Jpn. J. Toxicol. Environ. Health
衛生化学
44 (2) 116—121 (1998)

—Note—

HPLC/Chemiluminescence Detection of Methamphetamine and Amphetamine in Black and White Hair Samples

Nariaki TAKAYAMA,*,[a] Seishi TANAKA,[a] Ryoichi KIZU,[b] and Kazuichi HAYAKAWA[b]

Forensic Science Laboratory, Ishikawa Prefectural Police Headquarters,[a] 2-1-1 Hirosaka, Kanazawa 920-8553, Japan and Department of Hygienic Chemistry, Faculty of Pharmaceutical Sciences, Kanazawa University,[b] 13-1 Takaramachi, Kanazawa 920-0934, Japan

(Received October 20, 1997)

Black and white hair samples were obtained from black-, gray- (*i.e.*, a mixture of black and white hair) and white-haired methamphetamine (MA) users, and MA and amphetamine (AP) were determined by HPLC using chemiluminescence detection (CL-HPLC). MA and AP were detected in black hair, and were found in the part of the hair that grew during the period of MA use. In the same subjects, MA concentrations were lower in white hair than in black hair and AP was not detected in white hair. This difference may be related to the affinity of MA and AP for melanin.

Key words —— methamphetamine; amphetamine; black and white hair; HPLC; chemiluminescence detection; dansyl chloride

Introduction

In recent decades, abuse of methamphetamine (MA) and amphetamine (AP) has become a serious problem in Asian and Pacific countries including the United States. MA and AP are covered control by the Stimulant Drug Control Law in Japan. MA is used by most addicts, and AP is detected in body fluids as its major metabolite. The period during which MA and AP can be detected in urine samples is usually up to about 10 d after MA use. However, evidence of MA use remains in the hair for several years if the part of the hair that grew during the period of its use was available. Therefore, scientists have begun to use hair samples to confirm MA use. To detect MA in hair samples, GC-MS,[1-11] immunoassay (EIA, ELISA),[12,13] and HPLC[14] have been mainly used.

The hair of most Japanese men and women is black, while the hair of older people is sometimes gray (*i.e.*, a mixture of black and white hair) or completely white. Therefore, it is important to clarify any differences in MA and AP concentrations between black and white hair samples used to confirm MA use. Differences in the

concentrations of haloperidol,[15] ofloxacin,[16] nicotine,[17] phencyclidine[18] and cocaine,[19,20] in black and other color (*i.e.*, brown, blond and white) hair have been reported, but there is no information about MA. Recently, we have developed a highly sensitive HPLC method using chemiluminescence detection (CL-HPLC) for determining MA and AP in a single hair.[21] This method makes it possible to compare black and white hairs obtained from the same person. In this report, we analyzed black and white hair samples obtained from subjects, 6 black-haired, 5 gray-haired and 1 white-haired, and found that the concentration of MA in white hair was much lower than that in black hair.

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.

Hair Samples —— Each single hair (not including the root) was cut at approximately 0—1 mm above the skin surface. Black hair samples were taken from 6 black-haired MA users, arrested by the Ishikawa Prefectural Police under "Voluntary Presentation". Both black and white hair samples were taken from 5 gray-haired MA users, arrested by the Ishikawa Prefectural Police under "Voluntary Presentation" or a "Personal Search Warrant". Five strands of white hair were taken from 1 completely white-haired MA user, arrested by the Ishikawa Prefectural Police under "Voluntary Presentation". Their sex, age and user status (addict, non-addict or unknown) of the subjects are given in Table 1. Addicts were defined as heavy users and non-addicts were defined as occasional users. Subjects were classified into the

Table 1. Subjects whose Hair Samples were used in This Study

Sample No.	Hair Condition	Hair Length	Sex[a]	Age	User[b] Status	Days after MA Use
1	Black	51 cm	F	25	A	23
2	Black	7.5	M	38	A	11
3	Black	7.4	M	23	A	16
4	Black	6.5	M	43	A	30
5	Black	10	F	23	A	9
6	Black	11	M	24	A	21
7	Gray	6.5	M	30	A	4
8	Gray	2.0	M	49	A	20
9	Gray	4.0	M	42	A	21
10	Gray	2.5	M	35	un	un
11	Gray	5.0	M	44	N	60
12	White	15	M	52	A	20

a) F, female ; M, male. b) A, MA addict ; N, non-addict ; un, unknown of MA addict or non-addict.

two groups based on a combination of their own statements and investigations by the police. The latter included such information as testimony by the subject's family and criminal records of MA use. Control black hairs were collected from a healthy individual who had not taken any legal or illegal drug for several months before sampling. Each single hair of the same subject was approximately the same length, and hair samples of the 12 subjects and one control ranged in length from 2 to 51 cm and weighed from 0.12 to 3.99 mg. Hair samples were stored in a refrigerator for 1—30 d until analysis.

CL-HPLC System and Conditions —— The CL-HPLC system consisted of two LC-10A pumps (Shimadzu, Kyoto, Japan), a DGU-3A on-line degasser (Shimadzu), an SIL-10A auto-injector (temperature 4℃, 20 μl loop ; Shimadzu), a CTO-10A column oven (temperature 40℃ ; Shimadzu), an L-column ODS (4.6 mm i.d.×250 mm ; Chemicals Inspection & Testing Institute, Tokyo, Japan), a CLD-10A chemiluminescence detector (temperature 30℃, reaction coil 0.5 mm i.d.×600 mm ; Shimadzu), an SCL-10A system controller (Shimadzu), a CBM-10A communications bus module (Shimadzu), an FMV-5120DPC personal computer (Fujitsu, Tokyo, Japan) and CLASS-LC10 software (Shimadzu).

The mobile phase was a mixture of 1 mM imidazole buffer (adjusted to pH 7.0 with nitric acid) and acetonitrile (2 : 3, v/v). The flow rate was 1.0 ml/min. The post-column chemiluminescence reagent solution was prepared by dissolving 0.5 mM bis(2,4,6-trichlorophenyl) oxalate (TCPO) and 150 mM hydrogen peroxide in acetonitrile. Its flow rate was 1.0 ml/min. Other conditions were as previously reported.[21]

Washing of Hair, Extraction and Derivatization —— Hair samples were alternately washed with distilled water and methanol, 5 times each.[5] 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 5 N HCl (20 : 1, v/v), and allowed to stand at room temperature overnight.[6] 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 100 mM carbonate buffer (pH 9.0) and 0.1 ml acetone containing 1 mM dansyl chloride (DNS-Cl), and the solution was heated at 45℃ for 1 h. The reaction mixture containing DNS derivatives was immediately placed in an auto-injector apparatus (temperature 4℃), and an aliquot of the mixture was then subjected to CL-HPLC.[21]

Calibration Curve —— A single control hair (0.3—0.7 mg) was washed and cut as described above. After spiking with a standard solution prepared by dissolving MA and AP at 0.1—50 ng in distilled water, the above extraction and derivatization procedures were performed. Calibration curves prepared by the external standard method for both MA and AP over the range of 0.2—100 ng/mg were linear with correlation coefficients of 0.991 and 0.990, respectively. The intermediate precisions of "within-run" assays for MA and AP (each 2 ng/mg) were 6.8% and 6.3%, respectively (n=5), and the intermediate precisions of "between-run" assays were 4.6% and 5.2%, respectively (n=10).

Results and Discussion

Both MA and AP were detected in all the black hair samples of the 11 MA users (except No. 12). The AP/MA ratio of black-haired subjects (Nos. 1—6) was 0.17 ± 0.10, and that of the black hair from gray-haired subjects (Nos. 7—11) was 0.16 ± 0.06. This result suggests that there is no difference in the black hairs of black-haired and gray-haired subjects in terms of the incorporation ratio of MA and AP. These values were similar to those in a previous report.[22] We also analyzed white hair samples of 6 MA users (4 addicts, 1 non-addict and 1 unknown). MA was detected in 4 samples (Nos. 7—10 in Table 2) and not detected in 2 samples (Nos. 11 and 12 in Table 2). AP was not detected in any of these samples. In the same subjects (Nos. 7—11 in Table 2), the mean concentrations of MA in white hair were much lower than those in black hair. Typical chromatograms of extracts of black and white hair samples from an MA user (No. 9) are shown in Fig. 1. MA and AP were detected in the former but not in the latter. Considering that the AP concentration was lower than that of MA in the black hair samples, the AP concentration in white hair seemed to be below the detection limit of

Table 2. Results of Hair Analyses of Black and White Hairs

Sample No.	Black Hair		White Hair	
	MA ng/mg	AP ng/mg	MA ng/mg	AP ng/mg
1	0.30±0.07(n=3)	0.10±0.03(n=3)	ns	ns
2	9.80±1.41(n=3)	0.80±0.14(n=3)	ns	ns
3	19.5±0.70(n=3)	5.50±1.41(n=3)	ns	ns
4	7.30±0.71(n=3)	0.90±0.07(n=3)	ns	ns
5	8.80±3.75(n=3)	1.00±0.36(n=3)	ns	ns
6	29.0±4.24(n=3)	3.20±0.81(n=3)	ns	ns
7	1.37±0.50(n=3)	0.51(n=1)	0.50(n=1)	ND(n=3)
		0.40(n=1)	0.39(n=1)	
		ND(n=1)	trace(n=1)	
8	89.7±22.6(n=5)	9.22±4.46(n=5)	24.4(n=1)	ND(n=2)
			15.0(n=1)	
9	10.2±4.86(n=5)	1.88±0.97(n=5)	0.20(n=1)	ND(n=5)
			trace(n=3)	
			ND(n=1)	
10	23.5±12.1(n=5)	2.87±1.06(n=5)	1.10(n=1)	ND(n=5)
			0.40(n=1)	
			trace(n=2)	
			ND(n=1)	
11	4.81±2.53(n=5)	1.18±0.34(n=5)	ND(n=3)	ND(n=3)
12	ns	ns	ND(n=5)	ND(n=5)

ns, no hair sample ; ND, not detected.

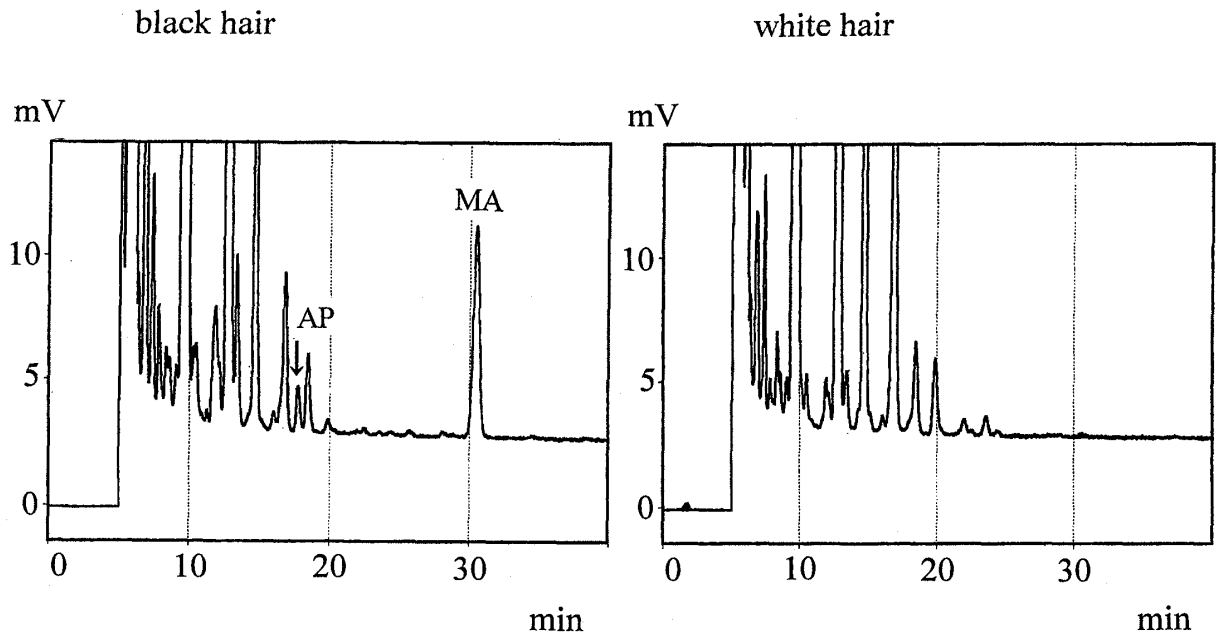

Fig. 1. Typical Chromatograms of Extracts of Black and White Hair Samples from an MA User (No. 9)

the CL-HPLC method. Sample No. 12 was taken from a white-haired subject. This person had a record showing 5 episodes of MA use, but had recently stopped using MA. According to the self-report and police investigation, his last use of MA was at least 20 d before the hair sampling. Because the growth rate of Japanese male scalp hair is ordinarily 0.39—0.44 mm/d[23] and the length of the hair root is 3—4 mm, we consider that the sample was adequate for the detection of the drug. Nevertheless, MA was not detected.

AP is selectively incorporated into the pigment granules (*i.e.*, melanin) in the hair fiber of black-pigmented guinea-pigs.[24] Our results suggest that the color of the hair might affect significantly the concentration of MA and AP in hair. Hair samples from white-haired MA users may not be suitable specimens for analysis.

References

1) Ishiyama I., Nagai T., Toshida S., *J. Forensic Sci.*, **28**, 380 (1983).
2) Niwaguchi T., Suzuki S., Inoue T., *Arch. Toxicol.*, **52**, 157 (1983).
3) Suzuki S., Inoue T., Yasuda T., Niwaguchi T., Hori H., Inayama S., *Eisei Kagaku*, **30**, 23 (1984).
4) Suzuki O., Hattori H., Asano M., *J. Forensic Sci.*, **29**, 611 (1984).
5) Suzuki S., Inoue T., Hori H., Inayama S., *J. Anal. Toxicol.*, **13**, 176 (1989).
6) Nakahara Y., Takahashi K., Takeda Y., Konuma K., Fukui S., Tokui T., *Forensic Sci. Inter.*, **46**, 243 (1990).
7) Nakahara Y., Takahashi K., Shimamine M., Takeda Y., *J. Forensic Sci.*, **36**, 70 (1991).
8) Moriya F., Miyashi S., Ishizu H., *Arukoru Kenkyu To Yakubutu Izon*, **27**, 152 (1992).

9) Kauert G.F., Proc. Int. Symp. Forensic Science, Tokyo, 1993, p.63.
10) Kikura R., Nakahara Y., *Biol. Pharm. Bull.*, **18**, 267 (1995).
11) Nakahara Y., Takahashi K., Kikura R., *Biol. Pharm. Bull.*, **18**, 1223 (1995).
12) Aoki K., Kuroiwa Y., *J. Pharm. Dyn.*, **6**, 33 (1983).
13) Nakahara Y., Ishigami A., Takeda Y., Usagawa T., Uda T., *Eisei Kagaku*, **35**, 333 (1989).
14) Nagai T., Sato M., Nagai T., Kamiyama S., Miura Y., *Clin. Biochem.*, **22**, 439 (1989).
15) Uematsu T., Sato R., Fujimori O., Nakashima M., *Arch. Dermatol. Res.*, **282**, 120 (1990).
16) Uematsu T., Miyazaki N., Okazaki O., Nakashima M., *J. Pharm. Sci.*, **81**, 45 (1992).
17) Gerstenberg B., Schepers G., Voncken P., Volkel H., *Drug Metab. Dispos.*, **23**, 143 (1995).
18) Slawson M.H., Wilkins D.G., Foltz R.L., Rollins D.E., *J. Anal. Toxicol.*, **20**, 350 (1996).
19) Joseph R.E. Jr., Su T.P., Cone E.J., *J. Anal. Toxicol.*, **20**, 338 (1996).
20) Reid R.W., O'Connor F.L., Deakin A.G., Ivery D.M., Crayton J.W., 1996 SOFT meeting abstract, Denver, 1997, p.89.
21) Takayama N., Tanaka S., Hayakawa K., *Biomed. Chromatogr.*, **11**, 25 (1997).
22) Nakahara Y., *Forensic Sci. Inter.*, **70**, 135 (1995).
23) Saitoh M., Uzuka M., Sakamoto M., Kobori T., *"Advance in Biology of Skin,"* Vol. 9, Pergamon Press, Oxford, 1969, p.183.
24) Harrison W.H., Gray R.M., Solomon L.M., *Br. J. Dermatol.*, **91**, 415 (1974).