Toxicologie Analytique & Clinique (2014) **26**, 201—205

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ORIGINAL ARTICLE

Fatal alfentanil/morphine mixture: A case report

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Reçu le 27 août 2014 ; accepté le 28 août 2014
Disponible sur Internet le 17 septembre 2014

KEYWORDS
Alfentanil;
Morphine;
Anaesthetic drug;
Death;
Forensic

Summary A 23 year-old man, health care professional, was found dead in the toilets of a local hospital. Medical supplies for injection (syringe, needles) were found near the body at the scene, in a waste. External body examination revealed a single point of injection located at the left elbow crease and the lack of any traumatic injury. During examination, the pathologist collected cardiac blood and urine. These specimens were tested for ethanol, volatiles, pharmaceuticals and drugs of abuse, using headFspace GC/FID and GC/MS, Elisa, LC-DAD, GC/MS and LC/MS/MS. Ethanol tested positive in blood (0.99 g/L) and urine (0.19 g/L). Using a dedicated LC/MS/MS procedure, alfentanil was identified in both blood (19 ng/mL) and urine (25 ng/mL). Morphine was identified in blood, at 36 ng/mL (free morphine) and 39 ng/mL (total morphine). In urine, total morphine concentration was 81 ng/mL. No other drug was detected. Given the ratio (0.92) free morphine to total morphine in blood and the low concentrations of both alfentanil and morphine in urine, it was considered that the death occurred rapidly after drugs administration. The manner of death was considered as acute intoxication of both alfentanil and morphine, in presence of ethanol.

Introduction

Chemical dependency is a disease that can affect all professions. Among the health care professionals, people working with anaesthetic agents represent a specific group of concern. Numerous factors have been proposed to explain the high incidence of abuse among medical staff. They have easy access to a wide range of potent psychoactive drugs. Opioids promote rapid tolerance and dependence, particularly the highly lipid soluble

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http://dx.doi.org/10.1016/j.toxac.2014.08.002

agents (e.g. fentanyl). Diversion of these agents is relatively simple since only small doses will initially provide an effect desired by the abuser. Traditionally, opioids are the drugs of choice selected for abuse. Fentanyl and sufentanil are the most common, followed by meperidine and morphine. Other agents, which have been abused, include cocaine (in the USA), benzodiazepines (midazolam, flunitrazepam), and more recently propofol and sevoflurane [1—3].

There is no black market, but a direct supply by hospital deduction. Addicts can obtain drugs by substitution of prescribed drugs, use of residues of ampoules, false records in the prescription book, excessive/bogus prescription of drugs, or by resealing the content of ampoules with physiological serum. Excessive post-surgery pain for a patient that means incomplete anaesthesia can be considered as a suggestive sign of addiction in a particular hospital area [4,5].

Although there are numerous fatality reports in the scientific literature involving fentanyl, those dealing with alfentanil seem to be exceptional. A literature search on PubMed, performed on 20 August 2014 with the following keywords « alfentanil, overdose, fatality, death » was unable to produce any citation. Only Braithwaite et al. [6] published a case of a 27 year-old man found unconscious after self-injecting a mixture of alfentanil and midazolam where resuscitation failed. Specimens collected 2 days later showed the presence of 65 ng/mL alfentanil in the heart blood and 67 ng/mL in right femoral vein blood, with no apparent post-mortem redistribution. Dumestre-Toulet and Kintz presented in 2004 during the TIAFT-SOFT meeting a case involving a 46 year-old male nurse deceased (in the toilets of a hospital) from alfentanil acute intoxication, where blood and urine tested positive at 45.1 and 2.7 ng/mL, respectively in presence of ethanol (BAC at 1.32 g/L).

In order to document alfentanil fatalities, we present here the death of a hospital employee after injection of the drug.

Case report

A 23 year-old man (75 kg, 1.72 m), health care professional, was found dead in the toilets of a local hospital. Medical supplies for injection (syringe, needles) were found near the body at the scene, in a waste. External body examination revealed a single point of injection located at the left elbow crease and the lack of any traumatic injury (confirmed by radiology). Cyanosis of both arms and legs was remarkable. During examination, the pathologist collected cardiac blood and urine. The prosecutor in charge of the case did not request an autopsy.

Toxicological analyses

Ethanol was tested by headspace GC/FID on a PerkinElmer system (TurboMatrix 40 & Clarus 580) using a standard validated procedure. Volatiles were tested by headspace GC/MS on a Thermo system (Focus GC & DSQII) using a standard validated procedure. Elisa tests were achieved using Concateno Cozart Microplate kits using the recommendations of the manufacturer. Opiates were analyzed by GC/MS using a standard validated procedure [7].

Given the circumstances of death, anaesthetic agents were specifically tested by headspace GC/MS for propofol [8] and by a dedicated method for narcotics using LC/MS/MS.

Alfentanil analysis

Briefly, alfentanil was extracted from 0.2 mL blood and urine in presence of 2 ng of fentanyl-d_5 used as internal standard by 0.2 mL saturated ammonium chloride pH 9.5 buffer (adjusted with ammonia water) and 0.8 mL of dichloromethane/n-heptane/isopropanol (25/65/10, v/v). After extraction, centrifugation and evaporation to dryness, the residue was reconstituted in 50 μL of acetonitrile/ammonium formate 5 mM (13/87, v/v).

LC was performed using a Waters Acquity system. Chromatography was achieved using a Waters HSS C18 column (150 × 2.1 mm, 1.8 μm) eluted with a gradient delivered at a flow rate of 0.4 mL/min (from 13 % acetonitrile to 87 % formate buffer adjusted to pH 3.0 with formic acid 0.1 % to a ratio 95:5 % at 12.25 min). An injection volume of 10 μL was used in all cases. A Quattro Premier XE triple-quadrupole mass spectrometer (Micromass-Waters) was used for analyses. Ionization was achieved using electrospray in the positive ionization mode (ES+).

The following conditions were found to be optimal for the analysis of alfentanil and the IS: capillary voltage, 1.0 kV; source block temperature, 120 °C; and desolvation gas (nitrogen) heated to 350 °C and delivered at a flow rate of 800 L/h. In order to establish appropriate multiple reaction monitoring (MRM) conditions, the cone voltage was adjusted to maximize the intensity of the protonated molecular ion and collision induced dissociation (CID) of both species was performed. Collision gas (argon) pressure was maintained at 3.0×10^{-3} bar and the collision energy (eV) was adjusted to optimize the signal for the 3 most abundant product ions: transitions m/z 417.1 to 268.2, 197.1 and 314.2 and m/z 342.2 to 188.2 for alfentanil and the IS, respectively. Transition m/z 417.1 to 268.2 was used for quantitation. MassLynx 4.0 software was used for quantitation.

In blood, linearity was observed for alfentanil concentrations ranging from 0.1 to 40 ng/mL with a correlation coefficient of 0.999. Within-batch precision at 10 ng/mL was 17.1 %. The limit of detection was estimated to be 5 pg/mL, with a S/N ratio of 3. Under the chromatographic conditions used, there was no interference with the analytes by chemicals or any extractable endogenous materials present in blood or urine (matrix effect < 20 %).

Results and discussion

Ethanol tested positive in blood and urine at 0.99 and 0.19 g/L. This was the first issue in this case, given death occurred at workplace.

Elisa screening was positive for opiates. Morphine tested positive in blood, at 36 ng/mL (free morphine) and 39 ng/mL (total morphine). In urine, total morphine was identified at 81 ng/mL.