Hemolytic Anemia Following Attempted Suicide

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CASE

A 43-year-old African-American man with a history of hypertension, depression, and chronic alcohol abuse presented to the emergency service of an outside hospital complaining of chest pain. Laboratory testing indicated hepatocellular injury with aspartate aminotransferase (AST)2 of 14000 U/L (reference interval 5–41 U/L) and alanine aminotransferase (ALT) of 6400 U/L (reference interval 8–45 U/L). Because laboratory indicators of liver function worsened, the patient was reinterviewed and confessed to having attempted suicide 3 days prior by ingesting about one-half gallon (approximately 4 L) of vodka and one-half bottle of extra-strength acetaminophen.

The patient was administered a bolus of N-acetylcysteine (NAC) at the outside hospital and transferred to our institution because of concern for progression to fulminant hepatic failure. Laboratory tests were repeated and results were consistent with significant hepatocellular damage secondary to acetaminophen poisoning, including increased AST of 11 000 U/L (reference interval 8–48 U/L), ALT of 6510 U/L (reference interval 7–55 U/L), total bilirubin of 395 μmol/L (23.1 mg/dL) [reference interval 2–17 μmol/L (0.1–1.0 mg/dL)], and direct-bilirubin of 207 μmol/L (12.1 mg/dL) [reference interval <5.0 μmol/L (<0.3 mg/dL)]. Treatment with intravenous infusion of NAC was continued, and nitroglycerin was administered for chest pain.

Within 24 h of admission the patient exhibited dyspnea with low hemoglobin (Hb) oxygen saturation (89%). Chest x-ray findings were unremarkable, whereas laboratory tests indicated a borderline-low Hb of 107 g/L (13.7 g/dL) [reference interval 135–175 g/L (13.5–17.5 g/dL)] with 4% methemoglobin (reference interval <1.5%). The patient received high-dose oxygen treatment but remained hypoxemic.

At 48 h the patient continued to have pronounced hyperbilirubinemia with evidence of hemolytic anemia, which included low Hb of 7.5 g/dL with 6% methemoglobin, marked reticulocytosis of 11.5% (reference interval 0.6%–1.83%), nondetectable haptoglobin [1.4 μmol/L (<14 mg/dL); reference interval 3–20 μmol/L (30–200 mg/dL)], increased mean corpuscular volume (MCV) (97.8 fl; reference interval 81.2–95.1 fl), and increased lactate dehydrogenase (LD) (6870 U/L; reference interval 122–222 U/L). Moreover, results of a direct Coombs test were negative, although a routine peripheral blood smear revealed the presence of “bite cells.” An enzymopathy with a drug-induced acute episode of hemolytic anemia was suspected.

DISCUSSION

ACETAMINOPHEN POISONING AND TREATMENT

Acetaminophen (paracetamol) is a safe antipyretic-analgesic if a maximum dosage of 4 g/day is not exceeded. Ingestion of higher doses, however, can lead to acetaminophen poisoning with hepatotoxicity (10–15 g/day) and may be fatal (20–25 g/day). We estimated that this patient ingested approximately 15 g acetaminophen and thus was at risk for fulminant hepatic failure.

Acetaminophen has high oral bioavailability, with peak plasma concentrations occurring within 30–60 min after ingestion (1). Metabolism of therapeutic doses of acetaminophen occurs rapidly (t1/2 of 2 h) in the liver, wherein the majority (approximately 90%) of acetaminophen is conjugated to glucuronide or sulfate and excreted in the urine within 24 h of ingestion (2). The remaining 10% is metabolized via the cytochrome P-450 pathway to form N-acetyl-p-benzoquinone imine (NAPQI), a potent electrophile, which is rapidly converted to a nontoxic moiety and excreted via conjugation with reduced glutathione. Acetaminophen poisoning saturates the glucuronide and sulfate stores, resulting in abnormally high NAPQI production (2) (Fig. 1). High concentrations of NAPQI deplete reduced-glutathione reserves, causing NAPQI accumulation, liver damage, and possibly fulminant hepatic failure. Chronic alcohol abuse has a potentiating effect on acetaminophen-induced hepatotoxicity through inducing the cytochrome P-450 pathway and subsequent NAPQI production or...
decreasing the hepatic content of glutathione. Conversely, acute alcohol ingestion minimally affects the clinical course of hepatotoxicity after acetaminophen overdose (3, 4). Transaminases typically increase within 48–72 h after acetaminophen poisoning. Not surprisingly, the case patient’s transaminase concentrations were highly increased (AST 11000 U/L; ALT 6510 U/L) in a setting of chronic alcohol abuse, acute alcohol ingestion, and acetaminophen poisoning.

A well-known antidote for acetaminophen-induced NAPQI poisoning (secondary to acetaminophen poisoning) is administration of NAC. NAC is an acetylated-precursor of L-cysteine used in de novo synthesis of reduced glutathione. Therefore, NAC treatment facilitates repletion of reduced-glutathione stores, which are used to detoxify NAPQI (4). Administration of NAC within 8 h of acetaminophen poisoning is recommended to minimize NAPQI accumulation and hepatocellular damage.

**DIAGNOSIS OF HEMOLYTIC ANEMIA**

Hemolytic anemia is a common blood disorder typified by excessive erythrocyte destruction and is etiologically heterogeneous (5). Acquired causes of hemolysis include autoimmunity, microangiopathy, and infection. Conversely, hereditary hemolytic anemia is a result of erythrocyte enzymopathies, membranopathies, and/or hemoglobinopathies. Accordingly, a combination of clinical evidence and laboratory findings are essential for accurate diagnosis of hemolytic anemia and its underlying etiology.

Laboratory findings of low Hb, increased reticulocytosis, increased unconjugated bilirubin and LD, and decreased haptoglobin are suggestive of hemolytic anemia (5). Reticulocytosis occurs in the bone marrow as a response to erythrocyte depletion and is typically observed within 3–5 days after a decline in Hb. MCV may increase in response to marked reticulocytosis because the MCV of reticulocytes is slightly larger than that of erythrocytes. The destruction of erythrocytes results in release of LD and Hb. Liberated Hb may be broken down into heme and catabolized to unconjugated bilirubin by the spleen, or it may bind plasma haptoglobin. Excessive liberation of Hb quickly saturates plasma haptoglobin, and the complexes are rapidly cleared by the reticuloendothelial system, which can lead to low or undetectable plasma haptoglobin concentrations. The patient’s initial laboratory results indicated normal Hb [137 g/L (13.7 g/dL)], whereas concentrations of total bilirubin [395 μmol/L (23.1 mg/dL)] and direct bilirubin [207 μmol/L (12.1 mg/dL)] were markedly increased and consistent with hepatocellular injury secondary to acetaminophen overdose. Three days after admission, the patient exhibited persistent hyperbilirubinemia with additional signs of hypoxemia, as evidenced by low Hb [75 g/L (7.5 g/dL)] with 6% methemoglobin, marked reticulocytosis (11.5%), nondetectable haptoglobin [1.4 μmol/L (<14 mg/dL)], and increased concentrations of MCV (97.8 FL) and LD (6870 U/L). These laboratory findings provided evidence of acute hemolytic anemia.

Hematological test findings facilitate diagnosis of hemolytic anemia and its etiology (5). A positive direct Coombs test indicates the presence of antibodies or complement on the surface of erythrocytes and is the hallmark of autoimmune hemolysis. Direct Coombs test results were negative in the case patient; however, a peripheral blood smear revealed bite cells, which are indicative of oxidative damage to Hb (Fig. 2). Oxidation of Hb causes denaturation and precipitation of Hb intracellularly, with subsequent Heinz-body formation (cellular inclusions of damaged aggregated Hb). Heinz bodies are removed by the reticuloendothelial system, leaving erythrocytes with a missing section of cytoplasm seen as bite cells in routine blood smear (6). In this patient, the presence of bite cells together with laboratory findings indicated that hemolytic anemia was...
caused by an abnormal erythrocyte response to oxidative-stress and suggested an underlying enzymopathy.

**GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY**

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-linked disorder that principally affects males, with increased frequency detected in African, Asian, and Oceanic ethnic groups. G6PD deficiency is the most common enzymopathy associated with acute hemolytic anemia; however, most patients have no clinical or laboratory evidence of hemolysis until an acute event (e.g., infection, drug reaction, ingestion of fava beans) induces oxidative damage to Hb. In this regard, G6PD plays an important role in repletion of reduced-glutathione concentrations and minimizing oxidative damage. Specifically, G6PD catalyzes the first step in the hexose-monophosphate shunt of the glycolytic pathway by converting glucose-6-phosphate to 6-phosphogluconate, with NADP⁺ acting as an electron acceptor (i.e., NADP⁺ converts to NADPH). Accordingly, G6PD activity is critical in maintaining adequate concentrations of NADPH (8). In erythrocytes, NADPH is necessary to convert oxidized glutathione into its reduced form. Reduced glutathione plays a crucial role in reducing reactive oxygen species into hydrogen peroxide and subsequently water. Therefore, individuals with G6PD deficiency are more susceptible to oxidative damage that can result in damaged Hb and hemolytic anemia.

The role of G6PD in maintaining adequate NADPH stores is crucial during methylene blue treatment of methemoglobinemia. Specifically, NADPH is critical for reducing methylene blue to leukomethylene blue, which subsequently reduces methemoglobin to Hb (8). In the presence of G6PD deficiency, treatment with methylene blue is contraindicated because methylene blue can exacerbate methemoglobinemia by functioning as an Hb-oxidizing agent, resulting in hemolysis and hyperbilirubinemia. The case patient had 6% methemoglobin and was appropriately tested for G6PD activity before methylene blue treatment.

The patient exhibited abnormal G6PD enzyme activity (4.1 U/g Hb; reference interval 8.6–18.6 U/g Hb), activity that was 30%–50% of the adult reference range. Diagnosis of G6PD deficiency, however, requires that G6PD activity be <25% of the adult reference range. Interestingly, G6PD enzyme activity during an acute hemolytic episode reflects only the presence of surviving erythrocytes because young/immature reticulocytes have a high G6PD activity and therefore avoid

**POINTS TO REMEMBER**

- Acetaminophen poisoning rapidly saturates the normal metabolic clearance pathway (i.e., glucuronidation) and also reduces glutathione stores, leading to accumulation of NAPQI. Rapid treatment with NAC is required to minimize hepatocellular injury. Persistent increases in prothrombin time and serum bilirubin are indicative of a poor prognosis.
- A combination of clinical evidence, laboratory findings (low Hb, increased reticulocytosis, increased unconjugated bilirubin and lactate dehydrogenase, and decreased haptoglobin), and hematological test findings (peripheral blood smear, hemoglobin electrophoresis, direct Coombs test, erythrocyte enzyme tests) are required for accurate diagnosis of hemolytic anemia and its underlying etiology.
- G6PD deficiency predominantly affects males, with increased frequency in African, Asian, and Oceanic ethnic groups. G6PD deficiency is the most common enzymopathy associated with acute hemolytic anemia; however, most patients have no clinical or laboratory evidence of hemolysis until an acute event (e.g., infection, drug reaction, ingestion of fava beans) induces oxidative damage to Hb.
- Measurement of G6PD activity during an acute hemolytic episode reflects only the surviving erythrocytes and young reticulocytes that may evade hemolysis owing to G6PD activity that is higher than average. To accurately diagnose G6PD deficiency in a patient with acute hemolysis, G6PD enzyme activity must be retested after 2–3 months, when cells of all ages are again present.
- In the presence of G6PD deficiency, treatment with methylene blue is contraindicated because methylene blue can further exacerbate methemoglobinemia by functioning as an Hb-oxidizing agent, resulting in hemolysis and hyperbilirubinemia.
hemolysis (8). To accurately diagnose G6PD deficiency in a patient with acute hemolysis, it is important to retest G6PD activity after 2–3 months, when cells of all ages are again present (5). After discharge from the hospital, the case patient was referred to his home clinic for follow-up and retesting of G6PD activity.

The acute hemolytic anemia in this patient presented 3 days after admission to the referral clinic and 6 days after acetylsalicylic ingestion. Because of the absence of infection or ingestion of fava beans, we deduced that drug-induced oxidative-stress was likely the precipitant of hemolytic anemia with underlying G6PD deficiency. Acetaminophen at a therapeutic dose is considered a safe antipyretic-analgesic in patients with G6PD deficiency. However, the present case and others suggest that acetaminophen intake exceeding therapeutic doses likely induces hemolysis in patients with G6PD deficiency (9, 10). In the case patient, the acetaminophen poisoning was treated with NAC, which promotes resolution of the patient’s hemolytic anemia.

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References

Commentary
Ernest Beutler†

In the US, G6PD deficiency is most common among African-Americans; 11% of the X-chromosomes in this population bear the common G6PD A(–) mutation. Other mutations, usually more severe, are common among Americans of Southern European, Middle Eastern, and Asian origin. More than 5 million American males and nearly twice as many females are G6PD deficient, and many of these individuals are also at clinical risk for this X-linked trait.

Given the widespread occurrence of this condition, why do we not more frequently encounter drug-induced hemolytic anemia among our patients? The answer is that very few of the drugs used in the US today precipitate hemolysis in patients with G6PD deficiency. G6PD deficiency was discovered as a result of investigation of the hemolytic anemia produced by the antimalarial drug primaquine, which invariably produces hemolysis in G6PD-deficient individuals. Many other drugs have the potential to produce hemolysis in these patients (e.g., aspirin, acetaminophen, and trimethoprim/sulfamethoxazole). However, these drugs produce hemolysis in only a small minority of G6PD-deficient patients, or do so only when massive amounts of drug are ingested, as in the case described by Korpi-Steiner et al. Even in this case, it is not clear that the hemolysis was caused by acetaminophen. Cysteine produces oxidative stress when incubated with erythrocytes (1), and NAC, given to treat acetaminophen toxicity, very likely has the same effect. Massive hemolysis has been reported in another case in which this NAC was given to counteract an acetaminophen overdose (2). It is ironic that “antioxidative” drugs may cause hemolysis, but ascorbic acid administered in large amounts has been documented to do so (3). In the oxygen-rich environment of the erythrocyte, such
Acetaminophen (paracetamol) poisoning is, regrettably, quite common, and the clinical course and interventions are well recognized. It is salutary to be reminded of the rare, potentially fatal, complications that can arise after an apparently appropriate intervention. Such was the case in the patient described by Korpi-Steiner et al.

There are 2 principle mutations that affect the glucose-6-phosphate dehydrogenase (G6PD) gene. The A(–) type affects approximately 11% of African Americans and West Africans, and predisposes these individuals to primaquine sensitivity. The Mediterranean variant predisposes individuals to favism. Both types lead to susceptibility to drug-induced hemolytic anemia. Reduced G6PD activity caused by both mutations leads to NADPH deficiency, with consequent accumulation of methemoglobin because NADPH is a required cofactor in converting methemoglobin back to hemoglobin. Methemoglobin accumulation presents a clinical risk because treatment with methylene blue could exacerbate the hemolytic anemia, although methylene blue probably should not be used with methemoglobinemia of <20%.

Acetaminophen is not unique among xenobiotics in exposing susceptible individuals to hemolytic anemia. Oxidizing drugs, which often contain an aromatic amine moiety, can induce oxidative injury through the production of free radicals that cause structural damage to proteins, including hemoglobin; oxidation of the structurally critical cysteine (β93) will result in denaturation.

Xenobiotic-induced hemolytic anemia can also be caused by other less-clear mechanisms, examples being exposure to arsine, copper, or lead; these are not G6PD linked. Immune hemolytic anemia induced by a wide range of drugs is also a well-recognized phenomenon. Infection and envenomation are other causes to remember.

Being alert to the hematologic changes associated with hemolytic anemia following poisoning in patients in ethnic groups with a high prevalence of G6PD deficiency is essential. It should be remembered that although the majority of such individuals are hemizygous males, heterozygous females, depending on their chimerism, may have G6PD deficiency in as many as 80% of their erythrocytes.

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Commentary

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