CAPILLARY ELECTROPHORETIC ENANTHIOMER SEPARATION OF AMPHETAMINES AFTER THE ADMINISTRATION OF *L*-DEPRENYL TO MAN

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ABSTRACT: The enantiomeric analysis in urine collected from volunteers ingested with *l*-deprenyl by capillary electrophoresis (CE) using carboxy methylated-β-cyclodextrin (CMCD) as a chiral selector was investigated to compare the metabolic pattern of *l*-deprenyl with the metabolism of *d*-MA. *l*-Deprenyl and its metabolites, *l*-MA, *l*-AM and *l*-DMS were detected in human urine sample. The AM/MA ratio from *l*-deprenyl user (0.33 ± 0.03) was significantly higher than the ratio from MA abusers (0.20 ± 0.12).

KEY WORDS: Enantiomeric separation; Capillary electrophoresis; Deprenyl; Methamphetamine; Amphetamine.

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INTRODUCTION

l-Deprenyl (R(-)-N-methyl-N-(1-phenyl-2-propyl)-2-propargylamine; selegiline) is a selective and irreversible inhibitor of monoamine oxidase type B that has been widely used in the treatment of Parkinson's disease (PD). It has been reported that *l*-deprenyl is extensively metabolized by the liver, forming *l*-enantiomers of desmethylselegiline (DMS), methamphetamine (MA), amphetamine (AM) and their conjugated p-hydroxy derivatives. The predominant drug of abuse in Korea is a *d*-MA so that the urine from MA abusers usually contains unchanged *d*-enantiomer of MA and its metabolite, *d*-AM. *l*-Deprenyl is recently being marketed in Korea as a therapeutic drug of PD, but the metabolites of *l*-deprenyl, *l*-MA and *l*-AM, as well as *d*- and *dl*-MA (AM), are all controlled by the Korean government as illicit psychotropic drugs. It is, therefore, necessary to distinguish legitimate therapeutic drug use from illegal drug of abuse.

In the present study, using cyclodextrin-modified CE analytic method the metabolic pattern of *l*-deprenyl was compared with the metabolism of *d*- or *dl*-MA in urine collected from 5 healthy volunteers ingested with *l*-deprenyl. Urine samples were also col-

lected from 8 illegal drug abusers for the comparison of therapeutic drug (*l*-deprenyl) use with abuse drug (*d*-MA) use.

EXPERIMENTAL

Chemicals and reagents

l-Deprenyl•HCl was kindly provided by Cho Dang Pharmaceutical Co. (Seoul, Korea). *dl*-N-DMS•HCl was purchased from Radian Co. (Austin, TX). *d*-MA•HCl, *l*-MA•HCl, *dl*-MA•HCl, *d*-AM, *l*-AM and *dl*-methoxyphenamine•HCl were from Sigma Co. (St. Louis, MO). The chiral selector CMCD was purchased from Cyclolab (Budapest, Hungary). All other chemicals and solvents were used of analytical grade.

The standard stock solutions of *l*-AM, *d*-AM, *l*-MA, *d*-MA, *l*-deprenyl and *dl*-DMS were 25 μg/ml in distilled water. Working standards (0.25, 0.5, 1, 3 and 5 μg/ml) were prepared by dilution with water. The stock solution of an internal standard, *dl*-methoxyamphetamine, was 100 μg/ml in distilled water.

Apparatus

A P/ACE 5500 (Beckman, CA) CE system equipped with diode-array detector set at 203 nm was used for the separation and quantitation of metabolites of *l*-deprenyl and MA. Fused silica capillary (57 cm x 50 μm) with an effective length of 50 cm to the detector window was used.

Human subjects, drug treatment and urine sampling

Five Korean healthy male volunteers (28–32 years old, 58–70 kg) were ingested with a single oral 10 mg dose of *dl*-deprenyl•HCl and their urine samples were collected 6 times at 3, 6, 9, 12, 15 and 24 h after the ingestion. Urine samples from 8 *d*-MA abusers were obtained from National Institute of Scientific Investigation.

Calibration curve

Solutions for standard calibration curves were prepared by spiking drug free urine with the stock solutions of *l*-AM, *d*-AM, *l*-MA, *d*-MA, *l*-deprenyl, *dl*-N-DMS, (0.25, 0.5, 1, 3, 5 μg/ml) and *l*-methoxyamphetamine (5 μg/ml) as an internal standard. The recoveries of drugs from 1ml of urine spiked with standards (1 μg) were calculated from these curves.

Extraction of urine samples

Human urine (2–4 ml) samples were mixed with 50 μl of *dl*-methoxyamphetamine (100 μg/ml), 200 μl of 1N-NaOH and 200 μl of saturated NaCl, and extracted with 500 μl of ethylacetate by shaking for 30 min and centrifugation at 3,000 x rpm for 10 min.

The organic layer was transfered to a eppendorf tube (1.5 ml) and extracted with 50 µl of 0.1N-HCl. After removing the organic phase, the residual aqueous phase was analysed by CE.

RESULTS AND DISCUSSION

Fig. 1. Electropherograms of: a) extract of urine at 3 h after ingestion of *l*-deprenyl-HCl (10 mg); b) extract of *d*-MA abuser's urine.

Simultaneous identification of *l*-deprenyl, MA and their metabolites in human urine

Six calibration curves for each standard were linear over the concentration ranges studied (0.25–5 µg/ml), with correlation coefficients of 0.995 to 0.999. Optical isomers

of AM, MA and DMS were simultaneously detected by CE analysis using CMCD as a chiral selector.

Figure 1 is an electropherogram of a drug-free urine sample spiked with 1 μg/ml each of AM enantiomers (*l*-, *d*-), MA enantiomers (*l*-, *d*-), *l*-deprenyl and DMS enantiomers (*l*-, *d*-), showing that all standard compounds were well separated. The recoveries for *l*-AM, *d*-AM, *l*-MA, *d*-MA, *l*-deprenyl and *l*-DMS from rat urine spiked with the standard drugs were in the range of 92.0–100.0%. Reproducibility of migration times for each standard substance run three times was satisfactory at a concentration of 1 μg/ml with coefficients of variation of the migration times were 0.81–1.00%.

Comparison of the metabolites of *l*-deprenyl and MA in human urine

In human urine collected from healthy Korean male volunteers up to 24 h after oral ingestion of *l*-deprenyl•HCl (10 mg) as shown in Figure 1. The representative electropherogram of urine extract from *d*-MA abuser shows clear detection of *d*-MA and *d*-AM, and it can be easily distinguished from the electropherogram from *l*-deprenyl user. Urinary recoveries and concentrations of *l*-deprenyl metabolites in humans are summarized in Table I.

l-MA content excreted was larger than *l*-AM in 24 h urine after *l*-deprenyl ingestion, in which urinary *l*-AM content was higher than any other metabolites of *l*-deprenyl. Urinary recoveries of *l*-MA and *l*-AM were ranged from 12.26 to 21.18% and ranged from 3.23 to 8.54%, respectively. The ranges for *l*-AM/*l*-MA ratios as calculated from either their urinary recoveries or urinary concentrations were very similar. The *l*-AM/*l*-MA ratio for urinary concentration from *l*-deprenyl users were ranged from 0.28 to 0.36 (0.33 ± 0.03, n = 5), while the urinary *d*-AM/ *d*-MA ratio from MA abusers were ranged from 0.04 to 0.37 (0.20 ± 0.12, n = 8).

The present result shows that the ratio of AM to MA from *l*-deprenyl user was significantly higher than the ratio from *d*-MA abuser. Similar results could be derived from other studies for the metabolism of *l*-deprenyl and *l*-MA in humans. A study in which plasma levels of AM, MA and N-DMS were analyzed in a healthy male subject given a 10 mg oral dose of *l*-deprenyl has shown that the AM/MA ratio during 36 hr test period was 0.33. Studies using the Vicks Inhaler which contains *l*-MA have given urinary AM/MA ratios from 0 to 0.12. Very recently, Hasegawa et al reported that the values of AM/MA in the urine increased from 0.24 to 0.67 along with the time after *l*-deprenyl administration, while the urinary AM/MA was less than 0.24 in 74% of the MA abusers tested. Taken together with these results, our data confirm that the metabolic pattern of AM/MA ratio between *l*-deprenyl use and MA abuse is significantly different.

TABLE I. METABOLITE CONTENTS IN URINE COLLECTED FROM 5 HEALTHY ADULT MEN AFTER ORAL INTAKE OF *l*-DEPRENYL (10 mg) OR FROM 8 *d*-MA ABUSERS; COMPARISON OF *l*-DEPRENYL USE AND *d* -MA ABUSE [a]

Subject no.	Age	MA		AM		DMS		AM/MA ratio	
		Dose [%]	[μg/ml]	Dose [%]	[μg/ml]	Dose [%]	[μg/ml]	Dose [%]	[μg/ml]
l-DPN ingested									
1	28	16.5	0.58	7.21	0.16	1.11	0.16	0.44	0.28
2	30	14.8	0.28	4.84	0.09	1.31	0.16	0.33	0.32
3	32	12.3	0.44	3.23	0.16	0.97	0.09	0.26	0.36
4	30	21.2	0.74	8.54	0.26	1.14	0.16	0.40	0.35
5	29	18.6	1.01	6.89	0.35	1.27	0.21	0.37	0.35
Mean ± SD		16.7 ± 3.43		6.14 ±2.10		1.16 ± 0.14		0.36 ± 0.07	0.33 ± 0.03
d-MA abused									
1	uk		0.49		0.18				0.37
2	uk		1.90		0.46				0.24
3	uk		15.7		0.60				0.04
4	uk		106		7.96				0.07
5	uk		0.24		0.06				0.24
6	uk		6.76		1.57				0.23
7	uk		0.96		0.32				0.33
8	uk		12.1		1.37				0.11
Mean ± SD									0.20 ±0.12*

[a] Healthy human (all men) urine up to 24 h after *l*-deprenyl (*l*-DPN) ingestion and *d*-MA abusers' urine collected at unidentified time after intake of the drug was analyzed.
* $P < 0.05$ relative to AM/MA ratio from *l*-deprenyl user; uk – age unknown.

The curves of urinary excretion rate (μg/h) for *l*-deprenyl and its metabolites after oral dose of *l*-deprenyl to healthy volunteers are presented in Figure 2. Both *l*-MA and *l*-AM were excreted in urine largely during the time between 3 and 12 h with the highest level at 3–6 h, and then continuously excreted during 12–24 h after oral administration. This result indicates that the detection of a specific metabolite of *l*-deprenyl, *l*-DMS, could not distinguish the therapeutic *l*-deprenyl use from the illegal MA abuse, as also indicated in the recent study by Hasegawa et al.[2].

In conclusion, we performed the chiral separation of *l*-deprenyl and MA metabolites using a new cyclodextrin-modified CE analysis to compare the metabolic patterns of *l*-deprenyl and MA in human urine and to distinguish the illicit use of drug of abuse and the therapeutic drug use. Results indicate that the AM/MA ratio from *l*-deprenyl use is significantly higher than the ratio from MA use in human urine.

References:

1. Fitzgerald R. L., Ramos J. M. Jr, Bogema S. C., Poklis A., Resolution of methamphetamine stereoisomers in using drug testing: Urinary excretion of R(-)-methamphetamine following use of nasal inhalers, *Journal of Analytical Toxicology* 1988, vol. 12, pp. 255–259.
2. Hasegawa M., Matsubara K. , Fukushima S., Maseda C., Uezono T., Kimura K., Stereoselective analyses of selegiline metabolites: possible urinary markers for selegiline therapy, *Forensic Science International* 1999, vol. 101, pp. 95–106.
3. Hayes S. M., Liu J. R. H., Tsang W. S., Legendre M.G, Borni R. J., Pillion D. J. , Barnes S. , Ho M. H., Enantiomeric composition analysis of amphetamine and methamphetamine by chiral phase high performance liquid chromatography-mass spectrometry, *Journal of Chromatogr*aphy 1987, vol. 98, pp. 239–246.
4. Kim E. M., Kim S. C., Chung H. S., Yoo Y. C., Gas chromatographic analysis of optical isomers of methamphetamine and amphetamine after administration of *l*-deprenyl (selegiline) to rats, *Yakhak Hoeji* 1997, vol. 41, pp. 698–703.
5. Yoo Y. C., Chung H. S., Choi H. K., Kim E. M., Kim M. K., Kim S. C., Enantiomeric separation of methamphetamine abused in Korea, *Yakhak Hoeji* 1997, vol. 41, pp. 277–282.