Case Report

Tissue Distribution of Amphetamine Isomers in a Fatal Overdose


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Abstract

A young man (22 years old) died of a cardiorespiratory arrest a few hours following admission to the emergency department of a hospital. He was found lying seriously ill in the parking lot of a dance club. Screening of postmortem blood and urine with enzyme multiplied immunoassay (EMIT) detected only amphetamines, caffeine, and cotinine. Further screening of blood, urine, and stomach contents with thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) was negative for all three matrices. Specific conditions for amphetamines were used for the gas chromatographic (GC) screening (GC–mass spectrometric [MS] and GC–nitrogen-phosphorus detection). This resulted in the preliminary identification of amphetamine in both blood and urine. Confirmation of the presence of amphetamine in all available postmortem specimens was provided by mass and infrared spectral data (GC–MS and GC–Fourier transform infrared spectrometry) after derivatization. Quantitative results and differentiation between the enantiomers of amphetamine were obtained after chiral derivatization. The calculated concentrations disclosed amphetamine ingestion as the cause of this fatality.

Introduction

Amphetamine (racemic β-phenylisopropylamine) is the basic molecule of the amphetamines, which is a group of structurally related compounds with limited legitimate clinical use but vast potential for abuse, addiction, and toxicity (1).

Oral and intravenous abuse of amphetamine began around 40 to 50 years ago. Since then, the restricted legitimate supply of this controlled substance has led to the widespread production of amphetamine and other synthetic derivatives in clandestine laboratories.

The amphetamines are structurally very similar to the endogenous catecholamines (nor)epinephrine and dopamine. They act as indirect sympathomimetic compounds with prominent central nervous system activity (2). Abuse to combat fatigue or achieve euphoria or both (e.g., during nonstop dance parties as in this case) exposes the individual to potentially serious toxicity risks. The quantitative composition of drugs is often ignored by the inexperienced user and sometimes even by the dealer. Moreover, occasional use can lead to dependence, tolerance, and chronic abuse.

Case History

The decedent, a young man aged 22 years, was found on a Sunday afternoon, about 12:30, lying seriously ill in the parking lot of a dance club. He had been dancing almost nonstop from 3 a.m. until noon. He was admitted to the emergency department of a nearby hospital where he died of a sudden cardiorespiratory arrest approximately 4 h later. At admission he showed symptoms of drug intoxication: vomiting and alternating extreme aggressiveness and agitation with periods of sleepiness. In this state of confusion, he also claimed to have taken 2 g of heroin recently.

The autopsy was carried out one day after death. A marked cyanosis was observed externally. The body was slender, and no injection sites were found. The brain showed signs of congestion. The lungs displayed an outspoken vascular congestion and alveolar rupture on section. A pseudocolloidal pinkish liquid was found in the lungs and exogenous material, especially at the bronchial level. Histological findings demonstrated all signs corresponding with a terminal asphyxia. All other organs had a normal aspect and were thus unremarkable. No remnants of tablets were embedded in the stomach, which was almost empty and contained only a few milliliters of blood-resembling liquid. Because an intoxication was strongly suspected, postmortem samples of blood (prelevated peripherally), urine, stomach contents, liver, kidney, and brain were collected and stored at −20°C in glass jars so a thorough toxicological investigation could be performed.
Experimental

Solvents and reagents
All solvents were of HPLC-grade purity and were from Merck (Darmstadt, Germany). Heptafluorobutyric acid anhydride (HFBA) was obtained from Sigma (St. Louis, MO). Amphetamine was available from the standards collection at the Laboratory of Toxicology (University of Gent, Belgium). Ammonium hydroxide, (S)-(−)-N-(trifluoroacetyl)-prollyl chloride (L-TPC) 0.1M in dichloromethane, and the internal standard 4-phenylbutylamine (4-PBA) were all from Aldrich (Milwaukee, WI).

Drug screening
Postmortem blood and urine were analyzed following a previously described comprehensive screening process using enzyme multiplied immunoassay techniques (EMIT); radioimmunoassay (RIA) (3); and chromatographic techniques such as high-performance liquid chromatography with diode array detection (HPLC-DAD) following alkaline extraction (4), thin-layer chromatography (TLC) on Sunshine extracts (5) using a modified extraction of organic bases from urine with ether and 4N HCl instead of chloroform and 0.1N H2SO4, and gas chromatography with nitrogen-phosphorus detection (GC-NPD) (3) and with mass spectrometric detection (GC–MS) (6).

Amphetamine determination

Extraction step
GC–MS analysis. A solid-phase extraction procedure on Bond Elut Certify® 130-mg columns (Varian, Harbor City, CA) was used essentially as described by the manufacturer for urine. Briefly, samples were buffered at pH 6.0, applied on the solid-phase column, rinsed with buffer and methanol, and eluted with a freshly prepared 2% (v/v) ammonium hydroxide solution in ethyl acetate. Total sample volume was always adjusted to 5 mL with HPLC-grade water if the original sample volume was less than 5 mL. All tissue samples were homogenized with an Ultra-Turrax mixer after dilution with HPLC-grade water (1:1, w/w). Aliquots (2 g) of the tissue homogenates (stomach contents, liver, kidney, and brain) and of the diluted whole blood sample were ultrasonicated for 15 min before centrifugation at 2500 rpm for 10 min. Solid-phase extraction was performed on the obtained supernatant. At the end of the evaporation step under nitrogen, 50 μL of 4N methanolic HCl was always added to prevent the loss of the volatile amphetamine base.

GC–FID analysis. For the quantitation and the enantioselective differentiation of amphetamine in both its enantiomers (R)- and (S)-amphetamine, another derivatization procedure was used (7). The obtained extraction residue was dissolved in a mixture of 250 μL of n-hexane and 50 μL of the 0.1M L-TPC solution. After derivatization at 90°C for 5 min, samples were cooled, 50 μL of water and 50 μL of 2% (v/v) NH4OH were added, and the excess L-TPC was separated by centrifugation at 700 × g.

Both types of derivatives were also injected on the GC–FTIR system.

Chromatographic conditions

GC–MS system (6). For GC–MS analysis, a series 3400 Varian gas chromatograph was used (Varian, Sunnyvale, CA) in combination with a Finnigan MAT Magnum mass selective ion trap detector (Finnigan, San José, CA). A J&W Scientific (Folsom, CA) DB-5 capillary column (30 m × 0.25-mm i.d., 0.25-μm film thickness) was installed in the GC. The injector and the transfer line were held at 280 and 270°C, respectively. The carrier gas was helium at a flow rate of 0.8 mL/min. The initial oven temperature of 70°C was held for 1 min, programmed to 100°C at 30°C/min, and more slowly ramped to 270°C at 10°C/min. Samples (1 μL) were injected in the splitless mode.

GC–FID system (7). The column was an Ultra-2 (20 m × 0.32-mm i.d., 0.17-μm film thickness) from Hewlett-Packard (Palo Alto, CA). The injector and detector were held at 170 and 270°C, respectively. The analyses were started at an oven temperature of 60°C, immediately programmed at 30°C/min to 150°C, and then at 5°C/min to 260°C. The injector was used in the splitless mode, and 2-μL aliquots were injected.

GC–FTIR system (6). For GC–FTIR analysis, a Perkin Elmer AutoSystem GC was used (Perkin Elmer, Buckinghamshire, U.K.) in combination with a Perkin Elmer GC–IR system 2000 interface and an FTIR system 2000 detector. An HP Ultra-1 (Hewlett-Packard, Palo Alto, CA) capillary column (25 m × 0.32-mm i.d., 0.50-μm film thickness) was installed in the gas chromatograph. The PTV injector from Gerstel (Broekhuis, The Netherlands) was used in the splitless mode and programmed from 50 to 250°C at a rate of 12°C/s with a splitless time of 1 min. The initial oven temperature of 100°C was held for 1 min and then programmed to 270°C at 10°C/min. The light pipe was heated at a constant temperature of 225°C, and the carrier gas was helium at a flow rate of 0.95 mL/min.

Quantitative analysis

GC–MS. For the semi-quantitative data, calibration samples were prepared by spiking a blank postmortem specimen for each of the unknown postmortem matrices analyzed, except for stomach contents and brain. The added amounts of amphetamine were 5.0 and 25.0 μg/mL for whole blood and urine, respectively, and 10.0 and 5.0 μg/g for liver and kidney, respectively. No internal standard was added. The calibration samples were taken throughout the extraction and derivatization procedure in a manner similar to that used for the unknown samples.

GC–FID. Definitive quantitative data were obtained using three different calibration graphs: one in blank blood, one in blank urine, and one in blank liver homogenate. Fifty microliters of the 0.1 μg/μL internal standard (4-PBA) solution was added to each sample. The added levels of racemic
amphetamine were 0, 0.5, 1.0, 2.5, 5.0, and 7.5 μg/mL; 0, 0.5, 1.0, 2.5, 5.0, and 10.0 μg/mL; and 0, 0.25, 0.5, 1.0, 2.5, 5.0, and 7.5 μg/g, respectively. Again, calibration samples and unknowns were treated similarly.

Results and Discussion

The routine screening of blood and urine by immunoassay disclosed the presence of amphetamines and of toxicologically irrelevant levels of cannabinoids (only in urine, 30 ng/mL), ethanol (only in urine, 0.18 g/L), cotinine (blood, 0.5 μg/mL; urine, 3.8 μg/mL), caffeine (blood, 2.2 μg/mL; urine, 4.9 μg/mL), and insulin (blood, 3.1 μIU/mL). Opiates and the other commonly used drug classes and drugs were not present in blood or in urine. When blood, urine, and stomach contents were screened with the general HPLC and TLC methods, all results were negative.

Because drug abuse was strongly suspected from the case history and because amphetamines were found with EMIT, gas chromatographic screening conditions were focused on amphetamines. Using a specific solid-phase extraction procedure with methanolic HCl addition during evaporation and the specified chromatographic conditions as described previously, a large peak was detected in urine both with GC-NPD and GC-MS. This peak displayed the same retention characteristics and mass spectrum as an amphetamine standard. No other amphetamines could be demonstrated. The negative results for the HPLC and TLC screening were caused partly by the loss of the highly volatile amphetamine during nonadapted sample preparation and partly by the lack of detection sensitivity of both systems for amphetamine. Indeed, amphetamine displays characteristic but low UV absorption (8).

Additional peak-identification criteria were generated after derivatization of the extracts with HFBA. These extracts were injected on both GC-MS and GC-FTIR systems. The obtained mass and infrared (vapor-phase FTIR) spectra perfectly matched the corresponding spectra of mono-HFB-amphetamine. Derivatization dramatically increased the selectivity of the amphetamine mass spectrum. Indeed, whereas underivatized amphetamine shows extensive fragmentation with an unspecific base peak (m/z 44) (8), amphetamine–HFBA yields a spectrum with three characteristic prominent ions, that is, the molecular ion with a mass-to-charge ratio of 332, an ion at m/z 240, and the base peak with m/z 118 (Figure 1). The amphetamine–HFBA peak also eluted later than underivatized amphetamine (t_r = 7.0 min instead of 4.8 min). Moreover, superior chromatographic peak shape and thus increased sensitivity in comparison with underivatized amphetamine was observed. Therefore, HFBA derivatization of all available postmortem samples was performed and semiquantitative data were obtained as described in the Experimental section. All spectra were collected under electron impact conditions and in the scan mode using the sum of mass fragmentograms 332, 240, and 118 for quantitation. Results are summarized in Table I.

Finally, a newly developed enantioselective GC-FID method (7) was applied to obtain an extra identification criterion and the definitive quantitative data (Table I). Except for the brain tissue, the two sets of concentration levels showed good to excellent correlation. The difference for the brain tissue could be explained by the reported uneven distribution of amphetamine in this tissue (9). All sample extracts were also injected on the GC–FTIR system. The obtained infrared (vapor-phase FTIR) spectra perfectly matched the corresponding spectra of the derivatized (R)- and (S)-enantiomers of amphetamine (Figure 1). In all available postmortem samples, both the (R)- and (S)-enantiomers of amphetamine were present in virtually a 1 to 1 ratio. This enantiomeric ratio indicated the relatively recent intake of racemic amphetamine, as the (S)-form of amphetamine is known to be metabolized at a higher rate than the (R)-form (10). The absence of a predominating (R)-isomer suggested that samples could not have been taken at a late stage in the metabolic time profile.

The absence of injection sites at autopsy made the intravenous intake of the drug unlikely, whereas the presence of exogenous material in the lungs supported the hypothesis of intranasal drug abuse. The demonstration of amphetamine in stomach contents is not conclusive for the route of intake because this amount might be attributed to enterohepatic recirculation or aspiration or both.

Our quantitative values were compared with three original references from 1970 to 1975 (11–13). No recent data on amphetamine overdose were available. Reported fatal amphetamine blood levels ranged from less than 0.5 to 41 μg/mL following intravenous or oral amphetamine abuse or both. Although fatalities occur with amphetamine blood concentrations of less than 0.5 μg/mL, concentrations of 0.4 μg/mL and
higher are not uncommon in severe addicts with high tolerance. This wide range of fatal concentrations is also observed in urine and liver (ranging from 39 to 700 μg/mL and from 0.7 to 45 μg/g, respectively) (11–13). Reported kidney concentrations range from 4 to 8 μg/g (12). No brain concentrations from a fatality that was due to amphetamine were found.

Conclusion

After comparison with the cited values, it can be concluded from the demonstrated amphetamine concentrations (2.44 μg/mL in blood) that an acute amphetamine intoxication was the cause of this fatality. This rare case therefore illustrates the serious risk involved with amphetamine abuse.

Table I. Concentrations (or Total Amount for Stomach Contents) of Racemic Amphetamine and its (R)- and (S)-Enantiomers in Postmortem Specimens

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amphetamine (racemic)</th>
<th>(R)-Amphetamine</th>
<th>(S)-Amphetamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood*</td>
<td>2.44 (2.70)</td>
<td>1.26</td>
<td>1.18</td>
</tr>
<tr>
<td>Urine*</td>
<td>33.4 (32.4)</td>
<td>16.7</td>
<td>16.7</td>
</tr>
<tr>
<td>Stomach contents†</td>
<td>N.A. (49.1)</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Liver‡</td>
<td>11.7 (11.6)</td>
<td>6.07</td>
<td>5.64</td>
</tr>
<tr>
<td>Kidney‡</td>
<td>3.85 (2.22)</td>
<td>2.00</td>
<td>1.85</td>
</tr>
<tr>
<td>Brain‡</td>
<td>5.30 (13.4)</td>
<td>2.95</td>
<td>2.55</td>
</tr>
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</table>

* μg/mL
† μg/k
‡ μg/g
* N.A. = not analyzed.

Acknowledgments

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References


Figure 2. GC–FTIR reconstructed Gram-Schmidt chromatogram of the forensic urine extract after L-TPC derivatization. Vapor-phase FTIR spectrum of peak 1 is shown in the upper trace. Peak identification: 1, (R)-amphetamine-TPC; 2, (S)-amphetamine-TPC; and IS, internal standard.