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Ocfentanil overdose fatality in the recreational drug scene

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ABSTRACT

This paper describes the first reported death involving ocfentanil, a potent synthetic opioid and structure analogue of fentanyl abused as a new psychoactive substance in the recreational drug scene. A 17-year-old man with a history of illegal substance abuse was found dead in his home after snorting a brown powder purchased over the internet with bitcoins. Acetaminophen, caffeine and ocfentanil were identified in the powder by gas chromatography mass spectrometry and reversed-phase liquid chromatography with diode array detector.

Quantitation of ocfentanil in biological samples was performed using a target analysis based on liquid–liquid extraction and ultra performance liquid chromatography tandem mass spectrometry. In the femoral blood taken at the external body examination, the following concentrations were measured: ocfentanil 15.3 μg/L, acetaminophen 45 mg/L and caffeine 0.23 mg/L. Tissues sampled at autopsy were analyzed to study the distribution of ocfentanil. The comprehensive systematic toxicological analysis on the post-mortem blood and tissue samples was negative for other compounds.

Based on circumstantial evidence, autopsy findings and the results of the toxicological analysis, the medical examiner concluded that the cause of death was an acute intoxication with ocfentanil. The manner of death was assumed to be accidental after snorting the powder.

1. Introduction

Ocfentanil is a synthetic opioid and structure analogue of fentanyl. Ocfentanil (also called A-3217) was developed in the early 1990s in the attempt to obtain an analgesic opioid with less cardiovascular and respiratory effects. The activity of ocfentanil was studied in human volunteers, showing that ocfentanil is similar in action to fentanyl, given effective analgesia at 1 μg/kg and being approximately 2 times as potent as fentanyl. Nausea, itching and dose-dependent potentially life-threatening respiratory depression are reported side effects of fentanyl and analogues [1–5]. Ocfentanil is not approved for medical use.

To the author's knowledge, this paper describes the first reported death involving ocfentanil abused as a new psychoactive substance (NPS). The intoxication occurred in March 2015 in Belgium [6,7]. A target analysis on ocfentanil in the postmortem tissues was performed using liquid–liquid extraction and ultra performance liquid chromatography tandem mass spectrometry operating in multiple reaction monitoring mode.

2. Case history

A 17-year-old man was found dead in his home, seated and leaning forward on the toilet at 6:00 am. The victim was last seen alive at 22:30 pm when his parents went to sleep. No farewell letter was present. The parents stated that their son has left school at age 15 and never left the house. He stopped taken antidepressants 3 month before and was prescribed sleeping medication. The young man had a history of illegal substance abuse and was previously hospitalized with an acute intoxication due to the combined intake of cocaine and sleeping tablets. The abused products were purchased over the internet with bitcoins. Drug paraphernalia were found in the proximity of the victim: a brown powder (2.07 g) in a small zip-locked plastic bag which was lying on a card with a straw. Residue of a brown powder, similar to the powder in the bag, were present on the card.

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3. Materials and methods

3.1. Materials

Certified reference ocfentanil (*N*-(2-fluorophenyl)-2-methoxy-*N*-[1-(2-phenylethyl)-4-piperidyl]acetamide) was obtained from Viwit Pharmaceutical co., Ltd (Shanghai, China). The reference material fentanyl-d_5 100 μg/mL in methanol was from Cerilliant (Round Rock, Texas). Standard compounds were diluted in methanol (1 μg/mL) and stored at −18 °C. Methanol and acetonitrile were obtained from Fisher Chemical (Fisher Bioblock, Belgium). Formic acid 98–100% was purchased from Merck (VWR, Leuven, Belgium). Water was purified by a Milli-Q system obtained from Millipore. All solvents and inorganic chemicals were of analytical grade. The potassium carbonate solution was prepared by dissolving 13.6 g of anhydrous K_2CO_3 (VWR, Leuven, Belgium) into a 100 mL volumetric flask and made up to volume with water.

3.2. Instrumentation

The UPLC-MS/MS analysis was performed using a Acquity separations module coupled to the Acquity TQD mass detector equipped with ES interface (Waters Milford, MA, USA). Chromatographic separation was achieved using a Acquity UPLC HSS C18 column (150 mm length x 2.1 mm i.d., 1.8 μm particle size) with a HSS C18 Vanguard column (5 mm length x 2.1 mm i.d., 1.8 μm particle size) as guard column at 50 °C. The mobile phases consisted of 0.15% formic acid (A) and 0.15% formic acid in acetonitrile (B). The following gradient elution was used (runtime 15.00 min), starting with 13% B held for 0.50 min., increased to 50% B in 9.50 min., changed to 95% B in 0.75 min and held for 1.50 min., and finally changed back to initial conditions in 0.25 min and held for 2.50 min. The flow rate was 0.400 mL/min. The electrospray source was operated in the positive ionisation mode (ES+). Product ions were obtained by collision-induced dissociation which allowed the MS/MS to be operated in the multiple reaction monitoring (MRM) mode. The MRM transitions and conditions for the measurement of ocfentanil (retentiontime: 5.24 min.); 371.00/188.00 (qualifier) and 371.00/105.00; cone voltage 28 V; collision energy 24 V and 32 V, respectively; fentanyl-d5 (retentiontime: 6.26 min.): 342.45/188.25 (qualifier) and 342.45/105.10; cone voltage 40 V; collision energy 25 V and 38 V, respectively. Quantitations were carried out using the first transition (qualifier). For confirmation, the percent ratio of the second transition to the qualifier was calculated and monitored. The source temperature and desolvation gas (nitrogen) temperature were set at 150 °C and 400 °C, respectively. The gas flow was delivered at a rate of 800 L/h. The capillary voltage was 3.00 kV. Waters Mass-lynx system software Version 4.1 was used for instrument control and quantitation.

3.3. Samples

The brown powder in the small zip-locked plastic bag was submitted to the laboratory for analysis. Femoral blood, vitreous humor and a swab of the mucous membrane of the nose were taken at the external body examination by the medical examiner at 11:00 am. The blood samples contained respectively sodium fluoride and EDTA as preservative.

An autopsy was carried out 3 days later. Multiple samples were taken for toxicological investigation: cardial blood (without preservative and EDTA, respectively), urine, stomach content (40 mL), liver, kidney, brain tissue, bile and a hair sample from the scalp. The tissue samples were stored at −18 °C for 6 weeks until arrival of the reference compound ocfentanil and before the distribution study was performed.

4. Methods

4.1. Systematic toxicological analysis

The brown powder was subjected to the authors systematic toxicology identification scheme (ISO/IEC 17025:2005 accredited) based on analysis of freshly prepared methanolic sample solution on reversed-phase liquid chromatography with diode array detector (HPLC/PDA) and gas chromatography mass spectrometry (GC/MS) as previously described [8].

A comprehensive systematic toxicological analysis was performed on the post-mortem tissue samples to investigate for illegal drugs, medical drugs, alcohol, volatile substances and other poisons. Blood was analyzed for the presence of carboxyhemoglobin and cyanide. Screening for the presence of basic drugs in urine and stomach contents was performed by GC/MS and in blood by HPLC/PDA. Analysis for the presence of alcohol in blood, vitreous humor and urine and of other volatile substances in blood was performed by gas chromatography and static headspace gas chromatography with flame ionisation detector. The screening for the presence of illicit drugs of abuse and medical drugs (including opiates, amphetamines, methadone and metabolite, cocaine and metabolites, ketamine/norketamine, fentanyl/norfentanyl and medical analogues, cannabinoids, benzodiazepines, narcotic analgesics, antidepressant drugs and several new psychoactive substances) were investigated in blood and β-glucuronidase hydrolyzed urine using UPLC-MS/MS methods. Color spot tests on urine and gastric content were used to detect salicylates, acetaminophen, phenothiazines and imipramines.

Quantitative determination of acetaminophen (matrix-matched standard calibration using acetaminophen-d4 as internal standard) and caffeine (matrix-matched external standard calibration) in the blood taken at the external body examination (with EDTA as preservative) were performed using UPLC-MS/MS using the same liquid/liquid extraction and LC-conditions as applied for ocfentanil. The hair was analyzed on the presence for amphetamine, methamphetamine, MDMA, MDA, MDEA, cocaine, benzoylecgonine, norcocaine, cocaethylene, morphine, 6-monoacetylmorphine, codeine, tetrahydrocannabinol, cannabidiol, cannabinol, ketamine and norketamine. Hair specimen was decontaminated twice with methylene chloride. The proximal 6 cm-long hair section was homogenized by cutting with scissors into small pieces (< 1 mm). Twenty mg of hair were incubated overnight at 40 °C using 400 μL of methanol in the presence of the deuterated analogues as internal standard. An aliquot of the media was evaporated to dryness and injected into the UPLC-MS/MS in MRM mode (ES+). Each compound was identified based on two MRM transitions and quantified using a calibration curve (ISO/IEC 17025:2005 accredited).

4.2. Sample preparation and extraction

The biological tissue samples were submitted to toxicological examination. Liquid–liquid extraction was made after homogenization. Kidney, liver, stomach content (semi-solid), bile and brain tissue were homogenized in water at a ratio (m/m) of 1:2 or 1:5 by means of a Ultra Turrax® (IKA T18 basis). The swab of the **mucous membrane of the nose** was extracted in 0.5 mL ultrapure water. To a 0.5 mL aliquot of sample (blood, urine, vitreous humor, bile, extract of nose swab) or 0.5 g homogenized tissue sample, 5 μL of internal standard solution (fentanyl-d_5 1 μg/mL) was added. After addition of the internal standard solution (5 ng fentanyl-d5/sample), the samples were vortex mixed and allowed to equilibrate 30 min. prior to extraction. Alkalinization was obtained by addition of 1.0 mL potassium carbonate solution followed by agitation in a vortex mixer. Extraction was performed with 5 mL of