Immune Hemolytic Anemia and Thrombocytopenia Secondary to Quinidine: In Vitro Studies of the Quinidine-Dependent Red Cell and Platelet Antibodies

By Z. Zeigler, R. K. Shadduck, A. Winkelstein, and T. K. Stroupe

A patient developed immune hemolysis and thrombocytopenia while receiving quinidine. In vitro testing showed that ant Quinn dine antibodies of the IgG class were active against both cell lines. Complement- and drug-dependent platelet lysis occurred with either the patient's serum or his IgG fraction; there was no reaction with his IgM fraction. Drug-dependent red cell antibodies were studied by antiglobulin testing and analysis of the interaction of sensitized red cells with human monocytes in vitro. Antiglobulin testing revealed the presence of cell-adherent IgG, IgM, and complement; however, IgG-type red cell antibodies were of prime importance in producing red cell ingestion by monocytes. Donor red cells incubated with the drug and the patient's serum readily adhered to and were ingested by monocytes. The majority of antibody activity was found in the IgG fraction, whereas only a minor degree of phagocytosis was seen with the patient's IgM complement fraction. Using the monocyte system, IgG antibodies were detected on the cells, even after extensive washing. This suggests that red cell destruction in this syndrome may occur by a mechanism other than the innocent-bystander type.

QUININE and quinidine are known to cause both drug-induced immune hemolytic anemia and thrombocytopenia. Cellular destruction in these syndromes is believed to occur by means of an innocent-bystander mechanism wherein drug-antibody complexes passively adsorb to the cell membrane and induce the fixation of complement. The immune complexes are believed to dissociate from the red cells, as antiglobulin tests show only weak reactivity with specificity for complement components. Typically the antibodies responsible for hemolysis belong to the IgM class, those responsible for thrombocytolysis belong to the IgG class.

Evaluation of a patient who simultaneously developed hemolytic anemia and thrombocytopenia while receiving quinidine allowed for characterization of both drug-induced reactions.

CASE REPORT

A 72-yr-old male was admitted to Montefiore Hospital with an acute myocardial infarction. On the second hospital day he was started on quinidine sulfate, 200 mg every 6 hr, to control arrhythmias. His hospital course (Fig. 1) was complicated by fever from the second through the seventh hospital days. Chest x-ray and throat, blood, and urine cultures did not show any evidence for infection. On the seventh...
hospital day he developed a generalized petechial rash. Quinidine was discontinued, and he was treated with prednisone, 40 mg daily for 4 days.

His platelet count, which by evaluation of blood smear appeared normal on admission, fell to 13,000/µl on the seventh day. Simultaneously, his hematocrit decreased from an admission value of 45% to 28% on the ninth hospital day. There was no evidence for exogenous blood loss. A reticulocyte count at this time was 3.1%. The total bilirubin rose from an admission value of 0.5 mg/dl to 1.6 mg/dl, with an indirect fraction of 1.3 mg/dl. The patient was transfused with 2 units of packed red cells for relief of chest pain. Hematologic remission and a decrease in the indirect bilirubin level persisted after discontinuation of corticosteroid therapy.

The patient's blood smear showed occasional spherocytes; no fragmented cells were noted. By microscopic evaluation he had an increased fraction of large platelets; the percentage of platelets greater than 3 µ in diameter was 22% (normal range, zero to 14%), a finding consistent with destructive thrombocytopenia. Other studies, including haptoglobin, serum hemoglobin, urinary hemoglobin, prothrombin time, partial thromboplastin time, thrombin time, and fibrinogen level, were negative or normal. Fibrin degradation products were 10-40 mg/dl (normal, < 10 mg/dl). Direct and indirect antiglobulin tests obtained after discontinuation of quinidine were negative; unfortunately these tests were not performed during the period of drug therapy.

Special Studies

One-tenth milliliter of a 4%-5% suspension of donor or patient red cells, 0.2 ml of patient or ABO and Rh-compatible donor serum, and 0.1 ml of quinidine gluconate (8 mg/ml, Eli Lilly) were incubated at room temperature for 15 min. The samples were examined for hemolysis and spontaneous agglutination. Antiglobulin testing was performed using anti-non-gamma, anti-gamma Coombs reagents (Ortho Diagnostics) and anti-IgM antiserum (Meloy Laboratories). The concentration of quinidine used in these studies was in excess of the concentrations achieved in vivo. This concentration was chosen empirically because it gave the clearest separation between the patient's serum and controls in antiglobulin testing.

The anti-IgM serum was diluted and then standardized (vide infra). This was done because the anti-IgM serum used neat agglutinated human red cells presumably due to species antibodies. The
anti-IgM serum was standardized by two methods. Red cells containing the I antigen were sensitized with a subagglutinating titer of anti-I (Hyland Laboratories) at 4°C. These cells showed strong reactions at 4°C with the anti-IgM serum but were nonreactive with the anti-gamma serum. Additionally, Rh-positive red cells that were presensitized with anti-D serum showed 3+ reactions with IgG Coombs serum but were negative with the anti-IgM and the anti-non-gamma Coombs serum.

For in vitro studies with monocytes, mononuclear cells were separated from human peripheral blood by Ficoll-Hypaque density centrifugation. For in vitro studies with monocytes, mononuclear cells were separated from human peripheral blood by Ficoll-Hypaque density centrifugation. These cells (5 X 10⁸ cells/ml in RPMI-1640 with 24-mM Hepes buffer and 20% fetal calf serum) were allowed to adhere to glass coverslips in Leighton culture tubes for 60 min at 22°C. The adherent cells were washed three times, and fresh medium was added prior to the addition of sensitized cells. Analysis of latex-particle ingestion indicated that 85%-95% of the adherent cells were phagocytic.

Red cells for the monocyte assay were sensitized by incubation of 0.2 ml serum, serum fraction, or red cell eluate, 0.05 ml of quinidine gluconate (8 mg/ml), and 0.05 ml of a 50% suspension of patient or O Rh+ red cells at 37°C for 30 min. These cells were washed in saline three times and resuspended in culture medium. The sensitized cells were added to the monocytes in Leighton culture tubes in a 0.5% suspension and incubated 2 hr at 37°C. The coverslip was removed, and erythrophagocytosis and rosette formation were assessed microscopically. Scoring criteria were as follows: three or more adherent red cells/monocyte was considered to be positive for rosette formation; cytoplasmic ingestion of one or more red cells/monocyte was positive for phagocytosis.

Blocking experiments were performed by the addition of purified human IgG, final concentration 1.4 mg/ml (Meloy Laboratories), or an equivalent volume of saline to the medium at the same time indicator cells were added. IgG fractions from the patient’s serum and donor serum were isolated by DEAE Sephadex column chromatography with 0.1-M Tris-HCl buffer, pH 7.0. Immunoelectrophoresis of this material using antihuman serum showed a single band that corresponded to human IgG. In addition, a partially purified IgM fraction was prepared by Sepharose 6B column chromatography using 0.1-M Tris, 0.5-M NaCl buffer, pH 7.8. These fractions contained no IgG by immunoelectrophoresis.

In vitro platelet lysis was assessed by a modification of the ⁵¹Cr release assay, as previously described. In these experiments 0.05 ml of heat-inactivated serum, serum fraction, or red cell eluate was tested. Eluates were prepared from donor red cells sensitized with patient serum and quinidine by incubation with an equal volume of 6% albumin at 56°C for 10 min. The cells were centrifuged, and the supernatant was used for sensitization of platelets and red cells in further studies of antibody specificity.

To determine if quinidine directly binds to erythrocytes, 0.15 ml of red cells (50% suspension) was incubated with 0.15 ml of quinidine gluconate (8 mg/ml) at 37°C for 30 min. These cells were washed in saline either three times or ten times prior to incubation with 0.3 ml of patient serum and 0.3 ml of fresh donor serum, a source of complement. After 30 min at 37°C these cells were washed three times, and antiglobulin testing was performed. The interaction of these cells with monocytes was also evaluated.

RESULTS

Neither patient nor donor erythrocytes were reactive with antiglobulin serum, nor could sensitization be induced after incubation of donor red cells with the patient’s serum. However, after the addition of quinidine the patient’s serum resulted in positive antiglobulin reactions with both anti-IgG and anti-IgM antisera (Table 1). Using complement antiserum, strongly positive reactions were consistently observed. No hemolysis or spontaneous agglutination was observed.

Table 2 summarizes data evaluating red cell interactions with monocytes. Red cells treated with patient serum or IgG or IgM fraction alone were consistently negative. However, red cells treated with patient serum and quinidine resulted in both erythrophagocytosis and rosette formation. Similar results were obtained using the patient’s IgG fraction and quinidine. By contrast, the IgM fraction and quinidine, tested alone or in the presence of fresh serum as a source of complement, promoted only a minor degree of phagocytosis. It is usually recognized that complement promotes adherence, as opposed to phagocytosis. However, phagocytosis has also previously been noted with red cells coated with IgM and comple-
Table 1. Anti-globulin Testing

<table>
<thead>
<tr>
<th></th>
<th>Anti-gamma Coombs (anti-IgG)</th>
<th>Anti-IgM</th>
<th>Anti-gamma Coombs (anti-C3,C4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Patient cells</td>
<td>0</td>
<td>ND*</td>
<td>0</td>
</tr>
<tr>
<td>2. Patient or donor cells + patient serum</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3. Patient or donor cells + donor serum ± Q†</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4. Patient or donor cells + patient serum + Q</td>
<td>1-3 + ‡</td>
<td>1-3 ‡</td>
<td>3 +</td>
</tr>
</tbody>
</table>

*ND = not done.
†O = quinidine gluconate.
‡Values obtained on three tests.

In this assay monocytes do not react with IgM-coated cells,\textsuperscript{27,28} and therefore the responses may be due to complement components contaminating the IgM fraction. Indeed, immunoelectrophoresis of the separated IgM fraction continued to show a band in the β\textsubscript{1C}-globulin region.

The interaction of patient serum-quinidine-treated red cells with monocytes was blocked by the addition of IgG (1.4 mg/ml) to the culture medium. Moreover, this inhibition could be circumvented by compatible fresh serum as a source of complement during the red cell sensitization phase (Table 3). This suggested that red-cell-bound anti-quinidine antibodies fixed complement to the target red cells, and in part the monocyte reactivity was attributed to bound complement.

Similar results were obtained in the platelet lysis assay (Table 4). The patient’s serum and IgG fraction promoted \textsuperscript{51}Cr release in the presence of quinidine; the patient’s IgM fraction had no effect.

To further characterize the reaction, a red cell eluate was prepared; this proved capable of sensitizing target cells in the monocyte test. However, it did not promote platelet lysis in the \textsuperscript{51}Cr release test. This suggests either that antiplatelet antibodies were different than those reacting against red cells or that the \textsuperscript{51}Cr release assay is less sensitive than the monocyte test.

To evaluate the reaction among red cells, antibody, and drug, incubation studies were carried out in two stages. Donor red cells were incubated with quinidine for 30 min at 37°C and then washed three times or ten times in saline prior to addition of serum. After 30 min of incubation with patient serum, antiglobulin testing and monocyte interactions were evaluated. The results are shown in Table 5.

Cells that were incubated simultaneously with drug and serum showed moderate

Table 2. Red Cell Sensitization

<table>
<thead>
<tr>
<th>Monocyte/Red Cell Interaction</th>
<th>Phagocytosis</th>
<th>Rosettes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Donor serum, IgG\textsuperscript{<em>} or IgM\textsuperscript{</em>} ± Q†</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2. Patient serum, IgG\textsuperscript{<em>} or IgM\textsuperscript{</em>}</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3. Patient serum + Q</td>
<td>76%</td>
<td>50%</td>
</tr>
<tr>
<td>4. Patient IgG\textsuperscript{*} + Q</td>
<td>61%</td>
<td>26%</td>
</tr>
<tr>
<td>5. Patient IgM\textsuperscript{*} + Q</td>
<td>12%</td>
<td>0</td>
</tr>
<tr>
<td>6. Patient IgM\textsuperscript{*} + Q + C\textsuperscript{‡}</td>
<td>17%</td>
<td>0</td>
</tr>
</tbody>
</table>

*iG and IgM refer to column-separated fractions from either the patient or normal donor.
†O = quinidine gluconate.
‡Compatible fresh serum was used as a source of complement.
IgG and IgM refer to column-separated fractions from either the patient or normal donor. Note: Fresh plasma (a source of complement) is coherent in this assay system.

Table 3. Blocking Experiments With IgG Added to the Medium

<table>
<thead>
<tr>
<th>Red Cell Sensitization</th>
<th>Addition to Medium</th>
<th>Phagocytosis</th>
<th>Rosettes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient serum* + Q</td>
<td>Saline</td>
<td>76 ± 8%†</td>
<td>50 ± 10%</td>
</tr>
<tr>
<td></td>
<td>IgG (1.4 mg/ml)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Patient serum*</td>
<td>Saline</td>
<td>72 ± 9%</td>
<td>3 ± 3%</td>
</tr>
<tr>
<td>(1:1 with saline) + Q</td>
<td>IgG (1.4 mg/ml)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Patient serum*</td>
<td>Saline</td>
<td>83 ± 3%</td>
<td>52 ± 3%</td>
</tr>
<tr>
<td>(1:1 with fresh serum) + Q</td>
<td>IgG (1.4 mg/ml)</td>
<td>63 ± 10%</td>
<td>41 ± 5%</td>
</tr>
</tbody>
</table>

*Patient fresh serum frozen at −20°C. Cells were sensitized with serum and quinidine. Q = quinidine gluconate.
†Values expressed as mean ± SEM from 3–6 experiments.

reactivity in all assays. Extensive washing of the treated cells (ten washings) decreased the responses in both assays; however, positive results were still obtained. In part, the overall reduction in complement-mediated antiglobulin tests as compared with that reported in Table 1 may have resulted from prolonged storage of patient serum prior to these assays.

**DISCUSSION**

The present case illustrates three aspects of quinidine-induced immune cytopenias. First, quinidine can cause simultaneous hemolytic anemia and thrombocytopenia. Although these syndromes are not uncommon, the simultaneous occurrence of both processes has rarely been reported. Second, it appears that IgG complement-fixing antibodies are the prime mediators of both types of cellular destruction. Antiquinidine antibodies of the IgG class are commonly associated with thrombocytopenia, whereas IgM antibodies have been typically but not invariably involved in the drug-induced hemolytic anemia. Third, the persistence of antiquinidine antibodies on the red cell membrane as detected in the monocyte receptor assay suggests that the innocent-bystander mechanism may not apply to all cases of quinidine hemolysis.

Human monocytes are closely related to macrophages and bear membrane receptors for IgG and for activated complements but not for IgM, under these experimental conditions. These cells can rosette with or phagocytize antibody- or complement-coated cells, and thus they may provide an in vitro assessment of "immune clearance." As shown in this study, donor cells that were incubated with

Table 4. Platelet Lysis (% Cr Release Assay)

<table>
<thead>
<tr>
<th>Percentage Immune Lysis</th>
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<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>1. Patient serum, IgG* or IgM*</td>
</tr>
<tr>
<td>2. Donor serum, IgG*, IgM* + Q</td>
</tr>
<tr>
<td>3. Patient serum + Q</td>
</tr>
<tr>
<td>4. Patient IgG* + Q</td>
</tr>
<tr>
<td>5. Patient IgM* + Q</td>
</tr>
<tr>
<td>6. Red cell eluate + Q</td>
</tr>
</tbody>
</table>

*IgG and IgM refer to column-separated fractions from either the patient or normal donor.
Note: Fresh plasma (a source of complement) is coherent in this assay system.
quinidine and either the patient's serum or IgG fraction adhered to and were ingested by monocytes. Blocking experiments indicated that IgG was the predominant antibody. However, complement seemed to enhance the reaction. These findings were confirmed by antiglobulin testing using monospecific antisera to IgG and to complement.

The differences observed using IgM and IgG fractions in the monocyte assay suggest that the latter was the more potent antibody and may have been responsible for the in vivo phenomenon. Similar results were observed in the platelet lysis assay; activity was present in the IgG fraction but not the IgM fraction. Results of studies to determine if the same antibody was involved in both processes were inconclusive.

Quinidine is believed to have little affinity for the red cell membrane. It is commonly believed to cause hemolysis by an innocent-bystander mechanism.14,15 This implies that the drug-antibody complex passively attaches to the red cells and in the process causes irreversible binding of complement. The antibody complexes are believed to dissociate rapidly so that antiglobulin testing usually shows only complement components.1,2,4,5,7

However, results from this study suggest that the innocent-bystander mechanism may not be the only process involved in quinidine hemolysis. Antiglobulin testing provided evidence for attachment of antiquinidine antibodies of both IgG and IgM classes to the red cell membrane. Moreover, a two-step assay procedure in which donor erythrocytes were extensively washed after quinidine exposure still showed reactivity with the patient's serum. This suggests that the antiquinidine antibodies in this patient were capable of directly reacting with drug-treated red cells. Thus passive adherence of immune complexes may not solely account for quinidine-induced hemolytic anemia.

REFERENCES

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Immune hemolytic anemia and thrombocytopenia secondary to quinidine: in vitro studies of the quinidine-dependent red cell and platelet antibodies

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