A case of fatal 2,4-dinitrophenol (2,4-DNP) intoxication with delayed onset methaemoglobinaemia

Sir,

2,4-dinitrophenol (2,4-DNP) is an industrial chemical with a range of commercial applications, including use as a precursor for the manufacture of dyes and explosives, as a wood preservative, and as a herbicide and insecticide.[1] The chemical was largely withdrawn from commercial use in the 1980s because of teratogenic concerns. There was interest in the drug in the 1930s because of weight loss effects; however, side-effects, mainly in the form of high fever, diaphoresis, nausea, vomiting and diarrhoea, peripheral neuropathy, cataracts, and rarely death, resulted in its withdrawal from the market 5 years later.[2]

Since the early 2010s, the drug has been marketed on the internet under a variety of brand names as a weight loss agent and body building supplement, and a number of deaths associated with its use or accidental exposure have subsequently been reported.[3]

The patient was a young adult male who together with a number of other persons ingested capsules thought to be 'ecstasy' or MDMA (methylenedioxymethamphetamine) together with a considerable quantity of alcohol. Around 6 hours later, the patient commenced vomiting and an ambulance was called; other persons present did not exhibit obvious toxic effects. Paramedics found the patient to be diaphoretic, anxious and agitated. He had a GCS of 15, sinus tachycardia (pulse 150), normal blood pressure and a respiratory rate of 42. His pupils were equal and reactive. Tympanic temperature was 36.0°C.

On arrival at a regional hospital, the patient had a GCS of 4, was agitated but not combative, not responding to verbal commands, and he had developed bilateral pinpoint pupils. His respiratory rate was 40−45, pulse 175−180 and he had a systolic blood pressure peaking at 170 mmHg. Oxygen saturation was 96% in room air. With the exception of an elevated serum lactate concentration of 5.1 mmol/L, initial blood gas and serum electrolyte, glucose, renal function and liver function values were within acceptable limits. There was no response to administration of naloxone. A diagnosis of sympathomimetic toxicity was made and the patient was administered intravenous fluids, droperidol and midazolam. Trismus developed, requiring insertion of a surgical airway. The patient declined over the space of about 20 minutes, with heart rate and systolic blood pressure increasing to 200 and 210, respectively, progressing to an asystolic cardiac arrest an hour after admission to the emergency department. Immediate cardiopulmonary resuscitation was instituted, with oxygen saturation varying between 75 and 90%. Resuscitation attempts were ceased after about 60 minutes. No obvious cause of death was identified, and the death was referred to the Coroner for medicolegal investigation.

A coronial autopsy was conducted 3 days after death. No obvious abnormalities were identified on external examination of the body. There were no significant decompositional changes. On internal examination of the body, the deceased was found to have dark brown skeletal musculature, and there was pronounced pulmonary congestion and oedema. No discernible cardiovascular or intracranial pathology was identified. Microscopy of sampled tissues was non-contributory.

Both antemortem hospital admission EDTA-preserved blood and peripherally obtained sodium fluoride/potassium oxalate preserved blood were submitted for toxicological testing. Testing of the postmortem blood specimen revealed a 2,4-DNP concentration of 60 mg/L, a blood alcohol of 0.090 g/100mL and the presence of medications administered during emergency treatment of the patient. There was a methaemoglobin saturation of 45% in the postmortem blood specimen, compared to a methaemoglobin saturation of 1% in the antemortem blood specimen.

The cause of death was given as 2,4-dinitrophenol toxicity.

In this case, 2,4-DNP was consumed on the mistaken assumption that MDMA had been purchased. 2,4-DNP is a highly toxic substance with multiple mechanisms of toxicity suggested, including an uncoupling of oxidative phosphorylation by blocking ATP synthase, thereby preventing conversion of ADP to ATP in mitochondria, stimulation of glycolysis with a resultant rapid rise in pyruvate and lactate, and potassium and sodium accumulation as a result of inhibition of cellular respiration.[3] In this case, there was an elevated lactate level, but no other obvious indication of profound metabolic disturbance on routine biochemical testing. Symptoms of overdose, which may be delayed by 12 hours, typically include tachycardia, hyperthermia, diaphoresis and tachypnoea, and less commonly pronounced muscle rigidity and methaemoglobinaemia have been reported, with the latter likely the result of direct or indirect oxidation of haemoglobin.[4,5] The reason for delayed onset of symptoms as well as the delayed production of methaemoglobin is not clear, but is likely the result of accumulation of metabolites causing progressive cellular toxicity, renal failure and oxidation of haemoglobin. Although generalised muscle rigidity was not described in this case, which when present is thought to be due to depletion of ATP,[5] the presence of pronounced trismus and the need to create a surgical airway suggests this metabolic derangement may well have been present in the terminal stages.

The methaemoglobin saturation rose from 1% on admission to hospital about 6 hours after ingestion of the drug to a level of 45% measured at autopsy, and was reflected by generally dark brown discolouration of the deceased's musculature at postmortem examination.

Given the preservation of both the antemortem and postmortem blood specimens, their similar refrigerated storage and transportation conditions, and concurrent expeditious testing of both specimens, it appears highly probable that any chances of artefactural postmortem methaemoglobin production has been minimised.[6] In this case, therefore, death may in part have been a consequence of unrecognised methaemoglobinaemia, given that arterial blood gas analysis can be expected to overestimate the pO_2 level, thereby masking severe tissue hypoxia.

Methaemoglobinaemia is more commonly associated with ingestion of drugs such as amyl nitrite, various anaesthetic agents, sulphonamides, aniline dyes and solvents such as nitroethane, but it has also been reported as a manifestation of 2,4-DNP ingestion. A high level of awareness of novel illicit substances of abuse is required in clinical toxicology and autopsy practice, given the difficulty in detection of many of these substances in blood.

Further, the presence of methaemoglobinaemia should be considered clinically in cases of atypical suspected psychostimulant overdose so that effective and appropriate treatment with reducing agents such as methylene blue can be promptly instituted.

Conflicts of interest and sources of funding: The author states that there are no conflicts of interest to disclose.

Johan Duflou

National Drug and Alcohol Research Centre, University of New South Wales, Sydney, NSW, Australia; Sydney Medical School, University of Sydney, NSW, Australia

Contact Prof Johan Duflou.
E-mail: jduflou@forensicmedicine.com.au

1. Baselt RC. *Disposition of Toxic Drugs and Chemicals in Man*. 11th ed. Seal Beach, CA: Biomedical Publications, 2017; 710–1.
2. Colman E. Dinitrophenol and obesity: an early twentieth-century regulatory dilemma. *Regul Toxicol Pharmacol* 2007; 48: 115–7.
3. Grundlingh J, Dargan P, El-Zanfaly M, Wood D. 2,4-dinitrophenol (DNP): a weight loss agent with significant acute toxicity and risk of death. *J Med Toxicol* 2011; 7: 205–11.
4. Lu Y, Jiang J, Huang W. Clinical features and treatment in patients with acute 2,4- dinitrophenol poisoning. *J Zhejiang Univ Sci B* 2011; 12: 189–92.
5. McGillis E, Arens A, Olives T, *et al*. Rapid-onset hyperthermia and hypercapnia preceding rigor mortis and cardiopulmonary arrest in a DNP overdose. North American Congress of Clinical Toxicology (NACCT) Abstracts 2018. *Clin Toxicol* 2018; 56: 994–5 (Abstr 136).
6. Varlet V, Ryser E, Augsburger M, Palmiere C. Stability of postmortem methemoglobin: artifactual changes caused by storage conditions. *Forensic Sci Int* 2018; 283: 21–8.

DOI: https://doi.org/10.1016/j.pathol.2019.03.006

MALT lymphoma with IgM paraprotein and bone marrow involvement mimicking Waldenström macroglobulinaemia

Sir,

Waldenström macroglobulinaemia (WM) is defined in the World Health Organization (WHO) classification as lymphoplasmacytic lymphoma (LPL) associated with serum monoclonal immunoglobulin (Ig) M and involvement of the bone marrow by lymphoma.[1,2] Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) is defined as a low-grade lymphoma composed of a heterogeneous population of small B cells. Patients with MALT lymphoma have an indolent clinical course, have bone marrow involvement in less than half of patients, and rarely have a serum IgM paraprotein. Here, we report two patients with MALT lymphoma who had advanced tumour stage and features of WM, including high levels of serum monoclonal IgM and bone marrow involvement by lymphoma.

Case 1 was 64-year-old woman with a history of chronic hepatitis B and C who presented with thyroid-associated orbitopathy—proptosis, swelling of eyelids and blurred vision (OU)—for 3 years. She went to the rheumatology outpatient department for dry mouth. Laboratory examination revealed a high serum IgM level (5740 mg/dL; normal range 40–230 mg/dL) and lymphocytosis (15,500/μL). Furthermore, a serum protein electrophoresis (SPEP) and immunofixation electrophoresis (IFE) showed a monoclonal IgM with λ light chain restriction (Fig. 1A). Computed tomography (CT) showed enlarged lymph nodes in the bilateral axillary, retrocaval, aortocaval and peritoneal regions and diffuse subcutaneous nodules over the chest and abdomen. No hyperviscosity-related symptoms were noted. Incisional biopsy of the right eyelid yielded a diagnosis of MALT lymphoma. Additional pathological examination of the bone marrow and left back subcutis showed involvement by lymphoma. The patient received three cycles of cyclophosphamide, vincristine and prednisone, and six additional cycles of therapy supplemented with rituximab for 6 months. The eyelid swelling improved greatly. Imaging studies showed regression of lymphomatous lesions. The serum IgM level decreased to 132 mg/dL. Follow-up bone marrow aspiration and biopsy were negative for lymphoma. At last clinical follow-up, the patient had been clinically stable for one year after completion of chemotherapy. Pathologically, the right eyelid specimen showed a dense infiltrate of lymphoid cells in a diffuse pattern (Fig. 1B). The tumour cells exhibited predominantly small irregular nuclei with inconspicuous nucleoli and pale (monocytoid) cytoplasm (Fig. 1B, inset). Small numbers of large lymphoid cells (centroblast-like) and plasma cells with Dutcher bodies were present (Fig. 1C). Immunohistochemical analysis showed that the neoplastic cells were positive for CD20 (Fig. 1D) and MNDA (Fig. 1E), but negative for CD5, CD10, and cyclin D1. The plasma cells were positive for monotypic, cytoplasmic λ light chain (Supplementary Fig. 1A,B, Appendix A). Karyotypic analysis of bone marrow aspirate showed 48,XX,+3,+12,t(14;18)(q32;q21.1)[2] (Fig. 1F), consistent with a diagnosis of MALT lymphoma. Paraffin-embedded eyelid tissue was analysed for *MYD88* L265P and *CXCR4* mutations using droplet digital polymerase chain reaction (ddPCR; Bio-Rad Laboratories, USA) which showed negative results (Supplementary Fig. 2, Appendix A).

Case 2 was a 66-year-old woman who had a history of gastric ulcer with *Helicobacter pylori* infection and second degree atrioventricular block treated by permanent pacemaker implantation. She experienced intermittent shortness of breath and epigastric discomfort on hunger for a long time, but did not pay much attention to the symptoms. During follow-up in the cardiovascular outpatient department, chest radiograph revealed consolidation of bilateral lung fields. The patient did not have fever or cough. Laboratory evaluation showed atypical lymphocytes in the peripheral blood smear and a high serum IgM level, 3,220 mg/dL. SPEP and IFE showed a monoclonal IgM with κ light chain restriction (Fig. 2A). The patient then underwent bronchoscopy and biopsy showed MALT lymphoma. Chest and abdominal CT scans showed lymphoma involving mediastinal and retroperitoneal lymph nodes, bilateral lungs and the stomach as well as splenomegaly. Bone marrow examination and gastric biopsy also revealed MALT lymphoma. The patient received three cycles of cyclophosphamide, vincristine and prednisone, and six additional cycles of therapy supplemented with rituximab, as well as triple therapy for *H. pylori* eradication. After 7 months, the patient's serum IgM level decreased to 742 mg/dL. Imaging studies showed obvious regression of lymphoma lesions as well as resolution of the splenomegaly.