NEW!

Vitamin-Induced Hemolysis

An African American man who received a vitamin infusion several days ago now has fever, dyspnea, nausea, and vomiting. He presents to the ED, where he is diagnosed with hemolytic anemia. The mechanism of xenobiotic-induced hemolysis, especially in patients with G6PD deficiency, is discussed.

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Case

A 47-year-old African American man presents to the ED with a 3-day history of fever, shortness of breath, nausea, emesis, dark urine, and progressive confusion. The symptoms began 1 day after he had received an infusion of a "vitamin complex" at his physician's office. The patient's medical history is significant for retroperitoneal fibrosis and multiple urologic procedures, for which he takes oxybutynin and tamsulosin.

The patient's outpatient physician, who practices both homeopathic and Western medicine, revealed that he had administered an infusion containing vitamin B and D complex, free amino acids, magnesium, and taurine. He did not comment on the preparation method.

What are the mechanisms by which xenobiotic-induced hemolysis occurs?

Hemolysis is the premature destruction of red blood cells in the circulation, usually before the completion of their typical 120-day life span. Hemolysis is commonly induced by xenobiotics, which cause destruction of red blood cells via either immune- or non–immune-mediated mechanisms.[1-3]

Immune-mediated xenobiotic-induced hemolysis results from an antigen-antibody reaction following drug exposure. This form of hemolysis is often categorized into three types, based on the xenobiotic's mechanism of action in inducing hemolysis. In a type I immune-mediated reaction, the xenobiotic acts as a hapten, binding tightly to the red blood cell membrane glycoprotein and stimulating formation of immunoglobulin G (IgG) directed against the drug. The antigen-antibody complex is removed by the splenic macrophages. Large doses of penicillin are needed to induce hemolysis via this mechanism. With a type II reaction, the xenobiotic binds the red blood cell membrane glycoprotein with low affinity and attracts IgM. The resulting antigen-antibody complex is targeted by the innate complement system. In contrast to a type I reaction, type II reactions require only small doses of xenobiotics to trigger hemolysis. Quinidine is an example of a xenobiotic that can cause a type II reaction. The type III reaction is an autoimmune process in which the xenobiotic is thought to alter the natural suppressor system, resulting in antibody formation against the cellular components of red blood cell membrane. α-Methyldopa classically induces hemolysis via this mechanism.

Non–immune-mediated hemolysis is induced by oxidants, nonoxidizing xenobiotics, microangiopathic processes, spider and snake venom, and osmotically active xenobiotics. Patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency are especially prone to oxidant-induced hemolysis. This enzyme indirectly restores cellular levels of glutathione, an antioxidant agent that protects the red blood cell against oxidative damage. In G6PD-deficient cells, oxidants induce cross-linking of sulfhydryl groups on globin proteins, unfolding the protein chain and resulting in precipitation of the hemoglobin molecules as Heinz bodies within the erythrocytes. Nonoxidants such as

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TABLE. Oxidant Xenobiotics That Are Associated With Hemolysis in Patients With G6PD Deficiency

Dapsone
Isobutyl nitrite
Methylene blue (high dose)
Nalidixic acid
Naphthalene
Nitrofurantoin
Phenazopyridine
Primaquine
Quinidine
Sulfacetamide
Sulfamethoxazole
Sulfapyridine
Toluidine blue
Trinitrotoluene

Adapted from Silvilotti.[2]

arsine, copper, and lead may directly damage hemoglobin or the red blood cell membrane or may deplete the cell's intrinsic reducing system spearheaded by glutathione. The Table lists oxidant xenobiotic agents that are associated with hemolysis in patients with G6PD deficiency.

Xenobiotics that induce microangiopathic hemolysis include ticlopidine, clopidogrel, cyclosporine, and tacrolimus. Platelet aggregates form throughout the vasculature, likely due to antibody-mediated deficiency of ADAMTS13, a metalloproteinase that breaks down large multimers of von Willebrand factor. Accumulation of ultra-high-weight Von Willebrand factor enhances the adhesion of platelets to the vascular endothelium, thus causing the characteristic platelet microthrombi in microcirculation. When red blood cells are forced through these narrow vessels, they undergo mechanical injury and become fragmented.[4] Venom of *Loxosceles reclusa* (brown recluse spider) leads to hemolysis via interaction of sphingomyelinase D with the red blood cell membrane. Crotalinae snake venom produces hemolysis via hemostatically active components that interfere with the coagulation cascade. Hypophosphatemia reduces phosphorylation of intracellular molecules, including ATP (adenosine triphosphate) and 2,3-diphosphoglycerate, resulting in reduced membrane deformability. Water may osmotically swell the erythrocyte, causing it to lyse.

>>FAST TRACK<<

G6PD deficiency affects approximately 7.5% of the world population, although the magnitude of the enzyme dysfunction varies widely.

What are the clinical implications of G6PD deficiency?

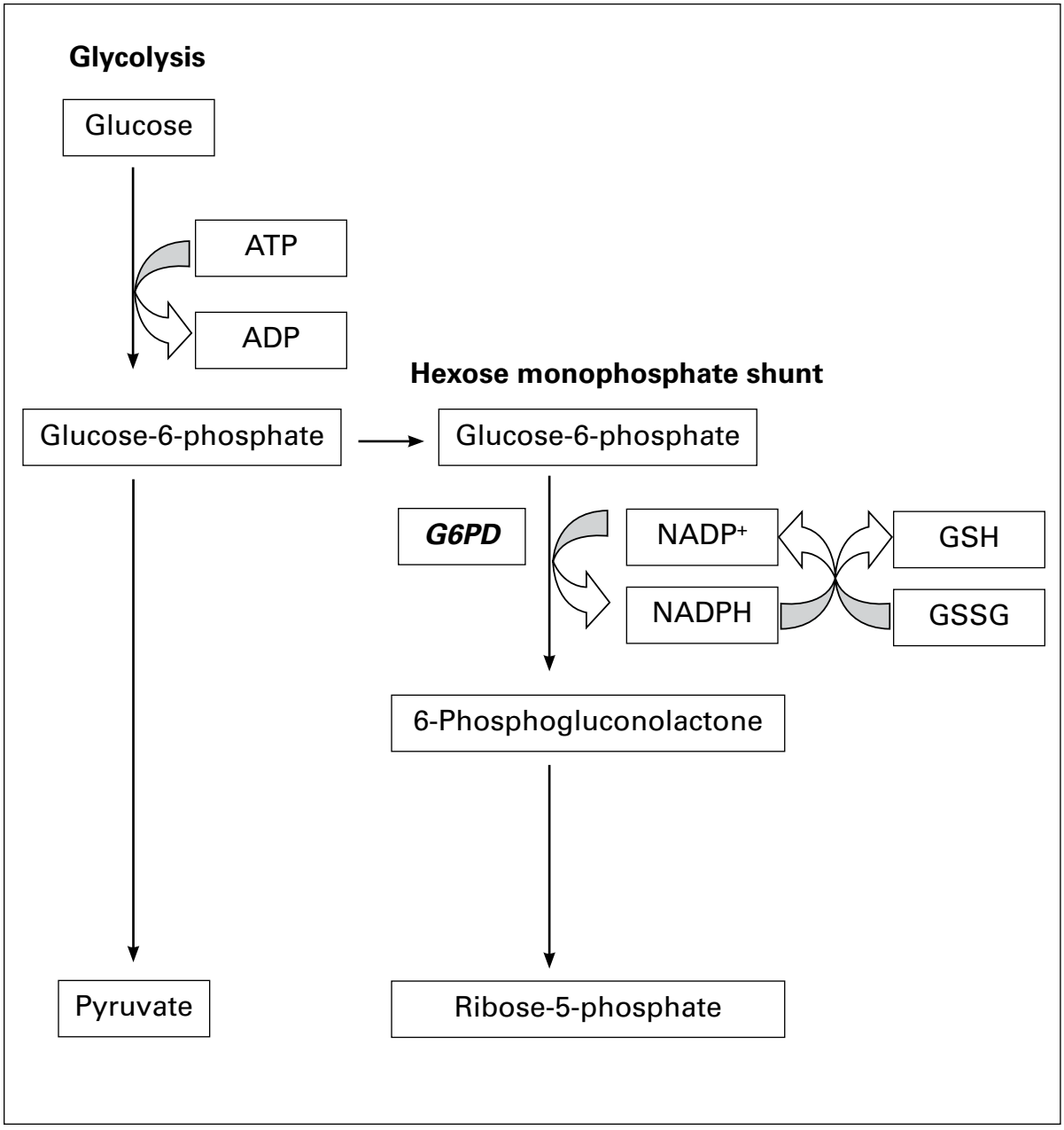
G6PD is an enzyme that generates NADPH (reduced form of nicotinamide adenine dinucleotide phosphate) via the hexose monophosphate shunt, a pathway that converts glucose to an energy source in red blood cells (Figure). Because NADPH is needed to regenerate glutathione, G6PD deficiency leaves the erythrocyte susceptible to oxidant-induced damage.

G6PD deficiency is an X-linked inherited disorder with variable phenotypic expression that produces a defect in the G6PD enzyme, reducing its half-life. Mutations in G6PD can also affect its affinity for substrate or its catalytic activity. G6PD deficiency affects approximately 7.5% of the world population, although the magnitude of the enzyme dysfunction varies widely.[2] The disorder is classified according to the degree of enzyme deficiency and the severity of hemolysis. Classes I and II occur mostly in persons of Mediterranean origin; these individuals have less than 10% of G6PD activity under normal conditions and experience fulminant hemolysis—classically, with exposure to fava beans and other oxidant stressors. Persons with class III G6PD deficiency (type A^-, a G6PD variant seen in persons of African origin, characterized by a significant decrease of G6PD enzyme activity in mature erythrocytes) have moderate (10% to 60%) deficiency and often present with limited hemolysis in response to certain drugs (eg, antimalarials) or infections. Class III G6PD deficiency affects approximately 11% of the African American population. The age at presentation is variable, and the first presentation of G6PD deficiency involves a hemolytic crisis.

How can diagnostic testing point to G6PD deficiency in a patient with acute hemolytic anemia?

Patients with G6PD deficiency may have either a normal hemoglobin level or anemia with unaffected

FIGURE. Hexose Monophosphate Shunt in Erythrocytes



ATP = adenosine triphosphate; ADP = adenosine diphosphate; G6PD = glucose-6-phosphate dehydrogenase; $NADP^+$ = oxidized form of nicotinamide adenine dinucleotide phosphate; NADPH = reduced form of nicotinamide adenine dinucleotide phosphate; GSH = reduced form of glutathione; GSSG = oxidized form of glutathione.

platelet and leukocyte counts (which are reduced in patients with aplastic anemia, for example), elevated total bilirubin levels with normal direct bilirubin levels (suggesting an elevated indirect bilirubin value), elevated lactate dehydrogenase levels, increased reticulocyte counts, and a negative Coombs test result.

The presence of a normal or decreased hemoglobin concentration depends on the degree of compensation via increase in reticulocytes. The Coombs reagent is an antiglobulin that is used in both the direct and indirect Coombs test. The direct test detects the presence of antibodies and/or complement

coating the erythrocytes. The indirect test identifies autoantibodies in the serum; a false-negative result may occur if there is only a small concentration of immunoglobulin present. The direct Coombs test is used to evaluate for antibodies induced by xenobiotics or autoimmune conditions, whereas the indirect Coombs test is typically used to screen for serum antibodies during blood cross-match procedures.

The peripheral blood smear may demonstrate Heinz bodies, or precipitated hemoglobin, in the erythrocyte. Alternatively, there may be "blister" cells present, ie, erythrocytes whose precipitated hemoglobin was removed during passage through the spleen. Blister cells are common in G6PD deficiency. "Bite cells" are erythrocytes with a precipitated Heinz body that appear as if a semicircular "bite" has been taken from the edge. This process often occurs via splenic macrophages. It is important to remember that bite cells generated during an acute hemolytic episode in G6PD deficiency may be seen in other hematologic disorders such as thrombotic thrombocytopenic purpura and hemolytic uremic syndrome.

>>FAST TRACK<<

The direct Coombs test is used to evaluate for antibodies induced by xenobiotics or autoimmune conditions, whereas the indirect Coombs test is typically used to screen for serum antibodies during blood cross-match procedures.

Serum G6PD concentration should not be measured for approximately 3 months after an acute hemolytic event. Testing before 3 months shows falsely elevated G6PD activity, since the remaining erythrocytes have adequate G6PD activity and have survived the oxidant stressor. That is, the older cells (with dysfunctional G6PD) have hemolyzed, leaving only the younger cells, which still have adequate enzyme activity.

Case continuation

The patient continued to improve clinically. He had a negative result on a direct Coombs antiglobulin test. His peripheral smear demonstrated "blister cells," which, as mentioned, are commonly noted in patients with G6PD deficiency. The patient was discharged from the hospital in stable condition. At 2-week follow-up, his hemoglobin level was 11.4 g/dL. His G6PD concentration was reduced upon recovery.

Can a patient have a G6PD deficiency–associated hemolytic crisis following a vitamin infusion?

G6PD-deficient erythrocytes hemolyze when faced with oxidants. Unfortunately, the literature on vitamin-induced acute hemolysis is sparse. There is a reported case of acute hemolysis in a 32-year-old man of Nigerian descent who was diagnosed with HIV infection and treated with a high dose of ascorbic acid.[5] Another case of G6PD deficiency–induced hemolysis was described in a patient treated with the homeopathic remedy *Acalypha indica* (Indian copperleaf, "acal").[6] Four cases of hemolytic crisis after topical application of henna have been described in G6PD-deficient children ranging in age from 20 days to 4 years.[7] It is unknown which minerals were included in the infusion received by the case patient; however, copper and many other transition metals are highly associated with hemolysis when administered parenterally. Properly formulated naturopathic products have little active ingredient and are generally not poisonous since the preparations are highly diluted. Life-threatening reactions associated with these products may be attributed to improper dilution, incorrect diluents, and/or contaminants or adulterants. □

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