Depletion of Coagulation Factors in Drug-Resistant Plasmodium Falciparum Malaria

By Lewis H. Dennis, James W. Eichelberger, Max M. Inman and Marcel E. Conrad

The development of the synthetic antimalarial drugs led most authorities to believe that malaria was no longer a military problem. It was thought that the weekly administration of a chloroquine-primaquine tablet would protect troops from all malarious infections. In 1959, chloroquine-resistant plasmodium falciparum malaria was reported from South America. Subsequently, drug-resistant malarial strains were found in Malaya, Thailand and Vietnam. Following the deployment of U. S. soldiers in the Central Highlands of South Vietnam, the incidence of chloroquine-resistant falciparum malaria soared despite an enforced program of chloroquine-primaquine prophylaxis. Treatment of this malaria was discouraging. Parasitemia persisted despite therapeutic doses of chloroquine and relapses frequently occurred after long-term quinine therapy. Soldiers were made ineffective for combat, causing their evacuation from Vietnam to the continental United States.

Falciparum malaria causes fever, chills, intravascular hemolysis and anemia and may progress to acute renal failure, shock and death. In 1917 Dudgeon and Clark reported a malarial outbreak in Salonica in which 57 per cent of afflicted soldiers died within 48 hours after the clinical onset of malaria. Postmortem examinations revealed renal thrombosis and extensive cerebral thrombosis with hemorrhage. In 1946, Spitz examined tissue sections from 50 U. S. soldiers who died with acute falciparum malaria during World War II and found many thrombi in cerebral vessels as well as glomerular congestion and thrombosis. Both studies reported focal necrosis of the liver, adrenals, and spleen with evident thrombus formation. The coexistence of hemorrhage and thrombosis in these patients led us to postulate that blood coagulation abnormalities might be important in the pathophysiology of malaria. The availability of many patients with acute and relapsed falciparum malaria led us to test this hypothesis.

Materials and Methods

The subjects of this study were 31 American soldiers from Vietnam hospitalized at Walter Reed General Hospital with chloroquine-resistant falciparum malaria. Patients were studied initially at the clinical onset of acute relapsed malaria, and at intervals during the next 21–28 days. Each subject was febrile and had P. falciparum in smears of the peripheral...
blood. They received various antimalarial drugs including quinine, chloroquine, Dapsone (DDS), Daraprim and sulfadiazine.

Blood for coagulation studies was drawn in plastic syringes. Serum, oxalated, and citrated platelet-poor plasma were prepared and studied. Citrated, pooled, platelet-depleted plasma was obtained from 10 normal male donors to establish normal dilution curves for tests of the one-stage prothrombin time and assay of Factors II, V, VII, VIII and X.

The one-stage prothrombin time was measured by the method of Quick,\(^4\) using rabbit brain thromboplastin.\(^5\) The partial thromboplastin time was performed by the method of Langdell et al.\(^5\) using liquid rabbit brain cephalin with ellagic acid as the plasma activator.\(^6\) Prothrombin (II) activity was determined by the method of Owren;\(^6\) labile factor (V), by the method of Stefanini;\(^7\) antihemophilic globulin (VIII), by the method of Bregna;\(^8\) stable factor (VII), by the method of Owren and Aas;\(^9\) and the Stypven time (X), by the method of Bachman et al.\(^10\) Platelets were counted by the direct method of Brecher and Cronkite.\(^11\) Fibrinogen concentration was measured by the method of Ratnoff and Menzie.\(^12\)

The euglobulin lysis time (ELT) was performed by a modification of the method of Iatridis and Ferguson.\(^13\) The observed range for complete lysis to occur in 50 normal males was between 4 and 7 hours. An ELT of more than 8 hours was considered prolonged.

The serial thrombin time (STT) was performed with the patients’ platelet-poor oxalated

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* Dade Reagents, Inc., Miami, Fla.
Fig. 2.—Assay of Factor V, VII and VIII are illustrated as per cent of the activity found in pooled normal blood. The stypven time is reported in seconds. The shaded area represents the range of values observed in 50 normal subjects. Each point is a determination in a patient during acute relapsed malaria and convalescence.

plasma incubated at 37 C. and human thrombin (Fibrinex,* 50 NIH U./ml.) as previously described. Values observed in 50 normal subjects were similar to those previously reported.

Immunodiffusion studies employing specific rabbit antisera against human fibrinogen and human fibrin were performed on agar gel slides by the method of Ouchterlony. The "Fi" test was performed using a suspension of antifibrinogen coated latex particles. One or 2 drops of serum was mixed with an equal quantity of "Fi" reagent on a glass slide; the presence or absence of agglutination within 3 minutes of gentle rotation at 20 C. was recorded. Serum for immunologic studies was obtained by clotting the test blood in a stoppered glass, 12 mm. test tube at 20 C. for 24 hours. Then the specimen was centrifuged at 2500 r.p.m. for 10 minutes. The serum was decanted and used for immunodiffusion studies and the "Fi" test. Normal serum prepared in the same manner was used in each study as a control. No positive reactions were recorded with sera from 50 normal donors.

Test sera were also treated with bovine thrombin solution (250 NIH U./ml.†) in a ratio of 2 parts serum to 1 part thrombin. Serum specimens were incubated with thrombin for

* Supplied by Ortho Diagnostics Div., Ortho Pharmaceuticals, Raritan, N. J.
† Supplied by Hyland Laboratories, Los Angeles, Calif.
‡ Supplied by Hyland Laboratories, Los Angeles, Calif.
§ Parke, Davis & Co., Detroit, Mich.
Fig. 3.—The results of the euglobulin lysis time, serial thrombin time and immunodiffusion studies in malarious patients are summarized above. The shaded area represents the range of determinations in 50 normal subjects. The numbers indicate the number of malarious patients that had normal (shaded) or abnormal studies during relapse or convalescence.

RESULTS

The results of coagulation studies in U. S. soldiers from Vietnam with drug-resistant falciparum malaria are shown in Figures 1–3. During the acute phase of illness most patients had (1) a significant depression in platelets, (2) a prolonged one-state prothrombin time and/or partial thromboplastin time, (3) prolongation of the euglobulin lysis time, and (4) an abnormal serial thrombin time. These coagulation abnormalities occurred before drug therapy was initiated and abated while patients received therapeutic doses of antimalarial drugs. During convalescence these tests became normal, except for a prolonged serial thrombin time and euglobulin lysis time. Chemical measurements of the plasma fibrinogen concentration showed a decrease in less than one-third of relapsed patients. In the remainder the fibrinogen value increased or was unchanged.

Patient’s sera reacted positively with antihuman fibrinogen antiserum during relapse; this reaction persisted in sera from most convalescent patients. Sera from approximately one-half of acute relapsed patients reacted positively with antihuman fibrin; this abnormality did not persist during convalescence. Similar immunodiffusion reactions were observed in malarious sera treated with excess...
Fig. 4.—The clinical course of this 25-year-old Caucasian male with relapsed chloroquine-resistant falciparum malaria. A therapeutic course of chloroquine produced symptomatic improvement but did not cure the parasitemia. The platelet counts were decreased to levels of 50,000 per cu. mm. twice during the period of study. The prothrombin time and partial thromboplastin time, all abnormal during the acute phase, improved as the clinical course improved. However, significant depletions of SPCA and AHG were apparent for several weeks. Fibrinogen degradation products were present throughout the period of observation as shown by the persistently positive immunodiffusion studies and abnormal serial thrombin times.

thrombin and the same sera untreated, but compared with normal control sera. Results of studies using the "Fi" reagent were similar in malarious sera treated with excess thrombin and the same untreated sera. However, this parameter
appeared relatively insensitive as compared to immunodiffusion studies and the STT.

Each blood specimen with an abnormal serial thrombin time reacted positively in immunodiffusion studies with antihuman fibrinogen, suggesting that the STT is a sensitive indicator of fibrinogen degradation products. This observation explains the frequent disparity between the STT and ELT and why the ELT becomes abnormal before the STT in patients whose primary defect is accelerated activation of plasminogen.14

Assay of individual coagulation factors in the plasma of patients with acute and relapsed malaria showed that the prolonged prothrombin time and partial thromboplastin time were caused primarily by a significant depletion of factors V and VIII. Significant depletion of Stuart Factor (X) and proconvertin (VII) was found in plasma specimens of seriously ill patients.

DISCUSSION

The pathogenesis of accelerated intravascular coagulation remains obscure. Hypercoagulable states have been induced in animals by the slow infusion of such procoagulants as thrombin and various thromboplastins, the use of endotoxin, as well as fats and oils.17 It has been postulated that this syndrome is commonplace and contributes to the pathophysiology of many diseases.18 Yet, the majority of human studies of this syndrome have reported a few seriously ill patients with life-threatening or far-advanced diseases.19

Most of our patients with acute falciparum malaria had thrombocytopenia, a decreased concentration of multiple clotting factors, and evidence of decreased plasminogen activation with accumulation of fibrinogen degradation products in the blood. The greatest variations from normal were found in blood specimens from seriously ill patients. Mild malarial relapses were associated with less marked coagulation abnormalities. During convalescence, coagulation tests became normal but the persistence of fibrinogen degradation products and the suggestion of decreased plasminogen activation in blood from many patients suggested that accelerated intravascular coagulation continued at a compensated rate.4

The clinical and laboratory response of 3 patients with blackwater fever to heparin provided preliminary evidence that accelerated intravascular coagulation added to the morbidity of falciparum malaria. Each patient showed clinical improvement and partial correction of coagulation tests within 12 to 24 hours after the initiation of heparin therapy (Fig. 5). In contrast, unheparinized patients with severe malaria did not show similar improvement in coagulation studies until 2 or 3 days after conventional antimalarial drug therapy was begun.

SUMMARY

United States soldiers with acute relapsed P. falciparum malaria had accelerated intravascular coagulation which was manifested by thrombocytopenia, a prolonged prothrombin time and partial thromboplastin time, a de-
Fig. 5.—This 31-year-old Caucasian male developed blackwater fever during the period of observation. Although he had malarial parasites in his peripheral blood, initial studies of first and second stage coagulation were normal. On day 4 he again became febrile, spiking to 104°F. Quinine therapy was begun. The patient developed hemoglobinuria at once and quinine was discontinued. Daraprim and sulfadiazine therapy were instituted. The marked decrease in platelets as well as consumption of coagulation factors were followed by the initiation of heparin intravenously. There was rapid improvement in first and second stage coagulation defects and a progressive increase in platelets. The administration of large doses of heparin between days 9 and 13 caused the inordinate prolongation of the partial thromboplastin time.

crease in multiple coagulation factors, and evidence of decreased plasminogen activation with an accumulation of fibrinogen breakdown products in the blood. These changes may be important in the pathophysiology of malaria and cause the hemorrhage and thrombosis found in many organs of patients dying with falciparum malaria.
SUMMARIO IN INTERLINGUA
Soldatos statounitese con acute rec'idivas de malaria a Plasmodium falciparum manifestava un accelerate coagulation intravascular evidente in thrombocytopenia, un prolongation del tempores de prothrombina e de thromboplastina partial, un declino in multiplice factores de coagulation, e signus de un reducute activation de plasminogeno in simultaneitate con un accumulation de productos decomponitori de fibrinogeno in le sanguine. Iste alterationes es possiblemente importante in le pathophysiology de malaria causante le hemorrhagia e le thrombose trovate in multe organos de patientes qui mori ab malaria a P. falciparum.

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