Quinine-Induced Immune Thrombocytopenia Associated With Hemolytic Uremic Syndrome: A New Clinical Entity


Three patients are described who developed severe thrombocytopenia, microangiopathic hemolytic anemia, and acute renal failure after ingestion of quinine. In one patient, the same clinical findings recurred several months later after another exposure to quinine. Serum from one patient contained quinine-dependent IgG antibodies reactive with the platelet glycoprotein (GP) Ib/IX complex. In the second and third cases, serum contained IgG and IgM antibodies reactive with both the GP Ib/IX and Ib/IIa complexes in the presence of quinine. Quinine appears to have induced both immune thrombocytopenia and the hemolytic uremic syndrome (HUS) in these individuals. Findings made in these cases may have implications for the pathogenesis of some forms of HUS.

© 1991 by The American Society of Hematology.

CASE REPORTS

Case 1. A 66-year-old man was admitted with a history of nausea, vomiting, and myalgia for the preceding 24 hours. He had taken prescribed quinine sulfate for nocturnal leg cramps occasionally for several years. Two days before admission, he took a single quinine tablet, after which he experienced chills, rigor, and diaphoresis lasting several hours. On admission, he was afebrile and had a normal blood pressure. Physical examination showed peripheral edema and petechiae on the lower extremities. Blood studies showed hematocrit 39%, reticulocytes 1.6%, white blood cells (WBC) 11,000/µL with a normal differential count and platelets 31,000/µL. The Coombs' test was negative. Numerous schistocytes were seen in the peripheral blood film. Hemoglobin (Hb) casts were present in the urine. Serum creatinine was 9.4 mg/dL, lactate dehydrogenase (LDH) 2,700 IU/L, and total bilirubin 2.2 mg/dL. Urinary output was 100 mL/d. Two blood cultures and a urine culture were negative. A diagnosis of adult HUS was made and the patient was treated with plasma exchange, 4,000 mL daily for 6 days, combined with hemodialysis. Dipyridamole, 75 mg four times daily; aspirin, 325 mg/d; and prednisone, 80 mg/d, were also administered. Over a 3-day period, LDH levels declined to 700 IU/L and schistocytes became infrequent in the peripheral blood film. However, renal failure persisted for 10 days, after which urinary output rose to 3,500 mL/d. The patient was discharged on day 12 with a stable hematocrit of 25%, platelet level 285,000/µL, creatinine 2.4 mg/dL, LDH 150 IU/L, and a normal urine output. Aspirin, prednisone, and dipyridamole were continued for 10 days on an outpatient basis. He remains well at the present time, 24 months after his initial illness.

Case 2. A 51-year-old woman was admitted following a 7-day illness characterized by nausea, vomiting, diarrhea, and myalgia. For several years, she had occasionally taken an over-the-counter compound containing quinine for nocturnal leg cramps. She took one of these tablets about a week before becoming ill and one 4 days before admission. Admission blood pressure was normal, and the remainder of the physical exam was unremarkable except for petechiae over the lower extremities and scattered ecchymoses. Blood studies showed hematocrit 26%, reticulocytes 3.8%, WBC 17,000/µL with left shift, and platelets 31,000/µL. The Coombs' test was negative. Many schistocytes were found in the peripheral blood film. Hb casts and free red blood cells (RBC) were present in the urine. Serum creatinine was 11.7 mg/dL, LDH 1,245 IU/L, and total bilirubin 2.3 mg/dL. Four blood cultures were negative. A diagnosis of adult HUS was made and the patient was treated with plasma exchange, 3,000 mL daily for 4 days, combined with hemodialysis. Dipyridamole, 75 mg four times daily; aspirin, 325 mg/d; and prednisone, 80 mg/d, were also administered. The platelet count became normal within 3 days of admission and renal function returned to normal over 2 weeks. The patient was discharged with LDH 225 IU/L and creatinine 3.3 mg/dL. She remains well at the present time, 20 months after the initial illness.

Case 3. A 51-year-old woman was admitted with a history of dyspnea and light headedness of several-days duration. Blood pressure and physical examination were normal except for scattered ecchymoses on the lower extremities. Blood studies showed hematocrit 41%, reticulocytes 3.1%, WBC 2,700/µL with normal differential, and platelets 7,000/µL. Numerous schistocytes were seen in the peripheral blood film. Many RBC were present in the urinary sediment. Serum creatinine was 1.0 mg/dL, LDH 2,438 IU/L, lactate dehydrogenase less than 5 mg/dL, and total bilirubin 2.2 mg/dL. Urinary output was normal, but the serum creatinine increased to 5.0 mg/dL by the fourth hospital day. A blood culture was negative. A diagnosis of HUS or possible thrombotic thrombocytopenic purpura (TTP) was made and the patient was treated with infusion of 14 U of fresh frozen plasma over 4 days; aspirin, 325 mg/d; and prednisone, 40 mg/d. Also administered was vincristine 2 mg intravenously (IV) on days 5 and 8. After 10 days, hematologic and renal indices returned to normal and she was discharged without medication (Fig 1).

Six weeks later, she was readmitted with vomiting, weakness,
confusion, and ecchymoses of several-days duration. At this time, the patient provided the additional history of taking over-the-counter quinine tablets periodically for muscle cramps and having ingested one of these tablets several hours before the onset of symptoms. Physical exam showed normal blood pressure and multiple petechiae and ecchymoses over the trunk, arms, and legs. Blood studies showed hematocrit 30%, reticulocytes 3.5%, WBC 6,100/µL with normal differential, and platelets 23,000/µL. Numerous schistocytes were again seen in the peripheral blood film. Occasional RBC were found in the urinary sediment. Serum creatinine was 4.4 mg/dL increasing to 8.0 mg/dL 2 days later, LDH 1,928 IU/L, haptoglobin less than 5 mg/dL, and total bilirubin 4.2 mg/dL. The patient was treated with 7 U of fresh frozen plasma over 2 days, prednisone 40 mg/d and 2 mg vincristine IV. She then developed mental confusion, severe headache, and vomiting. Although no localizing neurologic changes were observed, the diagnosis of TTP was entertained. She was treated with plasma exchange, 4,000 mL daily for 3 days, after which hematologic indices and blood chemistries returned to normal. The patient was discharged on day 16 and remains well 18 months later. Before discharge, she was questioned about quinine exposure before her first hospital visit. She did not remember taking the drug for leg cramps, but did recall ingesting several gin and tonic cocktails shortly before the onset of symptoms.

MATERIALS AND METHODS

Serum samples from the three patients were evaluated for drug-dependent, platelet-reactive antibodies by three methods, the 51Cr release test, antigen capture enzyme-linked immunosorbent assay (ELISA) (ACE) and modified ACE (MACE).

51Cr release test. Complement-dependent release of 51Cr from radio-labeled, papain-treated target platelets was determined as described by Cimo et al.3 ACE. Glycoprotein (GP) IIb/IIIa and GP Ib/IX complexes from detergent-solubilized platelets were "captured" by mouse monoclonal antibodies (MoAbs) AP-2 and AP-1, respectively, in wells of microtiter plates as previously described.6 Serum samples at various dilutions were incubated with the immobilized complexes in the presence and absence of quinine sulfate, 0.2 mg/mL, and incubated for 60 minutes at room temperature. The wells were then washed with phosphate-buffered isotonic saline, pH 7.4, containing quinine, 0.2 mg/mL, or, for controls, lacking quinine. Bound IgG was detected with biotin-labeled MoAb HB-43 specific for the Fc portion of human IgG, using avidin-biotin-alkaline phosphatase complex for quantitation. IgM was similarly detected with MoAb HB-57. HB-43 and HB-57 were produced from hybrid cell lines obtained from the American Type and Culture Collection, Rockville, MD. MACE. Use of this assay for detection of non-drug-dependent antibodies has been described previously.4 For detection of drug-dependent antibodies, 0.5 mL of serum was incubated with 4 x 107 platelets from a normal group O donor with and without quinine sulfate, 0.2 mg/mL, at room temperature for 30 minutes. The platelets were then washed three times with phosphate-buffered isotonic saline, pH 7.4, containing quinine, 0.2 mg/mL, at room temperature for 30 minutes. The platelets were then washed three times with phosphate-buffered isotonic saline, pH 7.4, containing drug at the same concentration as in the primary mixture, and were solubilized with 1% Triton X-100 (Sigma-Aldrich) in Tris saline, pH 7.4 at 4°C for 30 minutes. Lysate from 1 x 107 platelets was placed in wells of a microtiter tray containing 0.5 µg of fixed AP-1 or AP-2 MoAb and incubated for 60 minutes at 37°C to allow="capture" of GP Ib/IX and GP Ib/IIIa by the MoAbs. The trays were washed three times with phosphate-buffered saline (PBS), also containing drug at the same concentration as in the primary mixture. IgG and IgM immobilized with the captured GP complexes were then detected with biotin-labeled MoAb HB-43 and HB-57 as described above for ACE. Specificity of the ACE and MACE assays for Ig reactive only
with GPs immobilized by the MoAbs used has been shown in previous reports from our laboratory and by others using assays based on similar principles.

RESULTS

\(^{32}\text{Cr} \text{ release assay.} \) Serum from case 1 failed to release \(^{32}\text{Cr} \) from radiolabeled target platelets in the presence of quinine. However, strong positive reactions were obtained with serum from patients 2 and 3 (Table 1).

ACE. As shown in Fig 2, serum from patient 1 contained an IgG antibody that reacted weakly, but consistently, with GP Ib/IX complex in the presence of quinine. Quinine-dependent reactions against GP Ib/IIa were not detected using IgG and IgM-specific reagents. Serum from patients 2 and 3 (second hospital admission) contained drug-dependent IgG antibodies reactive with both the GP Ib/IX and the GP IIb/IIIa complexes. Drug-dependent IgM antibodies reactive with Ib/IX and IIb/IIIa were also detected in serum from these patients (not shown).

MACE. Drug-dependent IgG antibodies showing the same reaction patterns obtained with ACE were also shown by MACE, the major difference being that the ratio of optical density (OD) obtained in the presence of the drug to that obtained in control reactions lacking the drug was generally higher (Fig 3). This result was especially apparent with patient 1 in whom the ACE reactions were barely detectable. Drug-dependent IgG antibody reactive with GP Ib/IX in patient 1 increased in strength progressively during his first 5 hospital days (Fig 4). The same samples gave only borderline positive reactions against GP Ib/IX in ACE. Serum samples obtained on days 1, 3, 5, 12, 15, 24, and 30 (patient 2) and days 1, 2, 3, and 8 (patient 3) postadmission all gave similar drug-dependent reactions against GP Ib/IX and GP IIb/IIIa. Two to four months after discharge from the hospital, quinine-dependent IgG antibodies with the same reaction patterns as the acute phase sera were still detectable in each patient, but in lower titer.

DISCUSSION

The serologic findings made with serum from these patients leave little doubt that each had drug-induced, immunologically mediated thrombocytopenia (DITP). They were also diagnosed as having adult HUS on the basis of their acute renal failure and microangiopathic hemolytic anemia. In patient 3, findings suggestive of TTP were also transiently present. DITP and adult HUS are uncommon conditions and it seems unlikely that the observed association was coincidental, especially in the case of patient 3, in whom DITP and HUS occurred on two occasions after exposure to quinine.

Although much has been learned about HUS and the closely related condition, TTP, in recent years, the pathogenesis of these disorders is not yet fully understood. The lesions that appear to be responsible for many of the symptoms of HUS are microthrombi consisting largely of fibrin and platelets lodged in the capillaries of renal glomeruli. Two general theories of pathogenesis have been advanced. In the first, it is postulated that endothelial injury, predominantly in small vessels of the kidney, is caused by an endogenous or exogenous agent. Exposure of blood to damaged endothelium then leads to microthrombus formation and damage to RBC and platelets. The second theory holds that endothelial cells, platelets, and RBC are damaged independently by the same agent.
Arguments for and against these proposals were recently reviewed.12

Infection with bacteria or viruses appears to trigger HUS in children.14 Epidemiologic studies and related laboratory investigations have provided convincing evidence for an association between childhood HUS and infection with verotoxin-producing strains of Escherichia coli and the Shiga toxin of Shigella.14 In general, drugs have not been implicated in the pathogenesis of childhood HUS with the possible exception of the antibiotic metronidazole which was being administered to six children with HUS described in a recent report.12

Infections have rarely been implicated in the pathogenesis of HUS in adults, but the condition does appear to follow exposure to certain drugs. Women taking oral contraceptives,13 conjugated estrogens,14 or in the peripartum period15 appear to be susceptible, and numerous recent reports have linked HUS in adults to treatment of malignant disease with chemotherapeutic agents, especially mitomycin C.16-19 An etiologic role for direct damage to endothelium by mitomycin has been suggested, but not shown. HUS associated with cancer chemotherapy is difficult to treat and mortality exceeds 50%.16-19

We are unaware of any previous reports documenting the association of quinine-induced, immunologic thrombocytopenia with adult HUS, but quinine and several other drugs have been implicated as causing some of the hallmarks of HUS. Hemolytic anemia with acute renal failure, and sometimes thrombocytopenia in patients taking large doses of quinine as an abortifacient has been recognized for many years.20,21 In several reported cases, renal and hematologic symptoms developed as long as 3 days after exposure to quinine, suggesting the possibility of a sensitivity reaction, rather than a direct toxic effect of the drug.21 The combination of hemolytic anemia (lacking microangiopathic RBC morphology), thrombocytopenia, and acute renal failure appears to have been induced by aspirin,21 the rauwolfia derivative ajmaline,22 and doxepin23 in single instances. In these cases, drug-dependent RBC-reactive, but not platelet-reactive, antibodies were detected. In other reports, drugs were thought to have triggered acute renal failure with thrombocytopenia24,25 and acute renal failure with hemolytic anemia25,26

The three patients we studied differed from any previously described by exhibiting the typical findings of adult HUS: microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure, and in having platelet-reactive antibodies induced by the drug to which they were exposed (quine). The antibody in serum of patient 1 was of particular interest because it increased significantly in strength over the first 4 hospital days (Fig 4) and might not have been detected if only the admission serum sample had been tested. This phenomenon has been observed previously in patients with thrombocytopenia induced by quinine and quinidine.20

The association of drug-induced, immunologic thrombocytopenia and acute renal failure in the patients studied raises the intriguing question of whether quinine, in addition to stimulating drug-dependent, platelet-reactive antibodies, may have induced antibodies, drug-dependent or independent, with specificity for renal glomerular endothelium. To date, we have been unable to demonstrate Ig in these patients’ sera that bind to renal glomeruli in tissue sections in the presence or absence of quinine. However, it is possible that epitopes recognized by such antibodies might be destroyed by fixation or some other aspect of tissue processing. Indeed, the platelet-reactive, drug-dependent antibody in serum of patient 1 reacted strongly with one or more epitopes expressed on GP Ib/IX of intact platelets (MACE) but gave barely detectable reactions against GP Ib/IX complex isolated by immunoprecipitation (ACE) (Fig 1). The GP IIa molecule is known to be expressed in both platelets and endothelial cells21 and reports of a GP Ib-like protein in endothelial cells have appeared.21,22 Thus, it seems possible that the renal abnormalities seen in our patients might be mediated by drug-dependent antibodies reactive with target molecules on endothelial cells in renal glomeruli. It can be speculated that other forms of HUS associated with exogenous agents might have a similar pathogenesis and further studies to test this hypothesis seem warranted.

ACKNOWLEDGMENT
We are indebted to Dr William Deardorff for permission to study patient 3 and to the Word Processing Department of The Blood Center for the manuscript preparation.
REFERENCES

Quinine-induced immune thrombocytopenia associated with hemolytic uremic syndrome: a new clinical entity

JL Gottschall, W Elliot, E Lianos, JG McFarland, K Wolfmeyer and RH Aster