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Special Issue

A Fatal Intoxication of 2,5-Dimethoxy-4-Chloroamphetamine: A Case Report†

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Designer drugs appear to be increasing in popularity because of the ease of obtaining these constituents, the lack of ability to identify the substance(s) in routine drug screening, the appeal of the drug(s) being 'safe' due to them being marketed as a 'legal high' and possibly due to stronger restrictions that are being placed on prescription drugs. As components of designer drugs are identified and regulated by the DEA, new constituents, or analogs, of these designer drugs are being manufactured to circumvent legislation. 2,5-Dimethoxy-4-chloroamphetamine (DOC) is a substituted alpha-methylated phenethylamine and acts as a selective serotonin receptor partial agonist. There is limited literature on this particular compound and no literature that attributes death to use of this drug alone. We present a case of a 37-year-old male found at home lying face down next to a book titled 'Psychedelic Chemistry' by Michael Valentine Smith and in the early stages of decomposition. The decedent was a known methamphetamine abuser. A peripheral blood sample collected at autopsy was sent to toxicology for routine analysis. Results yielded negative for the drugs of abuse classes on the enzyme-linked immunosorbent assay screen but was positive for DOC during routine GC–MS analysis. A urine sample collected at autopsy was subjected to a routine urine liquid/liquid analysis via GC–MS, and the specimen was positive for DOC. Quantification analyses showed DOC concentration levels to be 377 ng/mL in iliac blood; 3,193 ng/mL in urine; 3,143 ng/g in liver and 683 ng/g in brain. DOC was not detected in the gastric contents. Caffeine was the only other compound detected in blood and urine. Due to the lack of literature, we believe that this is the first case where death can be attributed to DOC alone.

Introduction

Designer drugs appear to be increasing in popularity because of the ease of obtaining these constituents, the lack of ability to identify the substance(s) in routine drug screening, the appeal of the drug(s) being 'safe' due to them being marketed as a 'legal high' and possibly due to stronger restrictions that are being placed on prescription drugs. 2,5-Dimethoxy-4-chloroamphetamine (DOC) is a substituted alpha-methylated phenethylamine and acts as a selective serotonin receptor partial agonist. The psychedelic effects are suspected to act on the 5-HT_{2A} receptor with effects, as described by Alexander Shulgin, 'to include visuals and interpretive problems with knowing just where you are (1).' DOC was presumably first synthesized by Alexander Shulgin and is presented in his PIHKAL book as entry #64 DOC. Shulgin classifies DOC as an archetypal psychedelic, which he further defines as a certain drug that allows for increased personal insight and expansion of one's mental and emotional horizons (1). We present case circumstances, evidence collected at the scene and the results of the toxicology analysis so that other laboratories are aware of and can properly identify this particular compound that is being marketed as a designer drug. There is limited literature on this particular compound and no literature that attributes death to use of this drug alone. Due to the lack of literature, we believe that this is the first case where death can be attributed to DOC alone.

Case history

A 37-year-old male was found at home lying face down next to a book titled 'Psychedelic Chemistry' by Michael Valentine Smith and in the early stages of decomposition. The decedent was a known methamphetamine abuser and various drug paraphernalia was found at the scene. The evidence that was collected and submitted for testing included 2-fluoromethamphetamine, 4-fluoromethamphetamine, 4-fluoroamphetamine, 5-iodo-2-aminoindane, ethylphenidate, aluminum sulfate, a substance that tested positive for mitragynine, and another substance that tested positive for methylethcathinone. The first six bags of evidence collected were purchased online from a company in China and the label stated 'For NMR testing purposes only. Not for *in vivo* testing. Do not consume.' The other two substances were in unlabeled bags at the residence. Additionally, two other bags were collected and they were labeled Green Riau and Green Malay. Both substances were identified as a form of Kratom. The findings at autopsy included pulmonary edema and a subgaleal hemorrhage on the right parietal scalp.

Materials and methods

Whole blood, urine, gastric contents and tissues were collected at autopsy and sent to toxicology for analysis. A whole blood iliac sample was collected in a 10-mL gray top BD Vacutainer tube (BD; Franklin Lakes, NJ, USA) containing 100 mg sodium fluoride/20 mg potassium oxalate preservative and subjected to routine testing of the laboratory, which includes a volatile screen by gas chromatography using flame ionization detection (GC/FID), an abused drug screen by enzyme-linked immunosorbent assay (ELISA), which screens for 12 classes of drugs including amphetamines and methamphetamine, and a basic/acidic/neutral drug screen by gas chromatography–mass spectrometry (GC–MS). The urine sample was collected in a 10-mL red top BD Vacutainer tube (BD) that has no preservative and was subjected to routine analysis via GC–MS. The iliac blood that was collected at autopsy in a 6-mL purple top BD Vacutainer tube (BD) containing 10.8 mg K2 EDTA preservative was analyzed on the GC–MS upon positive identification during routine testing, and quantification values were obtained from this tube of blood. The quantification values from the blood, liver, brain and urine were obtained using an established GC–MS sympathomimetic amine

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(SMA) method in selected ion monitoring (SIM) mode. The gastric contents were subjected to analysis via GC–MS, operated in full-scan mode, upon identification of DOC in the blood and urine.

Determination of DOC

A certified reference standard for DOC was ordered from Cayman Chemical Company (Ann Arbor, MI, USA), and MDA-d_5 (internal standard) was ordered from Cerilliant® (Round Rock, TX, USA). Acetonitrile (ACN, HPLC-grade), methanol (HPLC-grade), ammonium hydroxide, methylene chloride (dichloromethane), 2-propanol (isopropanol, HPLC-grade) and sodium phosphate monobasic monohydrate (crystalline) were all purchased from Fisher Scientific (Pittsburgh, PA, USA). Sodium phosphate dibasic (anhydrous powder) was purchased from J.T. Baker Chemical Company (Phillipsburg, NJ, USA). De-ionized water was obtained from a Direct-Q UV3 water purification system (Millipore Corporation, Billerica, MA, USA). Heptafluorobutyric anhydride (HFAA or HFBA) and the clean screen extraction columns were ordered from United Chemical Technologies (UCT, Bristol, PA, USA). Drug-free bovine whole blood was obtained from the Herman Falter Packing Company (Columbus, OH, USA). Certified human drug-free urine with no preservatives was obtained from UTAK Labs (UTAK Laboratories, Inc., Valencia, CA, USA).

A stock solution of DOC was prepared in methanol at a 34.52 μg/mL concentration level. The working standard DOC solution, concentration 8.63 μg/mL, is prepared by adding one part of the stock solution to three parts methanol. A working stock solution of internal standard, MDA-d_5, was prepared in methanol at a 20 μg/mL working concentration. Calibrators and controls were fortified in a drug-free bovine blood matrix. One negative quality control (QC) was prepared with a 1-mL aliquot of drug-free bovine blood and 25 μL of internal standard. To an aliquot of 1 mL of the unknown whole blood sample, 25 μL of internal standard solution, MDA-d_5, was added. The urine sample was spiked with 25 μL of internal standard and was diluted using 100 μL of sample to 900 μL of certified human drug-free urine. The liver sample was homogenized at a 1:5 dilution and the brain was homogenized at a 1:2 dilution allowing 1 mL of sample to be added with the 25 μL internal standard. Each sample tube had 2 mL of de-ionized water and 3 mL of phosphate buffer added to it. After sample preparation, a solid-phase extraction procedure was performed. The columns were conditioned with methanol, followed by water and then adding phosphate buffer (each solvent was aspirated individually via gravity). The samples were then poured onto the extraction columns. Columns were then rinsed with de-ionized water, acetic acid and hexane, with aspiration in between each step. The drug was then eluted with an isopropanol/ammonium hydroxide/methylene chloride (20:2:78) basic elution solution. Once the drug was collected, the eluate was evaporated to dryness under a nitrogen stream and reconstituted with 100 μL of HFBA. The tubes were then capped, vortexed and allowed to sit at room temperature for 45 min. After incubation, samples were put back under a nitrogen stream for evaporation and reconstituted with 100 μL of acetonitrile. One microliter of the sample was injected onto the GC–MS.

GC–MS Instrumentation

The samples were analyzed on an Agilent 7890A GC/5975C MS (equipped with a Zebron® ZB-50 10 m × 0.18 mm ID × 0.18 μm mini-bore column with an additional 2 m guard) using an established SMA method in SIM mode. Both the injector and interface were both set at 250°C. Injections (1 μL) were made in splitless mode. Ethyl acetate and acetonitrile were used as the wash solvents, with a total of six pre- and postinjection syringe washes between samples. The oven temperature was held at 70°C for 2 min, ramped to 230°C at a rate of 20°C/min with no hold time, ramped to 240°C at a rate of 0°C/min with no hold time and then ramped to 270°C at a rate of 30°C/min. The total run time was 10.0 min. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The ion source was set at 230°C and the quadrupole was set at 150°C. A seven-point linear calibration curve was created for DOC, linear range 43.15–1294.5 ng/mL, and reflected a correlation coefficient of 0.99. The limit of detection and the limit of quantitation were the same concentration 43.15 ng/mL. Low and high positive QC samples (six replicates of each) were prepared at two concentration levels 431.5 and 1294.5 ng/mL, respectively. Intraday low QC had a −1.3% bias and the high QC had a 4.3% bias (both within an acceptable criterion of ±20% of the target value). Percent CV was 4.3 for the low QC and 5.9 for the high QC. Interday values have not been calculated as this is the first instance of this compound and the method is not expected to be routine. Retention times for DOC and MDA-d_5 were 3.60 and 2.57 min, respectively. The target ion for quantification of DOC was 185 with two qualifier ions of 212 and 240. MDA-d_5 had a target ion of 167 with one qualifier ion of 136. Acceptability of quantification was based on ion ratios being within ±20% of the target ion as well as retention time match ±0.02 min.

Results

The initial screening of iliac blood and urine during routine lab testing revealed the presence of DOC. The initial identification of DOC was made during the analysis of the basic, solid-phase extraction using GC–MS in a full-scan mode. The ions that were identified on the fragmentogram were 44, 77, 99, 105, 155, 186 and 229. The formula weight for DOC was calculated to be 266.16 and once the HCL salt was subtracted out the molecular weight was calculated to be 229.70. The 229 ion was indeed present in the fragmentogram. The volatiles screened for in the blood were not detected so the urine sample was not tested. The amphetamine and methamphetamine test kits, used during the ELISA drugs-of-abuse screen in blood, were not detected, reflecting that DOC does not cross-react with said compounds. Kerrigan reports that cross-reactivities were <0.4% for the DO-series drugs using commercial amphetamine, methamphetamine or methylenedioxymethamphetamine (MDMA) assays. Concentrations of 50,000 ng/mL were not sufficient to produce a positive response. This clearly demonstrates that although these kits are extremely effective for the target drugs for which they were intended (methamphetamine, MDMA and amphetamine), they cannot be used to reliably identify these designer drugs, even at concentrations that would greatly exceed those expected in case samples (2). Additionally, the blood sample had no detection of any of the other classes of compounds

screened. Quantitative analyses showed DOC concentration levels to be 377 ng/mL in iliac blood, 3,193 ng/mL in urine, 3,143 ng/g in liver and 683 ng/g in brain. DOC was not detected in the gastric contents of the decedent following a liquid/liquid extraction and full-scan GC–MS analysis. The evidence collected at the scene was subjected to a methanol extraction for qualitative identification using GC–MS. Once the compounds were identified, a whole blood sample from the decedent was mailed to NMS Labs in Willow Grove, PA, and subjected to the Bath Salts and Stimulants Designer Drugs-Expanded Blood Test #8756B, which included compounds identified in the evidence, as well as other amphetamine analogs. The findings on the report revealed that none of the compounds included in the #8756B panel were detected. Therefore, the decedent did not recently ingest any other compounds found at his residence. The cause of death was ruled acute intoxication by DOC (probable 'bath salts'). The manner of death was ruled accidental.

Discussion

A case report of a fatal ingestion of DOC along with a validated method has been presented. DOC is not well understood yet as its prevalence is uncommon. Research and subsequent literature is lacking and fatalities are minimal. The case presented here is the first reported fatality attributed to DOC. Additionally, no reports have been made regarding the ingestion of DOC or demise of an individual from the state of Ohio. DOC has been noted to present itself in Michigan, Florida, New Mexico, California, Kentucky, Kansas and Georgia (2). DOC is a compound that was presumed to have been originally synthesized by Alexander Shulgin, and the re-emergence of this substance appears to have begun. Shulgin reports a dosage range of 1.5–3.0 mg with duration time of 12–24 h. At the highest ingested dose (2.4 mg), Shulgin notes 'gentle images and fantasies along with a relaxed, good feeling.' He further states that 'the body and mind are very comfortable but at 24 h post-ingestion the sleep was not great, dreams were tight and he kept defending against trouble: the nervous system was too alert (1).' Many of the psychedelic phenethylamines derive their effects from their action as 5-HT_{2A} agonists. The profound hallucinogenic effect associated with several of these drugs is likely due to their strong affinity toward serotonin receptor sites (2, 5, 6). Although DOC is classified as a stimulant, we suspect the pulmonary edema and central depression were due to serotonin syndrome. One reference notes further study of serotonin syndrome has determined that overstimulation of primarily the 5-HT_{2A} receptors appears to contribute substantially to the condition (3). No reports of confirmed DOC use are available in the medical literature, although there have been previous user website reports concerning its use and the potential adverse effects (4). Hopefully, other laboratories can build upon the findings presented here and a compilation of results can be formed, relating concentration to a toxic or lethal amount, for future reference. Development of extraction protocols and sensitive confirmatory procedures using GC–MS are needed, because it is the most widely used technique (2). We feel that the findings presented here are of significant value to the Forensic Toxicology field and the general public alike. We believe that this is a good foundation on which future findings can be built upon.

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