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CHLOROQUINE-RESISTANT PLASMODIUM FALCIPARUM FROM VIET NAM

by

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Preliminary studies have been carried out upon a strain of Plasmodium falciparum from Viet Nam. This strain is referred to as the Viet Nam (Sn.) strain. Captain Sn. (34 years old, Caucasian), United States Marine Corps, had been stationed for almost one year near Nha Trang in South Viet Nam. He had been receiving 300 mg of chloroquine base weekly and had at no time been in Thailand. While stationed in Viet Nam, he developed his first acute clinical attack of malaria in late August 1962. On 27 August 1962, thick blood smears revealed asexual erythrocytic forms of P. falciparum. Subsequently, "standard dose" (1500 mg chloroquine base administered over three days) or greater-than-standard oral regimens of chloroquine were administered on three occasions: during the last week of August 1962, during the third week of September 1962, and during the first week of October 1962. The second course of chloroquine consisted of 2700 mg of chloroquine base administered over seven days (600 mg initially, 300 mg six hours later, and 300 mg daily for the next six days). On each occasion, administration of chloroquine resulted in a temporary abatement of symptoms and of overt parasitaemia. However, recrudescences (fever and positive blood smears) occurred two to three weeks after each of the first two courses of therapy. During the third week in October 1962 (two weeks after the third course of chloroquine) the patient was transferred to the United States Naval Hospital, Great Lakes, Ill., United States of America.¹

¹ We wish to express our appreciation to the members of the United States Armed Forces, particularly those of the United States Navy at the United States Naval Hospital, Great Lakes, Ill., United States of America, whose co-operation made possible initiation of these studies.

On 5 November and 6 November 1962 (four weeks after the third course of chloroquine) fever recurred and blood smears revealed asexual erythrocytic forms of P. falciparum. The studies presented in this report were initiated on 7 November 1962 by inoculation of infected blood from this patient into non-immune inmate volunteers at Stateville Penitentiary, Joliet, Ill., United States of America.

During the second and third weeks of November 1962, the patient (Captain Sn.) received 1935 mg of quinine sulfate (six doses of five grains each) daily for ten days and 50 mg of pyrimethamine daily for three days, concurrently. Radical cure resulted as evidenced by (1) no recrudescence of malaria in the patient and (2) subinoculation, on 5 December 1962, of 450 ml of the patient's blood into a non-immune volunteer who then failed to develop malaria during a follow-up period of three months.

Results of the chemotherapeutic studies carried out with volunteers are summarized in Table 1. The volunteers were healthy adult American males between 22 and 42 years old (mean age: 31 years); they weighed between 60 and 91 kg (mean weight: 73 kg). Two were Negroes; 18 were Caucasians. The non-immune status of every volunteer was established by excluding from the study; (1) all volunteers who, prior to entering the institution, had a history of malaria or of an illness even remotely suggesting malaria; (2) all volunteers who were born in or who had ever lived in or visited a malarious area; and (3) all volunteers who had previously had falciparum malaria as a part of studies at the institution. Methods of study are described in detail elsewhere (Alving, Craige, Pullman, Whorton, Jones & Eichelberger, 1948; Powell, Brewer, Alving & Millar, 1963). Thick blood smears were prepared and stained by the method described by Earle & Perez (1932); the asexual erythrocytic forms of P. falciparum contained in 0.1 mm^3 of blood were counted by examination of 90 oil-immersion fields (each 0.1 mm^2) and the counts were expressed in terms of 1.0 mm^3 of whole blood. Parasite counts noted in this report refer to trophozoites, not gametocytes; mature schizonts were very rarely seen. Drugs were given orally (with the exception of chloroquine administered intramuscularly to one volunteer) and under close supervision. Doses of all drugs are expressed in terms of free base. Samples of urine from volunteers

receiving chloroquine were examined frequently for the presence of chloroquine by the method of Haskins (1958). During the study it was at times necessary to administer small doses of quinine, 270 or 540 mg of quinine base, to prevent dangerously high levels of parasitaemia (e.g., several days after administration of chloroquine). Quinine gives a positive Haskins test; for this reason, Haskins tests were not done after quinine had been given to a volunteer who had received chloroquine.

Six volunteers were treated, during acute clinical attacks of malaria (when parasitaemia and fever of at least 38.5°C were present) with standard oral regimens of chloroquine (diphosphate) recommended for therapy of acute attacks in non-immune adults (600 mg chloroquine base the first day, 300 mg six hours later, and 300 mg on each of the next two days). The total dose of chloroquine base was 1500 mg. Chloroquine exerted remarkably little therapeutic effect. Fever and parasitaemia decreased transiently in five of the six volunteers, but in each of these five volunteers a lower-grade fever persisted and thick blood smears remained positive. In one volunteer, parasitaemia increased during administration of chloroquine. At the outset of therapy, counts of asexual erythrocytic forms (trophozoites) of P. falciparum in samples of blood from each of the six volunteers were 280, 720, 1310, 1560, 2220 and 16 960 per mm^3 , respectively. Forty-eight hours after initiation of chloroquine administration, the respective counts were 30, 440, 6400, 820, 1860 and 13 420 per mm^3 . All six volunteers had acute clinical attacks of malaria one to four days after completion of the administration of chloroquine. Haskins tests for the presence of chloroquine in the urine were negative prior to drug administration and strongly positive both during and after administration of chloroquine (when acute clinical attacks of malaria were occurring).

One volunteer received, during an acute clinical attack, a three-day course of chloroquine (diphosphate) administered orally in doses twice those listed above (total dose: 3000 mg chloroquine base). Fever (39°C initially) decreased, but a lower-grade fever (38 to 38.5°C) persisted. At the outset of therapy, the parasite count was 1000 per mm^3 . Thick blood smears proved negative on the third and fifth days after initiation of therapy, but overt parasitaemia recurred on the sixth day

after initiation of therapy. During the subsequent four days, the parasite count increased to 2270 per mm^3 and spiking fever (39°C) recurred. Haskins tests were negative prior to therapy; they were strongly positive both during chloroquine administration and six to ten days after initiation of therapy (at the time of recrudescence).

One volunteer was treated, during an acute clinical attack, with 240 mg of chloroquine base (300 mg of chloroquine dihydrochloride), thrice daily for three days, administered intramuscularly. The total intramuscular dose was 2160 mg of chloroquine base. Fever (40°C initially) decreased and temperature returned to normal five days after initiation of therapy. Parasitaemia (parasite count initially 230 per mm^3) decreased and thick blood smears proved negative three days after initiation of therapy. However, 10 days after initiation of therapy, thick blood smears again proved positive and, during the next two days, rapidly increasing parasitaemia (to 1010 per mm^3) and spiking fever (to 40.5°C) ensued. Haskins tests were negative prior to and strongly positive during and 10 days after administration of chloroquine.

These data indicate that asexual erythrocytic forms of the Viet Nam (Sn.) strain of P. falciparum are highly resistant to chloroquine.

The asexual erythrocytic forms of the Viet Nam (Sn.) strain of P. falciparum do not respond to the usual dosages of hydroxychloroquine, amodiaquine, mepacrine and proguanil (Table 1). These drugs were administered orally in doses identical to those employed in a previous study of a strain of chloroquine-resistant P. falciparum from Thailand (Powell, Brewer, Alving & Millar, 1963). Doses of these schizontocidal drugs that effect radical cure of infections with susceptible strains of P. falciparum failed to eliminate infections with the Viet Nam (Sn.) strain. Administration of these drugs resulted, at best, in only a temporary reduction in fever and parasitaemia. In addition, the asexual erythrocytic forms of the Viet Nam (Sn.) strain are resistant to 377-C-54 (Table 1), a hydroxy-naphthalene known to exert a schizontocidal effect against certain strains of P. falciparum and P. vivax in humans (Bruce-Chwatt & Charles, 1951; Singh, Ray, Sen Gupta & Misra, 1959) and against a strain of chloroquine-resistant P. berghei in mice (Hawking & Gammage, 1962).

The administration of pyrimethamine, 50 mg daily for three days, resulted in a radical cure of the infections in each of eight volunteers treated during acute attacks. Oral administration of 1620 mg of quinine base (three doses each consisting of ten grains quinine sulfate) daily for four days failed to effect radical cure of the infections in four volunteers (Table 1). Administration of this dose of quinine daily for seven days failed to effect radical cure in two of four volunteers. In contrast, ten-day courses of quinine (1620 mg base daily) effected radical cure of the infections in each of five volunteers. The relative tolerance of the asexual erythrocytic forms of the Viet Nam (Sn.) strain of P. falciparum to quinine is currently being investigated further.

The characteristics of the Viet Nam (Sn.) strain of chloroquine-resistant P. falciparum differ from the characteristics of other strains of chloroquine-resistant P. falciparum reported upon previously. A strain of chloroquine-resistant P. falciparum from Colombia, South America, has been studied extensively and has been found to be susceptible to proguanil, pyrimethamine and quinine (Moore & Lanier, 1961; Young & Moore, 1961; Young, 1961; Young, 1962; Powell, Brewer & Alving, 1963). The asexual erythrocytic forms of the Viet Nam (Sn.) strain do not respond to proguanil, so that this strain differs in this respect from the strain from Colombia. The asexual erythrocytic forms of a strain (JHK) of chloroquine-resistant P. falciparum from Thailand have been found to be resistant to all widely-used antimalarials except quinine (Young, Contacos, Sticher & Millar, 1963; Powell, Brewer, Alving & Millar). The asexual erythrocytic forms of the Viet Nam (Sn.) strain are sensitive to pyrimethamine and thus the Viet Nam (Sn.) strain differs in this respect from the (JHK) strain from Thailand.

Preliminary studies on the prophylactic effect of CI-501 against the Viet Nam (Sn.) strain of P. falciparum have been completed. CI-501 (4,6-diamino-1-(p-chlorophenyl)-1,2,-dihydro-2,2-dimethyl-s-triazine pamoate), a pamoic acid salt of a highly active (dihydrotriazine) metabolite of proguanil, is a new experimental drug of great interest (Thompson, Olszewski, Elslager & Worth; Schmidt, Rossan & Fisher). A single intramuscular dose (5 mg per kg) of CI-501 has been shown to protect volunteers against repeated challenges of *Chesson vivax* malaria for six

or more months (Coatney, Contacos, Elder & Kilpatrick). A single intramuscular injection of CI-501 (350 mg) was administered to three non-immune volunteers on 14 February 1963. One volunteer (weight 80 kg) received a preparation containing 25-micron-sized particles in an aqueous vehicle (X-9149); the second volunteer (weight 91 kg) received a preparation containing 25-micron-sized particles in a lipid vehicle (X-9150); and the third volunteer (weight 72 kg) received a preparation containing 1- to 5-micron-sized particles in a lipid vehicle (X-8607). A fourth individual who received no drug served as a control; this individual received a preparation consisting of only the aqueous vehicle for CI-501 (X-9164). Twenty days following administration of CI-501, each of the four volunteers were bitten (at the same time) by the same 10 Anopheles stephensi mosquitos heavily infected with the Viet Nam (Sn.) strain of P. falciparum. All four volunteers developed patency nine days after "bite". Acute clinical attacks followed. The course of parasitaemia in the three volunteers who received CI-501 did not differ significantly from the course of parasitaemia in the control individual treated with the aqueous vehicle. At the time of patency, the drug was present in the urine of the three volunteers who had received CI-501 (24-hour levels of urinary excretion were 8.6, 2.3 and 2.9 mg, respectively¹).

Conclusions

The asexual erythrocytic forms of a strain of Plasmodium falciparum from Viet Nam have been found to be resistant to standard therapeutic doses of chloroquine, hydroxychloroquine, amodiaquine, mepacrine and proguanil. Administration of these drugs during acute attacks of malaria resulted only in a temporary decrease in fever and parasitaemia. Radical cure of infections with this strain of P. falciparum was achieved, in each of eight volunteers, by the administration of 50 mg of pyrimethamine daily for three days. Radical cure was achieved in only two of four volunteers who received 1620 mg of quinine base (30 grains of quinine sulfate) daily for seven days. CI-501 did not exert a demonstrable causal prophylactic effect against the Viet Nam (Sn.) strain of P. falciparum in experiments in which this drug was administered in doses comparable to those reported to be effective against repeated challenges of *Chesson vivax* malaria.

¹ These determinations were carried out through the courtesy of Parke, Davis & Company, Research Laboratories, Ann Arbor, Michigan.

TABLE 1. VIET NAM (SN.) STRAIN OF PLASMODIUM FALCIPARUM
EFFECTS OF DRUGS AGAINST ASEXUAL ERYTHROCYTIC FORMS
(TREATMENT DURING ACUTE CLINICAL ATTACKS)

Drug	Dose mg base (orally)		Dura- tion of therapy (days)	No. treated	Therapeutic effects ^a			
	Daily	Total			No effect	Temporary effect		Radical cure
						Partial	Complete	
Chloroquine		1 500	3	6	1	5		
		3 000	3	1			1	
	720 ^b	2 160	3	1			1	
Hydroxychloroquine		1 500	3	1		1		
Amodiaquine		1 400	3	2		2		
Pyrimethamine	50	150	3	8				8
Mepacrine		2 198	7	2			2	
Proguanil	261	2 610	10	3		1	2	
377-C-54		1 500	3	1		1		
		2 500	3	1			1	
Quinine ^c	1 620	6 480	4	4			4	
	1 620	11 340	7	4			2	2
	1 620	16 200	10	5				5

^a Temporary partial effect = temporary reduction in fever or parasitaemia attributable to drug without occurrence of both normal temperature and negative blood smears.

Temporary complete effect = temporary reduction in fever and parasitaemia attributable to drug with transient occurrence of both normal temperature and negative blood smears.

Radical cure = complete eradication of parasites from the body as evidenced by a lack of recrudescence during a follow-up of at least 60 days.

^b Administered intramuscularly

^c 1620 mg quinine base = 1935 mg quinine sulfate (30 grains)

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