Cross-Reactivity of Cefotetan and Ceftriaxone Antibodies, Associated With Hemolytic Anemia, With Other Cephalosporins and Penicillin

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Key Words: Cross-reactivity; Drug-induced immune hemolytic anemia; Cefotetan antibody; Ceftriaxone antibody; Antibiotics

Abstract

Most drug-induced immune hemolytic anemias since the late 1980s have been caused by the second- and third-generation cephalosporins, cefotetan and ceftriaxone, respectively. Cross-reactivity of cefotetan and ceftriaxone antibodies with other cephalosporins or penicillin has been studied only minimally. We tested 7 serum samples previously identified to contain cefotetan antibodies and one serum sample previously identified to contain ceftriaxone antibodies against 9 other cephalosporins, penicillin, and 7-aminocephalosporanic acid in the presence of RBCs and also used hapten inhibition to indicate cross-reactivity. Serum samples containing cefotetan antibodies showed some cross-reactivity with cephalothin and cefoxitin (and to a much lesser extent with penicillin and ceftazidime). The ceftriaxone antibodies showed very weak cross-reactivity with cefotaxime, cefamandole, and cefoperazone. There was very little cross-reactivity between cefotetan antibodies and the drugs tested in the present study. We have no data to determine whether the in vitro data relate to in vivo reactivity.

Drug-induced immune hemolytic anemia (IHA) occurs rarely, with an estimated incidence of about 1 case per million of the population.1 In the 1970s, 67% of drug-induced IHA was due to methyldopa and 23% to penicillin.2 Since the late 1980s, 88% of drug-induced IHA that our laboratory has studied has been due to the second- and third-generation cephalosporins, cefotetan (75%) and ceftriaxone (13%).3,4

Table II shows that first-generation cephalosporins rarely caused IHA. The clinical and serologic manifestations in these cases were similar to penicillin-induced IHA.2 In contrast, 3 second-generation cephalosporins and 4 third-generation cephalosporins have been associated with numerous cases of IHA (Table 1). In some cases, severe intravascular hemolysis occurred, which was supported by serologic findings that indicated an “immune complex” mechanism (the patient’s serum reacts with untreated RBCs in the presence of drug) rather than the “drug adsorption” mechanism (the patient’s serum reacts with drug-coated RBCs). Fatal hemolysis occurred in 1 (33%) of 3 cases due to cefoxitin, 11 (17%) of 64 cases due to cefotetan, and 9 (47%) of 19 cases due to ceftriaxone.

The extent of cross-reactivity of cefotetan and ceftriaxone antibodies with RBCs in the presence of other cephalosporins and penicillin is poorly documented. In a few reports, the patient’s serum samples containing cefotetan (n = 9) or ceftriaxone (n = 3) antibodies were tested by the drug adsorption or immune complex methods against 1,7,10,242,8,12,25,263,274,28 or 518 different antibiotics. We present results of testing 7 cefotetan antibody samples and 1 ceftriaxone antibody sample against 9 other cephalosporins, penicillin, and 7-aminocephalosporanic acid, the basis for semi-synthetic cephalosporins.
Materials and Methods

Antibodies

Antibodies studied were 7 cefotetan antibodies previously shown to react with cefotetan-treated RBCs and untreated RBCs in the presence of cefotetan and 1 ceftriaxone antibody previously shown to react with untreated RBCs in the presence of ceftriaxone. The source of complement was a pool of serum samples from healthy individuals that were separated, pooled, and frozen at −70°C on the day the serum samples were obtained.

Drugs

The following drugs were studied: cefotetan disodium (Cefotan, Zeneca Pharmaceuticals, Wilmington, DE), ceftriaxone sodium (Rocephin, Roche Laboratories, Nutley, NJ), cephalothin sodium (Keflin, Eli Lilly, Indianapolis, IN), cefazolin sodium (Kefzol, Eli Lilly), cefamandole nafate (Mandol, Eli Lilly Italia, Florence, Italy), cefoxitin sodium (Mefoxin, Merck, West Point, PA), cefotaxime sodium (Claforan, Hoechst-Roussel, Somerville, NJ), cefoperazone sodium (Cefobid, Roerig, Pfizer, New York, NY), penicillin G potassium (Pfizerpen, Roerig, Pfizer), ceftizoxime (Fortaz, Glaxo Wellcome, Research Triangle Park, NC), and cefepime hydrochloride (Maxipime, Bristol Myers Squibb, Princeton, NJ). The 7-aminocephalosporanic acid was obtained from Sigma Chemical (St Louis, MO).

Antibody Detection Using Drug-Treated RBCs

Penicillin-treated and cephalothin-treated RBCs were prepared by previously described methods. Briefly, 40-mg/mL solutions of penicillin G and the cephalosporins were prepared in a 0.1-mol/L concentration of sodium barbital (Sigma) buffer, pH 9.8, or phosphate-buffered saline (PBS), pH 7.3, respectively. Group O RBCs were added, incubated for 1 to 2 hours at room temperature (penicillin) or 37°C (cephalosporins), washed, and suspended to 3% to 5% (vol/vol) for use.

Ceftriaxone has never been shown to bond to RBCs by the aforementioned methods. A previously described method using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (Sigma) was used to try to bond ceftriaxone to RBCs. Briefly, 3 mL of ceftriaxone (6.7 mg/mL PBS), 0.1 mL 50% washed type O RBCs, and 1 mL freshly prepared carbodiimide (50 mg/mL in PBS) were incubated for 50 minutes at 4°C and then poured into 4°C PBS + 2% Na4EDTA (Sigma). Treated RBCs were washed 3 times and resuspended to 3% to 5% (vol/vol) in PBS for use.

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Six serum or plasma samples containing cefotetan (historic antiglobulin test titers ranged from 65,000 to 262,000) antibodies and a pool of normal “inert” plasma were tested, undiluted and/or diluted in PBS, against drug-treated and untreated RBCs by a saline (60 minutes at 37°C) anti-IgG (American National Red Cross, Washington, DC) method. The normal plasma was used as a control for nonspecific reactions with cephalosporin-treated RBCs.

Antibody Detection Using “Immune Complex” Method

Three serum samples that historically reacted to titers of 1,024 to 4,096 (antiglobulin test) when tested against untreated RBCs in the presence of cefotetan and 1 serum sample that reacted in the presence of ceftriaxone to titers of 80, 5,120, and 640 (hemolysis, agglutination, antiglobulin test, respectively) were used for testing in the presence of other drugs by a previously described method. Two
volumes of serum, 2 volumes of complement source, 2 volumes of drug solution (1 mg/mL in PBS), and 1 volume of 10% ficin-treated RBCs were incubated for 1 hour at 37°C. After examination for hemolysis, agglutination, or both, the RBCs were washed and tested with polyspecific antiglobulin serum (Ortho Diagnostic Systems, Raritan, NJ). Doubling dilutions of the 3 cefotetan antibody serum samples (in complement source) were tested in the presence of cefotetan to determine the hemolysin and antiglobulin test titers vs ficin-treated RBCs.

Hapten-Inhibition Studies
Two samples containing cefotetan antibodies were tested, at a dilution predetermined to react 2+ by antiglobulin test against cefotetan-treated RBCs, in the presence of different concentrations of drug by a modification of previously described hapten-inhibition methods.32,34 One volume of the cefotetan antibody dilution (1 in 20,000 in PBS) was incubated with 1 volume of drug (0.1, 1.0, and 10.0 mg/mL in PBS) for 1 hour at 37°C. One volume of 3% to 5% (vol/vol) cefotetan-treated RBCs was added, and the tests were reincubated for 1 hour at 37°C. After examination for agglutination, the RBCs were washed and tested with anti-IgG.

Results

Drug-Treated RBCs
Results are shown in Table 2. All 6 serum samples containing cefotetan antibodies showed reactivity with cephalothin- and cefoxitin-treated RBCs. Most samples containing cefotetan antibodies showed some weak reactivity with penicillin-treated RBCs. Weak reactivity was seen, using some serum samples, with some of the other drug-treated RBCs.

Immune-Complex Testing
Results are shown in Table 3. The 3 samples of cefotetan antibodies did not show hemolysis in the presence of any drug other than cefotetan. Only 1 sample containing cefotetan antibodies (sample 2) showed reactivity in the antiglobulin test in the presence of a drug other than cefotetan; this was cephalothin. The serum containing ceftriaxone antibodies hemolyzed enzyme-treated RBCs only in the presence of 1 drug other than ceftriaxone; this was cefotaxime. Weak agglutination and/or positive antiglobulin test results were seen in the presence of cefotaxime, cefamandole, and cefoperazone. A pool of fresh normal serum samples, tested in parallel, was nonreactive.

Hapten Inhibition
Results are shown in Table 4. Both samples of cefotetan antibodies were inhibited or partially inhibited by 10-mg/mL solutions of cefotetan and cephalothin; 1 was additionally inhibited by a 10-mg/mL solution of ceftazidime.

Discussion
Second- and third-generation cephalosporins are, by far, the most common cause of drug-induced IHA at present.3,4 We often are asked what would be the effect of giving other cephalosporins to patients who have (or previously had) drug-induced IHA due to cefotetan or ceftriaxone antibodies. We could find few data in the literature on the degree of cross-reactivity of various cephalosporins. The same question also

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* Positive results are shown in bold.
was posed some years ago regarding the various penicillins and the cross-reactivity of antibodies to penicillin with the first-generation cephalosporins (eg, cephalothin).

Most of the work relating to the latter question involved allergic reactions (eg, Could patients who were allergic to penicillin G receive other penicillins, or cephalothin?).\textsuperscript{35-40} Penicillins and cephalosporins share a basic beta-lactam structure.\textsuperscript{\textbullet} Antibodies can be directed to this basic structure or to side chains, which may be specific to a certain antibiotic. Most penicillin-sensitive patients develop antibodies to the beta-lactam nucleus; thus, extensive cross-reactions are observed with semisynthetic penicillins that have different side chains.\textsuperscript{43} Some patients develop antibodies exclusively against side chains (eg, a patient may be allergic to amoxicillin but not to penicillin G).

Cross-reactivity of penicillins and cephalosporins has been more controversial. Although there is evidence that antibody responses to penicillins and cephalosporins are closely linked,\textsuperscript{44} the incidence of reactions to cephalosporins in patients with a history of penicillin allergy is low. Novalbos et al\textsuperscript{40} reported that the risk of a patient with penicillin allergy having an allergic reaction to cephalosporins was very low if the cephalosporins had a side chain different from the responsible penicillin. Cephalosporin allergy in patients not allergic to penicillin has been reported, but the exact incidence is unclear.\textsuperscript{45} Cerny and Pichler\textsuperscript{45} believe that the interpretation of available data is controversial, and, although some believe it is safe to administer a cephalosporin to a patient with penicillin allergy, they still recommend an immunologically unrelated antibiotic for patients with penicillin or cephalosporin allergy.
There are some data in the literature relating to cross-reactivity of penicillin and various cephalosporin antibodies with respect to RBC interactions. Abraham et al³⁴ described a severe cutaneous reaction to cephalothin in a patient who previously had received penicillin repeatedly. The patient had a positive skin test with cephalothin but not with penicillin. The patient also had an antibody that reacted with cephalothin but not with penicillin-treated RBCs. The patient subsequently was given penicillin with no reaction. Petz⁴⁶ showed that penicillin and cephalothin antibodies usually cross-react with penicillin and cephalothin-treated RBCs, but in 40% of serum samples, strikingly dissimilar titers are present. When penicillin was administered to patients with antibodies to both types of treated RBCs, 55% showed an increased titer with penicillin-treated RBCs and 23% an increased titer with cephalothin-treated RBCs. Following cephalothin administration, 25% showed increased titers against cephalothin-treated RBCs and 14% with penicillin-treated RBCs. Spath et al⁴⁷ showed that serum samples from patients in whom positive direct antiglobulin tests developed following cephalothin administration reacted with penicillin and cephalothin-treated RBCs to a similar titer. Adsorption of 3 serum samples with penicillin-treated RBCs caused an almost complete loss of activity, a moderate loss of activity, and no significant decrease in titer, respectively, against cephalothin-treated RBCs.

The published reports of immune hemolytic anemia caused by cefotetan contain some reports of the cefotetan antibodies' being tested against RBCs treated with other cephalosporins and sometimes penicillin. One reported negative reactions with cefotaxime-coated RBCs,²⁷ and 3 reported negative reactions with cefazolin.¹⁰,¹²,²⁷ There are single reports of positive reactions with cefadroxil,²⁶ cefalexin,⁸ ceftriaxone,⁷ and cephalothin.²⁵ Two reported positive²⁶,²⁷ and 2 reported negative¹²,²⁵ reactions with penicillin-treated RBCs.

Compared with the other cephalosporins shown in Figure 1, cefotetan has a unique R₁ group. The portion(s) of the cefotetan molecule against which the patient’s antibodies are directed is unknown. Both cephalothin and cefoxitin showed the strongest cross-reactivity with cefotetan antibodies. Their R₁ and R₂ groups are very similar to each other but are quite different from those in cefotetan. Cefamandole, which has the same R₂ structure as cefotetan, was only weakly cross-reactive in our studies. Cefamandole, cefoperazone, and

Table 5

Results of Cefotetan Antibodies Tested Against Other Cephalosporin-Treated RBCs

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0, negative; +, positive; (+), weakly positive; —, not tested.

Figure 1 Chemical structures for 7-aminocephalosporanic acid (7-ACA), penicillin, the cephalosporin (eg, cephem) nucleus, and the R₁ and R₂ side chains found in the cephalosporins that were tested in the present study (modified from Mandell and Petr⁴¹ and O’Neil⁴²).
Cefotaxime showed weak cross-reactivity with ceftriaxone antibodies by immune complex testing. Only cefotaxime and ceftriaxone share a common R1 structure.

**Conclusions**

There are data in the literature proving that patients have a more severe hemolytic anemia (even fatal hemolytic anemia) when receiving a second dose of the drug causing the original hemolytic episode. It is not always clear whether drugs that are closely related will cause the same clinical effects. Although it usually is recommended that patients with cephalosporin-induced hemolytic anemia never take any of the cephalosporins again, there are few data to support that advice. Our results suggest that there is very little cross-reactivity with antibodies to the 2 most common cephalosporins (cefotetan and ceftriaxone) to cause drug-induced IHA and the other cephalosporins we tested. Whether these in vitro data relate to in vivo reactivity remains unknown.

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**References**
