Immune-Mediated Severe Hemolytic Crisis with a Hemoglobin Level of 1.6 g/dl Caused by Anti-Piperacillin Antibodies in a Patient with Cystic Fibrosis

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Abstract

We report a 23-year-old female patient with cystic fibrosis developing severe intravascular hemolysis with a minimal hemoglobin level of 1.6 g/dl after 7 days of treatment with piperacillin, consistent with an immune-mediated hemolytic crisis. Twenty days later, the patient could leave the hospital in good condition without any neurological deficit. To our knowledge, this is the lowest reported hemoglobin value caused by hemolytic anemia with intact survival. As piperacillin is commonly used in patients with cystic fibrosis, it is important to monitor the full-blood counts of patients during treatment with piperacillin and to be aware of the potential for hemolytic anemia to develop. Anti-piperacillin antibodies should be considered whenever these patients develop hemolytic anemia or a positive direct antiglobulin test (DAT). Furthermore, drug-fever under piperacillin application could be a warning sign for the development of hemolytic anemia.

Infection 2010; 38: 131–134
DOI 10.1007/s15010-009-9227-8

Introduction

Chronic bronchopulmonary infection, often associated with *Pseudomonas aeruginosa*, is the main cause of morbidity and mortality in cystic fibrosis. In consequence, colonization of the airways with *P. aeruginosa* is regularly treated with intravenous antibiotics in this condition. Likewise, when patients develop acute clinical worsening, they are often admitted to the hospital for supportive care and parenteral antibiotic therapy. In addition to other antipseudomonal agents such as ceftazidime, ciprofloxacin, colistin, meropenem, imipenem-cilastatin, aztreonam and aminoglycosides, piperacillin is commonly used to treat pseudomonal infections [1, 2]. Piperacillin is a semisynthetic ureidopenicillin with high in vitro activity against *P. aeruginosa* [3–5]. Among the reported complications of therapy with piperacillin are recurrent fever, rash and a serum sickness-like illness [1, 4, 6]. Because drug-fever may precede the development of more serious manifestations, e.g. hepatitis and exfoliative dermatitis, its recognition is important [1]. In patients with cystic fibrosis, the prevalence of adverse reactions to piperacillin appears to be high. This observation might be explained by the frequent use of complex penicillins in combination with a hyperimmune state in patients with cystic fibrosis [7]. Since the 1960s, hemolysis has been described as a serious complication of penicillin therapy [8–10]. The mechanism of piperacillin-induced immune hemolytic anemia is an antibody/hapten immune response. The hapten/protein complex is formed when piperacillin binds to surface proteins on erythrocyte-cell membranes. Penicillin-induced hemolysis, which usually takes place in the extravascular space, classically occurs after approximately 10 days of treatment [9, 10]. We report a severe piperacillin-associated hemolytic crisis with a minimal hemoglobin level of 1.6 g/dl in a patient with cystic fibrosis.

Case Report

Medical History

A 23-year-old female patient with cystic fibrosis, homozygous for ΔF508, was chronically colonised with *P. aeruginosa*. Pulmonary function testing showed the typical changes of advanced lung disease in cystic fibrosis (FEV1 36% predicted, FVC 46% predicted, RV/TLC 222% predicted). The Crispin–Norman score of the chest X-ray was 20, also indicating advanced disease. She had no sign of right heart failure. Because of her chronic pseudomonal colonisation, the patient had received several courses of intravenous antibiotic therapy. In addition, she was on a regular medication with oral ciprofloxacin and inhalational colistin twice daily. The patient had no immunohematologic diagnosis and had

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Received: July 4, 2009 · Revision accepted: September 28, 2009
Published online: ***March 5, 2010***

not received red blood cells (RBCs), thrombocytes or plasma expander before.

She was admitted to the hospital because of fatigue, headache, vomiting for several days, decreased appetite and loin pain. The physical examination revealed crepitations over both lungs, mild flank tenderness to palpation, unspecific abdominal pain and signs of dehydration. Blood analysis showed signs of inflammation (13,270 leukocytes/μl with 75% neutrophils, C-reactive protein [CRP] 7.0 mg/dl) and renal dysfunction (serum creatinine 2.7 mg/dl [normal: 0–0.95 mg/dl], endogenous creatinine clearance 33 ml/min/1.72 m^2 [normal: 88–128 ml/min/1.73 m^2] and uric acid 9.1 mg/dl [normal < 5.7 mg/dl]). Urinary output was 1.5 ml/kg/h. Her hemoglobin level (13.4 g/dl), serum electrolytes, urea, liver enzymes, immunoglobulin (Ig)G and IgE were normal. Urinalysis showed 1,218 leukocytes/μl with no RBCs, no epithelial cells and no casts. There was a mild proteinuria of 246 mg/m^2/day (normal < 150) but no glucosuria. Urine culture revealed no growth. Renal ultrasound showed enlarged kidneys with a diffusely increased echogenicity of the parenchyma, but no structural abnormalities and normal resistive indices in Doppler. In the patient's stool, norovirus could be detected. In a sputum culture, *P. aeruginosa* (mucoid) species were grown. They were susceptible to piperacillin and meropenem. The hantavirus serology was negative.

Based on the antimicrobial sensitivities of *P. aeruginosa* in the sputum and a suspected urinary tract infection, the patient was treated intravenously with piperacillin (260 mg/kg/day), meropenem (70 mg/kg/day) and tobramycin adapted for creatinine clearance (3.3 mg/kg/day). Oral ciprofloxacin was discontinued. Tobramycin was stopped on day 2 because her creatinine levels increased to a maximum of 3.1 mg/dl. Recombinant urate oxidase (rasburicase; 7.5 mg) was given for hyperuricemia and the patient received intravenous fluid resuscitation and furosemide for acute renal failure secondary to dehydration. The patient showed clinical improvement in response to this treatment. Her serum creatinine decreased, reaching 1.7 mg/dl on day 7 in hospital. Urinalysis normalised on day 6. CRP levels, which had reached a maximum of 11.0 mg/dl on day 3, declined to 2.9 mg/dl on day 8. Uric acid was not detectable on day 2 and was 2.5 mg/dl (2.4–5.7) on day 5.

On day 8, the clinical condition of the patient acutely worsened. She suddenly complained about headache and nausea. She was febrile (39°C), had a heart rate of 160/min and a low blood pressure of 88/43 mmHg. Her white blood cell count showed 35,100 leukocytes/μl, CRP was 2.9 mg/dl, creatinine 1.7 mg/dl, urea 66 mg/dl and uric acid 3.8 mg/dl. Partial thromboplastin time, prothrombin time and thrombocyte count were within normal ranges. Fibrinogen and D-dimers were elevated to 7.2 g/l (1.8–3.5) and 8 mg/dl (0–0.5), respectively. The hemoglobin concentration had decreased to 1.6 g/dl and the hematocrit level to 3.8%. The total bilirubin and lactate dehydrogenase (LDH) had increased to 3.2 mg/dl (0.1–1) and 700 U/l (< 250), respectively. Twelve hours later, her total bilirubin and LDH increased to a maximum of 8.4 mg/dl and 1,242 U/l, respectively. The serum haptoglobin concentration was < 10 mg/dl. Reticulocytes were 0.2%. Hemoglobinuria was noted and the patient's plasma was grossly icteric and contained hemoglobin. Hemagglutination, spherocytes, hyperchromasia, polychromasia, anisocytosis and nucleated red cells could be found in the peripheral blood (Figure 1), but no Heinz bodies in the bromocresyl green stain could be observed. Serological studies were performed by a standard gel technique (DiaMed, Cressier-sur-Morat, Switzerland). The direct antiglobulin test (DAT) was strongly positive (4+) and RBC-bound IgG and IgM could be demonstrated when

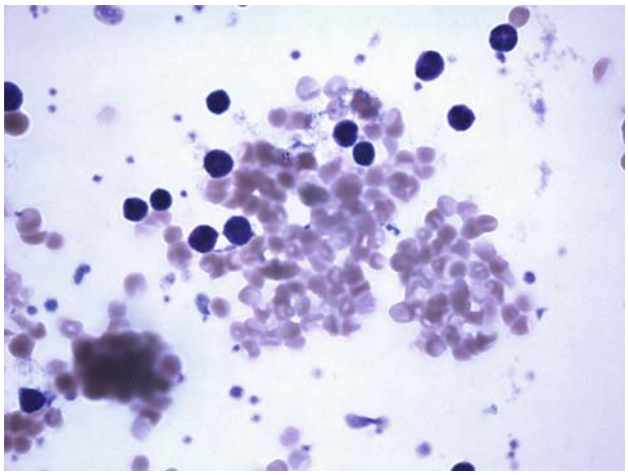

Figure 1. Peripheral blood film showing hemagglutination, hyperchromasia, polychromasia and anisocytosis of red cells (Pap staining, 1:1,000) in our patient.

tested in the acute phase of the hemolytic event. The serum reacted with all tested RBCs in the saline agglutination and in the indirect antiglobulin test. Further serological studies were carried out on a second sample taken about 1 week later. The DAT was still positive (polyspecific 1+) but showed only weak C3d (1+) and very weak IgG (±), but no IgM or IgA sensitization. Antibody screening test and eluate were negative. Drug-dependent antibodies were investigated in the presence and absence of meropenem and of piperacillin and its metabolites (drug immune complex method), as described previously [11]. Antibodies to piperacillin of the IgM- and IgG-type were detected in the serum of the patient, the latter reacting up to a titre of 1:256. One month later, the titre was 1:128 and, even 4 months later, antibodies to piperacillin could be detected. No antibodies to meropenem were detected.

All medications were discontinued, the patient received an emergency transfusion with one unit of packed RBCs, was intubated because of shock and loss of consciousness, and was transferred to the intensive care unit (ICU). In the treatment of the shock, catecholamines and a plasma expander were administered and three further units of packed RBCs were transfused. Prednisolone (2 × 10 mg/kg), ciprofloxacillin (17 mg/kg/day) and cefotaxime (85 mg/kg/day) were administered intravenously. Her hemoglobin concentration subsequently remained stable at 8.7 g/dl and mechanical ventilation could be stopped after 2 days. The patient could be transferred from the ICU on day 10. She left hospital on day 20 in good condition without any neurological deficit and with a hemoglobin concentration of 9 g/dl, hematocrit level of 25% and serum creatinine of 0.6 mg/dl.

Discussion

We reported on a patient who developed severe intravascular hemolysis after 7 days of treatment with piperacillin, consistent with an immune-mediated hemolytic crisis. To our knowledge, this is the lowest hemoglobin value caused by drug-induced hemolytic anemia with intact survival reported so far.

Drug-induced immune hemolysis can be caused by different mechanisms (summarized in [12–15]). Antibodies

to drugs can be categorised as being drug-dependent or drug-independent, depending upon whether or not the drug is required in the test system for detection. Drug-dependent antibodies can be subdivided into two categories based on serological and clinical characteristics: (1) those that react with drugs which bind firmly to cell membranes and can be detected by incubating the patient's serum or eluate with drug-treated RBCs ('drug adsorption' method); and (2) those that react with drugs which do not bind firmly to the cell membrane and are detected by incubating sera with drug and RBCs that have not been treated with the drug in advance ('immune complex' method). Antibodies to drugs in the former group tend to be associated with extravascular red cell destruction and those in the latter group with complement-mediated intravascular red cell destruction. In vivo, drugs such as penicillin and its derivatives become covalently linked to RBC membrane glycoproteins and, acting as haptens, may trigger antibody production. The treatment of such patients with the drug at very high doses sometimes leads to the coating of autologous RBCs with enough drugs to allow antibody binding that leads to hemolysis. This type of antibody can be detected by showing that it binds to RBCs which have been incubated with the suspected drug and washed afterwards.

Piperacillin has previously been described to be a cause of immune hemolytic anemia. *Johnson* et al. [17] described a patient with urosepsis who developed an immune hemolytic anemia caused by anti-piperacillin antibodies. The drug-dependent antibody was primarily IgM and reacted with piperacillin-treated RBCs as well as untreated RBCs in the presence of piperacillin. *Thickett* et al. [18] described a patient with cystic fibrosis who developed headache, nausea, fever, hemoglobinuria, decreased hemoglobin and haptoglobin, increased bilirubin and a positive direct antiglobulin test (DAT) after receiving piperacillin for 12 days. A third report of *Bressler* et al. [19] on anemia caused by piperacillin had no serological details to substantiate that the anemia was caused by immune hemolysis. *Arndt* et al. [12] described two further cases of immune hemolytic anemia caused by piperacillin in two patients with cystic fibrosis, one of which was fatal. Antibodies from both patients were detected by the 'immune complex' method; one also reacted with piperacillin-treated RBCs. In addition, *Broadberry* et al. and *Garcia Gala* et al. reported immune hemolysis due to piperacillin/tazobactam administration [20, 21].

In our patient, hemolytic anemia as a rare side-effect of rasburicase administration could also be considered [16, 22–24]. However, as the patient is female, relevant glucose-6-phosphate-dehydrogenase deficiency as one predisposing factor for this condition is unlikely. Moreover, rasburicase-induced hemolytic anemia develops within hours after exposure to the medication and not after 1 week, as in our case [16]. Besides, we did not find Heinz bodies in the bromocresyl green stain as indicators of methemoglobinemia. During the acute phase of the hemolytic event in our patient, a strongly positive DAT with positive anti-piperacillin IgM could be found, which has been shown to be associated with fatal or severe hemolytic anemia in patients treated with piperacillin [12, 18]. Also, the patient had no signs of gastrointestinal or urogenital bleeding.

In conclusion, it is important to monitor the full-blood counts of patients during treatment with piperacillin and to be aware of the potential for hemolytic anemia to develop. Furthermore, if the patient's history causes immune-mediated hemolysis, induced by a broad-spectrum antibiotic, probable, a screening of the patient's serum for antibiotic-specific antibodies should be considered before the administration of the medication. Hemolysis in our case was highly specific to piperacillin because no agglutination was observed in vitro with the other antibiotics that had been administered. Therefore, the latter still remain therapeutic options for our patient.

As piperacillin is commonly used in patients with cystic fibrosis, screening for anti-piperacillin antibodies should be taken into account whenever these patients develop hemolytic anemia or a positive DAT. Furthermore, drug-fever under piperacillin application could be a sentinel for the development of hemolytic anemia.

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