Cefotetan-induced immune hemolytic anemia due to the drug-adsorption mechanism

R.J. Eckrich, S. Fox, and D. Mallory

Positive direct antiglobulin tests (DATs) associated with cephalosporin therapy have been reported, but rarely were associated with immune hemolytic anemia (IHA). In 1989, we described the first case of IHA associated with cefotetan (Cefotan™), causing hemolysis by the drug-adsorption mechanism. We now report the full details of our investigation. The patient was a 23-year-old female with a 2 1/2 year history of chronic ulcerative colitis. After 4 days of therapy with cefotetan (2 g/day), her hematocrit (Hct) decreased from 34.3% to 23.3%. The reticulocyte count was 6.9%. The DAT was 2+ (IgG only), and the serum and an eluate were nonreactive with a panel of standard reagent red blood cells (RBCs). Cefotetan therapy continued and the patient was transfused with two units of RBCs. On day 6 of therapy, the patient experienced an anaphylactoid reaction attributed to sensitivity to cefotetan. Cefotetan therapy was discontinued, the patient was treated with corticosteroids and epinephrine, and she was transfused with an additional unit of RBCs. Her Hct rose to 34.1% prior to her discharge on day 11. Further investigation revealed that the patient’s serum and an eluate contained an antibody that reacted with cefotetan- and cephalexin-coated RBCs by the indirect antiglobulin test. In a monocyte monolayer assay, monocytes readily phagocytized cefotetan- and cephalexin-coated reagent RBCs but not uncoated reagent RBCs. The patient’s serum did not react by the so-called immune-complex mechanism when cefotetan or cephalexin was added to the patient’s serum + complement + RBCs. Immunohematology 1994;10:51-54.

Positive direct antiglobulin tests (DATs) associated with cephalothin therapy were first described by Gralnick et al.1 and Molthan et al.2 in 1967. The primary mechanism for these positive DAT reactions was attributed to nonspecific, nonimmune binding of cephalosporin-protein complexes to the red blood cell (RBC) surface. This mechanism was later referred to as "membrane modification" (MM) when it was shown that washed, drug-treated RBCs would adsorb many different proteins.3 In 1971, Gralnick et al.4 described the first two cases of immune hemolytic anemia (IHA) associated with cephalothin therapy. The mechanism for these cases of IHA was attributed to anti-cephalothin antibodies attaching to drug-coated RBCs in vivo, resulting in extravascular hemolysis. This mechanism is similar to the "drug adsorption" (DA) mechanism, associated with penicillin therapy,5,6 that causes positive DATs and, on occasion, IHA.

Although frequently the cause of positive DATs, IHA caused by first-generation cephalosporins has been documented in only five case reports.4,7-9 Since 1987, however, there has been an increase in the incidence of IHA associated with second- and third-generation cephalosporins, including cefamandole,10 cefoxitin,11 cefotaxime,12,13 cefazidime,14 and ceftriaxone.15

In 1989, we reported the first case of IHA associated with cefotetan (Cefotan™, Stuart Pharmaceuticals, Wilmington, DE) causing hemolysis by the DA mechanism.16 Since this report, there have been six others describing IHA associated with cefotetan causing hemolysis by the DA and/or immune-complex (IC) mechanisms,17-22 including two fatal cases.21,22 We now report the details of our investigation of the first such case.

Case Report

The patient was a 23-year-old female with a 2 1/2-year history of chronic ulcerative colitis. She had been transfused once, in 1987, with six units of RBCs for anemia following rectal bleeding. She had been treated with numerous medications, including sulfasalazine, metronidazole, prednisone, ferrous sulfate, and vitamin B12. She had been on oral contraceptives before her initial diagnosis and had also used topical hydrocortisone for treatment of an unrelated dermatologic problem. The patient had never been pregnant. She had no previous history of therapy with any cephalosporin antibiotics.

On May 1, 1988, the patient was admitted to the hospital for acute ileitis and possible appendicitis. Her hematocrit (Hct) on admission was 34.3%. All other laboratory values were within normal limits. Upon admission, she was treated with sulfasalazine (3 g/day) and cefotetan, intravenously, 2 g per day.

On May 5, 1988, the patient’s Hct had fallen to 23.3%, despite no evidence of active bleeding. Her uncorrected reticulocyte count was 6.9%. Her peripheral blood smear showed polychromasia, microcytosis, and
spherocytes. She was found to be group O, D+ and had a positive DAT (2+) due to IgG. Her serum and an eluate from her RBCs were nonreactive by the indirect antiglobulin test (IAT) with a panel of reagent RBCs. She was transfused with two units of RBCs, and her post-transfusion Hct rose to 30.0%. Since cefotetan was not yet suspected as the cause of the positive DAT and the unexplained anemia, the drug was continued.

On May 7, 1988, the patient experienced life-threatening acute upper respiratory distress caused by an anaphylactoid reaction that was attributed to hypersensitivity to cefotetan. The symptoms subsided following treatment with corticosteroids and epinephrine, and cefotetan was discontinued. Her Hct had decreased to 30.0% and she was transfused with an additional unit of RBCs, after which her Hct rose to 34.1%. Her Hct remained stable and she was discharged on May 11, 1988. Her DAT remained positive through June 6, 1988.

**Materials and Methods**

**Samples**

Serum and eluate from the patient's May 5, 1988, sample, and a sample of serum drawn on June 27, 1988, were forwarded to our laboratory for investigation of a possible drug-induced IHA.

**Indirect antiglobulin test (IAT)**

The IAT method used for all studies employed a polyspecific anti-human globulin (AHG) (Gamma Biologicals, Houston, TX) and/or a monospecific anti-human IgG (American Red Cross, Rockville, MD). No potentiators were used. The serum or eluate and reagent RBC mixtures were examined for agglutination at immediate spin, after a 30-minute incubation at 37°C, and after the addition of AHG reagent. All negative reactions were confirmed with appropriate antiglobulin control reagent RBCs.

**Titration studies**

Titration were performed on the June 27, 1988, sample by using serial dilutions of the patient's serum in normal saline with 3% bovine serum albumin (NS/BSA) by the IAT method described above. The strength of agglutination was scored by the method of Marsh.23

**Drug-coated reagent RBCs**

Separate aliquots of reagent RBCs were coated with cefotetan, cephalothin (Keflin™, Eli Lilly & Co., Indianapolis, IN), and penicillin G (Sigma Chemical Co., St. Louis, MO) by the method of Garratty.24 All drugs were diluted to a concentration of 40 mg per mL in barbital-buffered saline (BBS). For cefotetan and cephalothin, 1 mL of group O, washed, packed reagent RBCs was incubated in 10 mL of a drug-BBS solution for 2 hours at 37°C. For penicillin, 1 mL of washed, packed reagent RBCs was incubated in 15 mL of a drug-BBS solution for 1 hour at room temperature. The treated reagent RBCs were washed in NS and resuspended in modified Alsever's solution (American Red Cross). Controls were prepared by incubation of the RBCs in identical ratios and at identical temperatures in BBS with no drugs added.

**Detection of antibodies to drug-coated RBCs**

Two drops of serum or eluate were mixed with one drop of a 5 percent suspension of drug-coated or control reagent RBCs and tested by the IAT method described above. When testing cefotetan- or cephalothin-coated RBCs, the serum was diluted 1 in 20 with NS.

**Detection of drug immune-complex formation**

Cefotetan, cephalothin, penicillin, and sulfasalazine were diluted to 1 mg per mL in phosphate-buffered saline (PBS), pH 7.4. Tests for detection of immune complexes formed by these drugs and their respective antibodies were by the method of Garratty.24

**Monocyte monolayer assay (MMA)**

To assess the hemolytic potential of an antibody reacting by the DA mechanism, MMA studies were carried out using the method of Nance et al.25 Peripheral blood monocytes from two normal donors were incubated with cefotetan-coated, cephalothin-coated, penicillin-coated, and uncoated reagent RBCs incubated at 37°C with the patient's serum, as well as with drug-coated and uncoated reagent RBCs incubated in PBS. Following incubation in tissue culture well slides, the wells were washed, fixed, and stained, and the percentage of reactive monocytes, i.e., monocytes having phagocytized or adherent RBCs, was determined. The normal range is 0–3 percent reactive monocytes.

**Results**

**Serologic studies**

Results of the IAT using serum and eluate are summarized in Table 1. The serum and eluate contained an antibody that reacted strongly with both cefotetan- and cephalothin-coated reagent RBCs, but failed to react with penicillin-coated or uncoated RBCs. Titration and score values done with the June 27, 1988, sample were...
Cefotetan-induced hemolytic anemia

Table 1. Serologic results—indirect antiglobulin test

<table>
<thead>
<tr>
<th>Sample dates</th>
<th>Serology</th>
<th>Red blood cells (RBCs)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 5, 1988, and June 27, 1988</td>
<td>Serum antibody screen</td>
<td>Untreated RBCs</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cefotetan-treated RBCs</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cephalothin-treated RBCs</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Penicillin-treated RBCs</td>
<td>0</td>
</tr>
<tr>
<td>May 5, 1988</td>
<td>RBC eluate</td>
<td>Untreated RBCs</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cefotetan-treated RBCs</td>
<td>2+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cephalothin-treated RBCs</td>
<td>2+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Penicillin-treated RBCs</td>
<td>0</td>
</tr>
<tr>
<td>June 27, 1988</td>
<td>Serum titers/scores</td>
<td>Cefotetan-treated RBCs</td>
<td>64/46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cephalothin-treated RBCs</td>
<td>16/28</td>
</tr>
</tbody>
</table>

slightly higher for cefotetan-coated RBCs than for cephalothin-coated RBCs (Table 1).

No reactions were observed when the patient's serum was incubated with reagent RBCs in the presence of any of the implicated drugs, with or without added complement.

MMA studies

Monocytes from two normal donors strongly phagocytized the cefotetan-coated reagent RBCs incubated with the patient's serum (62% and 40% reactivity), and less strongly, but still significantly, the cephalothin-coated RBCs incubated with the patient's serum (29% and 11% reactivity). Phagocytosis of penicillin-coated or uncoated RBCs incubated with the patient's serum and of the drug-coated and uncoated RBC controls incubated in PBS were within the normal range (0-3% reactivity).

Discussion

While each reported case of IHA due to cefotetan has unique features, all reported cases have one feature in common: the DAT became positive and significant immune-mediated hemolysis became present after only a short initial exposure (<7 days) to the drug.16-22 This temporal relationship contrasts with the positive DATs and IHAs associated with penicillin or first-generation cephalosporins.4,6-9 In the latter cases, the DAT becomes positive after patients have received large doses of the drug daily for 7 to 10 days, and the IHA is less acute in its onset than that seen with drugs causing hemolysis by the IC mechanism, developing slowly over a period of 7 to 10 days.

An unusual clinical feature of the present case was the severe upper respiratory distress (anaphylactoid reaction) caused by cefotetan. In the cases of cephalosporin- and penicillin-associated positive DATs and IHA caused by IgG antibodies, the patients did not experience the signs of drug hypersensitivity usually associated with IgE antibodies. In this case, it is interesting to note that the IHA was a minor clinical problem relative to the anaphylactoid reaction. Indeed, it was not until after the patient's discharge that her physicians suspected that the anemia might have been drug-induced and asked for serologic proof.

An unusual serologic feature of this case is the lack of cross-reactivity with penicillin. In earlier reports, investigators noted that penicillin antibodies, in sera or eluates, often reacted with cephalosporin-coated RBCs and vice versa, that is, cephalosporin antibodies also reacted with penicillin-coated RBCs.1,4,8 In the present case, the cefotetan antibody in the serum failed to react with penicillin-coated RBCs. In reviewing the history of positive DATs and IHA associated with cephalosporins, we noted an interesting and alarming trend. With each successive generation of these drugs, the number and severity of hematologic complications have increased. There are now four reported cases of fatal IHA associated with second- and third-generation cephalosporins.11,15,21 This case, as well as the others cited here, support the importance of carefully monitoring the hematologic status of patients on therapy with second- or third-generation cephalosporins.11,15,21

This case, as well as the others cited here, support the importance of carefully monitoring the hematologic status of patients on therapy with second- or third-generation cephalosporins. The possibility of drug-induced IHA should be investigated immediately if the patient develops an unexplainable anemia and positive DAT, especially when the accompanying IAT is negative or, as in this case, a patient experiences an anaphylactoid reaction due to sensitivity to the drug. While these drugs play an important role in fighting or preventing infections caused by gram-negative organisms, they also possess the potential to cause serious complications.

References


Notice to Readers: All articles published, including communications and book reviews, reflect the opinions of the authors and do not necessarily reflect the official policy of the American Red Cross.