CASE REPORT

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Methamphetamine and Amphetamine Derived from the Metabolism of Selegiline

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ABSTRACT: Routine methamphetamine testing identified a urine specimen with inconsistent screening and confirmation results. The methamphetamine RIA screening test (Diagnostic Products Corporation) indicated a borderline positive specimen, while the achiral confirmatory GC/MS result showed 4690 ng/mL of methamphetamine and 1895 ng/mL of amphetamine. Analysis of the specimen after derivatization with S(-)-N-trifluoroacetylprolyl chloride showed only the presence of l-amphetamine and l-methamphetamine. It was later learned that the individual providing the specimen had been taking Selegiline.

Selegiline, (-) propynylmethamphetamine, is a monoamine oxidase inhibitor used for the treatment of Parkinson's disease. It is sold under the trade name Eldepryl. Its major metabolites are l-methamphetamine, l-amphetamine and N-desmethylselegiline.

Urine specimens from other Selegiline users were obtained and analyzed. A characteristic metabolic pattern was noted, exemplified by a ratio of l-methamphetamine to l-amphetamine of about 2.8. This is in contrast to what is observed in the urine of individuals who ingest pure l-methamphetamine, such as with Vicks Inhaler, where the l-methamphetamine to l-amphetamine ratio in the urine is usually greater than 8. Caution is advised when interpreting methamphetamine results without using a chiral identification technique.

KEYWORDS: forensic science, toxicology, methamphetamines, amphetamines, selegiline

The identification of users of drugs of abuse by analysis of urine specimens has become a major undertaking in the military, as well as in the civilian workplace. While the accuracy of the analytical tests are assured by forensic procedures, quality assurance programs, proficiency testing, certification, and outside inspections, the differentiation between intentional use and inadvertent appearance of drugs in the urine specimen remains a major concern [*1,2*]. For this reason, the federal certifying agency, the Substance Abuse and Mental Health Service Administration (SAMHSA), requires that a Medical Review Officer (MRO) review the subject's medical record before a final determination is made for release to the employer. The appearance of drugs of abuse metabolically derived from prescription or non-prescription medicines requires that MROs and professional toxicologists be thoroughly familiar with the pharmacology of a wide variety of drugs. Of particular concern are those from which amphetamine and/or methamphetamine have been identified in the urine of individuals taking prescription medications such as benzphetamine, famprofazone, dimethylamphetamine, biphetamine, selegiline, fencamine, furfenorex or clobenzorex [*1,3–8*]. Amphetamine and methamphetamine have also been identified in the urine of users of the over-the-counter Vicks Nasal Inhaler [*9–12*].

Since some prescription drugs are enantiomerically pure, identification of the parent drug can be simplified by identifying the specific methamphetamine and amphetamine enantiomers. Certain prescription drugs metabolize to give the d-stereoisomers, while other prescription drugs, such as Eldepryl, metabolize to the l stereoisomers. The drug furfenorex metabolizes to both the d- and l-stereoisomers [*3*]. Vicks Inhaler contains only l-methamphetamine which is partially metabolized to l-amphetamine [*9–12*]. Urine from amphetamine abusers usually contains d-stereoisomers or d,l-racemic mixtures [*10,12*].

The relative concentration levels of amphetamine and methamphetamine may further aid in identifying the parent drug. For example, studies have indicated that in the urine of Vicks Inhaler users, the l-amphetamine concentration is almost always less than 1000 ng/mL, the l-methamphetamine concentration is much greater than the l-amphetamine concentration, and the l-methamphetamine concentration is almost always less than 12,000 ng/mL [*10*].

To improve differentiation between amphetamine abuse and legal drugs yielding l-stereoisomers, manufacturers of drug screening reagents have developed antibodies with greater specificity towards d-amphetamine or d-methamphetamine compared to the l-isomers. Coupled with the fact that the concentration of l-isomers from over-the-counter products such as Vicks' Inhaler are generally low, the probability that a laboratory will screen l-amphetamine or l-methamphetamine above a cutoff of 500 or 1000 ng/mL or d-amphetamine of d-methamphetamine is reduced.

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Case History

Our laboratory was alerted to an unusual situation when the screening and GC/MS results were compared for a randomly collected urine specimen (SA-1) that was positive for amphetamine and methamphetamine. The screening results (Diagnostic Products Corporation (DPC) Methamphetamine RIA, with a 500 ng/mL cutoff) indicated a positive for methamphetamine (interpolated value of 645 ng/mL), while the GC/MS confirmation showed a much higher concentration of 4690 ng/mL of methamphetamine and 1895 ng/mL of amphetamine. The specimen had a specific gravity of 1.025, a pH of 5.07, and did not show any signs of adulteration.

To eliminate potential cross-reactivity due to ephedrine and pseudoephedrine, the methamphetamine screening test was repeated with the addition of sodium periodate (13). The RIA counts per minute (cpm) for the specimen were equivalent to 235 ng/mL of d-methamphetamine, which was below the cutoff of 500 ng/mL. Screening the specimen with the Roche double antibody amphetamine RIA, with or without periodate, resulted in a finding with an interpolated concentration of 215 ng/mL, which was also below the 500 ng/mL cutoff.

Repeat GC/MS using a heptafluorobutyric anhydride (HFBA) as the derivatization reagent again confirmed the high levels of methamphetamine and amphetamine.

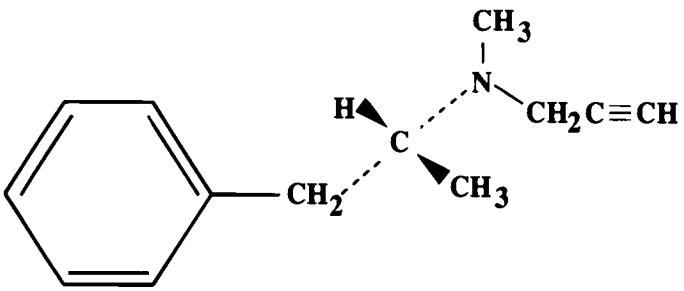
The GC/MS analysis of the specimen was then repeated using an optically active derivatizing reagent, (S)(-) N-trifluoroacetylprolyl chloride. This chiral resolving agent indicated the presence of only l-methamphetamine and l-amphetamine.

Discussion with the individual who provided the specimen revealed that he had been taking selegiline. Selegiline (Eldepryl, Movergan, 1-deprenyl), structure shown in Fig. 1, is an irreversible monoamine oxidase inhibitor, used in the control of Parkinsonism [*6,7,13,14*]. It is excreted into the urine as l-methamphetamine ($t_{1/2}$ = 20.5 h), l-amphetamine ($t_{1/2}$ = 17.5 h) and as the N-demethylated metabolite, norselegiline (N-desmethylselegiline) ($t_{1/2}$ = 2.0 h) [*14,15*].

Experimental

A voluntary follow-up specimen (SA-2) was obtained one month later from the same individual who had provided the first random specimen (SA-1). A second subject who was using selegiline was identified and provided an additional urine specimen (SB-1). Another specimen (SC-1) was also received for study.

Specimens were screened with the Coat-a-Count methamphetamine coated-tube radioimmunoassay kit (Diagnostics Products Corp, Los Angeles, CA), or with the Roche double antibody RIA



Selegiline (Deprenyl, Eldepryl)

FIG. 1—*Structure of selegiline.*

TABLE 1—*Semi-quantitative screening results derived from the amphetamine and methamphetamine specific RIA assays with and without Sodium Periodate (ng/mL).*

Specimen	Amp[a] RIA	Amp RIA + Periodate	Met[b] RIA	Met RIA + Periodate
SA-1	215	215	645	235
SB-1	172	150	109	106
SC-1	70		83	
Eldepryl	33	22	80	134

[a]Amphetamine, Roche double antibody RIA.
[b]Methamphetamine, Diagnostics Products Corp coated tube RIA.

both using a cutoff value of 500 ng/mL of d-methamphetamine. Some of the RIA assays were repeated with the addition of 50 μL of 0.15 M sodium periodate. Semi-quantitative values were calculated by interpolation between the 0 and 250 ng/mL or 500 ng/mL and 1000 ng/mL known controls and calibrators.

The specimen were quantitated in a Hewlett-Packard 5890 gas chromatograph fitted with a Hewlett-Packard 5970 mass selection detector. GC/MS analysis of the 4-carbethoxyhexafluoroacetic acid was by the method of Hornbeck and Czarny [*16*]. Stereoisomers were differentiated by derivatizing with (S) (-) N-trifluoroacetylprolyl chloride [*17*].

A 70 mg tablet containing 2.5 mg of Eldepryl (selegiline HCl) [*14*] was obtained from the Navy pharmacy for base line comparison. The tablet was crushed and dissolved in 0.5 mL of water and centrifuged. The supernate was added to 9.5 mL of certified negative urine to give a final concentration of 250,000 ng/mL of selegiline. The selegiline solution was tested with the amphetamine and methamphetamine RIA kits with and without the addition of sodium periodate.

Results and Discussion

Screening results from SA-1, SA-2, SB-1, SC-1 and the selegiline solution are summarized in Table 1. The only specimen to screen above the 500 ng/mL cutoff, was SA-1. The addition of sodium periodate to specimen SA-1 significantly reduced the apparent concentration of methamphetamine.

Phenylpropanolamines such as ephedrine and pseudoephedrine cross-react with antibodies in the methamphetamine RIA assay. The combination of l-methamphetamine and phenylpropanolamines in specimen SA-1 probably provided sufficient cross-reactivity towards the methamphetamine antibodies to cause a positive result. Addition of sodium periodate to specimen SA-1 oxidized the unidentified phenylpropanolamine(s) to non-cross-reacting material, which resulted in a significant decrease in apparent methamphetamine concentration in the RIA assay.

TABLE 2—*Enantiomeric GC/MS analysis[a] of urine from selegiline users.*

Specimen	d-Amp[b]	l-Amp	d-Met[c]	l-Met
SA-1		1895		4690
SA-2		342		829
SB-1		867		2332
SC-1		915		2490

[a]Concentration determined using the 4-carbethoxyhexafluoroacetic acid derivative, enantiomeric composition determined using the (S) (-)N-trifluoroacetylprolyl chloride derivative.
[b]Amphetamine.
[c]Methamphetamine.

The other specimens from selegiline users and the solution made from the selegiline tablet did not show any significant differences when assayed with or without periodate. This suggests that the difference seen with and without periodate for specimen SA-1 in the methamphetamine screening assay was not inherently due to selegiline itself or to selegiline metabolites. N-desmethylselegiline is not expected to be oxidized by periodate under these conditions and was not measured in any of the specimens.

The concentrations of the four specimens by GC/MS are summarized in Table 2. While all of these specimens have methamphetamine concentrations greater than 500 ng/mL and specimens SA-1, SB-1, and SC-1 have concentrations greater than 1000 ng/mL, only specimen SA-1 screened positive above the 500 ng/mL cutoff. The reported cross-reactivity of the DPC methamphetamine RIA kit used in this experiment was 6.6% (at 1000 ng/mL) for l-methamphetamine and 1.9% (at 1000 ng/mL) for l-amphetamine. While the apparent cross-reactivity of 13.7% for specimen SA-1 was higher than expected, part of the increase could be due to other phenylpropanolamines that might have been present in the urine. This is consistent with the fact that the addition of sodium periodate reduced the cross-reactivity of specimen SA-1 as seen in Table 1.

Amphetamine/Methamphetamine Ratios

Kikura et al., have shown that the ratio of l-amphetamine to l-methamphetamine could be used to distinguish l-methamphetamine use from l-deprenyl (selegiline) use in mice [*16*]. The urinary [Amphetamine]/[Methamphetamine] ([Amp]/[Met]) ratio after l-methamphetamine use was less than 0.1 for the first 24 hours. Levels were not reported for more than 24 hours. The urinary [Amp]/[Met] ratios after selegiline use ranged from 0.13 to 0.3 during the first 24 hours, from 0.15 to 0.41 for 24 to 48 hours and from 0.27 to 0.81 for 48 to 72 hours. In a similar manner, metabolism of l-methamphetamine and selegiline have been studied in humans. Studies using the Vicks Inhaler, which contains l-methamphetamine have given urinary [Amp]/[Met] ratios from 0 to 0.12 [*10,11*]. Plasma levels of amphetamine, methamphetamine and N-desmethylselegiline were studied in a healthy male subject given a 10 mg oral dose of selegiline [*14*]. Throughout the 36 hour testing period the ratio of amphetamine to methamphetamine remained about 0.33.

The four urine specimens obtained from current users of selegiline in this study have [Amp]/[Meth] ratios ranging from 0.37 to 0.42. These observed [Amp]/[Met] ratios are consistent with the data from Reimer et al. and Kikura et al. for selegiline metabolism [*14,18*]. [Amp]/[Met] ratios from selegiline users are generally higher than [Amp]/[Met] ratios from users of l-methamphetamine.

Conclusion

The fact that users of prescription drugs such as selegiline, Vicks' Inhaler, famprofazone and clobenzorex can metabolize to amphetamine or methamphetamine has the potential for confusing intentional drug abuse and innocent excretion of these metabolically derived drugs into the urine. This ambiguity along with the fact that unidentified phenylpropanolamines also cross-react with methamphetamine antibodies, increases the likelihood of a positive screen. This situation clearly demands the need for careful interpretation of the test results. Interpretation is greatly enhanced when the concentration of the specific stereoisomers have been identified. The ratio [Amp]/[Met] in the urine has the potential to distinguish selegiline use from l-methamphetamine use.

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