

Ceftriaxone Induced Immune Hemolytic Anemia: Detection of Drug-dependent Antibody by Ex-vivo Antigen in Urine

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There have been a few reported cases of immune hemolytic anemia induced by ceftriaxone. We encountered a patient with immune hemolytic anemia that seemed to be stimulated by a degradation product of ceftriaxone. The patient's direct antiglobulin test was positive only for C3d, and no ceftriaxone-dependent antibodies were detectable in the patient's serum. To demonstrate the presence of the ceftriaxone-induced antibodies, an ex-vivo antigen in urine was obtained from the patient. In addition, we prepared a 1 mg/mL suspension solution of ceftriaxone, and group AB serum as a complement source. Using several combinations of the above reactants, the indirect antiglobulin test was performed. Only the indirect antiglobulin test using the patient's serum with the ex-vivo urine antigen was found to be positive. Other combinations were not reactive. To our knowledge, this is the first reported case in Korea, in which the causative antibody appeared to be stimulated solely by a degradation product of ceftriaxone.

Key words: Ceftriaxone, drug-dependent antibody, immune hemolytic anemia, ex-vivo antigen

INTRODUCTION

Ceftriaxone is a third generation cephalosporin, which has a triazene substituent at the 3rd position of the nucleus, resulting in a mean elimination half-life of 8 hours.¹ Administration of the drug once or twice daily has been effective for patients with meningitis, while once a day dosage has been effective in treating other infections.² About half the administered dose can be re-

covered from the urine in unchanged form, and the remainder appears to be eliminated by biliary secretion.³ Adverse reactions of ceftriaxone administration have included diarrhea, skin rash, thrombocytopenia, eosinophilia, transient neutropenia, serum hepatic enzyme elevation, biliary sludge, and rarely, hemolytic anemia.⁴ Ceftriaxone induced immune hemolysis has been reported in immunocompromised patients or patients with hematologic diseases.⁴⁻⁷ We encountered a patient with ceftriaxone induced immune hemolytic anemia that seemed to be stimulated by a degradation product of ceftriaxone in urine.

CASE REPORT

On February 23rd, 2000, a 46-year-old man was admitted to the emergency center with multiple trauma due to a motor vehicle accident. Upon physical and radiological evaluation, he was found to have a pelvic bone fracture, facial bone fracture, basal skull fracture, and a bilateral tibiofibular open compound fracture. His initial hemoglobin level was 12.1 g/dL and his hematocrit was 34.5%. A daily dose of 2g ceftriaxone (Triaxone, Hanmi Pharmaceutical Co., Ltd., Seoul, Korea) was administered to prevent wound infection. The patient underwent surgery the following morning. His right lower extremity was amputated, and the operation was completed successfully with no further bleeding. Sixteen units of packed RBC were transfused until the operation was completed. After the operation, he was transferred to the general ward. There were no post-operative complications except for mild fever. On the 6th day of hospitalization, sudden

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cardiac arrest developed. Fortunately, he was resuscitated successfully and transferred to the intensive care unit. Laboratory evaluation revealed a drop in his hemoglobin level from 9.1 g/dL one day before the episode to 5.4g/dL. The serum total bilirubin was 12.1 mg/dL, LDH 1,980 IU/L, AST 503 IU/L, ALT 314 IU/L. The serum haptoglobin level was 7.25 mg/dL and also significant hemoglobinuria was noted. On a peripheral blood smear, many spherocytes (20-30/HPF) and nucleated RBCs (5/100 WBC) were found. Because immune hemolytic anemia was suspected, look back procedures for blood group mismatch were performed. These procedures were all negative except for the fact that the direct antiglobulin test (DAT) was positive. The DAT was 2+ positive with anti-C3d and negative with anti-IgG. The antibiotics being administered were changed because drug-induced immune hemolytic anemia was strongly suspected. Thereafter the patient recovered from his severely anemic status with only four units transfusion of packed RBC (Fig. 1). To confirm the drug-induced immune hemolytic anemia, we performed ceftriaxone-related serologic tests according to the standard methods.⁸ To demonstrate the presence of the drug-dependent antibody using the ex-vivo urine antigen, the following reagents were prepared. Ceftriaxone (Triaxone, Hanmi Pharmaceutical Co., Ltd., Seoul, Korea) was dissolved in 0.9% NaCl solution to prepare a 1 mg/mL

suspension solution of the drug in normal saline. Ex-vivo antigen in urine was obtained from a volunteer patient receiving daily doses of ceftriaxone as part of the treatment for a simple Colles' fracture. Group AB fresh serum as a complement source was prepared from the healthy blood donor. The screening cell (DiaMed AG Diagnostic and Medical products, Murten, Switzerland) was used as normal O indicated RBCs. The patient's serum was obtained on the 7th day of admission when the patient's clinical status was at its worst, at the point when the most severe hemolytic attack occurred. We prepared the test mixtures as shown in Table 1. To 3 drops of each test mixture, 1 drop of normal 3% RBCs was added, and the indirect antiglobulin test was performed. Table 1 shows that the patient's serum was not reactive with normal 3% RBCs either in the presence of or in the absence of ceftriaxone drug solution. There was no reaction when normal serum was tested with normal 3% RBCs and ex-vivo urine antigen. However, the reactivity of the patient's serum with ex-vivo urine antigen and RBCs was 2+.

DISCUSSION

Many drugs are capable of causing antibody-mediated immune hemolytic anemia. The prevalence of cases of cephalosporin-induced immune

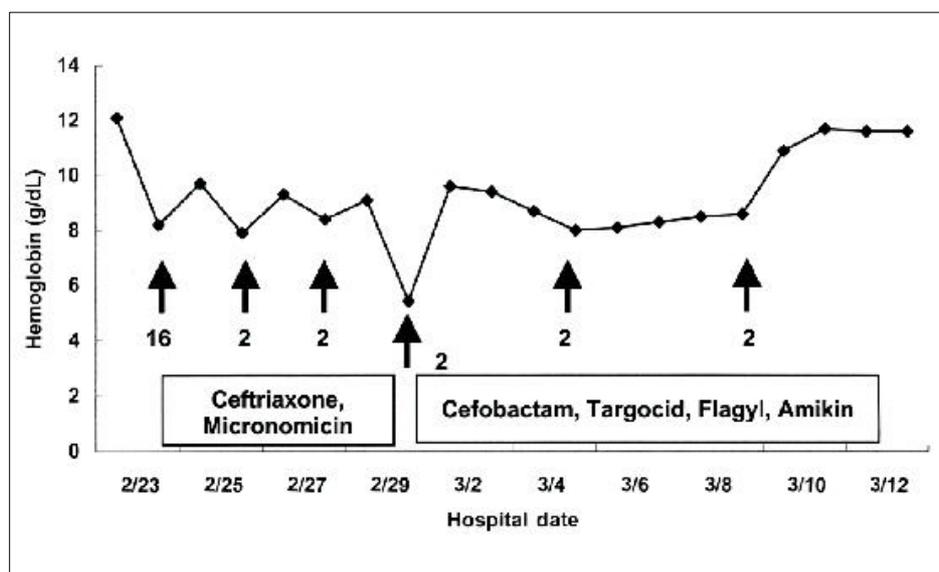


Fig. 1. Hemoglobin changes during admission. (↑: packed RBC transfusion, number: the number of unit transfused).

Table 1. Reactivity of Patient's Serum with Normal O RBC's in Various Reagent Conditions

Test reagent mixtures	IAT
patient's serum + O red cells	-
patient's serum + drug + O red cells	-
patient's serum + drug + complement + O red cells	-
patient's serum + urine (ex vivo urine Ag) + complement + O red cells	2+
normal's serum + urine (ex vivo urine Ag) + complement + O red cells	-

red cell destruction appears to be increasing. Second- and third-generation cephalosporins have been implicated as drugs causing immune hemolysis, yet for most of these drugs, only a few examples of such cases have been reported. The drug-antibody immune complexes can also activate complement and cause intravascular hemolysis.⁷⁻⁹ Several cases of fatal immune hemolysis related to ceftriaxone have been reported. These cases can occur by means of the immune complex mechanism.^{3-6,10} This mechanism is the least frequent occurrence of drug-induced immune mediated red cell destruction. Acute intravascular hemolysis with hemoglobinemia and hemoglobinuria is the usual occurrence, and the drug must be present *in vitro* for the presence of the antibody in the patient's serum to be able to be demonstrated. The presence of immune drug/anti-drug complexes may be demonstrable by serologic testing in the presence of the drug. Our case shows the classic clinical features of drug-dependent immune hemolytic anemia. A severe hemolytic episode was observed, and the DAT was positive only for C3d. However, ceftriaxone-dependent antibodies were not demonstrable in his serum. With some drugs, antibodies are directed against metabolites of the drug rather than against the native drug itself. Many authors have tried to prove the drug-induced immune hemolytic reaction with ex-vivo antigen in urine following unsuccessful attempts to demonstrate drug dependent antibodies in the serum. We found similar reports in the literature concerning fatal immune hemolysis.^{11,12} Our case, however, did not lead to acute renal failure and, fortunately, had a favorable outcome. In our case, discontinuing the ceftriaxone and avoiding blood transfusions, in spite of the continued presence of the drug or active metabolites in the patient's

blood circulation, turned out to be life saving. Steroid treatment was unnecessary. To our knowledge, this is the first reported case in Korea, in which the causative antibody appeared to be stimulated solely by a degradation product of ceftriaxone.

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