We present a fatal case involving the combined ingestion of amphetamine, 3,4-methylenedioxymethylamphetamine, 3,4-methylenedioxyamphetamine, and paramethoxyamphetamine. Various postmortem specimens (e.g., several blood samples, urine, and tissue samples) were analyzed to study the distribution of the compounds and their metabolites in the human body. Quantitation took place using liquid chromatography–sonic spray ionization–mass spectrometry after pretreatment with a liquid–liquid extraction. The medico-legal findings were compatible with a disseminated intravascular coagulation induced by hyperthermia caused by the simultaneous intake of the amphetamine analogues.

Abstract

We present a fatal case involving the combined ingestion of amphetamine, 3,4-methylenedioxymethylamphetamine, 3,4-methylenedioxyamphetamine, and paramethoxyamphetamine. Various postmortem specimens (e.g., several blood samples, urine, and tissue samples) were analyzed to study the distribution of the compounds and their metabolites in the human body. Quantitation took place using liquid chromatography–sonic spray ionization–mass spectrometry after pretreatment with a liquid–liquid extraction. The medico-legal findings were compatible with a disseminated intravascular coagulation induced by hyperthermia caused by the simultaneous intake of the amphetamine analogues.

Introduction

The amphetamine-based designer drugs, especially popular in the “rave” environment (e.g., clubs or dance parties), have always been a great challenge for forensic toxicologists. In an effort to keep up with the clandestine drug laboratories, we must be alert for very fast changes in the molecular structure of the basic drug. Hence, we are strained to continuously search for the unknown.

A recent evolution in this field was the introduction of paramethoxyamphetamine (PMA) on the Belgian illicit drug market (1). However, this methoxylated phenylethylamine derivative (molecular formula: C_10H_15NO, Figure 1) was already sold on the street during the 1970s. Within a few years, the drug was associated with several fatalities and earned the street-name “death”, which led to its temporary disappearance from the drug scene (2). In the early 1990s, it first re-emerged in Australia, which again led to a number of fatal cases (3–5). In 1998, it was spotted on the European market (6) and more recently in Belgium (1), the United States (7), and Canada (8). As do its structurally related compounds 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethylamphetamine (MDMA, Figure 1), and 3,4-methylenedioxymethylamphetamine (MDEA), PMA exhibits hallucinogenic properties. However, it has been postulated that it is more toxic than MDMA because of its potent effect on serotonergic transmission (7). Furthermore, PMA is closely related to another new ring-substituted amphetamine, 4-methylthioamphetamine (4-MTA), which was also identified in a few fatalities (9–11). Doses of less than 50 mg of PMA, without co-ingestion of other drugs or alcohol, induce symptoms similar to MDMA such as increased heart rate, blood pressure, and respiratory rate; elevated body temperature; erratic eye movements; muscle spasms; nausea; and visual hallucinations. Higher doses are considered lethal, especially when taken with other amphetamine derivatives, cannabis, cocaine, prescription medication, or alcohol. Symptoms in severe intoxications can be cardiopulmonary related (e.g., cardiac arrhythmia, pulmonary edema), but vomiting, renal failure, hyperthermia, convulsions, and coma prior to

Figure 1. Structural formulas of PMA and MDMA.
death can also occur (7). The drug is sold in tablets, capsules, or powder form, and its appearance and cost are comparable to MDMA. Analysis of tablets revealed that “ecstasy” tablets can contain not only PMA but also paramethoxymethylamphetamine (PMMA) (12). Because of its great similarity to the popular and well-known MDMA, it has been mistakenly ingested as “ecstasy”, sometimes with lethal consequences. In the last few years, an increasing number of fatal intoxications involving PMMA have been reported (1,3–8). However, to our knowledge, data on PMA tissue concentrations are scarce (13).

We present a fatal case of the combined use of amphetamine, MDMA, MDA, and PMA. The complete toxicological findings of the specimens, analyzed by liquid chromatography–sonic spray ionization–mass spectrometry (LC–SSI-MS) after appropriate liquid–liquid extraction, are presented and the distribution of the drugs is discussed. To the best of our knowledge, post-mortem drug distribution of PMA in combination with MDMA has barely been explored in humans.

**Case History**

A young man was found dead at home, lying on a divan. It was warm in the room, and the ambient temperature was about 25°C. The man was naked, and his clothes were close by as though he just had undressed. The body temperature had reached the ambient temperature. The lividities were fixed, the rigor mortis had almost disappeared, and greenish coloration of the whole abdominal wall was noticed. Slight mummification of lips, nose tip, fingers, and toes was found. The eyeballs were depressed and dehydrated. These findings, correlated with the police information, revealed that the postmortem interval was about three days. The body weighed 56 kg and was 175 cm tall (body mass index: 18.3). The face showed no petechiae. Apart from a few slight recent excoriations on the arms, which were consistent with slight blunt trauma (e.g., fall or blow), nothing unusual was observed.

During internal inspection, moderate putrefaction and congestio of all organs was found. Both lungs weighed 1410 g and showed obvious congestion and distinct edema. The left and right pleural cavity contained about 150 and 100 mL bloody fluid, respectively. Somewhat vinous colored pericardial fluid was present (about 10 mL). The heart weighed 315 g and showed, apart from a few Tardieu spots, no anomalies macroscopically. The liver weighed 1160 g, and slight steatosis, congestion from the amphetamine mixture.

**Experimental**

**Materials**

PMA was synthesized in house according to a described procedure (14). MDMA (ecstasy) and MDA were purchased from Sigma-Aldrich (Bornem, Belgium). Amphetamine (AMP) and ephedrine (internal standard) were available from the collection of our laboratory (Laboratory of Toxicology, Ghent University, Belgium). All reagents and chemicals were of analytical grade (Merck-Eurolab, Leuven, Belgium). Solvents were all of high-performance liquid chromatography (HPLC) gradient grade and were also purchased from Merck-Eurolab.

An individual standard solution of 1 g/L of each compound was prepared in methanol and stored in the dark at −20°C until use. Under these conditions, all solutions proved stable for more than six months. A 2-µg/mL solution of internal standard, ephedrine, in methanol was also prepared. We decided to use this compound because of its structural similarity and sufficient separation from the amphetamine mixture.

**Analysis of biochemical parameters in vitreous humor**

Glucose, lactate, and potassium were determined in vitreous humor using enzymatic tests and specific electrodes on a routine automatic analyzer instrument.

**Initial drug screening**

Routine systematic toxicological analysis was performed on the samples to investigate for illegal drugs, medical drugs, alcohol, volatile substances, and other poisons. Immunoassay screens (ADx) were performed to test for amphetamines, cannabinoids, opiates, methadone, and benzoylcegonine in urine and for barbiturates and tricyclic antidepressants in blood. Radioimmunoassay was used to screen for LSD in urine and for morphine and benzodiazepines in blood. Color spot tests on urine and gastric content were used to detect salicylates, acetaminophen, phenothiazines, and imipramines. Postmortem blood was analyzed for the presence of carboxyhemoglobin and cyanide. Gastric content and urine were screened for the presence of basic drugs and hydrolyzed benzodiazepines; blood was screened for the presence of acidic and neutral drugs by thin-layer chromatography. Gas chromatography–mass spectrometry (GC–MS) was used to screen the urine and the gastric content for the presence of basic drugs. Blood was screened by HPLC with photodiode-array detection (HPLC–DAD). Analysis for the presence of alcohol and other volatile substances in blood and urine was performed by headspace GC with a flame-ionization detector (GC–FID) (10).

**LC–MS instrument**

Chromatography was carried out using a LaChrom separation module (Merck, Darmstadt, Germany) including an L-7100 low-pressure gradient pump, L-7200 autosampler (100-µL injection loop), L-7360 column oven, and D-7000 interface. The system uses the LC/3DQ-MS System Manager Software running under Windows NTTM version 4.0 on a Compaq Deskpro EN.

All MS experiments were carried out on the M-8000 ion-trap-based MS from Merck equipped with an on-axis SSI interface operated in positive ion mode.
**Method**

*Sample pretreatment.* Blood and urine were used as received. Tissue samples were homogenized after appropriate dilution with water using an Ultra-Turrax homogenizer from IKA (Staufen, Germany). Most of the postmortem samples were extracted according to a liquid–liquid extraction procedure previously developed in our laboratory (15). However, for the more complicated (greasy or degraded) matrices such as adipose tissue, stomach content, and different brain parts, a liquid–liquid extraction with back-extraction was applied (16).

*LC–MS.* Chromatographic separation was achieved on a Hypersil BDS phenyl column (100 × 2.1-mm i.d., 3-µm particle size) protected by a Hypersil BDS phenyl guard column (7.5 × 2.1-mm i.d., 3-µm particle size), purchased from Alttech (Lokeren, Belgium). A gradient program with water and acetonitrile, both containing 0.001 vol% formic acid was used. The complete and detailed LC–SSI-MS method is described in a previous paper (17).

*Specimen collection.* Toxicological analyses were performed on blood collected from the subclavian vein, femoral and iliac vein, inferior vena cava, right atrium, aorta ascendens, pulmonary artery, and right and left pulmonary vein. Left atrial and ventricular blood (right and left) were not available. Other specimens analyzed include urine, vitreous humor, pericardial fluid, bile, stomach content, liver, spleen, iliosposas muscle, and abdominal adipose tissue. For pleural fluid, cardiac muscle, lungs, and kidneys, separate sampling of the left and the right occurred. In addition, brainstem, cerebellum, and brain lobes were sampled. All samples were stored in the freezer (–20°C) until analysis.

*Calibration.* Calibration curves were prepared in blank matrices of blood, urine, brain homogenate, and liver. Each calibrator sample (1 mL) was spiked with 50 µL of the 2-µg/mL internal standard solution and with the compounds of interest, resulting in a final concentration of 10–1000 ng/mL (blood and urine) and 20–2000 ng/mL (tissues). The spiked compound levels for each calibration graph were 0.1, 0.4, 1.0, 2.0, 4.0, and 10.0 µg/mL for each matrix. After extraction of a 1-mL aliquot of each sample according to the previously mentioned liquid–liquid extraction, samples were analyzed and calibration curves were created using quadratic regression analysis (in view of the limited linear dynamic range of the mass analyzer). Quantitation of the autopsy samples was performed by comparing the peak-area ratio of each specific compound and the internal standard against the calibration curve. All samples were analyzed twice. First, 1 mL of the available postmortem matrix (blood and urine) was spiked with the internal standard solution and analyzed. Following these orientating preliminary results, a second extraction was performed on appropriately diluted sample specimens.

**Results and Discussion**

**Routine biochemical parameters**

The urinary pH and creatinine concentration in this case were 7.5 and 0.3 g/L, respectively. The potassium level in the vitreous humor was 38.9 mmol/L. The vitreous glucose and lactate concentrations were 3 and 309 mg/dL (sum value of 312 mg/dL), respectively, excluding hypoglycemia.

**Drug screening**

The initial drug screening (including GC–MS) revealed the presence of MDMA in blood and urine and amphetamine in urine. In addition, nicotine and caffeine were demonstrated in blood and urine. The results of the screening tests for the presence of other relevant drugs or medications were all negative.

The GC–MS run showed a small broad peak with a mass spectrum corresponding to PMA. However, this was initially considered not toxicologically relevant because the peak area/height was relatively low and because it was thought to be a methylated artefact of a p-hydroxylated metabolite of amphetamine.

**LC–MS**

The LC–SSI-MS method was completely validated and proved to fulfill analytical standard criteria (17).

The toxicological findings for amphetamine, MDMA, MDA, and PMA in the different autopsy samples are summarized in Table I. The ratios of vitreous humor to femoral blood level for PMA, MDMA, MDA, and AMP were 1.29, 1.45, 1.33, and 1.47, respectively.

**Postmortem findings and toxicological/biochemical data**

Referring to the situation on the scene and the autopsy findings, the mechanism of death is consistent with a DIC caused by hyperthermia. Even in the 1970s, the hyperthermic effect of PMA has been investigated, and in mice, it was found that this effect was not as pronounced as for 3,4-methylenedioxyamphetamine (MDA) (18). Hyperthermia was a frequently seen symptom in patients who presented to the Emergency Department after ingestion of PMA (19) and was also described in a few fatalities (4). In addition, hypoglycemia and hyperkalemia have been described as typical in PMA intoxications (19). Analysis of the vitreous humor in this case revealed a high potassium level. This value could reflect an antemortem hyperkalemia but, referring to the postmortem interval of about three days (20) and the dehydration of the eyes, this potassium concentration should be interpreted with caution. The glucose and lactate sum value of 312 mg/dL as such is not consistent with hypoglycemia. According to the study of Sippel and Möttönen (21), sum values lower than 160 mg/dL are compatible with a hypoglycemia. However, in cases experiencing a prolonged and/or intense agony, high sum values were noticed (22) and as a result, the sum value in this case can be correlated to the DIC caused by hyperthermia as the mechanism of death.

As for MDMA, there is no consensus about the lethal blood level, but in general, a blood MDMA concentration higher than 1.0 µg/mL can be potentially lethal (23). For PMA, blood levels greater than 0.5 µg/mL are likely to induce toxic effects (3). We believe that in this case, referring to the considerable MDMA and PMA levels in particular, both concentrations are definitely capable of inducing death. In the cases published by Byard et al. (5), blood PMA levels between 0.24 and 4.9 µg/mL were found. A PMA blood level up to 5.7 µg/mL has even been reported (6).

Only after incorporation of the LC–MS technique did it be-
came clear that the peak and the concentration of PMA were toxicologically relevant. The poor GC properties of PMA resulted in a broad peak. This was eliminated by the LC–MS procedure, in which it became clear that substantial amounts of PMA were present in various matrices.

In this case, the body distribution of the four amphetamine-related drugs was fairly comparable (Table I). Our data also confirm that blood sampled from the femoral vein should be considered as the “gold standard” for blood sampling. However, when this sample is not available, blood from the nearby iliac vein is also appropriate. A gradient from the inferior vena cava and the iliac vein to the femoral vein was noticed. This “diffusion gradient” can be explained by the relatively high levels in the liver. The huge concentrations in the bile are consistent with death the person urinated. In addition, for PMA, it was shown (28). However, it is not known when and how many times prior to death the person urinated. In addition, for PMA, it was shown that in humans, an average of 15% is eliminated as unchanged drug (29).

By these data, it has been confirmed that the concentrations of amphetamine derivatives in adipose tissue are very low (16).

**Table I. Distribution of PMA, MDMA, MDA, and AMP**

<table>
<thead>
<tr>
<th>Sample</th>
<th>PMA (µg/L)</th>
<th>MDMA (µg/L)</th>
<th>MDA (µg/L)</th>
<th>AMP (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subclavian blood</td>
<td>2012</td>
<td>1917</td>
<td>614</td>
<td>239</td>
</tr>
<tr>
<td>Femoral blood</td>
<td>1634</td>
<td>1129</td>
<td>436</td>
<td>198</td>
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<tr>
<td>Vena iliaca blood</td>
<td>1618</td>
<td>1421</td>
<td>493</td>
<td>203</td>
</tr>
<tr>
<td>Inferior vena cava blood</td>
<td>2058</td>
<td>1801</td>
<td>507</td>
<td>218</td>
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<tr>
<td>Right atrial blood</td>
<td>2058</td>
<td>1624</td>
<td>574</td>
<td>248</td>
</tr>
<tr>
<td>Pulmonary artery blood</td>
<td>2952</td>
<td>2212</td>
<td>475</td>
<td>279</td>
</tr>
<tr>
<td>Left pulmonary vein blood</td>
<td>2120</td>
<td>1693</td>
<td>526</td>
<td>261</td>
</tr>
<tr>
<td>Right pulmonary vein blood</td>
<td>2427</td>
<td>1941</td>
<td>610</td>
<td>329</td>
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<tr>
<td>Aorta ascendens blood</td>
<td>2031</td>
<td>1726</td>
<td>558</td>
<td>252</td>
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<td>Right pleural blood</td>
<td>1794</td>
<td>1375</td>
<td>446</td>
<td>207</td>
</tr>
<tr>
<td>Left pleural blood</td>
<td>3814</td>
<td>3243</td>
<td>912</td>
<td>450</td>
</tr>
<tr>
<td>Pericardial fluid</td>
<td>2373</td>
<td>2335</td>
<td>615</td>
<td>318</td>
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<tr>
<td>Vitreous humor</td>
<td>2101</td>
<td>1633</td>
<td>577</td>
<td>292</td>
</tr>
<tr>
<td>Urine (µg/L)</td>
<td>932</td>
<td>791</td>
<td>369</td>
<td>522</td>
</tr>
<tr>
<td>Bile (µg/L)</td>
<td>50,012</td>
<td>25,420</td>
<td>11,655</td>
<td>9425</td>
</tr>
<tr>
<td>Muscle of the right cardiac ventricle (µg/g)</td>
<td>2176</td>
<td>1650</td>
<td>469</td>
<td>235</td>
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<tr>
<td>Muscle of the left cardiac ventricle (µg/g)</td>
<td>2422</td>
<td>1815</td>
<td>293</td>
<td>290</td>
</tr>
<tr>
<td>Right lung, upper lobe (µg/kg)</td>
<td>4614</td>
<td>2580</td>
<td>925</td>
<td>592</td>
</tr>
<tr>
<td>Right lung, median lobe (µg/kg)</td>
<td>4460</td>
<td>2535</td>
<td>1023</td>
<td>693</td>
</tr>
<tr>
<td>Right lung, lower lobe (µg/kg)</td>
<td>3164</td>
<td>1281</td>
<td>676</td>
<td>395</td>
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<tr>
<td>Left lung, upper lobe (µg/kg)</td>
<td>3742</td>
<td>2138</td>
<td>661</td>
<td>475</td>
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<tr>
<td>Left lung, lower lobe (µg/kg)</td>
<td>4390</td>
<td>2358</td>
<td>875</td>
<td>543</td>
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<tr>
<td>Liver (µg/kg)</td>
<td>8904</td>
<td>6657</td>
<td>744</td>
<td>857</td>
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<tr>
<td>Stomach contents (µg/L)</td>
<td>73,103</td>
<td>33,168</td>
<td>14,308</td>
<td>5478</td>
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<tr>
<td>Right kidney (µg/kg)</td>
<td>5669</td>
<td>4058</td>
<td>3888</td>
<td>746</td>
</tr>
<tr>
<td>Left kidney (µg/kg)</td>
<td>4716</td>
<td>3411</td>
<td>2891</td>
<td>534</td>
</tr>
<tr>
<td>Spleen (µg/kg)</td>
<td>4390</td>
<td>3050</td>
<td>1454</td>
<td>666</td>
</tr>
<tr>
<td>Iliopsoas muscle (µg/kg)</td>
<td>1654</td>
<td>1528</td>
<td>592</td>
<td>221</td>
</tr>
<tr>
<td>Abdominal adipose tissue (µg/kg)</td>
<td>131</td>
<td>317</td>
<td>44</td>
<td>67</td>
</tr>
<tr>
<td>Brain, frontal lobe (µg/kg)</td>
<td>4081</td>
<td>2258</td>
<td>919</td>
<td>330</td>
</tr>
<tr>
<td>Brain, temporal lobe (µg/kg)</td>
<td>4188</td>
<td>2289</td>
<td>1035</td>
<td>358</td>
</tr>
<tr>
<td>Brain, parietal lobe (µg/kg)</td>
<td>4040</td>
<td>2514</td>
<td>1026</td>
<td>773</td>
</tr>
<tr>
<td>Brain, occipital lobe (µg/kg)</td>
<td>3357</td>
<td>1932</td>
<td>918</td>
<td>910</td>
</tr>
<tr>
<td>Brainstem (µg/kg)</td>
<td>3200</td>
<td>1951</td>
<td>761</td>
<td>346</td>
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<tr>
<td>Cerebellum (µg/kg)</td>
<td>2371</td>
<td>978</td>
<td>491</td>
<td>664</td>
</tr>
</tbody>
</table>

Conclusions

In summary, a fatal poisoning in which considerable blood levels of MDMA and PMA were found, is presented. We conclude that the man died of disseminated intravascular coagulation, induced by hyperthermia caused by the combined ingestion of amphetamines. In addition, the postmortem distribution of these amphetamine derivatives in the human body was discussed and proved once more that peripheral blood sampling is recommended.

The results also indicate that because of the poor GC proper-
ties of PMA, under specific conditions, this compound can be erroneously overlooked. However, systematic toxicological analysis based on the combination of both GC and HPLC can reveal the presence of PMA in biological matrices without any difficulty.

Acknowledgments

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