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RICHARD N. ROSSAN, MARTIN D. YOUNG, AND DAVID C. BAERG
Gorgas Memorial Laboratory, P.O. Box 2016, Balboa Heights, Canal Zone

Abstract. Three standard antimalarial compounds were tested against trophozoite or sporozoite induced infections of the Panamanian Achiote strain of *Plasmodium vivax* in two species of monkeys. In *Saimiri sciureus* (24 subjects) and *Aotus trivirgatus* (11 subjects), parasite clearance from the peripheral blood averaged 3 days after initiating chloroquine therapy (total dose of 25 mg base/kg body weight over 3 days or single dose of 10 mg base/kg). Trophozoite induced infections were cured in all of 10 *Saimiri* and all of 6 *Aotus*, as indicated by the absence of relapses. Relapses did occur in 3 of 11 tests with *Saimiri* and 3 of 5 tests with *Aotus* against sporozoite induced infections. Subpatent periods ranged from 38 to 111 days among intact and splenectomized hosts. This is the first chemotherapeutic evidence for the persistence of exoerythrocytic stages of *P. vivax* in New World monkeys. Pyrimethamine (single dose of 1 mg/kg) cured trophozoite induced infections in all of five *Saimiri* hosts. Radical cure of sporozoite induced infections was accomplished in each of six trials with chloroquine (25 mg base/kg) plus primaquine (1 mg base/kg for 14 days). The primary attack or relapse was treated. These models warrant further investigation in chemotherapy.

With the advent of the adaptation and transmission of *Plasmodium vivax* to *Aotus trivirgatus* (the night monkey),[1,2] this new model and others became available for diverse investigations in malaria. Of particular importance is that the evolution of these systems has offered the opportunity to study the chemotherapeutic response of human malarias to standard, as well as experimental, antimalarial compounds in a small easily manipulable host, rather than directly in man.

Panamanian squirrel monkeys, *Saimiri sciureus* (= *oerstedii*), recently shown to be susceptible to infections induced by trophozoites or sporozoites of *P. vivax*,[3,4] served as the primary host in the current investigations. *Aotus* monkeys were utilized in limited companion trials.

The objectives of these studies, incorporating the new models, were to establish the following: 1) curative activity of the blood schizontocides, chloroquine or pyrimethamine, against trophozoite induced infections; 2) response of sporozoite induced infections to chloroquine alone; and 3) radical curative properties of chloroquine plus a tissue schizontocide, primaquine, against sporozoite induced infections.

MATERIALS AND METHODS

All data were acquired with the Achiote strain of *P. vivax*, passaged in New World monkeys since 1966.[5] Citrated blood, containing 10^7 parasites from the homologous donor species, was inoculated intraperitoneally for each trophozoite induced infection.

Sporozoites were obtained via infected *Anopheles albimanus* subsequent to feeding only on *Aotus*, as *Saimiri* are poor hosts for infecting mosquitoes.[4] Sporozoites were introduced by the interrupted bite technique (*Saimiri*) or by intravascular inoculation (*Aotus*). No attempts were made to standardize the sporozoite inoculum, as all those available were used; the number of positive mosquitoes per recipient ranged from 5 to 142 ($\bar{x} = 45$), with average gland sporozoite densities between 100 and 1,000.

Routinely, blood films were obtained daily throughout the test periods. Parasites were stained with Giemsa and enumerated by the Earle-Perez method. Trophozoite induced infections were treated when the parasitemia first

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TABLE 1

Chemotherapy of trophozoite induced infections of Plasmodium vivax *(Achiote strain) in unaltered* Saimiri sciureus

Experiment no.	Monkey no.	Treatment initiated: Patent day	Treatment initiated: Parasitemia per mm³	Response: Days to clear	Response: Negative days examined*
		Chloroquine (10, 10, 5 mg base/kg in 3 days)			
1	5885	3	1,100	3	52
	6409	5	1,040	4	175
	5881	7	1,160	2	15
	5890	7	1,760	2	21
	5882	8	940	2	67
	5900	8	3,850	3	73
		Chloroquine (10 mg base/kg in 1 day)			
2	5895	5	1,120	2	126
	5896	6	1,160	3	38
	5886	7	1,070	3	194
	6386	8	1,590	1	161
		Pyrimethamine (1 mg/kg in 1 day)			
3	5203	6	1,190	6	176
	5879	6	2,610	6	24
	5986	6	3,060	5	17
	5988	7	4,210	5	72
	5982	13	5,150	4	11

		Untreated controls: Primary attack: Patent period (days)	Primary attack: Maximum parasitemia per mm³	Relapse(s): Subpatent period(s) (days)	Negative days examined after final patent period
1	5892	24	87,130	40	103
	5876	39†	69,420	–	–
2	5897	24	40,280	12 ; 33	23
	5898	55	76,530	–	22
3	5921	33†	37,540	–	–
	5889	45	93,210	17 ; 31	105

* No relapses occurred in treated monkeys.
† Died during patency after maximum parasitemia.

approximated 1,000 per mm³, usually during the 1st week of patency, although some of these infections were treated during the 2nd week at higher parasitemias. Drug therapy was initiated for sporozoite induced infections also during the ascending phase, at parasitemias of at least 1,000 per mm³, except in two *Aotus* (6252 and 6894); these infections were at lower levels (after peak parasitemia) when treated. All drugs were given orally via gastric tube. Chloroquine and primaquine were administered as the diphosphate salt. One or more months after treatment of the primary attack, splenectomy was done to provoke parasite relapse in sporozoite induced infections.

The test monkeys were of Panamanian origin and free of naturally acquired malaria, as shown by examination of preinoculation blood films. Aspects of the husbandry of *Saimiri* and *Aotus* have been discussed previously.[4, 5]

RESULTS

No evidence of drug toxicity at the highest dosages used was seen in uninfected *Saimiri* and *Aotus* as measured by body weight change and overt host activity.

TABLE 2

Chemotherapy of trophozoite induced infections of Plasmodium vivax *(Achiote strain) in unaltered* Aotus trivirgatus

Monkey no.	Treatment initiated		Response	
	Patent day	Parasitemia per mm³	Days to clear	Negative days examined*
	Chloroquine (10, 10, 5 mg base/kg in 3 days)†			
6109	8	1,560	3	40
6120	5	7,750	5	28
6124	6	3,180	3	56
6013	6	4,410	3	283
6012	7	2,560	3	314
5546	14	20,670	2	44

* No relapses occurred in treated monkeys.

† Two additional *Aotus* served as untreated controls. They died on patent days 30 and 49 and showed maximum parasitemias of 72,330 and 45,330 per mm³, on days 8 and 10, respectively.

Trophozoite-induced infections

At the level of inoculum used, the prepatent periods in *Saimiri* ranged from 1 to 25 days ($\bar{x}$ = 6 days).

With a total chloroquine dose of 25 mg base/kg of body weight or 10 mg base/kg, parasite clearance from the peripheral circulation in *Saimiri* occurred within 1 to 4 days (Table 1). There was essentially no difference between the rate of clearance with either treatment. No relapses* were then seen during observation periods extending to 175 and 194 days, respectively.

After pyrimethamine in *Saimiri* at 1 mg/kg, parasites disappeared from the peripheral blood more slowly (4 to 6 days), and none was detected during subsequent observation periods of up to 6 months.

Normal infections and multiple attacks occurred among inoculated, untreated subjects serving as controls in each of the above experiments. Subpatent periods for the first relapse ranged from 12 to 40 days. These relapse patterns were comparable to those previously reported by us for unaltered *Saimiri*, in which the infections relapsed one or more times in 19 of 28 (68%) monkeys and showed a mean primary subpatent period of 29 days.[4]

Among the *Aotus*, prepatent periods ranged from 1 to 6 days ($\bar{x}$ = 3 days).

The higher dosage of chloroquine (25 mg base/kg) against the infections in *Aotus* (Table 2) gave results similar to those obtained for *Saimiri*. Parasite clearance was effected in 2 to 5 days and there were no relapses during subsequent periods reaching 314 days.

While both control *Aotus* died during patency, a compilation of relapse frequencies for the Achiote strain in this host species (unpublished data) showed at least one relapse to occur in 48 of 106 (45%) intact, surviving monkeys, with an average primary subpatent period of 19 days. From 2 to 7 relapses occurred in 12 hosts, with subpatent periods of 3 to 85 days.

Sporozoite induced infections

The prepatent periods in *Saimiri* hosts varied from 13 to 42 days ($\bar{x}$ = 30 days).

For radical curative studies with *Saimiri* (Table 3), parasite clearance was effected with either 25 or 10 mg/kg of chloroquine within 2 to 6 days, except the infection in one monkey (6270) which required 13 days. At the higher dose, 2 animals (6840 and 6969) experienced relapses. Both had been splenectomized during the post-treatment period, with parasite reappearance 54 to 84 days thereafter. One intact host (6270) of 2 which received the lower chloroquine dose, relapsed after 46 days. Blood films from 4 of the remaining 8 *Saimiri* were negative during a follow-up period exceeding the longest interval to relapse (114 days), and the infections were presumably cured. The other 4 animals were not followed long enough to determine if cured.

The relapses in 6840 and 6969 and the primary

* The term relapse indicates a renewed manifestation of malaria infection, whether initiated from surviving erythrocytic or exoerythrocytic forms. This is in accord with the Report of a Drafting Committee, *Terminology of Malaria and of Malaria Eradication*, W.H.O., Geneva, 1963.

TABLE 3

Chemotherapy of sporozoite induced infections of Plasmodium vivax *(Achiote strain) in* Saimiri sciureus

Monkey no.	Treatment initiated: Patent day	Treatment initiated: Parasitemia per mm³	Response and post treatment examination, Days from initiation of treatment to: Parasite clearance	Splenectomy	Relapse	End of test
			Chloroquine (10, 10, 5 mg base/kg in 3 days)			
6840	7	1,080	3	30	114	130
6969	15	7,530	3	45	99	107
6873	18	7,620	4	29	–	362C*
7166	20	1,800	5	35	–	288C
5915	10	1,030	5	63	–	537C
6296	15	7,620	3		–	611C
6963	11	7,260	4	–	–	30†
6975	15	4,290	3	–	–	16†
5912	11	1,010	6	–	–	18†
			Chloroquine (10 mg base/kg in 1 day)			
6270	19	1,260	15	–	59	125
6298	13	1,870	2	–	–	18†
			Chloroquine (10, 10, 5 mg base/kg in 3 days) plus primaquine (1 mg base/kg in 14 days)			
6840R‡	14	24,680	6	NA§	–	368C
6969R	9	1,430	5	NA	–	187C
6392	11	1,670	2	56	–	251C
6844	17	1,380	3	58	–	209C
6374	16	2,670	4	59	–	563C

Untreated controls

	Primary attack: Patent period (days)	Primary attack: Maximum parasitemia per mm³	Relapse(s): Subpatent period(s) (days)	Negative days examined after final patent period
5984	30	17,620	15; 17; 34; 35; 50	597
4792	30	260	8; 51; 3	654
6577	87	7,680	43	211
6964	30	2,300	18	†
4767	15	<10	40	531
7173	35	840	–	37
4685	17	<10	–	449
5198	14	<10	–	357

* C, cured.
† Died.
‡ R, relapse treated.
§ NA, not applicable—previously splenectomized.

attack in three intact *Saimiri* were treated with chloroquine (25 mg/kg) plus primaquine (1 mg base/kg for 14 days); the latter animals were splenectomized approximately 2 months after the initiation of treatment. The infections did not recur throughout the test periods of 187 to 563 days. This indicates radical cure.

Five of the 8 untreated (control) infections in the intact *Saimiri* relapsed from 1 to 5 times (Table 3). Of these relapsing infections, the primary attack in 2 monkeys was low grade (<1,000 parasites per mm³) and the relapses also showed low maximum parasitemias. The subpatent periods following the primary attack ranged from 8 to 43 days, thus being shorter than in the treated *Saimiri* (but similar to those seen in the untreated control trophozoite induced infections—Table 1).

In comparative studies with *Aotus*, the prepatent periods ranged from 8 to 29 days ($\bar{x} = 20$ days).

Chloroquine against these infections in *Aotus*

TABLE 4

Chemotherapy of sporozoite induced infections of Plasmodium vivax *(Achiote strain) in* Aotus trivirgatus

Monkey no.	Treatment initiated		Response and post treatment examination Days from initiation of treatment to:			
	Patent day	Parasitemia per mm³	Parasite clearance	Splenectomy	Relapse	End of test
Chloroquine (10, 10, 5 mg base/kg in 3 days)						
6252	31	360	6	52	84*	320
6090	8	29,000	2	275	–	332C†
6092	12	28,910	3	–	41	50
6092R‡	9	1,340	1	–	91*	483
6894	21	90	2	–	–	30§
Chloroquine (10, 10, 5 mg base/kg in 3 days) plus primaquine (1 mg base/kg in 14 days)						
5798	6	3,080	3	61	–	188C
Untreated control						
	Primary attack		Relapses			Negative days examined after final patent period
	Patent period (days)	Maximum parasitemia per mm³	Subpatent periods (days)			
6151	29	45,380	5; 22; 36; 49			39§

* Each animal experienced additional relapses during the test period. Subpatent periods were as follows: 6252—18, 21, 24, 7; 6092R—28, 15, 18, 85, 56.
† C, cured.
‡ R, relapse retreated.
§ Died.

(Table 4), eliminated the parasitemias in 1 to 6 days. In contrast to the other experiments, the parasite densities in this series upon initiation of treatment varied considerably, from 90 to 29,000 per mm³. These differences in parasite levels did not influence the number of days required for clearance. Relapses appeared in 1 of 2 splenectomized and 1 of 2 intact subjects. In the first monkey (6252), the relapse occurred 52 days after splenectomy. No additional treatment was given, and the animal experienced four more relapses during the 320-day observation period. The intact monkey (6092) sustained two relapses, each after chloroquine therapy. Treatment was not repeated and five other relapses occurred during the 483-day test period.

The primary attack in a fifth *Aotus* (5798) was treated by the combined chloroquine-primaquine regimen. This monkey was splenectomized 2 months later. No parasites were seen during the 188-day observation period.

The infection in the untreated intact control *Aotus* relapsed four times, and showed subpatent periods within the usual ranges seen for trophozoite induced infections.

DISCUSSION

There are no references in the literature on the experimental chemotherapy of sporozoite induced infections of vivax malaria in monkeys. One report has presented the activity of blood schizontocides against the New Guinea Chesson and Vietnam-Palo Alto strains of *P. vivax* in *Aotus*.[5] Cure of trophozoite induced infections was achieved by a total dose of 17.5 mg/kg of chloroquine or 4.4 mg/kg of pyrimethamine (Chesson).

In our experiments with the Achiote strain in *Saimiri* monkeys, a total dose of chloroquine base as low as 10 mg/kg cured trophozoite induced infections. Pyrimethamine cured Achiote *P. vivax* in *Saimiri* monkeys at a single dose of 1 mg/kg, which is approximately ¼ of that previously reported for Chesson vivax in *Aotus*.[5]

Vivax relapses did occur after chloroquine alone was administered against sporozoite induced infections in both *Saimiri* and *Aotus*. Parasitemias reappeared in 6 of 16 tests. Since the trophozoite stages are eliminated by chloroquine at the dosages used, these findings are the first chemotherapeutic evidence for the persistence of exo-

erythrocytic stages of *P. vivax* in New World monkeys.

Apparent radical cure was achieved with the chloroquine-primaquine regimen in each of 6 trials. All attempts to provoke relapse failed. Moreover, the sporozoite concentrations, used for infecting the subjects treated with the combined therapy, were comparable with those used to infect monkeys administered chloroquine alone and which showed subsequent relapse.

The results thus far with chloroquine and pyrimethamine correspond to those obtained in man. Primaquine at four times the usual dose in man radically cured the infections. However, further testing is warranted to verify the efficacy of the 8-aminoquinoline in these systems. Future trials with this drug should approximate the human dosages. It appears that both species of monkeys can serve as models for drug investigations.

As a corollary, this study has provided information on the relapse characteristics of the Achiote strain. In *Saimiri* and *Aotus*, the first relapse of treated sporozoite induced infections appeared from 125 to 159 days and 63 to 139 days, respectively, after inoculation. These ranges are similar to those reported by Contacos et al.[7] for another Panamanian vivax strain in human volunteers after chemotherapy, viz. 94 to 226 days. The persistence of infection, as shown by parasitemias occurring after inoculation as long as 483 days for *Aotus* and 308 days for *Saimiri*, demonstrate the need for long term survival of test monkeys for complete evaluation of chemotherapeutic agents.

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