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Case Report

MDMA Intoxication in Potential donor with cardiac arrest

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Abstract

Amphetamine and its derivatives' consumption is still an important public health issue, namely in terms of compounds variability and disposition to consumers. However, some of them, like MDMA, still live in the illicit market, with continuous success. Nevertheless, there is always new information and data on MDMA intoxication, both in vivo and in post-mortem context.

The authors report an intoxication case with MDMA, in an 18 years old male, considered a potential organ donor after a cardiac arrest. Whole blood samples were collected in vivo, at the Emergency Room (ER), and post-mortem, at the National Institute of Legal Medicine and Forensic Sciences.

After a general screening procedure, samples were extracted by SPE (OASIS® MCX), followed by GC-MS analysis. The whole blood post-mortem sample was positive for lidocaine (< 500 ng/mL), compatible with the ER intervention, and positive for MDMA (2278 ng/mL) and MDA (49 ng/mL), while whole blood samples collected in vivo (during the maintenance of the individual under advanced life support), were positive for MDMA (504 ng/mL to 1918 ng/mL) and MDA (20 ng/mL to 89 ng/mL). Samples were negative for other substances, namely ethanol, other drugs of abuse and medicines.

Results interpretation is pivotal to understand the behaviour of the substance. Thus, in this case, MDMA post-mortem behaviour should be carefully evaluated, considering as possible influencers, in the specific context of the case, the time lapse between death verification, maintenance of the advanced life support and body manipulation for organ collection purposes. Also referred and discussed is the ante-mortem/post-mortem ratio of MDMA obtained values, compared with literature references. There is no doubt that death was due to MDMA intoxication, but information from the analysis performed on the in vivo samples suggests that this type of sample should also be considered, in a complementary role, whenever possible.

Key-words: MDMA, organ donor, forensic toxicology

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA), or "Ecstasy", is a derivative of methamphetamine. It represents a class of psychoactive agents called entactogens, which produce feelings of closeness to others and empathy, well-being, and insightfulness, alongside with the physical effects of enhanced energy, endurance, sociability, and sexual arousal (1).

MDMA is typically consumed orally, being used alone or mixed with other substances, including ethanol. Adverse effects are generally known, and can be very different, being coma, hyperpyrexia and cardiovascular compromise the most usual ones. However, MDMA-related deaths are unpredictable, both in terms of ingestion quantities as in terms of acute or chronic consumption (2).

Cardiac arrest (CA) is a catastrophic event with a high mortality rate. In some cases, intervention by extracorporeal CPR (eCPR: extracorporeal cardiopulmonary resuscitation) does not avoid an anoxic brain injury, evolving to brain death (BD) and, potentially, to organ donation (3). In Portugal, the law determines that every citizen is a potential organ donor, unless clearly expressed by the individual when alive, through a specific written form, under the Portuguese Blood and Transplantation Institute management. Thus, whenever an individual is considered dead, mainly in ER or Intensive Care Units, he/she becomes eventually a potential donor.

In this paper, the authors report an intoxication case with MDMA in an 18 years old male, who was considered a potential organ donor, after a cardiac arrest. The obtained values, both "in vivo", under eCPR, and post-mortem are discussed, along with some discussion on the ante-mortem/post-mortem ratio, among other potential issues.

2. Case Report

An emergency call was received (9 a.m.) reporting that a 18 years old male was found uncouncious in his car, with some signs of violence inside it. An intoxication suspicion was considered. At 9.17 a.m., an emergency team arrived and observed the existence of stridor, mydriasis and hiperthermia (tympanic temperature: 42ºC). He was brought to the Hospital Emergency Room, and was declared death at 10.13 a.m., classified as a potential organ donor, and was maintained under eCPR until organ collection, which was performed between 2 p.m. and 4 p.m.. Autopsy was performed in the next day, at the Forensic Pathology Service of the North Branch of the National Institute of Legal Medicine and Forensic Sciences. Whole blood samples were collected in vivo, at the Hospital Emergency Room (ER), in different moments, as well as post-mortem, during the autopsy procedure.

3. Material and Methods

3.1. Materials, standards and chemicals

Pure MDMA, MDA and deuterated internal standards (MDMA-D_5 and MDA-D_5) were purchased from Cerilliant Corporation (Round Rock, TX, USA). Working solutions at concentrations of 0.5 mg/L and 5 mg/L were prepared in methanol and stored at -20ºC. Methanol (gradient grade), HCl 37%, KH_2PO_4, dichloromethane, 2-propanol and NH_4^+ were purchased from Merck (Darmstadt, Germany). The derivatization reagent [MBTFA: N-Methyl-bis(trifluoroacetamide)] was purchased from Macherey-Nagel (Duran, Germany). OASIS® MCX columns were purchased from WATERS™ (Milford, MA, USA). The analytical apparatus included an Agilent 6890 GC, coupled to an Agilent 5973n MS.

3.2. Samples preparation and analysis

A method for amphetamine-type substances was applied. Twenty-five µL of internal standard solution (50 ng/mL per sample) were added to 4 mL of KH_2PO_4 0,1M buffer solution. A 500

µL whole blood aliquot, previously agitated in a roller mixer for 15 min, was added to the above mixture. The sample was vortexed and centrifuged at 4000 RPM, for 30 min. SPE procedure, using OASIS MCX columns, included the following steps: cartridge conditioning with 2 mL of methanol and 2 mL of deionised water. The sample was loaded onto the conditioned cartridge and allowed to drain at a 1–2 mL/min flow. Washing steps were performed sequentially with 2 mL of deionised water, 2 mL of HCl 0,1M, 3 mL of a dichloromethane/methanol solution (70:30, v/v), 3 mL of n-hexane and vacuum drying was performed for 15 min. After that, an elution step was performed, with 2 mL of dichloromethane/2-propanol/NH_4^+ (78:20:2, v/v/v) mixture. Samples were evaporated to dryness at 45ºC, in a TurboVap evaporator. After evaporation, 60 µL of BSTFA:TMCS (99:1) were added, and the sample consequently vortexed. The derivatization was performed at 65ºC for half an hour, followed by instrumental analysis using GC–MSD single quad (Table 1 and Table 2). This method was previously validated and applied to routine determination of amphetamines (data not published). Some of the validation parameters are briefly described at Table 3.

4. Results and discussion

The *in vivo* samples were collected after death verification and under eCPR. The post-mortem sample was collected at autopsy. The obtained values are shown in Table 4. The post-mortem whole blood sample was also positive for lidocaine (< 500 ng/mL), compatible with advanced life support intervention. All the samples were negative for other substances, namely other illicit compounds, ethanol and medicines.

Results interpretation is always a quest with specific issues, deep related to each case. Firstly, the presence of MDA and MDMA is compatible with MDMA consumption, with MDA as a

metabolite, with a much lower concentration. Both compounds' ratio (0,021) at the post-mortem level supports this assumption, when compared with the literature (4,5).

On the other hand, postmortem/antemortem (PM/AM) values ratio is also an issue, which has been discussed for some compounds. The literature refers that there is an apparent rise in MDMA concentrations in blood after death, regardless the postmortem collection site (5). However, the subsequent increasing of the concentration may vary, depending on the anatomical site, indicating possible redistribution. In other similar situations, with Hospital admission prior to death, Elliott reports ratios between 1.1 and 2.8 (5).

However, and in this particular case, the eCPR maintenance can be a problem, since it allows further MDMA metabolization, even after when death is declared. Thus, it must be noted that MDMA concentration increases to a maximum of 1918 ng/mL and decreases again, in an approximately two hours time-lapse. In a typical metabolic profile, MDMA shows a t_{max} (time after ingestion, at which the plasma concentration is maximal) of 2 hours and a $t_{1/2}$ from 5 to 10 hours (6,7). Considering that medical intervention started about 1.5 – 2 hours before the first sample collection, the results seem consistent to these reference values.

After eCPR maintenance, organ collection procedure started about two hours after the last *in vivo* collected sample. It is known that MDMA and other amphetamine-type stimulants are fond to some post-mortem redistribution, even though mainly observed in heart blood, as it is more easily exposed to post-mortem redistribution and can, therefore, be misleading in the interpretation of a toxic or lethal post-mortem concentration (8). On the other hand, and due to the fact that MDMA can accumulate in various tissues and organs (8), a procedure like an organ collection can potentially alter, in a dramatic way, the "usual" course of MDMA post-mortem behavior.

In practical terms, it is generally accepted that MDMA blood concentration, alone, is not an unequivocal sign of the death mechanism, with cases reporting values from 600 ng/mL to

84000 ng/mL, depending, among other factors, on age, health general status, (in)existence of symptoms related with hypersensitivity reactions and route of administration (9,10). Moreover, fatalities are not apparently directly linked to dosage and ingestion time-lapse (10). Still, the detected concentration is compatible with an intoxication, when compared to the existing literature (11,12). Moreover, and according to the literature, the obtained PM/AM ratio is also consistent with an MDMA intoxication (5), allowing to conclude that the death was due to a MDMA accidental intoxication, and that, in this case, the organ collection procedure did not change the course of facts in a dramatic way.

5. Conclusion

The obtained results suggested MDMA intoxication as the cause of death, based on the identification of the substance, the obtained value, and histological alterations, namely the presence of edema in the pulmonary tissue histology. The different values obtained during the mechanical ventilation and other life-support measures show that MDMA was still being metabolized. Thus, information on this procedure should always be transmitted to the medico-legal experts. Post-mortem interval is an important point of discussion, concerning toxicological results and interpretation. In this particular case, the increased MDMA concentration observed in the PM sample, and after studying the PM/AM ratio, is coherent with previous studies. Nevertheless, the body manipulation needed for organ collecting procedures can alter PM redistribution, influencing the quantitative result for the substance.

Therefore, MDMA post-mortem behaviour should be carefully evaluated, considering as possible influencers, in the specific context of the case, the time lapse between the death verification, the maintenance of the advanced life support and the body manipulation for organ collection purposes. The post-mortem/ante-mortem ratio of MDMA obtained values, compared with literature references, also supports the conclusions. No doubt that death was

due to MDMA intoxication, but information from the in vivo samples analysis suggests that this type of sample should also be considered, in a complementary role, whenever possible. Consequently, the authors consider that communication between the medico-legal services and Hospital departments can be decisive, in order to obtain samples collected as closer to the event as possible, as well as all the medical data available, in order to know all the procedures that were performed in the victim which can influence the final outcome, to determine in a more effective way the cause of death.

References

[1] Valtier, S., Phelix, C.F., Cody, J.T. (2007) Analysis of MDMA and its Metabolites in Urine and Plasma Following a Neurotoxic Dose of MDMA. Journal of Analytical Toxicology, 31, 138–143.

[2] Greene, S.L., Dargan, P.I., O'Connor, N., Jones, A.L., Kerins, M. (2003) Multiple Toxicity From 3,4-Methylenedioxymethamphetamine ("Ecstasy"). American Journal of Emergency Medicine, 21(2), 121-124.

[3] Casadio, M.C., Coppo, A., Vargiolu, A., Villa, J., Rota, M., Avalli, L., Citerio, G. (2017) Organ donation in cardiac arrest patients treated with extracorporeal CPR: A single centre observational study. Resuscitation, 118, 133–139.

[4] Liu, R.H., Tiu, H.C., Lin, D.L. (2006) Distribution of Methylenedioxymethamphetamine (MDMA) and Methylenedioxyamphetamine (MDA) in Postmortem and Antemortem Specimens. Journal of Analytical Toxicology, 30, 545-550.

[5] Elliott, S. (2005) MDMA and MDA Concentrations in Antemortem and Postmortem Specimens in Fatalities Following Hospital Admission. Journal of Analytical Toxicology, 29, 296-300.

[6] Jones, A.W. (2008) Elimination Half-Lives of Benzoylecgonine and MDMA in an Apprehended Driver. Journal of Analytical Toxicology, 32, 197-198

[7] Maurer, H.H., Bickeboeller-Friedrich, J., Kraemer, T., Peters, F.T. (2000) Toxicokinetics and analytical toxicology of amphetamine-derived designer drugs ('Ecstasy'). Toxicology Letters, 112–113, 133–142.

[8] De Letter, E.A., Bouche, M.P.L.A., Bocxlaer, J.F.V., Lambert, W.E., Piett, M.H.A. (2004) Interpretation of a 3,4-methylenedioxymethamphetamine (MDMA) blood level: discussion by means of a distribution study in two fatalities. Forensic Science International, 141, 85–90.

[9] Verschraagen, M., Maes, A., Ruiter, B., Bosman, I.J., Smink, B.E., Lusthof, K.J. (2007) Post-mortem cases involving amphetamine-based drugs in the Netherlands Comparison with driving under the influence cases. Forensic Science International, 170, 163–170.

[10] Cox, D.E., Williams, K.R. (1996) 'ADAM' or 'EVE'? - a toxicological conundrum. Forensic Science International, 77, 101-108.

[11] Kłys, M., Rojek, S., Woźniak, K., Rzepecka-Woźniak, E. (2007) Fatality due to the use of a designer drug MDMA (Ecstasy). Legal Medicine, 9, 185–191.

[12] Kaye, S., Darke, S., Duflou, J. (2009) Methylenedioxymethamphetamine (MDMA)-related fatalities in Australia: Demographics, circumstances, toxicology and major organ pathology. Drug and Alcohol Dependence, 104, 254–261.

Table 1 - Instrumental conditions.

	GC-MSD Single Quad
GC	AGILENT 6890N
MS	AGILENT 5973N
Chromatographic separation	J&W Scientific 5-ms, 30 m x 0,25 mm x 0,25 µm $T_{injector}$: 250ºC 90ºC for 1 min, to 200ºC at 25ºC/min, plateau for 4 min to 290ºC at 30ºC/min, plateau for 2 min
Detector	Direct interface; Internal Ionization by EI; $T_{transferline}$: 280ºC $T_{quadrupole}$: 150ºC; SCAN mode, dwell time 1 sec/scan; $T_{ionization}$: 230ºC.

Table 2 – MS SIM conditions (quantitation ion underlined)

Compound	Target-ions (m/z)
MDA	135, <u>162</u>, 275
MDA-D5	167
MDMA	135, <u>154</u>, 162
MDMA-D5	158

Table 3

Validation data.

Validation parameter	MDA	MDMA
LOD (ng/mL)	10	10
LLOQ (ng/mL)	25	25
Recovery (%)	90	89
Linearity / workrange (ng/mL)	25 - 1000	25-1000
Wheighting factor	$1/x^2$	$1/x^2$

Table 4 – Sampling time and obtained results

Sampling time	MDA (ng/mL)	MDMA (ng/mL)	Ratio PM/AM
10H45 – ER – AM	Neg	504	4.52
11H34 – ER – AM	89	1918	1.19
12H48 – ER – AM	20	1359	1.68
Day 2 – 14H00 – PM	49	2278	-

ER – Emergency Room (*in vivo*); AM – *Antemortem*; PM – *Postmortem*