Distribution Study of 3,4-Methylenedioxy-methamphetamine and 3,4-Methylenedioxy-amphetamine in a Fatal Overdose

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Abstract

In this study, regional tissue distributions of the amphetamine analogue 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”) and its metabolite 3,4-methylenedioxyamphetamine (MDA) in a fatal overdose are presented. Quantitation of MDMA and MDA levels occurred in blood samples taken centrally (right and left heart and main adjacent great vessels) and peripherally (subclavian and femoral blood). In addition, MDMA and MDA concentrations were determined in cardiac and iliopsoas muscle, both lungs, liver, both kidneys, spleen, the four brain lobes, cerebellum and brainstem, and adipose tissue. Finally, MDMA and MDA levels were determined in serum, vitreous humor, urine, and bile. For all samples, a fully validated high-pressure liquid chromatography procedure with fluorescence detection was used. The found substances were also identified with liquid chromatography–tandem mass spectrometry. Our data confirm that blood sampling from an isolated peripheral vein is recommended for MDMA and MDA. In addition, the vitreous humor MDMA level indicates that this fluid can be an interesting alternative when a suitable blood sample is missing. Considering the substantial differences in concentrations in blood samples taken from various sites in the body and the high levels in some tissues (e.g., in liver), we conclude that the influence of postmortem redistribution should be taken into account in the interpretation of toxicological data when an appropriate peripheral sample cannot be obtained or when blood samples are not available because of putrefaction.

Case History

One morning, a 23-year-old man was found unconscious in a bar. He was sitting on a chair, resting with his head upon his forearms on the table in front of him. The emergency team attempted intensive reanimation, which failed. Upon examination 28 h postmortem, the body weighed approximately 100 kg and was 186 cm in length. In the pocket of the decedent, a small amount of white powder was found. Some vomit was noticed on his t-shirt, and his boxer shorts were soiled with urine. During further external examination, many vibices were observed in the postmortem lividity located on the upper thorax and back, and his face was strongly cyanotic. Obvious congestion of the conjunctivae and intermediary pupils was present. A fresh puncture wound was seen on the right arm, but inquiry revealed that it occurred during the reanimation attempt. During internal inspection, signs of intensive reanimation, including a sternal fracture, were found. Numerous Tardieu spots were observed on the pericardium and both pleurae. Both lungs weighed 1620 g, and, upon sectioning, some emphysema, severe congestion, and moderate edema were found. The heart...
weighed 405 g, and an aberrant course of the superior vena cava, a persistence of the left superior caval vein, was noticed. The stomach contained a brownish liquid without food fragments, and the mucosa showed a few pin-point ulcerations. The brain weighed 1545 g, and, apart from slight edema and congestion of the white matter, nothing unusual was observed. The remaining organs showed no obvious anomalies macroscopically, except for congestion.

On histologic examination, pronounced pulmonary congestion, hemorrhagic edema, a slight intra-alveolar infiltration with a few polymorphonuclear cells and some leucocyte sludging in the pulmonary veins were found. Groups of alveolar macrophages were seen, although staining with Prussian blue was negative and there were no signs of pulmonary hypertension. The liver showed slight fatty infiltration, and a few peripancreatic lymphocyte infiltrates were found. In the caudate nucleus and the nucleus lentiformis, a few venulae were surrounded by a lymphocyte infiltration. The hippocampus showed no marked hypoxicemic lesions. No obvious pre-existing disease was identified histologically.

Because drug abuse was suspected and amphetamines could be involved, appropriate samples for a distribution study were taken. Sampling included all possible central and peripheral blood samples, stomach contents, urine, bile, and vitreous humor. Several small tissue fragments were taken at random throughout the organs. The samples of cardiac and iliopectoralis muscle, both lungs, both kidneys, liver, spleen, abdominal adipose tissue, all four lobes of the cerebrum, cerebellum, and brainstem were preserved at −30°C until analysis.

Materials and Methods

Reagents and materials

All reagents and chemicals were of analytical grade and were obtained from Aldrich (Gillingham, U.K.) unless stated otherwise. Solvents were of HPLC grade from Merck (Darmstadt, Germany). Pure MDA, MDMA, and 3,4-methylenedioxyethylamphetamine (MDEA) standards were obtained from Sigma (St. Louis, MO). 3,4-Methylenedioxyethylpropylamphetamine (MDMPA) was synthesized by in-house following a procedure described earlier (17). Stock solutions of these active substances were prepared by dissolving 10 mg of the pure compound in 10 mL of methanol. Appropriate dilution with methanol yielded the working solutions containing all three compounds. All concentrations of the standards are expressed as the free base. The stock solutions were stored in the dark at −20°C and were stable for at least 1 year. Working solutions were stored under the same conditions as the stock standards but discarded after 6 months.

Drug screening

A comprehensive screening was performed on blood, urine, and stomach contents. Screening methods used were the enzyme-multiplied immunoassay technique (EMIT®), radioimmunoassay (RIA), and various chromatographic techniques, including high-performance liquid chromatography–diode-array detection (HPLC–DAD) (following extraction under alkaline conditions), thin-layer chromatography (TLC) on Sunshine extracts, and gas chromatography–mass spectrometry (GC–MS), as described previously (18).

Apparatus

The HPLC unit was composed of a ternary low-pressure gradient pump and an autosampler with a 25-µL loop (Kontron Instruments, Milano, Italy) equipped with a solvent degassing module (Shodex, Tokyo, Japan). A spectrofluorometric detector (RF-10Axl, Shimadzu, Kyoto, Japan) linked to a Kromasystem 2000 data system (Kontron Instruments) was used for data acquisition and storage.

The MS analyses were carried out on a Micromass Q-TOF hybrid MS (Micromass, Wythenshawe, U.K.) equipped with an orthogonal electrospray source (Z-spray) and a Waters Alliance 2790 separation module (Waters, Milford, MA) integrated with the Q-TOF instrument.

Isolation of the compounds

Serum, whole blood, vitreous humor, and urine samples (250 µL) were extracted with 8 mL of hexane/ethylacetate (7:3, v/v), after the addition of 50 µL of the internal standard solution (containing 400 ng/mL MDMPA for water, serum, whole blood, and vitreous humor and 5 µg/mL MDMPA for urine), dilution with 1 mL of H2O, and adjustment of the pH with 0.5 mL of 1M aqueous K2CO3 (brought to pH 9.5 with 37% HCl). Samples were mixed on a rotary mixing device (10 min) and centrifuged for 15 min (1200 × g). The organic layer was transferred to a test tube containing 50 µL methanolic HCl (5M acetylchloride in methanol) and evaporated using a Turbovap® evaporator (Zymark, Hopkinton, MA) at 35°C under nitrogen.

Tissue samples were homogenized after a 1:4 dilution in water (1 mL of the homogenate corresponds to 250 mg tissue) using an Ultra-Turrax homogenizer from IKA (Staufen, Germany). The resulting homogenate of the tissue samples as well as bile and stomach contents were extracted using a liquid–liquid extraction with back extraction that was especially developed for the analysis of amphetamines from degraded postmortem samples. After addition of the internal standard (containing 400 ng/mL MDMPA) to 1 mL tissue homogenate or 250 µL bile or stomach contents, 1 mL of water was added, and the pH was adjusted with 0.5 mL of the 1M aqueous K2CO3 solution. Subsequently, the samples were extracted with 8 mL of hexane/ethylacetate (7:3, v/v). To that end, samples were mixed on a rotary mixing device (10 min) and centrifuged for 15 min (1200 × g). The organic layer was transferred to a test tube containing 1 mL of 1M hydrochloric acid. After mixing on a rotary mixing device (10 min) and centrifuging for 15 min (1200 × g), the organic layer was discarded. The aqueous layer was brought to pH 9.5 with 2 mL of 2M aqueous K2CO3 (also brought to pH 9.5 with 37% HCl) and again extracted with 8 mL of hexane/ethylacetate (7:3, v/v). After mixing on a rotary mixing device (10 min) and centrifuging for 15 min (1200 × g), the organic layer was transferred to a test tube containing 50 µL methanolic HCl (5M acetylchloride in methanol) and evaporated at 35°C under nitrogen. The dry residues from both extraction procedures were redissolved in 125 µL of HPLC eluent A (for all matrices except for
Photograph of the page reveals a document discussing the analysis of drugs in various samples, including blood, serum, urine, vitreous humor, and tissue. The text describes the methodology used for these analyses, including the use of liquid chromatography-mass spectrometry (LC-MS) and mass spectrometry (MS) to detect and quantify drugs.

The document highlights the importance of accurate and precise measurements, with a focus on the detection of methylenedioxyamphetamines (MDA, MDMA, MDEA) and methylethylketoxime (MDMPA) in different matrices.

Key points:
- **Chromatography**: Chromatographic separation was achieved on a Hypersil BDS C18 column. The mobile phase was a 0.1M solution of ammonium acetate in HPLC-grade water (90%), methanol (5%), and acetonitrile (5%) (Eluent A) or in methanol (45%), acetonitrile (45%), and HPLC-grade water (10%). An isocratic part (100% A) of 6 min, a linear gradient from 0 to 70% B within 14 min was used.
- **Fluorescence detection**: The MS instrument was set to pass precursor ions of the selected mass (180.1 for MDA, 194.1 for MDMA, 208.1 for MDEA, and 236.1 for MDMPA) to the hexapole collision cell (using argon as the collision gas for collision-induced dissociation (CID)) and product ion spectra were acquired with the TOF analyzer. The TOF analyzer was scanned over m/z 100 to 250 with a 3-s integration time.

**Results**

**Drug screening**: The routine screening of blood and urine by immunoassay techniques disclosed the presence of a high level of amphetamines in urine only (68.4 µg/mL), toxicologically irrelevant levels of cotinine (6.9 and 1.0 µg/mL) urine and blood, respectively, caffeine (22.9 and 3.9 µg/mL), urine and blood, respectively, and trace amounts of benzoylecgonine (only present in urine, 0.7 µg/mL). Headspace GC analysis demonstrated the absence of ethanol in blood and urine. The analysis of blood, urine, and stomach contents using general purpose HPLC–DAD, GC–MS, and TLC methods as well as a method developed for the determination of cocaine and metabolites (19,20) in urine and blood confirmed the results found by the preliminary screening. Simultaneously, it revealed the presence of MDMA in blood, urine, and stomach contents. For additional confirmation, we developed a fully quantitative LC assay for the determination of the methylenedioxyamphetamine in all specimens available.

**Analytical performance**: Calibration curves were constructed for MDA (metabolite of MDMA, present in the majority of the matrices) and MDMA. The linearity ranged from 10 to 1000 ng/g for tissues; from 2 to 1000 ng/mL for blood, serum, and vitreous humor; and from 0.1 to 5 µg/mL for urine. The correlation coefficients in the different matrices ranged from 0.982 (kidney homogenate) to 0.998 (spleen homogenate) for MDA and from 0.976 (liver homogenate) to 0.999% (serum) for MDMA. The limits of detection, which were determined by analyzing decreasing concentrations of the compounds added to blank matrices, were 0.8 ng/mL for MDA and MDMA in whole blood, serum, and vitreous humor; 2 ng/g for MDA and MDMA in tissue samples; and 2 ng/mL for MDMA in urine. The limit of quantitation, which was defined as the lowest concentration that could be quantitated with an imprecision of < 20%, was 2 ng/mL for whole blood, serum, and vitreous humor; 10 ng/g for tissue samples, and 0.1 µg/mL for urine. Reproducibility (within-day and between-day, n = 6) was tested at low, medium, and high concentration levels in whole blood, serum, water (substitute for vitreous humor), and tissue (brain tissue) and was found to be < 20% in all cases. All samples were assayed in parallel using LC–MS–MS, and the obtained MS data confirmed the proper identities of the target compounds.
**Toxicological findings**

The toxicological findings are summarized in Table I. The MDMA and MDA tissue-to-blood ratios were calculated using the femoral blood level as reference. The ratio of blood-to-serum MDMA levels in the subclavian vein and in the aorta are 0.83 and 0.54, respectively. The corresponding ratios for MDA are 0.90 and 0.85, respectively.

**Discussion**

The autopsy findings, including macroscopic features (such as increased lung weight) and the microscopic examination (pronounced pulmonary congestion and edema), are consistent with an acute to subacute cardio-pulmonary failure. From the purely physiological point of view, the aberrant course of the superior vena cava (persistence of the left superior caval vein) was not important, as the outlet of the vena cava superior was also present in the right atrial cavity. Referring to the toxicological data, we can conclude that the cardio-respiratory insufficiency was caused by the sympaticomimetic mechanism of MDMA. Indeed, electrical instability of the heart has been described in this anomaly (21).

We present a detailed distribution of MDMA and MDA concentrations in this fatal overdose. Possible mechanisms of redistribution are presented in Figure 1.

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**Table I. Distribution of MDMA and MDA**

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>MDMA (µg/mL)</th>
<th>MDA (µg/mL)*</th>
<th>Ratio: fluid or tissue to femoral blood MDMA level</th>
<th>Ratio: fluid or tissue to femoral blood MDA level</th>
</tr>
</thead>
<tbody>
<tr>
<td>subclavian blood</td>
<td>3.5</td>
<td>0.090</td>
<td>1.13</td>
<td>0.97</td>
</tr>
<tr>
<td>femoral blood</td>
<td>3.1</td>
<td>0.093</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>vena iliaca blood</td>
<td>3.5</td>
<td>0.191</td>
<td>1.13</td>
<td>2.05</td>
</tr>
<tr>
<td>inferior vena cava blood</td>
<td>4.8</td>
<td>0.199</td>
<td>1.55</td>
<td>2.14</td>
</tr>
<tr>
<td>right atrial blood</td>
<td>5.5</td>
<td>0.185</td>
<td>1.77</td>
<td>1.99</td>
</tr>
<tr>
<td>right ventricular blood</td>
<td>5.7</td>
<td>0.296</td>
<td>1.84</td>
<td>3.18</td>
</tr>
<tr>
<td>left atrial blood</td>
<td>7.6</td>
<td>0.274</td>
<td>2.45</td>
<td>2.95</td>
</tr>
<tr>
<td>blood from the aorta serum</td>
<td>4.4</td>
<td>0.151</td>
<td>1.42</td>
<td>1.62</td>
</tr>
<tr>
<td>(blood from the aorta)</td>
<td>4.2</td>
<td>0.100</td>
<td>1.35</td>
<td>1.08</td>
</tr>
<tr>
<td>vitreous humor</td>
<td>3.4</td>
<td>0.060</td>
<td>1.10</td>
<td>0.65</td>
</tr>
<tr>
<td>urine</td>
<td>170.9</td>
<td>4.000</td>
<td>NR†</td>
<td>NR†</td>
</tr>
<tr>
<td>bile</td>
<td>14.2</td>
<td>0.320</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>cardiac muscle</td>
<td>14.0</td>
<td>0.346</td>
<td>4.52</td>
<td>3.72</td>
</tr>
<tr>
<td>right lung</td>
<td>12.5</td>
<td>0.446</td>
<td>4.03</td>
<td>4.80</td>
</tr>
<tr>
<td>left lung</td>
<td>18.9</td>
<td>0.609</td>
<td>6.10</td>
<td>6.55</td>
</tr>
<tr>
<td>liver</td>
<td>26.2</td>
<td>1.203</td>
<td>8.45</td>
<td>12.94</td>
</tr>
<tr>
<td>stomach content</td>
<td>118.1</td>
<td>0.448</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>right kidney</td>
<td>12.1</td>
<td>2.700</td>
<td>3.90</td>
<td>29.03</td>
</tr>
<tr>
<td>left kidney</td>
<td>13.9</td>
<td>3.022</td>
<td>4.48</td>
<td>32.49</td>
</tr>
<tr>
<td>spleen</td>
<td>10.0</td>
<td>0.264</td>
<td>3.22</td>
<td>2.84</td>
</tr>
<tr>
<td>iliopsoas muscle</td>
<td>4.5</td>
<td>0.144</td>
<td>1.45</td>
<td>1.55</td>
</tr>
<tr>
<td>adipose tissue</td>
<td>0.4</td>
<td>&lt;LOQ</td>
<td>0.13</td>
<td>NR</td>
</tr>
<tr>
<td>brain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>frontal lobe</td>
<td>17.4</td>
<td>0.296</td>
<td>5.61</td>
<td>3.18</td>
</tr>
<tr>
<td>temporal lobe</td>
<td>14.9</td>
<td>0.252</td>
<td>4.81</td>
<td>2.71</td>
</tr>
<tr>
<td>parietal lobe</td>
<td>17.1</td>
<td>0.362</td>
<td>5.52</td>
<td>3.89</td>
</tr>
<tr>
<td>occipital lobe</td>
<td>12.9</td>
<td>0.256</td>
<td>4.16</td>
<td>2.75</td>
</tr>
<tr>
<td>brainstem</td>
<td>13.2</td>
<td>0.220</td>
<td>4.26</td>
<td>2.37</td>
</tr>
<tr>
<td>cerebellum</td>
<td>11.7</td>
<td>0.225</td>
<td>3.77</td>
<td>2.42</td>
</tr>
</tbody>
</table>

* for tissues: µg/g.
† NR: not relevant.
Our data (Table I) confirm that a peripheral blood sample is strongly recommended and femoral blood remains the most representative. When femoral blood is not available, blood from the vena subclavia or vena iliaca may be appropriate; however, cardiac blood samples and left atrial blood in particular should be avoided. The site-dependent differences in heart blood concentrations have previously been observed for methamphetamine (12). In our case, the left atrial MDMA level was the highest of all and can probably—or at least partially—be explained by diffusion from both lungs via the venae pulmonales. It is not excluded that the high MDMA level in cardiac muscle is also correlated with the high MDMA concentration in the adjacent lungs, but diffusion from the stomach content volume could also be speculated. The obvious difference in MDMA and MDA levels between both lungs could be explained by post-mortem diffusion out of the high reservoir of these substances present in the stomach content volume. Indeed, the stomach is only separated from the left lung by the diaphragm. As the gastric mucosa is easily influenced by the autolytic process, postmortem diffusion to the closely adjacent organs can be assumed. The relatively high MDMA level in vena cava inferior blood can be correlated with diffusion out of the kidneys and the liver. Indeed, the MDMA concentration in the liver was the highest of all organ levels. As the liver seems to be an important “reservoir” of MDMA, this organ can be assumed to be capable of inducing considerable postmortem redistribution at increasing postmortem intervals. As a result, blood sampling near the liver (e.g., inferior vena cava blood or blood from the right heart) should be avoided.

The MDMA and MDA levels in liver and bile can point to an elimination by biotransformation or by excretion via the bile. However, MDA levels in the kidneys were higher than the corresponding MDA liver concentrations, which may indicate that the impact of tubular reabsorption should not be overlooked because the MDA level in urine was rather low. In our case, because the MDA blood and tissue levels (also the ratios as given in Table I) are mainly consistent with the MDMA distribution, though with a considerable difference in size, we can assume that MDA acts as a metabolite of MDMA. Referring to the MDA amounts, our data confirm the results of previous studies, namely MDA is not a major metabolite in humans (22–24).

The ratio of vitreous humor to femoral blood MDMA level of 1.1 was consistent with previous research in rabbits (25), indicating that equilibration was attained. This assumes that vitreous humor can be a suitable alternative when an appropriate blood sample is lacking, but this should be confirmed with larger series. In addition, when a significant degree of putrefaction has already taken place, thus making blood and vitreous humor sampling impossible, quantitation of MDMA in iliopsoas muscle can give relevant information (see values in Table I).

The MDMA levels in the various brain regions demonstrate regional differences with the highest levels being in the frontal and parietal lobes. In our data, these regional differences are similar for MDA. Because the sampling method was different, our results cannot be compared with recently published findings (16). Finally, the high cerebral levels are concordant with the strong neuropharmacologic effects of “ecstasy”.

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