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Clinical vignette

Prednisolone-induced immune hemolysis: a case report

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Background: Drug induced immune hemolysis is potentially serious. Most commonly antibiotics are responsible, while immunosuppressive drugs have been reported rarely.
Objectives: To report a rare case of suspected prednisolone-induced immune hemolysis.
Methods: A 59-year-old male patient with hemoglobin H disease and lymphoma developed hemagglutination and immune hemolytic anemia 20 days after receiving prednisolone.
Results: A direct antiglobulin test was positive for C3d. A test indicated prednisolone-dependent red blood cell antibody of the "immune complex" type. Tests with all other medications administered to the patient were negative. Dexamethasone was given to the patient without any reaction, as consistent with in vitro test results.
Conclusions: Physicians should be aware of possible prednisolone-induced complement-mediated immune hemolysis by an immune complex mechanism and request appropriate diagnostic tests as indicated.

Keywords: Drug-induced immune hemolysis, prednisolone

Drug-induced immune hemolysis (DIIHA) is a potential complication from drug administration and can be a serious condition. The estimated prevalence is approximately 1 in 1 million of the population [1]. Because the laboratory tests for this condition are not generally available, the true prevalence may be higher. The severity of this condition varies from mild to severe cases that need medical treatment and drug cessation. There are more than one hundred drugs which are listed to cause DIIHA, such as antibiotics and chemotherapeutics. Cephalosporins and piperacillin have been the leading culprits for this entity [1, 2].

Suggested mechanisms for DIIHA include drug-independent antibodies, drug-dependent antibodies and nonimmunologic protein adsorption. Some drug-dependent antibodies can be detected by testing drug-treated red blood cells (RBCs) ("drug-adsorption type"); other antibodies can only be detected by testing in the presence of the drug ("immune complex type") [3, 4]. Regardless of the mechanism, patients with DIIHA typically present with a positive direct antiglobulin test (DAT).

Corticosteroid is an immunosuppressive agent and is very unlikely to cause an autoimmune phenomenon. Therefore, it has been rarely reported as a cause of DIIHA. Hydrocortisone has been shown to induce intracellular hemolysis [5] and hemagglutination [6]. To our knowledge, prednisolone has not yet been reported to cause DIIHA.

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Learning objectives

Physicians should be aware of DIIHAs and the possibility of prednisolone-induced complement-mediated immune hemolysis by an 'immune complex' mechanism, and request appropriate diagnostic tests as indicated. Immunohematologic tests may be helpful for confirmation of diagnosis and selection of alternative drugs.

Case description

A 59 year-old male patient presented with bulky axillary lymphadenopathy. His lymph node pathology was compatible with diffuse large B-cell lymphoma; stage IAX, according to the Ann Arbor staging system. The patient had a medical history of hemoglobin H disease, but he had rarely received blood transfusion. His baseline hemoglobin level was 10 gm/dL, mean corpuscular volume (MCV) was 61 fL and he had mild splenomegaly. The patient was scheduled to receive the CHOP regimen for lymphoma treatment. The CHOP regimen consisted of cyclophosphamide 750 mg/m^2, vincristine 1.4 mg/m^2, doxorubicin 50 mg/m^2 and prednisolone 100 mg for 5 days every 3 weeks.

Twenty days after his first course, he developed progressive fatigue during hospitalization. A physical examination revealed markedly pale conjunctivae and nonicteric sclera. A complete blood count showed hemoglobin 6.6 gm/dL, hematocrit 3%, MCV 70 fL, WBC 5.59×10^9/L, and platelets 231×10^9/L. Blood chemistry found the total bilirubin 0.4 mg/dL (normal range 0.1–1.1 mg/dL), direct bilirubin 0.1 mg/dL (normal range 0.1–0.5 mg/dL), lactate dehydrogenase 834 U/L (313–618 U/L). Peripheral blood smear showed multiple clumps of RBC agglutination as shown in **Figure 1**. Because of the agglutination of RBCs, a reticulocyte count could not be conducted. Neither red serum nor dark urine was noticed.

The direct antiglobulin test result was positive 1+ for C3d, but negative for IgG. An indirect antiglobulin test was negative. The patient was diagnosed as having autoimmune hemolytic anemia. After RBC transfusion of 1 unit was administered, the anemic symptoms gradually improved. Cold agglutinin titer was 128 (normal range < 32). Immunoglobulin levels were within normal range; IgG 1340 mg/dL (normal range 700–1600 mg/dL), IgM 217 mg/dL (normal range 40–230 mg/dL) and IgA 185 mg/dL (normal range 70–400 mg/dL). The next 5 courses of chemotherapy were continued, but prednisolone was replaced by dexamethasone. The patient did not develop immune hemolytic anemia after the drug replacement.

This case report and any associated retrospective review of patient records was approved by the Ethics Committee of Chulalongkorn university (IRB No. 146/59, approval No. 008/2016) and has been sufficiently anonymized so as not to cause harm to the patient or their family. The patient has provided written informed consent for the publication of their case.

Testing drug-treated RBCs

To prepare drug-treated RBC, 400 mg of the drugs were dissolved in 10 mL of phosphate-buffered saline (PBS) and then 1 mL of group O red cells added [7]. In a separate tube, control cells were prepared by adding 1 mL of untreated red cells (without the drug) to 10 mL of PBS. Both tubes were incubated for 1 hour at 37°C with occasional mixing. Cells were washed three times and prepared as a 5% suspension in PBS. Two sets of tubes were labeled (drug-treated and untreated) for each sample to be tested: patient's serum, patient's eluate, PBS, and pooled plasma. We modified the American Association of Blood Banks Technical Manual method using a gel (LISS/Coombs gel card, screening I and II, Bio-Rad Laboratories, Diamed, Cressier, FR, Switzerland) instead of a tube test. Salama et al. described the gel method for drug antibody detection [8]. We added 25 μL of each sample to the appropriate tube and added 50 μL of RBC (drug-treated and untreated set) to the gels. Gels were incubated at 37°C for 60 min. The tubes were centrifuged and examined for hemolysis and agglutination.

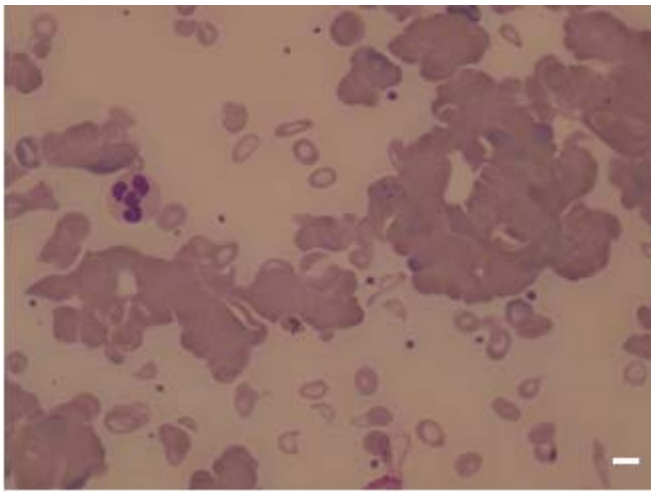

Figure 1. Clumps of red cell agglutination from blood smear of the patient. Bar approx. 10 μm.

Testing in the presence of soluble drug

Drugs were prepared as a 1 mg/mL solutions in PBS and then centrifuged to remove any particulate matter [7]. We prepared 2 sets (untreated and enzyme-treated) of 6 gels for the following test mixtures: (a) patient's serum + drug, (b) patient's serum + PBS, (c) patient's serum + complement (normal serum) + drug, (d) patient's serum + complement (normal serum) + PBS, (e) Normal serum + drug, and (f) normal serum + PBS. We used papain for enzyme-treated red cells. We added 50 μL of each component to the appropriate gel, followed by 50 μL of a 5% saline suspension of the untreated group O reagent red cells to one set of tubes, and 50 μL of a 5% saline suspension of enzyme-treated group O reagent red cells to the second set of tubes. The components were mixed and incubated at 37°C for 1 to 2 hours. The tubes were centrifuged and the reaction examined.

Immunohematologic test

Complement mediated hemolysis is usually the main mechanism for the 'immune complex' formation type of DIIHA, but only a rare cause in the drug adsorption type. To confirm the specific drug that caused complement mediated immune hemolysis, we performed an indirect antiglobulin test for all of the drugs given simultaneously to the patient. They were cyclophosphamide (Baxter), vincristine (Pfizer), doxorubicin (Pharmacia), and prednisolone (generic, Patar Lab). Specific tests for detection of drug antibodies in DIIHA were performed using both techniques, which were testing drug-treated RBCs (drug absorption type) and antibody testing in the presence of drug (immune complex type) [7, 9].

We prepared the drug-treated RBCs for the drug absorption type test by incubating the drug with group O RBCs for 1 h. Subsequently, we performed the indirect antiglobulin test using a gel card technique. We used both the drug-treated and the untreated negative control tubes. Samples tested included the patient's serum, patient's eluate, PBS, and pooled plasma. All 4 drugs suspected of causing DIIHA gave negative results.

The drugs suspected of causing DIIHA were prepared as a 1 mg/mL solutions in PBS for the immune complex method. Pooled group O RBCs, in 5% suspension, were either incubated with a proteolytic enzyme or left untreated. Two sets of group O RBCs (enzyme-treated and untreated) were tested in 6 panels according to this method. An indirect antiglobulin test for 2 sets of group O RBCs using a gel-test was performed. Only prednisolone gave a positive result as shown in **Table 1**. The immune complex method showed a positive result only in the mixture of the patient's serum, complement, prednisolone, and enzyme-treated RBCs, subsequently identified as anti-C3. The untreated RBC panels all showed negative results. The tests were also conducted for the other 3 drugs (cyclophosphamide, vincristine, and doxorubicin), which gave negative results for both enzyme-treated and untreated red cells. A further test using dexamethasone instead of prednisolone, was also negative (**Table 1**).

Table 1. Results of tests using the immune complex method with prednisolone, dexamethasone, cyclophosphamide, vincristine, and doxorubicin (and enzyme-treated red blood cells)

Drug	Patient's serum				Normal serum	
	With drug	With PBS	With complement and drug	With complement and PBS	With drug	With PBS
Prednisolone	negative	negative	positive (1+)	negative	negative	negative
Dexamethasone	negative	negative	negative	negative	negative	negative
Cyclophosphamide	negative	negative	negative	negative	negative	negative
Vincristine	negative	negative	negative	negative	negative	negative
Doxorubicin	negative	negative	negative	negative	negative	negative

PBS, phosphate-buffered saline

Discussion

The results of the test using the immune complex method suggested that prednisolone caused complement-mediated immune hemolytic anemia. Hydrocortisone-associated hemagglutination has also been reported [6]. In this and a related case, IgM antibody was found in the serum, but it did not cause in vivo or in vitro hemolysis [6, 10]. Hydrocortisone-induced intravascular hemolysis was positive in both drug-treated RBC and immune complex methods [5].

Because the present patient did not show any signs and symptoms of respiratory tract infection, infectious associated cold agglutinin disease [11] e.g., *M. pneumoniae* is less likely despite that the cold agglutinin titer was slightly elevated. Clinical onset and the specific immunohematologic test supported a diagnosis of prednisolone-induced immune hemolytic anemia. We hypothesized that prednisolone induced cold agglutinin antibody, which caused agglutination of the RBCs and resulted in immune hemolysis by complement activation. Subsequently, the C3d-coated red cells were processed through splenic sequestration. Because the complement pathway was not complete, the patient did not show intravascular hemolysis. This splenic sequestration showed slow onset causing a gradual drop in hemoglobin concentration (from 10 g/dL to 6.6 gm/dL), therefore the indirect bilirubin level was not increased.

Matinengo et al. reported that hydrocortisone-dependent antibodies also reacted with other corticosteroid products, including dexamethasone, methylprednisolone, and betamethasone [5]. Because our patient did not develop immune hemolysis after being switched to dexamethasone, the antibody in this case appeared to be specific to prednisolone. Dexamethasone showed negative results in the in vitro test suggesting a role of this drug antibody detection method in predicting clinical hemolysis.

The excipients in the prednisolone tablets, produced by Patar Lab pharmaceutical manufacturer (Bangkok, Thailand), are starch, lactose, erythrosine, and magnesium stearate. Magnesium stearate is widely used as the lubricant for compressed tablets, while erythrosine is also commonly used for the color of the tablets. Lactose was reported to cause hemaglutination in a healthy donor [12], but this donor did not develop symptomatic hemolytic anemia. Pure prednisolone was not tested, but these 4 excipients are unlikely to be a cause of immune hemolysis.

In the immune complex mechanism, the 3 proposed components are: (1) drug, (2) RBC membrane, and (3) antibody. The drug-dependent antibody has been reported to be sometimes specific to the subtype of RBC antigen such as Rh, Kell, or Kidd blood group [13, 14]. Unfortunately, we did not analyze the RBC subtype in the present patient. Because corticosteroid-induced DIIHA is rarely encountered, some host factors, especially specific RBC alloantigens, should be further explored. Autoimmune hemolytic anemia (AIHA) is rarely associated with lymphoma except in chronic lymphocytic leukemia (CLL) [15, 16]. The prevalence of non-Hodgkin's lymphoma, excluding CLL, associated with AIHA is approximately 0.23%–2.6% [6, 17] Generally, AIHA occurs in non-Hodgkin's lymphoma while in the active disease phase, but not after treatment. AIHA is reported to be a rare complication in Thalassemia including hemoglobin H disease [18]. Immune activation may be caused by sequestered RBCs in the spleen [19] and by red cell fragmentation [20]. AIHA associated with Thalassemia requires steroids for treatment, but in the present patient the hemoglobin level gradually improved after blood transfusion and prednisolone cessation. Following the clinical presentation of this patient, neither lymphoma nor hemoglobin H disease were considered to be the cause of AIHA.

We recommend clinicians be aware of possible prednisolone-induced immune hemolysis. Immuno-hematologic tests may be helpful to confirm diagnosis and selection of alternative drugs.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

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