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An application of general unknown toxicological screening in emergency medicine of new psychoactive drugs poisoning – a case report

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An analytically confirmed case of acute and combined designer drugs intoxication of a 28-year-old man hospitalized in coma (Glasgow Coma Scale score 3) and the application of toxicological analyses in the clinical medicine is reported. The patient was admitted with reduced level of consciousness and generalized tonic-clonic seizures, sinus tachycardia with heart rate of 120-140 beats/min and blood pressure of 210/120 mmHg, dilated pupils, respiratory acidosis (pH 7.27, bicarbonate 21.1 mmol/L and base excess – 2.9), cyanosis, an ineffective breathing. An urgent toxicological analysis was performed, which covered a detailed analytical strategy using immunoassay (multi-drug panel), headspace-gas chromatography with flame ionization detector (HS-GC-FID) for toxic alcohols, and general unknown screening by gas chromatography coupled to mass spectrometry (GC-MS). Three different drugs, deschloro-N-ethyl-ketamine (eticyclidone or 2-oxo-PCE or O-PCE), 3-methoxyeticyclidine (3-MeO-PCE) and 4-chloro-2,5-dimethoxyamphetamine (DOC) were identified in the urine sample. The presence of these drugs was also confirmed in the blood sample. The results from the urgent toxicological analysis were used for an adequate treatment of the patient. The patient's heart rate and blood pressure were normalized 48 hours after hospitalization. Seizure activity was minimized and spontaneous respiration was restored 4-5 days later. Mental status was normalized on the 10^{th}-12^{th} day of hospitalization.

Keywords: urgent toxicological analysis; general unknown screening; new psychoactive substances (NPS); 2-oxo-PCE, 3-MeO-PCE, DOC

INTRODUCTION

New psychoactive substances (NPS) or so-called designer drugs are functional or structural analogues of narcotic and psychotropic drugs of abuse (DoA). These substances are designed to mimic the psychological and/or pharmacological properties of the "traditional" drug such as tetrahydrocannabinol, opiates, amphetamines, etc. [1, 2]. Recently, they are available on the black market and freely sold over the Internet as safe and legal substitutes of controlled drugs. At the same time, the wide variety of different NPS makes them difficult to test in forensic and clinical practice due to the negative toxicological screening in routine drug analysis.

Deschloro-N-ethyl-ketamine (eticyclidone or 2-oxo-PCE or O-PCE, Figure 1a) and 3-methoxyeticyclidine (3-MeO-PCE, Figure 1b) belong to the arylcyclohexylamines and are eticyclidine (PCE)-like derivatives. Drugs in this class possess N-methyl-D-aspartate (NMDA) receptor antagonist activity with variable effects at several other receptors ($5HT_2$-, D_2- and σ-receptors, etc.) [3-5]. Both, 2-oxo-PCE and 3-MeO-PCE, are associated with ketamine (KET)-like dissociative and mild sympathomimetic effects, but differ in potency and duration of action. Clinical features of patients exposed to either of these drugs include impaired consciousness, convulsions, hypertension, tachycardia, etc.

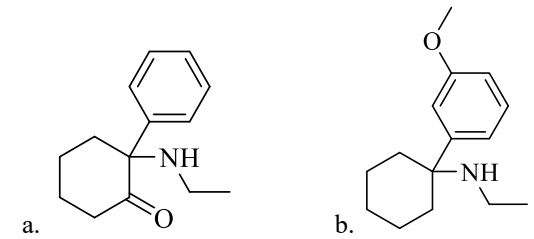

Figure 1. Chemical structure of: **a.** deschloro-N-ethyl-ketamine (eticyclidone or 2-oxo-PCE or O-PCE) and **b.** 3-methoxyeticyclidine (3-MeO-PCE).

4-Chloro-2,5-dimethoxyamphetamine (4-chloro-2,5-DMA or DOC, Figure 2) is a ring-substituted phenethylamine analog, that belongs to the 4-substituted-2,5-dimethoxyamphetamines (DO*x*) series. This class NPS are long-acting psychedelic

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drugs that act as highly selective 5-HT_2 (serotonin) receptor partial agonists [6-8]. They produce potent hallucinogenic effects and dysphoria, with minimal sympathomimetic activity and prolonged duration of action. Symptoms include hallucinations, convulsions, hypertension, tachycardia, and mydriasis.

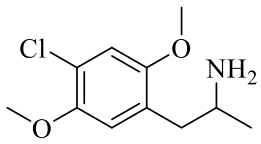

Figure 2. Structure of 4-chloro-2,5-dimethoxy-amphetamine (DOC).

The present case report describes a non-fatal exposure to 2-oxo-PCE (Figure 1a), 3-MeO-PCE (Figure 1b) and DOC (Figure 2) with severe clinical symptoms as coma, prolonged seizures and acute cardiovascular problems. The three drugs are analytically confirmed by a general unknown toxicology screening using gas chromatography-mass spectrometry (GC-MS) of the patient's blood and urine samples. The report also highlights the usefulness of this procedure in cases involving recreational DoA and has a direct impact on the clinical management of individual patients.

CASE DESCRIPTION

A 28-year-old man with a history of various drug abuse (6-7 years) was found unconscious. He was admitted to the hospital by ambulance cyanotic with generalized tonic-clonic seizures and ineffective breathing, sinus tachycardia with a heart rate of 120-140 beats/min and hypertension (210/120 mmHg blood pressure). On arrival at the emergency department (ED), he had a Glasgow Coma Scale (GSC) score of 3 out of 15. His body temperature was 37.4°C. The patient's pupils were bilaterally symmetrically dilated (6.0 mm) and unresponsive to light. His initial electrocardiography confirmed sinus tachycardia with normal QRS duration and corrected QT interval. He had respiratory acidosis (pH 7.27, bicarbonate 21.1 mmol/L and base excess – 2.9 mEq/L).

A complete blood count (CBC) and some basic biochemical and hemostatic tests were performed. Most of the results were within the reference ranges, except for these parameters, presented in Table 1. An urgent toxicological analysis was also performed (described below). Blood and/or urine samples were analyzed by headspace-gas chromatography with flame ionization detector (HS-GC-FID) for alcohols and by panel immunoassay for multiple drugs, as well as by gas chromatography coupled to mass spectrometry (GC-MS) for general unknown screening of DoA.

The patient was intubated (continuous mandatory ventilation (CMV), FiO_2 70%) and transferred to the intensive care unit (ICU). Complex therapy included: clonidine, nitroglycerin, dexamethasone, mannitol, torsemide, paracetamol, diazepam, ceftriaxone, gastro- and hepato-protectors (esomeprazole, L-ornithine-L-aspartate, ademetionine), vitamins (B_1 and B_6).

Table 1. Some of the patient's biochemical and hemostatic test results and their reference ranges.

Parameter	Patient's result	Reference range
White blood cells (WBC), $\times 10^9$/L	25.3	4.5 ÷ 9.5
Neutrocytes, $\times 10^9$/L	19.8	1.0 ÷ 6.8
INR, sec	1.3	0.8 ÷ 1.2
Activated partial thromboplastin time (aPTT), sec	20	32 ÷ 51
Blood glucose (Glu), μmol/L	7.4	2.8 ÷ 5.6
Serum iron (sFe), μmol/L	8.5	12.5 ÷ 26.7
Total bilirubin (TBil), μmol/L	37.1	3.4 ÷ 21.0
Aspartate aminotransferase (AST), U/L	154	14 ÷ 36
Alanine aminotransferase (ALT), U/L	92	10 ÷ 40
Creatinine kinase (CK), U/L	461	< 200
Creatine kinase myocardial band (CK-MB), U/L	108	< 25
Lactate dehydrogenase (LDH), U/L	691	< 460
Troponin I (TPN I), ng/mL	0.1	< 0.01
α-amylase (α-AMY), U/L	244	< 96
Uric acid (UA), μmol/L	762	208 ÷ 387

Additionally, piracetam (from the second day) and phenobarbital (from the third day) were added to the therapy.

On the fourth day of hospital stay, sedation therapy was discontinued. The ventilation mode was changed from CMV to synchronized intermittent mechanical ventilation (SIMV) and, after two hours of adaptation, to adaptive support ventilation (ASV). The patient was disoriented and agitated. Six hours later his condition deteriorated, so he was again anaesthetized, relaxed and placed on SIMV. Pupils were still mydriatic.

On the fifth day, the patient was extubated and supported with T-tube, but due to severe psychomotor agitation, he was sedated and intubated again.

Finally, the patient was extubated on the sixth day. The mental status remained problematic in the following days (agitation, disorientation, amnesia, verbal and motor response suppression, etc.). The patient was discharged on day 12 in a stable condition and mental status (on long-term therapy with valproic acid and quetiapine).

MATERIALS AND METHODS

All reagents and solvents were of analytical grade or chromatographic grade, respectively. Acetonitrile (MeCN), ethylacetate (EtOAc), n-propanol (n-PrOH), sodium hydroxide (NaOH), and anhydrous magnesium sulphate ($MgSO_4$) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Deionized water (DI, Q-Front N HPLC QF-2303, Adrona, Latvia) with a resistivity of 18.2 MΩ×cm and a conductivity of 0.055 μS was used in all experiments as needed.

The patient's blood sample (fluoride/oxalate vacuum tube with grey cap) was analyzed for alcohols (ethanol and methanol) by HS-GC-FID (7890B GC, 7697A HS, Agilent Technologies, USA). In a 10 mL headspace vial, 100 μL blood was added to 500 μL of internal standard solution – 0.2 g/L n-PrOH. The vial was immediately sealed with headspace crimp aluminum caps equipped with PTFE silicon septum and then HS-GC-FID analyzed. Details of acquisition and processing methods can be found below.

GC conditions: helium carrier gas; 1.5 mL/min constant flow rate; 300°C FID temperature; FID gas – hydrogen 30 mL/min, air 300 mL/min and nitrogen 10 mL/min; split type injector, 200°C; 40:1 split ratio; DB-WAX Ultra Inert chromatographic column (30 m × 0.25 mm × 0.25 μm); temperature program – 40°C for 4 min, then raised at a rate of 50°C/min to 110°C, held for 1 min.

HS parameters: 85°C oven temperature; 95°C temperature of the sample valve; 110°C transfer line temperature; 10 min vial equilibration; 1 mL loop size; 1 min injection duration. Total run time was 6.3 min. Blood alcohol was determined using linear calibration curve ranged from 0.00 to 4.00 g/L ethanol (blank sample and standard solutions; Medichem SA, USA).

A multi-panel immunoassay from AllTest Biotech Co (Hangzhou, China) was used to screen the patient's blood (heparin vacuum tube with green cap) and urine (container) samples for DoA. The rapid blood test consists of nine parameters – tetrahydrocannabinol (THC), amphetamine (AMP), methamphetamine (MET), 3,4-methylenedioxy-methamphetamine (MDMA), cocaine (COC), opiates (OPI), methadone (MTD), benzodiazepines (BZD), and barbiturates (BAR) with the following corresponding cut-offs: 35, 80, 70, 50, 50, 40, 40, 100, and 100 ng/mL. The rapid urine test consists of 10 parameters – THC, AMP, MET, MDMA, COC, OPI, MTD, BZD, BAR, and tricyclic antidepressants (TCA) with the following respective cut-off values: 50, 1000, 1000, 500, 300, 2000, 300, 300, 300 and 1000 ng/mL.

General unknown screening by GC-MS (7890B GC, 5977A MSD, Agilent Technologies, USA) was also performed on patient blood (heparin vacuum tube with green cap) and urine (container) samples. 2 mL blood or 6 mL urine were deproteinized with 4 mL or 1 mL MeCN and then 2 mL or 1 mL 1 M NaOH were also added, respectively. After that, liquid-liquid extraction (LLE) with 7 mL of EtOAc was carried out. The resulting samples were vortex mixed (Vortex, Boeco, Germany) and centrifuged (3 min, 3000 rpm; Centrifuge T54, MLW, Germany). The organic layers were transferred to a clear tube, dried with $MgSO_4$, evaporated to dryness under nitrogen and reconstituted with 100 μL EtOAc. Then, the samples were analyzed by GC-MS equipped with DB-1701 capillary column (30 m × 0.25mm × 0.25 μm). MS Library searching was performed using PMWTox3N, DD2011, and NIST 2011. For details on the acquisition and processing methods, see below.

GC parameters: 1 μL injection volume; splitless mode; column temperature profile – 50°C for 1 min, to 150°C for 10°C/min, to 280°C for 8°C/min, held for 15 min, helium carrier gas, 1.5 mL/min column constant.

MS parameters: 270°C transfer line temperature; 250°C temperature of the front inlet; 230°C ion source temperature; filament delay 4.5 min; 50-500 Da mass range; full-scan mode; 70 eV electron energy.

RESULTS AND DISCUSSION

The importance of general unknown toxicological screening in clinical medicine significantly increased in the recent years. Access to a large number of DoA and NPS is now a diagnostic challenge to confirm or exclude an abuse or poisoning in emergency medicine. Nowadays, we perform a general unknown screening as a routine diagnostic tool, which includes a large number of well-known compounds (such as ethylene glycol, prescription drugs as paracetamol, bisoprolol, etc., and popular DoA) as well as screening of unknown compounds using coupled chromatography-mass spectral methods.

In the case described, based on the patient's manifestation of KET-like dissociative and sympathomimetic effects and reported history of recreational drug abuse, clinical suspicion of NPS intoxication was raised. Therefore, a typical screening workflow for the identification of alcohols and DoA in blood and/or urine samples was performed to determine the cause of intoxication. Analytical detection of NPS has always been a challenge due to the protean nature and diverse chemical structures of these drugs.

The blood sample was tested for toxic alcohols. The result was negative for the presence of ethanol and methanol.

The urine sample was first profiled by multi-drugs immunoassay and then analyzed by GC-MS for common drugs and DoA. The 10-parameter rapid immunoassay was positive for OPI and THC. The general unknown screening analysis revealed 2-oxo-PCE, 3-MeO-PCE plus their metabolites and DOC. In fact, the initial breakthrough in the current case came from a match to the three NPS compounds in the available mass spectra libraries. Morphine was also detected. Figure 3 generally illustrates the abundance of each NPS compound. The mass spectra of the three identified NPS compounds are shown in Figure 4.

The 9-parameter multi-drugs immunoassay of the blood sample was positive for THC only. 2-oxo-PCE, 3-MeO-PCE and DOC were also confirmed in the patient's blood by GC-MS analysis. The quantitative analysis of these drugs was not performed due to the lack of an analytical standard (reference material). OPI were not found in the blood. These data, along with the morphine identified in the urine sample, suggested that morphine had been used in the past. However, the amount of THC metabolite (11-nor-9-carboxy-Δ^9-tetrahydrocannabinol) found was 219 ng/mL, indicating recent use. Quantitative analysis of unmetabolized THC was not performed due to technical incapability.

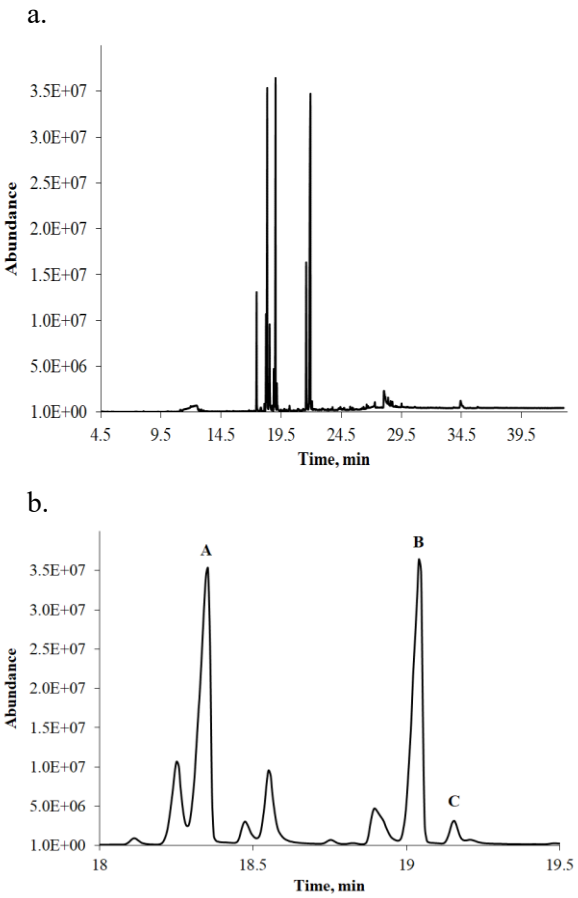

Figure 3. GC-MS chromatogram of the urine sample. **a.** Full total ion chromatogram (4.5-43.8 min), **b.** Total ion chromatogram from 18.0 to 19.5 min. A: 2-oxo-PCE, B: 3-MeO-PCE, C: DOC.

NPS compounds were not found in the patient's blood on the second day of hospitalization and in urine on the seventh day.

Cases of intoxication with any of the three NPS identified in the present patient have been reported in the literature [9-18]. Some of them have ended fatally. Frequently, different NPS are found together, and are either co-consumed with prescription or other illicit drugs, and ethanol. Clinical effects include mainly neurological (agitation, delirium, abnormal behavior and convulsions) and cardiovascular effects (hypertension, tachycardia). Although excessive cardiovascular stimulation is seen, respiratory depression remains the most life-threatening complication. The medical management of such patients is essentially based on symptomatic treatment with hyperhydration (to stimulate excretion) and tracheal intubation (to prevent respiratory failure). Clinical biochemical variables (especially cytolysis markers, creatinine, and CK/CK-MB) and vital signs (arterial oxygen

saturation, heart rate, and blood pressure) must be closely monitored.

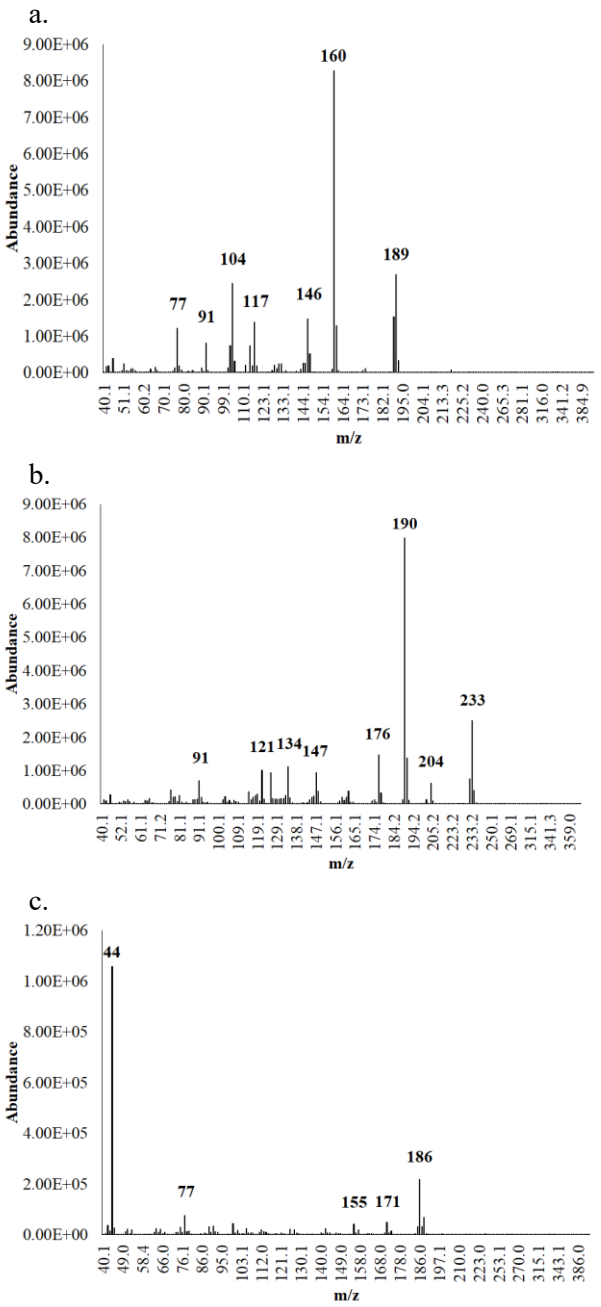

Figure 4. Mass spectra of **a.** 2-oxo-PCE, **b.** 3-MeO-PCE, and **c.** DOC.

This report illustrates the significance of the toxicological analyses in the emergency clinical medicine, particularly in the identification of NPS. Clinical suspicion was initially raised when the patient presented to the ED with persistent seizure activity, ineffective spontaneous breathing, and decreased consciousness with signs of cardiovascular toxicity. However, the diagnosis of NPS intoxication was accepted only after the toxicological findings were available to answer the initial clinical question.

CONCLUSIONS

The case presents an analytically confirmed combined 2-oxo-PCE, 3-MeO-PCE and DOC intoxication of a 28-year-old man in critical condition with symptoms of cardiovascular toxicity, as well as continuous seizure activity, ineffective spontaneous breathing and reduced consciousness.

Immediately after the patient's hospitalization, an urgent toxicological analysis was performed, including blood and urine toxic alcohols (HS-GC-FID), and also blood and urine drug screening (immunoassay and GC-MS). Ethanol and methanol were not found in the biological samples. A multi-panel immunoassay was positive for THC in the blood sample and for OPI and THC in the urine. The general unknown screening analysis in urine revealed the presence of 2-oxo-PCE, 3-MeO-PCE plus their metabolites and DOC. Identification of these compounds was performed by mass spectral library search. Morphine was also detected in urine, but not in blood. The general unknown screening of the blood sample confirmed the presence of the three NPS drugs – 2-oxo-PCE, 3-MeO-PCE, and DOC. The main THC metabolite (11-nor-9-carboxy-Δ^9-tetrahydrocannabinol) was also found in blood.

The toxicological findings, available to answer the patient's clinical condition, were used first to confirm the diagnosis of NPS intoxication and then to monitor and guide the patient's appropriate treatment.

The NPS drugs were not detected in the blood 48 h after hospitalization and in the urine – on the seventh day, which corresponded to the normalization of heart rate and blood pressure on the second day, and minimization of seizure activity and recovery of spontaneous respiration 4-5 days later. The mental status of the patient normalized on the 10^{th}-12^{th} day of hospitalization.

In general, the present study demonstrates the powerful role of toxicological analysis in cases of recreational DoA intoxication and the application of general unknown toxicological screening in emergency medicine. Therefore, toxicological testing in cases such as the one currently presented should be encouraged and required.

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