Acute Nitrobenzene Poisoning with Severe Associated Methemoglobinemia: Identification in Whole Blood by GC-FID and GC-MS

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Abstract

A rare fatal case of self-poisoning with nitrobenzene following oral ingestion is reported. On presentation to the hospital, severe methemoglobinemia (70%) was observed in an 82-year-old male who had ingested 250 mL of an unknown substance in the previous 24 h. Methylene blue and exchange transfusion were the therapeutic methods applied in the treatment of the methemoglobinemia. Forty-eight hours after ingestion, a blood sample was collected in ICU and sent to our laboratory. We detected that the blood contained 3.2 µg/mL of nitrobenzene. The determination of nitrobenzene was performed using the combination of GC-FID for screening analysis and quantitation and GC-MS for confirmation of the obtained results.

Introduction

Nitrobenzene (NB), also known as nitrobenzol, mirbane oil, or essence of mirbane, is a pale yellow oily liquid with an odor of bitter almonds. NB has a melting point of 5.85°C and a boiling point of 210.9°C. It is slightly soluble in water, readily soluble in organic solvents such as alcohol, ether, and benzene, and very soluble in lipids. It was first synthesized in 1834 by reacting benzene and fuming nitric acid and was made commercially in England by 1856. It serves as an intermediate in the synthesis of aniline dyes, a solvent for the manufacture of cellulose ethers and acetate, a flavoring agent, a perfume for soap, and in the rubber industry (1).

NB can be absorbed by humans following oral, inhalation, or dermal exposure. When introduced into the blood, it is metabolized by reduction to aniline, which induces methemoglobinemia (MetHb). NB oxidizes the iron in hemoglobin to form methemoglobin, this reduces the oxygen carrying capacity of the blood and also impairs oxygen delivery to the tissues. Acute oral exposure of NB has resulted in MetHb, cyanosis, anemia, and neurological effects including headache, nausea, vertigo, confusion, unconsciousness, apnea, coma, and death (2). Signs and symptoms may be delayed several hours because some chemicals do not directly produce MetHb, but require biochemical transformation to toxic metabolites.

MetHb imparts a chocolate hue to the blood. The diagnosis should be suspected when a blood sample is brown colored and does not redden on exposure to air. The aetiology of MetHb may be congenital because of deficiency in cythocrome b5 reductase and structural abnormalities in the hemoglobin molecule or could be acquired as the result of the oxidant stress from various drugs or chemicals, most commonly nitrates or nitrates (3). Concentrations of methemoglobin up to 20% are generally well tolerated, although values above 40% are associated with cardiorespiratory symptoms. Life threatening MetHb is rare and is usually the result of acute poisoning by oxidizing compounds (4). Lethal concentrations of methemoglobin are probably greater than 70%.

Numerous cases of acute nonfatal and fatal poisoning because of the ingestion or dermal absorption of NB have been reported in the scientific literature of the 1970s, or earlier (5-9) at a time when the chemical was a common constituent of soaps, shoe dyes, inks, and other household products (10). More recently, one case has been reported in Germany (11) and three in India (12-14). However, NB poisoning is an uncommon cause of death nowadays in occidental countries. We report a severe MetHb case after an acute ingestion of NB. The substance was analyzed in the blood sample by gas chromatography with flame ionization detector (GC–FID) and gas chromatography–mass spectrometry (GC–MS), following a toxicological screening procedure used routinely in our laboratory. The analytical method was validated and described in this paper.

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Case History

A telephone consult was made to our Poison Control Center (PCC) from the Intensive Care Unit (ICU) of Móstoles Hospital (Madrid, Spain) concerning an 82-year-old male who had intentionally ingested, in the previous 24 h, approximately 250 mL of a substance that the family alleged to be “White Spirit”. The ingestion occurred in a mechanical garage owned by the patient’s son, and the container was unlabeled. At admission into the hospital, the patient had, as the main clinical features, a metabolic coma of toxic origin (possibly autolytic), a MetHb of 70%, and anuric renal failure. He also suffered severe cyanosis mainly in acra areas, mild hypotension, and Glasgow coma score 6. He was admitted into the ICU and was intubated under mechanical ventilation. Computed Axial Tomography was consistent with hypoxic isquemic encephalopathy. The chest radiograph showed a pattern consistent with bronchoaspiration. He was treated with oxygen 100%, activated charcoal, and methylene blue, as well as exchange transfusion. Significant blood parameters revealed: pH, 7.36; pCO₂, 27 mmHg; pO₂, 138 mmHg; bicarbonate, 15.7 mEq/L; leukocytes, 12,920 cells/mL; hemoglobin, 12 g/dL; hematocrit, 39%; glucose, 174 mg/dL; urea, 49 mg/dL; creatinine, 13.8 mg/dL; sodium, 136 mEq/L; potassium, 4.2 mEq/L; and CPK, 83 μg/mL.

Because the clinical features did not correspond to a case of poisoning with “White Spirit”, a differential diagnosis was made in our PCC with acquired causes of MetHb, and a toxicological screening was suggested. In ICU, a nasogastric tube was introduced and a thick liquid with solvent odour was extracted. A blood sample was taken and sent for analysis to the National Institute of Toxicology with instructions to investigate the presence of solvents.

The patient had hemodynamic instability refractory to intravenous liquids and vasopressor agents (dopamine and noradrenaline). Shock and renal failure persisted, so continuous venovenous hemofiltration was initiated. In spite of methylene blue administration, MetHb persisted at 30%. Therefore, exchange transfusion was made with reduction to 18%. However, refractory shock with asystolia and death occurred three days after admission.

Experimental

Materials

Sodium sulfate, diethyl ether, and methanol of analytical grade were obtained from Scharlau (Barcelona, Spain). The NB and N-octylbenzene (internal standard, IS) were purchased from Fluka-Sigma Aldrich (Buch, Switzerland). Stock solutions (1 mg/mL) were prepared by dissolving the appropriate amount of each substance in methanol. These stock solutions were stored in glass tubes at −25°C.

In order to perform the validation of the method, a pool of citrated human whole blood samples provided by Hospital 12 de Octubre (Madrid, Spain), were tested to verify the possibility for use as blanks. No interferences were found for the studied compound and the samples were kept frozen at −25°C until used.

Instrumentation

A P-Selecta Centric S centrifuge was obtained from Selecta (Barcelona, Spain). GC–flame ionization detection (FID) analysis of the extracts was performed on a model 5890 Series II gas chromatograph equipped with a flame ionization detector, an autosampler 7890A, and linked to a 3396A integrator (all from Hewlett-Packard, Avondale, PA).

A 25-m × 0.20-mm i.d. fused-silica capillary column coated with cross-linked dimethylsilicone (0.1-μm film thickness) (Hewlett-Packard) was employed. The carrier gas was helium (Air Liquid, Madrid, Spain) delivered at a column head pressure of 22 psi. The injection port and detector temperatures were 280°C and 300°C, respectively. The split ratio was 1:24. The column temperature was initially held at 40°C for 3 min and then increased to 280°C at 10°C/min. The total chromatographic time, including 2 min of equilibration time, was 29 min. The detector gases were hydrogen and air (Air Liquid), delivered at flow rates of 40 and 400 mL/min, respectively. Insert liners silanized with dimethylchlororosilane (Supelco) and packed with Supelco silanized glass wool (Bellefonte, PA) were used.

GC–MS analysis was performed on a model 5791 mass-selective detector linked to an MS Chemstation Series II (all from Hewlett-Packard). The autosampler, gas chromatograph, and column were as mentioned above. The MS was operated in the electron impact mode using an electron energy of 70 eV and a full scan mass-to-charge (m/z) ratio range of 35–650 amu. Transfer line and ion-source temperatures were both maintained at 280°C. The presence of NB was confirmed using identical chromatographic conditions.

Sample preparation and extraction

Liquid–liquid extraction was performed in a 10-mL screw-capped glass tube by adding 1 mL of diethyl ether (cold at 4°C) and 15 mg of anhydrous sodium sulfate to 3 mL of whole blood added with 100 μL IS solution (N-octylbenzene methanolic solution of 100 μg/mL), vortex mixed for 3 min, centrifuged at 4000 rpm for 10 min, and cold after being centrifuged at 4°C. The organic layer was then collected and transferred to a gas vial and 3 μL were injected first for GC–FID screening analysis and later for GC–MS confirmation of the obtained results.

Validation of the method

The method described here was validated by adding NB to human whole blood (100 mL) to achieve 0.1, 0.3, 1, 3, and 5 μg/mL. The blood was sonicated for 5 min at room temperature then submitted to liquid–liquid extraction using the extraction procedure described previously and quantitated by GC–FID.

Calibration curves were prepared with standard solutions of NB in diethyl ether. The concentrations were 0.3, 1, 5, 10, and 20 μg/mL and the concentration of the IS was fixed at 10 μg/mL. NB to IS area ratios were measured, and the calibration curves were generated from least-squares linear regression. The regression lines were used to calculate the absolute recoveries (n = 6) of NB from spiked blood at five concentration levels.
The intra-assay precision was assessed at five concentration levels by the extraction and analysis on the same day of six spiked blood samples for each level. The interassay precision was assessed by analyzing, on two different days, a set of ten spiked blood samples at each concentration.

The limit of detection (LOD) and limit of quantitation (LOQ) were determined as the lowest concentration, giving a response of three- and ten-times, respectively, the average of the baseline noise defined from six control samples.

The linearity of the method for NB was checked by preparing six replicates of the calibration curves at five different concentrations ranging from 100–5000 ng/mL, by the addition of known amounts of NB to human whole blood.

The sensitivity of GC–MS as a confirmation technique was also assessed by analyzing (n = 3) the lowest NB blood spiked levels (0.1, 0.3, and 1 µg/mL), in the total ion chromatogram (TIC) and selected-ion monitoring (SIM) modes.

Results and Discussion

Assay characteristics

A comprehensive toxicological screening for solvents was performed in the blood sample. This included ethanol and volatile analysis by headspace GC–FID. The blood GC–FID screening analysis using the described method revealed the presence of 3.2 µg/mL of NB. This quantitation was performed using a blood calibration curve in the range of 0.1–5.0 µg/mL. During the development of the GC–FID analysis, propofol, an anesthetic and hypnotic administrated in the hospital, was also detected. The presence of other hydrocarbons and volatile solvents were discarded. The obtained results were confirmed by GC–MS analysis in TIC mode. Figures 1 and 2 show the GC–FID chromatogram and a zoom of the TIC obtained in the GC–MS analysis of the patient’s blood, respectively. Figures 3 and 4 show the GC–MS mass spectrum of analyte peaks eluting at 8.21 and 12.80 min and the resulting match of the computerized library searches, respectively.

The results of the validation of the GC–FID analytical method for NB detection are shown in Table I. No interferences were found, and the time for the chromatographic analysis was 27 min for one sample. Recoveries in the studied range were more than 93.0%, with intra- and interassay precisions of less than 8.4% and 12.0%, respectively. The LOD and LOQ were 79 ng/mL and 263 ng/mL, respectively. An excellent linearity (r² =

Figure 1. GC-FID chromatogram of the patient’s blood obtained at the hospital 48 h after the ingestion of a commercial product. Detected are: 1 = NB, 2 = propofol, 3 = IS, 4 = ionol, and 5 and 6 = fatty acids.

Figure 2. Zoom of the TIC in GC–MS analysis of the patient’s blood. 1 = NB, 2 = propofol, 3 = IS, 4 = ionol.

Figure 3. GC–MS mass spectrum of analyte eluting at 8.21 min (A) and nitrobenzene from data system reference library (B).
0.999) was observed from LOQ up to 5.0 μg/mL. The sensitivity study of GC–MS as confirmation technique should be confirmed in GC-MS, using SIM mode. The remainder concentrations studied could be confirmed using TIC mode.

For toxicological screening in our method, N-octylbenzene was chosen as the IS because it is not present in commercial products, as far as we know. Its chemical structure makes it suitable for use as an IS of a wide variety of hydrocarbon compounds (Figure 5). According to our toxicological analytical experience, it has a very good extraction behavior [the recovery obtained for this standard was 103% with a relative standard deviation (RSD) of 5%].

As far as we know, there are very few reports describing analytical methods for NB detection in biological specimens (11,23). The methods that have been developed to test NB in biological samples such as blood or gastric content are just briefly described, and no validation data has been reported; Ewert et al. (11) used dichloromethane and Shormakov et al. (23) used toluene as the extraction solvent.

**Poisoning characteristics**

This is an unusual case of voluntary ingestion of a substance that had MetHb as the main clinical manifestation. Relevant features of the case are the uncertainty of the etiologic agent and the erroneous information given by the patient’s relatives. The product mentioned as the cause of the poisoning was “White Spirit”, which is actually a mixture of aromatic hydrocarbons with boiling points in the range of 150–200°C and sold as a substitute of turpentine. They have not been described as a cause of MetHb, therefore nitrated organic solvents were suspected by our PCC.

From 1991 to 2000, Spanish PCC reported 5000 exposures to products able to produce MetHb. According to our experience, when this sign was already present, causes of poisoning were aniline dye derivatives (present in shoe or clothes dyes and inks), phenols, creosotes, nitrates (such as fertilizers and nitrates), and mothballs. The main routes of exposure were respiratory and cutaneous, and intoxication affected mainly workers. Other less common etiology of MetHb, although accidents are frequently reported, include an artificial fingernail remover (15) and herbicides (monolinuron, metobromuron) (16,17), even diesel fuel additives. MetHb has also been described after dermal contact with an octane booster containing 80% aniline and 20% toluene (18). Etiology of poisoning with all these products is mainly accidental, but suicidal cases are also described (9,11,17).

Nitro and amino derivatives of aromatic hydrocarbons are used in the chemical industry in synthetic dyes, but significant acute damage because of them is rare in Spain. In our country, NB or its derivatives are present at low concentration in household products (metal and furniture polish) and at higher concentrations in industrial products. NB is also used illegally to improve combustion in car races; this could be the reason for its presence in a mechanical garage. Only two other cases of NB poisoning in adults had been reported to our PCC in the past 10 years. Routes of exposures were inhalation of vapor and dermal and ocular contact with vapor and liquid. They occurred in the workplace (in a printmaking industry) and in the University (a laboratory in the chemistry department).

The metabolism route of NB is oxidation to p-nitrophenol and reduction to aniline, which is further oxidized to p-aminophenol. Nitrosobenzene and phenylhydroxylamine, highly toxic compounds, are believed to be produced as intermediates in the reduction of nitrobenzene to aniline, which oxidizes hemoglobin to methemoglobin. NB and its intermediary prod-
ucts are eliminated via the lungs and kidneys partly unchanged and partly after reduction and conjugation (19). Only 13–16% of a dose is excreted in the urine as p-nitrophenol, and probably less than 10% as p-aminophenol. Both substances are eliminated in the form of sulfate or glucuronide conjugates. The half-life of these substances is between 2 and 20 days, which explains the duration of MetHb (20) and the possibility of analytical detection after 48 h of ingestion. Unfortunately, no other samples such as urine or gastric contents were submitted for toxicological analysis, so a distribution study was not possible. Our patient suffered a severe poisoning with progressive deterioration leading to fatality. Contributing factors could be the delay in hospital arrival and the old age, because elders are at greater risk of developing MetHb. On the other hand, NB is slowly eliminated in the presence of renal failure causing prolonged MetHb. There could also be a delayed release of NB from stores in the adipose tissue and gastrointestinal tract, because of its high liposolubility. Formation of MetHb by NB occurs more slowly than with aniline, but cyanosis is more persistent. However this analytical method was also validated for aniline (LOD =107 ng/mL), this compound was not detected in the blood sample. Methylene blue is recommended for reversal of the often severe MetHb that results from nitrobenzene exposure (5,21,22). There is some evidence to suggest that exchange transfusion may be beneficial when methylene blue is ineffective. However, in spite of all therapeutic interventions, the patient died four days after exposure.

Conclusions

A rare and fatal case of self-poisoning with methemoglobinemia after the ingestion of NB is reported. Clues for diagnosis were the history of chemical ingestion at a garage and the clinical characteristics. NB is a substance rarely present in commercial products in our country. Therefore, a general toxicology screening for substances causing MetHb was undertaken. The toxin was rapidly identified and quantitated by a combination of GC–FID/GC–MS screening method in daily use in our laboratory. To date, this is the first case of NB poisoning for which the analytical data are described and the analytical method was also described and validated. These results complete the previous data on NB in humans and highlight the importance of an accurate analytical identification for a more comprehensive understanding of unusual compounds involved in poisonings in occidental countries.

References


