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CASE REPORT

T. Saito · S. Takeichi · M. Osawa · N. Yukawa
X.-L. Huang

A case of fatal methemoglobinemia of unknown origin but presumably due to ingestion of nitrate

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Abstract A case of fatal methemoglobinemia (MetHb-emia) which was presumably due to ingestion of nitrate is presented. An unidentified man was taken to a local emergency hospital suffering from deep cyanosis and 7 h later he was found dead in the hospital bed. The post-mortem examination of the blood revealed a methemoglobin (MetHb) concentration of 78% and the concentrations of nitrate and nitrite were 1.50 and 0.76 μg/mL, respectively. Capillary gas chromatography coupled to mass spectrometry (GC-MS) and capillary gas chromatography with a nitrogen-phosphorus detector (NPD) were used to detect nitrates and nitrites in the blood.

Key words Nitrates · Nitrites · Methemoglobinemia · GC-NPD

Introduction

Methemoglobin (MetHb) is an oxidation by-product of hemoglobin, in which the sixth coordination position of ferric iron is bound to a water molecule or to a hydroxyl group. MetHb cannot serve as an oxygen carrier, therefore high MetHb concentrations bring about severe tissue hypoxia. MetHb-emia can be congenital or acquired. Acquired MetHb-emia is caused by various mild oxidizing agents and most cases of fatal MetHb-emia are induced as accidental poisoning, which is usually the result of ingestion of water containing nitrates, and food containing nitrites. Nitrites can directly oxidize hemoglobin to MetHb and MetHb-emia typically develops within 1 h after sodium nitrite ingestion [1, 2]. In contrast, nitrates cannot directly oxidize hemoglobin, however, the ingestion of water with a high nitrate concentration may also result in MetHb-emia due to conversion of nitrate to nitrite by intestinal bacteria. The conversion proceeds slowly to MetHb-emia [3], therefore, the rate of MetHb production by nitrates is slower when compared to nitrites.

Acute poisoning by nitrates is infrequent, but potentially severe and there is a lack of reports in the literature. We present a case of a hospitalized man who died from severe MetHb and to the best of our knowledge, this is the first report of toxicological data of nitrates poisoning in a forensic case. Nitrates and nitrites were identified and quantified using GC-MS and GC-NPD, respectively. This is a very rare case of intoxication such as intravenous injection of India ink into the vein [4] and accidental hydrogen sulfide poisoning [5]. Little is known of the underlying mechanism of this type of intoxication which could be important for the forensic toxicologist or emergency physician.

T. Saito (✉) · S. Takeichi · M. Osawa · N. Yukawa
X.-L. Huang
Department of Forensic Medicine,
Tokai University School of Medicine, Bohseidai,
Isehara, Kanagawa 259-1193 Japan
Fax +81-463-92-0284

Case history

An unidentified man about 70 years of age was admitted to a local emergency hospital. He presented with deep cyanosis and associated dyspnoea and on arrival, he was drowsy but had a normal EEG. The patient was hospitalized under a diagnosis of dehydration. On admission, no treatment for MetHb-emia was administered and the next morning he was found dead in the hospital bed. No information on the medical or drug history of the patient were available. At autopsy, no particular morphological changes were noted except that the blood was tinted a chocolate brown color. Samples of cardiac blood and gastric contents were collected and stored at –80 °C until toxicological analysis.

Materials and methods

Reagents

3,4-Dimethylnitrobenzene (4-nitro-o-xylene, 4NOX) and 2-nitromesitylene were purchased from Aldrich (Milwaukee, Wisc.). All other chemicals and reagents were of analytical grade (Wako, Osaka, Japan).

MetHb assay

MetHb assay of a blood sample was performed by the spectrophotometric method described by Rodkey et al. [6].

Extraction procedure

Nitrate

In this study, a previously published method for the analysis of nitrates in urine [7] was modified for blood and gastric contents. Blood and gastric contents (0.2 mL) were pipetted into a 1.5 mL polypropylene centrifuge tube with an attached cap, followed by 20 µL of a 1 mg/mL acetone solution of 4NOX (internal standard) (I.S.) and 0.2 mL saturated silver acetate solution. After mixing, using a vortex (2 min) and centrifugation (5 min at 3000 rpm), 0.2 mL of the supernatant was transferred to another 1.5 mL polypropylene centrifuge tube. After the addition of 0.5 mL of concentrated sulfuric acid and 0.5 mL of mesitylene, the tube was capped and mixed by vortexing for 2 min. The tube was centrifuged and the upper layer was transferred to another 1.5 mL polypropylene centrifuge tube. Approximately 20 mg of anhydrous Na_2CO_3 was added to the mesitylene, which was then mixed by vortex and centrifuged for 2 min. A 1µL volume of the mesitylene solution was injected directly into the GC column.

Nitrite

The analysis of nitrite was performed using an oxidant to convert NO_2^- to NO_3^-. The difference in concentration between nitrate and total oxidized nitrate would appear as a nitrate concentration. Oxidation was performed by the use of a 0.01 N H_2O_2 solution.

Briefly, blood and gastric contents (0.2 mL) were pipetted into a 1.5 mL polypropylene centrifuge tube with an attached cap, followed by 70 µL 0.01 N hydrogen peroxide solution. The subsequent procedures which were carried out were identical to those performed with nitrate.

Demonstration

A model 5890 Series II Hewlett Packard GC coupled with a model 5971 mass spectrometer was used. Splitless injection was employed (split value off-time of 1 min) and the mass selective detector was used at 70 eV. The temperatures of the injector port and transfer line were 250 °C and 280 °C, respectively. A 30 m × 0.25 mm fused-silica capillary column DB-1 (dimethyl polysiloxane, 0.25 µm film) (J&W Scientific, Folson, Calif.) was used. The column oven temperature was programmed to rise from an initial temperature of 100 °C to 130 °C at 5 °C/min and kept at 130 °C for the final 3 min.

Quantitation

The GC system consisted of a GC (Hewlett Packard 5890 Series II) and a NPD detector. The injection and detector temperatures were 250 °C and 270 °C, respectively. A DB-1 wide-bore capillary column (dimethyl polysiloxane; 15 m × 0.53-mm internal diameter, 1.5 µm film) (J&W Scientific, Folson, Calif.) was used. The column oven temperature was programmed to rise from an initial temperature of 100 °C to 125 °C at 5 °C/min and was maintained at 125 °C for the final 2 min.

Quantification of nitrate and nitrite was done by plotting peak-area ratios (drug-I.S.) against the concentration of standards to produce standard curves and the results of the case samples were compared with the calibration plot.

Results and discussion

The MetHb concentration in the blood of the victim was 78%, which was higher than the previously reported range of fatal concentrations [8, 9] which were 75, 80 and 76%. The preliminary drug screening of blood and gastric contents performed with GC-MS, was negative for aniline, nitrobenzene and acetaminophen, but clearly demonstrated the presence of nitrates and nitrites. Nitrates and nitrites were demonstrated after the mesitylene derivative 2-nitromesitylene which was derived by condensation of mesitylene with NO_3^- (Fig. 1). Mass spectral analysis of 2-nitromesitylene revealed the prominent ions m/z 91, 148 and 165 (Fig. 2). Figure 3 shows a GC-NPD chromatogram of 2-nitromesitylene (nitrate) and the internal standard. Quantification by GC-NPD was made because this was found to give a better correlation with the drug concentrations and limit of detection than GC-electron impact MS. The concentrations found in the blood and gastric contents are presented in Table 1 and the blood nitrite level was above the range of fatal concentrations of 0.55 and 1.0 µg/mL previously reported [1, 9]. In the present case, the high nitrate concentration found in the stomach indicated oral ingestion.

Nitrites and nitrates have been determined in blood samples by GC-MS [10, 11], high-performance liquid chro-

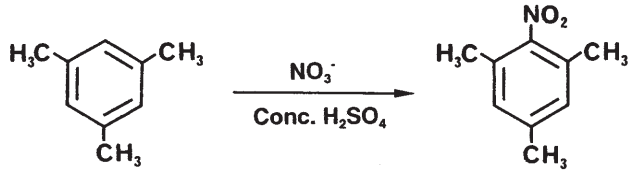

Fig. 1 The condensation of mesitylene to 2-nitromesitylene with NO_3^-

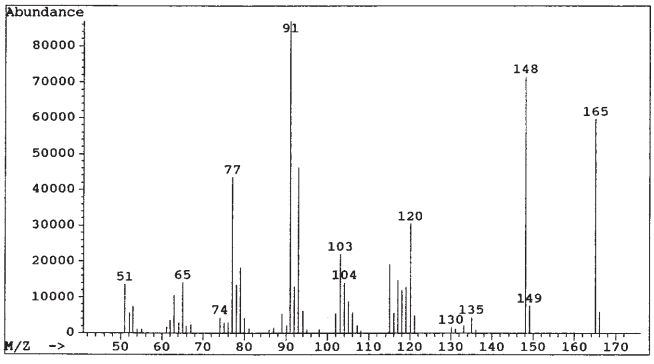

Fig. 2 Electron impact mass spectrum of 2-nitromesitylene

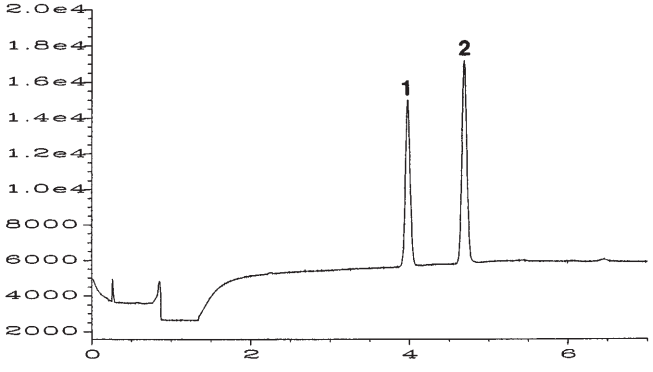

Fig. 3 GC-NPD chromatogram of the oxidized extracts from the blood sample. Peak 1, 2-nitromesitylene (nitrate); Peak 2, 3, 4-dimethylnitrobenzene (I.S.)

Table 1 Nitrate and nitrite concentration in blood and gastric contents from the victim

Concentration (μg/mL)	Nitrite	Nitrate
Blood	0.76	1.5
Gastric contents	N.D.	20.3

N.D. = not detected

matography system with a nitrite-Griess reagent reaction [12, 13] and ion chromatography [14]. The method for nitrate derivatives by GC-negative ion chemical ionization (NICI) [10, 11] is more sensitive, but too demanding for routine screening tests whereas the extraction method used in this study is simple and rapid. For the analysis of the GC-NPD, calibration curves in the range 0.3–5.0 μg/mL were found to be linear for nitrate and nitrite. Correlation coefficients were r^2 of 0.998 for nitrate and r^2 of 0.997 for nitrite and the limit of quantitation of the assay was 300 ng/mL for nitrates and nitrite. Recovery was determined at three concentrations (0.5, 1.0 and 10.0 μg/mL nitrate) by comparison with extracted samples (as 2-nitromesitylene) and pure standard and the mean recovery was 78%. The values for intra-assay accuracy and precision were determined from the analysis of five measurements at each concentration (0.5, 1.0 and 10.0 μg/mL nitrate) within a single analysis batch. Interassay values were determined from seven analytical runs performed on separate days. Coefficients of variation for both intra- and interassay precision experiments were less than 8%. Moreover, the present method using GC-NPD is useful in forensic toxicology as evidenced in an actual case.

Usually, blood samples left at room temperature for more than 4 h may show oxidation of nitrite to nitrate [13]. In the case of MetHb-emia, MetHb concentrations of the blood were stable at –80 °C or –196 °C [15] therefore our samples were stored at –80 °C.

Serious MetHb-emia caused by nitrate is not rare, but fatalities seem to be exceptional and between 1945 and 1970, about 160 deaths were described in the literature [16]. However, recent reports of fatal MetHb-emia due to nitrates are rare and for this reason the diagnosis and treatment of MetHb-emia are not well known. The diagnosis of MetHb-emia is suspected when a patient is cyanosed and does not respond to oxygen therapy but has no significant cardiopulmonary disease.

In general, the normal MetHb level is less than 1% and levels greater than 70% are usually lethal, although one survival has been reported with a MetHb level of 75% [17]. In acquired MetHb-emia, the rate of hemoglobin oxidation is accelerated, surpassing the reducing capacity of the erythrocytes [18]. If the diagnosis is delayed, nitrates may cause MetHb-emia that rapidly leads to death. Therefore, a rapid and sensitive assay for MetHb-emia is essential, especially in an emergency and in cases of unexplained cyanosis.

Carlson and Shapiro [19] have described a case of MetHb-emia from water contaminated by soil nitrates, where methylene blue was used to treat the patient and the reduction of MetHb to hemoglobin was complete within 30 min. In the present case, no tests were performed for abnormal hemoglobin or glucose-6-phosphate dehydrogenase levels. Thus the possibility of an endogenous MetHb-emia remains uncertain. Although toxicological data on nitrite fatalities have only been rarely reported, death is generally associated with blood concentrations > 0.55 μg/mL [1, 7] and it is obvious that a blood nitrite concentration of 0.76 μg/mL is substantially above the toxic concentration.

In the present case, despite the confirmation of MetHb-emia due to nitrates, the circumstances of the death are still obscure. However the intake of well water or fertilizer containing nitrate could have caused the MetHb that led to death. Death might have been avoided if the patient had been treated with methylene blue but the attending physician was not aware of the MetHb-emia. Every physician should therefore be aware of these facts and patients should be transported to hospitals capable of treating these conditions.

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