Rifampin-Induced Thrombocytopenia: Diagnosis by a Novel In Vitro Lymphocyte Toxicity Assay

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Key words: rifampin, thrombocytopenia, hemolysis, lymphocyte toxicity assay

Several cases of rifampin-induced thrombocytopenia have been reported in the literature [1]. It was recently demonstrated that many of the rifampin-induced adverse events have an allergic origin. While non-reaginic anti- rifampin antibodies were detected in the serum of patients, anaphylactic reactions due to the use of the drug seem to be immunoglobulin E mediated [2].

Re-challenge and de-challenge of a drug are established tools but not accepted techniques for the diagnosis of adverse drug events. We report here the case of a woman who developed severe thrombocytopenia, hemolytic anemia and high serum levels of IgE, without renal function impairment. Her condition was evaluated by use of a modified in vitro lymphocyte toxicity assay.

Patient Description

A 40 year old woman (47 kg weight) of Ethiopian origin was admitted for the investigation of fever (38°C) and dry cough that had persisted for the previous 2 weeks. Her past history was unremarkable and she was not taking any fixed medications. On physical examination her body temperature was 38.2°C and she had diffuse wheezing and dry crackles in the lungs. The relevant laboratory findings are shown in Table 1. Anti-hepatitis B and C antibodies were found. Hepatitis B surface antigen was not detected. An enzyme-linked immunosorbent assay test for human immunodeficiency virus was negative, as was the viral load test.

A chest X-ray on admission demonstrated alveolar infiltration in the left and right lungs, a finding compatible with an infective process. A purified protein derivative (1/100) skin test was negative. A sputum specimen revealed positive acid-fast bacilli, and a specific gene probe identified groups of Mycobacterium tuberculosis hominis bacteria. Isoniazide 300 mg/day, pyrazinamide 1,500 mg/day, ethambutol 1,200 mg/day and rifampin 600 mg/day were prescribed.

On the day that rifampin was discontinued a direct Coombs' test (anti-IgG positive, anti-C3 traces) was positive; slight erythrocyte fragments and polychromatophilia were observed in a peripheral blood smear. A qualitative test for urine hemoglobin was slightly positive. Normal numbers of megakaryocytes without the presence of abnormal cells were found in the bone marrow aspiration that was mixed with peripheral blood. A bone marrow biopsy performed on admission did not demonstrate granulomata and no abnormal cells were present. The histological specimen showed mild hypercellularity and a mild increment of megakaryocytes was found. The IgE serum concentration was 320 KU/ml (normal up to 183 KU/L).

Forty-five days after cessation of the rifampin therapy her platelet count rose to 98 x 10^9/ml. She was admitted to the emergency room because of acute gastroenteritis. Her blood count showed hemoglobin 13.4 g/dl, white blood cells 4.8 x 10^9/ml, platelets 189 x 10^11/ml and mean corpuscular volume 87.0 fl.

An in vitro lymphocyte toxicity assay was performed to establish the possible relationship between the prescribed medications and the adverse events. A fresh whole-blood sample was layered on a Ficoll-Paque density gradient, and interphase cells were collected, layered on 20% sucrose and then suspended in a Heps-buffered medium. The pellet was re-suspended to yield 10^6 cells/reaction (viability at this stage was 95%). Lymphocytes were collected by centrifugation, the supernatant was aspirated, and NADPH, G6P and G6PD (Sigma, USA) were added. The cells were counted and re-suspended again at a density of 2 x 10^6/ml in tubes. Untreated lymphocytes were considered as controls. The lymphocytes treated with different drugs without microsomes comprised the second set of controls, and lymphocytes re-suspended with drugs and canine micro-

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1IgE = immunoglobulin E
Table 1. Laboratory data

<table>
<thead>
<tr>
<th>Date</th>
<th>Platelets (x10^9/ml)</th>
<th>Hb (gr/dl)</th>
<th>Retic (%)</th>
<th>MCV (fl) (80–94)</th>
<th>LDH (IU/L) (60–225)</th>
<th>GGT (IU/L) (5–60)</th>
<th>AST (IU/L) (5–40)</th>
<th>ALT (IU/L) (5–40)</th>
<th>AP (IU/L) (30–115)</th>
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Figures in parentheses represent normal values.
** Isoniazide, rifampin, ethambutol, pyrazinamide.
Retic = reticulocytes, LDH = lactate dehydrogenase, GGT = gamma-glutamyl transferase, AST = aspartate aminotransferase, ALT = alanine aminotransferase, AP = alkaline phosphatase, IVIG = intravenous hyperimmune gammaglobulin.

somtes (Promega, USA) were considered as the test cells. Each test was done in triplicate, and 24 hours later cells were washed and plated onto microplates at 37°C. Formazan (MTT, 100 µl) was added for 2 hours, the toxicity assay was performed and the results read by an ELISA reader at 595–650 nm. The percentage of cells displaying cytotoxicity, compared to control cells treated only with the drug, was calculated. The normal result for this test is <15% cytotoxicity, between 15% and 20% of cytotoxicity is undetermined, and above 20% is frankly positive. The cytotoxicity results were 61% for rifampin and 15% for ethambutol. Pyrazinamide and isoniazide revealed no cytotoxic response. The control group was established to be 15% cytotoxicity.

Comment

We present a woman who developed severe and persistent rifampin-induced thrombocytopenia, hemolytic anemia, slight intravascular hemolysis and high serum levels of IgE, without renal function impairment. The use of a modified in vitro lymphocyte toxicity assay allowed us to reach the diagnosis of this drug-induced cytotoxicity phenomenon, without performing the classical de- and re-challenge procedure.

It can be assumed that the mechanism/s of rifampin-induced thrombocytopenia and hemolysis are due to reactive metabolite/s and/or rifampin metabolites such as the desacetyl rifampin and/or formylrifampin, which may covalently link to host blood cell proteins and form hapten-protein conjugates. The resulting anti-drug antibody damages the cell membrane. In addition, the aberrant expression of hapten-conjugate on cellular membranes in conjunction with major histocompatibility complex I molecules may lead to a cytotoxic response against the blood cells.

The resultant immune responses may give rise to rifampin-induced immune hemolytic anemia, intravascular hemolysis, hemoglobinuria and/or thrombocytopenia. The increased sensitivity to reactive metabolites of a drug, in particular those produced by microsomal oxidation, seems to be inherited. The presence of rifampin-dependent antibodies of the IgM class, which had anti-I specificity against erythrocytes and an IgG anti-rifampin antibody with complement-fixing capability, has been described [3].

Other possible mechanisms involved is the presence of a Fab terminal anti-
drug antibody that binds to the GPIb/IX or to the GPIb/IIa platelet receptor, causing drug-induced thrombocytopenia such as in quinidine-induced thrombocytopenia [4]. Our patient’s delayed recovery from thrombocytopenia after discontinuation of the drug and the later reappearance of severe thrombocytopenia may be attributed to the generation of rifampin-associated IgG antibodies (PAILgG) [5].

The relevance of lymphocytotoxicity assay to those mechanisms is based on the reactive drug metabolite/s, which are generated in vitro using canine microsomes as a source of cytochrome P-450. Human lymphocytes from patients with suspected hypersensitivity reactions are used, as surrogate target cells form in vitro rechallenge. If the cells are susceptible to damage by reactive metabolites, the in vitro toxicity can be measured by using MTT, which is converted into a

References

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Toxic Delirium due to Datura Stramonium

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Key words: Datura stramonium, anticholinergic toxidrome, tropane alkaloids, toxic delirium

Altered mental status and delirium are common and challenging problems in emergency medicine. Since the differential diagnosis is wide a systematic approach is necessary to reach the correct diagnosis and prescribe the appropriate treatment. Toxic delirium is one of the common causes, and poisoning from anticholinergic tropane alkaloids (or belladona alkaloids: scopolamine, hyoscymine and atropin) one of its possible etiologies. Many plants contain these alkaloids.

We present two patients with tropane alkaloid poisoning due to Datura stramonium taken as a recreational hallucinogen, and describe the clinical manifestations. Physicians should be alert to the variable clinical picture in order to avoid pitfalls in diagnosis and treatment.

Patient Descriptions

Two brothers, 17 and 16 years old, were brought to the emergency department by ambulance. On arrival they were uncooperative and in agitated delirium. Physical examination revealed tachycardia (140 and 120 per minute), blood pressure 140/70 and 120/55 mmHg, respirations 18/min, and rectal temperature 37 and 37.7°C respectively. Both of them had mydriasis and their skin was warm and dry. One of them had flushed skin and dry mucosa. Their veins were not scarred and no signs of venous injections were noted. Their chests were clear, and apart from the tachycardia their hearts seemed normal. There was no obvious evidence of trauma. They were not communicating and were in a state of agitated delirium with purposeless movements. No focal or meningeal irritation signs were found. Blood glucose, oxytemy, and blood chemistry were normal.