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Case Report

Acute 5-(2-Aminopropyl)Benzofuran (5-APB) Intoxication and Fatality: A Case Report with Postmortem Concentrations

Iain M. McIntyre*, Ray D. Gary, Amber Trochta, Susan Stolberg and Robert Stabley

County of San Diego Medical Examiner's Office, 5570 Overland Ave., Suite 101, San Diego, CA 92123, USA

*Author to whom correspondence should be addressed. Email: iain.mcintyre@sdcounty.ca.gov

A 20-year-old man, a college student, became unresponsive in front of his girlfriend. He was known to consume alcohol and take an unknown drug at some point while in attendance at a local music festival earlier in the day/evening. Upon arrival of emergency personnel, he was noted to be asystolic and apneic. Despite aggressive medical intervention by emergency personnel and at a local hospital emergency room, he was pronounced deceased within 1.25 h of initial medical attention. Postmortem blood initially screened positive for methamphetamine by ELISA. An alkaline drug screen detected 5-(2-aminopropyl)benzofuran (5-APB) which was subsequently confirmed and quantified by a specific GC-MS SIM analysis following solid-phase extraction. Concentrations were determined in the peripheral blood (2.5 mg/L), central blood (2.9 mg/L), liver (16 mg/kg), vitreous (1.3 mg/L), urine (23 mg/L) and gastric contents (6 mg). No other common amphetamine-like compound was detected, although 5-(2-aminopropyl)-2,3-dihydrobenzofuran (5-APDB) was *presumptively* identified in both peripheral blood and urine. Alcohol, the only other drug identified, was confirmed at a concentration of 0.02% (w/v).

Introduction

5-(2-aminopropyl)benzofuran (5-APB) is a recreational drug commonly referred to as 'benzofury'. A phenylethylamine analog, it has positional isomers named 4-APB, 6-APB and 7-APB which have been isolated and differentiated chromatographically and by mass spectrometry (1). These compounds are also considered analogs of 3,4-methylenedioxymethamphetamine (MDA) (2) (see Figure 1). The effects described following doses of 50–120 mg (orally or intranasally) are euphoria, empathy and color enhancement. Side effects include nausea, vomiting, jaw clenching and sleep (3).

The pharmacology and toxicity of 5-APB are not fully understood. 5-ABP has been shown to interact with the dopamine transporter, act as an agonist at 5-HT2A and 5-HT2B receptors, and inhibit monoamine transmitters (4, 5)—therefore consistent with it having both stimulant and hallucinogenic properties similar to other phenylethylamine like stimulants. Compounds that block the dopaminergic active transporter and the norepinephrine transporter, such as 5-APB, also often affect the cardiovascular system, increase blood pressure and increase heart rate (6). High doses and chronic intake, therefore, are likely to cause cardiotoxicity (4).

Although there are descriptions of toxicity, and even drug-related deaths (principally in Europe) (4), there are scant reports of biological concentrations. Chan and colleagues (7) showed a urine concentration of 2.0 mg/L for the 6-APB isomer in a case of toxicity combined with cannabinoids and the synthetic cannabinoid receptor agonist JWH-122. There are no reported concentrations for 5-APB.

We report for the first time a death attributed solely to 5-APB with postmortem concentrations in peripheral blood, central blood, liver, urine, vitreous humor and gastric contents.

Methods

Case report

The decedent was a healthy 20-year-old male. He was reported to have partaken of alcoholic beverages during the day and evening while attending a music festival on his college campus. Eyewitness accounts stated that the decedent also admitted he had taken a drug at some point in the day at the festival. After leaving the festival at midnight, his friend noticed him to 'freeze' at times during their walk back to their apartment. The friend also stated he was 'starting to reach out into the air grabbing at stuff.' He had clenched teeth and was having difficulty breathing. After returning to his apartment with his girlfriend he was witnessed to collapse and become unresponsive—he was noted to be talking 'gibberish' minutes before collapsing. A 9-1-1 call was placed at 0123 h. Upon arrival of emergency personnel, he was noted to be asystolic and apneic. Despite aggressive medical intervention by emergency personnel and at a local hospital emergency room, he was pronounced deceased. The time of death was recorded at 0231 h in the emergency room—within 1.25 h of initial medical care. Examination at the scene of death documented foam and blood exiting the decedent's oral cavity. A 'Chinese research chemical' along with some e-cigarettes were located in the dorm room by the campus police.

The autopsy was begun at 0945 h on the same day as the reported death, and documented white foam in the trachea, marked congestion and edema of the lungs (right lung, 1,070 grams; left lung, 1,240 grams), and congestive splenomegaly. There was a focal contusion of the tongue and of the lower lip. There was no evidence of natural disease or acute trauma to independently account for the decedent's death. There was mild enlargement of the pituitary gland but no monomorphic cell population on histological examination. Comprehensive toxicology screening was requested.

Postmortem specimen collection

All specimens analyzed were collected at autopsy at the San Diego County Medical Examiner's Office. Peripheral blood (~20 mL) was drawn from the left common iliac vein (blood returning from the leg and visually identified in the pelvis at autopsy) and stored in standard glass tubes containing sodium fluoride (100 mg) and potassium oxalate (20 mg). Central blood was

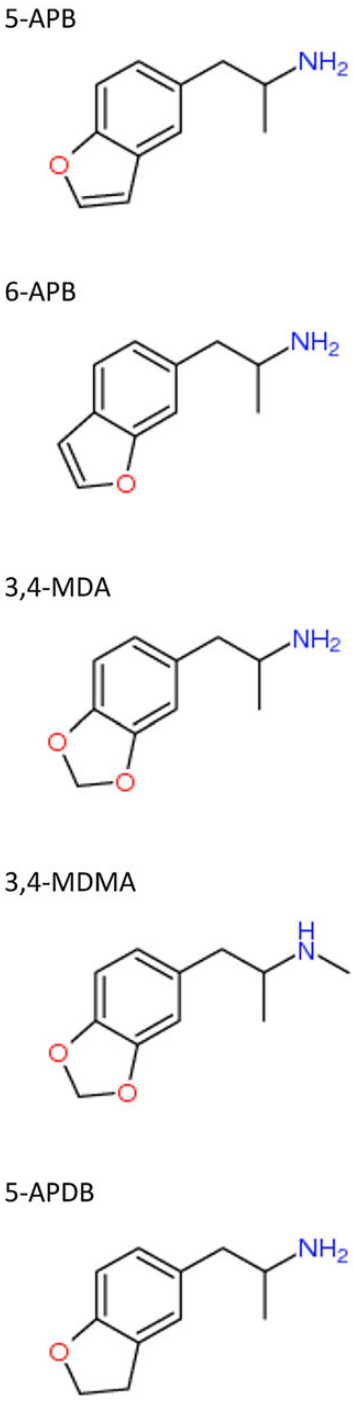

Figure 1. Chemical structures.

collected from the intrapericardial inferior vena cava and placed in identical tubes. Vitreous humor samples were withdrawn from the eye with a syringe and stored in a glass tube without preservative. Urine was collected into in a four ounce container without preservative. Sections of the upper right lobe of liver were collected and also stored in a four ounce container without preservative. The entire gastric contents were collected and stored in a four ounce container without preservative. All samples were stored at 4°C until analyzed.

Toxicology

A comprehensive toxicological screening regimen was requested and performed. Postmortem blood was screened for alcohol and volatile compounds (GC-FID headspace), drugs of abuse by ELISA (cocaine metabolite, opiates, methamphetamine, benzodiazepines, cannabinoids, fentanyl, phencyclidine, oxycodone, methadone, zolpidem, carisoprodol and buprenorphine) (Immunalysis Inc., Pomona, CA, USA), an alkaline drug screen by GC-MS following solid-phase extraction (SPE) and an acid/neutral drug screen with HPLC-photodiode array detection following specimen precipitation with acetonitrile. Positive results were confirmed and quantified by subsequent and specific techniques.

Alkaline drug screen (GC-MS)

The drug screening procedure (8) has been utilized by this laboratory for over 6 years. It consists of a routine SPE technique utilizing SPEWare Trace B extraction cartridges. Two milliliters of calibrators, controls and casework were extracted after the addition of cyclizine (1.0 μg: internal standard) and ascorbic acid (200 μL, 2% solution). Samples were then precipitated with zinc sulfate (5 mL, 5% methanolic solution) and treated with sodium acetate buffer (4 mL, pH 6.0). SPE cartridges were pretreated with 3 mL each of methanol, deionized water and (2 mL) sodium acetate buffer before the addition of samples. Following the extraction of samples, the SPE cartridges were washed with 3 mL each deionized water, (2 mL) acetic acid (0.1 M) and methanol. Cartridges were dried for 3 min and the specimens were eluted with dichloromethane/IPA/14.8 M ammonium hydroxide solution (78/20/2). Samples were then evaporated (30°C, under a stream of nitrogen) and reconstituted with 150 μL of ethyl acetate. One microliter (splitless) of each extract was then injected on to the GC-MS system to attain separation and identification of alkaline drugs. A 15 m, 0.25 mm diameter, 0.25 μm film thickness analytical column (Phenomenex Zebron, ZB-5MS) was used with helium as the carrier gas (1.1 mL/min). The inlet temperature of the gas chromatograph (Agilent Technologies, 7890A) was 250°C, and oven temperature was initially 85°C, ramped 40°C/min up to 170°C (held 4 min), then 40°C/min to 190°C (held 5 min) and finally 10°C/min up to 300°C (held 7 min). The MS Aux was 280°C. The mass selective detector (Agilent Technologies, 5975C) was set in scan mode with a solvent delay of 2.64 min. Peak identification was determined by relative retention time (relative to the internal standard), and then mass spectral matching from a commercial MS library (at least 70% match). The retention time of 5-APB under the listed conditions was 3.1 min.

5-APB confirmation and quantitation analysis (GC-MS SIM)

Materials

Solvents (dichloromethane, methanol, ethyl acetate, isopropanol and acetone) were EMD Chemicals OmniSolv® grade, purchase through VWR International (Radnor, PA, USA). Pentafluoropropionic Anhydride (PFPA) was obtained from Sigma-Aldrich (St Louis, MO, USA). Ammonium hydroxide

(ACS) and glacial acetic acid (ACS) were obtained from VWR International. Zinc sulfate heptahydrate (Certified ACS) was obtained from Fisher Scientific (Pittsburg, PA, USA) and anhydrous sodium acetate (14.8 M, GR ACS Mallinckrodt) was obtained from VWR Inc. 5-APB and 5-APB-D5 were obtained from Cerilliant (Round Rock, TX, USA). SPE columns were Trace-B® from SPEWare Corp. (Baldwin Park, CA, USA). GC column Zebron-1MS was purchased from Phenomenex® (Torrance, CA, USA).

Aqueous working standard containing 1.0 mg/L of 5-APB and internal standard containing 1.0 mg/L of 5-APB-D5 were prepared. A linear calibration curve from 0.02 to 1.0 mg/L produced using five calibrators (0.02, 0.05, 0.25, 0.50 and 1.0 mg/L) were made by diluting the working standard. Linearity was achieved by applying a linear least squares calibration curve ($r^2 \geq 0.99$). All calibrators were prepared in deionized water. Whole-blood controls containing 0.10 and 0.40 mg/L of 5-APB were independently prepared using porcine blood as a matrix, and were run with the calibrators and case specimens. Additionally, both blank (extract containing no additives) and negative control (extract containing only internal standard) specimens were extracted to confirm the lack of interference and/or contamination.

Extraction

The analytical procedure employed was a slightly modified version of previously described and validated techniques (9, 8). 5-APB was extracted using a SPE procedure. A 2.0 mL sample was extracted for all calibrators, controls and casework (blood, vitreous, liver, urine and gastric). Working internal standard (0.25 mL, 5-APB-D5) was added to all tubes. Five milliliters of 5% zinc sulfate/methanol solution was added to each tube. The tubes were then vortexed and centrifuged at 2,400 rpm for 10 min. The supernatant was transferred to new tubes and buffered with 4 mL 0.1 M sodium acetate buffer (pH 6.0). The SPE columns were conditioned by adding sequentially 2 mL each of ethyl acetate, methanol and acetate buffer (pH 6.0). The buffered supernatant was added to the SPE columns and allowed to flow through at 2–5 mL/min. Columns were then washed by adding sequentially 2 mL each of deionized water, 0.1 M acetic acid, methanol and ethyl acetate. Columns were dried at maximum pressure (40 psi nitrogen) for 2 min. Compounds were eluted with 2 mL elution solvent (dichloromethane/isopropanol/ammonium hydroxide, 78/20/2) using gravity flow. The extracts were evaporated in screw top tubes at room temperature under a stream of nitrogen until just dry. Derivatization was accomplished by adding 50 μL PFPA, capping tightly, vortexing and allowing to stand at room temperature for 20 min. After derivatization, samples were diluted with 200 μL ethyl acetate, mixed by vortexing and then transferred to autosampler vials.

Instrumentation

One microliter splitless injections were made onto an Agilent Technologies 6,890 gas chromatograph. The analytical column was a Phenomenex Zebron ZB-1MS (15 m, 0.25 mm diameter, 0.25 μm film thickness) with helium as the carrier gas (1.1 mL/min). The oven was programmed to an initial temperature of 50°C for 1 min, ramped 15°C/min to 200°C and then held at 300°C post run for 3 min. An Agilent Technologies 5,973 MSD operating in selective ion mode (SIM) was utilized for detection. The GC-MS was controlled by Chemstation software. The 5-APB retention time under these conditions was 8.9 min. The total chromatography time per injection was 11 min.

Ions monitored for 5-APB include 158*, 190 and 321 while those for the internal standard 5-APB-D5 include 163*, 194 and 326 (*quantitative ions).

Validation

The limit of detection (LOD) was 0.01 mg/L and limit of quantitation (LOQ), determined from the lowest calibration concentration, was 0.02 mg/L. Control samples, prepared independently at 0.10 and 0.40 mg/L in whole-blood, measured 0.11 and 0.44 mg/L, respectively. Any potentially significant matrix extraction effects were negated by the use of deuterated (5-APB-D5) internal standard.

Results and discussion

The initial methamphetamine ELISA screen was positive, however, none of the routine compounds examined by subsequent GC-MS amphetamine confirmation were identified. The compounds normally detected and quantified with the confirmation method include methamphetamine, amphetamine, methylenedioxymethamphetamine (MDMA), methylenedioxyamphetamine (MDA), ephedrine, pseudoephedrine, phentermine and phenylephrine. This method, previously described (9), has limits of detection and quantitation of 0.01 and 0.02 mg/L, respectively, for all compounds. The ELISA screen, established with 50 ng/mL of methamphetamine as a reference, provided a positive result with about 33% binding compared with a negative sample (100% binding). In this case, the central blood demonstrated 18% binding—a clearly positive finding.

5-APB was initially identified from the SWGDRUG Mass Spectral Library (http://www.swgdrug.org) with the GC-MS alkaline drug screen following the extraction of peripheral blood—subsequently confirmed with extraction and a full mass spectral scan of a pure stock of the compound. It was detected at a retention time of 3.1 min (internal standard cyclizine at 8.8 min) with ions of 44, 131, 77, 132 and 175. Later specific GC-MS SIM quantitation (method described earlier) confirmed concentrations in the peripheral blood (2.5 mg/L), central blood (2.9 mg/L), liver (16 mg/kg), vitreous (1.3 mg/L), urine (23 mg/L) and gastric contents (6 mg). This urine concentration was markedly higher than an earlier report of toxicity with the 6-APB isomer (7) —about 12 times greater—perhaps affirming the magnitude of the dose ingested in the current case.

Interestingly, 5-(2-aminopropyl)-2,3-dihydrobenzofuran (5-APDB) (Figure 1) was also *presumptively* identified from the SWGDRUG Mass Spectral Library in the peripheral blood (alkaline drug screen: RT 3.5 min; ions 44, 134, 133, 77 and 177) and in urine (GC-MS), with a substantially smaller peak area compared with 5-APB—*a pure stock of this compound was not obtained to confirm chromatographic retention which is required for definitive drug confirmation.* It is most likely that this compound was an added constituent to the ingested material, but whether this was a consequence of an intentional or rather accidental (contaminant from synthesis) nature remains unknown at this time.

In the case reported herein, the central blood to peripheral blood (C/P) ratio was 1.16, the liver to peripheral blood (L/P)

ratio 6.4 L/kg and the vitreous to peripheral blood ratio 0.52. Given recent information documenting the L/P ratio as a marker for postmortem redistribution (PMR), these data suggest minimal potential for 5-APB PMR: established on criteria that ratios less than 5 L/kg indicate little to no propensity towards PMR, while ratios exceeding 20–30 L/kg are indicative of a propensity for significant PMR (10, 11).

After a comprehensive toxicology screening, the only other compound detected was ethanol at 0.02% (w/v). Based on the circumstances, autopsy findings, histology and toxicology results, the cause of death was certified due to acute 5-APB intoxication. The manner of death was certified as accident.

In conclusion, the present case describes the first postmortem 5-APB concentrations reported to date. Furthermore, this compound—at the concentrations reported—was determined to be the sole cause of death. Also of note was the positive reactivity on the methamphetamine ELISA drug screen. First revealed by ELISA, then confirmed by a routine alkaline GC-MS screen, concentrations were quantified by a specific GC-MS SIM analysis. A structurally similar compound (5-APDB) was also *presumptively* identified in peripheral blood and urine; perhaps a contaminant from synthesis. 5-APB is not expected to be prone to substantial postmortem redistribution.

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