Intravascular Hemolytic Anemia in a Patient with Antibodies Related to Meropenem

Satoko Oka, Hiroshi Shiragami and Masaharu Nohgawa

Abstract

A 76-year-old woman treated with meropenem developed intravascular hemolytic attacks. A direct antiglobulin test was positive for C3d and IgG, and drug-dependent antibody testing indicated that the antibodies were indeed drug-dependent and reacted with drug-treated RBCs and RBCs in the presence of the drug. To our knowledge, this is the first reported case in which the causative antibodies related to meropenem were identified. This case highlights the importance of maintaining a high level of suspicion for drug-induced immune hemolytic anemia in patients with explained hemolysis as well as conducting specialized serologic testing.

Key words: immune hemolytic anemia, drug-dependent antibody, meropenem

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Introduction

Drug-induced immune hemolytic anemia (DIIHA) is a rare condition in which exposure to a specific drug induces a sudden drop in the number of circulating red blood cells (RBCs) via an immune-mediated process (1). Although reactions are variable, DIIHA can be life-threatening (2). The estimated incidence of this disorder is approximately 1 per 1 million patients per year, although this is likely an underestimate, as many cases are unappreciated or misdiagnosed, often as warm-type autoimmune hemolytic anemia (AIHA). The mechanisms by which drugs interact with the immune system to induce the formation of RBC antibodies are not fully understood. Drug-induced antibodies may be of two types: drug-dependent and drug-independent (3, 4). Drug-dependent antibodies usually demonstrate positivity on direct antiglobulin tests and negativity in solution, while drug-independent antibodies are serologically indistinguishable from warm autoantibodies and similarly steroid-responsive.

More than 100 drugs have been reported to induce DIIHA (5). Of these, 42% are antimicrobials, 16% are nonsteroidal anti-inflammatory agents, 13% are anti-neoplastics and 6% are anti-hypertensives/diuretics. The most frequently associated agents are antibiotics, such as second- and third-generation cephalosporins.

We herein report the case of a patient who developed DIIHA associated with the formation of antibodies against meropenem, a beta-lactam antibiotic that belongs to the subgroup of carbapenems. To our knowledge, this is the first reported case in which the causative antibodies related to meropenem were identified.

Case Report

A 76-year-old woman with no past medical history presented to the emergency room with a fever and abdominal pain lasting for seven days. Computed tomography of the abdomen showed gallbladder distention without wall thickening. Laboratory tests demonstrated a white blood cell count of 13.3×10^9/L, RBC count of 381×10^10/L, hemoglobin (Hb) concentration of 110 g/dL (Figure) and platelet count of 229×10^9/L. The serum lactate dehydrogenase (LDH) level was 573 U/L (normal range: 106-211 IU/L), the aspartate transaminase level was 133 U/L (normal range: 5-40 U/L), the alanine transaminase level was 151 U/L (normal range: 5-35 U/L), the alkaline phosphatase level was 1,425 U/L (normal range: 104-338 U/L), the gamma-glutamyltransferase level was 307 IU/L (normal range: 8-64 IU/L) and the C-reactive protein level was 17.8 mg/dL (nor-
According to the Tokyo Guidelines for the Management of Acute Cholangitis and Cholecystitis (TG13) diagnostic criteria, the patient was diagnosed with moderate acute cholecystitis. Biliary drainage was carried out, and a daily dose of 2 g of meropenem (Meiji Seika, Tokyo, Japan) was administered. After several days of treatment, the patient’s symptoms were gradually relieved. On the 10th day of the administration of meropenem, she complained of a sudden episode of lumbar pain accompanied by macroscopic hemoglobinemia, shivering and fever. Laboratory studies were significant for a RBC count of 275×10ⁱ⁰/L and Hb level of 76 g/L (which had been 110 g/L on admission) (Figure). A peripheral smear showed spherocytes. The LDH level was 3,274 U/L and the serum total bilirubin (T-Bil) level was 2.9 mg/dL (0.3-1.0 mg/dL), of which the indirect bilirubin (I-Bil) value was 2.0 mg/dL (0.3-0.7 mg/dL). Other significant findings included increased reticulocytes at 54%, an elevated Cre level and a decreased haptoglobin level of <10 mg/dL (36-195 mg/dL). A direct antiglobulin test (DAT) was positive for both C3d and immunoglobulin G (IgG). A diagnosis of AIHA was suspected, and treatment with 50 mg of prednisolone was therefore commenced. Since the possibility of DIIHA could not be ruled out, the dose of meropenem was discontinued, and the patient was treated with aggressive hydration therapy and blood transfusions (4 units of red blood cells in total). The hemolysis subsequently stopped, and the RBC, Hb, T-Bil, LDH and Cre values gradually recovered. The Hb level and reticulocyte count ultimately normalized five weeks after the onset of symptoms.

Serologic studies [indirect antiglobulin test (IAT) and DAT] were performed according to the test tube method (6). The patient’s ethylenediaminetetraacetate (EDTA)-washed RBCs were tested with anti-IgG (Immucor Gamma, Tokyo, Japan) and anti-C3d antibodies (Ortho-Clinical Diagnostics, Tokyo, Japan). The eluate was subjected to the acid method in order to remove RBC-bound antibodies (Immucor Gamma).

Testing for the presence of meropenem antibodies was performed with meropenem-treated RBCs (meropenem-coating method) and untreated or ficin-treated RBCs in the presence of a solution of meropenem (meropenem-solution addition method).

**Meropenem-coating method**

Group O donor RBCs were incubated with meropenem (Meiji Seika) in an attempt to prepare drug-coated RBCs for testing. The methods included incubating 40 mg/mL solutions of meropenem prepared in pH 7.2 phosphate-buffered saline (PBS) with 1/10 volume of RBCs for one hour at 37°C.

The patient’s serum was tested with the meropenem-treated and untreated RBCs according to a previously described test tube method (7). Briefly, two drops of serum were incubated with one drop of meropenem-treated or control untreated RBCs. After one hour of incubation at 37°C, tests were performed for hemolysis and agglutination. Antiglobulin tests were performed using anti-IgG and -C3d antibodies. As negative controls, pools of normal sera were tested in parallel with the patient’s serum.

**Meropenem solution addition method**

Ficin was used to prepare enzyme-treated RBCs. The patient’s serum was treated with and without the addition of pooled fresh normal sera as a source of complement in the

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**Figure.** Clinical course of the laboratory data. DAT: direct antiglobulin test
Table 1. DAT and Antibody Results on Patient with RBC Samples.

<table>
<thead>
<tr>
<th>Patient's information</th>
<th>Sample date</th>
<th>Anti-IgG</th>
<th>Anti-C3</th>
<th>Serum meropenem antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>meropenem started</td>
<td>day 0</td>
<td>0</td>
<td>0</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>day 9</td>
<td>micro+</td>
<td>1+</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>day 10</td>
<td>1+</td>
<td>1+</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>day 12*</td>
<td>1+</td>
<td>3+</td>
<td>detected</td>
</tr>
<tr>
<td></td>
<td>day 30</td>
<td>1+</td>
<td>1+</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>day 56**</td>
<td>0</td>
<td>0</td>
<td>not detected</td>
</tr>
</tbody>
</table>

DAT: not tested; *Sample 1; **Sample 2

Table 2. RBC Coating Method.

<table>
<thead>
<tr>
<th>Patient's serum</th>
<th>Pooled normal sera</th>
<th>Patient's eluate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>37 °C AGT</td>
<td>37 °C AGT</td>
</tr>
<tr>
<td>RBCs</td>
<td>Sample 1/2</td>
<td>Sample 1/2</td>
</tr>
<tr>
<td>meropenem-treated (40 mg/15 mL)</td>
<td>3+/0</td>
<td>3+/0</td>
</tr>
</tbody>
</table>
| The patient's serum, eluate, and eluate last wash were tested against meropenem-coated RBCs. Samples 1 and 2 were collected 2 and 46 days after cessation of meropenem.

Table 3. Meropenem Solution Addition Method.

<table>
<thead>
<tr>
<th>Reactions</th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>37 °C AGT</td>
<td>37 °C AGT</td>
</tr>
<tr>
<td></td>
<td>untreated</td>
<td>untreated</td>
</tr>
<tr>
<td>meropenem</td>
<td>2+</td>
<td>0</td>
</tr>
<tr>
<td>serum + meropenem</td>
<td>2+</td>
<td>0</td>
</tr>
<tr>
<td>serum + PBS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>serum + C+ meropenem</td>
<td>3+</td>
<td>4+</td>
</tr>
<tr>
<td>serum + C+ PBS</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C+ meropenem</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C+ PBS</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The patient's serum was tested in the presence of meropenem solution or PBS, with and without complement against untreated RBCs. Samples 1 and 2 were collected 2 and 46 days after cessation of meropenem. C: complement

* No reactions were seen with or without meropenem, with or without complement, against when control was tested.

Days after the cessation of meropenem administration (Sample 1). DAT was performed, and RBCs were found to be strongly (3+) reactive with the anti-C3d antibodies and weakly (1+) reactive with the anti-IgG antibodies (Table 1). No alloantibodies to common RBC antigens were detected when the serum was tested against reagent RBCs on immediate spinning at a room temperature of 37°C and the albumin-IgG-antiglobulin test (AGT) phase. In addition, the eluate was nonreactive when tested against a panel of reagent RBCs. Meanwhile, direct agglutination of meropenem-treated RBCs occurred in the patient’s serum (3+) (Table 2). Furthermore, the patient’s serum reacted with untreated RBCs (3+) and ficin-treated RBCs (4+) in the presence of meropenem for agglutination and the antiglobulin test (Table 3). A reduction of agglutination was noted after DTT treatment, indicating that the antibodies belonged to the IgM class. No reactions were seen with the negative controls (Table 2, 3).

A second sample was collected from the patient 46 days after the cessation of drug therapy (Sample 2). Consequently, DAT was negative for anti-C3d and anti-IgG antibodies (Table 1), and the patient’s serum was nonreactive with meropenem-treated RBCs and untreated and ficin-treated RBCs in the presence of meropenem (Table 2, 3). No reactions were seen with the negative controls (Table 2, 3).

Discussion

DIIHA is a rare form of hemolytic anemia that can be caused by a broad spectrum of drugs. Antibody development and hemolysis may occur any time during drug treatment, from immediately after initiation to many months later (1, 9). Numerous beta-lactamase inhibitors have been implicated as triggers of acute antibody-mediated hemolysis (1). The present case clearly documents the immune etiology of meropenem-induced hemolytic anemia and emphasizes the importance of providing close laboratory monitor-
ing in patients receiving meropenem. A high level of vigilance is required on the part of clinicians when treating patients with a history of prior exposure to or prolonged initial therapy with meropenem, as the clinical and laboratory signs of hemolysis may manifest at any time during therapy. In adult patients, immune hemolysis typically develops days to weeks after exposure to the medication, compared to within minutes in children. Because there are no published data regarding detailed laboratory monitoring for this rare reaction, the need for monitoring is a clinical decision that should be made by the treating physician.

The current patient developed severe anemia, with symptoms that can be attributed to intravascular immune hemolysis, such as lumbar pain, red urine and severe anemia. DAT was strongly positive for IgG, pointing to the presence of an autoantibody, as well as C3d, which may indicate drug induction. The initial detection of panagglutinin suggested a warm-type autoimmune process; however, the eluate was nonreactive. Obtaining a careful clinical history, including medications and their temporal relationship with the onset of hemolysis, is critical because positive DAT findings with a negative eluate are commonly associated with DIIHA, the passive transfer of ABO antibodies (10), such as anti-A or anti-B, due to prior out-of-group plasma or platelet transfusion (11), intravenous immunoglobulin therapy (12) or hemolytic disease of the newborn or fetus (13). In this case, we reviewed the patient’s medication profile and recent transfusions, although the possibility of DIIHA could not be ruled out; thus, meropenem was discontinued and a further work-up was initiated.

Drug-induced antibodies may be of two types: drug-dependent and drug-independent (3). Drug-independent antibodies against RBCs are detected in vitro on DAT and IAT in the absence of the drug. These antibodies are directed against components of the RBC membrane and the in vitro and in vivo characteristics are identical to those of RBC autoantibodies found in cases of warm IgG-mediated AIHA (3). In contrast, drug-dependent antibodies react in vitro only in the presence of the drug, either bound to RBCs or added to the patient’s serum. These antibodies are directed against the drug only or a combination of the drug and RBC membrane antigens (3, 4).

The serum antibodies discovered in the evaluation of this patient were drug-dependent, as they reacted only in the presence of meropenem or with RBCs treated with meropenem. The antibodies were IgM, activated the complement system and induced intravascular lysis. These findings are thought to indicate that meropenem may bind loosely to RBCs in vivo, thus becoming immunogenic and stimulating the production of antibodies. After antibody production is initiated, immune complexes form (consisting of the antibody and meropenem) and in turn bind non-specifically to other RBCs, ultimately leading to the activation of the complement system. With respect to detecting hemolysis or sensitization by complement, the addition of fresh normal serum to the patient’s serum was important for identifying hemolysis because the patient’s sample had been stored for several weeks prior to testing.

Drugs containing beta-lactamase inhibitors also contain antibiotics, which can cause DIIHA via well-described mechanisms (14, 15). Examples of these drugs include tazobactam plus piperacillin and sublactam plus ampicillin. Therefore, one or both mechanisms may be involved in the onset of hemolytic anemia associated with these drugs. Arndt et al. reported that sera obtained from random patients and blood donors contain IgM agglutinins reactive with meropenem-treated RBCs in vitro (16). Meropenem may also show the characteristics of more than one mechanism of action. However, it is not clear why only a subset of patients with drug-induced RBC antibodies experience severe hemolytic reactions.

In conclusion, meropenem should be used with caution due to its rare but potentially serious adverse effect of hemolysis. There is at present no way of identifying individuals at risk of developing this complication. In patients who present with acute immune hemolysis, it is important to obtain a careful history of drug exposure, identify potential sensitizing medications and, where appropriate, perform confirmatory testing in order to prevent the inadvertent reinduction of the syndrome at a later time. Early recognition of DIIHA and the initiation of supportive care are likely to improve the outcome.

The authors state that they have no Conflict of Interest (COI).

References


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