Determination of L-Methamphetamine: A Case History

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Abstract

Methamphetamine was detected in a 77-year-old male who had a history of congestive heart failure. Using a modification of a previously reported method, trifluoroacetyl-L-prolyl chloride was used to derivatize sympathomimetic amines to allow separation and identification of individual enantiomers. The L-enantiomer of methamphetamine and a trace amount of D-amphetamine were found in blood and urine specimens from this case. Further investigation revealed the decedent had bronchial asthma and regularly used a Vicks Inhaler, which contains L-methamphetamine as the active ingredient.

Introduction

Sympathomimetic amines such as amphetamine and methamphetamine contain a single asymmetric carbon and are found as optically pure dextrorotatory (d, +, S) or levorotatory (l, −, R), or as mixtures of the two. In the case of methamphetamine, the d-enantiomer has a greater CNS stimulatory effect than its l-counterpart and is therefore more prone to abuse (1). Specific sympathomimetic amines, which are prone to abuse, are amphetamine, methamphetamine, and the so-called “designer” amphetamines, 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxyamphetamine, and methylene-dioxyethylamphetamine. Identification of enantiomers is possible using chiral derivatizing agents (1-3), chiral columns (4), or a combination of the two for analyses. This report describes the detection of the L-enantiomer of methamphetamine in an individual who died of cardiovascular disease. A previously reported method for enantiomer analysis (2,3) was modified to use solid-phase extraction (SPE) prior to derivatization for analysis of blood, urine, and vitreous humor.

Case history

An Emergency Medical Service team responded to the residence of a 77-year-old male who was having difficulty breathing (initial oxygen saturation was 69%). A breathing treatment of oxygen plus albuterol and Atrovent (ipratropium bromide) was administered; oxygen saturation improved to 96%. Although improved, the patient remained in poor condition and was transported to the emergency department of a local hospital. He was later placed in the ICU, and his condition was guarded. Approximately 12 h after admission, he became unresponsive and expired. His medical history included bronchial asthma, atrial flutter, and congestive heart failure. He was reportedly not taking any medications, but he was administered furosemide (40 mg IV), nitroglycerine infusion (30 μg/kg/min), dexamethasone (10 mg IV), enoxaparin (30 mg), diltiazem (125 mg), famotidine (20 mg), levalbuterol (0.63 mg/3 mL), azithromycin (500 mg/10 mL), ceftriaxone (50 mL of 5%), and midazolam (2 mg) while in the hospital. The cause of death was ruled arteriosclerotic cardiovascular disease. Samples of blood, urine, and vitreous humor were collected for toxicology analyses, but an autopsy was not performed.

Overall results of toxicology testing

Initial screens of urine were performed using Toxilab, immunoassay (FPIA with Abbott’s TDX, and Labstix (Bayer); blood was screened for volatiles using headspace gas chromatography (GC)–flame-ionization detection [model 7694, Hewlett-Packard (HP) and model 5890, series II, Agilent Technologies]. Positive results were confirmed using GC–mass spectrometry (MS) (model 6890N/5973, Agilent Technologies). Antemortem blood was positive for cotinine and caffeine (not quantitated), a trace amount [below the 0.05 μg/mL limit of quantitation (LOQ)] of doxylamine, and methamphetamine. Urine was positive for acetaminophen, nicotine, cotinine, caffeine, diltiazem, doxylamine, and methamphetamine. Identification of methamphetamine was accomplished following acetylation with acetic anhydride (m/z 58, 91, and 100, retention time approximately 2.71 min; amphetamine: m/z 86, 91, 118; retention time approximately 2.05 min); however, this procedure did not allow identification of specific enantiomers. The presence of methamphetamine raised the possibility of illicit drug use (d-methamphetamine), which was judged atypical
because of the age of the deceased. A previously reported method (2,3) that used trifluoroacetyl-L-prolyl chloride as a derivatizing agent was used to establish which enantiomeric form(s) of methamphetamine had been detected.

Materials and Methods

All chemicals used in this analysis were of reagent-grade quality or better. Derivatizing agent, trifluoroacetyl-L-prolyl chloride (t-TPC), was obtained from Sigma-Aldrich Chemical (St. Louis, MO). Standards for l-amphetamine, d-methamphetamine, and d,l-methamphetamine-d5, were obtained from Cerilliant (Round Rock, TX). Controls for sympathomimetic amines were prepared commercially as Amphetamine Plus®. Whole Blood Control (UTAK Labs, Valencia, CA). Specimens were collected in Vacutainer® tubes, (blood samples in gray tops and vitreous and urine in red tops). All of these specimens were collected by external puncture using needles and syringes.

Extraction of sympathomimetic amines was accomplished using SPE columns (ASDAU020, United Chemical Technologies, Bristol, PA) (5) in the following way: internal standard (d,l-methamphetamine-d5, final concentration 250 ng/mL) was added to specimens (2 mL of blood, urine, or vitreous), which in turn were mixed, diluted with distilled water (4 mL), and adjusted to pH 6 with 1.0M phosphate buffer (2 mL). The diluted specimens were added to the SPE columns, and both neutral and basic drugs were extracted onto the sorbent bed. Neutral drugs were eluted with 3 mL of methylene chloride/isopropyl alcohol/ammonium hydroxide (78:20:2). Eluents were dried under nitrogen and resuspended in 50 µL of acetonitrile. Fractions containing sympathomimetic amines (SMA) were dried at room temperature to minimize evaporative losses. In our laboratory, the SMA extracts were previously derivatized with acetic anhydride (50 µL for 1 h at 100°C). This derivatization method did not allow separation of enantiomers. The enantiomers in SMA extracts could be separated by substituting a simple derivatization as previously described (2,3). Briefly, 50 µL of t-TPC was added to extracts, and the mixture was allowed to stand at room temperature for 15 min. The extract was evaporated under a stream of dried nitrogen. The dried extracts were then resuspended in 2 mL of n-butylchloride and washed with 0.01M NaOH (2 mL) to help eliminate excess reagent. The mixture was separated by centrifugation, and the top layer was removed and dried under a stream of nitrogen. This final fraction was resuspended in 50 µL acetonitrile and (1 µL) injected onto the GC-MS.

Chromatographic analysis was performed as previously described (2,3) using an HP 5890 series II GC with an HP 970 MS and 7673 autoinjector. The MS was operated in SIM mode monitoring m/z 237, 251, and 255 for amphetamine, methamphetamine, and methamphetamine-d5, respectively. The analysis for both methamphetamine and amphetamine was linear from 5 to 1000 ng/mL. The LOQ was 5 ng/mL, and the limit of detection was 1 ng/mL for methamphetamine and amphetamine. These same values were obtained when the procedure was previously reported (3).

Results and Discussion

Methamphetamine is most often associated with illicit drug use, so finding this drug in a 77-year-old man raised concerns. The presence of the drug was confirmed, and identification of enantiomers was performed. Only the l-enantiomers were present in the specimens collected from the deceased. Figure 1 shows the chromatograms of amphetamine and methamphetamine extracted from heart blood and vitreous humor, compared to known (d and l) standards for these drugs.

Under the conditions used, the retention times for l- and d-amphetamine and l- and d-methamphetamine were 12.46, 13.01, 15.71, and 15.94, respectively. An internal standard of d,l-methamphetamine-d5 had retention times of 15.59 and 15.82 min for the l- and d-enantiomer. Amounts of l-methamphetamine present in blood, urine, and vitreous humor are shown in Table I. The concentration determined for vitreous humor is a calculated value, in that there was insufficient specimen to perform the analysis without making a dilution. Levels of the methamphetamine metabolite, l-amphetamine, were detectable but below the LOQ.

It is not possible to draw any conclusions about trends in the biological disposition of l-methamphetamine, considering this single case. Why the concentrations of amphetamine and methamphetamine in antemortem blood were lower than were found in postmortem samples is not clear. Only l-enantiomers of methamphetamine and amphetamine were detected, which...
led to the conclusion that this was not an example of illicit drug use. The toxicology report spurred further investigation of the case, which produced a family member who reported that the deceased routinely used a Vicks Inhaler for his asthma. Use of the inhaler was not known initially because it was not found with the deceased's personal effects.

Several reports (1,2,6–10) have described the determination of methamphetamine and amphetamine enantiomers in urine following Vicks Inhaler use. Those laboratories that have been most instrumental in the detection of licit and illicit use of methamphetamine have been drug-testing laboratories, often affiliated with the military. Substance Abuse and Mental Health Service Administration cutoffs for methamphetamine confirmation require that urine samples must contain methamphetamine at or above 500 ng/mL and the amphetamine metabolite to also be greater than or equal to 200 ng/mL to report the sample as positive (11). Using these guidelines, the SMA results of the present case would not have been reported. The urine concentration detected in this case was 10-fold lower than the peak concentration of a pooled urine sample of a volunteer in a study where subjects “were given one Vicks Inhaler and were instructed to take several deep inhalations approximately every 20 minutes for the next 6 hours” (1). This protocol exceeded the instruction for use of the Vicks Inhaler, which is two inhalations in each nostril, not more often than every 2 h. An earlier report indicated that the amount of l-methamphetamine that a single inhalation would deliver was 21 ng and that 17 inhalations were required to detect l-methamphetamine in urine by immunoassay (8). To our knowledge, this is the first report of blood and vitreous concentrations of l-methamphetamine and l-amphetamine from therapeutic use of a Vicks Inhaler.

Compared to drug testing laboratories, distinguishing specific enantiomers of methamphetamine is less common in laboratories concerned primarily with postmortem analysis. Without the aid of special derivatizing agents or chiral columns, it is not possible to identify the specific optical isomer involved in toxicology cases. Not all cases require that the specific optical isomer be established. But, when necessary, laboratories must be able to demonstrate which isomers are present so that misinterpretation of results can be avoided. We were pleasantly surprised at how simple this derivatization was to accomplish, and how completely the separation was achieved. In addition to the Vicks Inhaler, selegiline, a monoamine oxidase inhibitor, effective against Parkinson’s disease, is metabolized to l-methamphetamine (10,12–14). Famprofazone, a component of the analgesic formulation Gewodin, is metabolized to both the l- and d-enantiomers of methamphetamine and amphetamine (15,16).

<table>
<thead>
<tr>
<th>Specimen</th>
<th>l-Methamphetamine (ng/mL)</th>
<th>l-Amphetamine (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital blood</td>
<td>detected; &lt; LOQ</td>
<td>not detected</td>
</tr>
<tr>
<td>Femoral blood</td>
<td>5.2</td>
<td>detected; &lt; LOQ</td>
</tr>
<tr>
<td>Heart blood</td>
<td>5.1</td>
<td>detected; &lt; LOQ</td>
</tr>
<tr>
<td>Urine</td>
<td>313</td>
<td>detected, not quantitated</td>
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<tr>
<td>Vitreous humor</td>
<td>20.8</td>
<td>detected; &lt; LOQ</td>
</tr>
</tbody>
</table>

Table I. Distribution of l-Methamphetamine in Collected Specimens

References