attached to the gut wall.

Three cases (1243, 1300, and 1301) which had relapses after the initial 25-mg dose were again challenged with the drug (Tables 1 and 3). Patient

TABLE 2. EFFECT OF INITIAL AND SUBSEQUENT TREATMENT WITH PYRIMETHAMINE ON SPOROGONOUS CYCLE OF *PLASMODIUM FALCIPARUM* IN *ANOPHELES FREEBORNI*

Patient [a]	Contact with drug [b]	Quantity of drug (mg)	Percentage of mosquitos infected										
			hours before (—) and after (+) drugging										
			—24	—4 to 0	+4	+8	+12	+16	+20	+24	+48	+72	+96
			First treatment										
1287	1	25	77	69	0 [c]	0 [c]	0 [c]	0 [c]	0 [c]	0 [c]	0 [c]	0 [c]	0
1296	1	25	50	40	0	0	0 [c]	0 [c]	0 [c]	0 [c]	0 [c]	0	0
1299	1	50	90	94	0 [c]	0 [c]	0 [c]	0 [c]	0 [c]	0 [c]	0 [c]	0 [c]	0 [c]
1295	1	100		80	0 [c]	0 [c]	0	0	0	0	0	0	0
1301	1	25	45	85						0	0	0	0
			Subsequent treatment										
1301	2	25		100						100	100	75	100
1301	3	50	65	93						100	100	100	75
1309	3	25	15	64						87	70	70	53
1309	4	25		89						80	94	80	100
1309	5	50	87							100	93	10	100 [d]
1309	6	100	100	100						90	100	100	100
1309	7	100	100	87						93	100	93	

[a] All Panama strain, except patient 1295 (McLendon strain)

[b] Number of times the particular line of parasites had been treated

[c] Some mosquitos with abnormal, small, retarded or degenerated oocysts which never matured or produced sporozoites

[d] Reading for 120 hours

TABLE 3. INFLUENCE OF PYRIMETHAMINE TREATMENT UPON PARASITAEMIA AND MOSQUITO INFECTIONS WITH *PLASMODIUM FALCIPARUM* (PANAMA STRAIN)

Drug day	Patient 1300						Patient 1301						Patient 1309					
	parasites per ml			average number of oocysts [a]	gut infection (%)	gland infection (%)	parasites per ml			average number of oocysts [a]	gut infection (%)	gland infection (%)	parasites per ml			average number of oocysts	gut infection (%)	gland infection (%)
	total	asexual	gametocytes				total	asexual	gametocytes				total	asexual	gametocytes			
−1	140	140	0				9 810	6 450	3 360	10	43	50	5 150	5 100	50	2	20	10
0	63 839	63 839	0 [b]				14 075	10 925	3 150 [b]	90	90	80	2 740	2 680	60 [b]	3	80	25
1	220	220	0				3 520	1 320	2 200	R			1 200	1 090	110	5	90	60
2	10	10	0				3 660	0	3 660	R			580	500	80	11	67	63
3	10	0	10				3 750	0	3 750	R			220	190	30	15	70	70
4	350	0	350	0	0		3 060	0	3 060	R			460	280	180	45	30	100
5	640	0	640				3 725	0	3 725	R			1 000	820	180	34	80	75
6	4 930	0	4 930	0	0		2 210	0	2 210	R			2 956	2 856	100	10	90	67
7	8 569	0	8 569				2 764	0	2 764	R			2 710	2 660	50	30	100	80
8	8 703	0	8 703				1 960	0	1 960				8 740	8 710	30			
9	7 760	0	7 760				840	0	840	R			7 850	7 790	60			
10	12 975	0	12 975	R			670	0	670				8 599	8 569	30			
11	7 650	0	7 650				560	0	560				1 090	1 050	40			
12	6 120	0	6 120				130	0	130	R			2 060	2 000	60			
13	4 200	0	4 200	0	0		300	0	300				620	560	60	5	73	20
14	4 290	0	4 290				180	0	180				500	290	210	7	60	67
15	3 900	0	3 900				180	0	180	0	0		930	300	630			
16	2 220	0	2 220				120	0	120				1 530	270	1 260 [b]	87	92	80
17	1 980	0	1 980	2	40	0	60	0	60				2 766	1 626	1 140	19	90	60
18	1 840	680	1 160				100	0	100				3 683	2 255	1 428	27	100	80
19	2 040	1 290	750	0	0		30	0	30	0	0		7 010	5 600	1 410	50	80	80
20	9 650	9 050	600				120	0	120				3 033	1 509	1 524	57	100	70
21	23 390	23 280	110 [b]	0	0		40	0	40				8 110	6 500	1 610	101	100	100
22	7 040	6 930	110	0	0		50	10	40	0	0		1 890	870	1 020	70	100	60
23	6 752	6 752	0	0	0		50	0	50				2 800	1 230	1 570 [c]			
24	10 564	10 564	0	0	0		150	130	20				1 390	340	1 050	82	100	100
25	4 380	4 370	10	0	0		80	60	20				1 640	570	1 070	71	100	80
26	12 995	12 975	20	0	0		3 700	3 690	10	0	0		2 100	330	1 770	4	16	0
27	1 400	1 320	80	0	0		890	860	30				1 800	690	1 110			
28	5 660	5 130	530 [c]	0	0		14 315	14 315	0				2 250	1 150	1 100	73	100	100
29	700	70	630	0	0		400	400	0	0	0		3 213	1 785	1 428	47	100	100

30	800	560	240	0	0		4 510	4 510	0				1 710	630	1 080[d]	105	100	100
31	410	140	270	0	0		1 110	1 110	0				1 160	270	890	124	100	80
32	380	50	330	0	0		8 310	8 250	60				960	110	850	43	100	100
33	500	30	470	0	0		5 350	5 280	70	0	0		1 220	120	1 100	25	100	100
34	640	160	480	0	0		8 160	7 000	1 160				1 300	160	1 140	56	100	100
35	490	90	400	0	0		2 340	480	1 860[b]	50	100	100	470	40	700	45	100	100
36	320	60	260	0	0		2 220[e]	580	1 640	82	100	100[e]	1 090	60	1 030	58	100	100
37	370	270	100	0	0		2 510[e]	80	2 430	216	100	100[e]	870	20	850	45	100	100
38	1 490	1 080	410	1	5		3 490	930	2 560	283	100	40	1 220	470	750[d]	38	80	100
39	6 060	5 760	300				2 070	210	1 860	108	100	100	1 990	1 350	640	23	90	100
40	7 220	7 140	80				4 200	2 100	2 100	29	80	30	1 670	1 170	500	8	100	100
41	5 920	5 810	110	0	0		4 560	2 910	1 650	63	100	38	680	310	370	7	100	80
42	7 820	7 790	30				4 530	2 640	1 890[c]	129	100	75	730	70	660	5	100	80
43	5 365	5 355	10				1 400	60	1 340	25	100	100	310	0	310	3	50	60
44	2 240	2 240	0				1 310	270	1 040	66	100	100	300	0	300	2	50	60
45	9 521	9 521	0	0	0		990	20	970	50	100	100	170	0	170	1	40	20
46	3 390	3 340	50				400	60	340	22	70	80	280	0	280			
47	2 580	2 430	150[c]	0	0		420	60	360	9	100	90	390	220	170			
48	590	400	190	4	50	50	250	30	220	13	100	60	290	70	220			
49	540	80	460	47	50	0	430	130	300				360	140	220			
50	550	10	540	4	70	80	540	300	240	4	70	20						
51	610	30	580	4	40	0	680	420	260									
52	510	0	510	1	10		120	60	60									
53	320	10	310	2	60	40	190	180	10									
54	440	30	410				40	20	20									
55	650	100	550				350	320	30									
56	410	40	370	3	80	50	120	110	10									
57	1 530	930	600				100	100	0									
58	380	200	180				0	0	0	1	8	0						
59	1 800	1 680	120	1	20	33	50	20	30									
60	460	320	140				40	20	20									
61	1 740	1 620	120[c]	1	23	0	20	20	0									
62	160	110	50	0	0		30	30	0									
63	420	290	130	0	0		70	70	0									
64	50	20	30	0	0		70	60	10									
65	90	40	50				50	40	10									
66	20	0	20	0	0		10	10	0									
67	160	120	40				60	60	0									
68	170	110	60[d]	0	0		320	320	0									
69	80	40	40	0	0		160	150	10									
70	60	10[f]	50	0	0		260	250	10									

[a] R = retarded abnormal oocysts
[b] 25 mg pyrimethamine after mosquitos fed
[c] 50 mg pyrimethamine after mosquitos fed
[d] 100 mg pyrimethamine after mosquitos fed
[e] Transmitted by mosquitos to patient 1309
[f] Cleared on day 72 and relapsed on day 86

1243 received 50 mg 19 days after the first dose; the parasitaemia increased from 150 per ml to 6740 per ml four days later. The infection was symptomatic 13 days after the second dose of pyrimethamine, at which time it was terminated, with a normal response, by chloroquine.

Patients 1300 received 25 mg as his second dose of pyrimethamine three weeks after the first dose (Tables 1 and 3). The asexual parasitaemia was not reduced below the clinical threshold. It was not until the sixth dose of pyrimethamine, consisting of 100 mg, was given that the asexual parasites were temporarily eliminated from the blood-stream; they returned 18 days later.

Mosquitos feeding on this patient did not become consistently infected until after the fourth dose of pyrimethamine (50 mg). Just before the drug was given the mosquitos were negative. The following day and subsequently for the next two weeks, they were infected. They became negative following the fifth dose (50 mg) but this could have been due more to the low gametocytaemia than to the drug.

When the parasites returned following the initial dose of drug in patient 1301, they were challenged twice more without being eliminated. The gametocytes became virtually completely resistant to the drug at the second dose (25 mg); mosquitos were infected at a very high rate 24 hours after the second dose was given and subsequently (Tables 1, 2, and 3; Fig. 1).

Mosquitos infected on patient 1301 one and two days after the second dose of pyrimethamine transmitted the infection to patient 1309. This patient received pyrimethamine on five occasions in increasing amounts without much adverse effect on the asexual parasites and with no effect upon the infectivity of the gametocytes (Tables 1, 2 and 3; Fig. 2). Even after the 100-mg doses, the mosquitos were heavily infected. The resistant quality of the infection in this patient appeared to be undiminished from that in the donor patient. The retention of the resistant character through the mosquito transmission is evident.

Discussion

The response of two strains of *P. falciparum* to the first contact with pyrimethamine was very rapid as to both schizontocidal and sporontocidal effects. None of the three cases treated with 100 mg or of the four cases treated with 50 mg relapsed. The sample is not large enough to establish this as representative of the relapse rates with those dosages.

Most of the cases treated with 25 mg relapsed. The parasites tested (all of the Panama strain) showed resistance to the drug on the next contact 19 to 35 days later. In some cases the drug exerted some slight effect; in others little, if any. The sporontocidal effect of the drug appeared to be lost even faster than the schizontocidal effect in contrast to a complete

inhibition within four hours after the first dose. Subsequent to the first dose of drug, increasing the amount up to four times that initially given had no sporontocidal effect and frequently no schizontocidal effect.

These experiments indicate the point where the sporontocidal effect first becomes obvious. Morphologically the gametocytes do not appear to be adversely affected by the drug. They exflagellate as usual and progress in the cycle as far as the early oocyst stage on the mosquito gut. There the first evidence of drug action appears in the form of the loss of organization of the cell contents. Furthermore, most of the oocysts do not enlarge beyond the earliest visible stage, representing three to four days' normal growth. A few became slightly larger but never appeared normal, and mature oocysts were never found. These findings are similar to those of Shute & Maryon (1954), who used a West African *P. falciparum.*

After the resistance appeared, none of these sporontocidal effects occurred. The parasites completed the sporogonous cycle in a normal manner.

Although pyrimethamine exerts a sporontocidal effect upon susceptible gametocytes, it does not appear to prevent their occurrence or greatly to modify their morphology, and it may not reduce their densities (see patient 1300 following first drug dose). It is therefore apparent that the presence of gametocytes in blood smears following pyrimethamine has no significance as to their potential infectivity.

The conditions of these experiments might be duplicated in programmes of mass drug distribution when populations are given pyrimethamine without regard to the intensity of existing parasitaemias.

In our experiments, we found resistance developing when the intervals between doses were less than one month. In East Africa, pyrimethamine-resistant parasites have appeared when the drug was given to populations at six-month intervals (Jones, 1954) and at monthly intervals (Clyde & Shute, 1954). Rollo (1955) suggested that the resistance by *P. falciparum* may have been due to widely spaced (monthly) dosages and that when pyrimethamine is used for continued prophylaxis it should be given at intervals not greater than one week. But Clyde & Shute (1957) found in Tanganyika that even with weekly adult doses of 50 mg resistant *P. falciparum* appeared after five months. On the other hand, it is of interest that in one area in Tanganyika resistance did not appear when school-children were given weekly doses of pyrimethamine for 37 consecutive weeks (Clyde, 1957).

RÉSUMÉ

Poursuivant leurs recherches sur la résistance des parasites du paludisme à la pyriméthamine, les auteurs ont étudié l'effet du médicament sur *P. falciparum.* Seize malades atteints de neurosyphilis, infectés par *P. falciparum* (souches McLendon et Panama) ont été traités par des doses uniques de pyriméthamine. L'effet schizontocide et sporontocide de la première dose a été rapide.

Sept cas infectés par transfusion et traités, trois par 100 mg et quatre par 50 mg, n'ont pas présenté de rechutes. Cinq des sept cas traités par 25 mg ont eu des rechutes.

Le traitement des rechutes avec des doses de 25 mg ou de 50 mg, et plus tard de 100 mg, n'ont eu pratiquement aucun effet schizontocide ou sporontocide. Dans un cas, le parasite résistant a été transmis à un autre sujet par piqûre de moustique. L'infection qui en est résultée a également résisté au médicament.

Les auteurs concluent que, dans les conditions de cette étude, la résistance de *P. falciparum* à la pyriméthamine peut se produire rapidement après une seule dose de 25 mg. Elle se manifeste lors de la seconde administration du médicament, pour le traitement des rechutes. Un accroissement de la dose ne surmonte pas la résistance. Le caractère résistant se maintient chez le *Plasmodium* transmis par piqûre de moustique.

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