Case Report

Analytical Findings in a Suicide Involving Sodium Azide

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Abstract

A 47-year-old laboratory assistant ingested approximately 9 g of sodium azide powder and died 4 h later at a hospital. A high-performance liquid chromatographic method using diode-array detection has been developed for the determination of an azide benzoyl derivative in blood (after a simple deproteinization) and in several tissues (after homogenization in a neutral buffer and deproteinization of the supernatant). The blood concentration in this case was lower than those previously published. The highest azide concentration was found in lung tissue. A complete toxicological screening revealed the presence of cyanide in blood, which has been previously reported twice, but for the first time, it was confirmed by mass spectrometry. Whether the production of cyanide in the presence of azide took place in vivo or postmortem remains unknown; the nature of the metabolic pathway involved also remains unknown.

Introduction

Sodium azide, a highly toxic chemical, is used in industry as an explosive, in cars as a safety bag (AIRBAG®) inflation reagent, and in chemical and biomedical laboratories as an antifungal and conservative for biological samples and diagnostic reagents. It has also been used as a potent hypotensive agent (1), but clinical trials in the 1950s were abandoned because of side effects.

Sodium azide normally occurs as a white powder, is highly soluble in water, and has a $pK_a$ of 4.6. In acidic solutions, it is rapidly converted to hydrazoic acid, a toxic and pungent gas. In humans, sodium azide is rapidly absorbed after ingestion (1), through the skin, and, together with hydrazoic acid, by inhalation. It has been shown in vitro, on rat liver mitochondria, that it acts by uncoupling mitochondrial oxidative phosphorylation by cytochrome oxidase inhibition (2). It also inhibits horse liver catalase (3). The LD$_{50}$ by the oral route in white mice is 27 mg/kg (4).

Case History

A 47-year-old male laboratory technician intentionally ingested approximately 9 g of sodium azide powder at 8 p.m. He was found several minutes later, vomiting and confused. When he arrived at the hospital emergency department at 9:30 p.m., he showed no other clinical signs. A gastric lavage was immediately performed, and cardiac and blood pressure monitoring were established. The patient quickly fell into a coma, and he showed signs of laryngeal dyspnea. At 10:15 p.m., bradycardia and a drop in blood pressure necessitated treatment with atropine and...
ephedrine. The patient was then intubated and mechanically ventilated. A few minutes later, electrical shocks and vaso-
pressive drugs (adrenaline and isoprenaline) had to be admin-
istered because of cardiac arrest. A continuous supraventricular arrhythmia followed for some minutes, and there were electrocardiographic signs of myocardial ischemia. A second heart arrest then occurred; resuscitation was ineffective. Death was declared at 11:45 p.m.

The autopsy was performed on the following day. A deep cyanosis of the face and the fingers and purple and bluish lividities on the body were noted. The lungs were congested, and pleura, like epicardium, presented petechia. The liver bled when sectioned. Meninges were congested, and the brain was very pale. Gastric contents and bile, kidney, liver, and brain samples were collected for toxicological analysis. Blood sampled during the sectioning of the thoracic wall, which probably came from congested intercostal veins (called thoracic blood), and from the suprahepatic veins (suprahepatic blood) was also collected for analysis.

Experimental

Analytical reagents. Sodium azide (99% pure) was purchased from Merck (Paris, France); HPLC-grade acetonitrile was obtained from Carlo-erba (Nanterre, France); benzoyl chloride was purchased from Sigma (St. Quentin Fallavier, France); tris-(hydroxymethyl)-methylamine (Tris) (99% pure) was obtained from Aldrich (St. Quentin Fallavier); and RP Normapur KH₂PO₄ was purchased from Prolabo (Fontenay-sous-Bois, France). A 1-g/L stock solution of sodium azide was prepared in deionized water and kept frozen. Working solutions were obtained by dilution of the stock solution in deionized water.

Procedure. Solid tissues were pretreated prior to liquid–liquid extraction as follows: 20 g of tissue was homogenized in 40 mL 1M Tris buffer, pH 7.4, then separated into two equal parts. To one part was added 10 mg of subtilisin, and hydrolysis was performed at 37°C for 2 h. Then, after centrifugation, 1 mL of the supernatant of each hydrolyzed and nonhydrolyzed tissue extract was further processed like liquid biological samples. In a 10-mL glass tube, 1 mL of liquid sample was deproteinized with 1 mL acetonitrile, shaken for 15 min on an oscillating agitator, and centrifuged at 900 × g for 5 min. The supernatant was transferred in another 10-mL glass tube containing 50 μL 1M Tris buffer, pH 4.6, and 50 μL of a 100-g/L benzoyl chloride methanolic solution. After shaking for 15 min, extracts were incubated at ambient temperature for 15 min. Fifty microliters was then injected into the chromatograph.

Chromatography. HPLC was performed using a reversed-phase isocratic method with a Waters 501 pumping system and a 7125 Rheodyne® injector equipped with a 20-μL loop. A column (150 × 4.6-mm i.d.; 5-μm particle size) (Waters S.A., St. Quentin en Yvelines, France) packed with Novapack C18 was used. The mobile phase was a 0.05M KH₂PO₄ Tris buffer (pH 4.6)–acetonitrile mixture (55:45, v/v). The analysis was performed at ambient temperature and at a flow rate of 1.1 mL/min.

![Figure 1.](https://academic.oup.com/jat/article-abstract/20/2/134/713430)

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**Figure 1.** (A) Chromatogram and (B) UV spectrum of sodium azide as benzoyl derivative, obtained from one-fifth diluted postmortem blood.
Detection. A Waters 991 diode-array detector (Waters S.A.) continuously recorded the ultraviolet (UV) spectrum from 200 to 400 nm. The spectrum of an eventual peak in the chromatogram of an unknown sample was statistically compared with the recorded spectrum of a derivatized azide standard. Chromatograms were represented at 252 nm, and benzoylazide (azide derivative) concentrations were computed, comparatively to a calibration graph, at the same wavelength.

Validations. To study linearity and reproducibility, test samples were prepared at 0, 500, 1000, 2000, and 5000 µg/L by adding 100 µL of an appropriate working solution to 900 µL of whole blood. Each of these standards was prepared, extracted, and analyzed daily for 5 days. Repeatability was assessed on 500-, 1000-, and 5000-µg/L standard concentrations by performing six different extractions and quantitations of a same sample test on the same day. All the calculations were performed from chromatographic peak areas. Percent recovery was directly obtained from these same experiments, as peak-area ratio between each extract and a respective nonextracted derivatized solution of sodium azide, corresponding to 100% extraction. Accuracy was evaluated by comparison of mean computed concentrations with target ones.

Drug screening. A screening on more than 400 drugs and toxicants in blood samples and gastric contents has been performed by means of a double detection method using HPLC–DAD and gas chromatography–mass spectrometry (GC–MS), after two separate nonselective liquid–solid extractions, for acidic and basic compounds, respectively. More sensitive chromatographic screenings have also been performed, using GC–MS after acetylation for neuroleptics; GC for ethanol and barbiturates; HPLC–DAD for alpidem, zolpidem, and zopiclone; and spectrophotometry for carboxyhemoglobin and methemoglobin. Cyanides have been assayed in blood by means of a colorimetric technique measuring cyanocobalamin formed from hydroxocobalamin (19). Confirmation was performed by a headspace GC–MS method, based on a previously published GC method with thermoionic detection (20). This previous method utilized blood cyanide conversion in an acidic medium to volatile hydrogen cyanide in a tightly closed vial. Then, hydrogen cyanide was immediately derivatized to cyanogen chloride in the headspace of the vial by reaction with chloramine T.

Results and Discussion

By using our present method, the chromatographic retention time of benzoylazide was approximately 10 min, and its UV spectrum showed a maximal absorbance at 252 nm (Figure 1). The limit of detection was slightly lower than 200 µg/L, as the signal-to-noise ratio measured for this concentration was better than 3. Within-day coefficient of variation (CV) values were better than 10 and 5% for the 500- and 5000-µg/L sodium

### Table I. Repeatability and Reproducibility of Sodium Azide Determination in Whole Blood

<table>
<thead>
<tr>
<th>Added concentration (µg/L)</th>
<th>Repeatability (n = 6)</th>
<th>Reproducibility (n = 5)</th>
<th>Calculated concentration (µg/L)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Repeatability (%)</td>
<td>Reproducibility (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>area [%]</td>
<td>CV [%]</td>
<td>area [%]</td>
<td>CV [%]</td>
</tr>
<tr>
<td>500</td>
<td>0.0180</td>
<td>6.95</td>
<td>0.0176</td>
<td>14.45</td>
</tr>
<tr>
<td>1000</td>
<td>0.0345</td>
<td>4.56</td>
<td>0.032</td>
<td>10.08</td>
</tr>
<tr>
<td>2000</td>
<td>-</td>
<td>-</td>
<td>0.0655</td>
<td>9.29</td>
</tr>
<tr>
<td>5000</td>
<td>0.1968</td>
<td>3.23</td>
<td>0.186</td>
<td>6.05</td>
</tr>
</tbody>
</table>

* CV = Coefficient of variation.

### Table II. Present and Previously Published Sodium Azide Concentrations in Postmortem Samples

<table>
<thead>
<tr>
<th>Study</th>
<th>Gastric contents (µg/g)</th>
<th>Intestinal contents (µg/g)</th>
<th>Blood (µg/L)</th>
<th>Bile (µg/L)</th>
<th>Liver (µg/L)</th>
<th>Kidney (µg/L)</th>
<th>Brain (µg/g)</th>
<th>Lung (µg/g)</th>
<th>Urine (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kozlicka-Cajdzińska et al. (1966)</td>
<td>867</td>
<td>429</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>NF*</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Klug et al. (1987)</td>
<td>58,900</td>
<td>7360*</td>
<td>85</td>
<td>–</td>
<td>6.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Peclet et al. (1991)</td>
<td>8.8*</td>
<td>–</td>
<td>40</td>
<td>–</td>
<td>NF</td>
<td>–</td>
<td>–</td>
<td>NF*</td>
<td>–</td>
</tr>
<tr>
<td>Lambert et al. (1995)</td>
<td>754</td>
<td>–</td>
<td>262</td>
<td>1283</td>
<td>14</td>
<td>205</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Present study</td>
<td>1036</td>
<td>–</td>
<td>7.45, 8.3*</td>
<td>21.4</td>
<td>NF (&lt;0.4)</td>
<td>NF (&lt;0.4)</td>
<td>2.7</td>
<td>17.6</td>
<td>–</td>
</tr>
</tbody>
</table>

* NF = Not found.
* This value was found in the duodenum; no azide was found in the jejunum.
* Total content value given in grams.
* Value found in thoracic blood.
* Value found in suprahepatic blood.
azide concentrations, respectively. Interday CV values were less than 15 and 10% for the same respective concentrations (Table I). Accuracy, based on the interday results, showed a 19% overestimation of the 500-μg/L theoretical concentrations. On the other hand, accuracy was very good for the 1000-μg/L target concentration. This could be due in part to the moderately increased extraction recovery at 500 μg/L (Table I), which directly affects the calculated concentration and is probably caused by the lack of a chemical analogous as internal standard. For this reason, the limit of quantitation retained in this study was 500 μg/L, although repeatability and reproducibility were still satisfactory below this concentration.

Regression on 20 points (five concentrations from 500 to 5000 μg/L, four determinations per concentration) was excellent ($r = 0.9937$, $p < .001$), and nonlinearity, assessed by ANOVA, was insignificant. Linearity has even been verified from 500 up to 50,000 μg/L on two different days (seven concentrations, one determination per concentration); correlation coefficients were 1.0000 and 0.9998, respectively.

The lack of an internal standard was due to the difficulty of rapidly finding a chemical analogue of azide, as these forensic results could not be delayed. Nevertheless, on the condition of the above range limitation and accurate filling of the injector loop, the present method is precise, reproducible, and accurate.

Sodium azide concentrations were measured in gastric contents, thoracic blood, suprahepatic blood, bile, and various tissues (Table II). Most of these concentrations were greater than the calibration range of the method, so biological liquids or supernatants of ground tissues had to be diluted before a new extraction. Enzymatic hydrolysis of ground tissues did not improve azide recovery.

We could find only four papers presenting postmortem azide concentrations in the digestive tract, blood, or tissues. These values are also represented in Table II. The large concentration variability between studies is not directly representative of the actual ingested doses, as some subjects had vomited or been lavaged or both after ingestion. Also, the time between ingestion and death was not always known.

Blood concentrations varied from 8 to 262 mg/L, the lowest value being ours and the highest having been obtained by Lambert et al. (16) with a very similar analytical method.

Unfortunately, clinical signs and time between ingestion and death were unknown in the latter case.

We failed to detect any azide in the liver and kidneys, notwithstanding extractions at different pH values, with or without enzymatic hydrolysis; this was opposed to a high concentration found in the bile. The absence of azide in the liver was confirmed by two of the other four studies (5,15), whereas low concentrations were found in the two studies that reported the highest blood concentrations (8,16). Only Lambert et al. (16) determined sodium azide in the kidneys, at a concentration almost as high as blood concentrations (205 μg/g and 262 μg/mL, respectively), but as in our case, no urine could be sampled. Peclet and Ponton (15), who reported a fairly high blood concentration (40 mg/L), found no azide in urine, but they used direct spectrophotometry, which has a low sensitivity.

We reported here for the first time azide concentrations in the brain and lung. The former was low, and it was noticeable that the latter was higher than the concentration in blood or in other tissues. It suggested that azide is either preferentially bound to lung tissue or preferentially excreted in expired gas as hydrazoic acid.

Among the other drugs or toxicants analyzed, the only findings were ethanol in gastric contents (0.79 g/L), probably by postmortem production as it was absent in blood, and cyanide in blood (0.38 mg/L). The presence of cyanide was confirmed by GC-MS; the single-ion chromatogram (m/z 61) of CNCI is shown in Figure 2. This finding has been reported twice before in the literature: the cyanide concentration was 1.6 mg/L (unknown method) in the plasma of a male who had ingested 1.2-2 g of sodium azide (11) and 9 mg/L (Koenig's colorimetric method) in the blood of a female who had ingested an unknown but high dose, as suggested by the obvious rapidity of death (16). Whether the production of cyanide in the presence of azide took place in vivo or postmortem remains unknown, as well as the nature of the metabolic pathway involved.

Reported deaths with sodium azide were usually related to voluntary ingestion of the equivalent of 1.5-20 g of the powder form, when it was known or could be evaluated. Delay before death ranged from 40 min (5) to 3½ days (12), during which neurological signs (agitation alternating with coma), dyspnea, lactic acidosis, and cardiac rhythm disorders (resistive to most treatments) were always found. Clinically, this case was thus very similar to these reports. Moreover, the 4-h delay before death, the vomiting, and the gastric lavage were in agreement with the low azide concentrations in blood and tissues as compared with the few other documented studies.

References


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