Fatal Poisoning With a New Phenylethylamine: 4-Methylthioamphetamine (4-MTA)

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Abstract

There has been much publicity in the United Kingdom regarding a new phenylethylamine-based compound called 4-methylthioamphetamine (4-MTA), also known as para-methylthioamphetamine (p-MTA), MTA or “Flatliner”. Chemically, 4-MTA is an amphetamine derivative and is a non-neurotoxic potent serotonin-releasing agent and reversible inhibitor of rat monoamine oxidase-A. Analysis of postmortem blood and urine specimens in a case implicating 3,4-methylenedioxymethamphetamine revealed the presence of 4-MTA at a concentration of 4.6 mg/L in femoral blood and 87.2 mg/L in the urine. The concentration of 4-MTA in perimortem blood was measured at 4.2 mg/L. This is the first reported case of death involving 4-MTA in the United Kingdom and the first case known to involve 4-MTA only.

Introduction

4-Methylthioamphetamine (4-MTA), also known as para-methylthioamphetamine (p-MTA) or MTA (Figure 1), was first synthesized in 1992 by Nichol et al. (1) as a possible non-neurotoxic serotonin-releasing agent which may have clinical potential. Chemically, 4-MTA is an amphetamine derivative but animal studies have suggested that it exhibits different effects compared to amphetamine (1). These studies also indicated that 4-MTA is a dose-dependent, potent serotonin-releasing agent, and reversible inhibitor of rat monoamine oxidase-A (MAO-A). It is non-neurotoxic at behaviorally relevant doses, and at high doses, it causes serotonergic behavior in rats, including hind limb abduction, flat body posture, reciprocal forepaw treading, and salivation. Further studies appeared to suggest it had a delayed reaction compared to the 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy)-related compounds studied, reducing the blood pressure of the rats but not significantly reducing the heart rate (2).

Although 4-MTA is derived from the classic phenylethylamine structure, it does not appear in the work by Shulgin and Shulgin (3). Its use as a possible drug of abuse was first encountered in 1997 when the compound was seized from illegal drug laboratories in The Netherlands (4). Gas chromatography–mass spectrometry (GC–MS) and nuclear magnetic resonance (NMR) data were published in 1998 concerning a seizure of “SS The One And Only Dominator” in the U.K. (5). It was found that these tablets (14 mm in diameter) contained 138 mg 4-MTA and 99 mg caffeine. The packaging indicated that Ginkgo-biloba, Siberian ginseng, and vitamins B3, B12, and C were also present. There were subsequent seizures in the U.K. of tablets apparently having the street name Flatliners. These tablets were half-scored and resembled known Ecstasy-type tablets in size (9.2 mm in diameter). Analysis of the tablets indicated the presence of (on average) 100 mg 4-MTA only and a very low concentration of impurities (6). At present, 4-MTA is in the process of becoming a controlled substance in the U.K. under the Misuse of Drugs Act.

Only one previous case involving 4-MTA has been reported (4). In 1997, 4-MTA was detected at a concentration of 1.5 mg/L in the blood in a death in The Netherlands; however, 1.5 mg/L of amphetamine was also present in the postmortem blood (4). Therefore, because of the limited number of instances where 4-MTA has been encountered, the exact mechanism of 4-MTA poisoning has yet to be ascertained.

This paper describes the analytical findings in the first reported death in the U.K. involving 4-MTA and the first known death involving 4-MTA in the absence of any other drug. Analysis was performed by GC–MS, enzyme-multiplied immunoassay technique (EMIT), high-performance liquid chromatography with diode-array UV detection (HPLC–DAD) and gas chromatography with nitrogen-phosphorus-specific detection (GC–NPD).
Case History

A 22-year-old male in previously good health had attended a "rave" (outdoor music event) with a group of friends. Various witnesses, later interviewed by the police, reported his appearance and behavior throughout the night (7). At 22:00 on the night of the rave, he appeared to be "fine". Half an hour later, witnesses noticed he was beginning to suffer from stomach cramps and was "fairly sick". Three hours later, he had not improved and was shaking. This continued during the night, and at 06:15 the following morning, he was shaking, sweating, and had trouble speaking. At 07:00, he was unable to stand. He was described as being very hot, shaking, and moaning, and his eyes were rolling back. At 10:00, witnesses reported that the victim was sweating, experiencing breathing problems, foaming at the mouth, and having convulsions. He collapsed and was taken to the hospital; however, resuscitation was unsuccessful, and he died at 12:58. Circumstantial evidence suggested that he had taken seven or eight Ecstasy-type tablets, but surprisingly none of the witnesses had seen him take anything. A post-mortem examination two days after death revealed no evidence of natural disease. Specimens of body fluids taken at post-mortem and blood taken during attempted resuscitation (perimortem) were submitted for toxicological analysis.

Experimental

Chromatographic equipment

HPLC-DAD analysis was performed using an M480 High Precision pump, a column oven, a Gina 50 autosampler, and a UVD340S diode-array detector, all from Gynkotek (Macclesfield, Cheshire, U.K.), with an X-Act 4-channel degasser from JouR Research. A Waters Spherisorb S5OD-CN 4.6-mm x 150-mm cartridge column (Watford, U.K.) protected by a 4-mm x 10-mm guard column of Spherisorb S5OD2 was used for the analysis. Data acquisition was handled by a Gynkotek Chromel computer package running on an Elanex Pentium PC with the diode-array detector recording spectral data between 200 and 595 nm.

The GC system was an Ai GC 94 (Ai Cambridge Ltd., Cambridge, U.K.) fitted with a nitrogen-phosphorus-specific detector (NPD) and a flame ionization detector (FID). The GC was fitted with a CTC Analytics A200SE autosampler (Zwingen, Switzerland). The column was a DB5, 25-m x 0.32-mm capillary column with a 0.52-μm film thickness (J&W Scientific, Folsom, CA).

GC-MS analysis was performed using a Hewlett-Packard (Bracknell, U.K.) HP 6890 GC system fitted with an HP 6890 series mass selective detector (MSD). A Hewlett-Packard HP5 MS capillary column (30 m x 0.25 mm, 0.25-μm film thickness) was used.

Materials

The 1.0M triethylammonium phosphate buffer (pH 3.0) was supplied by Fluka (Gillingham, Dorset, U.K.), and the HPLC-grade acetonitrile was supplied by Rathburns Chemicals Ltd. (Walkerburn, Scotland). The HPLC-grade 1-chlorobutane and isopropanol (propan-2-ol) were obtained from Fisher Scientific Int. (Loughborough, U.K.). Fenfluramine hydrochloride was supplied by Sigma-Aldrich (Poole, Dorset, U.K.). Diethylpropion was obtained from Wyeth Laboratories (Maidenhead, Berkshire, U.K.). Pure 4-MTA hydrochloride was obtained from Drs. Huizer and Poortman (Forensic Science Laboratory, Rijswijk, The Netherlands) and was used to prepare reference and calibration standards for the formal identification and quantitation of 4-MTA in the specimens analyzed. 4-MTA calibration standards (0.5, 1, 2.5, 5, and 10 mg/L) were prepared in blank equine plasma and blank human urine.

Extraction methods for biological specimens

For HPLC-DAD screening purposes, 0.5 mL 0.2M Na₂CO₃ (pH 10) solution was added to 0.5 mL of blood/urine followed by 5 mL of 1-chlorobutane in a 12-mL polypropylene tube. After 3 min shaking and 3 min centrifuging at 4500 rpm, the supernatant was transferred to a second tube. Drugs were extracted into 100 μL 0.05M H₂SO₄, and after 3 min shaking and 3 min centrifuging at 3500 rpm, the supernatant was aspirated and 100 μL of the acid layer was transferred into a vial for injection. The injection volume was 30 μL.

For the HPLC-DAD quantitation procedure, 0.5 mL 10 mg/L fenfluramine internal standard (in 0.2M Na₂CO₃ solution) was added to 0.5 mL of sample/standard, followed by extraction with 5 mL 1-chlorobutane and back extraction with 100 μL 0.05M H₂SO₄ as for the screening procedure detailed here previously. The injection volume was 20 μL.

For GC-NPD screening purposes, 0.2 mL saturated NH₄Cl (adjusted to pH 9 with 0.88M NH₄OH) solution was added to 0.5 mL of blood/urine followed by 5 mL of 1-chlorobutane/isopropanol (9:1) in a polypropylene 12-mL tube. After 3 min shaking and 3 min centrifuging at 4500 rpm, the supernatant was transferred to a second tube. H₂SO₄ (0.5 mL, 0.02M) was added to the solvent and vortex mixed for 1 min. The solvent was aspirated, and the acid layer was transferred into a dreyer tube. Drugs were extracted into 100 μL butyl acetate (containing 10 mg/L hydrocarbon markers) after the addition of 100 μL 5M NaOH solution. After vortex mixing for 2 min, the butyl acetate layer was transferred into a vial for injection. The injection volume was 1 μL.

For the GC-NPD quantitation procedure, 100 μL 5M NaOH was added to 100 μL of sample/standard followed by addition of 100 μL 10 mg/L diethylpropion internal standard (in water) and extracted with 100 μL butyl acetate. The injection volume was 1 μL.

Chromatography conditions

For HPLC-DAD screening, a previously described method based on single-step gradient elution (0-70% acetonitrile in 15 min, holding at 70% acetonitrile for 3 min) was used (8). The screening procedure was based on 20% acetonitrile isocratic elution conditions at a flow rate of 2 mL/min. For GC-NPD screening a thermal ramp was used (100°C to 320°C in 28.5 min) with a homologous series of hydrocarbons (C10-C36)
detected using flame ionization to obtain retention index marker values. For GC–NPD quantitation, isothermal conditions were used with the oven temperature maintained at 140°C.

**Results and Discussion**

**Qualitative analyses**

The 4-MTA obtained from The Netherlands was used to obtain GC–NPD, GC–MS, and HPLC–DAD analytical data, including retention index values (RI) for GC (RI = 1571) and HPLC (RI = 214), mass spectral data (Figure 2, molecular ion = 181), and UV spectral data (UV maxima = 205 and 255 nm).

GC–NPD, GC–MS, and HPLC–DAD screening of the postmortem blood and urine specimens from the femoral vein detected only 4-MTA. Using HPLC–DAD, 4-MTA (RI = 214) was identified by retention index value (8,9) and by UV spectral matching (Figure 3). The results also indicated the presence of two possible metabolites of 4-MTA. These compounds have been confirmed as probable metabolites after being detected in three subsequent cases involving 4-MTA intoxication in which the patients survived (10). It is proposed that the earliest eluting compound (RI = 113) is possibly a sulfoxide and the later eluting compound (RI = 181) may be hydroxy-MTA. Only one 4-MTA associate was observed when using GC–NPD, and it had an RI value of 1785, compared to 1571 for 4-MTA parent compound. Immunoassay screening (EMIT) for common drugs of abuse (cannabis, cocaine, methadone, opiates, benzodiazepines, and amphetamines) in the urine detected the presence of an amphetamine-class drug. However, confirmation by GC and HPLC did not indicate the presence of amphetamine; therefore, this result was thought to be due to the potential cross-reactivity of 4-MTA with the amphetamine monoclonal antibody because of their chemical structural similarities. An investigation into the cross-reactivity of 4-MTA with the amphetamine EMIT assay indicated that some degree of cross-reactivity occurred. From the results it appeared that a false-positive amphetamine result could be obtained if the concentration of 4-MTA in the urine was greater than 8 mg/L in the absence of amphetamine or other amphetamine-related substances (10).

Drug screening of the postmortem blood and urine by GC and HPLC–DAD detected no other drugs or drug metabolites (including ethanol). Because of the limited volume of post-mortem blood available, preliminary drug screening was not performed on this specimen.
Quantitative analyses

**HPLC–DAD analysis.** Under 20% acetonitrile isocratic conditions, 4-MTA eluted at 2.57 min, and the 10 mg/L fenfluramine internal standard eluted at 5.37 min (Figure 4). A linear calibration curve was produced from the 0.5, 1, 2.5, and 5 mg/L 4-MTA plasma standards. Duplicate perimortem and postmortem blood samples were analyzed in addition to duplicate 1:2 dilutions. The mean 4-MTA concentrations measured in the perimortem blood and the postmortem femoral blood were 4.3 mg/L and 4.6 mg/L, respectively. Subsequent analyses based on a linear calibration curve produced from 0.5, 1, 2.5, 5, and 10 mg/L 4-MTA urine standards determined the concentration of 4-MTA in the postmortem urine to be 87.2 mg/L.

**GC–NPD analysis.** Under isothermal conditions (140°C), 4-MTA eluted at 4.26 min, and the 10 mg/L diethylpropion internal standard eluted at 3.33 min. A linear calibration curve was produced from the 0.5, 1, 2.5, and 5 mg/L 4-MTA plasma standards. Duplicate perimortem and postmortem blood samples were analyzed in addition to 1:2 dilutions. The mean 4-MTA concentrations measured in the perimortem blood and the postmortem femoral blood were 4.0 mg/L and 4.6 mg/L, respectively. The concentration of 4-MTA in the postmortem urine specimen was not quantitated by GC–NPD.

Overall, particularly in the postmortem femoral blood, the GC–NPD and HPLC–DAD results showed good agreement. Table I shows a summary of the quantitative analyses.

**Conclusions**

As 4-MTA is a relatively new compound, there is the possibility of misidentification or omission during drug screening. This paper describes an analytical approach to the investigation of a fatality due to 4-MTA and reports the first published measurements of perimortem blood in addition to postmortem blood and urine 4-MTA concentrations. Although this is only one case where both perimortem and postmortem blood specimens are available, the results suggest that there is little difference in the concentration in perimortem and postmortem femoral blood. This could indicate that 4-MTA may not undergo significant postmortem redistribution and, like amphetamine, may have a relatively low volume of distribution. Compared to other amphetamine-related compounds (11), a blood concentration over 4 mg/L would be considered to constitute an overdose (e.g., p-methoxyamphetamine and methamphetamine) or at least excessive usage (e.g., MDMA and amphetamine). In the absence of any other findings, the coroner concluded that death was probably due to 4-MTA intoxication and recorded a verdict of accidental death.

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**References**

3. A. Shulgin and A. Shulgin. *Phenylethylamines I Have Known and*...