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Regular paper

Severe clinical toxicity caused by 25I-NBOMe confirmed analytically using LC-MS-MS method

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Rhabdomyolysis is a relatively rare, but potentially serious complication of various diseases. Muscular injury and resultant release of electrolytes, myoglobin and other enzymatic proteins e.g. creatine kinase (CK) into circulation may result in multi-organ failure requiring an extensive treatment. Non-traumatic causes of rhabdomyolysis, like poisonings, appear to be much more frequent than traumatic causes. We present the case of a patient who developed exceptionally massive rhabdomyolysis, with CK up to 516 455 U/l, after ingestion of a relatively small dose of a novel psychoactive substance known as "Alice in Wonderland". Laboratory quantification was performed using a validated LC-MS/MS method in a whole blood sample.

Key words: 25I-NBOMe, novel psychoactive substances, designer drug, party pill, rhabdomyolysis, multi organ failure

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Abbreviations: 25I-NBOMe, 2,5-dimethoxy-4-iodo-N-(2-methoxybenzyl)phenethylamine; HS/GC-MS, head-space gas chromatography mass spectrometry; HPLC-FL, fluorescent high performance liquid chromatography; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantitation; NPS, novel psychoactive substances; CT, computed tomography; CVVHD CiCa, continuous venovenous hemodialysis with citrate calcium; iHD, intermittent hemodialysis; CK, creatine kinase; LDH, lactate dehydrogenase; AlAT, alanine aminotransferase; AspAT, aspartate aminotransferase; CRP, C reactive protein; INR, international normalized ratio; APTT, activated partial thromboplastin time.

INTRODUCTION

Compound 25I-NBOMe was created in research laboratories as a potent serotonin (5-HT2A) receptor agonist, but because of its high hallucinogenic properties it has been used as an alternative to other drugs such as lysergic acid diethylamide. This has been associated with severe toxicity and fatalities. Prevalence of 25I-NBOMe use and acute toxicity for the years 2010–2015 are given by Wood (Wood *et al.*, 2015).

Rhabdomyolysis is a condition characterized by damage of muscular tissue with resultant release of electrolytes, myoglobin and other enzymatic proteins, such as CK, lactate dehydrogenase (LDH), alanine aminotransferase (AlAT) and aspartate aminotransferase (AspAT), into circulation. This may lead to hypotonia and renal failure due to complex process of fluid escape from circulation to the injured muscles (Huerta-Alardin *et al.,* 2005; Bosch *et al.,* 2009).

Rhabdomyolysis may be of traumatic or non-traumatic origin. The former is associated with crushing, burning, stenosis or occlusion of blood vessels with resultant ischemia, strenuous physical exercise, prolonged seizures or immobilization. The non-traumatic rhabdomyolysis, which appear to be at least 5 times more frequent, may be a consequence of toxic injury caused by medications, illicit drugs, plant toxins, animal poisons, electrolyte and metabolic disorders, infections, neuroleptic malignant syndrome, dermatomyositis and polymyositis. It is usually the result of multiple contributing factors. (Efstratiadis *et al.,* 2007; Keltz *et al.,* 2013).

It is noteworthy that despite the fact that rhabdomyolysis can result from exposure to at least 150 various medications and toxins, it is usually a consequence of toxic injury caused by illicit drugs and alcohol (Melli *et al.,* 2005).

Most patients do not show the classical triad of symptoms associated with rhabdomyolysis, i.e. swelling, muscle pain and discoloration of urine.

The individuals with rhabdomyolysis typically present muscle weakness, pain in isolated muscle groups and/or poor general condition. Biochemical abnormalities include increased activity of CK, observed as early as 4-6 hours after the exposure to an etiological factor of rhabdomyolysis. According to Sharon (Sharon, 2005), the activity of CK in patients with massive rhabdomyolysis ranges between 10 000 U/l and more than 100 000 U/l.

We report a case of a 20-year-old male who developed massive rhabdomyolysis with a maximum serum concentration of CK exceeding 500 000 U/l (normal limit: < 200 U/l) and multi-organ failure after a recreational use of a party pill known as "Alice in Wonderland".

According to the best of our knowledge only Luckoor (Luckoor *et al.,* 2017) described such an extreme increase in CK activity.

Toxicological studies have detected 25I-NBOMe alone as a substance responsible for the clinical picture and the course of intoxication. Laboratory quantification was performed using a validated LC-MS/MS method in a whole blood sample.

MATERIALS AND METHODS

Clinical part

A 20-year-old Caucasian male patient with no significant past medical history was admitted to a hospital after a seizure episode he experienced during a party. According to the witnesses, just before the

Table 1. The most important abnormalities in physical examination and main treatment strategy

System	Main physical findings	Main treatment strategy
Glasgow Coma Score	4 points on (E1+V1+M2)	Intubation
State of consciousness	Deeply unconscious	
Neurological condition	Presented with periodical strains and seizure episodes	Antiepileptic treatment
Cardio-circulatory system	Supraventricular tachyarrhythmia, paroxysmal atrial fibrillation, sinus bradycardia, hypotension	Metoprolol and amiodarone, intensive fluid repletion, continuous infusion of noradrenaline
Respiratory system	Tachypnea, hypoxia, acute respiratory failure	Mechanical ventilation, tracheotomy
Renal function	Oliguria, gross hematuria, anuria	CVVHDF CiCa, iHD
Temperature	Hyperthermia, up to 40°–41°C	Antipyretic, mechanical cooling
Muscular system	Generalized muscle edema, Interfascial tightness syndrome	Supportive treatment.
Hematology.	Coagulation disturbances. anemia, thrombocytopenia	23 units Fresh Frozen Plasma, 10 units Frozen Erythrocytes, 3 units Platelet Concentrate,
Metabolism status	Catabolism	Parental nutrition with Kabiven Peripheral emulsion for 30 days, enteral nutrition
Infectious complications	Infection (carbapenem-resistant *Acinetobacter baumannii*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) in bronchoalveolar lavage fluid	Wide-spectrum antibiotic therapy (gentamycin, colistin)

Legend: CVVHD CiCa; Multifiltrat, continuous venovenous hemodialysis with citrate calcium; iHD; Fresenius, intermittent hemodialysis procedure

attack, the male took an illegal psychoactive agent of unknown name. No traces of opiates, amphetamine, methamphetamine, cocaine, phencyclidine and tetrahydrocannabinol were found in routine toxicological screen of the urine sample. Due to unclear etiology of the patient's condition, comprehensive differential diagnostics were implemented, including computed tomography (CT) of the head and lumbar puncture. None of these tests revealed any significant abnormalities. The most important abnormalities in physical examination, main treatment strategy and biochemical results were shown in Table 1 and Table 2.

The muscle swelling gradually resolved beginning on the 14th day of the hospital stay, and a pulse could be determined on both radial arteries. The status of the patient stabilized during subsequent days, and then gradually improved. Also, normalization of fluid, electrolyte and metabolic balance was observed, along with the return of a spontaneous lower limb mobility in response to touch and acoustic stimulation. After discontinuing the mechanical ventilation, the rehabilitation was intensified, which resulted in a gradual normalization of the patient's motor function.

Analytical part

Analysis of biological material

Reagents and materials. 25I-NBOMe and mephedrone-D_3, used as an internal standard (IS) were purchased from Cerilliant (Round Rock, Texas, USA). Acetonitrile (MeCN) (HPLC-grade) and formic acid (98–100%) were bought from Merck (Warsaw, Poland).

Blank blood samples used for validation of the method and preparing controls were obtained from

Table 2. Laboratory work-up showed as below

Chosen lab tests	Units	Normal range	The largest deviation
pH	–	7.35–7.45	6.4
pCO_2	mmHg	35–48	105
lactate concentration	mmol/l	0.5–1.6	above the detection limit >30
Potassium	mg/dl	3.5–5.1	5.2
Calcium	mg/dl	8.8–10.2	7.72
Phosphorus	mg/dl	2.5–4.5	6.2
CK	U/l	< 200	516 455
Creatinine	mg/dl	0.7–1.2	2.73
AlAT	U/l	5–37	2899
AspAT	U/l	5–37	6722
Total /direct bilirubin	mg/dl	<1.2/<0.3	7.09/4.57
INR	–	0.8–1.2	3.67
Prothrombin activity	%	80–120	27
APTT	s	26–37	75
APTT ratio	–	0.80–1.20	2.25
D-dimer	ng/ml	<500	55 342
Fibrynogen	g/l	1.80–3.50	1.58
Antithrombin III	%	80–120	29
CRP	mg/l	<5.0	154.7

Legend: INR, international normalized ratio; APTT, activated partial thromboplastin time; CK, creatine kinase; AspAT, aspartate aminotransferase; AlAT, alanine aminotransferase; CRP, C reactive protein

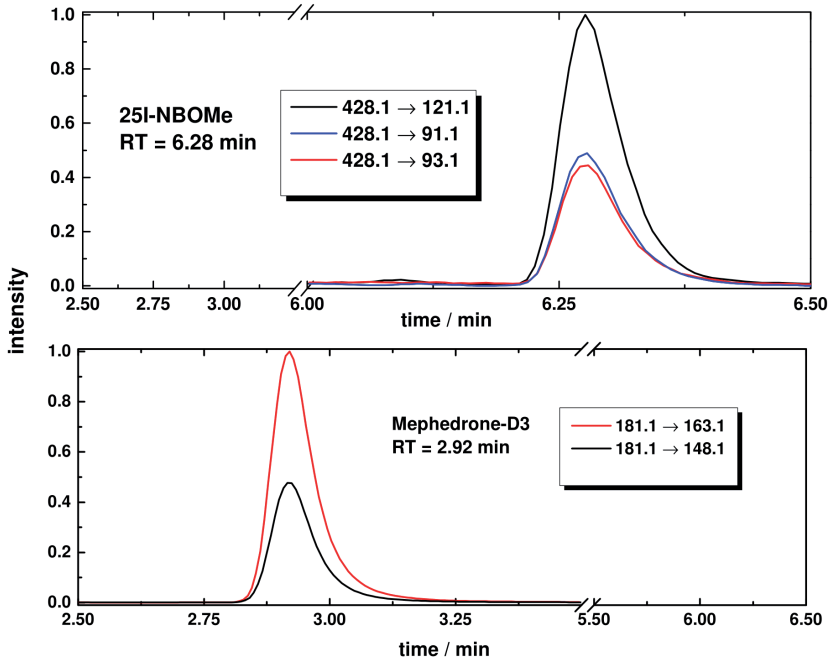

Figure 1. The MRM chromatograms of 25I-NBOMe (0.24 ng/ml) in the blood sample of the patient, and IS (Mephedrone-D3 at 50 ng/ml), obtained with the targeted method.

a regional blood donation center. The blank blood samples were screened for common drugs of abuse, including 143 NPS (Adamowicz *et al.,* 2015), and results were negative. Biological material was stored at –20°C before the analysis.

Standards, calibrators and controls preparation. Stock solutions of 25I-NBOMe (1 mg/ml) were stored at –20°C. Calibrators were prepared in 0.2 ml of drug-free blood samples by spiking them with 25I-NBOMe to the concentrations of 0, 0.1, 0.2, 0.4, and 0.5 ng/ml. Control samples of 25I-NBOMe at 0.1 and 0.5 ng/ml, negative blood controls and mephedrone-D_3 as IS at the concentration of 50 ng/ml were also prepared.

Detection, identification and quantification of 25I-NBOMe. 25I-NBOMe was detected and identified in blood of the patient using LC-MS/MS multianalyte method developed by Adamowicz (Adamowicz *et al.,* 2015). This method fulfilled the requirements for screening and qualitative methods as advised by Scientific Working Group for Forensic Toxicology (SWGTOX) (SWGTOX, 2013), where the limit of detection (LOD) for 25I-NBOMe was set at 0.09 ng/ml in the experiments performed in this article. For quantitative analysis the method was validated as follows. Five microliters of 2 µg/ml methanolic solution of IS were added to the blood samples (0.2 ml) and placed in Eppendorf vials to obtain a final concentration of 50 ng/ml. For all calculations, the ratio of areas of analyte to IS was used. The samples were precipitated with MeCN. To this end, 600 µl of MeCN were added to each sample in 200 µl portions, and after each addition, the samples were vortex mixed for 10 s. Next, the samples were mixed for 5 min and centrifuged at 13 000 rpm for 5 min, followed by the transfer of the supernatant into 2 ml glass vials. Next, supernatant was evaporated to dryness under air at 37°C. The dry residues were dissolved in 100 µl of 0.1% formic acid in water (v/v) and transferred to inserts placed in autosampler vials. The compound was quantified using five-point blood calibration curves.

Instrumental analysis

The blood extracts were analysed using an Agilent Technologies 1200 series liquid chromatograph coupled to a 6460 Triple Quad mass spectrometer. Separation was achieved on a Zorbax SB-C18 (2.1 mm × 50 mm, 1.8 µm) column (Perlan Technologies, Warsaw, Poland) maintained at 25°C. The mobile phase consisting of 0.1% formic acid in MeCN (v/v) and 0.1% formic acid in water (v/v) was composed under the following gradient conditions (shown in relation to MeCN content): 0 min – 10%, 6 min – 100%, 7 min – 10%, and 14 min – 10%. The flow rate was 0.3 ml/min. The injection volume was 10 µl. Dynamic multiple reaction monitoring (dMRM) with positive ion detection was applied. In targeted assay the precursor and three fragment ions of 25I-NBOMe **428.1 → 121.0**, 428.1 → 93.0, 428.1 → 91.0, and of mephedrone-D_3: **181.1 → 163.1**, 181.1 → 148.1, 181.1→ 91.1 (the quantifier shown in bold) were selected for monitoring. Retention time of 25I-NBOMe was 6.28 min, relative retention time – 2.92 min, total analysis time – 14 min. The mass detector parameters were as follows: capillary voltage: 3000 V, gas flow (N_2): 10 l/min, and gas temperature: 325°C, sheath gas flow: 10 l/min, sheath gas temperature: 325°C, nebulizer pressure: 40 psi, cycle time: 500 ms, minimum dwell time: 38.17 ms, maximum dwell time: 246.50 ms, and retention time window: 2 min. The fragmentor voltages and collision energies for 25I-NBOMe were: 118 V and 20, 36 and 56 V, and for mephedrone-D_3: 87 V and 8 and 20 V, respectively.

Toxicological results

Targeted method validation parameters. Results of validation of LC-MS/MS method for a quantitative assay for 25I-NBOMe analysis in blood were as follows. The direct method was found to be selective for

25I-NBOMe. No interfering peaks were observed in the extract of analyte-free blood collected from five persons. Interferences with common drugs typically taken in combination by the drug addicts, and other 141 NPS, were tested and could be excluded due to different retention time and MRM transitions. The LOD was estimated as signal to noise, S/N=3, and it was 0.09 ng/ml for the transition with the lowest intensity (Adamowicz *et al.,* 2015). The five-point blood calibration curve was prepared (number of replicates for each level, n=3) in the range of 0.1–0.5 ng/ml. The value of limit of quantitation (LOQ) was assumed to be the lowest point from the calibration curve, and it was 0.1 ng/ml. The assay was linear in the range of LOQ to the highest calibrator, which was 0.5 ng/ml. The matrix effect at the concentration of 0.5 ng/ml (n=3) was calculated by comparing the response (analyte area/IS area) measured for 25I-NBOMe and blank blood extract samples. Matrix effect for 25I-NBOMe was 76% on average, and showed moderate signal suppression.

Carryover effect should be always considered when working with sub-nanogram quantities. Two main actions were taken to monitor it. First, we analyzed a blank blood sample before the case sample, since the injection of solvent is usually ineffective, and second, we compared the quantitative results of two consecutive case samples. The routine handling of the case samples was separated from the calibration samples.

The reconstituted extracts were stable for a period of more than 24 h at room temperature.

The clinical implications

The MRM chromatogram of the blood sample of the patient, obtained by the targeted method, is presented in Fig. 1. The concentration of 25I-NBOMe in blood of the patient, determined by direct analysis using validated LC-MS/MS method, was 0.24 ng/ml on admission to the hospital. Initial toxicological screens and analyses were performed on the blood samples, including volatiles by head-space gas chromatography mass spectrometry (HS/GC-MS), immunoassay for several classes of drugs, and a screen for other pH-dependent (acidic and alkaline) extractable drugs by GC-MS. Additionally, a targeted analysis for LSD, using fluorescent high performance liquid chromatography (HPLC-FL), was also performed. No volatiles, drugs, or LSD were detected.

DISCUSSION

Recently, numerous cases of severe poisonings and fatal intoxications, especially in teenagers and young adults who have consumed blotter papers containing 25I-NBOMe and/or ingested or smoked other new psychoactive substances (NPS), have been reported all over the world. (Wood *et al*., 2015).

Use of 25I-NBOMe in both liquid and powder form has been described, with many potential routes of administration, including inhalation of vapor, nasal insufflation, oral ingestion, sublingual/buccal administration, and intravenous injection (Weaver *et al.,* 2015).

The most common clinical presentations were sympathomimetic toxidrome (tachycardia, hypertension, mydriasis, agitation, and hypokalemia) plus hallucinations, bizarre behavior, and persistent seizure activity likely associated with serotonergic toxicity (Rose *et al.,* 2012).

Among others, rhabdomyolysis is a relatively common complication of severe NBOMe toxicity (Halberstadt. 2017). One of rhabdomyolysis markers is high activity of creatine kinase in blood (exceeding the normal upper limit 10-25 times, Linares *et al.,* 2009).

A small active dose of 25I-NBOMe and its analogs leads to low concentrations of the compound in the organism, therefore analytical methods to detect and quantify this substance in bio-samples must be highly accurate, sensitive and specific. The most suitable method for this purpose is high performance liquid chromatography with tandem mass spectrometry (LC-MS/MS) (Johnson *et al.,* 2014).

Up to now, limited data have been published concerning concentrations of 25I-NBOMe in intoxicated and fatally poisoned persons, especially after just a single ingestion of this drug. Hill et al. identified, but without quantification, 25I-NBOMe in plasma samples from seven patients (Hill *et al.,* 2013). In the serum specimen drawn from an 18-year-old male admitted to an emergency department, 25I-NBOMe was detected in concentration of 0.76 ng/ml (Rose & Poklis *et al.,* 2013). In 2014 Poklis presented first postmortem case of 25I-NBOMe intoxication documented by toxicological analysis of tissues and body fluids (Poklis *et al.,* 2014). Hermanns-Clausen described a case of a rapid degradation of 25I-NBOMe metabolites in serum after mistaken intake of its concentrated solution. At 50 minutes and 13 hours after the drug ingestion, the concentrations of 25I-NBOMe in serum were 34 and 4.2 ng/ml, respectively (Hermanns-Clausen *et al.,* 2017). In the postmortem heart blood taken from a 16 year old male, who was partying with friends and used a drug that was spotted on blotter paper, the toxicological analyses revealed 25I-NBOMe present at concentration of 19.8 ng/ml (Shanks *et al.,* 2014).

CONCLUSIONS

Validated LC-MS/MS method is the effective tool to measure the concentration of 25I-NBOMe in blood.

25I-NBOMe appears to be potent with a reported concentration of 0.24 ng/ml in blood and can result in life-threatening overdose.

The use of new psychoactive substances containing 25I-NBOMe may result in massive rhabdomyolysis associated with multi-organ failure, sepsis, and severe coagulopathies.

Early CVVHD CiCa seems to be efficient in normalizing disorders associated to muscle injury and treating complications of severe rhabdomyolysis.

Declaration of interest

The authors declare no conflict of interest.

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