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PLASMODIUM FALCIPARUM MALARIA IN PANAMA RESISTANT TO 4-AMINOQUINOLINE DRUGS*

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Abstract. Fifty Panamanian patients with 69 attacks of *Plasmodium falciparum* malaria were treated with chloroquine and amodiaquine, 4-aminoquinoline drugs. The cure rate was only 22%. Failures were represented by relapses, persistence of the parasitemia, or an increase in the parasitemia following administration of the drug. Some cases were cured when subsequent attacks were treated with the same drug. The high failure rate of 78% indicates resistance. This is the first report of 4-aminoquinoline drug resistance in *P. falciparum* malaria in a country north of Colombia in the Western Hemisphere. Patients given quinine sulfate 2 grams daily for 10 days were cured.

Resistance of human malaria parasites to the 4-aminoquinoline drugs was first suggested by the findings of Moore and Lanier (1961), who reported failures to cure with chloroquine two men who had contracted *Plasmodium falciparum* in Colombia.[1] Conclusive evidence that these strains were resistant to chloroquine was shown experimentally by Young and Moore (1961).[2] Later, these parasite strains were demonstrated to be resistant also to amodiaquine hydrochloride and hydroxychloroquine, closely related members of the 4-aminoquinoline group.[3]

Following this, strains of *P. falciparum* resistant to chloroquine were reported from Brazil, and other South American countries. The nearest to Panama of the reported sites of resistance is at Curiche, on the Pacific coast of Colombia, just below the Panama border.[4] So far as we know, proven resistance to 4-aminoquinoline drugs has not been reported in countries north of Colombia in the Western Hemisphere.

Previous work with Panama strains both in naturally acquired cases[5,6] and in experimental infections[7] has never produced solid evidence of 4-aminoquinoline resistance. There has been a considerable number of *P. falciparum* malaria cases treated in Panama during the past several years with various indications of drug resistance.

The responses to treatment of several hundred cases of *P. falciparum* malaria have been studied by us in Panama. From this, enough reliable information has been obtained to show definitely that resistance to chloroquine and amodiaquine drugs is present in local strains of these parasites.

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MATERIALS AND METHODS

The patients were from several sources but primarily the nearby Children's and Santo Tomás Hospitals. Some of the patients were referred to the Gorgas Memorial Laboratory clinic by physicians or came in voluntarily from the field study areas.

Thick blood films were stained by the Giemsa technique. The number of parasites per mm^3 of blood was calculated by the Earle-Perez method.[8] The parasites found in a volume of blood representing 0.1 mm^3 were counted and the number converted to parasites per mm^3. Generally, when no parasites were found in the first 0.1 mm^3 of blood examined the entire thick film was examined; if positive for parasites, this was expressed as less than 10 per mm^3 of blood.

Usually patients were examined daily during the 1st week after treatment. As many of the patients were in crowded hospitals, it was not possible to keep them for long observation periods. Thus the number of daily blood examinations varied. Upon release, the patients often would not return regularly to the laboratory for follow-up examinations. Sometimes they would return when the malaria recurred, but often not on the 1st day or so of the renewed clinical attack. In many cases the patients did not return for subsequent examinations after the 1st week, so that often the true relapse or failure rate could not be determined accurately.

The drugs used were chloroquine phosphate, amodiaquine hydrochloride, and quinine sulfate.

TABLE 1

P. falciparum *response to chloroquine and amodiaquine in 50 patients*

Attack treated	Number of patients with adequate follow-up of 28 days*			
	Treatment	Failure	Cure	Failure (%)
CHLOROQUINE				
Adults				
1st	5	4	1	80
2nd	2	2	0	100
Subtotal	7	6	1	86
Children				
1st	9	8	1	89
2nd	3	2	1	67
Subtotal	12	10	2	83
TOTAL	19	16	3	84
AMODIAQUINE				
Adults				
1st	21	17	4	81
2nd	9	8	1	89
3rd	1	1	0	100
4th	1	1	0	100
Subtotal	32	27	5	84
Children				
1st	15	9	6	60
2nd	2	2	0	100
5th	1	0	1	0
Subtotal	18	11	7	61
TOTAL	50	38	12	76
Combined total of chloroquine and amodiaquine rates:				
Chloroquine	19	16	3	84
Amodiaquine	50	38	12	76
GRAND TOTAL	69	54	15	78

* Chloroquine + amodiaquine: Adult dose, 1.5 g base in 3 days; children, 37.5 mg/kg total in 3 days.

The chloroquine and amodiaquine tablets contained 150 mg of the base. Because of the difficulty in administering tablets, children were given amodiaquine in a sweet liquid suspension (Basoquin®) containing 30 mg of the base per cc. The routine adult dose of the drugs was 1.5 g total of the base given over a 3-day period, equivalent to 25 mg/kg for a 60 kg adult. Single 600 mg doses were given on days 1 and 2 and 300 mg on day 3. Because of the impression that children require higher doses of the drug than adults, they were given total doses over 3 days amounting to 37.5 mg per kg. On the 1st and 2nd days they received single doses of 15 mg/kg each and on the 3rd day 7.5 mg/kg. Quinine sulfate was given to some cases. The adult base was 650 mg three times daily for 10 days. Doses to children were reduced according to weight.

Blood smears were taken at 24-hour intervals following the first dose of drug.

Failure is defined as persistance of parasitemia following treatment or the reappearance of parasitemia after a temporary disappearance. In cases where the parasitemia disappeared, the patients were examined at intervals over a 28-day period and in some instances longer. Adequate follow-up is designated for those patients followed at intervals for at least a 28-day period. As some of the cases were not followed for 28 days, the total attacks treated are greater than those with adequate follow-up. Cure is defined as absence of parasitemia for a period of 28 days or longer after treatment.

RESULTS

One hundred six persons with a total of 139 attacks of *P. falciparum* malaria were treated with chloroquine or amodiaquine. Sufficient data for evaluation were obtained from 50 patients with 69 attacks of malaria (Table 1). The failure rate for chloroquine was 84%. Although the children were given relatively higher doses of chloroquine by weight, the failure rate was similar to the adult rate.

TABLE 2

Susceptibility and grades of resistance according to the WHO scheme. Response of first attacks only

Response*	Chloroquine		Amodiaquine		Total		
	Adults	Children	Adults	Children	Adults	Children	Total
S	1	1	4	6	5	7	12
RI	4	4	6	8	10	12	22
RII		4	7	1	7	5	12
RIII			4		4	0	4
	5	9	21	15	26	24	50

* S, Parasitemia cleared in 7 days, no relapse; RI, parasitemia cleared in 7 days followed by reappearance; RII, parasitemia reduced but not cleared; RIII, no marked clearance or an increase of parasitemia.

TABLE 3

RIII type of P. falciparum *resistance to amodiaquine*

Patients	Days after amodiaquine treatment started						Days after quinine treatment started	
	0	1	2	3	4	5	To clear	Follow-up
	Parasites per mm³							
AF14	6,640	10,550	3,950	5,350	1,650	6,110*	4	33
AF18	260	280	1,510	100	1,730	260*	5	55
AF21	6,560	4,860	350	8,650	260*		4	26
AF23	101,590	890	745,920*				6	20

* Quinine treatment started.

The failure rate for amodiaquine was 76% and was higher in adults than in children. The responses of second and subsequent attacks to treatment varied; some were cured, others were not.

Criteria for determining resistance were first suggested by Young and Eyles in 1963.[9] These were modified variously to a scheme developed by World Health Organization[10] to show grades of resistance from RI, lowest level of resistance, to RIII, highest level of resistance. A compilation of our results according to these grades show the following percentages of the responses of the 50 first attacks: RI, 44; RII, 24; RIII, 8; Sensitive (S), 24 (Table 2).

Of particular interest are the four adults with RIII response to the initial treatment (Table 3). These were all re-treated within 6 days or less following the initial treatment with amodiaquine because of the increasing severity of the illness of the patients.

Patient AF14 had about the same number of parasites on day 5 as on day 0 (1st day of therapy). AF18 had about 7 times as many parasites on day 4 as on day 0. AF21 had more parasites on day 3 than day 0. Patient AF23 had a 7-fold increase of parasites to the very dangerous level of about 750,000 per mm³ on day 2.

There were 14 adults and 2 children who, following failures with chloroquine and/or amodiaquine, were treated with quinine on their second, third or fourth attacks (Table 4). The adults received 2 g quinine sulfate salt per day, in 3 divided doses, for 10 days; the children lesser amounts according to weight.

Six were followed for 12, 14, 20, 23, 26, and 27 days, respectively. The other 10 were followed from 33 to 81 days. None relapsed during the follow-up observation period and the quinine treatments appeared to be curative.

DISCUSSION

Malaria has been endemic in Panama for several hundred years. The prevalence of the various species has varied from time to time. Until recently, in the central area of Panama, near the Canal Zone, most of the malaria has been caused by *Plasmodium vivax* infections. Surveys which

TABLE 4

Responses to quinine of relapses or failures after previous chloroquine and/or amodiaquine treatments

Patient	Parasites/mm³	Days	
		To clear parasitemia*	Followed
ADULTS			
Treatment of second attack			
AF12	120	4	37
AF14†	6,110	4	33
AF15	100	4	67
AF16	190	3	53
AF18†	260	–	55
AF19	270	–	72
AF21†	260	3	26
AF22	250	3	14
AF23†	745,920	6	20
C25	3,580	6	81
C31	140	–	73
Treatment of third attack			
AF11	190	4	23
AF13	360	–	27
AF18	260	–	55
CHILDREN			
Treatment of second attack			
CAF1	2,730	7	46
Treatment of fourth attack			
CAF6	270	3	12

* Blood not examined daily during the week after treatment.
† Had received amodiaquine shortly before. See Table 3.

have been done for many years in the villages along the Chagres river by the staff of the Gorgas Memorial Laboratory have shown that *P. falciparum* infections were scarce or absent since 1960.

P. falciparum infections have increased recently in this area.[11] Clinical cases began to appear in 1969 and many were reported in 1970. The proportion of *P. falciparum* to total cases increased from 2.3% in 1969 to 64.2% in 1970.

It soon became apparent that some of the treated cases were responding inadequately to the 4-aminoquinoline drugs, or appeared to be relapsing. However, the difficulty of long-term follow-up and even of supervision of the drug administration caused considerable delay in acquiring a large number of cases with dependable observations.

In the 50 patients studied, the failure rates of the attacks treated with the 4-aminoquinoline drugs were high. Of the first attacks, in about one-third the parasitemias were not eliminated from the blood streams. About one-half of the total number treated showed a reappearance of the parasites after a temporary clearance.

A comparison of the responses to chloroquine can be made between the present results and those obtained some 15 years ago. A strain of *P. falciparum* from El Limón, Gatún Lake, near the Transisthmian highway in Central Panama was studied in 1956.[7] This malaria was induced in 23 mental patients for the treatment of neurosyphilis. The response of the infections to 1.5 g of chloroquine was rapid, all of the parasitemias being cleared within 2 to 5 days, averaging 3.5 days. Only one relapsed, giving a cure rate of 94%. This is in contrast to the present study where only 2 of the 15 first attacks were cured, giving a cure rate of 13%.

The nearest to Panama of the previously reported sites of resistance of *P. falciparum* to 4-aminoquinoline drugs is Curiche, Colombia some 24 km south of the Panama border on Humboldt Bay on the Pacific coast. Resistance was found there in 1967.[4]

One of the earliest indications of chloroquine resistance in Panama was in our Patient CF1 in April 1969. This man contracted his malaria at Santa Fe, Darien Province, Panama about 160 km northwest of Curiche, Colombia and 160 km east of Panama City. He relapsed twice after 1.5 g chloroquine, at 36 and 43 days, respectively. He was cured with quinine sulfate, 20 g total over a 10-day period, as indicated by a follow-up period of 52 days.

About this time, cases of *P. falciparum* also began to occur along the Transisthmian highway in Central Panama near Panama City. Some of these early cases were not cured by the chloroquine treatment, which led to the present study.

In some of our present cases, the initial treatment with a 4-aminoquinoline drug did not cure but subsequent treatments with the same drug did cure. An interesting example was a child (CAF10). The first attack was treated with amodiaquine, the next three attacks with other drugs, and the fifth attack again with amodiaquine. The follow-up of the latter over 78 days without a recurrence of parasites indicated a cure.

The subsequent cures with drugs that previously failed suggests that the age of the infection plays a part in the cure rate of these resistant malarias. This might be related to the increasing amount of immunity, presumably as a result of the multiple attacks.

In the 1956 study,[7] one patient whose malaria was induced by infected mosquitoes had a relapse after 47 days following treatment with 1.5 g of chloroquine under conditions where reinfection could not have occurred. This relapse was never satisfactorily explained and may indeed be the first documented failure of a 4-aminoquinoline drug to cure *P. falciparum*. This strain of malaria came from a town on the Transisthmian highway near the locale of the present outbreak of *falciparum* infections which are showing drug resistance. Although interesting to speculate, it is not possible to determine whether the present resistant strains moved northward from the known resistant areas in Colombia or if their origin was in the area of Panama where the El Limón strain studied in 1956 came from, the two most logical possibilities.

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