— Original Article —

HPLC/Chemiluminescence Detection of Methamphetamine and Amphetamine in Hair

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We have developed a highly sensitive HPLC method using chemiluminescence detection (CL-HPLC) for determining methamphetamine (MA) and its metabolite, amphetamine (AP), in a single hair. MA and AP were extracted from a single hair with a solution of methanol and hydrochloric acid, evaporated to dryness, dansylated, and subjected to CL-HPLC. Bis(2,4,6-trichlorophenyl)oxalate and hydrogen peroxide were used as post column reagents. This method was used for the determination of MA and AP in hair samples obtained from 44 MA users. Of these, 31 samples showed both MA and AP, 8 samples showed only MA and 5 samples showed neither MA nor AP. Seven MA users whose hair samples contained both MA and AP, neither compound was detected in the urine. Because the present method can be performed on a single hair, it is easier to procure samples and to obtain the subjects' willingness to provide samples.

Key words : methamphetamine; amphetamine; hair analysis; HPLC/chemiluminescence detection; dansyl chloride.

1. Introduction

Hair analysis has begun to be used for the detection of drug use in forensic science¹⁻¹⁰⁾. Methamphetamine (MA) and its metabolite, amphetamine (AP), can be detected in urine for about 7-10 days after its use by gas chromatography/mass spectrometry (GC/MS). However, it has been possible to detect MA and AP in hair even after several years¹¹⁻²⁰⁾.

We have developed a highly sensitive HPLC/chemiluminescence detection (CL-HPLC) method using dansyl derivatives of MA and AP in a single hair²¹⁾. The mechanisms of dansyl derivatization and chemiluminescence reaction are shown in Fig. 1. The detection limit of MA was 2 pg, suggesting that this method was five times more sensitive than the GC/MS/SIM (selective ion monitoring) method using stable isotope dilution^{5,17)}. In this study,

Dansyl derivatization

Chemiluminescence reaction (proposed)

Fig.1 The mechanisms of dansyl derivatization and chemiluminescence reaction.

we analyzed hair samples from 44 MA users utilizing the CL-HPLC method. The results were compared with those of urine analyses and with the status of the subjects to clarify the usefulness of the present method for the detection of MA use.

2. Materials and methods

Chemicals

MA hydrochloride and AP sulfate were obtained from Dainippon Pharmaceutical (Osaka, Japan) and Takeda Pharmaceutical (Osaka, Japan), respectively. All other chemicals used were of analytical grade.

Subjects

Forty-four subjects were examined. Of the subjects, 36 MA users were arrested by the Ishikawa Prefectural Police and sampled under "Voluntary Presentation", 7 MA users were arrested by the Ishikawa Prefectural Police under a "Personal Search Warrant", and one MA addict was sampled

at an autopsy at the Department of Legal Medicine of Kanazawa University, Faculty of Medicine. Their sex, age and user status (addict, non-addict and unknown) are described in Table 1. The subjects were classified into addicts, defined as users who used MA successively, and non-addicts, based on their own statements and investigations by the police. The investigations included such information as testimony by the subjects' family and criminal records of MA use.

Hair samples

Two or more hairs were taken from the top of the head of each of the 36 "Voluntary Presentation" subjects and the one deceased subject. Fifty hairs were taken from each of the 7 "Personal Search Warrant" subjects (10 hairs from each of the top, fore, back, right and left sides). All hairs were snipped off approximately 0-1 mm above the skin surface and did not include the roots. The length and weight of the hair samples from a particular subject were approximately the same length, but among the 44 subjects, they ranged from 2 to 51 cm and from 0.12 to 3.99 mg, respectively. The hair samples were stored in a refrigerator for 1-30 days until analysis.

CL-HPLC system and conditions

The HPLC system (Fig. 2) consisted of two LC-10A pumps (Shimadzu, Kyoto, Japan), a DGU-3A on-line degasser (Shimadzu), an SIL-10A auto-injector (temperature 4°C, 20 µl loop; Shimadzu), a CTO-10A column oven (temperature 40°C; Shimadzu), an L-column ODS (4.6 mm i.d. 250 mm; Chemicals Inspection and Testing Institute, Tokyo, Japan), a CLD-10A chemiluminescence de-

Table 1
Subjects whose hair samples were used in this study

Sample No.	Sex *	Age	User ** status	Presentation ***
1	M	28	A	V
2	M	30	A	V
3	F	29	A	V
4	M	27	A	V
5	M	32	A	D
6	F	25	A	V
7	M	29	A	V
8	M	35	A	V
9	F	38	A	V
10	F	32	A	V
11	M	38	A	V
12	M	23	A	V
13	M	43	A	V
14	M	30	A	V
15	F	23	A	V
16	F	17	A	V
17	M	32	A	P
18	F	24	A	V
19	M	49	A	V
20	M	24	A	V
21	M	42	A	V
22	M	26	un	P
23	M	35	un	P
24	M	32	A	V
25	M	31	A	V
26	M	51	A	V
27	M	62	A	V
28	M	31	A	V
29	M	30	A	V
30	M	35	A	V
31	F	46	A	V
32	F	22	A	V
33	M	44	N	P
34	M	48	A	V
35	M	29	N	V
36	M	32	A	P
37	F	20	N	V
38	M	26	A	P
39	F	17	N	V
40	F	44	A	V
41	F	20	A	V
42	M	48	A	P
43	M	52	A	V
44	F	25	A	V

* M, male; F, female.

** A, MA addict; N, non-addict;

un, unknown of addict or non-addict.

*** V, voluntary presentation; D, deceased subject; P, personal search warrant.

Fig. 2 CL-HPLC system and conditions.

tector (temperature 30°C, reaction coil 0.5 mm i.d. 600 mm; Shimadzu), an SCL-10A system controller (Shimadzu), a CBM-10A communications bus module (Shimadzu), a FMV-5120DPC personal computer (Fujitsu, Tokyo, Japan) and CLASS-LC10 software (Shimadzu).

The mobile phase was a mixture of 1mM imidazole buffer (adjusted to pH 7.0 with nitric acid) and acetonitrile (2:3, v/v). The flow rate of the mobile phase was 1.0 ml/min. The post-column chemiluminescence reagent solution was prepared by dissolving 0.5mM bis(2,4,6-trichlorophenyl)oxalate (TCPO) and 150mM hydrogen peroxide in acetonitrile. The flow rate of the post-column reagent was also 1.0 ml/min.

Hair analysis by CL-HPLC

Hair samples were washed with distilled water and methanol alternately 5 times each¹⁹. Each single hair was weighed and cut into pieces about 3 mm long. The sample was sonicated for 1 h with 0.5 ml of a mixture of methanol and 5M HCl (20:1, v/v), and allowed to stand at room temperature overnight. After the hair was removed by filtration, the filtrate was evaporated to dryness under a nitrogen stream. The residue was dissolved in 0.1 ml of 100mM carbonate buffer (pH 9.0) and 0.1 ml of acetone containing 1mM dansyl chloride (DNS-Cl), and the solution was heated at 45°C for 1 h. The reaction mixture containing DNS derivatives was immediately placed in an auto-injector apparatus (temperature 4°C) and an aliquot of the mixture was then subjected to CL-HPLC.

Urine analysis by GC/MS

Urine (10 ml) was alkalized and extracted 3 times with chloroform - isopropanol (3:1, v/v). A drop of acetic acid was added to the extracted solution, and then the mixture was evaporated to dryness under a nitrogen stream. The residue was dissolved in 0.2 ml of ethyl acetate and 0.2 ml of trifluoroacetic anhydride (TFA), and the solution was heated at 55 °C for 15 min. The reaction mixture containing TFA derivatives was evaporated to dryness under a nitrogen stream. The residue was dissolved in 0.1 ml of ethyl acetate containing 500 ng/ml of diphenylmethane (internal standard). An aliquot of the mixture was then subjected to GC/MS.

3. Results and discussion

We analyzed hair samples of 44 MA users, 38 of whom were addicts, 4 of whom were non-addicts and 2 of whom were unknowns (Table 1). Figure 3 shows three typical chromatograms; No. 20 shows both MA and AP, No. 25 shows only MA, and No. 30 shows neither MA nor AP. Subjects were separated into two groups based on the results of the urine analyses except for No. 44 whose urine was not analyzed. MA was detected in the urine of 31 subjects (Nos. 1-31, urine (D) group), while MA was not detected in the urine of 12 subjects (Nos. 32-43, urine (ND) group) as shown in Table 2.

In the urine (D) group, 23 hair samples (Nos. 1-23) showed both MA and AP. In the urine (ND) group, 7 hair samples (Nos. 32-38) also showed both MA and AP. The concentrations of MA and AP of these 30 samples were in the ranges from 0.27 ng/mg to 89.7 ng/mg and from 0.08 ng/mg to 9.22 ng/mg, respectively. The AP/MA ratio (mean $\pm$ SD) was 0.19 ± 0.11 (max. 0.42, min. 0.04).

In the urine (D) group, 5 hair samples (Nos. 24-28) showed only MA. In the urine (ND) group, 3 hair samples (Nos. 39-41) also showed only MA. The concentration of MA of these 8 samples was in the range from 0.15 ng/mg to 7.81 ng/mg. Considering the concentration of MA of these 8 samples was lower than that of the above first group, and the concentration of AP was lower than of MA, the concentration of AP of these samples might have been lower than the detection limit. The fact that MA was detected in the hair in 10 of the 12 subjects of the urine (ND) group demonstrates the large advantage of the hair analysis.

These 10 hair samples of the urine (ND) group were from 6 addicts and 4 non-addicts as described in Table 1. Generally, subjects who used MA at least 7-10 days before the urine sample is taken will give a negative urine analysis (urine (ND) group) and a positive hair analysis, assuming that hair and urine were collected at the same time. Hair samples of 2 addicts (Nos. 34 and 36) and 2 non-addicts (Nos. 35 and 37) in the urine (ND) group were cut into two pieces at 2 cm from the bottom of the hairs, and both parts were separately analyzed. MA and

AP were detected in both parts of the hair sample of the 2 addicts, and in the bottom parts of the hair samples of the 2 non-addicts. Neither MA nor AP was detected in the top parts of the latter 2 samples. These results mean that the 2 addicts (Nos. 34 and 36) continued to take MA for a long period (over 2 months) and that 2 non-addicts (Nos. 35 and 37) used MA only within the 2 months before sampling. Thus, the present CL-HPLC method is capable of clarifying the history of MA administration utilizing only a single hair.

However, 5 hair samples not only of the urine (ND) group (Nos. 42 and 43) but also of the urine (D) group (Nos. 29-31) showed neither MA nor AP in spite of the fact that these subjects were all addicts. The growth rate of scalp hair in Japanese men is ordinarily 0.39-0.44 mm/day²²⁾ and hair root length in epidermis and dermis is 3-4 mm. This suggests that it would take newly consumed MA at least 7-10 days to move from the hair root to the surface of the scalp, where it could then be detected.

Fig. 3 Typical chromatograms of extracts of hair samples.

Table 2
MA and AP concentrations in hair samples

Sample No.	Days *		Hair **			Urine ***
	urine	hair	MA ng/mg	AP ng/mg	AP/MA	
1	1	7	24.4±3.20	1.64±0.29	0.07	D
2	1	5	4.46±1.42	0.88±0.75	0.20	D
3	1	3	5.13±4.43	0.40±0.28	0.08	D
4	3	3	27.6±15.3	2.28±0.25	0.08	D
5	1	1	27.3±9.36	3.02±0.87	0.11	D
6	4	4	4.40±3.19	0.16±0.07	0.04	D
7	1	7	6.71±3.89	1.37±0.44	0.20	D
8	4	25	21.9±5.49	1.34±0.34	0.06	D
9	2	25	0.53±0.33	0.20±0.08	0.38	D
10	1	16	0.27±0.19	0.10±0.05	0.37	D
11	1	11	9.80±1.41	0.80±0.14	0.08	D
12	2	16	19.5±0.70	5.50±1.41	0.28	D
13	1	30	7.30±0.71	0.90±0.07	0.12	D
14	2	4	1.37±0.50	0.45±0.07	0.33	D
15	2	9	8.80±3.75	1.00±0.36	0.11	D
16	2	2	0.33±0.15	0.08±0.02	0.24	D
17	1	2	6.88±4.99	1.05±0.45	0.15	D
18	3	9	1.86±0.81	0.33±0.05	0.18	D
19	3	20	89.7±22.6	9.22±4.46	0.10	D
20	1	21	29.0±4.24	3.20±0.81	0.11	D
21	3	21	12.8±6.06	2.39±1.14	0.19	D
22	un	(2)	25.2±5.86	2.46±0.64	0.10	D
23	un	(2)	23.5±12.1	2.87±1.06	0.12	D
24	1	20	3.81±1.77	ND		D
25	1	1	2.71±0.43	ND		D
26	9	43	0.40±0.29	ND		D
27	2	2	1.38±0.56	ND		D
28	1	10	7.81±2.73	ND		D
29	3	13	ND	ND		D
30	2	16	ND	ND		D
31	2	24	ND	ND		D
32	16	16	0.77±0.27	0.24±0.09	0.31	ND
33	52	60	4.81±2.53	1.18±0.34	0.24	ND
34	13	17	2.50±1.29	1.05±0.35	0.42	ND
35	10	10	1.64±0.41	0.40±0.28	0.24	ND
36	15	20	3.17±1.75	0.93±0.35	0.29	ND
37	6	20	0.47±0.21	0.12±0.09	0.26	ND
38	7	17	6.28±1.37	0.59±0.19	0.09	ND
39	10	18	0.21±0.09	ND		ND
40	7	8	0.15±0.13	ND		ND
41	5	8	1.74±0.80	ND		ND
42	120	120	ND	ND		ND
43	20	20	ND	ND		ND
44	-	23	0.30±0.07	0.10±0.03	0.33	-

*, days between last use and sampling (urine and hair)
(2), 2 days after urine sampling; -, no sample.

**, mean ± SD; n=2-10; ND, not detected.

***, D, both MA and AP were detected; ND, neither MA nor AP was detected; -, not analyzed.

However, these 5 hair samples were collected at least 13 days after the last use as shown in Table 2. Thus, sampling too soon could not explain why the results were negative. In the case of the subject who had the longest time between last use and sampling (No. 42), a single hair with length of 7 cm long was taken at 120 days after the last use. Thus, sampling too late could not explain why the result was negative. Possible reason for the failure to detect MA or AP in these 5 hair samples include decolorant treatment, permanent wave treatment, hair dye treatment and white hair. The effects of these hair treatments and white hair on the absorption/release of MA and AP are now being studied.

In addition to the above advantages, the present CL-HPLC method which requires only a single hair,

facilitates procurement of samples, as subjects are more willing to accede to requests for small numbers of hairs. A "Personal Search Warrant" is necessary for the collection of 50 hairs and an "Evidencing Disposition Warrant" is necessary for chemical analysis that destroys the collected hairs. The data obtained by the proposed method can be used to support requests for these warrants.

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