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Forensic Science International 135 (2003) 110–114

Forensic Science International

www.elsevier.com/locate/forsciint

# Tissue concentrations of MDMA and its metabolite MDA in three fatal cases of overdose

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Received 3 April 2003; accepted 14 April 2003

**Abstract**

The recreational use of amphetamine derivatives has become increasingly popular in our country in past recent years. Their use is especially common among young people participating in dance parties known as ''raves.'' As a direct consequence of their increased use, the number of fatal cases in which these compounds have been involved have increased dramatically since the second half of the last decade. In our laboratory, we have registered 25 cases related to amphetamine derivatives use since 1996. Three of them were deeply studied and the results obtained are presented in this paper. This information may be useful for the interpretation of the results obtained in toxicological analysis in the cases in which death may be attributed to MDMA use.


*Keywords:* MDMA; MDA; Death and tissue concentration

## 1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) are ring-substituted amphetamine derivatives. Both of them, commonly known as ''design drugs,'' are potent CNS stimulants and structurally related to the hallucinogen mescaline. MDMA was first synthesized by Merck Company in 1914. Its street names are Ecstasy, ''XTC'' or ''Adam.''

MDMA is a synthetic compound that has become increasingly popular in recent years as a recreational drug due to its entactogen properties such as euphoria, friendliness, sociability, closeness, and empathy and also to the widespread belief that it is a safe drug. However, this drug also has serious toxic effects, both acute and chronic, that resemble those provoked by other amphetamines.

MDMA is almost always swallowed and is prepared as single-dose tablets. A normal dose ranges between 50 and 150 mg of MDMA. Tolerance to MDMA appears after a repeated use, increasing the doses to 5–10 tablets [1]. However, little is known about MDMA ability to cause addiction although it has been demonstrated that MDMA can produce a psychosis similar to the one produced by metamphetamine. MDMA effects appear between 20 min and 1 h after its consumption, longing between 4 and 6 h. MDMA is readily absorbed from the intestinal tract and reaches its peak concentration in the plasma about 2 h after oral administration. Elimination of the drug from the body is moderately slow, its half-life being between 7 and 8 h. Almost 65% of the dose is eliminated unchanged in urine. MDMA is metabolized by *N*-demethylation to MDA by hepatic microsomal enzymes and to ring hydroxylated metabolites [2,3].

MDMA exherts its action on serotoninergic system in three ways: increasing the serotonine liberation, inhibiting its recapitation by presynaptic neurones and acting as an agonist of 5-$HT_2$ receptors. MDMA also increases the activity of noradrenergic and dopaminergic systems by blocking their re-uptake by presynaptic receptors. In overdose cases, symptoms are a consequence of an excess of sympatic stimulation, which are similar to those observed in amphetamine overdose: tachycardia, hypertension, hyperthermia, rhabdomyolysis, renal failure and brain edema [4]. MDMA interference with the serotoninergic mechanism of thermoregulation is the cause of hyperthermia.

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0379-0738/$ – see front matter 
doi:10.1016/S0379-0738(03)00179-8

The incidence of deaths associated with MDMA has been increasing in recent years in Spain [5]. As it has been previously reported [6], fatalities usually occur among young attendants at rave parties, which involve all-night dancing with high-tech music.

## 2. Cases histories

Since 1996 our laboratory has received 25 cases of death related to amphetamine derivatives consumption. Three of them were selected to perform the present study due to their common characteristics. All of them have died after attending a rave party in which they needed medical assistance, we received samples from the hospital while they were alive and after the autopsy and finally the drugs of abuse found were MDMA and its metabolite MDA.

### 2.1. Case 1

A 19-year-old woman who arrived at hospital with the following symptoms: tachycardia sinusal, hyperthermia, muscular rigidity, renal impairment, rhabdomyolysis, brain edema and coma. She died 24 h later. Her friends reported that she had taken "Ecstasy" during a party.

### 2.2. Case 2

A 19-year-old man who arrived at hospital after being attended by an urgency team in a rave party and died 7 h later. His friends reported the consumption of "design drugs." The symptoms observed were: loss of conscience, seizures, midriatic and arreactive pupils, profuse sweating, sphynters relaxation, hyperthermia (41.6 °C), hypotension (70/40 mmHg), tachycardia (160 pul/min), anuria, rhabdomyolysis, myoclonus, coagulopathy and apnea. Exitus was due to multiorganic failure.

### 2.3. Case 3

A 20-year-old man who arrived at hospital after being attended by an urgency team in a rave party and died 19 h later. His friends reported the consumption of "design drugs." The symptoms observed were: loss of conscience, seizures, midriatic and arreactive pupils, profuse sweating, sphynters relaxation, hyperthermia (41.6 °C), hypotension (70/40 mmHg), tachycardia (180 pul/min), anuria, rhabdomyolysis, myoclonus, coagulopathy and apnea. Exitus was due to multiorganic failure. His father took a tablet similar to those he had used to the hospital in order to be analyzed.

## 3. Materials and methods

Reactives and solvents used were of analytical grade (Merck, Barcelona, Spain). Analytical standards were purchased from Promochem (Austin, TX, USA). Screening of drugs of abuse was performed in urine specimens by means of homogeneous enzymeimmunoassay CEDIA® (Microgenics, Manheim, Germany) following the instructions given by the manufacturers [7]. All specimens were extracted by means of SPE (Bond-Elut certified, Varian® Harbor City, CA, USA), following the normal procedure in our laboratory [8,9].

Table 1
Ions employed in gas chromatography–mass spectrometry

| Compound analyzed | Ions monitored |
|---|---|
| MDMA | 254[a], 389 |
| MDMA-$d_3$ | 257[a], 392 |
| MDA | 162, 375[a] |
| MDA-$d_3$ | 164, 377[a] |

[a] Ions employed in quantitation.

A Varian CP-3800 (Walnut Creek, CA, USA) gas chromatograph (GC) fitted with a NPD detector was used for a general screening. The column used was a fused-silica capillary Ultra 1 column (200 μm × 0.33 mm × 25 m) coated with methyl siloxane. Carrier gas was helium at a rate of 1.7 ml/min. Injector and detector temperatures were 280 and 300 °C, respectively. Initial oven temperature was 60 °C, maintained for 2 min, increasing at 12 °C/min to 290 °C staying at this temperature for 10 min. Injection volume was 1 μl.

The presence of MDMA and its metabolite MDA was confirmed and quantitated by GC–MS. Dry residues of the obtained extracts were heated for 20 min at 60 °C with 50 μl of the derivatization reagent heptafluorobutyric anhydride. Deuterated compounds were used as internal standards.

A Hewlett-Packard (Palo Alto, CA, USA) GC model 5890 II equipped with a MS detector HP 5973 working in electron impact (EI) mode was used for quantitation of derivatives under selected ion monitoring (SIM) conditions. Ions monitored are shown in Table 1. The column used was a fused-silica capillary Ultra 1 column (200 μm × 0.33 mm × 25 m) coated with methyl siloxane. Carrier gas was helium at a rate of 1.7 ml/min. Injector temperature was 280 °C. MS source and MS quadrupole temperatures were 230 and 250 °C, respectively. Initial oven temperature was 60 °C, maintained for 3 min, increasing at 12 °C/min to 290 °C staying at this temperature for 10 min. Injection volume was 1 μl.

The tablet received in case 3 was analyzed, after being extracted with chloroform, by means of gas chromatography (GC) with NPD detector working in the same conditions described above. Results were confirmed by GC–MS.

## 4. Results

Table 2 shows specimens received from hospital and from the autopsy, while Table 3 shows MDMA and MDA

Table 2
Specimens analyzed in the presented cases

| | Specimens from hospital | Specimens from autopsy |
|---|---|---|
| Case 1 | Blood, urine | Bile, liver, hair, brain, lungs and kidney |
| Case 2 | Blood, urine, urine (8 h) | Blood, vitreous humor, gastric content, bile, liver |
| Case 3 | Blood, urine | Blood, vitreous humor, gastric content, bile, liver, brain, lungs |

concentrations in each specimen analyzed of the three cases studied.

A drug of abuse screening (opiate, cocaine metabolites, amphetamines/Ecstasy, benzodiazepines, barbiturates, methadone, propoxyphene, cannabis and LSD) was performed in all specimens of urine received by means of CEDIA® enzymeimmunoassay. All of them gave positive results for the presence of amphetamine/Ecstasy derivatives, in cases 2 and 3 positive results were obtained for benzodiazepines.

In case 1, we also received a sample hair of 16 cm length, which was divided in three parts. MDMA was detected in all of them, the concentrations being (ng/g): 9.78 (0–3 cm), 5.78 (4–6 cm) and 15.14 (7–16 cm). In case 3, we also received a tablet with a weight of 144.70 mg being 61.64 mg MDMA.

Neither ethanol nor another drug of abuse was detected in samples analyzed. However, in cases 2 and 3, we detected some drugs, which have been administered at the hospital. In case 2, we detected atracurium, lidocaine, dypirone, midazolam and doxylamine. In case 3, we detected atracurium, dypirone, papaverine, midazolam, methylprednisolone and clyndamicine.

## 5. Discussion

In the three cases studied in the present paper, consumers have died after attending a rave party in the same way that has been reported in most cases. Deceased people in the three cases studied presented a common set of symptoms when arrived at hospital. These symptoms were common amongst people who died after using MDMA: hyperthermia, water intoxication with hyponatremia, cerebral edema and hypertension. All of them are capable of causing death alone or in combination [4,10].

One of the consequences of the use of MDMA at raves is profuse sweating as a result of both the vigorous physical activity and the pharmacological action of the drug on the thermoregulatory mechanism. Large amounts of sodium can be lost in sweat, and if the dancers drink large amounts of water in order to avoid overheating, the result is frequently hemodilution and resulting hyponatremia. Furthermore, MDMA increases vasopresin release decreasing water elimination from the body. An excessive water intake can lead to passage of the water form the blood to tissues, including the brain. Also, renal impairment is a frequent symptom in MDMA intoxication. All these factors alone or in combination could explain the water intoxication with hyponatremia and brain edema that are a common cause of death in MDMA overdose [11].

It is thought [4] that the hyperpyrexic pattern of toxicity is due to a combination between MDMA effects, intense phsysical activity and the hot environment in which the consumption takes place. The final consequence is a fulminant hyperthermia. In the most severe cases, the marked elevation of body temperature initiates a series of interrelated effects, such as rhabdomyolysis, renal failure, liver damage and disseminated intravascular coagulopathy.

MDMA cardiovascular toxicity is also a frequent cause of death. MDMA produces hypertension with a consequent risk of ruptured blood vessels and internal hemorrhage and

Table 3
MDMA and its metabolite MDA concentrations in studied cases

| Specimen analyzed | Case 1 | | Case 2 | | Case 3 | |
|---|---|---|---|---|---|---|
| | MDMA | MDA | MDMA | MDA | MDMA | MDA |
| Blood from hospital (mg/l) | 0.17 | 0.03 | 1.09 | 0.08 | 0.04 | N.D. |
| Urine (mg/l) | N.D.[a] | N.D. | 7.99 | 1.18 | 32.00 | 0.74 |
| Urine 8 h (mg/l) | N.A.[b] | N.A. | N.A. | N.A. | 49.06 | 6.90 |
| Blood autopsy (mg/l) | N.A. | N.A. | 3.18 | 0.06 | 0.28 | 0.05 |
| Vitreous humor (mg/l) | N.A. | N.A. | 0.89 | N.D. | N.D. | N.D. |
| Gastric content (mg/l) | N.A. | N.A. | N.D. | N.D. | N.D. | Traces |
| Bile (mg/l) | Traces | N.D. | 1.41 | 1.96 | 1.23 | 27.49 |
| Liver (mg/kg) | 0.18 | 0.05 | 4.86 | 0.24 | 5.13 | 0.26 |
| Brain (mg/kg) | 0.14 | N.D. | | | 8.42 | 0.41 |
| Kidney (mg/kg) | 0.05 | Traces | | | | |
| Lungs (mg/kg) | 1.46 | 0.44 | | | 2.64 | 0.03 |

[a] N.D.: not detected.
[b] N.A.: not analyzed.

tachycardia, with a consequent cardiac workload and a resulting risk of heart failure.

The reported effects of MDMA vary according to the dose and frequency and the duration of use. In general, the effects desired by most users are those produced by low doses on single occasions. However, MDMA unwanted effects are subject of a great interindividual variability.

The usual ''recreational'' dose of MDMA produces blood levels in the range of 0.1–0.25 mg/l. Most of the cases of serious toxicity or fatality have involved blood levels ranging from 0.5–10 mg/l, that is, up to 40 times higher than the usual recreational range. However, in revised literature we have found cases in which consumers have survived to a MDMA overdose (40–50 tablets) without any symptom while others died after taking 1–3 tablets, with serum levels between 0.1 and 0.4 mg/l, that is overlapping the normal range and a little above it [4]. This is an important point, because it demonstrates the degree to which the seriousness of the effects can be dependent on environmental factors other than drug concentration.

In the same way, our findings are in good agreement with these data: in cases 1 and 3, blood concentrations are within the normal range for recreational users while in case 2 it is in the range of the concentrations reported for fatalities caused by MDMA (Table 3).

The wide range of blood concentration found in deceased people are probably due to MDMA metabolism. Fallon et al. [12], reported that MDMA has a non-linear pharmacokinetics. This lack of linearity is due, in part, to the stereoselective metabolism of MDMA, which is present in two enantiomeric forms. MDMA isomers differ in the rate at which they are converted to their corresponding MDA metabolites. *S*-enantiomer is more potent but is eliminated more rapidly. This non-linear kinetics makes blood concentration interpretation and its correlation with the observed effects more difficult. Another factor, which may contribute to the non-linear pharmacokinetics, is the enzyme saturation at relatively low concentration of the drug. Consequently, as the dose is increased and the higher affinity enzymes are saturated, disproportionately large increases in blood concentration occur.

MDMA secondary effects range from mild to severe, being in some cases lethal. As drug is synthesized illegally, MDMA tablets content vary from 50 to 150 mg [4]. The MDMA content of the tablet analyzed in case 3 is within this range. We can only estimate the amount consumed of MDMA in case 3. In this case, we can suppose a consumption of at least 60 mg of MDMA (MDMA content of analyzed tablet). However, blood concentration was lower than that by De la Torre et al. [13], for a similar consumption.

The forementioned factors demonstrate that it is not possible to establish a direct relationship between MDMA blood concentrations and toxic effects [4,10].

MDMA is metabolized by *N*-demethylation to the active compound MDA. Four hours after swallowing 50 mg of MDMA, MDA is detected in blood in a concentration that is approximately 40% of the dose [14]. However, in the three cases we have studied MDA/MDMA relationship is much lower.

Table 4
MDA/MDMA relationship in studied cases

| Specimen analyzed | MDA/MDMA | | |
|---|---|---|---|
| | Case 1 | Case 2 | Case 3 |
| Blood (alive) | 0.18 | 0.42 | |
| Urine | | 0.15 | 0.023 |
| Urine 8 h | – | – | 0.14 |
| Blood (autopsy) | – | 0.02 | 0.18 |
| Bile | | 1.72 | 22.34 |
| Liver | 0.28 | 0.02 | 0.05 |
| Brain | 0 | – | 0.05 |
| Lungs | 0.30 | – | 0.01 |

A MDA/MDMA urine concentration relationship lower than 0.15 establishes MDMA consumption [3]. Therefore, we can assure that in the three cases presented (Table 4), the drug consumed was MDMA and the MDA detected came from its metabolism.

MDMA physicochemical properties explain its preferential distribution to brain and liver (Table 3). Once the individual has died, the MDMA present in these tissues is redistributed to blood. This phenomenon known as postmortem redistribution [11] is responsible for the increase observed in blood concentration after the death in cases 2 and 3 (Table 3).

MDMA liver concentration in case 2 is two-fold higher than the blood concentration; while in case 3 the later is almost 18-fold higher. These findings are similar to what was published by Drummer [3] in 2001. According to other cases, the bile concentrations are not always higher than postmortem blood (case 3). This kind of relationship can not be considered in case 1 because we did not receive postmortem blood.

In cases 1 and 3, liver and brain concentrations are similar, which is in agreement with other cases reported [4,6]. Table 3 also shows that vitreous humor and antemortem blood have similar MDMA concentrations, indicating that equilibration was reached. Therefore, it can be thought that vitreous humor is not affected by MDMA postmortem distribution [15] and can be used as an alternative specimen when an appropriate blood sample is lacking.

Nowadays, hair analysis is used to demonstrate a chronic consumption of drugs of abuse. We only received hair specimen in case 1. The analysis of this specimen showed a chronic abuse of MDMA from 16 months before the death.

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