대한진단검사의학회지 제29권 제6호 2009
Korean J Lab Med 2009;29:578-84
DOI 10.3343/kjlm.2009.29.6.578

Ceftizoxime과 Cefobactam에 의한 면역용혈성 빈혈 1예

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A Case of Immune Hemolytic Anemia Induced by Ceftizoxime and Cefobactam (Sulbactam/Cefoperazone)

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Simultaneous drug-induced immune hemolytic anemia (DIIHA) caused by multiple drugs is rare. We report a case of a patient who developed DIIHA caused by 2 drugs. The patient's serum exhibited agglutination of ceftizoxime- or sulbactam-coated red blood cells (RBCs; via a drug-adsorption mechanism) and of uncoated RBCs in the presence of sulbactam (via an immune-complex mechanism). Although ceftizoxime is known to exhibit a positive reaction by an immune-complex method with or without reactivity with drug-coated RBCs, this patient's antibodies were reactive only against drug-coated RBCs. On the other hand, sulbactam, which is known to cause hemolytic anemia by nonimmunologic protein adsorption, exhibited positive reactions in tests with both drug-coated RBCs and in the presence of sulbactam. This is the first report of DIIHA due to a sulbactam-cefoperazone combination and the fourth report of DIIHA due to ceftizoxime. Owing to the patient's complicated laboratory results, DIIHA was suspected only at a late stage. We propose that for the prompt diagnosis of DIIHA, tests for all possible causative drugs should be conducted by 2 methods. *(Korean J Lab Med 2009;29:578-84)*

Key Words : *Drug-induced immune hemolytic anemia, Beta-lactamase inhibitor antibody, Cephalosporin antibody, Ceftizoxime antibody*

INTRODUCTION

Drug-induced immune hemolytic anemia (DIIHA) can be caused by more than 100 types of drugs. Although DIIHA is a well-known condition, it cannot easily be diagnosed and it is usually misdiagnosed [1, 3]. In the recent reported cases, more than 50% [2], or possibly as much as 80% of DIIHA were due to second- or third-generation cephalosporins [4]. However, only 3 reports have described ceftizoxime as a cause of DIIHA [5-7]. There have been many cases where positive results were obtained in direct antiglobulin tests (DATs) because of beta-lactamase inhibitors such as sulbactam, tazobactam, and clavulanate. Among these cases, hemolytic anemia was noted only in a few cases and was considered to be caused by nonimmunologic protein adsorption [3, 8, 9]. However, cefobactam-a combination of sulbactam and cefoperazone-has not been implicated in any

Received : June 11, 2009 Manuscript No : KJLM09-075
Revision received : August 31, 2009
Accepted : November 9, 2009
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case of DIIHA. Furthermore, simultaneous immune responses to more than one drug are exceptionally rare [2].

We report a case of a patient who exhibited drug-induced antibodies with unusual mechanisms of reactivity against both ceftizoxime and cefobactam. Therefore, we propose that experiments should be conducted on both drug-coated red blood cells (RBCs) and uncoated RBCs for a patient with DIIHA, in the presence of each possible causative drug that the patient has been administered.

CASE REPORT

1. Patient history

A 49-yr-old woman was transferred to our hospital for the treatment of a large skin injury she had incurred during a traffic accident. In the previous hospital, DATs yielded negative results before antibiotic treatment was initiated. On admission to our hospital, the patient's hemoglobin (Hb) level was 5.7 g/dL, and her absolute reticulocyte count was 86.6×10^3 cells/μL (reticulocyte proportion, 4.8%) (Table 1). Contrary to the negative DAT results obtained at the previous hospital, the DAT performed at our hospital yielded positive results for anti-IgG antibodies (3+) and negative results for anti-C3d antibodies on hospitalization day (HD) 6. Furthermore, the unexpected antibody screening and identification tests revealed panagglutination with a positive auto-control. Ceftizoxime treatment (1g q 12 hr, iv), which was administered from the time of admission to HD 10, was discontinued and replaced with cefobactam treatment (1g q 8 hr, iv). On HD 18, vancomycin (1g q 12 hr, iv) was included in the regimen to treat a cutaneous wound infection (Fig. 1).

Table 1. Patient's laboratory results at the time of admission

Test	Patient's results	Reference interval
Hematological values		
Hemoglobin (g/dL)	5.7	12.0-16.0
Hematocrit (%)	18.0	37.0-47.0
RBC count ($\times10^6/\mu$L)	2.08	4.0-5.4
Mean corpuscular volume (fL)	91.1	80.0-98.0
Mean corpuscular hemoglobin (pg)	31.4	27.0-33.0
MCHC (g/dL)	34.5	33.0-37.0
RDW (%)	13.4	11.5-14.5
Absolute reticulocyte count ($\times10^3/\mu$L)	86.6	20.8-109.6
Reticulocyte proportion (%)	4.8	0.5-2.31
Total bilirubin (mg/dL)	0.5	0.2-1.2
Lactic dehydrogenase (IU/L)	462	225-455
Direct antiglobulin test*	Positive	Negative
Haptoglobin (mg/dL)	<7.81	30.0-200.0

*The direct antiglobulin test was performed on HD 4.
Abbreviations: MCHC, mean corpuscular hemoglobin concentration; RDW, Red cell distribution width.

The Hb levels, which increased (from 5.7 g/dL to 6.8 g/dL) from the day of admission to HD 6, did not continue to increase but instead decreased to 6.1 g/dL on HD 16. The absolute reticulocyte count and the proportion of reticulocytes (%) decreased to 42.8×10^3 cells/μL (2%) on HD 4 but increased to 73.2×10^3 cells/μL (3.5%) on HD 12 and 166.2×10^3 cells/μL (7.5%) on HD 19. The haptoglobin level was undetectable on HD 12. The serum total bilirubin level remained within the reference interval. Because the patient's DAT results were positive and her Hb level did not increased, we considered the possibility of warm autoimmune hemolytic anemia (AIHA) or drug-induced hemolysis even though there were no episodes of bleeding for about 20 days. The patient had no secondary causes of warm AIHA, such as lymphoproliferative disorders, autoimmune disorders, infections, or tumors [10]. Despite the patient's previous history of RBC transfusion, the possibility of panagglutination due to the previous transfusion could be ruled

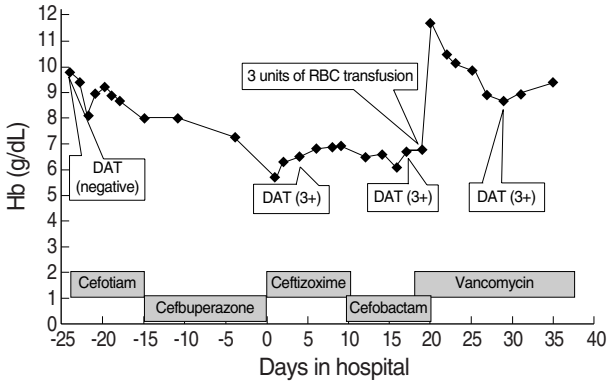

Fig. 1. Changes in the hemoglobin levels depending on the drugs administered and the days of hospitalization. The day the patient was admitted to our hospital was considered as hospitalization day 0.
Abbreviations: DAT, direct antiglobulin test; Cefobactam, cefoperazone/sulbactam.

out since she did not have multiple alloantibodies or antibodies to high-incidence antigens [11]. Further, panagglutination had disappeared by HD 12. Therefore, we explored the cause of DIIHA by conducting experiments for every drug the patient had been administered, including cefotiam and cefbuperazone, which were administered at the previous hospital, because DIIHA may have developed in the previous hospital (Fig. 1).

After performing tests for DIIHA and confirming the presence of ceftizoxime- and cefobactam-dependent antibodies on HD 20, cefobactam was discontinued and only the vancomycin administered as a treatment. The patient's Hb level began to increase, and she was discharged on HD 38. DATs continued to yield positive results (3+) until HD 29.

2. Blood samples and drugs for antibody testing

Blood was collected in serum separation tubes at various time points during the hospitalization period. Most of the drugs considered for drug-dependent antibody testing were exactly the same as those that were intravenously administered to the patient, except for sulbactam and cefoperazone, which were purchased from other companies but had the same constitutional formulas and no additives. To determine the effect of IgG on the reactivity of beta-lactamase-coated RBCs, the serum IgG level was measured using nephelometry (Dade Behring BN II nephelometer, Mahburg, Germany).

3. Antiglobulin testing

DATs and unexpected antibody screening and identification by indirect antiglobulin tests (IATs) were performed by the gel card method (DiaMed, Cressier sur Morat, Switzerland), according to the manufacturer's instructions.

Hemolysis was not visually evident in the patient's serum. The DATs first yielded positive results (3+) for monospecific anti-IgG antibodies on HD 4, and the expression of these antibodies remained strongly positive (3+) on HDs 19 and 29; however, the results for monospecific anti-C3d antibodies were negative. Panagglutination, which was detected by the gel card method on HD 6, was not detected either by the tube method on HD 12 or by the gel card method on HD 19. The serum IgG level was 1,800 mg/dL (reference interval, 700-1,600 mg/dL).

4. Testing of drug-coated RBCs

The tested drugs were cefotiam (Fontiam; Hanmi Pharmaceutical, Seoul, Korea), cefbuperazone sodium (Tomiporan; HanAll Pharmaceutical, Seoul, Korea), ceftizoxime (Epocelin Inj; Dong-A Pharmaceutical, Seoul, Korea), cefoperazone/sulbactam (Cefobactam; Hanmi Pharm, Seoul, Korea), cefoperazone (Cefozone; Kukje Pharmaceutical, Seongnam, Korea), sulbactam (Pfizer Korea; Seoul, Korea), and vancomycin (Dong-A Pharmaceutical, Seoul, Korea). The drugs were dissolved in phosphate-buffered saline (PBS) at pH 7.3 and washed; packed normal group-O RBCs were then added to the drug solutions, as per the reference protocol [12]. Briefly, control RBCs were taken in a separate tube, and PBS without any drugs was added to the tube. All tubes were incubated, and the cells were then washed. The saline suspension of drug-coated RBCs was mixed with the patient's serum or with normal AB sera that were obtained from 3 donors and pooled. A saline suspension of uncoated RBCs mixed with the patient's serum was used as a control (Table 2). To minimize nonspecific adsorption by proteins, serum obtained from the patient on HD 19 was diluted (1:2) and used for tests with sulbactam and cefoperazone. The eluate from HD 12 was made by the heat elution method,

Table 2. Testing of drug-coated RBCs

Drugs	Drug-coated RBCs + patient's serum	Drug-coated RBCs + normal AB serum	Uncoated RBCs + patient's serum
Ceftizoxime	2+	0	0
Cefobactam	2+	0	0
Sulbactam	1+*	0	0*
Cefoperazone	0*	0	0*
Cefotiam	0	0	0
Cefbuperazone	0	0	0

Reactions were observed after incubation at 37℃ and during the antiglobulin phase.
*Tested with serum diluted 1:2.
Abbreviation: RBC, red blood cell.

and tested for ceftizoxime- or cefobactam-coated RBCs. Each tube was incubated, centrifuged, and examined for hemolysis or agglutination, after which a polyspecific anti-human globulin (AHG) reagent was added to the tubes (Ortho Diagnostic Systems, Raritan, NJ, USA).

Agglutination was not detected by the immediate spin method in any of the experiments. However, it was detected both after incubation at 37℃ and after the addition of antiglobulin sera in the tubes containing ceftizoxime (2+), cefobactam (2+), and sulbactam (1+) but not in those containing cefoperazone alone. Normal serum and the eluate yielded negative results. Thus, ceftizoxime and sulbactam were deemed responsible for the positive reactions noted with drug-coated RBCs (Table 2).

5. Testing in the presence of drugs

The patient's serum was tested using an immune-complex method, according to the reference protocol [12]. In brief, the patient's serum, a pool of fresh normal sera from 3 group AB donors (for supplemental complement), and either the test drug or PBS were incubated either with uncoated RBCs or with RBCs coated with 0.1% ficin (Sigma Chemical Co., St. Louis, MO, USA). Serum obtained on HD 19 was diluted (1:2) and used for tests with sulbactam and cefoperazone. The eluate obtained by heat elution was tested for reactivity against ceftizoxime and cefobactam. After incubation, the sample was examined by the tube method to detect agglutination and hemolysis; this was followed by washing and the addition of the polyspecific AHG reagent. The intensity of the agglutination was assessed by 3 experts.

There was no agglutination or hemolysis in the presence of drugs when reacted with uncoated RBCs; however, tests with 0.1% ficin-treated RBCs showed weak reactions in tubes containing the patient's serum with or without drugs. Stronger reactions were observed in tubes containing the patient's serum and cefobactam after incubation at 37℃ and during the antiglobulin phase, as indicated by the formation of weak immune complexes involving cefobactam. On the other hand, in tests with diluted serum, a 1+ reaction was noted in the presence but not in the absence of sulbactam. Thus, sulbactam was deemed responsible for the positive reaction noted in the presence of drugs (Table 3). Negative results were obtained with the eluate.

DISCUSSION

DIIHA is a very rare and under-diagnosed condition [1, 3, 13]. In practice, when clinicians suspect DIIHA, they simply discontinue treatment with the possible causative drugs and switch to other drugs. However, DIIHA can occur again on treatment with the new drugs. The best way to avoid this complication is to confirm DIIHA by *in vitro* analysis if the patient has prolonged anemia and DIIHA is suspected. DIIHA caused by multiple drugs is extremely rare. In one previous study, only 1 of 73 DIIHA cases were found

Table 3. Testing in the presence of drugs

Drugs	Reagents in the tubes					
	Pt's serum	Yes	Yes	Yes	Yes	No	No
	AB serum	No	No	Yes	Yes	Yes	Yes
	PBS	No	Yes	No	Yes	No	Yes
	Drug	Yes	No	Yes	No	Yes	No
Ceftizoxime		1+	1+	1+	1+	0	0
Cefobactam		2+	1+	2+	1+	0	0
Sulbactam		NT	NT	1+*	0*	0	0
Cefoperazone		NT	NT	0*	0*	0	0
Cefotiam		NT	NT	0	0	0	0
Cefbuperazone		NT	NT	0	0	0	0

The drugs and reagents were reacted with ficin-treated RBCs, and the results were assessed after incubation at 37℃ and during the antiglobulin phase.
*Tested with serum diluted 1:2
Abbreviations: Pt's, patient's; AB serum, pooled normal AB serum for complement; PBS, phosphate-buffered saline; NT, not tested.

to be caused by multiple drugs [2]. Except for HIV-associated IHA [14], no specific condition has been shown to be associated with the production of antibodies against multiple drugs. The patient in the present case had been healthy before being admitted to the hospital after the car accident.

The mechanisms of drug-dependent IHA can be classified into 2 types: those wherein drug-coated RBCs show positive reactions and those wherein an immune-complex method shows positive reactions with uncoated RBCs in the presence of drugs [3]. In the previous studies on DIIHA caused by ceftizoxime, 3 patients developed antibodies that could only be detected by the immune-complex method, and one patient developed antibodies that could be detected with both drug-coated RBCs and the immune-complex method [5-7]. The present report is the first to describe a case where the patient's antibodies against ceftizoxime reacted only with drug-coated RBCs; this result revealed that the antibodies to ceftizoxime do not necessarily react via a single specific mechanism.

Sulbactam is known to cause hemolytic anemia through nonimmunologic protein adsorption onto RBCs, wherein drug-coated RBCs react with normal sera as well as the patient's serum [8, 9]. However, in the present case, reactivity was noted only in tubes containing the patient's serum but not in those lacking it; this finding ruled out the possibility of nonimmunologic protein adsorption. Beta-lactamase inhibitors have never been shown to react with RBCs in the presence of drugs. Interestingly, our patient's sera exhibited positive reactions in 2 different test methods. To minimize the nonspecific adsorption by proteins such as albumin, we used diluted serum in the tests with sulbactam or cefoperazone-coated RBCs and detected reactivity even with the decreased concentration. Furthermore, because we detected positive reactions in tubes containing enzyme- treated RBCs and the patient's serum with or without drugs (Table 3), we performed tests with the patient's diluted sera to identify the specific differences in reactivity when sulbactam or cefoperazone was present or absent. Reactivity was noted only in tubes containing the patient's serum and sulbactam; this finding confirmed the activity of the sulbactam-induced antibodies.

Because a high plasma IgG level is known to enhance nonimmunologic protein adsorption [3, 8], we determined the IgG level in the patient's serum in order to rule out the possibility of nonimmunologic protein adsorption. The patient's IgG level was only slightly higher than the upper limit of the normal range; this level may not have been adequate to influence the results of IATs with drug-coated RBCs, as observed in experiments conducted by Broadberry et al. [8].

When tests were requested for DIIHA on HD 18, we were confused by the previous results of panagglutination on HD 6. This finding may be attributable to the presence of circulating drug-antibody immune complexes or weak autoantibodies. Because panagglutination was not observed on HD 12, a diagnosis of DIIHA could not be ruled out on the basis of *in vitro* experiments.

We suspect that the patient had lost some blood when she was at the previous hospital; this blood loss may have been responsible for the low Hb level and elevated reticulocyte count noted on admission to our hospital. However, there was no episode of bleeding after admission to our hospital, and the Hb level did not increase without any probable cause. After DIIHA was confirmed, treatment with the 2 causative drugs was discontinued, and the Hb level began to increase without transfusion or the administration of erythropoietin. The patient was discharged for outpatient follow-up once her Hb level increased beyond 9.0 g/dL. Given that the patient was healthy without any chronic disease or apparent cause of anemia after admission to our hospital, DIIHA may have been responsible for the delayed increase in the Hb levels and the need for prolonged hospitalization.

In one reported case, antibodies against 3 drugs-all cephalosporin drugs (cefotetan, cefuroxime, and cefotaxime)-were detected [2]. Sulbactam and cephalosporins have the same basic beta-lactam structure. However, in the present case, we ruled out the possibility of antibodies directed against this basic structure because the other 3 cephalosporins (cefoperazone, cefotiam, and cefbuperazone; Table 2, 3) did not react with the patient's serum. Furthermore, sulbactam and ceftizoxime do not have similar side chains. Although

more data and experiments are required for identifying the immunologic predisposing conditions for DIIHA or cross-reactivity, it seems likely that the findings of the present study were the result of a coincidence.

We cannot entirely explain the patient's anemic state for the following reasons. We did not have precise information regarding bleeding episodes or hemolytic events that may have occurred while the patient was at the previous hospital; therefore, we could not definitively identify the cause of anemia on admission. Despite the negative reaction noted with cefotiam and cefbuperazone and the lack of any previous report on DIIHA caused by these 2 drugs, we could not rule out the possibility of DIIHA at the time of admission, owing to the delay in obtaining serum, which may have led to the disappearance of antibodies. The overall reactions noted with enzyme-treated RBCs on HD 12, without the addition of drugs (Table 3), may have been because of the presence of circulating drug-antibody immune complexes or weak autoantibodies with enhanced sensitivity. The eluate obtained by heat elution did not exhibit reactivity in either of the methods used. If the patient had warm AIHA, IATs would have revealed the presence of autoantibodies that reacted with all RBCs. The fact that the eluate was nonreactive suggests the presence of drug-specific antibodies. In our laboratory, both the heat elution method and the glycine-acid elution method are routinely used. Further, all antibodies can be detected with the former method, although the sensitivity of this method is slightly lower than that of the glycine-acid method and heat elution is not the best method for detecting IgG auto- or alloantibodies. Nevertheless, we could not perform glycine-acid elution because only a very small amount of RBCs could be obtained from the patient.

In conclusion, we report a case of a patient with IHA caused by a ceftizoxime-induced antibody, which was detected by testing drug-coated RBCs, and a sulbactam-induced antibody, which was detected by testing drug-coated RBCs and by the immune-complex method. When a patient shows signs of hemolytic anemia, all drugs that may have caused IHA should be tested in order to rapidly diagnose DIIHA. Further, switching to another drug may hasten the recovery process.

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