Lethal poisonings with AH-7921 in combination with other substances

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ABSTRACT

AH-7921 is a synthetic μ-opioid agonist, approximately equipotent with morphine. We report the death of two young individuals after ingestion of AH-7921 in combination with other psychoactive drugs.

In the first case a young man died shortly after ingesting Internet drugs. Toxicological analysis of post mortem peripheral blood revealed AH-7921 (0.43 mg/L), 2-FMA (0.0069 mg/L) and 3-MMC (0.0021 mg/L) as well as codeine (0.42 mg/L), codeine-6-glucuronide (0.77 mg/L) and acetaminophen (18.7 mg/L).

The second case involved a young female found dead at home. The only positive finding at medicolegal autopsy was needle marks. Toxicological analysis revealed AH-7921 (0.33 mg/L), methoxetamine (MXE) (0.064 mg/L), etizolam (0.27 mg/L), phenazepam (1.33 mg/L), 7-aminonitrazepam (0.043 mg/L), diazepam (0.046 mg/L), nordiazepam (0.073 mg/L), and oxazepam (0.018 mg/L) in blood.

In both cases intoxication with AH-7921 in combination with other psychoactive drugs was considered to be the cause of death.

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1. Introduction

An increasing number of new psychoactive substances (NPS) have appeared on the drug market during the last decade. These drugs are synthetic, semi-synthetic or natural compounds, and often sold as “legal” alternatives to illicit drugs [1]. Several countries have introduced measures to control some of these substances, but the substances are continuously modified to avoid existing laws. By replacing one atom with another, a new and maybe unregulated substance with unknown properties is created. The effects may be similar, but the new substances may also have unexpected and different effects [2].

Since 2005 almost 300 NPS have been detected by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), the two most numerous groups being the synthetic cannabinoids and the cathinones. The last couple of years the diversity of NPS has increased and several different derivatives of medicinal substances are emerging on the marked [1,3,4]. Little is known regarding acute and long-term effects of these drugs [5]; however some information exists on the NPS that probably are the most widely used.

AH-7921 was reported for the first time by EMCDDA in 2012 [3]. AH-7921 has also been detected in synthetic cannabinoid products [6]. Since mid-2012, several European countries have reported the presence of AH-7921 in small seizures done by customs or police. The substance is only regulated by law in Sweden (March 2014) (The European information system and database on new drugs (EDND)). AH-7921 (3,4-dichloro-N-[(1-dimethylamino)cyclohexyl]benzamide) is a μ-opioid receptor agonist which was developed in 1974 [7] and patented as a potential analgesic agent in 1976 [8]. Its analgesic activity in mice is reported to be equal or slightly higher than morphine [9,10]. Animal studies have shown that AH-7921 is approximately equipotent to morphine with respect to respiratory depression, antinociception, sedation, decrease in pupil diameter, Straub tail, decrease in body temperature and inhibition of gut propulsion [11]. The pharmaceutical company did not continue further development of AH-7921, and data on use by humans is limited to recent poisoning cases and informal discussion fora on the Internet [8].

In this article we report two cases from Norway where AH-7921 was involved in fatal intoxications, in combination with other drugs.
2. Ethics

Approval to publish this case report has been given by the Public Prosecutor and the Council of Confidentiality and Research (appointed by the Norwegian Ministry of Justice).

3. Case reports

3.1. Case 1

A man in his early twenties was involved in a minor traffic accident. He was admitted to hospital and discharged the following day, as there was no sign of serious injury. He was prescribed a combination drug of codeine and acetaminophen (30 mg codeine/400 mg acetaminophen). He told his girlfriend that he had taken six of these tablets. She also observed that he had ingested some powder from zip-lock bags marked 3-MMC (3-methylmethcathinone) and 4-FMA (4-fluoromethamphetamine), bought on the Internet.

After having ingested the prescribed tablets as well as the powders, he had to lie down on the floor. Within 5 min he was snoring and it was impossible to wake him up. Shortly thereafter, his lips turned bluish. An ambulance was summoned and the paramedics started resuscitation, but did not succeed.

A medicolegal autopsy was performed five days post mortem. The lungs were edematous (weight 2080 g), apart from this there were no significant findings by gross or microscopic examination nor by neuropathological examination.

3.2. Case 2

A young female was found dead by her boyfriend in their home. Used needles and small plastic bags labeled “AH-7921” and “etizolam” were found in several waste bins in the house. A medicolegal autopsy was requested and performed, which showed needle marks in various stages of healing on the right cubital fossa, and the Department of Forensic Genetics and Forensic Toxicology, Hospital – Trondheim University Hospital, Trondheim, Norway, was analyzed at the Department of Clinical Pharmacology, St. Olavs Hospital – Trondheim University Hospital, Trondheim, Norway.

We present the analytical methods for case 1, which was analyzed at the Norwegian Institute of Public Health (NIPH). Case 2 was analyzed at the Department of Clinical Pharmacology, St. Olavs Hospital – Trondheim University Hospital, Trondheim, Norway, and the Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, Linköping, Sweden.

4. Analytical methods

Whole blood from the femoral vein was collected during autopsy in 25 mL Sterilene® polystyrene tubes with polyethylene screw caps (Bibby Sterilin, Staffordshire, UK) containing 0.3 mL 67% (w/v) potassium fluoride solution. All post-mortem blood samples received at NIPH are routinely screened for a selection of benzodiazepines and z-hypnotics, opiates and opioids, psychostimulants and THC by ultra-perforative substances was performed as well as an untargeted Q-TOF LC/MS screening. UPLC–MS/MS: As expected we found a peak for the 4-MMC transition, but the retention time was slightly different (0.05 min), supporting the information given that the sample contained 3-MMC. 3-MMC was confirmed, whereas for 4-FMA the retention time turned out to be different and the ion ratio between the two transitions differed with almost 50% compared to the reference standard. We thus suspected that this could be either 2-FMA or 3-FMA instead of 4-FMA. When all the FMA isomers were compared the substance was finally identified as 2-FMA. Q-TOF LC/MS: A large peak was found in addition to 3-MMC and 2-FMA.

Comparison with a large database, containing more than 7500 toxicologically relevant substances [15], gave no hit for the compound. Internet search of the molecular formula yielded many possibilities, among these, AH-7921.

We also received aliquots of the white powder from the two plastic bags from National Criminal Investigation Service, Norway. They were analyzed using the above mentioned technique and were found to contain 2-FMA and 3-MMC, but no trace of AH-7921.

4.1. Determination of 2-FMA, 3-MMC, and AH-7921

Chemicals: 2-FMA, 3-FMA, and 4-FMA were supplied by Chiron (Chiron AS, Trondheim, Norway), 3-MMC and AH-7921 by Cayman Chemical Company (Michigan 48108, USA) and amphetamine-d11 (internal standard) was supplied by Cerilliant® (Austin, TX, USA). Methanol (MeOH, LC–MS Chromasolv®) was purchased from Fluka (Sigma–Aldrich Norway AS, Oslo, Norway), AnalAr® ammonium formate from BDH Laboratory Supplies (Poole, England), ethylacetate PA from Chemi-Teknik (Chem-Teknik, Oslo, Norway), and heptane from VWR (VWR International AS, Oslo, Norway). Deionized water was obtained from a Milli-Q UF Plus water purification system (Millipore, Bedford, MA, USA). Human whole blood was supplied by the Blood Bank at Oslo University Hospital, Norway.

Stock solutions of 2-FMA, 3-FMA, 4-FMA, 3-MMC, and AH-7921 were prepared in methanol and working standards in water. Five calibration samples were prepared from whole blood spiked with working standard solutions (0.0017–0.033 mg/L for 2-FMA, 0.0018–0.035 mg/L for 3-MMC, and 0.033–0.66 mg/L for AH-7921). Quality control (QC) samples were prepared independently at two concentration levels (0.0025 and 0.025 mg/L for 2-FMA; 0.0027 and 0.027 for 3-MMC; and 0.049 and 0.50 mg/L for 7-AH7921). Sample preparation (0.5 mL blood) was an alkaline (borate buffer pH 11, 0.25 mL) liquid–liquid extraction with 1.2 mL ethylacetate/heptane following evaporation to dryness. The dry residue was then reconstituted with 100 µL of 10 mM ammonium formate buffer (pH 3.1) and MeOH mixture (90:10 v/v).

The samples were analyzed using a UPLC–MS/MS method on a Waters ACQUITY UPLC-system (Waters Corporation, Milford, MA, USA). Chromatographic separation was achieved using an Acquity UPLC HSS T3 column (2.1 x 100, 1.8 µm). The mobile phase consisted of 10 mM ammonium formate buffer pH 3.1 (A) and methanol (B), with a gradient from 2.5% B to 100% B. Total run time was 16 min. A Waters Quattro Premier XE tandem mass spectrometer, equipped with a Z-spray electrospray interface, was used for all analyses. Positive ionization was performed in the multiple reaction monitoring (MRM) mode, with two transitions for the analytes (2-FMA: 168.1 > 109.0/137.1; 3-MMC: 178.2 > 145.1/160.1, and AH-7921: 329.2 > 284.2/144.9) and one transition for amphetamine-d11 (147.0 > 98.0) Quantification was performed with TargetLynx using MassLynx 4.1 software. The retention time for 2-FMA, 3-MMC, AH-7921, and amphetamine-d11 were 4.26, 4.77, 8.73, and 3.61 min, respectively. The calibration curves were linear with correlation coefficients greater than 0.997 for all analytes. QC-samples (two replicates at each level) had less than 14% deviation from nominal values.

The blood sample was analyzed against separately made calibrators for 2-, 3- and 4-fluoromethamphetamine. The mean of
recess and ion ratios for the calibrators and QC-samples were 4.26 min/4.060, 4.17 min/2.583, and 4.17 min/2.008, respectively.

5. Toxicological findings

In case 1 toxicological analyses showed AH-7921 0.43 mg/L, codeine 0.42 mg/L, codeine-6-glucuronide (C6G) 0.77 mg/L, acetaminophen 19 mg/L, 2-FMA 0.0069 mg/L, and 3-MMC 0.0021 mg/L in peripheral whole blood. In case 2 toxicological analysis revealed AH-7921 0.33 mg/L, methoxetamine 0.064 mg/L (MXE), etizolam 0.27 mg/L, phenazepam 1.33 mg/L, 7-aminonitrazepam 0.043 mg/L, diazepam 0.046 mg/L, nordiazepam 0.073 mg/L, and oxazepam 0.018 mg/L in peripheral whole blood.

6. Discussion and conclusion

We report two fatal accidental poisonings involving AH-7921, found in concentrations at 0.33 and 0.43 mg/L. In the first case the deceased had also ingested prescribed codeine/acetaminophen and the Internet drugs 2-FMA and 3-MMC. In the second case the deceased had also consumed benzodiazepines and the Internet drugs 2-FMA and 3-MMC. In the second case the deceased had also ingested prescribed codeine/acetaminophen found in concentrations at 0.33 and 0.43 mg/L. In the first case the deceased had also consumed benzodiazepines and the Internet drug MXE. Edematous lungs were found in one of the cases, a typical finding seen in opioid overdoses [16]. AH-7921 is known to exert μ-receptor agonist effects approximately equipotent to morphine [8,9]. The AH-7921 concentrations measured in both cases may thus be consistent with significant and potentially lethal respiratory depression.

The toxicity of 2-FMA, 3-MMC, MXE, and AH-7921 in humans is poorly defined, and no toxic or lethal ranges have been established. It is thus difficult to know if the concentrations found in our cases are higher than what might be seen after recreational use of these substances, in persons with no signs of intoxication.

Kronstrand et al. have reported a concentration of 0.81 mg/kg in femoral blood in a fatal AH-7921 poisoning [17]. The substance was found in combination with 10 mg/kg gabapentin (an antiepileptic and analgesic substance). The cause and manner of death was concluded to be an accidental drug overdose with AH-7921. They also quantified AH-7921 in four other cases with concentrations in blood/serum ranging between 0.03 mg/kg and 0.99 mg/kg. Further information was not provided in these cases. Vorce et al. [18] have reported a fatality were only AH-7921 was detected. They measured the AH-7921 concentration to be 9.1 mg/L in peripheral blood. Elliott et al. [4] found AH-7921 concentrations of 0.05, 0.58, and 4.46 mg/L in post-mortem femoral blood in three fatalities. No case histories are given, and it is not stated whether they were mono-intoxications.

The concentrations of 0.43 mg/L and 0.33 mg/L, respectively, in our cases are within the range seen by Elliott et al. and Kronstrand et al. [4,17], but lower than the concentrations found in the lethal cases reported by Kronstrand et al. and Vorce et al. [17,18]. Other substances were detected simultaneously in our cases; this complicated the determination of the contribution of the toxic effects of the various substances.

In case 1 it was reported that the deceased had to lie down on the floor shortly after ingestion of the prescribed tablets (codeine/acetaminophen) and the powder bought on the Internet. This was followed by snoring and loss of consciousness. Snoring is a typical phenomenon after administration of high doses of opioids [19]. The concentration of codeine was slightly above the therapeutic level, while the acetaminophen concentration was within the therapeutic range. Codeine is metabolized to CG8 in the liver, and the metabolite is normally present in blood in much higher concentrations than codeine [20]. In our case, the CG8 concentration was only twice as high as the codeine concentration, a finding consistent with death shortly after ingestion.

Two additional NPS were found which might be of importance regarding the cause of death. 2-FMA is a stimulant phenethylamine related to methamphetamine. Because of its chemical properties it can be assumed that 2-FMA has typical central stimulant effects. In Europe, only Finland and Germany have reported seizure of this substance in the form of white powder in January 2011 and 2013, respectively (EDND). There is no reference data in the literature concerning toxicity and serum concentrations of 2-FMA.

3-MMC is a synthetic ring-substituted cathinone. It is a positional isomer of methedrone, differing only in the position of the methyl group on the phenyl ring. These ring-substituted cathinone derivatives are assumed to have effects similar to that of cocaine, amphetamine, and MDMA. Several of these substances have been associated with severe toxicity and death [21]. Seizures of white and off-white powder identified as 3-MMC have been reported in several European countries since late 2012 (EDND). The Swedish National Institute of Public Health has reported several mixed intoxications with 3-MMC. One of these mixed intoxication cases also involved a combination of AH-7921 and 3-MMC (EMCDDA).

In case 2 the forensic pathologist concluded that the cause of death was intoxication with AH-7921 in combination with several benzodiazepines and MXE. The measured concentrations of etizolam and, in particular, phenazepam were high, with etizolam being detected at a concentration more than 10 times higher than reported peak plasma concentrations after ingestion of therapeutic doses [22,23], and the phenazepam concentration being more than 50 times higher than reported peak whole blood concentrations after ingestion of high therapeutic doses [24]. However, a recent study from Finland reviewing phenazepam findings in forensic toxicology casework showed that higher phenazepam concentrations than the concentration detected in our case 2 were measured both among drivers apprehended for driving under the influence of drugs, and in medicolegal autopsy cases where phenazepam was not considered to be the sole cause of death [25]. Indeed, the majority of the medicolegal autopsy cases were accidental opioid overdoses.

MXE (2-(3-methoxyphenyl)-2-(ethylamino)cyclohexanone) is a derivative of ketamine. Both NMDA receptor blockade and dopamine reuptake inhibition are assumed to be responsible for its action, although formal pharmacology has not been determined [26]. Several European countries have reported seizures of tablets, capsules or white powder, and MXE is controlled by law in several European countries (EMCDDA). Several intoxications and deaths involving MXE have been reported [4,27–30]. A case series of three individuals who suffered acute toxicity related to the analytically confirmed use of MXE describes a “dissociative/catatonic” state similar to that seen with ketamine and clinical features of acute sympathomimetic toxicity with significant tachycardia and hypertension [31].

In our two cases the deceased had ingested the semi synthetic opioid AH-7921 in combination with other NPS, opioids and benzodiazepines. The contribution of AH-7921 to the cause of death in these cases is difficult to assess, and further information about this substance is needed. However, it seems prudent to alert care-takers and health professionals of this potent drug available through Internet retailers.

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