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The Haemolytic Effect of Various Regimens of Primaquine with Chloroquine in American Negroes with G6PD Deficiency and the Lack of an Effect of Various Antimalarial Suppressive Agents on Erythrocyte Metabolism*

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In view of the fact that increased resistance to drugs by malaria parasites in some parts of the world may lead to increasing use of a combination of primaquine with chloroquine for chemotherapy, studies were made on the severity of the haemolytic anaemia induced by 45 mg primaquine in American Negroes with glucose-6-phosphate dehydrogenase deficiency. It was found that twice-weekly administration of primaquine induced more haemolysis than once-weekly administration, and that administration once weekly for 4 weeks and twice weekly thereafter resulted in a degree of anaemia falling between those produced by the other regimens. Anaemia was not induced in controls with no G6PD deficiency. One volunteer developed an intercurrent infection that was treated with salicylates; his haemolysis was markedly intensified, but whether by the infection, the salicylates or both could not be determined.

In a conjoint study, the administration of six malaria-suppressive drugs had no detectable effect on the activities of several erythrocyte enzymes or on the levels of adenosine monophosphate, diphosphate or triphosphate.

I. THE HAEMOLYTIC EFFECT OF VARIOUS REGIMENS OF PRIMAQUINE WITH CHLOROQUINE IN AMERICAN NEGROES WITH G6PD DEFICIENCY

This study was conducted to obtain information on the severity of the haemolytic anaemia induced in Negro male subjects deficient in glucose-6-phosphate dehydrogenase (G6PD) by various regimens of primaquine combined with chloroquine.[3] Considerable information has accumulated on dosage response in terms of haemolytic anaemia after daily primaquine administration (Kellermeyer et al., 1961) but little information is available concerning the haemolytic effects from once-weekly (Cahn & Levy, 1962; Alving et al., 1960) or twice-weekly primaquine administration. One of the presently recommended antimalaria regimens (Alving et al., 1960) calls for administration of once-weekly doses of 45 mg of primaquine [4] combined with 300 mg

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[3] Haemolysis from primaquine+chloroquine combinations in Negroes deficient in G6PD is thought to result solely from the primaquine.

[4] All dosages in this paper are given in terms of mg of base.

chloroquine. It is possible that increased resistance of malaria parasites in some areas of the world may result in an increased frequency of administration of this drug combination (Young & Moore, 1961; Powell et al., 1963, 1964a, 1964b). It is important to have information on the degree of increased haemolysis in G6PD-deficient individuals resulting from the increased dose of drug. Accordingly, comparative evaluation of the haemolytic effects of various drug regimens has been carried out.

METHODS, SUBJECTS, AND EXPERIMENTAL DESIGN

Deficiency of G6PD was detected by use of the methaemoglobin reduction test (Brewer et al., 1960). Haematocrit and haemoglobin determinations, and reticulocyte counts, were performed by standard haematological methods. The subjects for these studies were all volunteer inmates of the Southern Michigan Prison, Jackson, Mich., USA. Approximately 500 Negro inmates were screened for G6PD deficiency in order to detect deficient subjects for the primaquine+chloroquine study. The available enzyme-deficient subjects, along with an equal number of non-deficient controls, were divided into three groups of 8 subjects each, and were given drug according to the following plan:

Group I: administration of 45 mg primaquine and 300 mg chloroquine once weekly.

Group II: administration of 45 mg primaquine and 300 mg chloroquine twice weekly.

Group III: administration of 45 mg primaquine and 300 mg chloroquine once weekly for 4 weeks, then twice weekly.

The subjects were given their medication on Monday mornings for once-a-week programmes, and on Monday and Thursday mornings for twice-a-week programmes. The medication was a combined 45-mg primaquine and 300-mg chloroquine tablet as supplied by the US Army Medical Research and Development Command. Blood samples (5 ml in ethylene-diamine-tetracetic acid) were taken thrice weekly for haematocrit and haemoglobin determinations. Normal individuals were maintained on their programme for a total of 8 weeks. G6PD-deficient individuals were maintained on their programmes a sufficient period of time to permit return of their haemoglobin and haematocrit levels to pre-drug, baseline values; this occurred by 8 weeks in most men and by 10 weeks in all volunteers. Three baseline values were obtained for each volunteer prior to drug administration. All G6PD-deficient volunteers were of the Negro race. Controls were from both the Negro and the Caucasian races.

RESULTS

Drug administration to non-deficient subjects did not result in haemolysis. The results of the studies of the haemolytic effect of the three regimens of primaquine+chloroquine in G6PD-deficient subjects are summarized in Table 1. In this table, the baseline averages for haemoglobin and haematocrit values are given, followed by the nadir values. The nadir has been arbitrarily designated as the lowest value and the value preceding and the value following the lowest value. This is followed in the table by the average of the three nadir values. The figures in the next column, the decrease in haemoglobin and haematocrit values, were determined by subtracting the nadir average from the baseline average. The day of the nadir is also shown, counting day zero as the first day of drug administration. In Group III, one deficient subject (Case No. 39) had an unusually severe reaction, apparently provoked by an intercurrent febrile illness, which was treated by salicylates.

Group I (administration of drug once weekly). Most subjects had a relatively minimal, asymptomatic haemolytic episode. One subject, No. 4, complained of an upset stomach, nervousness and loss of sleep early during haemolysis. Haematological recovery was complete in 8 weeks in all volunteers.

Group II (administration of drug twice weekly). In general, the haemolytic episodes were slightly more acute in onset and slightly more marked than in Group I. Symptoms, however, were minimal. One subject, No. 19, developed symptoms of an upper respiratory infection with no apparent exacerbation of haemolysis. Haematological recovery was complete in 8 weeks, except in one case in whom recovery took 10 weeks.

Group III (administration of drug once weekly for 4 weeks followed by twice weekly). The purpose of this group was to find out whether the somewhat more acute haemolytic anaemia associated with the twice-weekly drug dose of Group II could be avoided by the preliminary administration of once-weekly doses for 4 weeks in order gradually to destroy a portion of the susceptible red cells (the older red cells). In general, this objective was achieved. The volunteers tolerated the subsequent regimen of

TABLE 1

RESULTS IN NEGRO MALES WITH G6PD DEFICIENCY RECEIVING 45 mg PRIMAQUINE AND 300 mg CHLOROQUINE IN DIFFERENT REGIMENS

Case No.	Parameter measured [a]	Average of 3 baseline values	Nadir after drug (lowest point and values on each side)			Average of 3 lowest values	Decrease (baseline average minus average of 3 lowest values)	Day of nadir [b]
			Group I: Administration once weekly					
4	Hb	14.4	11.8	11.8	12.3	11.9	2.5	9
	Hct	45	39	37	40	39	6	11
6	Hb	13.1	12.8	12.3	12.6	12.6	0.5	7
	Hct	41	41	40	41	41	0	9
8	Hb	16.0	14.1	13.8	14.4	14.1	1.9	7
	Hct	46	43	42	44	43	3	16
10	Hb	14.7	13.8	12.8	13.3	13.3	1.4	7
	Hct	46	42	40	41	41	5	9
11	Hb	14.3	12.6	11.1	12.6	12.1	2.2	11
	Hct	45	40	38	42	40	5	11
12	Hb	15.2	14.3	13.3	13.3	13.6	1.6	32
	Hct	45	43	42	43	43	2	18
13	Hb	14.1	12.2	12.0	12.6	12.3	1.8	11
	Hct	46	40	37	38	38	8	7
16	Hb	15.0	15.4	13.3	14.3	14.3	0.7	9
	Hct	48	47	44	46	44	4	16
			Group II: Administration twice weekly					
17	Hb	14.5	12.8	12.6	12.8	12.7	1.8	11
	Hct	45	40	39	39	39	6	7
18	Hb	16.3	13.1	12.3	13.1	12.8	3.5	11
	Hct	50	43	42	42	42	8	9
19	Hb	14.7	12.3	10.8	11.5	11.5	3.2	14
	Hct	45	40	35	35	37	8	14
26	Hb	14.9	12.5	12.2	13.1	12.6	2.3	9
	Hct	45	39	38	39	39	6	7
29	Hb	15.3	14.9	13.6	15.1	14.5	0.8	7
	Hct	50	45	45	49	46	4	18
30	Hb	15.1	11.6	11.3	11.8	11.6	3.5	11
	Hct	47	37	36	36	36	11	9
31	Hb	14.7	12.8	11.2	11.3	11.8	2.9	9
	Hct	44	40	39	42	40	4	11
32	Hb	13.6	13.1	11.5	12.0	12.2	1.4	14
	Hct	43	37	35	36	36	7	9
			Group III: Administration once weekly for 4 weeks, then twice weekly					
35	Hb	15.9	14.6	12.8	13.8	13.7	2.2	9
	Hct	49	45	41	43	43	6	9
36	Hb	13.6	12.9	12.6	13.1	12.9	0.7	11
	Hct	44	41	39	41	40	4	9
37	Hb	14.7	14.6	12.5	13.1	13.4	1.3	4
	Hct	43	39	38	40	39	4	7
38	Hb	15.8	14.9	11.8	12.6	13.1	2.7	9
	Hct	47	44	34	40	39	8	9
39	Hb	13.2	10.8	9.2	9.5	9.8	3.4	32
	Hct	43	30	29	30	30	13	35
44	Hb	13.6	11.5	11.0	12.0	11.5	2.1	14
	Hct	45	39	37	40	39	6	42
45	Hb	15.7	14.6	13.3	15.1	14.3	1.4	37
	Hct	46	45	39	45	43	3	9
46	Hb	12.5	10.5	10.2	11.0	10.6	1.9	14
	Hct	39	37	34	36	36	3	9

[a] Haemoglobin (Hb) in g/100 ml and haematocrit (Hct) in vol. %.

[b] Day of first drug administration is day zero.

TABLE 2
COMPARISONS OF TREATED GROUPS DETAILED IN TABLE 1

	Haemoglobin values (g/100 ml)				Haematocrit values (vol. %)			
	Gp I	Gp II	Gp III	Gp IIIA[a]	Gp I	Gp II	Gp III	Gp IIIA[a]
Mean nadir	13.0	12.5	12.4	12.8	41	39	39	40
Mean decrease	1.6	2.4	2.0	1.8	4	7	6	5

[a] Group IIIA is the same as Group III, but with the elimination of Subject No. 39, whose severe response was probably brought about by certain factors in addition to primaquine administration.

twice-weekly drug with a less acute fall in haemoglobin and haematocrit levels than volunteers in Group II. However, this group contained an exceptional subject, No. 39, who underwent a marked haemolytic reaction concomitant with fever and chills, believed to be due to an intercurrent infection which was treated by salicylates. This subject will be further commented upon in a later section. Omitting Case No. 39, haematological recovery took place in 8 weeks in 2 volunteers, in 9 weeks in 2 subjects and in 10 weeks in 3 subjects.

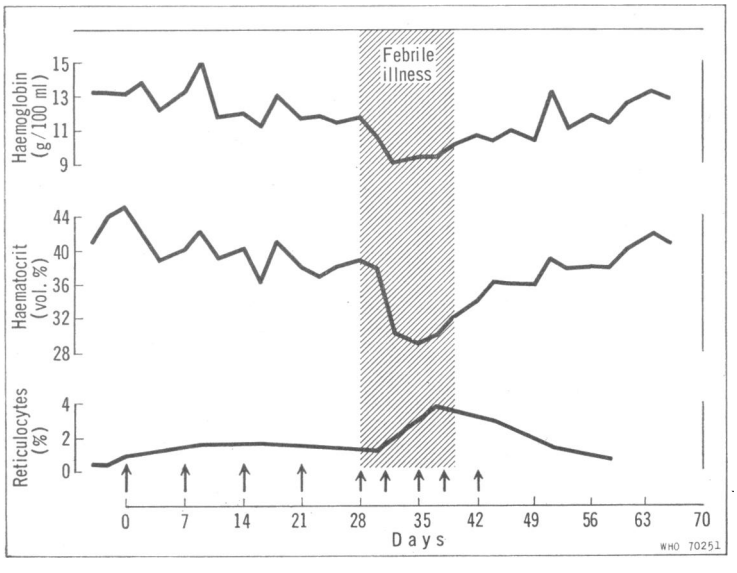
The data from the three groups are summarized for comparative purposes in Table 2. Haemolysis was most severe in Group II, and mildest in Group I; it was intermediate in Group IIIA (Group IIIA is group III omitting Case No. 39). However, the differences between the three groups were not marked.

Because of its unusual interest, the haemolytic reaction in subject No. 39, which was apparently intensified by infection, salicylate administration, or both, is shown in detail in the accompanying figure.

DISCUSSION

As expected, the toxicity, in terms of haemolysis, of twice-weekly doses of primaquine+chloroquine is somewhat greater than that observed with once-weekly doses, but does not seem such as to preclude the use of the regimen. It would appear that the temporary anaemia resulting from twice-weekly doses would be well tolerated in the vast majority of healthy American Negroes deficient in G6PD. The preliminary administration of drug once a week for a short time (such as 4 weeks; Group III) appears significantly to mitigate the haemolytic reaction of subsequent twice-weekly drug administration.

Two points deserve special comment. One is the marked apparent potentiation of the haemolytic effect of primaquine during the febrile illness treated with salicylates in subject No. 39. It is difficult to sort out the factors responsible for the severe reaction in this subject. It is known that infection or salicylates alone (Kellermeyer et al., 1958; Szienberg et al.,



HAEMOLYTIC EFFECTS OF AN INTERCURRENT FEBRILE ILLNESS, SALICYLATE ADMINISTRATION AND PRIMAQUINE + CHLOROQUINE ADMINISTRATION IN A NEGRO WITH G6PD DEFICIENCY[a]

↑ indicates administration of 45 mg primaquine and 300 mg chloroquine.

[a] The temperatures of the patient during the period indicated varied from 100°F to 104°F (37.8°C to 40°C). Salicylate administration, mostly in the form of aspirin, averaged about 2 g daily during this interval. A diagnosis regarding the etiology of the fever was not established.

1960) can precipitate haemolysis in enzyme-deficient individuals. It is possible that the factors of primaquine, salicylates, and infection all played roles in the severity of the haemolytic reaction in this individual. Primaquine was continued in this subject for a time after the development of increased haemolysis in order to develop some conception of what might occur in the field. While this episode was not life-threatening in this hospitalized and carefully observed subject, it is conceivable that under certain clinical circumstances, anaemia of this severity could play an important role in the outcome of a case. The possible development of a severe haemolytic reaction, such as that in subject No. 39, should be kept in mind by physicians, particularly during the first 8 to 10 weeks of administration of primaquine+chloroquine to a large group of individuals. It is doubtful that, had this febrile episode occurred one month later, it would have produced such a severe anaemia. We would recommend that in the usual clinical situation, if an individual develops an intercurrent infection or other disease requiring administration of a haemolytic drug, the primaquine be discontinued. Parasitaemia, if present, can be controlled by chloroquine alone.

The second point of importance is the response to be expected from the administration of primaquine+chloroquine to either G6PD-deficient Caucasians or Asians. This has not been well studied, but the enzyme deficiency is more severe in these groups than in the Negro, and the haemolytic reaction from a given dose of drug may also be more severe. It should be borne in mind that the deficiency occurs in about 0.1% of Anglo-Saxons, but reaches a very high frequency in some Caucasian ethnic groups. The administration of twice-weekly doses of primaquine+chloroquine could bring on a rather severe reaction in these individuals.

II. THE LACK OF AN EFFECT OF VARIOUS ANTIMALARIAL SUPPRESSIVE AGENTS ON ERYTHROCYTE METABOLISM

The effects of various antimalaria suppressive agents on certain aspects of erythrocyte metabolism have been studied. It has been previously shown that the quantitative level of erythrocytic adenosine triphosphate (ATP) affects the course of falciparum malaria (Brewer & Powell, 1965). It seemed possible that those antimalarials acting on the red cell stages of the parasite might be acting through an effect on the metabolism of the host red cell, rather than on the parasite itself.

METHODS, SUBJECTS AND EXPERIMENTAL DESIGN

The volunteers for these studies were inmates of the Southern Michigan Prison, Jackson, Mich., USA. The activities of G6PD and 6-phosphogluconate dehydrogenase (6PGD) in haemolysates were determined by the method of Glock & McLean (1953) as described by Zinkham & Lenhard (1959). Pyruvic-kinase activity in haemolysates was measured by the method of Tanaka et al. (1962) as modified by Powell & DeGowin (1965). Glutathione-reductase activity was measured by the method of Long & Carson (1961). Adenosine triphosphate was measured by a method previously described (Brewer & Powell, 1966) and adenosine diphosphate (ADP) and adenosine monophosphate (AMP) were measured by the "test combination" method of Boehringer Co., Mannheim, Germany.

The study of the effects of suppressive antimalarial drugs on erythrocytic metabolism was carried out by first ascertaining baseline G6PD, 6PGD, pyruvic-kinase, and glutathione-reductase activities, and ATP, ADP and AMP levels in red cells of 6 individuals. Then, each volunteer was given a therapeutic course of one of 6 suppressive antimalarials. During and after drug administration, the activities of the several enzymes, and the levels of ATP, ADP, and AMP were measured.

RESULTS AND DISCUSSION

One of each of 6 suppressive drugs was administered to one volunteer subject in the following dosage schedules:

(1) Chloroquine: 900 mg (600 mg initially, 300 mg 6 hours later) the first day; 300 mg daily for the next 2 days (total dose: 1500 mg).

(2) Hydroxychloroquine: schedule identical to that of chloroquine (total dose: 1500 mg).

(3) Amodiaquine: 600 mg the first day; 400 mg daily for the next 2 days (total dose: 1400 mg).

(4) Pyrimethamine: 50 mg daily for 3 days (total dose: 150 mg).

(5) Mepacrine: 785 mg (5 doses of 157 mg) the first day; 78.5 mg thrice daily for the next 6 days (total dose: 2198 mg).

(6) Quinine: 1494 mg (3 doses of 398 mg) daily for 7 days (total dose: 10 458 mg).

No changes were observed in any of the volunteers in haemolysate G6PD, 6PGD, pyruvic-kinase, and glutathione-reductase activities, or in levels of erythrocytic ATP, ADP and AMP.

The lack of an effect of various suppressive agents on the metabolic parameters measured suggests that these drugs do not owe their antimalarial properties to effects on these parameters of the host red cell.

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RÉSUMÉ

On a étudié chez 24 volontaires atteints de carence en glucose-6-phosphate déshydrogénase (G6PD) et chez 20 volontaires normaux les manifestations hémolytiques succédant à l'administration de primaquine (45 mg) associée à la chloroquine (300 mg). Trois schémas médicamenteux ont été utilisés: une dose hebdomadaire; une dose bihebdomadaire; une dose hebdomadaire pendant 4 semaines, puis une dose bihebdomadaire.

Les sujets normaux n'ont présenté aucun signe d'hémolyse. Chez les volontaires carencés, un effet hémolytique modéré (diminution du taux d'hémoglobine de 1,6 g/100 ml) a été observé après administration hebdomadaire. L'anémie a été légèrement plus intense (hémoglobine: —2,4 g/100 ml) et d'apparition plus rapide après administration bihebdomadaire. Enfin l'application du 3e schéma a provoqué chez les sujets carencés une anémie de type intermédiaire (hémoglobine: —1,8 g/100 ml). Chez un volontaire carencé soumis à la prise bihebdomadaire d'antipaludiques, le traitement salicylé d'une affection fébrile intercurrente a déterminé une hémolyse notablement plus accentuée, dont la pathogénie n'a pu être complètement élucidée.

D'autre part, six antipaludiques servant au traitement suppressif: chloroquine, hydroxychloroquine, amodiaquine, pyriméthamine, mépacrine et quinine, ont été expérimentés également sur des volontaires. On n'a constaté aucune influence sur l'activité des enzymes érythrocytaires, comme la G6PD et la 6PGD (phospho-6-gluconate déshydrogénase) de même que sur les taux de tri-,di- et monophosphate adénosine.

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