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Adam (MDMA) and Eve (MDEA) misuse: an immunohistochemical study on three fatal cases

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Abstract

Three fatal cases of MDMA/MDEA misuse have been examined. These referred to white males between 19 and 20 years of age, in which post-mortem toxicology showed the presence of MDMA (in one case), MDEA (in one case) and both (in one case). The clinical data were analysed and the histopathological findings were studied following immunohistochemical investigations. A complete immunohistochemical study has made it possible to demonstrate rhabdomyolysis and myoglobinuria with alterations of the organs typical of a DIC. Clinical, histopathological and toxicological data suggest that severe or fatal complications following ecstasy ingestion could be related to idiosyncratic response.

Keywords: Ecstasy; Postmortem pathology; Immunohistochemistry

1. Introduction

MDMA, or 'Ecstasy', and 3,4-methylenedioxyethylamphetamine (MDEA, or 'Eve') have emerged as popular recreational drugs of abuse over the last decade. In Western Europe, due to the erroneous belief that these are relatively safe hallucinogens, the use

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of such substances as mood enhancers has steadily increased, especially in discotheques, during the 1990s [1]. Recreational use of these drugs has spread in Italy in the last few years [2].

Pharmacological studies indicate that these substances produce a mixture of central stimulant and psychedelic effects, many of which appear to be mediated by brain monoamines, particularly serotonin and dopamine [3].

Recent well-publicised reports of deaths resulting from MDMA/MDEA abuse at 'rave' parties have led to an increased understanding of the pathology of their misuse. Toxic effects and the occasional death following ring substituted amphetamine misuse have been reported but postmortem data are lacking [4] previous to a recent review which illustrates seven fatal cases with a detailed examination of the post-mortem findings [5]. Additionally, while deaths due to MDMA/MDEA have been reported, none of these reports have on focused on immunohistochemical investigation. Three cases of death following the ingestion of these substances have been here examined to better define the histopathological findings related to MDMA/MDEA intoxication. One case has been reported previously [2]. In all cases we were able to carry out a complete immunohistochemical studies on autopsy specimens.

2. Methods

2.1. Case 1

B.F., 19 years of age, was seen ingesting numerous tablets of Ecstasy in a discotheque the night of June 15th, 1996, for the entire duration of the party until early morning. On June 16th, in the late morning, he began to experience respiratory difficulty, uncoordinated movements, generalised hypertonia and hyperpyrexia (40.6°C). He was transported to the local hospital where the following hematochemical values were registered at 18:00 h: PT 50%; PTT 52.7″; TT 24″; AT III 102%; fibrinogen 172 mg%; WBC 15 900/mm^3; RBC 5 410 000/mm^3; PLT 191 000/mm^3; HB 15.2 g/dl; HCT 46%; BP 100/50 mmHg; cardiac rate 165/min.

The patient was given artificial ventilation; an arterial blood gas analysis at 19:33 h revealed: pH 7.326; pCO_2 37 mmHg; pO_2 89,8 mmHg; HCO_3 18.8 mmol/l; SBC 19.4 mmol/l; So_2 96.3%.

He was diagnosed disseminated intravascular coagulation (DIC) and received therapy with heparin bolus 2000 IU (25 IU/kg) and heparin infusion at 7 IU/kg/h (12 500 IU for 24 h) together with the administration of three bags of plasma.

At 23:30 h he suffered a severe loss of blood from the oral cavity and injection wounds: BP 60/30 mmHg, PR 40/min., PT 21%, PTT 227.9″, TT 199.4″, AT III 75%, fibrinogen 2 mg%, WBC 17 000/mm^3, RBC 3 230 000/mm^3, HB 9.1 g/dl, HCT 28%, PLT 74 000/mm^3, uraemia 58.3 mg/dl, creatinine level 3.83 mg/dl, K 5.37 mEq/l, Na 141 mEq/l, CPK 7395, CPK MB 50, AST 222, ALT 112.

At 01:50 h he suffered a cardiac arrest unresponsive to cardiopulmonary resuscitation.

2.2. Case 2

C.C., 20 years of age, went with some friends to a discotheque, where he remained for some hours and where he was seen to ingest numerous tablets of Ecstasy.

When he returned home around 02:00 h on December 26th, he told his mother that he felt feverish. The armpit temperature was established at 40°C and he immediately went to bed. At 12:00 h he was found dead, his pillow soaked with blood.

2.3. Case 3

On May 19th,1996 at 15:00 h, R.L., 19 years of age, was found unconscious near a discotheque. After being carried to the Intensive Care Unit of the local hospital, the following clinical and laboratory data were established: PT 52%; PTT 55,4″; TT 29″; fibrinogen 150 mg%; WBC 11 200/mm^3; RBC 5 220 000/mm^3; PLT 180 000/mm^3; HB 14g/dl; HCT 43%; BP 90/50 mmHg; PR 170/min; T 40.5°C.

The clinical course progressively worsened and at 15:00 h on May 20th diffused subcutaneous petechiae appeared; the patient sustained convulsions and treatment with dopamine was started because of progressive hypotension: BP 60/40 mmHg; PR 60/min; PT 20%; PTT 210″; TT 195″; fibrinogen 5 mg%; WBC 15 000/mm^3; RBC 3 200 000/mm^3; HB 9.8 g/dl; HCT 30%; PLT 90 000/mm^3; uraemia 74 mg/dl; creatinine level 5.05 mg/dl; K 5.6 mEq/l; Na 138 mEq/l; CPK 8200; CPK MB 40; AST 110; ALT 90; T 40.9°C.

At 19:00 h on May 20th he was pronounced dead.

Amphetamines were detected in the urine of the subjects by immunoenzymatic screening. Toxicological analyses by solid–liquid extraction and gas chromatography–mass spectrometry analysis were therefore carried out to identify and quantify the individual substances present in the biological fluids and organs. Table 1 shows the concentrations of MDMA, MDEA and MDA (metabolite of MDMA) in current cases.

Table 1
Toxicological data

	Case 1		Case 2			Case 3
	MDMA (μl/ml or g)	MDA (μl/ml or g)	MDMA (μl/ml or g)	MDA (μl/ml or g)	MDEA (μl/ml or g)	MDEA (μl/ml or g)
Urine	31.00	0.85	263.13	5.25	183.73	16.1
Blood	7.15	0.25	0.18	0.12	1.59	Detected
Liver	5.10		13.23	0.17	10.68	0.42
Kidney	8.70	0.97	9.81	1.36	8.04	0.98
Lung	6.75		10.70		8.03	0.24
Brain	7.10		12.79		8.43	0.21
Spleen	5.00		9.17		7.05	0.56
Bile	2.50		27.34		21.93	1.37

2.4. Immunohistochemical staining

Immunohistochemical investigation of the kidney and the muscle structures was performed utilising polyclonal anti-myoglobin antibodies (Dako, Germany) [6,7]. Lungs were examined utilising polyclonal anti-fibrinogen antibodies (Calbiochem, USA). Liver structures were examined utilising polyclonal anti-fibrinogen antibodies (Calbiochem, USA), anti-FDP-D (AGC, USA), and anti-FDP-E antibodies (ICN Biomedicals, USA) [8]. Sections were counterstained, dehydrated, coverslipped and observed in a Leitz Aristoplan optical microscope.

2.5. Other tests

A routine microscopic histopathological study was performed by using formalin-fixed paraffin embedded tissue sectioned at 4 μm and stained with haematoxylin–eosin, periodic acid-Schiff and phosphotungstic acid–haematoxylin (PTAH).

3. Results

3.1. Pathological findings

The morphological data together with the autopsy findings revealed diffused subserous petechiae, polyvisceral stasis and the following histo-pathological alterations:

3.1.1. Brain

Massive oedema and signs of neuronal hypoxia were present in all cases; in one case perivascular ring haemorrhages, especially in the cortical zone, were observed.

3.1.2. Heart

Coagulative myocytolysis was present in two cases. We observed plurifocal foci of myocells with hypercontraction of the whole myocell and myofibrillar rhexis with anomalous deeply eosinophilic cross-bands formed by hypercontracted sarcomeres. More advanced stages of coagulative myocytolysis (alveolar or healing patterns) and old myocardial fibrosis were absent or minimal. In one case areas of subendocardial haemorrhage were also noticed.

3.1.3. Lung

All cases revealed subpleural and intra-alveolar haemorrhage with severe oedema and, in two cases, microthrombotic formations inside lung capillaries.

3.1.4. Liver

In two cases there was evidence of microvescicular steatosis and in one case clear centrilobular necrosis around the central veins. Liver cells in the central zones revealed coagulation necrosis with precipitation of fibrin in the whole area affected by necrosis. There were occasionally fibrillar or fine granular fibrin thrombi and Kupffer cells

ingesting fibrin in sinusoids around the area of necrosis. The PTAH staining method was revealed to be suitable for organized fibrin molecules, but not for fibrin molecules during the process of either polymerization or degradation. In contrast an indirect peroxidase antibody method using anti-fibrinogen, anti-FDP-D and anti-FDP-E antibodies was suitable for the latter, but not for the former [8]. Post-mortem virology was not performed in these cases.

3.1.5. Kidney

Fibrin thrombi in the renal glomeruli were observed in two cases. All glomeruli thrombi were already organised and were stained prominently by PTAH. In one case acute tubular necrosis was observed. H&E examination revealed numerous reddish-brown granular and amorphous casts within the renal tubules. In all the cases the presence of tubular casts was studied with immunohistochemical technique, thus demonstrating their myoglobinic nature (Figs. 1 and 2).

3.1.6. Muscle

In two cases hypercontracted fibers with an absence of the striations and cystic cavities adjacent to zones of fiber ruptures were present (Fig. 3a). The immunohistochemical investigation for myoglobin performed according to Fechner and coworkers [7,9] showed accumulation of the protein in the breakage areas of the fibres especially on the surface of the contraction caps (Fig. 3b).

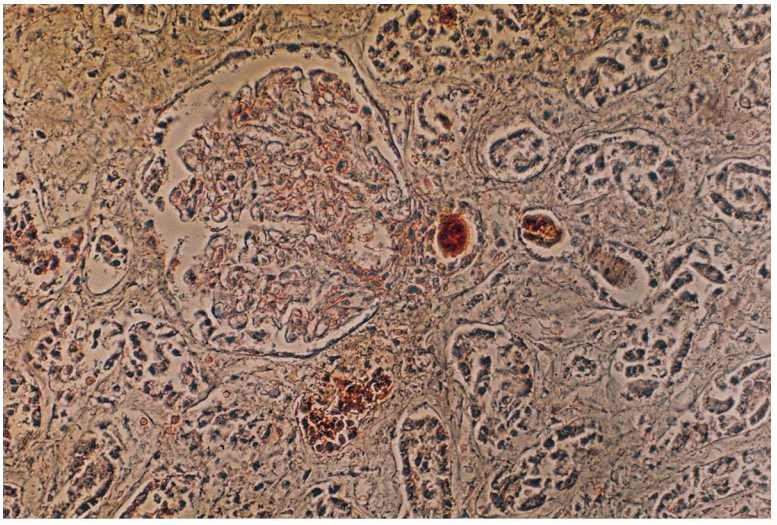
4. Discussion

There are substantial similarities in the clinical and histopathological findings of the fatal cases herein presented. Two deaths were caused by single intoxication, respectively, from MDMA and MDEA and the third from both MDMA and MDEA. No other drugs were detected.

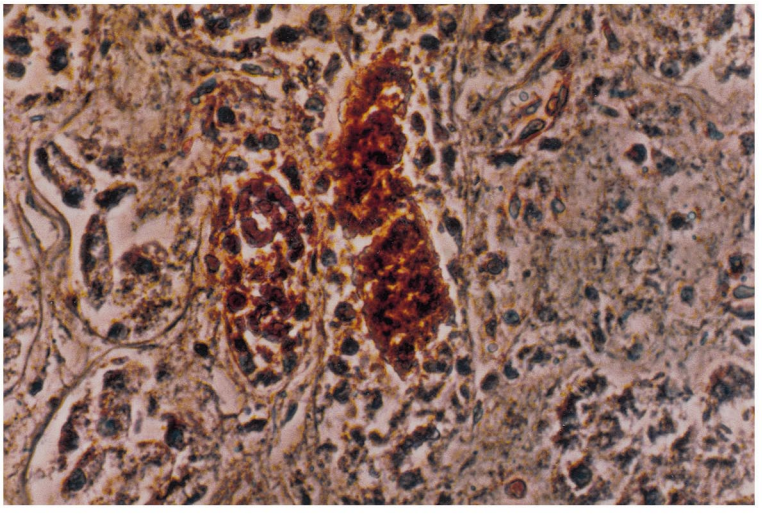
In the present study the pathological data are in accordance with a well studied and documented clinical entity, i.e. hyperthermia and disseminated intravascular coagulation [10–12]; however the macro and microscopic findings have not been definitively classified or defined yet.

After the onset of deaths related to MDMA ingestion [13], reports have been compiled about fatal arrhythmia caused by the ingestion of MDMA [14] or cases of hyperthermia followed by DIC [15–19]. Clinical proofs of hyperthermia, rhabdomyolysis and DIC are also evident in deaths caused by MDEA intoxication with findings of subserous haemorrhage and severe polyvisceral stasis [20–24]. Similar pathological findings are described in the cases of death due to combined intoxication of MDMA and MDEA [2,25,26].

A report describes acute myocardial infarction associated with amphetamine abuse [27]. Potential explanations include coronary vasospasm, excessive catecholamine discharge resulting in ischemic myocardial necrosis, and catecholamine mediated platelet aggregation with subsequent thrombus formation. The syndrome closely resembles acute myocardial infarction due to cocaine abuse [3].



(a)



(b)

Fig. 1. (a) Kidney: immunohistochemistry stain for myoglobin revealed distinct staining of the tubular casts (PAP 128×). (b) High magnification: the tubular casts showed strong antimyoglobin immunostaining (PAP 274×).

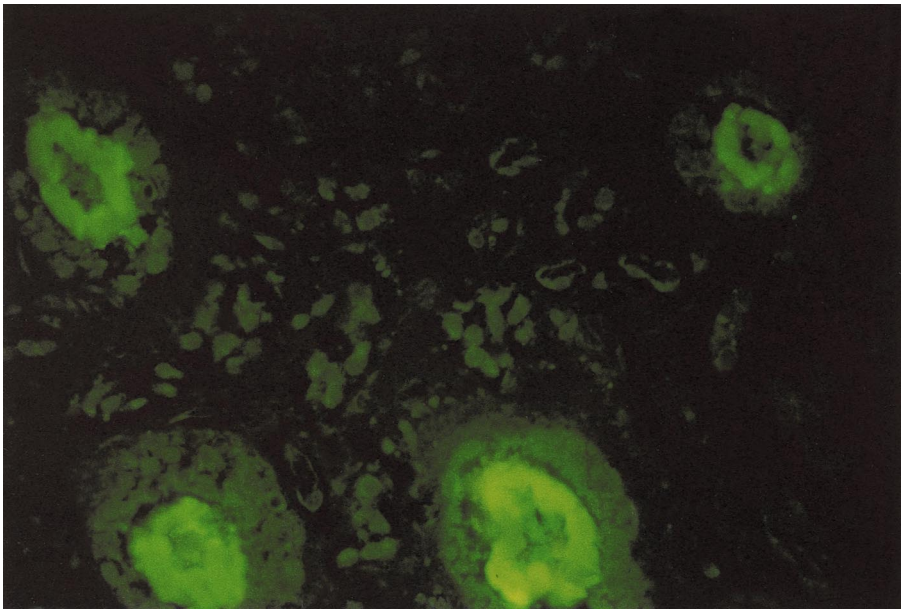


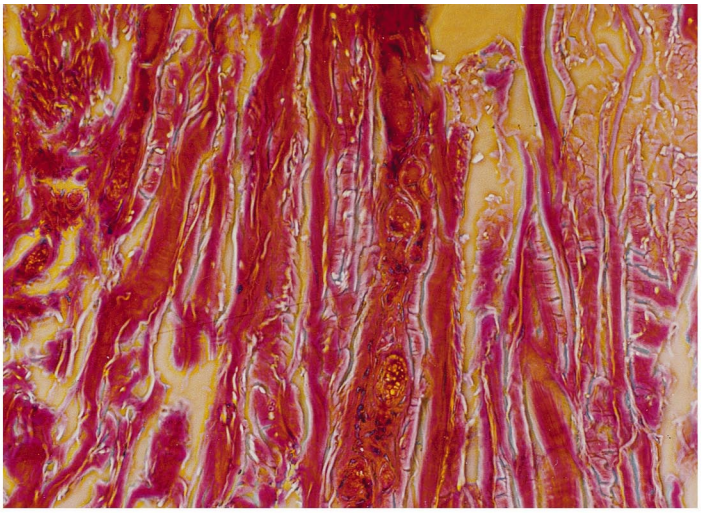
Fig. 2. Myoglobin in renal tubules (immunofluorescence polyclonal antibody against myoglobin, 274×).

A comparison of our findings with presently available related literature reveals slight differences in histopathological findings, particularly of the heart, liver and kidneys as compared to those described in previous reports. The types of myocardial necrosis found in our cases were coagulative myocytolysis. No histological signs of infarct necrosis were detected. Coagulative myocytolysis, even if confined to few myocells, can be interpreted as a histological sign of adrenergic overdrive [28]. Coagulative myocytolytic changes may be related to the type and length of survival, particularly in subjects predisposed to cardiac adrenergic response. It remains to be established if coagulative myocytolysis is due to unspecific agonal stimuli or drug action. In other words, any attempt to evaluate its meaning requires the recognition of older stages, the determination of survival time, and the exclusion of reanimative procedures.

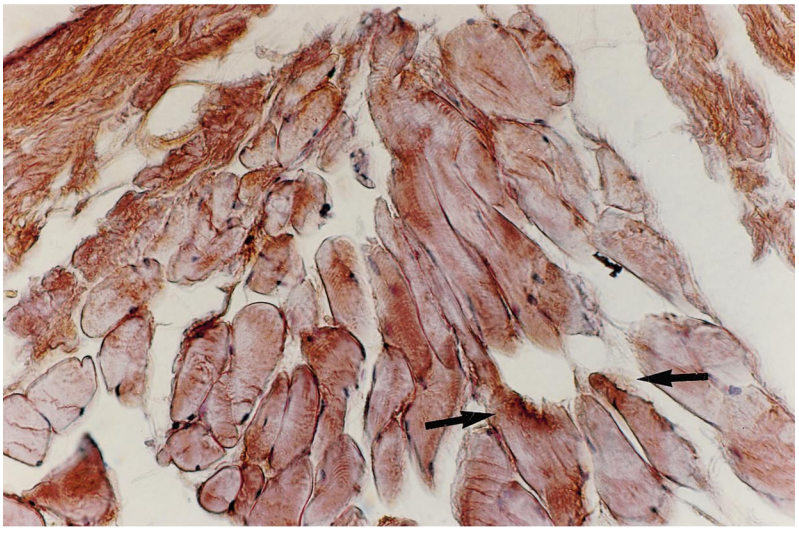
In two cases, the study of muscle samples revealed hypercontracted fibers with disruption of the cell architecture, typical pathological changes observed in deaths due to malignant hyperpyrexia [29]. Malignant hyperthermia is a rare, inherited abnormal susceptibility to certain drugs, mostly inhalational anaesthetics [30].

It is possible that individual susceptibility to the adverse effects of the amphetamine derivatives exists, with deficient demethylation of MDMA by debrisoquine hydroylase (CYP2D6), being shown in certain individuals. The absence of CYP2D6, a member of the cytochrome P450 superfamily of enzymes, in 5–9% of whites, may be another factor explaining apparently idiosyncratic or severe responses to the drug [31].

Rhabdomyolysis and myoglobinuria are often described [10,32]. The presence of myoglobin in the kidney has been reported in a histopathological study only [2]. In a



(a)



(b)

Fig. 3. (a) Muscle: hypercontracted fibres and cystic cavities adjacent to zones of fiber ruptures were present (Phaco 128×). (b) Muscle: immunohistochemical stain for myoglobin showed positive reaction (arrows) in the breakage areas of the fibres. Depletion of myoglobin in lighter fibres (PAP 63×).

recent review, myoglobin was not detected in the only two cases in which the kidneys were examined [5]. We were able to demonstrate the presence of myoglobin in the proximal tubules in all the three cases here examined. Two cases presented mi-

crothrombosis of the pulmonary and renal microcirculation related to DIC. Finally, in two cases has been possible to demonstrate a hepatic microvescicular steatosis and aspects of centrilobular necrosis related to DIC in one case.

The genesis of the hepatic damage caused by MDMA/MDEA still remains unexplained and does not seem to be related to the dose or frequency of drug ingestion. Idiosyncratic reactions or individual susceptibility are likely pathogenic causes of the hepatic pictures described [33,34]. The quality of Ecstasy tablets should also be considered due to the established and documented presence of toxic contaminants [5].

The increase in cases of toxicity due to MDMA and drugs sold as 'Ecstasy' deserves to be publicised for various reasons. First, it is not possible to establish the cause of severe or fatal complications following the ingestion of Ecstasy with the data available at present. Individuals who experience such an adverse reaction have often used the drug previously without problems [10]. Chemical and toxicological analysis of post-mortem biological material shows variable concentrations of amphetamines, suggesting hypersensitivity in the cases of low doses. Individual susceptibility to ring substituted amphetamines may be related to its metabolism in the liver [35] and can explain severe or fatal responses to the drug [31]. Second, clinicians should be aware of the pattern of toxicity so as to be able to perform correct diagnoses and treatments. Finally, from a diagnostic standpoint, in all ring-substituted amphetamines-related deaths, a complete immunohistochemical study should be performed especially on muscle [36] and kidney [37] samples.

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