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Case Report

A fatal case of paramethoxyamphetamine poisoning and its detection in hair

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ABSTRACT

Paramethoxyamphetamine (PMA) is a phenethylamine derivative that is structurally related to 3,4-methylenedioxymethamphetamine (MDMA), but has higher toxicity than MDMA. Here, we report a fatal intoxication case involving PMA. A 36-year-old man was found dead in a hotel room. Toxicological analysis revealed that PMA concentrations were 0.57 and 0.59 mg/L in peripheral and heart blood, respectively. Ketamine and diazepam were also detected in his blood. Based on toxicological results and autopsy findings, the cause of death was determined to be acute fatal intoxication with PMA. Hair analysis using gas chromatography/mass spectrometry was performed and PMA was detected at a concentration of 20.1 ng/mg after methanol extraction for 20 h. This is the first report of the determination of PMA concentration in the hair from a drug abuser.

1. Introduction

Paramethoxyamphetamine (PMA) is a phenethylamine derivative that is structurally and pharmacologically similar to 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). Since PMA first appeared on the illicit drug market in the 1970s, it has gained popularity as a recreational drug among young people because of its MDMA-like hallucinogenic properties [1]. PMA is typically administered as tablets, capsules, or powder and is often sold as ecstasy. PMA exerts its effects by stimulating the release of neurotransmitters such as serotonin and dopamine and by inhibiting the reuptake of neurotransmitters [2]. Similarly to other illicit substituted amphetamines, PMA has been observed to have an abuse potential [3]. PMA shows a delayed onset of action compared with MDMA, resulting in its overdose [1]. The clinical effects of PMA intoxication include tachycardia, agitation, seizure, hyperthermia, and renal failure [1]. While these symptoms are also observed in MDMA abuse, PMA is more toxic [4]. In the 1970s, several cases of PMA-related deaths were reported in Canada and the United States, which led to the street name "death" of PMA [5,6]. Thus, PMA was controlled by legislation in some countries and disappeared from the drug market. However, since the mid-1990s, the reappearance of PMA has become a considerable threat to public health. Fatal intoxications related to PMA were reported in South Australia in 1998 [7], followed by additional cases in the United States, Canada, and Europe [6,8,9].

In Korea, PMA has been controlled under drug regulation acts since 1980 and there has been no report of PMA detection in biological specimens or seized materials in forensic cases. Here, we present toxicological findings from biological specimens obtained from a fatal case of PMA intoxication. Furthermore, we report a validated analytical method for determining the PMA concentration in hair. To the best of our knowledge, this is the first report of PMA quantification in a drug abuser's hair.

2. Case report

2.1. Case history

The deceased was a male, aged 36 years, who was found dead by his partner in a hotel room. He checked into the hotel with his boyfriend, and they fell asleep after intercourse. His friend woke up after approximately 4 h and found that he was not breathing. He called the emergency services and attempted CPR, but the patient was not recovered. This case was reported to the police and consulted for determination of the cause of death. According to documents from the police, the deceased complained of an uncomfortable feeling before falling asleep. His past history of disease or drug abuse was unknown.

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2.2. Autopsy findings

The deceased appeared to be well-nourished. Upon external examination, no injuries or injection marks were identified. Upon internal examination, severe atherosclerosis was identified in the coronary artery of the heart, but the myocardium showed no necrosis or fibrosis. Pulmonary edema and congestion were observed in the lung. No pathological findings or injuries were identified in other internal organs. Samples were collected for toxicological analysis from the heart blood, peripheral blood, gastric contents, urine, and hair (approximately 1–2 cm in length) during postmortem examination.

3. Materials and methods

3.1. Chemicals and reagents

PMA, ketamine, norketamine, 3,4-methylenedioxyamphetamine (MDA)-D_5, ketamine-D_4, and norketamine-D_4 were purchased from Cerilliant (Round Rock, TX, USA). Sodium carbonate, sodium bicarbonate, and trifluoroacetic anhydride (TFAA) were obtained from Sigma–Aldrich (St. Louis, MO, USA). All chemicals were of analytical grade.

3.2. Blood and urine sample preparation

Blood or urine (1 mL) was fortified with 25 μL of 10 μg/mL MDA-D_5, ketamine-D_4, and norketamine-D_4 as internal standards, followed by the addition of 2 mL of 0.1 M sodium carbonate buffer (pH 9.5). Extraction was performed using 5 mL of ethylacetate. After centrifugation, the organic phase was evaporated to dryness at 45 °C under a stream of nitrogen and the residue was derivatized with 50 μL of TFAA for 20 min at 65 °C. The reaction mixture was removed under nitrogen and reconstituted in 50 μL of ethylacetate prior to gas chromatography/mass spectrometry (GC/MS) analysis.

3.3. Hair sample preparation

PMA, ketamine, and norketamine were extracted using a previously described method [10] with minor modifications. Briefly, the hair sample was washed with 2 mL of methanol followed by 2 mL of distilled water and 2 mL of methanol twice, respectively. The sample was then cut and weighed (*ca.* 10 mg), and 3 mL of 1% HCl in methanol was added. After fortification with 25 μL of 1 μg/mL MDA-D_5, ketamine-D_4, and norketamine-D_4, the samples were incubated for 20 h. The extract was evaporated to dryness at 45 °C under a stream of nitrogen. Fifty microliters of ethylacetate and 50 μL of TFAA were added to the residue, and the mixture was incubated at 65 °C for 30 min. Excess TFAA was removed under a stream of nitrogen and reconstituted in 50 μL of ethylacetate for GC/MS analysis.

3.4. GC/MS analysis

Analysis was performed using an Agilent 6890/5975N GC/MS system (Agilent, Santa Clara, CA, USA). The GC was equipped with a fused-silica HP-5MS capillary column (30 m × 0.25 mm × 25 μm) from Agilent. The GC oven temperature was set to 100 °C for 1 min. The oven temperature was increased to 160 °C at a rate of 15 °C/min and maintained at 160 °C for 3 min. Next, the temperature was increased to 280 °C at a heating rate of 30 °C/min and held for 10 min. The injector temperature was 250 °C and the injection volume was 1 μL. The GC/MS was operated in the selected ion monitoring (SIM) mode. The monitored ions for analytes were as follows: PMA, *m/z* 121, 148, 190; ketamine, *m/z* 270, 262, 305; norketamine, *m/z* 284, 275, 256; MDA-D_5, *m/z* 167, 136; ketamine-D_4, *m/z* 274, 266; norketamine-D_4, *m/z* 288, 279 (underlined ions were used for quantitation).

3.5. Method validation

Validation of the analytical methods was performed previously described [11] by determining the following parameters: selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy. Selectivity was determined using 10 different drug-free samples. Linearity was verified by spiking blank samples with the analytes over the concentration range of 50–1000 ng/mL for blood and urine and 0.5–10 ng/mg for hair, respectively. When the analyte concentration exceeded the upper limit of linearity, the specimens were diluted with blank samples. LODs and LOQs were estimated from drug-free samples spiked with decreasing concentrations of analytes. LOD was defined as the lowest concentration at which an acceptable qualifier/quantifier ion ratio was produced and the signal-to-noise ratio was greater than 3. LOQ was determined as the lowest concentration that provided acceptable precision (coefficient of variation $\leq$ 20%) and accuracy (within ±20%). Precision and accuracy for blood and urine were evaluated from five replicates of the samples at two quality control (QC) concentrations (low and high). Low and high QC values were 150 and 750 ng/mL for blood and urine, respectively. Intra- and inter-day precision and accuracy for hair were estimated using five replicates at low and high concentrations on five different days. The low and high QC values for hair were 1.5 and 7.5 ng/mg, respectively. Extraction recovery was determined at low and high QC concentrations by comparing the analyte peak areas of the extracts from five different sources fortified with analytes before and after extraction.

4. Results and discussion

Validation data are summarized in Tables 1 and 2. No interfering signals were observed at the retention times of the analytes or internal standards. Calibration curves were linear over the tested concentrations and the average correlation coefficient (r^2) values were greater than 0.994 for all analytes. The LODs of PMA and ketamine for both blood and urine were 5 and 50 ng/mL, respectively, and the LODs of norketamine for blood and urine were 25 and 10 ng/mL, respectively. The LOQs were 50 ng/mL for all analytes. Within-batch precision and accuracy for blood and urine was satisfactory at all tested QC concentrations. Precision was 1.5–12.5% and 2.2–8.5% in the blood and urine, respectively. Accuracy ranged from −8.1 to 5.2% and from −4.7 to 5.6% in the blood and urine, respectively. Upon processing *ca.* 10 mg of hair, the LODs of both PMA and norketamine were 0.25 ng/mg and their LOQs were 0.5 ng/mg. Both the LOD and LOQ of ketamine were 0.5 ng/mg. Precision and accuracy were within the acceptance criteria [11]. Intra- and inter-day precision values were 2.8–5.0% and 3.7–7.0%, respectively. Intra- and inter-day accuracy ranged

Table 1
Precision and accuracy for PMA, ketamine and norketamine in human blood and urine (n = 5).

Sample	Analyte	Precision (CV, %)		Accuracy (bias, %)	
		Low	High	Low	High
Blood	PMA	6.8	1.5	2.5	−6.6
	Ketamine	4.9	2.4	−8.1	5.2
	Norketamine	12.5	2.0	4.6	−5.3
Urine	PMA	8.5	6.3	2.3	−4.7
	Ketamine	5.5	3.3	5.5	5.6
	Norketamine	7.0	2.2	2.0	5.5

CV, coefficient of variation.

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