Fatal Intoxications Associated with the Designer Opioid AH-7921

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AH-7921 ([3,4-dichloro-N-[(1-dimethylamino)cyclohexylmethyl]benzamide) is a designer opioid with ~80% of morphine’s µ-agonist activity. Over a 6-month period, we encountered nine deaths where AH-7921 was involved and detected in blood from the deceased. Shortly after the last death, on August 1 2013, AH-7921 was scheduled as a narcotic and largely disappeared from the illicit market in Sweden. AH-7921 was measured by a selective liquid chromatography–MS-MS method and the concentrations of AH-7921 ranged from 0.03 to 0.99 μg/g blood. Six of our cases had other drugs of abuse on board and most had other medications such as benzodiazepines, antidepressants and analgesics. However, the other medicinal drugs encountered were present in postmortem therapeutic concentrations and unlikely to have contributed to death. In addition to the parent compound, we identified six possible metabolites where two N-deethylated dominated and four mono-hydroxylated were found in trace amounts in the blood. In conclusion, deaths with AH-7921 seem to occur both at low and high concentrations, probably a result of different tolerance to the drug. Hence, it is reasonable to assume that no sharp dividing line exists between lethal and non-lethal concentrations. Further, poly-drug use did not seem to be a major contributing factor for the fatal outcome.

Introduction

Designer drugs have historically been associated with central stimulants and especially ecstasy-analogs (1–7). However, during recent years, designer drugs within several of the established drugs of abuse categories have been encountered. Synthetic cannabinoids have dominated (8–14) but also designed substitutes for ketamine (15), benzodiazepines (16, 17) and cocaine (18) have emerged. Opioids were not late to follow with isolated clusters of deaths attributed to the tramadol metabolite O-desmethyltramadol, laced in Kratom leaves (19). The latest addition to the designer opioids was 3,4-dichloro-N-[(1-dimethylamino)cyclohexylmethyl]benzamide (AH-7921) where a fatal intoxication was recently reported by Vorce et al. (20). As is common with many designer drugs, AH-7921 was actually synthesized and evaluated many years ago but never reached the pharmaceutical market. However, the presence of AH-7921 in online purchased designer drugs has been reported from 2012 and onwards (21, 22). AH-7921 acts as an agonist on the opioid µ-receptor with a potency ~80% of morphine (23). Another study in mice showed that AH-7921 produced antinociceptive effects, decreased respiratory rate and decreased pulse rate and also lowered the body temperature more efficiently than morphine at same dose. In addition, AH-7921 suppressed abstinence syndrome during morphine withdrawal (24). In animals experiments, Brittain et al. found that the minimum oral dose for complete pain suppression by AH-7921 were 1.25 mg/kg for canine and 13.8 mg/kg for Rhesus monkey (23). In mice, AH-7921 showed stronger respiratory depression than morphine predicting untoward effects also in humans. AH-7921 may also have abuse potential, since its pharmacological effects are similar to those of morphine. On internet sites (www.erowid.org), the duration has been reported to be ~4 h, with a peak after 1.5 h. The analysis of AH-7921 in biological samples has been described by Vorce et al. using GC–MS (20) and by Soh and Elliott using LC-TOF (25).

In this paper, we report nine deaths where the designer opioid AH-7921 was involved and give some insight to its pathology and metabolism.

Case descriptions

Over a 6-month period, we encountered nine deaths where the µ-agonist AH-7921 was involved and detected in blood from the deceased.

Case 1
A 27-year-old male known drug addict was found dead at home laying on the kitchen floor. In the apartment, there were powders and equipment for intravenous injections, indicating an ongoing abuse of drugs. He was seen alive 2 days before he was found dead. Autopsy revealed pulmonary edema, a tablet remnant in the stomach together with fresh and older needle marks on the right arm. The weight of the lungs was 1,211 g. Tryptase level was high, 528 μg/L.

Case 2
A 26-year-old male was found dead on a sofa at his apartment. Empty beer cans but no pharmaceutical drugs were found at the scene. He had psychiatric problems for 3 years recently, but according to the relatives had not been depressed. When last contacting the psychiatry care, 5 days before he was found dead, he said he felt normal, was well off and was taking his medications. Autopsy revealed pulmonary edema, and signs of aspiration pneumonia. The weight of the lungs was 1,712 g.

Case 3
A 24-year-old male was taken to hospital by ambulance because of a suspected drug overdose. Twenty-four hours later he died. Autopsy revealed pontine hemorrhages and anoxic brain injury.

Case 4
A 45-year-old male living with his mother was found dead in the bathroom. Accordingly to relatives, he was self-medicating with analgesics because of back pain. Autopsy findings were unremarkable. The weight of the lungs was 1,167 g.
**Case 5**
A 26-year-old male with a history of psychiatry disease, narcolepsy and ADHD was found dead in his bed. Autopsy revealed pulmonary congestion. The weight of the lungs was 1,254 g.

**Case 6**
A 34-year-old male was found dead on a sofa at his apartment. He had not been seen for 4 days. Several pharmaceuticals as well as designer drugs were found at the scene. He was a known drug addict and was hospital treated due to intoxication the previous year. Autopsy findings were unremarkable. Hair analysis suggested previous use of tramadol but was negative for AH-7921. The weight of the lungs was 792 g.

**Case 7**
A 27-year-old male was found unresponsive in his bed by his girlfriend. According to his relatives, he had suffered from psychiatric problems for a long time and had been using drugs. Autopsy revealed extended lungs and bronchopneumonia. The weight of the lungs was 2,146 g.

**Case 8**
A 25-year-old male was found unresponsive sitting in his room. He was a known drug user and had been depressed. Autopsy revealed pulmonary congestion, acute bronchitis, pneumonia and brain edema. The weight of the lungs was 1,256 g.

**Case 9**
A 22-year-old male was found by the personnel at a treatment facility. Beside him there was Lyrica tablets and a white powder labeled 'AH-7921'. Autopsy revealed some tablets in the stomach, possible needle marks in both arms, brain edema and pulmonary congestion. The weight of the lungs was 1,590 g.

**Experimental**
Routine postmortem toxicology was performed in femoral blood using a targeted screening for pharmaceuticals and drugs of abuse with liquid chromatography time-of-flight (LC-TOF) technology (26). An in-house database comprising nearly 550 drugs was used for determination of primary target compounds relevant for postmortem toxicology. Data were extracted by the 'Find by Formula' algorithm with a match tolerance for mass error of ±10 ppm, retention time deviation ±0.15 min and area of ≥30,000 counts. Identification was based on scoring of retention time, accurate mass measurement and isotopic pattern (mass, abundance and spacing). Medications and drugs of abuse screened positive with LC-TOF were quantified in femoral blood using in house methods. Analysis of alcohols and acetone were screened positive with LC-TOF were quantified in femoral blood (mass, abundance and spacing). Medications and drugs of abuse with liquid chromatography time-of-flight (LC-TOF) technology (26). An in-house database comprising nearly 550 drugs were analyzed to investigate the selectivity. Matrix effects were investigated qualitatively by infusion of analyte when injecting negative samples (N = 4). Calibration model was determined analyzing triplicates at eight levels (0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 0.7 and 1.0 µg/g). A mean within 10% of the target value was considered acceptable for validation.

**Quantification of AH-7921**
AH-7921 was obtained from Cayman Chemical Company (Ann Arbor, MI, USA) and the internal standard EDDP-d3 was obtained from Cerilliant (Round Rock, TX, USA).

To 0.5 gram of blood 50 µL of internal standard (1.0 µg/mL of EDDP-d3 in methanol) was added and the blood was precipitated with 1.0 mL of 0.075% formic acid in acetonitrile:ethanol (90:10). After mixing, the sample was centrifuged for 10 min at 5,000 rpm and a 100-µL aliquot was transferred to an autosampler vial and 3 µL were injected.

The analysis was performed on an AT6460 triple quadrupole instrument using an electrospray interface. The analytical column was an Agilent Zorbax Eclipse Plus C18 (2.1 × 50 mm) with 1.8 µm particle size. Mobile phase A was 0.05% formic acid in 10 mM ammonium formate and phase B was 0.05% formic acid in methanol run in a linear gradient from 5% B to 70% B within 3 min at a total flow of 0.5 mL/min. For AH-7921, two transitions were measured (329/284 and 329/173) and one transition for the internal standard (282/235).

Validation was performed as proposed by Peters et al. for methods used in case reports (27). Ten postmortem blood samples with varying degrees of decomposition and 10 samples from living subjects without addition of internal standard, a blood sample with internal standard as well as blood samples fortified with 45 different drugs of abuse were analyzed to investigate the selectivity. Matrix effects were investigated qualitatively by infusion of analyte when injecting negative samples (N = 4). Calibration model was determined analyzing triplicates at eight levels (0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 0.7 and 1.0 µg/g). A mean within 10% of the target value was considered acceptable for validation.

<table>
<thead>
<tr>
<th>Case</th>
<th>BWI</th>
<th>Time of death</th>
<th>AH-7921 (µg/g)</th>
<th>Other drugs in femoral blood (µg/g)</th>
<th>Cause of death</th>
<th>Manner of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23.6</td>
<td>December, 2012</td>
<td>0.81</td>
<td>Gabapentin 10</td>
<td>Intoxication</td>
<td>Accident</td>
</tr>
<tr>
<td>2</td>
<td>32.1</td>
<td>January, 2013</td>
<td>0.99</td>
<td>Amphetamine 4.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>34</td>
<td>February, 2013</td>
<td>0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Ethanol 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Intoxication</td>
<td>Accident</td>
</tr>
<tr>
<td>4</td>
<td>28.1</td>
<td>March, 2013</td>
<td>0.20</td>
<td>Alimemazine 0.08</td>
<td>Intoxication</td>
<td>Accident</td>
</tr>
<tr>
<td>5</td>
<td>42.4</td>
<td>April, 2013</td>
<td>0.30</td>
<td>Alprazolam (positive) 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Intoxication</td>
<td>Uncertain</td>
</tr>
<tr>
<td>6</td>
<td>19.7</td>
<td>May, 2013</td>
<td>0.08</td>
<td>Ethanol 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Intoxication</td>
<td>Uncertain</td>
</tr>
<tr>
<td>7</td>
<td>21.8</td>
<td>June, 2013</td>
<td>0.16</td>
<td>Amphetamine 0.04</td>
<td>Intoxication</td>
<td>Accident</td>
</tr>
<tr>
<td>8</td>
<td>29.1</td>
<td>June, 2013</td>
<td>0.35</td>
<td>3-Methylmethcathinone (positive)</td>
<td>Intoxication</td>
<td>Accident</td>
</tr>
<tr>
<td>9</td>
<td>24.5</td>
<td>July, 2013</td>
<td>0.43</td>
<td>Bupropion 0.40</td>
<td>Intoxication</td>
<td>Accident</td>
</tr>
</tbody>
</table>

*BWII, Body mass index.*
<sup>a</sup>Heart blood.
<sup>b</sup>Ethanol reported at g/L.
establishing the calibration range. Five replicates at three levels (0.02, 0.20 and 0.70 μg/g) were used to estimate repeatability and accuracy.

**Metabolite investigations**

LC-QTOF analysis was performed on an Agilent 6540 quadrupole TOF mass spectrometer (Agilent Technologies, Kista, Sweden) equipped with a JetStream interface in combination with an Agilent 1290 Infinity UHPLC instrument (Agilent Technologies). Mobile phase A consisted of 0.05% formic acid in 10 mM ammonium formate and phase B of 0.05% formic acid in acetonitrile. Separation was achieved within 12 min by a linear gradient chromatography at a 0.5 mL/min flow rate on an ACQUITY UPLC HSS T3 column, 150 × 2.1 mm, 1.8 μm (Waters, Stockholm, Sweden) maintained at 60°C. Ions were generated in positive ion single MS mode (2 GHz), m/z range 50–1,000. Ion source parameters: drying gas 6 L/min at 300°C and sheath gas 10 L/min at 375°C. Data acquisition were performed using MassHunter Acquisition B.04.00 and evaluation was performed using MassHunter Qualitative Analysis B.06.01. Fragmentation investigation on AH-7921 was done by flow injection analysis on a pure standard solution (0.1 μg/mL in methanol). Fragmentation data were evaluated in MassHunter Molecular Structure Correlator B.05.00 software.

**Results and discussion**

The quantitation method for AH-7921 proved simple and accurate. The selectivity tests revealed no interfering peaks at the retention time of the analyte or internal standard. The qualitative matrix effect studies showed matrix effects at 1.75 min and

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**Figure 1.** Upper panel shows MS–MS spectra of AH-7921. Middle panel shows AH-7921 and the two demethylated metabolites and lower panel shows the hydroxylated metabolites found in Case 5.
1.95 min in putrefied autopsy samples but no matrix effects after 1.95 min. The retention time of the internal standard was 2.11 min and that of AH-7921 2.23 min. Calibration model was determined to be best fitted to a quadratic function using 1/X weighting and the range was determined to 0.01–1.0 μg/g blood. The repeatability tests showed coefficients of variation of 5% at 0.02 and 0.20 μg/g, and 1% at 0.70 μg/g. Accuracy was 105–115%, 94–103% and 101–104%, respectively.

In this case series, eight of the deceased were signed out as intoxications and one aspiration caused by intoxication. In four cases, the manner of death was uncertain and in five it was accidental. Table I shows the toxicological findings and the cause and manner of death for each case. The AH-7921 concentrations ranged from 0.03 to 0.99 μg/g blood but there was no case that presented with only AH-7921. In comparison to Vorce et al. (20) the concentrations in our nine cases were much lower but still the pathologist considered these deaths attributed to AH-7921 alone or in combination with other drugs. The signs of opioid toxicity are few; however, pulmonary edema and heavy lungs are commonly seen in opioid overdose deaths even though the mechanisms are incompletely understood (28). The lung weights in seven of the present cases were higher than the reference range of 1.142 g (29) which is compatible with, but not conclusive for, acute intoxication with the μ-agonist AH-7921. In one case, the lungs were donated prior to autopsy and the weights could not be measured.

Deaths from acute overdose of opioids often present with a wide range of blood concentrations and it has been suggested that rather than a lethal concentration, there are other factors that contribute to the death. Factors include poly-drug use, tolerance, allergic reactions and contributing pathology.

Case 1, for example, had only a therapeutic concentration of gabapentin present in femoral blood together with an AH-7921 concentration of 0.81 μg/g. The paraphernalia at the scene suggested that he could have injected AH-7921 and died suddenly. This was also in agreement with high levels of tryptase that might indicate an acute allergic reaction known to occur in deaths related to intravenous injection of narcotics (30).

Poly-drug use has been postulated as a major risk factor in opioid deaths, especially for buprenorphine as very few buprenorphine-only fatal intoxications have been reported (31–33). Six of the cases had other drugs of abuse on board and medications such as benzodiazepines, antidepressants and analgesics were also commonly found. Even though they were found in therapeutic concentrations their presence may have contributed to the delay in death. Pyrazolam and 3-methylmethacatinone were not quantified in cases 4, 5 and 8 and their contribution to death cannot be disregarded.

Another major risk factor in opioid deaths is reduced drug tolerance (29, 34). Due to the scarce literature on AH-7921, we could only speculate on this matter when interpreting the concentrations of the present designer opioid. Even if a majority of the cases were known drug addicts, no information of previous use of AH-7921 was available. Interestingly, only one case presented with other opioids in blood, namely tramadol and codeine (Case 4). In Case 6, hair was analyzed and pointed towards previous use of tramadol but not AH-7921. The other cases might have been naïve opioid users which could have contributed to the fatal outcome. In summary, the interpretation of emerging opioids such as AH-7921 seems as difficult as the more established ones.

Table II
Peak Information from MS–TOF and MS–MS–TOF Analysis of Case 5

<table>
<thead>
<tr>
<th>Peak ID</th>
<th>Compound</th>
<th>Molecular formula</th>
<th>Theoretical mass (M+H)+</th>
<th>RT (min)</th>
<th>Fragments (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH-7921</td>
<td>C16H21Cl2N2O2</td>
<td>329.1182</td>
<td>6.973</td>
<td>284.0593, 201.9812, 189.9815, 172.9545, 95.0849</td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>N-Desmethyl-AH-7921</td>
<td>C15H20Cl2N2O</td>
<td>315.1025</td>
<td>6.781</td>
<td>284.0607, 201.9818, 189.9815, 172.9552, 95.0854</td>
</tr>
<tr>
<td>M2</td>
<td>N, N-Didesmethyl-AH-7921</td>
<td>C14H18Cl2N2O</td>
<td>301.0869</td>
<td>6.523</td>
<td>294.0805, 201.9820, 189.9826, 172.9566, 95.0854</td>
</tr>
<tr>
<td>M3</td>
<td>Hydroxylated AH-7921</td>
<td>C15H22Cl2N2O3</td>
<td>345.1131</td>
<td>5.442</td>
<td>ND</td>
</tr>
<tr>
<td>M4</td>
<td>Hydroxylated AH-7921</td>
<td>C15H22Cl2N2O3</td>
<td>345.1131</td>
<td>5.802</td>
<td>ND</td>
</tr>
<tr>
<td>M5</td>
<td>Hydroxylated AH-7921</td>
<td>C15H22Cl2N2O3</td>
<td>345.1131</td>
<td>8.591</td>
<td>ND</td>
</tr>
<tr>
<td>M6</td>
<td>Hydroxylated AH-7921</td>
<td>C15H22Cl2N2O3</td>
<td>345.1131</td>
<td>9.713</td>
<td>ND</td>
</tr>
</tbody>
</table>

Table III
Metabolite Peak Areas in Relation to the Area of AH-7921

<table>
<thead>
<tr>
<th>Case</th>
<th>AH-7921 (μg/g)</th>
<th>Area% a</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
<th>M6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.81</td>
<td>24</td>
<td>1.2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>0.99</td>
<td>39</td>
<td>5.5</td>
<td>ND</td>
<td>ND</td>
<td>0.3</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.03</td>
<td>867</td>
<td>169</td>
<td>ND</td>
<td>ND</td>
<td>8.5</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.20</td>
<td>279</td>
<td>22</td>
<td>ND</td>
<td>ND</td>
<td>1.4</td>
<td>9.4</td>
<td></td>
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<tr>
<td>5</td>
<td>0.30</td>
<td>128</td>
<td>27</td>
<td>0.3</td>
<td>0.2</td>
<td>0.6</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.08</td>
<td>646</td>
<td>189</td>
<td>ND</td>
<td>ND</td>
<td>3.7</td>
<td>ND</td>
<td></td>
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<tr>
<td>7</td>
<td>0.16</td>
<td>562</td>
<td>91</td>
<td>ND</td>
<td>ND</td>
<td>3.4</td>
<td>ND</td>
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</tr>
<tr>
<td>8</td>
<td>0.35</td>
<td>140</td>
<td>26</td>
<td>0.5</td>
<td>ND</td>
<td>1.3</td>
<td>ND</td>
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</tr>
<tr>
<td>9</td>
<td>0.43</td>
<td>47</td>
<td>14</td>
<td>ND</td>
<td>ND</td>
<td>0.5</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

ND, not detected.
aArea metabolite per area AH-7921.
AH-7921. Unfortunately, they all had very low abundance, ~1% of the AH-7921 peak area and no MS–MS spectra could be obtained. Hydroxylated metabolites may be conjugated with glucuronic acid but when investigated we found no peaks corresponding to the hydroxyl metabolite glycones.

Over a 6-month period, we encountered nine deaths where the μ-agonist AH-7921 was involved and detected in blood from the deceased. Shortly after the last death, on August 1 2013, AH-7921 was scheduled as a narcotic and disappeared from the illicit market in Sweden.

Conclusions
In conclusion, deaths with AH-7921 seem to occur both at low and high concentrations, probably a result of different tolerance to the drug. Hence, it is reasonable to assume that no sharp dividing line exists between lethal and non-lethal concentrations. Further, poly-drug use did not seem to be a major contributing factor for the fatal outcome. The interpretation of emerging opioids such as AH-7921 seems equally difficult as the more established ones and should include a comprehensive drug screening, including hair analysis to investigate poly-drug use and possible tolerance to opioids.

References


