Persistence of cefotetan on red blood cells

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BACKGROUND: Cefotetan can cause severe immune hemolytic anemia that may persist long after the drug is discontinued. To study the binding of cefotetan to RBCs, patients who received cefotetan were followed and tested for the presence of antibody to cefotetan.

STUDY DESIGN AND METHODS: Patients receiving cefotetan were identified from pharmacy and nursing records. Blood samples obtained for routine hematology tests were analyzed. Cefotetan binding to patients' RBCs was tested using a previously characterized high-titer anticefotetan serum by gel technique. To determine the minimum amount of drug necessary for binding to occur, RBCs were incubated with serial dilutions of cefotetan at pH 7.4.

RESULTS: Sixty patients receiving 1 to 25 g IV (median, 2 g) of cefotetan were followed for 1 to 123 days (median, 18 days). All were initially positive, for cefotetan on RBCs. Positivity persisted for up to 98 days after the last dose of drug. Fifteen patients became negative during follow-up. The first negative sample occurred at Day 30 to 123. Using the midpoint between the last positive and first negative to estimate of the duration of positivity, we estimate that cefotetan remains RBC-bound for 16.5 to 92 days (median, 67.5 days). During the follow-up period, five patients developed anticefotetan detectable in the serum. Twenty patients receiving other cephalosporin antibiotics showed no specific reactivity of their RBCs with anticefotetan. In vitro studies showed a minimum necessary drug concentration of 1 μmol/L at physiologic pH, which was not significantly altered by RBC pretreatment with ficin, sialydase, or DTT.

CONCLUSIONS: Cefotetan is tightly bound to RBCs after intravenous administration and remains detectable for weeks after the last dose. Antibodies to cefotetan may occur in about 8 percent of patients receiving the drug. The minimum necessary concentration for RBC binding is low compared to an estimated plasma concentration of 240 μmol/L from a single IV dose of 1 g.

Cefotetan is a second-generation cephalosporin with a broad spectrum of activity against Gram-negative rods. It is commonly used for preoperative prophylaxis before abdominal surgery and Caesarian section. Like other cephalosporins, cefotetan can cause immune hemolytic anemia (IHA). Serologic studies have suggested that cefotetan is the most common cause of antibiotic-induced IHA.[1] A review of cefotetan-induced hemolytic anemia reported to the USFDA and the WHO identified 85 cases since 1985.[2] Of these cases, 76 percent were female, 59 percent had received the drug for surgical prophylaxis, 30 percent received the drug for 1 day or less, and 18 percent had a prior exposure to cefotetan. Fifteen fatalities were identified. Within the fatal cases of IHA, the treatment length ranged from 1 to 11 days. Eleven of the fatal cases had no prior history of exposure to the drug.

Cefotetan-induced IHA may occur several weeks after the last dose.[3,4] We have previously observed prolonged hemolysis in cefotetan-induced IHA after withdrawal of the drug. Although cefotetan is know to have a plasma $t_{1/2}$ of 3 to 4 hours,[5] which is relatively long compared to other cephalosporins, the persistence of IHA for days or weeks is unexplained. We hypothesized that cefotetan may remain bound to RBCs after expected clearance of the drug from plasma. We have studied the binding of cefotetan to RBCs in vivo and in vitro to test this hypothesis.

MATERIALS AND METHODS

Inpatients receiving cefotetan at the University of Michigan Medical Center were identified from pharmacy

ABBREVIATION: IHA = immune hemolytic anemia.

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records. When there was any question of dosage, medication administration records were checked. Blood samples were obtained after routinely ordered hematology tests were performed. The Institutional Review Board of the University of Michigan Medical School approved the study.

Cefotetan-coated cells were prepared, as previously described, by incubation of 1 mL RBCs with 465 mg cefotetan disodium (AstraZeneca, Wilmington, DE) dissolved in 15 mL of boric acid buffer, pH 9.6 to 10, for 1 hour at 37°C.[6] Drug-coated RBCs were stored in Alsever's solution until used. For detection of cefotetan on RBCs, we used a previously characterized antibody from a patient with cefotetan-induced IHA (IgG titer = 250,000). The serum reacted strongly with cefotetan-coated RBCs at a 1 : 500 dilution and did not react with uncoated RBCs regardless of ABO group. Testing was performed using ID-Micro Typing System™ IgG Gel cards (Ortho Clinical Diagnostics, Raritan, NJ). For each sample analyzed, RBCs were washed and suspended in MTS Diluent 2™ at 0.8 percent, and 50 µL was dispensed into each of two gel columns. To one column, 25 µL of the 1 : 500 dilution of anticefotetan were added, and 25 µL of 6 percent albumin were added to the other column as a control for IgG coating of patient RBCs. IgG Gel cards were incubated for 15 minutes at 37°C, centrifuged, and read according to the manufacturer's directions. Cefotetan-coated RBCs were included in each batch as a positive control, and matched uncoated RBCs were included as a negative control. When the albumin control was positive, indicating that the RBCs had a positive DAT, the patient's serum was tested neat against cefotetan-coated RBCs and control RBCs to detect anticefotetan.

For in-vitro studies, cefotetan disodium was prepared immediately before use in PBS pH 7.4. Five percent suspensions of normal donor RBCs were prepared in PBS. Serial twofold dilutions of cefotetan were prepared, taking into account the RBC diluent volume, with final concentrations of 100 to 0.39 µmol/L (62-0.24 mg/L). RBCs were incubated with drug for 1 hour at 37°C, washed with PBS, and tested with anticefotetan at 1 : 500 dilution by gel technique as above. Experiments were performed in three replicates using four different normal donor RBC samples. In some experiments, RBCs were pretreated with ficin, sialydase, or DTT according to published methods before incubation with cefotetan.[6]

RESULTS

Sixty patients receiving cefotetan were identified. All received intravenous doses of the drug. The total dosage range was 1 to 25 g (median, 2 g). The follow-up time ranged from 1 to 123 days (median, 18 days).

Twenty patients not receiving cefotetan were also tested. Seven patients received ceftriaxone, four received cefazolin, one received ceftazidime, and one received cefepime. One patient who received ceftriaxone had a positive test with both anticefotetan and 6-percent albumin. This patient had a 1+ positive DAT with anti-IgG, negative with anti-C3. She had a history of dematomyositis, rheumatoid arthritis, and chronic HBV infection. There was no history of cefotetan exposure. An RBC eluate was not performed. None of the others manifested a positive test with anticefotetan or 6-percent albumin.

All patients receiving cefotetan manifested a positive test of 1+ strength or greater with anticefotetan on the first sample obtained after with drug was begun. Positivity persisted for up to 98 days after the last dose was given (Fig. 1). During the follow-up period, a negative sample was obtained on 15 patients. The first negative sample occurred at Day 30 to 123. Using the midpoint between the last positive and first negative to estimate the duration of positivity, we estimate that cefotetan remains RBC-bound for 16.5 to 92 days (median, 67.5 days) (Fig. 2).

During the follow-up period, five patients developed anticefotetan antibodies detectable in the plasma. Anticefotetan was first detected 5 to 78 days (median, 54 days) after the first dose. One of these patients had a previous documented exposure to cefotetan; however, complete records were not available for all patients. Chart reviews were performed. No patient had a clinically evident hemolytic reaction. All five patients had decreases in Hb level at the time that anticefotetan was detected from 0.1 to 1.7 g per dL (median, 1.2 g/dL). Serum bilirubin concentration was measured in three patients after anticefotetan was detected and was normal in all cases. Antibody titration was performed in one case. The anticefotetan titer was 2560. Three of these patients received prophylactic doses of 1 to 4 g, whereas the other two received 2 g per day for 10 and 12 days. There was no

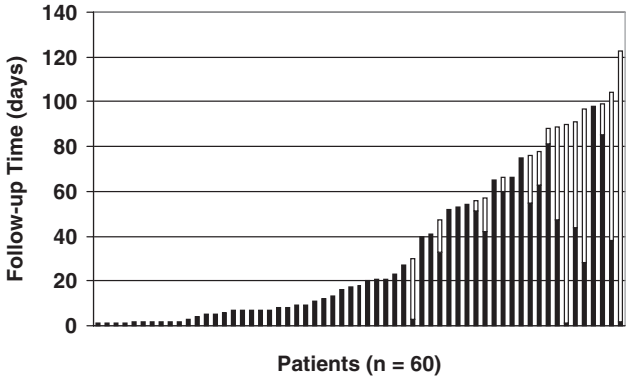

Fig. 1. Length of follow-up. The bars represent the length of follow-up from the last drug dose for each patient in the study. The top of the black bar indicates the last positive sample tested, and the top of the white bar indicates the first negative sample tested. The length of the white bars indicates the interval during which cefotetan became undetectable on circulating RBCs.

apparent difference between antibody producers and nonantibody producers in the dose of cefotetan administered (Fisher's exact test, p = 0.12).

Eight patients received RBC transfusions during the follow-up period for which paired pre- and posttransfusion samples were available. The transfusions occurred from 2 to 66 days after the last drug dose. In seven of these, there was no change in reactivity strength of the posttransfusion RBCs with anticefotetan, as compared to a pretransfusion sample. With one transfusion occurring 52 days after the last dose, the pretransfusion sample was 1+ with anticefotetan, and the posttransfusion sample did not react. No mixed-field reactivity was seen after any transfusion, including four cases where the pretransfusion reactivity was 3+ or greater.

We also performed in-vitro studies in an effort to determine which membrane proteins might be involved and the minimum concentration for cefotetan binding to RBC. At physiologic pH, 1+ reactivity occurred at approximately 1 µmol/L (Fig. 3). Pretreatment of RBCs with ficin or DTT did not alter cefotetan binding (minimum positive concentration, 0.78 µmol/L). Sialydase pretreatment caused a slight diminution in agglutination strength (minimum concentration, 1.63 µmol/L).

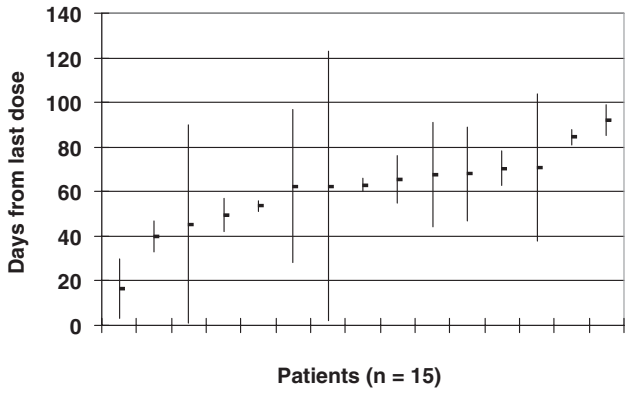

Fig. 2. Time from last positive to first negative sample. Results for the 15 patients in whom cefotetan testing became negative during follow-up. The bottom of each bar indicates that time from the last drug dose that the last positive test occurred. The top of each bar indicates the occurrence of the first negative test. The midpoints of the intervals cluster about 64 days.

DISCUSSION

These data indicate that cefotetan readily becomes tightly bound to RBCs when administered at prophylactic or therapeutic doses. After an intravenous dose of 1 g, the peak serum concentration is approximately 150 mg per L or 242 µmol/L.[5] This is well in excess of the minimal concentration of 1 µmol/L we found for in-vitro RBC binding. Cefotetan appears to remain bound to RBCs for most, if not all, of the life span of the cell. This markedly prolonged persistence may account for observed hemolytic reactions that continue for weeks after the drug is withdrawn.

In addition, we found that 5 of 60 patients (8%) in this study produced detectable anticefotetan. Although these patients had at most mild hemolysis, and none had a clinical reaction, this is a strikingly high rate of immune response. To our knowledge, this is the first study to prospectively identify immune responses to cefotetan. If further investigations confirm a high rate of antibody production, then testing previously exposed patients before administration of cefotetan again may be warranted.

We did not observe a dual cell population in patients who were transfused after the last dose of cefotetan. It is possible that our method was not sufficiently sensitive to detect a minor population of RBC that did not react with anticefotetan. Alternatively, it may be possible that transfused RBCs acquired cefotetan from previously coated cells in vivo. Further studies with highly sensitive methods such as flow cytometry will be necessary to distinguish between these possibilities.

The mechanism of cefotetan binding to the RBC membrane is unclear. Membrane proteins sensitive to ficin degradation, sialic acid residues, sulfhydryls, and free amines are not likely to be involved. A slight difference between untreated and sialydase-treated RBCs was observed. However, the preponder-

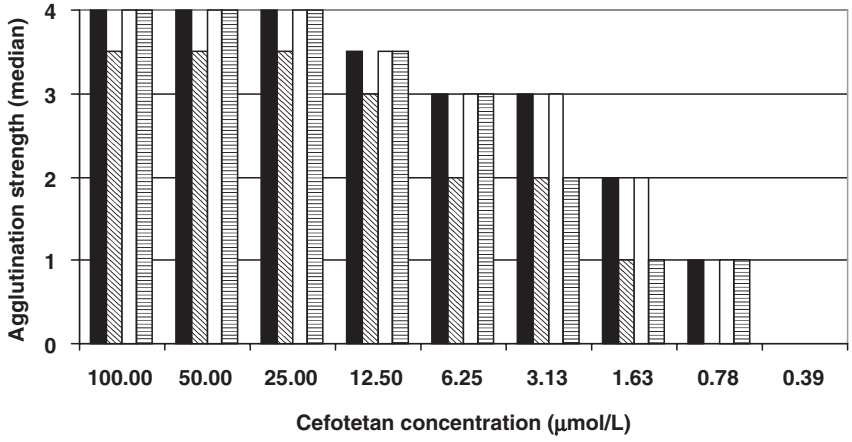

Fig. 3. In-vitro treatment of RBCs with cefotetan. RBC-coating by cefotetan at varying concentrations of drug. Results are given as the median agglutination strength of three replications using four different normal donor RBCs. Pretreatment of RBCs with sialydase, ficin, or DTT did not substantially alter cefotetan binding to RBCs. ■, untreated; ▨, sialydase; □, ficin; ▤, DTT.

ance of cefotetan binding is clearly not dependent on sialic acid.

We conclude from these data that cefotetan binds tightly to RBCs of patients given typical doses and that the drug persists on RBCs for about 9 weeks. Furthermore, the incidence of antibody production to cefotetan may be as high as 8 percent. Given the common use of cefotetan, there may be a substantial number of patients at risk of IHA if given a second dose of cefotetan.

REFERENCES

1. Arndt PA, Leger RM, Garratty G. Serology of antibodies to second- and third-generation cephalosporins associated with immune hemolytic anemia and/or positive direct antiglobulin tests. Transfusion 1999;39:1239-46.
2. Viraraghavan R, Chakravarty AG, Soreth J. Cefotetan-induced haemolytic anaemia: a review of 85 cases. Adv Drug React Roxicol Rev 2002;21:101-7.
3. Garratty G, Leger RM, Arndt PA. Severe immune hemolytic anemia associated with prophylactic use of cefotetan in obstetric and gynecologic procedures. Am J Obstet Gynec 1999;181:103-4.
4. Ray EK, Warkentin TE, O'Hoski PL, Gregor P. Delayed onset of life-threatening immune hemolysis after perioperative antimicrobial prophylaxis with cefotetan. Can J Surg 2000;43:461-2.
5. Martin C, Thomachot L, Albanses J. Clinical pharmacokinetics of cefotetan. Clin Pharmacokinet 1994;26: 248-58.
6. Judd WJ. Methods in immunohematology. 2nd ed. Durham, NC: Montgomery Scientific, 1994.