An Unusual Case of Toluene-Induced Metabolic Acidosis

Cyrenius M. Jone and Alan H. B. Wu

We used gas chromatography–mass spectrometry to analyze serum specimens from a patient whose metabolic ketoacidosis resulted from sniffing a toluene-containing substance; we also analyzed a sample of the abused substance itself and a serum specimen from a normal subject. The blood samples from the patient showed abnormally high concentrations of acetoacetic, azeleic, benzoic, and 3-hydroxybutyric acids, and a smaller amount of hippuric acid than in the control serum. The ketone bodies and benzoic acid appeared to be factors contributing to her acidosis and ketosis. Analysis for volatile solvents in the patient’s serum revealed a low concentration of methyl acetate, in contrast to the high concentrations of toluene and bis(2-ethylhexyl) phthalate and the trace concentration of xylene in the abused substance. We consider this case unusual because the presence and concentration of specific ketoacids was unexpected in light of what was known about the patient.

Additional Keyphrases: gas chromatography–mass spectrometry • metabolic ketoacidosis • abused drugs

Solvent abuse, which can produce a temporary state of euphoria, has gained popularity among young adults and teenagers (1, 2). Sniffers have inhaled a wide variety of chemical products (1, 3) because of the presence of solvents in these easily available and inexpensive products. Toluene, one of the most commonly abused solvents, is present in glues, paints, and other chemical products. Solvent sniffing has been demonstrated to cause a number of abnormalities, including high-anion-gap metabolic acidosis (4, 5); distal renal tubular acidosis, which is a normal-anion-gap metabolic acidosis (4, 5); Fanconi’s syndrome (5, 6); muscular weakness; gastrointestinal and neuropsychiatric syndromes (7); and renal diseases (8). Sudden death can also occur from cardiac arrhythmia in acute intoxication (9).

High-anion-gap metabolic acidosis is believed to result from accumulation of metabolites of the solvent inhaled (4, 5). However, these metabolites are not routinely identified in an emergency situation. In this study, we retrospectively performed gas chromatography–mass spectrometry (GC-MS) to identify the solvent metabolites and the organic acids present in serum samples from a patient who had high-anion-gap metabolic acidosis after sniffing a toluene-containing chemical product.

Case History

A 20-year-old Latino female was admitted to the emergency room at Hermann Hospital in Houston, TX. Her chief complaints were chest pain radiated to her left arm, shortness of breath, headache, occasional tachycardia, photophobia, and generalized muscle weakness. Moreover, she had been nauseated and anorexic for two days before hospital admission. She admitted to chronic abuse of organic solvents and alcohol, and stated that the frequency of solvent abuse had increased in the previous week. She denied using any other drugs, which was confirmed by a negative urine drug screen, and no ethanol was detected.

On physical examination, she showed a pulse of 122/min, a respiration rate of 36/min, a temperature of 98.2 °F, and a blood pressure of 130/71 mmHg, with no physical abnormalities. Relevant laboratory data at admission are summarized in Table 1. The low pH and total CO₂ indicate the extent of her metabolic acidosis. Calculations from electrolyte concentrations revealed a high anion gap. The test for acetoacetic acid in serum, based on the reaction with nitroprusside, was positive at a 1:16 dilution. The other laboratory data showed that the patient had normal renal and hepatic function, and she appeared nutritionally sound. However, the concentration of uric acid in serum slightly exceeded the reference limit. Serum specimens obtained 5 and 12 h after admission were also reactive with nitroprusside at 1:16 and 1:4 dilutions, respectively.

Materials and Methods

Samples: At admission, and 5 and 12 h after admission, blood was drawn into tubes containing no anticoagulant. After the blood had clotted, it was centrifuged and the serum was decanted and stored at –20 °C until GC-MS analysis. The first serum sample was also used for the analysis of volatiles. The substance the patient inhaled was identified as a product for shining shoes (Texas Super Coat, Houston, TX). For GC-MS analysis, we obtained a sample of this product from the Houston Police Department Crime Laboratory.

GC-MS analysis of volatiles in the patient’s serum: We deproteinized the serum sample by centrifuging 1 mL of it in a micropartition system (Amicon Co., Lexington, MA) at 500 × g for 10 min in a centrifuge with a fixed-angle rotor. After centrifugation, we injected 1 μL of the ultrafiltrate directly into a GC (Varian 3400, Palo Alto, CA)-MS (Model Incose 50; Finnigan Mat, San Jose, CA) system for analysis. The GC column, 1.5 m × 3.0 mm, was packed with Porapak Q, a styrene and divinylbenzene copolymer, 100–120 mesh (Waters Associates, Milford, MA), and helium was the carrier gas at a flow rate of 30 mL/min. The injector temperature was 250 °C. The initial column temperature, 160 °C, was held for 10 min, then increased to 245 °C at a rate of 20 °C/min without final holding. The mass spectral data were compared with those from the National Bureau of Standards by an on-line computer.

GC-MS analysis of the abused substance: We injected the original abused substance (0.2 μL) directly into a GC-MS equipped with a 15-m SE-54 fused-silica column (JW Scientific, Folsom, CA). The analysis was performed under two different conditions. In one, the column temperature was programmed to increase from 30 to 120 °C at a rate of 5 °C/min after injection of the sample; in the other, the temperature was programmed from 100 to 290 °C at 20 °C/min, with initial and final holding times of 1 and 5 min, respectively.

GC-MC analysis of metabolites and organic acids in the serum: We mixed three drops of 6 mol/L NaOH with 1 mL of
serum, added 0.5 mL of a solution of ethoxylamine (0.1 g/mL in pyridine), and heated the mixture at 60 °C for 30 min. After cooling the sample, we added 30 drops of 12 mol/L HCl, followed by solid NaCl until the sample was saturated. We then added 10 μg of a solution of 4-chlorobenzoinic acid in methanol (the internal standard), and extracted the sample with 3 mL of ethyl acetate. The organic and aqueous phases were separated by centrifugation and the organic phase was transferred to a test tube for evaporating the solvent. To the sample residue, we added one drop of pyridine and 150 μL of pure N₂O-bis(trimethylsilyl)trifluoroacetamide. After 30 min at 60 °C, we injected 2 μL of the derivatized sample into a GC-MS equipped with a 60-m column of DB-1 fused silica (J&W Scientific); the initial temperature of 110 °C was held for 2 min, then increased at 2.5 °C/min to 250 °C, which was held for 6 min.

For quantification, we analyzed pure standards of acetocetic (40 μg), azelaic acid (10 μg), benzoic acid (10 μg), 4-chlorobenzoinic acid (10 μg), hippuric acid (10 μg), 2-hydroxybutyric acid (10 μg), 3-hydroxybutyric acid (10 μg), and palmitic acid (10 μg) in methanol directly, using the same cavity column conditions. To calculate the amount of each of the compounds in the serum, we compared the ion currents generated by both the unknown and known samples and the ion current produced by the internal standard.

**Results**

GC-MS analysis for volatiles revealed methyl acetate in the patient's serum and toluene, xylene, and bis(2-ethylhexyl) phthalate in the abused substance. Table 2 lists the major organic compounds identified in the patient's serum by GC-MS. The representative chromatographic tracings are shown in Figures 1 and 2. We also found a trace of acetone in the serum sample. When the column temperature was programmed to increase from 30 to 120 °C at a rate of 5 °C/min, acetone, toluene, and xylenes were detected. Bis(2-ethylhexyl) phthalate was detected only after the column temperature was programmed to increase from 100 to 290 °C at 20 °C/min. Other, unidentified peaks were present in the GC tracings from the analysis of organic acids and the abused substance.

**Table 2. Major Organic Acids Present in the Patient's Serum Samples**

<table>
<thead>
<tr>
<th>Compounds Identified by GC-MS</th>
<th>Concentrations, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Admission</strong></td>
<td><strong>5 h</strong></td>
</tr>
<tr>
<td>Acetocetic acid</td>
<td>N.D.</td>
</tr>
<tr>
<td>Azelaic acid</td>
<td>0.88</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>8.11</td>
</tr>
<tr>
<td>Hippuric acid</td>
<td>N.D.</td>
</tr>
<tr>
<td>2-Hydroxybutyric acid</td>
<td>1.39</td>
</tr>
<tr>
<td>3-Hydroxybutyric acid</td>
<td>7.50</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>2.64</td>
</tr>
</tbody>
</table>

*Sample analyzed after storage at -20 °C for six months from the day of collection.
*Sample analyzed after storage at -20 °C for one month from the day of collection.
*Sample analyzed after storage at -20 °C for six months from the day of collection.

N.D. = not detected; i.e., <50 ng/mL.

**Discussion**

Our results demonstrate that toluene is metabolized to benzoic acid in humans, and this metabolite can be detected in the serum. In this patient, benzoic acid was initially present at a high concentration and gradually decreased with time. Our results are in agreement with reports that benzoic acid is detected in the urine of persons exposed to toluene (10, 11). Benzoic acid can also originate from sources other than toluene metabolism (12), e.g., from food preservatives and intestinal flora. Lawson et al. (13) showed that a

![Fig. 1. Gas-chromatographic tracing of the organic acids from the specimen obtained 5 h after hospital admission](image)
small amount of benzoic acid and hippuric acid can be detected in the urine of some but not all normal individuals; however, the serum of our normal subject contained neither acid. Although we do not know whether benzoic and hippuric acids were normally present in our patient before toluene inhalation, it is unlikely that a high concentration of benzoic acid is normal for this individual.

In contrast to finding the toluene metabolite, we were unable to detect the presence of toluic and methylhippuric acids, metabolites of xylene (10). Because the original abused substance contained only a small quantity of xylene, perhaps these metabolites did not accumulate significantly for detection. Also, neither toluene nor xylene was detected in the serum sample in the analysis for volatiles. Possibly, by the time of blood sampling, these two compounds had cleared from the circulation. Finally, despite high concentrations of phthalate in the abused substance, this compound was not volatile enough to enter the patient's respiratory tract, and we did not expect to detect it.

Although much 3-hydroxybutyric acid was present in the serum specimens of our patient, acetoacetic acid was found only in the sample obtained 5 h after admission. Because this sample was analyzed after a brief storage at -20 °C, whereas the other two samples were analyzed after storage for six months, the acetoacetic acid may have deteriorated during the long storage. All original specimens were positive for ketone bodies, as indicated by the positive reaction with sodium nitroprusside: the sample taken at the time of admission, the 5-h samples diluted 16-fold, and the 12-h specimen diluted fourfold. Unfortunately, repeat examination of these samples six months later no longer gave positive results.

Alcoholic ketoacidosis (14), diabetic ketoacidosis (15), and extreme starvation (16) are clinical conditions that can lead to ketosis. Laboratory findings for this patient were not consistent with any of these conditions. Although the patient did admit to chronic alcohol use, her gamma-glutamyltransferase activity, a marker for chronic alcohol use (17), was only 2 U/L, below the reference range (5–24 U/L in our laboratory). In addition, results for her other liver enzymes do not suggest that she had alcoholic ketoacidosis. The patient did not appear to have diabetic ketoacidosis because her blood glucose was only 570 mg/L. Furthermore, her acidosis was corrected without insulin therapy. Although the patient was slightly hypoglycemic, extreme starvation was unlikely, because her concentrations of total protein, albumin, triglyceride, and cholesterol were within normal limits. Moreover, fasting for as long as five days would not suffice to increase the total ketone bodies to the concentrations observed in our patient on admission and 5 h later (16, 18). Fery and Balasse (18) showed that, after a 16-h fast, concentrations of acetoacetic and 3-hydroxybutyric acids in serum were 0.06 ± 0.01 and 0.14 ± 0.04 mmol/L (mean ± SE), respectively, in one group of six subjects, whereas in another group under similar conditions, the respective concentrations were 0.09 ± 0.01 and 0.22 ± 0.04 mmol/L. In subjects who fasted for five days, these concentrations were 1.15 ± 0.08 and 4.52 ± 0.19 mmol/L, respectively.

Judging by the low blood pH and serum bicarbonate, a positive ketone test, and a high anion gap in the serum, the patient was diagnosed to be in metabolic acidosis secondary to solvent abuse. Her high-anion-gap acidosis contained two components, benzoic acid and ketone bodies. According to Fischman and Oster (4), ketone bodies are not usually detected in urine or sera in toluene-induced high-anion-gap acidosis, especially with ketone concentrations of this magnitude. Ketone bodies are generated from metabolism of fatty acids (19), and we will not even speculate on how inhalation of toluene could lead to accelerated fatty acid metabolism.

In comparison with 3-hydroxybutyric acid, the concentration of acetoacetic acid in this patient was unusually high. Generally, in ketoacidosis, the concentration of 3-hydroxybutyric acid is higher than that of acetoacetic acid because acetoacetic acid is converted to 3-hydroxybutyric acid (20) in the presence of NADH. We do not know why a high concentration of acetoacetic acid was observed in this patient. Perhaps, during the detoxification of toluene, so much NADH was consumed that only a limited amount was available for the conversion of acetoacetic acid to 3-hydroxybutyric acid. Although the mixed-function oxidase that oxidizes toluene generally requires NADPH, not NADH, (21), Lehninger reported (22) that the following reaction can be catalyzed by pyridine nucleotide transhydrogenase (EC 1.6.1.1):

\[
\text{NADH} + \text{NADP}^+ \rightleftharpoons \text{NADPH} + \text{NAD}^+
\]

If this reaction does take place at a fast rate, NADH will not accumulate. However, we are not able to confirm whether this reaction had occurred in the present case.

In conclusion, the patient's clinical presentation and the clinical laboratory findings were indicative of acidosis. The GC-MS analysis showed that the acidosis was caused by ketone bodies and the metabolite of toluene. The presence of ketone bodies and a higher concentration of acetoacetic acid relative to 3-hydroxybutyric acid is an unusual feature in toluene-induced acidosis. The relationship between inhalation of toluene and production of ketone bodies is not clear. We have demonstrated the utility of GC-MS in a clinical situation and confirmed that benzoic acid is a metabolite of toluene metabolism in humans.

We thank the Analytical Chemistry Center at the University of Texas Medical School at Houston, Richard M. Caprioli, Director, for the GC-MS analyses.

References
2. Lowenstein LF. Recent research into glue sniffing—extent of the