Case report

Antidote treatment for cyanide poisoning with hydroxocobalamin causes bright pink discoloration and chemical–analytical interferences

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A B S T R A C T

Here we report the case of a 70-year-old woman who committed suicide by cyanide poisoning. During resuscitation cases, she underwent an antidote treatment by hydroxocobalamin. Postmortem investigations showed marked bright pink discoloration of organs and fluids, and a lethal cyanide blood concentration of 43 mg/L was detected by toxicological investigation. Discolouration of hypostasis and organs has widely been studied in forensic literature. In our case, we interpreted the unusual pink coloration as the result of the presence of hydroxocobalamin. This substance is a known antidote against cyanide poisoning, indicated because of its efficiency and poor adverse effects. However, its main drawback is to interfere with measurements of many routine biochemical parameters. We have tested the potential influence of this molecule in some routine postmortem investigations. The results are discussed.

A R T I C L E   C O R R E S P O N D E N C E

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1. Introduction

Cyanide poisoning has been widely studied in forensic medicine and toxicology and can be considered as a classical issue in legal medicine. Because of its declining use in the industry, accidental poisoning is nowadays rare in developed countries. Cases of self-poisoning can still occasionally be observed, especially within particular occupational groups [1,2]. The most frequent source of cyanide poisoning in developed countries is from smoke inhalation [3].

Several antidotes with different mechanisms of action, side effects and efficiency have been proposed and used in cases of cyanide poisoning [4]. In recent times hydroxocobalamin has shown superior clinical efficacy, safety and tolerability compared to other molecules so that its use is nowadays considered as the first choice in cases of suspected cyanide poisoning [4]. In the literature, the use of hydroxocobalamin has been repeatedly associated to bright pink discoloration of human fluids and to analytical interferences in a clinical setting.

We here report a case of suicidal cyanide poisoning who had undergone an antidote therapy with hydroxocobalamin.

2. Case report

A 70-year-old woman was found gasping in her bed by her husband. Shortly before, he had heard her moving from the bedroom to the kitchen and coming back to bed after a few minutes. After calling the emergency medical service, the man started cardiopulmonary resuscitation. At arrival of the emergency staff, the woman showed cardiopulmonary arrest. Resuscitation manoeuvres were undertaken by the emergency service and at this moment the husband found a phial of cyanide in the washbowl of the kitchen and informed the present physicians. Antidote therapy with 5 g hydroxocobalamin was given but cardiopulmonary resuscitation remained unsuccessful. A farewell letter was later found in the apartment. The woman was a retired laboratory assistant known for a depression and she had already in the past expressed the idea of committing suicide by ingestion of cyanide. The public prosecutor ordered a medico-legal autopsy and toxicological analyses, as it is usual in cases of unnatural death in the Canton of Geneva, Switzerland. The body was refrigerated at 4 °C shortly after death and the medicolegal investigation was performed approximately 72 h later.

At external examination the body was that of 70-year-old Caucasian woman, 165 cm in height and 63 kg in weight. Hypostasis was normally developed at the dorsal parts of the body. Their colour was diffusely bright red, but showed normal red-blush colour at the fingernails and under defibrillator electrodes after removal (Fig. 1ESM).
At autopsy, the organs and tissues diffusely showed a bright pink coloration, which was especially evident at the inner aspect of the scalp, the dura mater, the visceral aspect of the pericardium and pleurae, the tracheobronchial mucosa, the ureters and the urothelium (Figs. 1, 2ESM, 3ESM and 4ESM). Such a peculiar colour also characterized the collected body fluids (Fig. 2). The gastric mucosa showed a diffuse brownish coloration. Other pathological findings were moderate pulmonary and cerebral oedema.

At histology the gastric mucosa showed some focal haemorrhages as well as signs of autolysis. Moreover, a hydropic degeneration of the pericentrolubular hepatocytes was observed.

Systematic toxicological analyses were performed on blood and urine. Comprehensive drug screening, including illicit and licit drugs, was performed on blood and urine by gas chromatography–mass spectrometry (GC–MS), and on blood by liquid chromatography with diode array detection (LC-DAD). Moreover, screening of volatile substances was performed on blood by headspace chromatography–flame ionization detection (HS-GC-FID). Cyanide determination in blood was performed by HS-GC–MS (adapted from [5–7]) and by microdiffusion and spectrophotometric detection (adapted from [8,9]). K$^{13}$C$^{15}$N was used as internal standard for the HS-GC–MS method. Blood samples spiked with different CN concentrations were used as control. The limit of detection was 0.1 mg/L, and the lower limit of quantification was 0.25 mg/L. Equation of the calibration curve was $y = 0.674x + 0.004$ with a regression coefficient of $r^2 = 0.998$. Precision and accuracy of the method were evaluated at different concentrations (0.5, 2.5 and 5.0 mg/L). The precision was 8.1%, 2.9%, and 1.9%, respectively. The accuracy was 105.6%, 100.1%, and 101.1%, respectively.

Concerning the spectrophotometric method, the limit of detection was 0.1 mg/L, and the lower limit of quantification was 0.3 mg/L. Equation of the calibration curve was $y = 1973x + 706$ with a regression coefficient of $r^2 = 0.99$. Precision and accuracy of the method were evaluated at different concentration (0.5 mg/L and 8.0 mg/L). The precision was 5.1% and 6.3%, respectively. The accuracy was 148% and 98%, respectively. Cyanide blood concentration was 43 mg/L by HS-GC–MS method and 42 mg/L by spectrophotometric detection. Presence of cyanide was revealed in the content of the phial found by the husband in the kitchen, using HS-GC–MS. Low concentration of bromazepam (10 μg/L) was detected in blood by liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS). This infratherapeutic concentration was consistent with former consumption included in the medical treatment of the victim. Moreover, traces of lidocaine due to resuscitation cares were also detected in blood by GC–MS.

Biochemistry analyses were also performed on post-mortem serum, which was obtained by the collection of the supernatant after centrifugation of samples of peripheral blood (3000 rpm for 5 min). The following results were obtained: ASAT = 3758 U/L, ALAT = 990 U/L, γGT = 107 U/L, CDTI = 1.6%, lactic acid = 3.66 mmol/L, CK = 18,307 U/L, and bilirubin < 10 μmol/L. Vitreous humor was also analysed and the following results were obtained: lactic acid = 10.4 mmol/L, CK = 134 U/L, and bilirubin < 10 μmol/L.

### 3. Discussion

In cases of accidental cyanide poisoning (mainly by smoke inhalation) or even in cases of suicidal cyanide poisoning (mainly by ingestion), patients may undergo urgent medical treatment and hospitalisation. In cases of fatal poisoning a medico-legal autopsy is often ordered and the forensic pathologist, besides the pathological changes due to the intoxication, has also to deal with the pathological changes induced by the administered therapies. Hydroxocobalamin has been recently widely studied [3,10,11]. It shows many of the characteristics of the ideal cyanide antidote: it has a rapid onset of action, neutralizes cyanide without interfering with cellular oxygen use, it has great tolerability and safety profiles, it is safe for use in smoke-inhalation victims, it is not harmful when administered to non-poisoned patients, and it is easy to administer [4]. This molecule is supposed to bind cyanide to form cyanocobalamin (vitamin B12), a non-toxic substance that is excreted in urine. As no major adverse effects have been reported, this therapy can be given in cases of suspect cyanide poisoning in the pre-hospital treatment, also to patients in which the actual poisoning is only presumed and analytical confirmation will be available only in a later phase [4].

In cases of cyanide poisoning numerous textbooks still report a cherry-red discolouration of the lividity because of high oxyhaemoglobin as a consequence of the action of the cyanide on the cellular respiration with prevention of the oxygen uptake [12,13]. Indeed the validity of this notion is controversial [2]. In our case the bright red colour of the hypostasis was interpreted as a postmortem reoxygenation facilitated by humidity and refrigeration of the body before autopsy. At internal examination we observed a peculiar and intense pink discolouration of the organs and body fluids. In numerous clinical reports, the most common adverse event attributed to the administration of hydroxocobalamin is a bright pink or bright red colouration of the skin, plasma and urine [4,10,11,14,15]. In a series of 69 patients treated with hydroxocobalamin, Borron et al. [3] reported chromatura in 6 cases and pink or red skin discolouration in 4 cases. In our case the
The main drawback of hydroxocobalamin reported in the literature is its analytical interference (spectral or chemical interactions) with the measurement of many biochemical parameters. Gourlain et al. [16] reported interferences in determination of creatine kinase (CK) and bilirubin due to hydroxocobalamin, using two different analysers. Vest et al. [17] showed hydroxocobalamin to be responsible for interferences in the measurement of creatinine, lactic acid, bilirubin, CK, alanine-amino-transferase (ALAT), aspartate-amino-transferase (ASAT) and phosphorus. Furthermore Pamidi et al. [18] reported that hydroxocobalamin and cyanocobalamin were both responsible for interferences on haemoglobin measurements including total haemoglobin, oxy–haemoglobin, carboxy–haemoglobin, met–haemoglobin and hydroxy–haemoglobin, using four different co–oximetry analysers. They also observed that the source of these interferences was an error in measurement algorithms and not due to cobalamin–induced changes of the haemoglobin. Recently interferences in the measurement of 73 different parameters on nine analysers were investigated [19]. In 64% of the analyses interferences on at least one instrument occurred. All instruments yielded some results that were affected by hydroxocobalamin at concentrations equivalent to a single therapeutic dose. Of all tests performed, 47% were biased with more than 10%.

In this case we decided to perform a double measurement of the cyanide concentration in order to double check the validity of the results and to evaluate the presence of eventual interferences. The first measurement was performed by head space extraction, and gas chromatography coupled with mass spectrometry (HS–GC–MS) and yielded a cyanide blood rate of 43 mg/L. The second measurement was performed by means of microdiffusion and spectrophotometric detection and yielded a result of 42 mg/L. The results were then comparable and we can conclude that they are reliable and that the presence of hydroxocobalamin did not interfere with both analyses.

For what concerns the biochemical investigations, five parameters were measured out of physiological ranges on serum (ASAT, ALAT, γGT, lactic acid and CK). Such variability can be influenced by postmortem changes due to factors linked to the cause of death (i.e. the duration of the agony) or independent from it (i.e. the postmortem interval) [20]. In our experience, the results we obtained are thus compatible with other cases with a short agony and similar postmortem interval. However, this is not an argument solid enough to state that no interferences occurred: to make more reliable statements we would have needed ante mortem data for comparison.

In conclusion, we think three main conclusions can be drawn from this case:

The forensic pathologist should know that in fatal cases of cyanide poisoning in which hydroxocobalamin was administered as an antidote, a bright pink discoloration of body fluids, mucosal and serous membranes is likely to occur and to be observed at postmortem investigation.

The presence of hydroxocobalamin does not seem to interfere with the measurement of cyanide blood concentrations by methods like HS–GC–MS and microdiffusion with spectrophotometry, commonly used in forensic toxicology.

Because of the known interferences in the investigation of common biochemical parameters, the results of postmortem biochemical investigations should be interpreted with much caution.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.forsciint.2012.08.019.

References