

The Effect of a Single Dose of Primaquine on the Gametocytes, Gametogony and Sporogony of *Laverania falciparum* *

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A number of studies have been carried out on the effect of several of the 8-aminoquinolines on gametogony and sporogony of Laverania falciparum, but only very limited work has so far been done on the effect of single doses of primaquine. The authors have sought to repair this omission with a study of 12 subjects showing gametocytes of L. falciparum who were treated with a single dose of 15 mg, 30 mg or 45 mg of primaquine base, according to age. Batches of Anopheles gambiae were fed on these gametocyte carriers before and up to 7 days after treatment.

It was found that a single dose of primaquine clears the blood of crescents in 4-8 days. In 5 out of 8 cases no gland infections were seen in A. gambiae fed on the first day after treatment; in 3 cases, however, sporozoites were found. After the first day following treatment no sporozoite infection of A. gambiae occurred in the 3 cases in which gland dissections were made. Feeding oöcyst-infected A. gambiae on a subject having received primaquine 3 and 4 hours before the feed had no effect on the development of the parasite.

Following treatment, gametogony was apparently normal but oökinetes did not penetrate the gut wall, showed staining differences after 24 hours, and appeared to be dead after 48 hours.

The knowledge that the 8-aminoquinolines eliminate the gametocytes of human malaria parasites is as old as the use of these drugs themselves (Mühlens, 1926). When pamaquine is given daily for three days, the gametocytes of *Laverania* (= *Plasmodium*) *falciparum* are eliminated in half a day to three days in naturally acquired infections in Africa (British Somaliland) (Dick & Bowles, 1947).

It has also long been known that during the time of persistence of gametocytes following pamaquine treatment, *Anopheles* can, in a few cases, still become infected on the first and even on the second day after treatment, *but not on subsequent days* (Barber et al., 1929; Jerace & Giovannola, 1933).

More specifically, a single dose of pamaquine will render the gametocytes of *L. falciparum* non-

infective to *Anopheles* after a maximum of two days and cause their disappearance from the blood within four to eight days (Whitmore et al., 1930; Jerace & Giovannola, 1933; Mackerras & Ercole, 1949). Primaquine was found to act similarly, by Jeffery, Young & Eyles (1956), but only in one trial was a single dose used by these authors. As single-dose treatment combining amodiaquine and primaquine has been used for the treatment of immigrants in some areas of Africa where malaria eradication programmes are under way (Alves, 1958), it seemed desirable to test the effect of single doses of primaquine upon sporogony of *L. falciparum* in a larger number of cases.

There appears to be some disagreement as to the site of action of the 8-aminoquinolines in regard to gametogony and sporogony. Pinto (1930) reported that pamaquine halted microgamete formation in *L. falciparum*. Mudrow-Reichenow (1953) reported that primaquine and pamaquine inhibited the microgamete formation of *P. cathemerium* five hours after administration. Jerace & Giovannola (1933) and Mackerras & Ercole (1949) agree that pamaquine does not always inhibit microgamete formation of

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L. falciparum at various times after administration. Mackerras & Ercole (1949) reported ookinete formation as "delayed" in feeds made nine to fifteen hours after drug administration. Attempts to obtain some more definite information on this point are also reported here.

MATERIALS AND METHODS

Colony-bred *A. gambiae* of a proper age were prepared for feeding by removing all food and water at least ten hours prior to applying them on the gametocyte carrier.

Test subjects with naturally acquired *L. falciparum* infections included five adults and seven children between three and seven years of age.

After the control feeding on day 0, a single dose of primaquine, 15-, 30- or 45-mg base (according to age), was administered to each subject. Subsequent mosquito feedings were made at intervals of one or two days up to the seventh day after treatment. Prior to each feeding gametocyte counts were made (Table 1). Examination for gametocytes continued daily until these had been absent for several days.

Gut dissections were usually started on the sixth or seventh day after the blood meal. If a series of gut dissections was negative for oöcysts, later gland dissections for sporozoites were dispensed with. When gut dissections were positive, later salivary gland examinations were usually made.

In order to determine the effect of primaquine on the developing parasite in the mosquito, a malaria-free adult was dosed with 45 mg of primaquine. Three hours and again four hours after treatment mosquitos with a three-day-old infection of *L. falciparum* (having fed three days earlier on a gametocyte carrier) were allowed to feed on the primaquine-treated subject.

On two occasions microgamete and ookinete production was examined in mosquitos having fed on untreated and on primaquine-treated subjects in order to locate the point of action of the drug.

All incubating mosquitos were kept at room temperature (72°-86°F; 22.2°-30.0°C) and a relative humidity rarely below 74% and fed on sugar-water and corn syrup.

RESULTS

Table 1

The numbers of crescents per mm³ on day 0 before treatment are given in the first line of Table 1.

TABLE 1
GAMETOCYTE COUNTS ON FIRST DAY (DAY 0) AND
SUBSEQUENT DAYS OF PRIMAQUINE TREATMENT

Day	Gametocytes per mm ³			Number of subjects
	High	Low	Mean	
0	2 310	69	618	12
1	2 296	38	475	11
2	1 101	19	352	7
3	435	11	142	7
5	132	22	77	2
7	34	0	17	2

Subsequently are shown succeeding counts made prior to each feeding. On day 1—the day following treatment—the average gametocyte count was diminished very little. Later counts became progressively smaller. The first day on which gametocytes were not found varied from four to eight days after treatment, with an average for eleven subjects of 5.4 days.

Table 2 and Table 3

Tables 2 and 3 show the effects of the primaquine treatment as indicated by developmental failure of the parasite in the mosquito.

While, as shown in Table 1, the gametocyte count remained in general high on the day following treatment, there was, on the other hand, a considerable drop in the percentage of infected mosquitos, as may be seen from Tables 2 and 3. Only three out of eleven subjects were able to infect *Anopheles* (including glands) on day 1 after treatment. One was a pregnant 16-year-old girl who had been given 30 mg and the two others were adults who had received 45 mg each. All three batches of mosquitos exhibited—in addition to normal oöcysts—some of the "retarded" or "disintegrated" oöcysts, the appearance of which is that of a small, abnormal or very young oöcyst; they never develop to maturity. Such oöcysts have been observed and depicted before (Shute & Maryon, 1954; Burgess & Young, 1959).

All gut infections in mosquitos fed on days 2, 3 and 4 after drug administration consisted of the

TABLE 2
GUT INFECTIONS IN MOSQUITOS FED ON FIRST DAY (DAY 0) AND SUBSEQUENT DAYS OF PRIMAQUINE TREATMENT

Day	Guts dissected	Positive guts	Percentage positive	Number of subjects	C.I. ^a		
					High	Low	Mean
0	307	237	77.2	12	9 950	156	4 334
1	316	86 ^b	27.2 ^b	11	3 820	0	467
2	185	1 ^c	0.5 ^c	7	3	0	0.5
3	224	13 ^c	5.8 ^c	7	36	0	5
5	67	1 ^c	1.5 ^c	2	2.5	0	1
7	57	0	0.0	2	0	0	0

^a C.I.= Calculated Index. The calculated number of oöcysts in 100 gut dissections (=average oöcysts per infected gut × percentage of positive guts).

^b Some retarded and degenerate oöcysts present in addition to normal oöcysts.

^c Oöcysts retarded and degenerate.

TABLE 3
GLAND INFECTIONS IN MOSQUITOS FED ON FIRST DAY (DAY 0) AND SUBSEQUENT DAYS OF PRIMAQUINE TREATMENT

Day	Glands dissected	Positive glands	Percentage positive	Number of subjects	C.I. ^a		
					High	Low	Mean
0	154	132	85.7	9	390	112	268
1	133	27	20.3	8 ^b	311	0	78
2	22	0	0.0	2			
3	18	0	0.0	1			

^a C.I.= Calculated Index (see Table 2). Instead of oöcyst numbers per gut the numbers of sporozoites per glands stated as plus signs are used in the C.I.:

+ = 1-10 sporozoites
 ++ = 11-100 sporozoites
 +++ = 101-1000 sporozoites
 ++++ = over 1000 sporozoites

^b All positive glands occurred in feeds from three subjects.

above abnormal oöcysts which are known to produce no gland infections (Table 3).

In order to determine the effect of primaquine on the developing parasite in the mosquito, *A. gambiae* were fed upon an untreated gametocyte carrier. Three days later these mosquitos were divided into three groups:

Group 1 remained unfed as a control.

Group 2 was refed on a malaria-free subject who had received 45 mg primaquine three hours before the feeding.

Group 3 was refed on the same malaria-free subject, who had received 45 mg primaquine four hours before the feeding.

Results of all dissections (guts and glands) were as follows:

	Total positive (%)
Group 1 (control)	34.6
Group 2 (three hours)	38.9
Group 3 (four hours)	51.6

TABLE 4
EFFECT ON SPOROGONY OF *L. FALCIPARUM* IN *A. GAMBIAE* OF PRIMAQUINE GIVEN ON DAY 0
TO CRESCENT CARRIERS

Patient	Age (years)	Prima- quine dose (mg base)	Crescents per mm ²	First day on which crescents absent	Day of feed	Number of guts dissected	Number positive	Percentage positive	Average number of oöcysts per positive gut	Percentage glands positive
(1) C.D.	4	30	1 431	6	0	55	19	34.5	39.2	None diss.
			175		3	22	0	0.0	0	" "
			0		7	21	0	0.0	0	" "
(2) C.D.	16 ^c	30	2 310	8	0	24	22	91.7	31.5	85.7
			2 296		1	23	23	100.0	38.2	77.8
			435		3	43	13	30.2	1.2 ^a	0.0
			132		5	40	1	2.5	1.0 ^a	None diss.
			34		7	36	0	0.0	0	" "
(3) S.W.	7	15	161	6	0	25	20	80.0	54.4	None diss.
			350		1	37	8	21.6	2.5 ^a	0.0
			146		3	30	0	0.0	0	None diss.
			22		5	27	0	0.0	0	" "
(4) L.P.	4	15	257	5	0	29	25	86.2	40.0 ^b	None diss.
			277		1	27	2	7.4	1.5 ^a	0.0
			31		3	28	0	0.0	0	None diss.
(5) S.S.	35	45	133	4	0	23	15	65.2	2.4	46.7
			68		1	37	1	2.7	1.0 ^a	None diss.
			11		3	40	0	0.0	0	" "
(6) F.G.	30	45	708	6	0	18	15	83.3	105.6	91.7
			568		1	14	11	78.6	2.0 ^a	54.5
			539		2	32	0	0.0	0	None diss.
			108		3	29	0	0.0	0	" "
(7) G.L.	7	15	482	6	0	17	17	100.0	99.5	94.7
			332		1	21	5	23.8	2.3 ^a	0.0
			202		2	17	0	0.0	0	None diss.
			85		3	32	0	0.0	0	" "
(8) A.B.	3	15	1 166	No data	0	21	13	61.9	41.4	80
			697		1	34	0	0.0	0	None diss.
			1 101		2	34	0	0.0	0	" "
(9) N.K.	5	30	140	5	0	28	27	96.4	15.2	88.9
			38		1	30	0	0.0	0	0.0
			19		2	39	0	0.0	0	0.0
(10) E.J.	Adult	45	69	5	0	32	31	96.9	13.7	100.0
			39		1	19	1	5.3	1 ^a	0.0
			24		2	10	0	0.0	0	0.0
(11) N.	6	30	181	4	0	11	11	100.0	70.2	71.4
			88		1	35	0	0.0	0	None diss.
			60		2	23	0	0.0	0	" "
(12) K.W.	Adult	45	429	4	0	24	22	91.7	94.6 ^a	95.2
			475		1	39	35	89.7	6.1 ^a	50.0
			520		2	30	1	3.3	1.0	None diss.

^a At least some retarded and disintegrated oöcysts.

^b Average number of oöcysts from only four dissections.

^c Pregnant seven months.

Gland infections were found only in Groups 2 and 3.

Apparently the drug ingested with the blood during the second feeding had no adverse effect on the oöcysts.

Microgamete production appeared to proceed normally after primaquine treatment. The number of oökinetes observed in mosquitos fed before treatment and in those which fed 24 hours after treatment were approximately equal when gametocytaemias were approximately equal. However, in mosquitos fed after treatment, oökinetes were substantially smaller and differed in their reaction to Giemsa stain. Forty-eight hours after feeding, the number of oökinetes in the lumen of the gut of mosquitos fed on primaquine-treated subjects exceeded the number found in mosquitos fed prior to drug administration, and these oökinetes stained pink throughout with Giemsa, no nucleus being visible. Oökinetes from mosquitos fed on untreated subjects stained normally.

DISCUSSION

A single dose of primaquine in the range from 15 mg base for children up to 45 mg base for adults slowly eliminates the gametocytes of *L. falciparum*.

Although the time of elimination may last up to four to eight days, only on the first day after primaquine administration in a few cases were gametocytes still infective to *A. gambiae* and capable of producing sporozoite infections. Only three such exceptions were observed in the reported series of 12 gametocyte carriers treated with a single dose of primaquine (Table 4). In none of the 12 subjects (which included a girl of 16 years, seven months pregnant, who received 30 mg base primaquine) was any toxicity or untoward side-effect noted. No recrudescences of gametocytaemia were seen. Thus it can be said that a single dose of primaquine is effective in eliminating *L. falciparum* gametocytes and preventing infection of mosquitos and therefore is a very useful additive drug for the treatment of malaria infections in immigrant populations entering malaria-free zones where anopheline vectors continue to be present.

The mode of action of primaquine on gametogony and sporogony appears to be similar to that recorded for pyrimethamine by Bray et al. (1959). Microgamete formation in the mosquito seemed to be normal and oökinetes appeared, but these did not find their way to the gut wall and by 48 hours were degenerating.

RÉSUMÉ

Il est connu que l'administration de pamaquine (l'un des antipaludiques du groupe des amino-8 quinoléines) pendant 3 jours consécutifs, fait disparaître les gamétocytes de *Laverania* (= *Plasmodium*) *falciparum* du sang des sujets qui ont contracté le paludisme en Afrique (Somalie britannique). En deux jours au maximum, les gamétocytes deviennent non infectieux et ils disparaissent du sang en 4-8 jours.

Les auteurs ont étudié l'effet d'un autre médicament du même groupe, la primaquine, sur 12 sujets infectés de *falciparum*. Ces malades ont été traités avec une dose unique de 15, 30 ou 45 mg de primaquine base, selon l'âge. On les a fait piquer par des lots d'*Anopheles gambiae* avant le traitement et jusqu'au septième jour après le traitement.

On a constaté qu'une dose unique de primaquine éli-

mine les croissants du sang en l'espace de 4 à 8 jours. Dans cinq cas sur huit, aucune infection des glandes salivaires n'a été observée chez les *A. gambiae* nourris le premier jour après le traitement; cependant, dans trois cas, on a trouvé des sporozoïtes. Au-delà du premier jour après le traitement, aucune infection à sporozoïtes n'est apparue chez *A. gambiae* dans les trois cas où l'on a disséqué les glandes salivaires. On a observé occasionnellement de petits oocystes retardés. En nourrissant des *A. gambiae* infectés d'oocystes sur un sujet ayant reçu de la primaquine 3 et 4 heures avant le repas sanguin, le développement du parasite n'a été affecté en aucune manière.

Après le traitement, la gamétogonie a paru normale, mais les ookinètes n'ont pas pénétré dans la paroi stomacale; ils ont montré des différences de coloration après 24 heures, et ont paru morts après 48 heures.

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