Persistently Increased Acetaminophen Concentrations in a Patient with Acute Liver Failure

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CASE

A 31-year-old woman was admitted into a regional hospital for abdominal pain, decreased appetite, malaise, confusion, and tea-colored urine. Investigations showed acute liver failure with a markedly decreased liver function characterized by greatly increased aminotransferases, bilirubin concentration, prothrombin time, and international normalized ratio. There was no history of liver disease or intake of herbal medicines or over-the-counter medications. Her condition worsened 2 days later, and she was transferred to our hospital for further management and the possibility of liver transplantation. A physical examination revealed a jaundiced woman in a fair general condition and with a soft but tender right upper quadrant with no guarding or rebound tenderness of the abdomen. She went into a semicomatose state 1 day later. Routine laboratory testing of a blood sample obtained on her arrival in the hospital revealed the following results: bilirubin, 1210 μmol/L (reference interval, 7–19 μmol/L); alanine aminotransferase, 6170 U/L (reference interval, 5–31 U/L); aspartate aminotransferase, 5080 U/L (reference interval, 12–28 U/L); alkaline phosphatase, 150 U/L (reference interval, 34–104 U/L); ammonia, 171 μmol/L (reference interval, 0–33 μmol/L); lactate dehydrogenase, 6830 U/L (reference interval, 200–360 U/L); prothrombin time, 39.7 s (reference interval, 11.3–13.2 s); international normalized ratio, 3.3; acetaminophen, 121 μmol/L (therapeutic up to 100 μmol/L). Other results were unremarkable. A serologic evaluation was negative for hepatitis A and B. The plasma acetaminophen concentration prompted the clinical suspicion of drug overdose, but she denied taking acetaminophen. The patient’s liver enzymes, prothrombin time, international normalized ratio, and acetaminophen concentrations were monitored on subsequent days. Her general condition and liver function gradually improved, but her plasma acetaminophen concentration remained >100 μmol/L. Failure of the liver to metabolize the drug was suspected, and liver transplantation was contemplated at that juncture.

DISCUSSION

DIFFERENTIAL DIAGNOSIS

Acute liver failure is defined as the rapid appearance of severe complications (hepatic encephalopathy and impaired protein synthesis) after the first signs of liver disease. These complications can be evidenced by a coagulopathy. Common causes for acute liver failure are toxic injury, idiosyncratic reaction to medication, alcoholic hepatitis, viral hepatitis, ischemic injury, and idiopathic (without an obvious cause). In many countries, the incidence of acute viral hepatitis has decreased markedly owing to the introduction of vaccines for hepatitis A and B, and the testing of the blood supply for hepatitis C. Drug-induced hepatitis is increasing and is the most common cause overall. For drug-induced hepatitis, acetaminophen is the drug that most commonly causes acute liver failure; it also causes the greatest absolute number of fatalities. Other drugs causing hepatitis include nonsteroidal antiinflammatory agents and anticonvulsants. In Southeast Asia, herbal remedy–induced hepatitis is common.

Apart from a detailed drug history, including the possible intake of herbal medicines or exposure to hepatotoxins, an initial laboratory evaluation of patients with acute liver failure should include testing for hepatitis A, B, and C. Other, less common causes of acute liver failure include autoimmune hepatitis and Wilson disease.
PHARMACOKINETICS OF ACETAMINOPHEN AND THE EFFECT OF OVERDOSE IN LIVER INJURY
At therapeutic doses, >90% of acetaminophen is metabolized by the liver to inactive, nontoxic sulfate and glucuronide conjugates that are subsequently excreted in the urine (1). Less than 5% is metabolized by cytochrome P450 2E1 to N-acetyl-p-benzoquinoneimine (NAPQI), a highly reactive and toxic intermediate (1–4) that occurs in the liver and kidneys. NAPQI is usually reduced to a nontoxic mercaptate conjugate by glutathione. In overdose situations, the sulfation and glucuronidation pathways become saturated, thereby causing the shunting of more acetaminophen down the P450 pathway to produce excessive NAPQI (3–5). The increase in NAPQI depletes the body’s glutathione stores. When the glutathione supply falls to <30% of usual, NAPQI binds nonspecifically to intracellular proteins, particularly those with sulfhydryl groups, causing cell dysfunction and death. Prompt treatment with the glutathione precursor N-acetylcysteine (NAC) can minimize or prevent hepatocellular damage, but its effectiveness diminishes rapidly by 12–24 h after acetaminophen exposure (6). Measurement of plasma acetaminophen in the acute stage can establish whether NAC treatment is indicated. Therefore, the plasma acetaminophen concentration should be measured as soon as possible for all cases of suspected drug overdose. For cases in which acetaminophen is undetectable and/or a history is not available, the measurement of acetaminophen adducts on serum proteins can be helpful in diagnosing acetaminophen toxicity (7).

METHODS OF ACETAMINOPHEN MEASUREMENT
Many methods, including various chromatographic and spectrometric techniques, have been described for the assay of acetaminophen. The latter are mostly automated in clinical laboratories. GLC and HPLC have proved to be reliable and accurate methods for quantifying acetaminophen concentrations in biological samples. These assays, however, involve lengthy analytical procedures that require expensive instrumentation and a high level of technical skill to perform. For the rapid assay of acetaminophen in the clinical setting, simple automated enzyme-coupled colorimetric methods are most commonly used. More recently, automated immunoassays have become available, but they are generally more expensive and have not been adopted as widely. In 2008, 1972 of the 3095 laboratories enrolled in the College of American Pathologists proficiency-testing program for acetaminophen used methods with a colorimetric measurement. In our laboratory, acetaminophen is measured with the Vitros system (Ortho Clinical Diagnostics), which is based on spectrophotometric principles.

FACTORS INTERFERING WITH ACETAMINOPHEN MEASUREMENT
Although enzyme-based assays are convenient and economical compared with immunoassays, they are generally more prone to interference from biological molecules, such as bilirubin and hemoglobin, which are present in patient samples. Bilirubin has considerable potential for interfering with spectrophotometric measurements because of its broad, intense absorbance in the ultraviolet and visible regions of the electromagnetic spectrum (8). The Vitros acetaminophen method is based on the enzymatic conversion of acetaminophen to p-aminophenol and subsequent reaction with o-cresol to form the blue-colored indophenol, which is measured by the change in absorbance at 600 nm. An increase in the background absorbance at 600 nm caused by the presence of bilirubin may contribute to a false increase in acetaminophen. According to the product literature, interference occurs at bilirubin concentrations >342 μmol/L. Some enzymatic assays are also subject to interference in the presence of therapeutic concentrations of NAC (9). In contrast, immunoassays are unaffected by the presence of NAC (9) and are less susceptible to interference by bilirubin and hemoglobin.

RESOLUTION OF THE CASE
Because the prolonged increase in plasma acetaminophen concentrations was inconsistent with the expected pharmacokinetics, we considered that bilirubin interference might be causing the falsely increased acetaminophen concentrations. We evaluated the use of plasma ultrafiltration to remove interfering substances before measuring acetaminophen with the enzymatic method. An ultrafiltrate is similar to plasma in its concentration of small molecules but is virtually free of proteins, including protein-bound bilirubin, hemoglobin, and lipoproteins (10). We purchased Centrifree® micropartition devices from Amicon Bioseparations/Millipore. We placed 1 mL of the patient’s plasma and a positive control in the reservoir of the device, centrifuged it in a fixed-angle centrifuge at 1000g–2000g for 10 min, collected the ultrafiltrate, and assayed for acetaminophen and bilirubin with the Vitros system. The results indicated that ultrafiltration with the Centrifree micropartition device removed the icteric interference (Table 1). The ultrafiltration data showed that the patient had not ingested acetaminophen. Her liver function gradually improved with supportive management, and she made an uneventful recovery after 3 weeks. The exact etiology for the acute hepatic derangement remains obscure, however. Taking over-the-counter health supplements containing potentially hepatotoxic ingredients is a possi-

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2 Nonstandard abbreviations: NAPQI, N-acetyl-p-benzoquinoneimine; NAC, N-acetylcysteine.
bility, although this cause was not apparent in the history given by the patient. A rarer viral hepatitis, such as that caused by the Epstein–Barr virus, has not been rigorously excluded in this case.

We recommend that bilirubin be measured in all samples for which an acetaminophen measurement by enzymatic methods has been requested. Ultrafiltration should be performed before acetaminophen measurement for samples with a bilirubin concentration greater than that for which interference has been reported for the assay.

Table 1. Acetaminophen and bilirubin concentrations.*

<table>
<thead>
<tr>
<th>Methodology</th>
<th>Sample</th>
<th>Tests</th>
<th>Concentration, μmol/L</th>
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</thead>
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<tr>
<td>Plasma, enzymatic assay</td>
<td>Patient sample</td>
<td>Acetaminophen</td>
<td>121 115 104</td>
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<td></td>
<td>Bilirubin</td>
<td>1210 1170 960</td>
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<tr>
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<td>Positive control</td>
<td>Acetaminophen</td>
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<tr>
<td></td>
<td></td>
<td>Bilirubin</td>
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</tr>
<tr>
<td>Ultrafiltrate, enzymatic assay</td>
<td>Patient sample</td>
<td>Acetaminophen</td>
<td>609 610 607</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bilirubin</td>
<td>&lt;2 &lt;2 &lt;2</td>
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<td>Bilirubin</td>
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<tr>
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<td>Patient sample</td>
<td>Acetaminophen</td>
<td>612 613 612</td>
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<tr>
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<td>Positive control</td>
<td>Acetaminophen</td>
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* The lowest reportable acetaminophen concentration measurable with the Vitros chemistry analyzer is 30 μmol/L.

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References