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Research Article

STUDIES ON THE CHEMOTHERAPY OF THE HUMAN MALARIAS. II. METHOD FOR THE QUANTITATIVE ASSAY OF SUPPRESSIVE ANTIMALARIAL ACTION IN FALCIPARUM MALARIA [1, 2, 3]

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INTRODUCTION

It has been demonstrated in vivax malaria that the therapeutic response to quinine is closely related to the mean plasma quinine concentration achieved during a standard testing procedure (1). In addition, the susceptibility of the erythrocytic phase to quinine has been shown to be a stable characteristic of the McCoy strain of *P. vivax* and to differ quantitatively from that of the Chesson strain.

The human malarias due to the two other common species of plasmodium differ from vivax malaria in their biological and clinical characteristics and in their responses to chemotherapeutic agents. Therefore, it seemed advisable to determine their susceptibilities to quinine as a basis for the comparative assay of other antimalarials. However, infections due to *P. malariae* proved to be impractical for therapeutic testing because of their long incubation periods, the low density of peripheral parasitemia, and the somewhat erratic course of the disease. Systematic studies were discontinued in view of these difficulties, together with the lesser importance of quartan malaria.

The present paper is concerned with studies of the susceptibility to quinine of the erythrocytic phase of two strains of falciparum malaria. All patients used in this study were neuro-syphilitics who presented no medical contraindication to therapeutic malaria. In general, the patients were those whose racial extraction or history of previous malaria made them unsuitable as experimental subjects in the vivax studies.

BLOOD-INDUCED MCCLENDON FALCIPARUM MALARIA

Falciparum (McClendon) [6] malaria, induced by an intravenous inoculum of 500,000 erythrocytic parasites, is characterized by a prepatent period of from two to 12 days (average five days). This is followed by an irregular, sustained fever and the rapid development of peripheral parasitemia which frequently reaches 50,000 per cu. mm. or higher by the second or third day after the first appearance of parasites in thick film preparations. In general, the onset of fever coincides with the first appearance of parasites in thick blood films. Only rarely does a patient achieve a parasite count as high as 10,000 per cu. mm. without fever during a primary attack.

Because excessive parasitemia and prolonged fever constitute a hazard to life in falciparum malaria, no deliberate attempt was made to observe

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[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and New York University.

[2] A portion of this work was presented at the meetings of the Federation of American Societies for Experimental Biology, March 11–15, 1946, Federation Proceedings, 1946, 5, 216.

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[6] The McClendon strain of *P. falciparum* utilized in these studies was obtained from Doctor Martin D. Young.

the natural course of the disease. However, the invasive nature of McClendon falciparum and its potential virulence were attested by the facts that parasites in excess of 500,000 per cu. mm. were not uncommon, in spite of the early administration of antimalarial therapy, and that one patient died with what was presumed to be cerebral malaria.

Therapeutic interruption early in the course of the infection, as with McCoy vivax, may modify a second exposure to the disease. The onset of fever during recrudescence or following reinoculation usually coincides with a higher parasite density than is the case with the primary attack. The development of tolerance to the parasite in secondary or reinoculation infections may be interpreted as an indication of immunity acquired during the primary attack.

Among 285 individuals who were inoculated with McClendon falciparum, only two failed to develop a primary attack of malaria. Furthermore, the immunity which developed during the initial attack or during the subsequent observation period was in no case sufficient to interfere with either the anticipated clinical recrudescence or a prompt development of a clinical attack following reinoculation. This is presumably attributable to the early stage of the infection at which therapy was administered.

Routine of therapeutic tests

Each subject was inoculated with 500,000 parasites from an untreated patient in the first or second day of fever. Thick blood films were examined daily throughout the period of observation and parasite counts were obtained when indicated.

Therapy was started on the first or second day of fever over 101° F., or when parasitemia reached 50,000 per cu. mm. in the absence of fever. Quinine was administered with dosage regimens designed to maintain a stable plasma concentration for a standard number of days. Each course of therapy was initiated by a priming dose and continued with smaller doses at four- to six-hour intervals. All doses are recorded in terms of quinine base. Blood samples for the estimation of the plasma quinine concentration were obtained at sufficiently short intervals to permit an appraisal of the mean concentration each day. The mean plasma drug concentrations referred to throughout this paper are the averages of the individual daily mean concentrations.

The observation period subsequent to therapy began on the day following the last effective plasma drug level. In the event of the complete disappearance of parasites and fever, the follow-up period was extended to at least 21 days, this interval being necessary to include the majority of renewals of clinical activity due to persistence of an erythrocytic phase. If, at the end of this period, there had been no evidence of renewed activity, reinoculation with one million parasites was performed and the patient followed until the recurrence of parasitemia. Therapeutic results were classified in three groups, as previously described for vivax infections (1).

The susceptibility of blood-induced McClendon falciparum malaria to quinine

The first series of observations involved the administration of quinine to 15 patients with blood-induced falciparum (McClendon) malaria (Table I). Stable plasma quinine concentrations were maintained for four days. Mean plasma quinine concentrations ranged from 2.1 to 10.4 mg. per liter. However, dosage regimens close to the upper limit of tolerance, when restricted to four days, result only in temporary therapeutic effects (Class II).

Therefore, therapeutic tests were performed in an additional 13 subjects in whom the period of effective plasma quinine concentration was extended to six days (Table II). Mean plasma drug concentrations ranged from 2.9 to 8.7 mg. per liter. Mean plasma quinine concentrations of 5.6 mg. per liter or higher, maintained for six days, consistently produced a permanent disappearance

TABLE I

The relationship between dosage and plasma concentration of quinine and therapeutic effect in four-day tests against blood-induced McClendon falciparum malaria

Patient	Daily dose	Mean plasma quinine concentration	Class of therapeutic effect		
			I	II	III
	grams (base)	*mg./L*			
You	1.50	10.4		x	
Jac	0.72	9.0		x	
Mor	1.50	8.3		x	
Ray	0.72	8.1			x
Ber	0.72	5.9		x	
Val	0.30	4.8		x	
Jun	0.15	4.7		x	
Jup	0.20	4.2		x	
Mor	0.30	3.6		x	
Yow	0.15	3.6		x	
Whi	0.15	3.5		x	
Gar	0.10	3.3		x	
Lun	0.20	3.2		x	
Sew	0.15	2.6	x		
Hop	0.15	2.1		x	

TABLE II

The relationship between dosage and plasma concentration of quinine and therapeutic effect in six-day tests against blood-induced McClendon falciparum malaria

Patient	Daily dose	Mean plasma quinine concentration	Class of therapeutic effect		
			I	II	III
	grams (base)	*mg./L*			
Mus	0.36	8.7			x
Asb	0.70–1.5	7.6			x
Rag	0.36–0.70	7.3			x
Mit	1.00–1.50	6.8			x
Lee	0.36–0.55	5.9			x
McC	1.00–2.10	5.8			x
Car	0.55	5.6			x
Cha	0.30	5.4		x	
Gri	0.50–0.80	5.0		x	
Gra	0.46	4.5			x
Wil	0.30	3.2		x	
Har	0.30	3.1		x	
Gre	0.30	2.9		x	

of parasites and fever (Class III). Plasma quinine concentrations between 2.9 and 5.4 mg. per liter resulted in five Class II effects and one Class III.

Further evidence of the importance of the duration of therapy in evaluating the quinine susceptibility of the erythrocytic phase of McClendon falciparum was obtained by a study of six patients in whom plasma quinine concentrations were maintained for eight days (Table III). Here, the mean concentrations ranged from 3.2 mg. per liter to 7.2 mg. per liter. In all but one, a permanent interruption of the disease was obtained. In spite of the limited number of therapeutic tests performed, it is apparent that the therapeutic response to quinine in McClendon falciparum malaria is a function of the duration of therapy as well as the plasma quinine concentration.

TABLE III

The relationship between dosage and plasma concentration of quinine and therapeutic effect in eight-day tests against blood-induced McClendon falciparum malaria

Patient	Daily dose	Mean plasma quinine concentration	Class of therapeutic effect		
			I	II	III
	grams (base)	*mg./L*			
Yat	0.72	7.2			x
Tai	0.72	6.1			x
Peo	0.72	4.1			x
Tho	0.72	4.1			x
Pow	0.72	4.1		x	
Ban	0.72	3.2			x

BLOOD-INDUCED COSTA FALCIPARUM MALARIA

The variation in quinine-susceptibility between different strains of vivax malaria (1) prompted an examination of the antimalarial effect of quinine against another strain of *P. falciparum*.[7] Sixteen patients were inoculated with Costa falciparum. The course of fever and parasitemia of Costa falciparum malaria is similar to that of the McClendon strain. In general, neither excessive parasitemia nor prolonged fever occurred as frequently in Costa falciparum infections as in McClendon malaria. Except for the strain of parasite used, the therapeutic testing procedure was the same as that previously described (1). Plasma quinine concentrations in the first group were maintained for six days.

Examination of the data in Table IV reveals that quinine administered for six days in nearly maximum tolerated doses does not generally produce a permanent interruption of the disease (Class III effect). There is no consistent re-

TABLE IV

The relationship between dosage and plasma concentration of quinine and therapeutic effect in six-day tests against blood-induced Costa falciparum malaria

Patient	Daily dose	Mean plasma quinine concentration	Class of therapeutic effect		
			I	II	III
	grams (base)	*mg./L*			
Hun	2.10	14.8		x	
Hen	2.40	13.5		x	
Riv	1.55	12.6			x
McN	2.40	12.0		x	
Ton	1.55	11.6		x	
Bon	1.55	11.1		x	
Gre	1.00	10.5			x
Ell	2.40	9.9		x	
Moo	1.55	8.2		x	
Alv	0.77	8.2		x	
McN	1.55	7.3			x
Smi	0.77	7.0			x
Pug	0.40	5.1		x	
Bat	0.40	4.4		x	
Hen	0.40	4.1		x	
Mor	0.40	3.8		x	

[7] The Costa strain was kindly furnished by Doctor Robert B. Watson. The strain was originally isolated by Doctor Mark F. Boyd.

TABLE V

The relationship between dosage and plasma concentration of quinine and therapeutic effect in eight-day tests against blood-induced Costa falciparum malaria

Patient	Daily dose	Mean plasma quinine concentration	Class of therapeutic effect		
			I	II	III
	grams (base)	*mg./L*			
Car	1.55	12.0			x
Chu	0.77	7.7		x	
Mar	0.77	6.5		x	
Lug	0.77	5.4			x
Ham	0.77	2.8	x		

lationship between plasma drug concentration and therapeutic effect. An exploratory study of five patients in whom plasma quinine concentrations were maintained for a period of eight days (Table V) presents similarly inconsistent results. It may be concluded that the resistance of the Costa strain to the suppressive antimalarial action of quinine is greater than that of the McClendon strain.

MOSQUITO-INDUCED COSTA FALCIPARUM MALARIA

Three patients were infected by the bites of *A. quadrimaculatus* mosquitoes whose salivary glands contained sporozoites of the Costa strain of *P. falciparum*. In other respects, the procedure of the therapeutic test was the same as that previously described for mosquito-induced vivax malaria (1).

The data are limited, but the results (Table VI) indicate a high degree of quinine resistance of the erythrocytic phase of the mosquito-induced disease, similar to that observed with the same strain when induced by the inoculation of infected blood.

TABLE VI

The relationship between dosage and plasma concentration of quinine and therapeutic effect in eight-day tests against mosquito-induced Costa falciparum malaria

Patient	Daily dose	Mean plasma quinine concentration	Class of therapeutic effect		
			I	II	III
	grams (base)	*mg./L*			
Kir	1.6	13.0		x	
Kih	1.6	10.2		x	
Cop	1.6	8.3			x

DISCUSSION

A standard testing procedure, modified in certain respects from that utilized for McCoy vivax malaria (1), was employed in determining the quinine-susceptibility of the McClendon strain of *P. falciparum*. This strain has a greater resistance to the suppressive antimalarial action of quinine than the McCoy strain of *P. vivax*, in terms of the plasma quinine concentration, oral dosage, or duration of therapy required to produce a given therapeutic effect. The difference between the McClendon strain of *P. falciparum* and the Chesson strain of *P. vivax* is less marked. Within the falciparum species, it has been demonstrated that the quinine-susceptibility of the erythrocytic phase differs significantly in different strains of the parasite. This evidence constitutes a quantitative expression of the clinical experience of many observers of falciparum malaria in endemic areas (2).

It is pertinent to note that resistance to the action of quinine is not necessarily related to the severity or virulence of the clinical disease. Costa falciparum, although more resistant to quinine than the McClendon strain, is nevertheless far less hazardous to life.

It is not intended that the appraisal of drug activity in blood-induced falciparum should replace studies utilizing vivax malaria. The two procedures are complementary in that they yield information on the comparative effectiveness of an antimalarial agent in both types of infection. It should be appreciated that, due to an apparent lack of a persisting tissue phase in falciparum malaria, the termination of the erythrocytic phase is tantamount to a cure.

CONCLUSIONS

1. The therapeutic response of blood-induced McClendon falciparum malaria to quinine has been shown to be related to the plasma quinine concentration and the duration of therapy, both of which factors are amenable to quantitative definition.

2. Therefore, blood-induced malaria due to this

strain should provide a suitable test object for the quantitative appraisal of the relative suppressive activities of antimalarial agents.

3. As in vivax malaria, the quinine-susceptibility of the erythrocytic phase differs in various strains of *P. falciparum*. Costa falciparum infections are more resistant to quinine than are those due to the McClendon strain.

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