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CASE REPORT

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Postmortem distribution of 3,4-methylenedioxy-*N*, *N*-dimethyl-amphetamine (MDDM or MDDA) in a fatal MDMA overdose

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Abstract In this manuscript, a newly identified compound, 3,4-methylenedioxy-*N*,*N*-dimethylamphetamine (MDDM or also called MDDA), was quantified. The substance was identified in the biological specimens of a 31-year-old man who died following a massive 3,4-methylenedioxymethamphetamine (MDMA) overdose. In addition, the postmortem distribution of the identified substance in various body fluids and tissues was evaluated. For MDDM quantitation, a formerly reported and validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method was adapted. The following quantitative results of the MDDM quantitation were obtained: Femoral blood, aorta ascendens, and right atrial blood contained 2.5, 21.7, and 11.6 ng MDDM/ml, respectively. In left and right pleural fluid and pericardial fluid, concentrations of 47.0, 21.7, and 31.9 ng/ml, respectively, were found. MDDM levels in urine, bile, and stomach contents were 42.4, 1,101, and 1,113 ng/ml, respectively. MDDM concentrations in lungs, liver, kidney, and left cardiac muscle ranged from 12.8 to 39.8 ng/g, whereas these levels were below the limit of quantitation (< LOQ) in right cardiac and iliopsoas muscle. In conclusion, for the first time, MDDM was unambiguously identified in a fatal MDMA overdose. MDDM was probably present as a synthesis by-product or impurity in the MDMA tablets, which were taken in a huge amount by the victim, or MDDM was ingested separately and prior to the MDMA overdose. A third option, i.e., the eventual formation of MDDM as a result of postmortem methylation of MDMA by formaldehyde, produced by putrefaction processes or during storage under frozen conditions, is also discussed. The MDDM levels, substantiated in various body fluids and tissues, are in line with the distribution established for other amphetamine derivatives and confirm that peripheral blood sampling, such as that of femoral blood, remains the "golden standard".

Keywords 3,4-Methylenedioxy-*N*,*N*-dimethyl-amphetamine · MDDM · MDDA · Postmortem distribution · Fatality

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Introduction

The abuse of amphetamine and its derivatives, such as 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine (MDEA), and 3,4-methylenedioxyamphetamine (MDA), is an important public issue, and fatalities are not infrequent in current forensic practice. These derivatives are, at present, well known, but some of them are less frequently encountered, such as *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB) [1] and benzodioxolylbutanamine (BDB) [2]. In addition, the toxicological analysis of the metabolites of amphetamine-derived designer drugs [3] in current practice is not always routinely performed. Recently, derivatives such as phenyl piperazine types were reported [4].

The derivative 3,4-methylenedioxy-*N*,*N*-dimethylamphetamine (MDDM, also called MDDA) has, at present, only seldom been reported in scientific literature, but was mentioned by Shulgin and Shulgin [5]. As this amphetamine analog has only recently been identified, the value of this compound is not yet fully elucidated. However, referring to the structure of MDDM, the potency of this substance is probably reduced, which was also indicated by Shulgin and Shulgin [5].

In this manuscript, the distribution of MDDM, which was incidentally found in a fatal MDMA overdose [6], is described. In Fig. 1, the chemical structures of MDMA and MDDM/MDDA are presented.

Case history

On 10 September, during a warm summer week, a 31-year-old man, known to have an alcohol abuse problem, was found dead at home next to his bed, in an advanced state of putrefaction. The apartment was locked and all windows were closed. The heating system was turned off, but, regretfully, the ambient temperature was not recorded.

At *external examination*, severe putrefaction signs were found, such as generalized bloating of the body, venous marbling, and epidermolysis. The combination of the external findings and the police inquiry pointed to a postmortem interval of about 7 days. The man weighed 85 kg and was 178 cm tall (body mass index=26.8).

At *autopsy*, 600 ml of bloody fluid was present in both pleural cavities, and putrefaction of all organs was confirmed. However, pulmonary and generalized visceral congestion could still be recognized, which was confirmed by the increased weight of the lungs (1,230 g). A somewhat enlarged heart (weighing 350 g), although without substantial coronary atherosclerosis, was found. The heart was not adhered to the pericardial sac, which contained about 3 ml of fatty pericardial fluid. The brain, liver, kidneys, and spleen weighed 1,300, 1,250, 325, and 120 g, respectively.

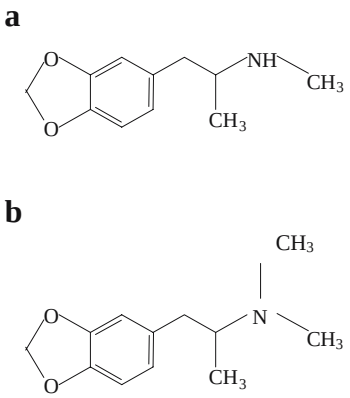

Fig. 1 Chemical structure of **a** 3,4-methylenedioxymethamphetamine (MDMA) and **b** 3,4-methylenedioxy-N,N-dimethylamphetamine (MDDM/MDDA)

A detailed *microscopical* diagnosis was disturbed by the pronounced putrefaction of all the tissues. However, significant cardiac scars (old infarction), as well as important brain lesions (such as a substantial hematoma), could be excluded. In addition, signs of pulmonary congestion and edema, as well as deep aspiration of vomit, were found. Histologically, a few Prussian blue-positive macrophages in the lung slides were observed, but hematoxylin eosin (H&E) stainings of the other organs, such as the liver, kidneys, and the pituitary gland, were inconclusive due to the marked putrefaction.

The brain was also very lytic, and therefore, sampling for toxicological investigation was inappropriate. In addition, the vitreous humor was not available for toxicological analysis.

Materials and methods

Determination of MDDM

For MDDM quantitation in various tissues and body fluids, a previously reported and validated liquid chromatography tandem mass spectrometry {LC-MS/MS [micromass quadrupole-time-of-flight (Q-TOF) hybrid mass spectrometer (electrospray ionization (ESI^+)]} method was adapted [7]. Recently, the performed procedure was reported in detail [8]. Extraction was based on liquid–liquid extraction under alkaline conditions [7], followed by a gradient-based LC-MS analysis on a Hypersil Base Deactivated Silica (BDS) phenyl column [8].

Materials

All reagents and chemicals were of analytical grade and were obtained from Aldrich (St. Louis, MO, USA). All solvents were of high-performance liquid chromatography (HPLC) grade and were provided by Biosolve (Valkenswaard, The Netherlands). The 3,4-methylenedioxymethylpropylamphetamine (MDMPA) was synthesized in-house [7] and was used as an internal standard. The MDDM standard was also synthesized in-house [8]. Stock solutions of these compounds (1.0 mg/ml) were prepared in methanol, stored at −20°C, and were stable for at least 1 year. Working solutions were also prepared by dilution of these stock solutions with methanol and stored under the same conditions, but were discarded after 6 months.

Calibration

Calibration samples were prepared in different blank matrices (blood, urine, bile, stomach contents, liver) and each consisted of at least seven calibrators, covering the range from 2 to 2,000 ng/ml, or nanograms per gram (for liver) (see Table 1).

Table 1 Calibration data for different matrices

Matrix	Range (ng/ml or ng/g)	Equation	R	LOQ	n
Blood	2–1,500	y=0.01349x+0.02622	0.993	2 ng/ml	8
Urine	2–2,000	y=0.01415x+0.02854	0.992	2 ng/ml	9
Stomach contents	10–2,000	y=0.00935x+0.07693	0.993	10 ng/ml	8
Bile	10–2,000	y=0.01045x+0.11973	0.994	10 ng/ml	8
Liver	10–1,500	y=0.01380x+0.08105	0.992	10 ng/g	7

LOQ limit of quantitation, *n* number of calibrators, *R* correlation coefficient

Toxicological screening

Routine systematic toxicological analyses were performed to investigate for illegal drugs, medical drugs, alcohol, volatile substances, and other poisons. The techniques applied have previously been described in detail [9].

Results

The MS/MS monitoring of the ion-transitions, *m/z* 208.10 to *m/z* 105+133+135+163, typical for MDEA, revealed another peak at a retention time of 17.6 min (retention time of MDEA was 15.5 min). Coinjection of the pure (in-house synthesized) MDDM together with a blood extract unequivocally identified the unknown compound as MDDM [8]. Calibrators containing increasing amounts of MDDM in various matrices were made. After weighted linear regression, correlation coefficients were greater than 0.990, indicating a good linearity. The lower limit of quantitation for MDDM was set at 2.0 ng/ml for blood and urine and at 10.0 ng/g for tissues. The relevant calibration data are presented in Table 1, while the MDDM levels in various fluids and tissues from this fatal overdose are presented in Table 2.

Table 2 Distribution of MDDM in various matrices

Sample	MDDM (ng/ml or ng/g)
Femoral blood	2.5
Aorta ascendens blood	21.7
Vena iliaca blood	<LOQ[a]
Right atrial blood	11.6
Pericardial fluid	31.9
Right pleural fluid	21.7
Left pleural fluid	47.0
Right lung upper lobe	13.5
Right lung median lobe	12.8
Right lung lower lobe	22.6
Left lung upper lobe	17.4
Left lung lower lobe	14.7
Left kidney	30.8
Right kidney	39.8
Urine	42.4
Stomach contents	1,113
Bile	1,101
Liver	20.3
Muscle of the left cardiac ventricle	13.1
Muscle of the right cardiac ventricle	<LOQ[a]
Iliopsoas muscle	<LOQ[a]

LOQ limit of quantitation
[a]LOQ for fluids was 2 ng/ml; LOQ for tissues was 10 ng/g

Due to the advanced putrefaction of the corpse, we were not able to analyze all the samples included in our standard protocol. However, cardiac blood concentrations and the aortic MDDM level (which is representative for the left ventricle blood concentration) were obviously higher than the peripheral blood sample (femoral blood). Substantial amounts of MDDM were also found in pleural exudates and pericardial fluid, lung tissue, stomach contents, bile, urine, and kidneys. The MDDM level in the iliopsoas muscle was below the limit of quantitation (10 ng/g for tissue samples). The routine toxicological analyses only demonstrated phenylethylamine in the liver, which confirmed the putrefaction.

Discussion and conclusions

The advanced putrefaction seriously hampered the investigation of this case. However, the substantial pleural exudations and the pulmonary and generalized visceral congestion were compatible with an acute to subacute cardiopulmonary failure. A huge femoral MDMA blood concentration of 13.5 μg/ml was found as the trigger for this mechanism of death [6]. The unknown substance was detected when screening some extracts (blood, urine, or tissue) for the presence of amphetamine and methylenedioxyamphetamine designer drugs, applying a previously described LC-MS/MS method [7]. Thorough investigation of the literature and of the obtained MS/MS spectrum (precursor and product ions) revealed that the initially unidentified molecule was MDDM [8]. Due to the presence of a 1,000-fold higher amount of MDMA and to the lack of selectivity, other techniques, such as HPLC-diode array detection (HPLC-DAD) and GC-MS, had failed to demonstrate the presence of the unknown designer drug. Although MDDM is classified within the "new synthetic drugs in the European Union" [10], this product has hardly been described, and therefore, the postmortem distribution was studied.

The toxicological data are in line with the distribution found for other amphetamine derivatives, such as MDMA [6, 11–13] and *para*-methoxyamphetamine (PMA) [14]. Recently, it has been pointed out that the lipophilicity of a

substance should influence the postmortem redistribution in the thoracic-abdominal compartment [15]. Pélissier-Alicot et al. studied the postmortem phenomena of three beta-blockers in a rabbit animal model, and showed furthermore that postmortem drug redistribution would be more influenced by the partition coefficient than by the volume of distribution [15]. However, for MDDM/MDDA, these pharmacokinetic parameters are, at present, unknown.

The results presented in Table 2 confirm that blood sampled centrally in the body, such as cardiac blood or aorta ascendens blood (which is representative for left ventricle blood), should be avoided and that peripheral blood sampling, such as the use of femoral blood, remains the "golden standard" for toxicological analysis [16]. Furthermore, vena iliaca blood can be appropriate, as well. The substantial MDDM levels in the pleural and pericardial fluid are compatible with postmortem diffusion from the lung tissues to these fluids. As the MDDM concentration in the left pleural cavity fluid was even higher than in the right thoracic cavity, postmortem diffusion of this component from the huge amount present in the stomach contents could be assumed. The latter could in part also account for the substantial amounts of MDDM in the pericardial fluid. Postmortem diffusion from the gastric residue has previously been described for, e.g., amitriptyline and paracetamol [17]. The high MDDM concentrations in liver and especially in bile point to hepatic biotransformation and excretion via the bile, which was demonstrated for other amphetamine derivatives, such as MDMA [2, 18, 19]. The substantial MDDM amounts observed in the urine and kidneys are proofs of urinary excretion, which was also previously described for amphetamine and derivatives [19].

A substantial difference in MDMA and MDDM concentrations (several micrograms per milliliter vs nanograms per milliliter, respectively) was disclosed [6]. Therefore, it can be assumed that MDDM was present as a synthesis by-product or impurity in the "ecstasy" tablets taken by the victim. Gimeno et al. substantiated that MDDM can be formed during the clandestine manufacture of MDMA by reductive amination of 3,4-methylenedioxyphenyl-2-propanone (MDP2P) by dimethylamine [20, 21]. This MDDM formation takes place because of the presence of dimethylamine as a contaminant in methylamine [22]. However, alternatively, MDDM could have been ingested by the victim some time before the MDMA overdose. Furthermore, we believe that, in this case, MDDM probably did not influence the mechanism of death, as complete methylation of the parent amphetamine molecule substantially reduces its potency. In addition to these two alternatives, a third possibility also has to be considered. Recently, it was described that methylation of MDMA by formaldehyde can produce MDDM/MDDA [23]. Several years ago, it was described that formaldehyde can be formed postmortem, along with other substances such as ethanol, methanol, and *n*-propanol [24, 25]. Furthermore, it has been demonstrated that the accumulation of formaldehyde can even occur in frozen storage conditions [26]. In the present case, the following mechanisms can then be hypothesized: demethylation of MDMA results in MDA and formaldehyde, and, subsequently, this formaldehyde reacts with the remaining MDMA to form MDDM/MDDA. As reported by Shakleya et al., this reaction is promoted in an acidic medium (e.g., stomach contents) [23]. However, in bile (pH 6.1–8.6 [27]) and, also, in other matrices, this reaction is probably more unlikely.

In conclusion, the MDDM concentrations in the various body fluids and tissues in this case are in line with the distribution found for other amphetamine derivatives [6, 11, 14] and confirm that peripheral blood sampling, such as that of femoral blood, remains the "golden standard."

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