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Detection of *Levorotatory* Methamphetamine and *Levorotatory* Amphetamine in Urine after Ingestion of an Overdose of Selegiline

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In this study, we measured the urine concentrations of methamphetamine and amphetamine as metabolites of selegiline after ingestion of an overdose of selegiline. A patient who had developed Parkinson disease took selegiline in a suicide attempt. Analysis by gas chromatography-mass spectrometry (GC-MS) with trifluoroacetic acid-derivatization revealed the presence of methamphetamine and amphetamine in the patient's urine at concentrations of 0.62 μg/ml and 0.25 μg/ml, respectively. To determine the stereospecificity of the methamphetamine and amphetamine, a urine sample was analyzed by GC-MS after derivatization with *N*-(trifluoroacetyl)-*l*-prolyl chloride. The methamphetamine and amphetamine were *levorotatory* in form. The ratio of the methamphetamine to amphetamine concentration in the urine was 2.5. This value is consistent with other case reports of ingestion of selegiline, which suggests that the methamphetamine to amphetamine concentration ratio in urine is useful information for indicating use of selegiline.

Key words——selegiline; metabolite of selegiline; methamphetamine; amphetamine; clinical analysis

INTRODUCTION

Selegiline hydrochloride (Deprenyl, Eldepryl) is a selective monoamine oxidase B inhibitor,[1,2] and is widely used as an anti-Parkinson agent. Methamphetamine, amphetamine and *N*-desmethyl selegiline are recognized as the main metabolites of selegiline. These metabolites occur stereospecifically as the *levorotatory* (*l*-) form, because the chiral center of selegiline is unaffected during metabolism. The *dextrorotary* (*d*-) forms of methamphetamine and amphetamine are commonly used as addictive drugs, but there are few toxicokinetic reports of selegiline intoxication. Therefore, analysis of methamphetamine and amphetamine in cases of selegiline intoxication is important in clinical and forensic medicine to distinguish between cases of selegiline and *d*-methamphetamine intoxication. In this study, we investigated the concentrations of *l*-methamphetamine and *l*-amphetamine as metabolites of selegiline in urine after ingestion of an overdose of selegiline.

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EXPERIMENTAL

Trifluoroacetic acid (TFA) derivatization of methamphetamine and amphetamine was performed according to the method described by the Japanese Society of Legal Medicine.[3] Briefly, 0.5 ml of 2M NaOH and 0.5 ml of internal standard (3-phenylpropylamine) were added to a 2 ml urine sample. The mixture was extracted with 10 ml of diethyl ether and the organic layer was back-extracted with 2 ml of 1M HCl. The aqueous layer (1.6 ml) was combined with 0.4 ml of 10M NaOH and the mixture was re-extracted with 1 ml of ethyl acetate. One drop of acetic acid was added to the organic layer (ethyl acetate) followed by concentration to approximately 0.1 ml under a stream of nitrogen. The concentrated ethyl acetate was derivatized with 100 μl of trifluoroacetic anhydride at 50°C for 10 min, and the mixture was evaporated to dryness under a stream of nitrogen. The residue was reconstituted with ethyl acetate and the solution was injected into the gas chromatography-mass spectrometry (GC-MS) system.

Derivatization of methamphetamine and amphetamine with *l*-*N*-(trifluoroacetyl)-*l*-prolyl chloride (*l*-

TPC) (Sigma-Aldrich, St. Louis, MO) was performed according to the method described by Hensley and Cody.[4] Briefly, a 2-ml urine sample was made alkaline (pH $\geq$10) with 1M NaOH and extracted with 5 ml of 1-chlorobutane. The organic layer was transferred to another tube and reacted with 50 μl of *l*-TPC for 15 min at room temperature. The organic layer was washed with 3 ml of 0.01M NaOH and evaporated under a stream of nitrogen at 50°C. The residue was reconstituted with ethyl acetate and the solution was injected into the GC-MS system. The optical purity of the *l*-TPC was 96.5%.

A PerkinElmer AutoSystem XL Gas Chromatograph and Turbomass Mass Spectrometer (PerkinElmer, Norwalk, CT) were used for GC-MS analysis. GC was performed with a DB-5MS column (30 m$\times$0.32 mm I. D.; J&W Scientific, Folsom, CA), with the temperature of the column oven maintained at 60 °C for 4 min and then programmed to 300°C at 20°C/min. The carrier gas was helium (1 ml/min). The temperatures of the injection port and ion source were kept at 250°C. The injection volume was set to 1 μl (TFA derivatives; split ratio (1:20), *l*-TPC derivatives; splitless). MS was performed in electron impact ionization mode at an ionization energy of 70 eV. Measurements were carried out in full scan mode (m/z 50–500). The m/z values of the monitored ions of the TFA and *l*-TPC derivatives of methamphetamine were m/z 154 and m/z 251, respectively, and those of the monitored ions of the TFA and *l*-TPC derivatives of amphetamine were m/z 140 and m/z 237, respectively.

Standard samples of *l*-methamphetamine, *l*-amphetamine and *d*-amphetamine were unavailable, but we used a urine sample of a patient who had abused *d*-methamphetamine and available standards of *d*-methamphetamine (Dainippon Sumitomo Pharma, Osaka, Japan) and racemic amphetamine (Japanese non-profit organization Kenkou Kiki Kanri Kyoukai, Hiroshima, Japan) to analyze the optical activity of methamphetamine and amphetamine in the urine of a patient who ingested selegiline.

RESULTS AND DISCUSSION

A 44-year-old male patient with Parkinson disease took about 30 mg of selegiline and other drugs including levodopa and cabergoline in a suicide attempt. He developed accidental hypothermia (32°C) and was given hyperthermia treatment, with recovery on the following day. We first tried to detect amphetamine analogs in the patient's urine using a Triage® DOA kit (Biosite, San Diego, CA), but no analogs were detected. However, methamphetamine and amphetamine were detected in the urine using TFA derivatization, and the retention time and mass fragmentation pattern of the TFA derivatives of methamphetamine and amphetamine in the patient's urine were consistent with those from spiked urine (Fig. 1). The concentrations of methamphetamine and amphetamine in the patient's urine on arrival at hospital were 0.62 μg/ml and 0.25 μg/ml, respectively, and the ratio of the methamphetamine concentration to the amphetamine concentration was 2.5. In GC-MS analysis, calibration curves for methamphetamine and amphetamine in urine were obtained by plotting the peak area ratio of each molecule relative to an internal standard. For both molecules, the calibration curves were linear over the range 0.05–2.0 μg/ml and the correlation coefficients were 0.999. The mean recovery rates were 73.2–77.8% and the coefficients of intra-day variation were 3.3–4.3% for 0.1 and 1.0 μg/ml of methamphetamine and amphetamine, respectively, in urine (n=7). The coefficients of inter-day variation were 5.3–7.9% for 0.1 and 1.0 μg/ml of methamphetamine and amphetamine in urine, and determination of the inter-day precision was performed five times each day on three separate days. The detection limit at a signal-to-noise ratio of 5 was 0.02 μg/ml for both methamphetamine and amphetamine.

We then used *l*-TPC derivatization to determine the stereospecificity of the methamphetamine and amphetamine in the patient's urine. The mass fragmentation patterns of *l*-TPC derivatives of methamphetamine and amphetamine in the patient's urine, in spiked urine, and in the urine of an abuser of *d*-methamphetamine are shown in Fig. 2. The retention time of the *l*-TPC derivative of methamphetamine in the patient's urine differed from those in the spiked urine and the *d*-methamphetamine abuser's urine, but the mass fragmentation pattern of the *l*-TPC derivative of methamphetamine in the patient's urine was consistent with those of the spiked urine and the *d*-methamphetamine abuser's urine. The mass fragmentation pattern of the *l*-TPC derivative of amphetamine in the patient's urine was consistent with those of the spiked urine and the *d*-methamphetamine abuser's urine, but the retention time of the *l*-TPC

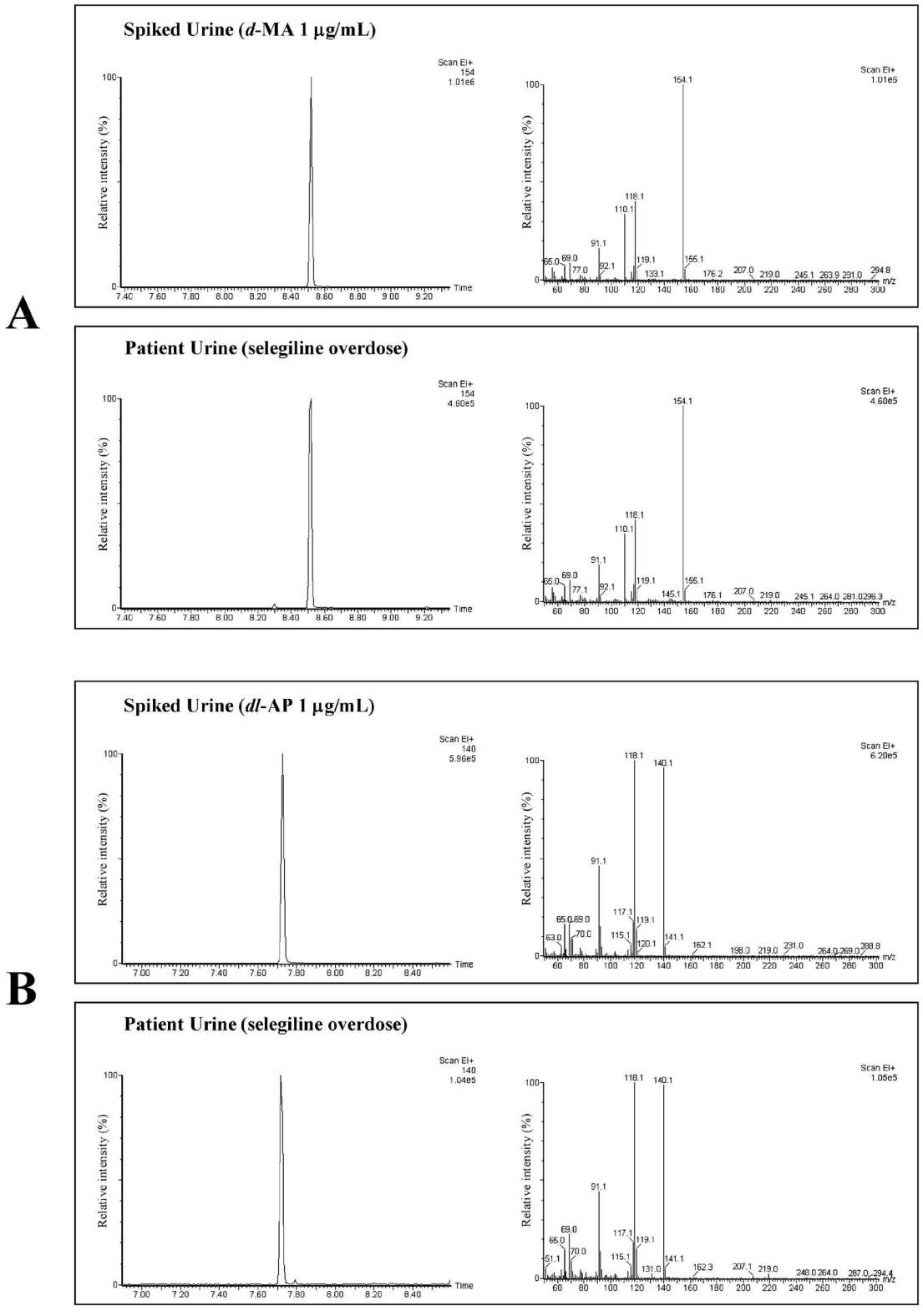

Fig. 1. Mass Chromatograms (Left Side) and Mass Spectra (Right Side) of TFA Derivatives of Methamphetamine and Amphetamine Extracted from Urine

MA: methamphetamine; AP: amphetamine. A. Mass chromatograms (m/z 154) and mass spectra of TFA derivatives of methamphetamine in spiked urine and the patient's urine. B. Mass chromatograms (m/z 140) and mass spectra of TFA derivatives of amphetamine in spiked urine and the patient's urine.

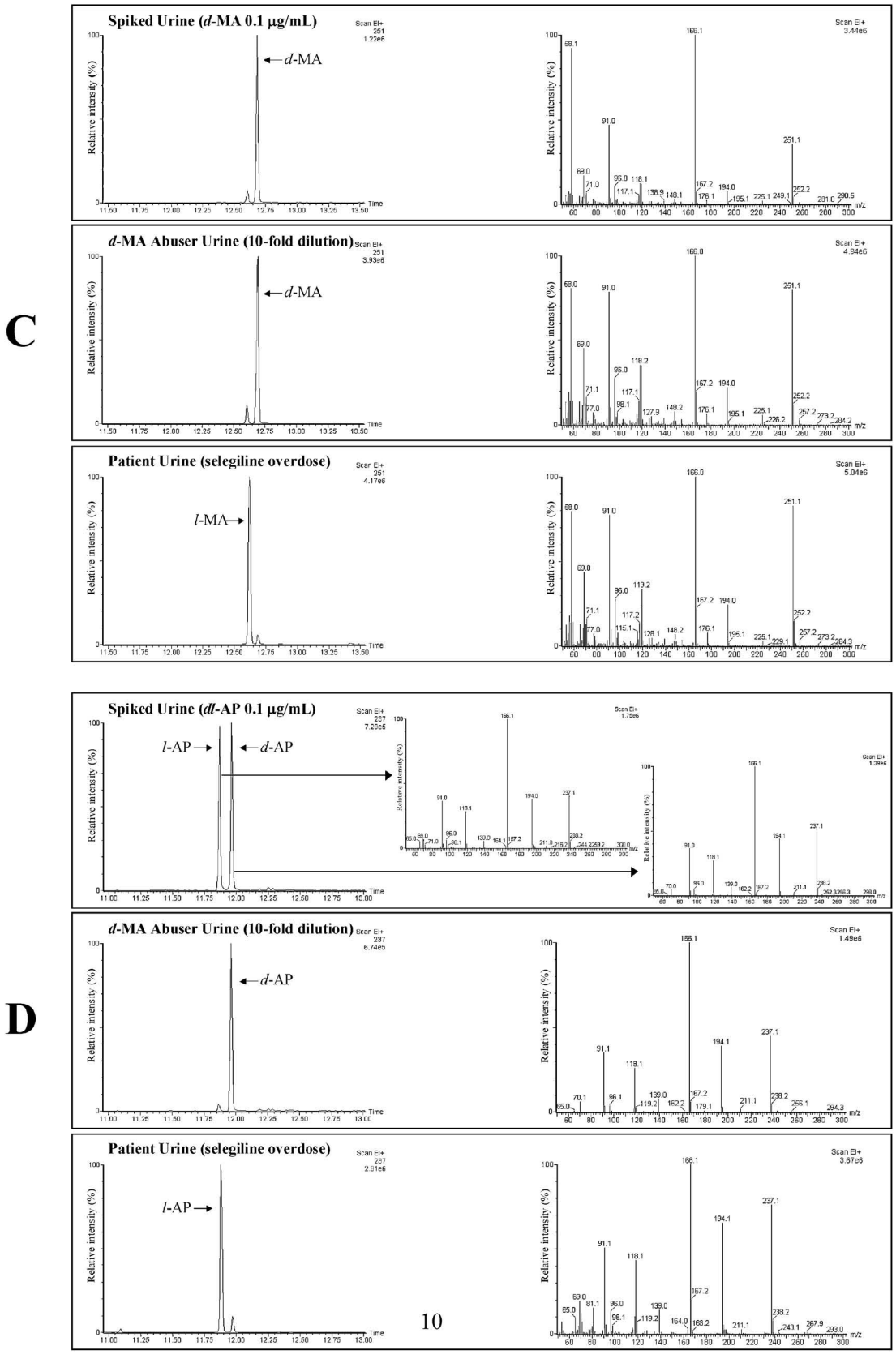

Fig. 2. Mass Chromatograms (Left Side) and Mass Spectra (Right Side) of *l*-TPC Derivatives of Methamphetamine and Amphetamine Extracted from Urine

MA: methamphetamine; AP: amphetamine. C. Mass chromatograms (m/z 251) and mass spectra of *l*-TPC derivatives of methamphetamine in spiked urine, urine from an abuser of *d*-methamphetamine, and the patient's urine. D. Mass chromatograms (m/z 237) and mass spectra of *l*-TPC derivatives of amphetamine in spiked urine, urine from an abuser of *d*-methamphetamine, and the patient's urine.

derivative of amphetamine in the patient's urine differed from that (*d*-amphetamine) in the *d*-methamphetamine abuser's urine. However, the retention time of the *l*-TPC derivative of amphetamine in the patient's urine was consistent with the first eluted substance (*l*-amphetamine) in the spiked urine. These results indicate that the methamphetamine and amphetamine in the patient's urine were of *l*-form stereospecificity.

The detection limits of *d*-methamphetamine and *d*-amphetamine in a Triage kit are 1000 ng/ml and 650 ng/ml, respectively,[5] and those for *l*-methamphetamine and *l*-amphetamine are 30000 ng/ml and 40000 ng/ml, respectively.[5] Therefore, analysis using the Triage kit is stereospecific, since the detection limits differ for the *d* and *l* forms of methamphetamine and amphetamine. The limits of detection may explain why methamphetamine and amphetamine in the patient's urine were not detected using the Triage kit.

In postmortem urine of two patients who ingested selegiline, the ratios of the methamphetamine to amphetamine concentration were 2.1 and 3.3, respectively.[6,7] In addition, the mean value of the ratio of the amphetamine to methamphetamine concentration in urine is 0.33 following oral administration of 10 mg of selegiline to humans, *i.e.*, a methamphetamine to amphetamine concentration ratio of 3.0.[8] These values are consistent with the results of the current study. *d*-Methamphetamine is metabolized to *d*-amphetamine by *N*-demethylation, whereas metabolism of selegiline to amphetamine has two pathways in humans.[9] First, selegiline can be converted to *l*-methamphetamine by *N*-depropynylation, and then *l*-methamphetamine is metabolized to *l*-amphetamine by *N*-demethylation. Second, selegiline can be converted to desmethylselegiline by *N*-demethylation, and then desmethylselegiline is metabolized to *l*-amphetamine by *N*-depropynylation. In mice, Nakahara *et al.*[10] have shown that demethylation of *l*-methamphetamine derived from selegiline is faster than that of *d*-methamphetamine, since the ratio of the methamphetamine to amphetamine concentration (a mean of 20) in animals administered *d*-methamphetamine was higher than that (a mean of 2) in animals administered selegiline. In humans, Takayasu *et al.*[11] have reported that the methamphetamine to amphetamine concentration ratios in urine in autopsy cases and emergency medical care cases of ingested methamphetamine ranged from 5.4–1149 (a mean of 114) and 1.9–69 (a mean of 20), respectively, and that these values were higher than in patients who ingested selegiline. The results of animal experiments may not reflect biological reactions in human, but it seems likely that demethylation of methamphetamine (*l*-form) in a patient who takes selegiline will be faster than that of methamphetamine (*d*-form) in a patient who abuses *d*-methamphetamine. Moreover, the methamphetamine to amphetamine concentration ratio in a patient who takes selegiline is likely to be lower than that in a patient who takes *d*-methamphetamine because metabolism of selegiline can occur *via* two pathways.[9] Therefore, our results and those of other reports suggest that the methamphetamine to amphetamine concentration ratio in urine provides useful information for distinguishing selegiline intoxication from *d*-methamphetamine intoxication. We note that this ratio is influenced by the time course after ingestion.[12,13] Since methamphetamine in use as an addictive drug in Japan may be present in the *d*-form or the *l*-form,[14] determination of the stereospecificity of methamphetamine and amphetamine is also necessary in cases of intoxication.

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