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Hair analysis for Drug Abuse XV. Disposition of 3,4-methylenedioxymethamphetamine (MDMA) and its related compounds into rat hair and application to hair analysis for MDMA abuse

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Abstract

In order to clarify the mechanism of drug incorporation into hair, disposition of 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine (MDEA), 3-methoxy-4,5-methylenedioxyamphetamine (MMDA) and metabolites of MDMA, 4-hydroxy-3-methoxyamphetamine (HMAP) and 4-hydroxy-3-methoxymethamphetamine (HMMA), into hair was investigated with an animal model. After the intraperitoneal administration of those six drugs to pigmented hairy rats (5 mg/kg/day, 10 days, $n = 3$), the parent compounds and their metabolites in the rat plasma (5, 15, 30, 60, 120, 360 min after administration) and in the newly grown rat hair for 4 weeks were determined by GC/MS-SIM. When the ratio of hair concentration to area under the concentration versus time curves (AUCs) in plasma was represented as an index of incorporation rate (ICR) of drugs into hair, the order of ICRs was HMAP < MDA < HMMA < MDMA < MDEA < MMDA. In the comparison between MDA, MDMA and MDEA, their ICRs increased according to the length of carbon branches from proton to ethyl at the *N* position. From the point of view that the ICRs of MMDA was 2.3 times as much as that of MDA, the methoxy group on the benzene ring seemed to serve as a positive factor for the ICR. However, the ICRs of 4-hydroxy-3-methoxy compounds, HMAP and HMMA, were lower in comparison with those of MDA and MDMA, respectively. On the other hand, the ICRs of MDA, MDMA and MDEA were 5.5–6.1 times larger than those of amphetamine, methamphetamine and ethylamphetamine, suggesting that the methylenedioxy group on the benzene ring raises their ICRs very positively. Moreover, in order to

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apply the results from the animal experiments to human cases, the scalp hair samples of seven MDMA abusers were analyzed. MDMA and its metabolites, MDA, were simultaneously detected in all the samples by GC/MS. In the two samples, MDEA was found in addition to MDMA and MDA. It was shown that a hair sample is a good specimen for the confirmation of retrospective use of methylenedioxyamphetamines.

Keywords: Hair analysis; Drug disposition; Methylenedioxyamphetamines; GC/MS; Drug abuse

1. Introduction

In recent years, hair analysis has rapidly progressed as a useful method for detecting and monitoring drugs over the long term. Hair analysis provides useful information on individual's drug history from weeks to months, while the information from blood and urine is limited in the view of time period. In our previous reports [1], we have demonstrated that incorporation of drugs of abuse into hair depends mainly on their physico-chemical properties and basic drugs with lipophilicity and high melanin affinity would be readily incorporated into hair. Moreover, it was found that the structural factors related to their lipophilicity and basicity clearly affect the drug incorporation into hair from blood [2].

In the last decade, 3,4-methylenedioxyamphetamines, including 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) and 3,4-methylenedioxyethylamphetamine (MDEA, Eve), have been reported on their popularity and their fatal poisonings in Europe and the USA [3–6]. Therefore, they have become drugs of interest in the field of forensic toxicology.

So far, four biotransformation pathways, *N*-demethylation, *O*-demethylation, deamination and conjugation, have been identified for MDMA in rats [7]. Regarding their metabolism in humans, Lin et al. [8] have identified 4-hydroxy-3-methoxymethamphetamine (HMMA) as a major metabolite as well as 3,4-methylene-dioxyamphetamine (MDA) and 4-hydroxy-3-methoxyamphetamine (HMAP) in the basic extract of urine from a fatally injured motorcyclist. Only a few reports have been published about hair analysis for methylenedioxyamphetamines. Moeller et al. [9] have reported the detection of MDMA in the hair of a culprit who took MDMA by GC/MS and Kintz et al. [10] reported the simultaneous determination of MDMA and MDA including amphetamine (AP) and methamphetamine (MA). Skopp et al. [11] also analyzed MDMA abuser's hair for the diagnosis of toxic hepatitis. However, disposition of these drugs into hair still remains unclear.

In this study, we describe a GC/MS method for identification and determination of four typical methylenedioxyamphetamines, MDA, MDMA, MDEA and 3-methoxy-4,5-methylenedioxyamphetamine (MMDA), and two 4-hydroxy-3-methoxy metabolites of MDMA, HMAP and HMMA, in rat plasma and rat hair. Then, the disposition of these drugs into rat hair from rat plasma is investigated by compar-

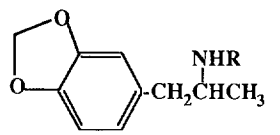
ison between concentrations in hair and areas under the concentration versus time curves (AUCs) in plasma in order to discuss the effect of structural factors on the incorporation of those drugs into hair. Furthermore, for the purpose of studying the disposition of MDMA and its related compounds into human hair, MDMA abusers' scalp hair samples are analyzed.

2. Materials and methods

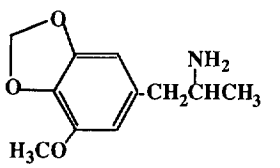
2.1. Materials

MDA was synthesized from 3,4-methylenedioxybenzaldehyde (Aldrich Chemical Co., USA) and nitroethane by the Ramirez method [12]. MDMA was prepared by *N*-methylation of MDA as reported by Fitzgerald et al. [13]. HMMA and HMAP were synthesized from 4-hydroxy-3-methoxybenzaldehyde (Aldrich Chemical Co., USA) according to the procedure reported by Lin et al. [7]. MMDA was prepared using 3-methoxy-4,5-methylenedioxybenzaldehyde (Lancaster Synthesis, Lancaster, UK) as described above for MDA. MDEA was synthesized from piperonylacetone (Tokyo Kasei, Tokyo, Japan) and ethylamine using sodium cyanoborohydride by the Braun method [14]. Deuterated compound, MDMA-d_4, was prepared from 3,4-methylenedioxy benzyl chloride obtained from 3,4-methylenedioxy benzyl alcohol [Wako Chemicals, Tokyo, Japan] with concentrated HCl and acetaldehyde-d_4 by modified Grignard reaction [15]. The structures of these drugs are shown in Fig. 1.

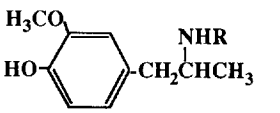
Pentafluoropropionic anhydride (PFPA) was purchased from Wako Chemicals (Tokyo, Japan) and Bond Elut Certify from Varian sample preparation products (Harbor City, CA, USA).



R	
-H	3,4-methylenedioxyamphetamine (MDA)
-CH3	3,4-methylenedioxymethamphetamine (MDMA)
-CH2CH3	3,4-methylenedioxyethylamphetamine (MDEA)



3-methoxy-4,5-methylenedioxyamphetamine (MMDA)



R	
-H	4-hydroxy-3-methoxyamphetamine (HMAP)
-CH3	4-hydroxy-3-methoxymethamphetamine (HMMA)

Fig. 1. The structures of methylenedioxyamphetamines and their metabolites investigated in this study.

2.2. Apparatus

Gas chromatograph-mass spectrometry (GC/MS) analysis was performed using a Hewlett Packard 5890 series-II gas chromatograph equipped with 7673A autosampler and MSD 5971. The gas chromatography (GC) was carried out with a 20 m × 0.25 mm i.d., 0.25 μm cross-linked methylsilicone fused silica column TC-1 (GL Sciences Inc., Tokyo, Japan). The injection port temperature was 200°C (splitless mode) and helium was the carrier gas (5.5 p.s.i. head pressure). The oven temperature was programmed from 60°C (held for 0.5 min) to 280°C at a rate of 20°C/min. Drugs in biological specimens were investigated by monitoring the selected ions as follows: m/z 135, 162, 325 for pentafluoropropionyl (PFP)-MDA, m/z 204, 162, 135, 339 for PFP-MDMA, m/z 218, 162, 135, 353 for PFP-MDEA, m/z 165, 192, 355 for PFP-MMDA, m/z 190, 310, 283 for $(PFP)_2$-HMAP, m/z 204, 310, 160 for $(PFP)_2$-HMMA and m/z 208 for PFP-MDMAd_4, respectively.

2.3. Animal experiments

Before drugs were administered, the back hair of the rats was shaved with an animal electric shaver (Daitoh Electric Machine Co., Tokyo, Japan). Male dark agouti (DA) pigmented rats, aged 5 weeks (100–120 g), were intraperitoneally administered once a day with MDA-HCl, MDMA-HCl, MDEA-HCl, MMDA-HCl, HMAP-HCl and HMAP-HCl (5 mg/kg) for 10 successive days ($n = 3$), respectively. The newly grown back hair was collected by the electric shaver 28 days after the first administration. Blood was collected at 5, 15, 30, 60, 120 and 360 min after injection with a capillary glass tube from the orbital vein plexus on day 1. The plasma samples were obtained by centrifugation at 10 000 g for 3 min and stored at −20°C until analysis. AUCs were calculated as described previously [16].

2.4. Human hair samples

MDMA abuser's hair samples 1–5 were offered from Dr. Tagliaro in Italy. Samples 6 and 7 were offered from Dr. Mieczkowski in the USA. All hair samples were collected from the posterior vertex of drug users.

2.5. Analytical methods

2.5.1. Plasma

To 200 μl of plasma of DA rat were added 100 μl of internal standard(IS) aqueous solution containing MDMA-d_4 at 1 μg/ml each and 500 μl of 0.1 M potassium hydrogen phosphate buffer (pH 6.0). After a Bond Elut Certify was pre-activated with MeOH and 0.1 M potassium hydrogen phosphate buffer (pH 6.0), the test solution was applied to the Bond Elut Certify and the column was washed with l ml of water, 1 ml of 0.1 M acetic acid and 1 ml of water, successively. The column was dried under vacuum for 5 min. After the column was rinsed with 1 ml of methanol and dried under vacuum for 2 min, 3 ml of MeOH-5 M HCl (20:1)

was passed through the column to elute the target drugs. Following evaporation of the solvent under a nitrogen stream, the residue was dissolved in 200 μl of PFPA/ethylacetate (1:1) and heated at 60°C for 20 min. The reaction solution was evaporated with a nitrogen stream and the residue was redissolved in 50 μl of ethylacetate. Two micro-litres of the ethylacetate solution was automatically injected into the GC/MS.

2.5.2. Hair

Rat hair samples and human scalp hair samples were washed three times with 0.1% sodium dodecyl sulfate (SDS) and water under ultrasonication. After the samples were dried under a nitrogen stream and precisely weighed (3–10 mg), they were extracted with 2 ml of MeOH-5N HCl (20:1) containing each IS as 100 ng of MDMA-d_4 for rat hair samples and 20 ng for human hair samples for 1 h under ultrasonication. Following storage at room temperature overnight, the hair was filtered off, and the filtrate was evaporated with a nitrogen stream and the residue was dissolved in 1 ml of 0.1 M phosphate buffer (pH 6.0). The solution was treated with Bond Elut Certify, derivatized and analyzed as above.

3. Results and discussion

3.1. Accuracy and precision of the methods

Under the chromatographic conditions used, there was no interference with all drugs or the deuterated internal standard by any extractable endogenous materials in the control rat plasma, control rat hair and control human hair. The calibration curves for determination of six drugs were constructed by analyzing the extracted and derivatized control specimens spiked with the standard solutions of these drugs and the deuterated internal standard, as mentioned above. The calibration curves for their drugs were linear over the concentration range 50–1000 ng/ml in rat plasma, 1.0–250 ng/mg in rat hair and 0.1–50.0 ng/mg in human hair, with correlation coefficients $r = 0.999$. The coefficients of variation of analysis ($n = 3$) for control human scalp hair spiked with the standard solution of MDA, MDMA, MDEA, MMDA, HMAP and HMMA at 1.0 and 10.0 ng/mg were in the range 0.32–12.04%. The values less than 0.1 ng/mg in the human hair for each drug were cut off.

3.2. Concentrations of drugs and their metabolites in rat plasma

After intraperitoneal administration of MDA, MDMA, MDEA, MMDA, HMAP and HMMA to DA rats at 5 mg/kg, concentrations of drugs in the rat plasma over 360 min were monitored using GC/MS. Time courses of the rat plasma concentrations of parent compounds and their metabolites are shown in Fig. 2. Half-lives of MDA, MDMA, MDEA and MMDA in the plasma were 87.8 ± 11.0, 69.4 ± 9.3, 94.9 ± 0.02 and 106.0 ± 14.0 min, respectively. Those of HMMA and HMAP were 18.3 ± 6.7 and 5.2 ± 0.04 min and these drugs were not detected in the plasma 120

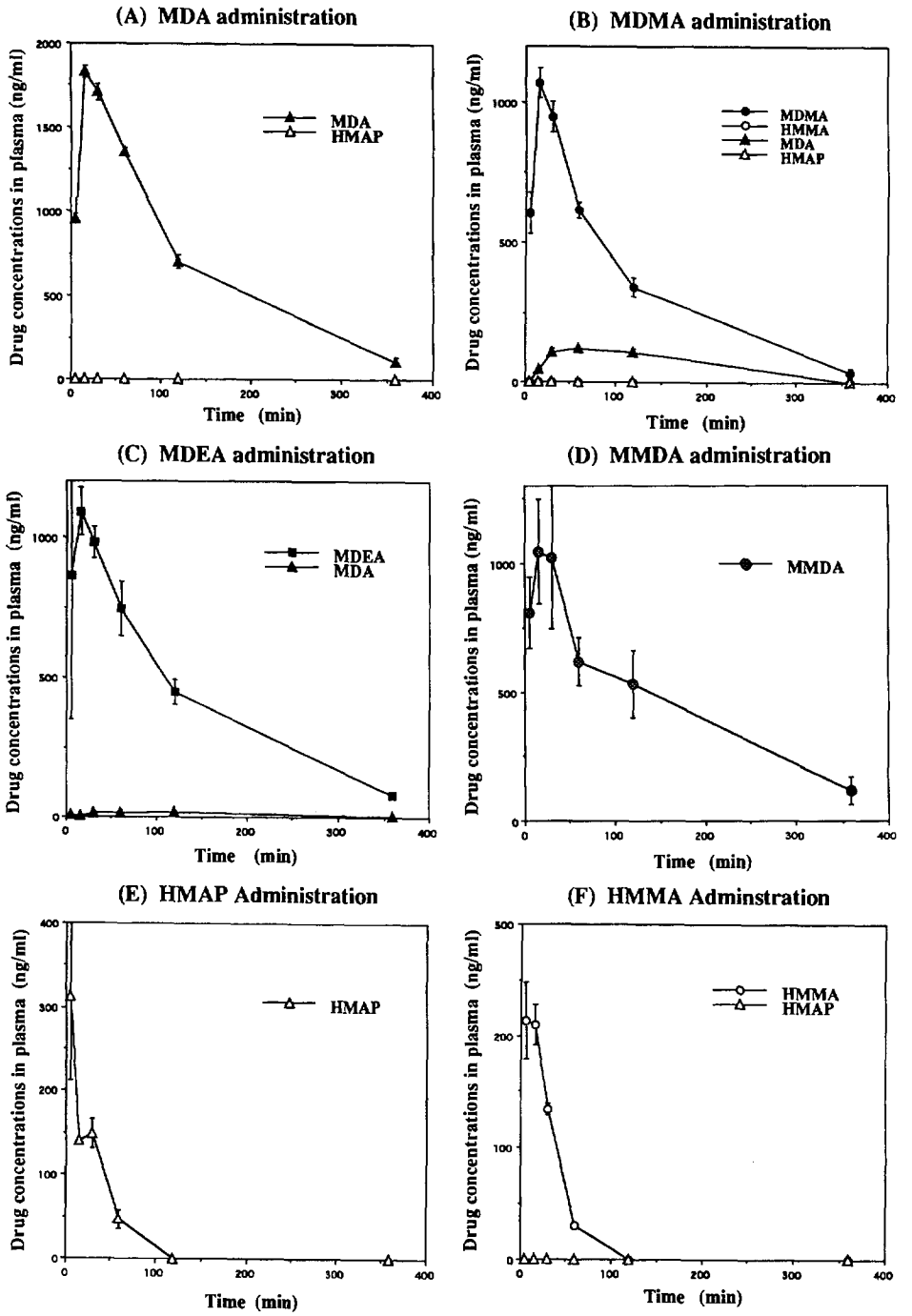


Fig. 2. The time courses of the rat plasma concentrations after administration of (A)MDA-HCl, (B)MDMA-HCl, (C)MDEA-HCl, (D)MMDA-HCl, (E)HMAP-HCl and (F)HMMA-HCl (i.p., 5 mg/kg, $n = 3$).

min after administration. Average peak plasma concentrations of MDA, MDMA, MDEA and MMDA were more than 1000 ng/ml 15 min after administration of these drugs, while those of HMAP and HMMA were only 200–300 ng/ml at 5 min.

The AUCs in rat plasma of these drugs are shown in Fig. 3. The plasma AUC of MDA was the highest (262.1 ± 9.9 μg·min/ml), followed by MMDA (182.9 ± 50.1 μg·min/ml), MDEA (164.5 ± 10.4 μg·min/ml) and MDMA (121.2 ± 16.0 μg·min/ml). Those of HMAP and HMMA were extremely low (10.0 ± 2.0 and 8.6 ± 0.2 μg·min/ml, respectively). This may be because these data were calculated with concentrations of only free forms of drugs in the plasma. MDA, *N*-dealkyl metabolite of MDMA or MDEA, was detected in the plasma of rat administered MDMA or MDEA, and their AUCs of MDA were 24.2 ± 2.2 μg·min/ml for MDMA administration and 4.4 ± 1.1 μg·min/ml for MDEA administration. On the other hand, only small amounts of HMMA and HMAP as metabolites of MDMA or MDA were detected in the plasma.

3.3. Drug concentrations in rat hair

The concentrations of each drug and their metabolites in the rat hair are shown in Fig. 4. GC/MS-SIM chromatograms of PFP-derivatized extracts from the rat hair are shown in Fig. 5. All parent compounds were detected in the rat hair. The concentration of MMDA was the highest level of all (215.4 ± 20.5 ng/mg). Those of MDA, MDMA and MDEA were 121.9 ± 27.5, 93.4 ± 10.9 and 138.4 ± 4.5 ng/mg, respectively. However, the concentrations of HMAP and HMMA in the hair of rat administered HMAP and HMMA were extremely low in comparison with those of methylenedioxy compounds, which were 3.4 ± 0.3 and 4.8 ± 0.8 ng/mg, respectively. MDA was simultaneously detected in the hair of rats adminis-

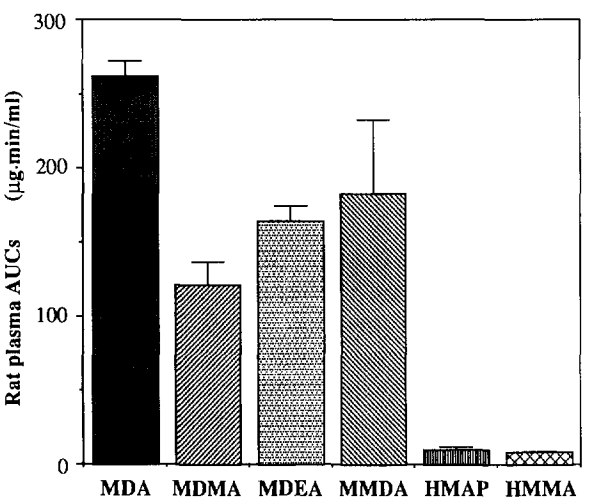


Fig. 3. The rat plasma AUCs of the six parent drugs after 5 mg/kg i.p. administration.

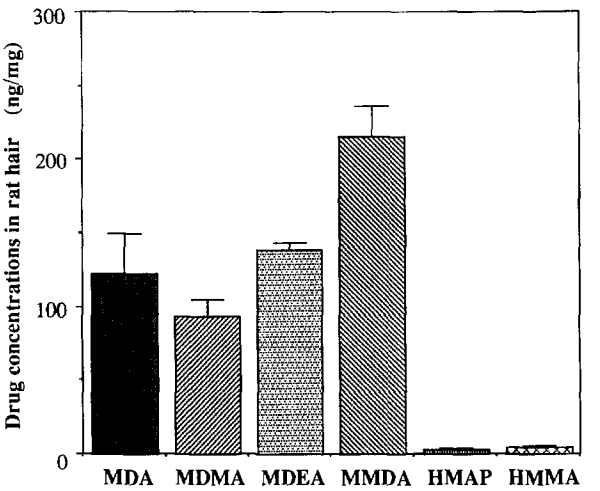


Fig. 4. The concentrations of the six parent drugs in newly grown rat hair after 5mg/kg (×10) i.p. administration.

tered MDMA and MDEA, while both HMAP and HMMA were not detected in the hair of rats administered MDA, MDMA or MDEA.

3.4. Incorporation rates of drugs into rat hair from rat plasma

We have proposed that the ratio of drug concentration in hair to AUC in plasma could be one of the indexes that indicate the incorporation rate (ICR) of drug into hair from plasma [1,2,16–21]. According to our proposal, ICRs of MDA, MDMA and MDEA which have proton, methyl and ethyl at N position were 0.55, 0.77 and 0.85, respectively. The ICRs increased according to the length of carbon branches at N-position (MDA < MDMA < MDEA) (Fig. 6). In a previous report [2], we have demonstrated that ICRs of AP analogs also increased from proton to propyl group at N position (AP < MA < ethylamphetamine (EAP) < propylamphetamine(PAP)). It is thought that these phenomena would be explained by increase of lipophilicity of compounds. The ICR of MMDA (*meta*-methoxy derivative of MDA) was 1.24, which is 2.3 times higher than that of MDA (MDA < MMDA). We have already reported [2] that the ICRs of *p*-methoxymethamphetamine and *o*-methoxymethamphetamine(methoxyphenamine) were 3.1–3.3 times as much as that of MA, though there was not as much difference for the ICRs when comparing the *para* and *ortho* position of the methoxy group. These facts suggested that the methoxy group on the benzene ring of the AP structure would serve as a positive factor for enhancing the ICR. Further, those of HMAP and HMMA which have 4-hydroxy and 3-methoxy groups on the benzene rings of AP and MA were 0.35 and 0.47, which are lower than those of MDA and MDMA, respectively (HMAP < MDA < HMMA < MDMA). In addition, the ICR of MDA which has a 3,4-methyl-

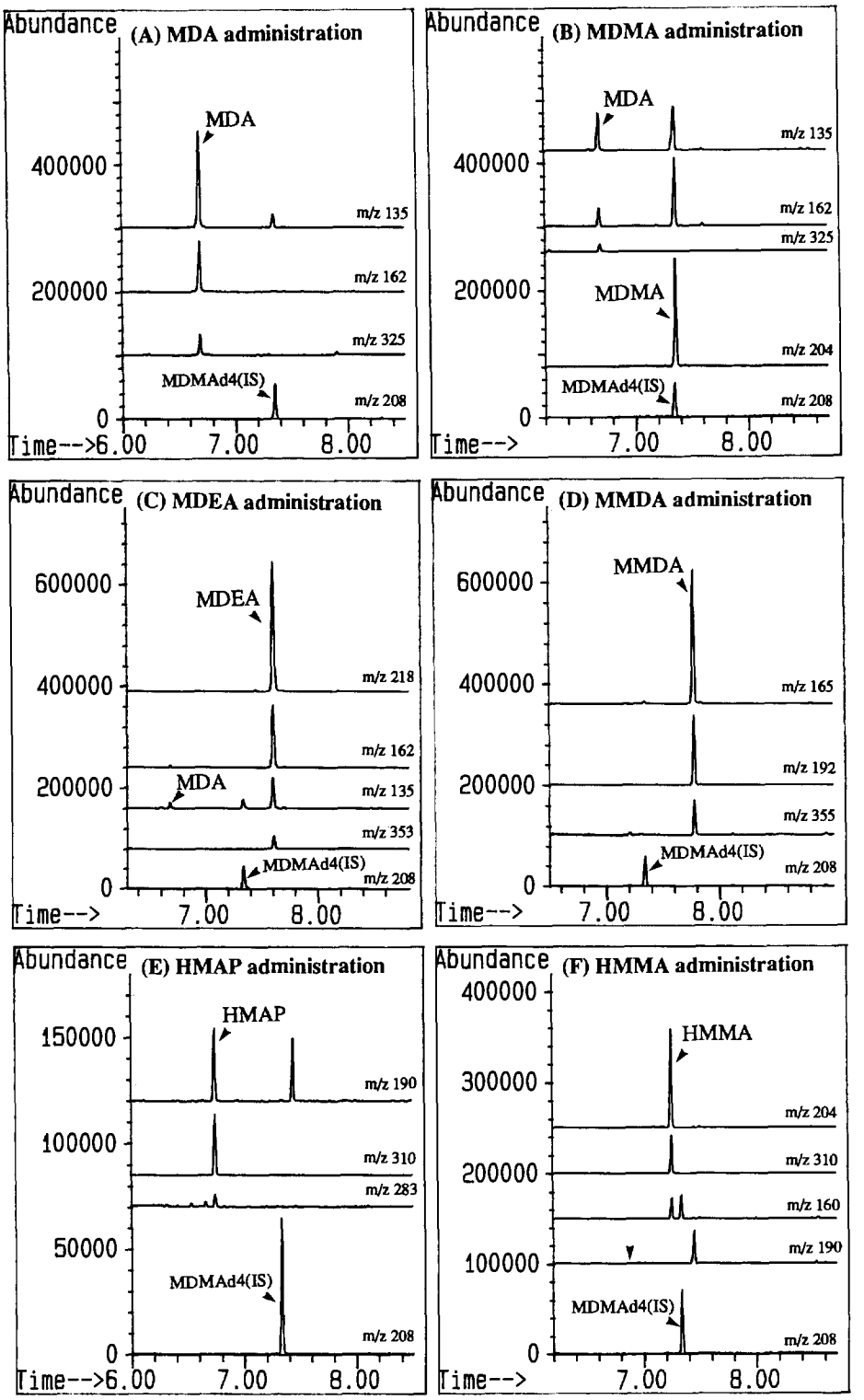


Fig. 5. GC/MS-SIM chromatograms of PFP-derivatized extracts from the rat hair after administration of (A)MDA-HCl, (B)MDMA-HCl, (C)MDEA-HCl, (D)MMDA-HCl, (E)HMAP-HCl and (F)HMMA-HCl (i.p., 5 mg/kg × 10 days).

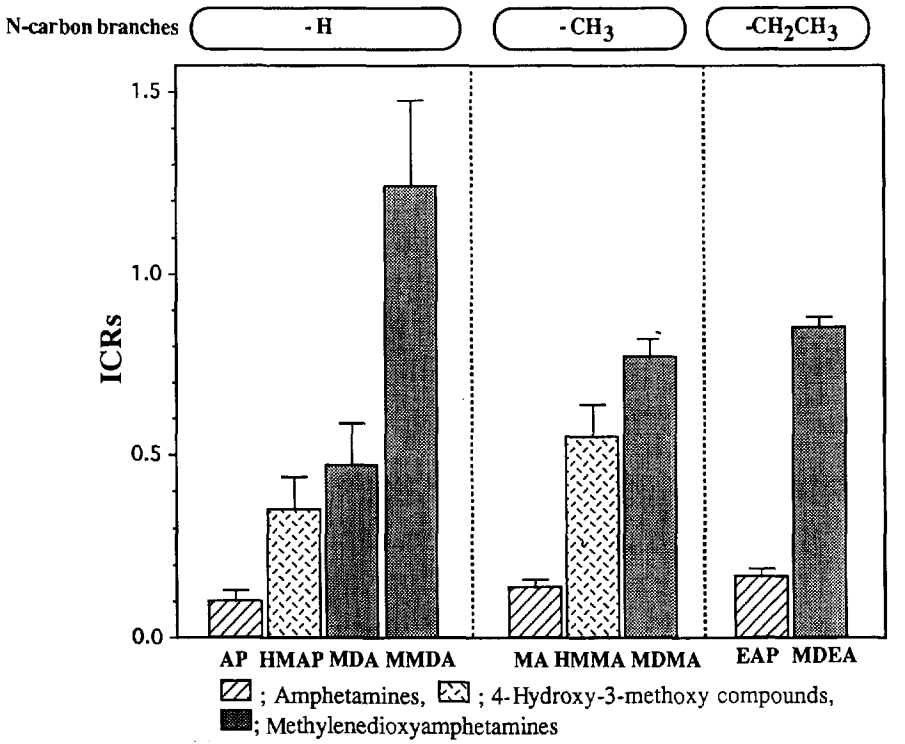


Fig. 6. The effect of length of N-carbon branches, methylenedioxy group and methoxy group on the ICRs.

enedioxy group to the benzene ring of AP was 5.5 times higher than that of AP (AP ≪ MDA), MDMA corresponding to MA analog was 5.9 times higher than that of MA (MA ≪ MDMA) and MDEA corresponding to EAP analog was 6.1 times higher than that of EAP (EAP ≪ MDEA). It is clear that the introduction of a methoxy group or methylenedioxy group to the benzene ring of AP structures raises their ICRs very positively. It is possible that the property to act as an electron donor of methoxy and methylenedioxy groups might affect ICRs.

In our previous report [1], we have already studied the ICRs of more than 20 drugs using animal model experiments. As a result, there were significant differences between their ICRs according to their chemical properties. Cocaine and phencyclidine have the highest ICRs (2–3), 6-acetylmorphine and MA have medium ICRs (0.1–1) and morphine, deprenyl and benzoylecgonine have low ICRs (< 0.1). In a case of methylenedioxyamphetamines, their ICRs were 0.55–1.24, which are classified into the level of medium or high ICRs. Furthermore, those of 4-hydroxy-3-methoxy compounds, HMAP and HMMA, were also classified into medium level (0.35 and 0.47, respectively). The hair concentrations of methylenedioxyamphetamines were the highest of all that we have previously studied under the same conditions, though their ICRs ([hair concentration]/[plasma AUC]) were lower than those of cocaine and phencyclidine.

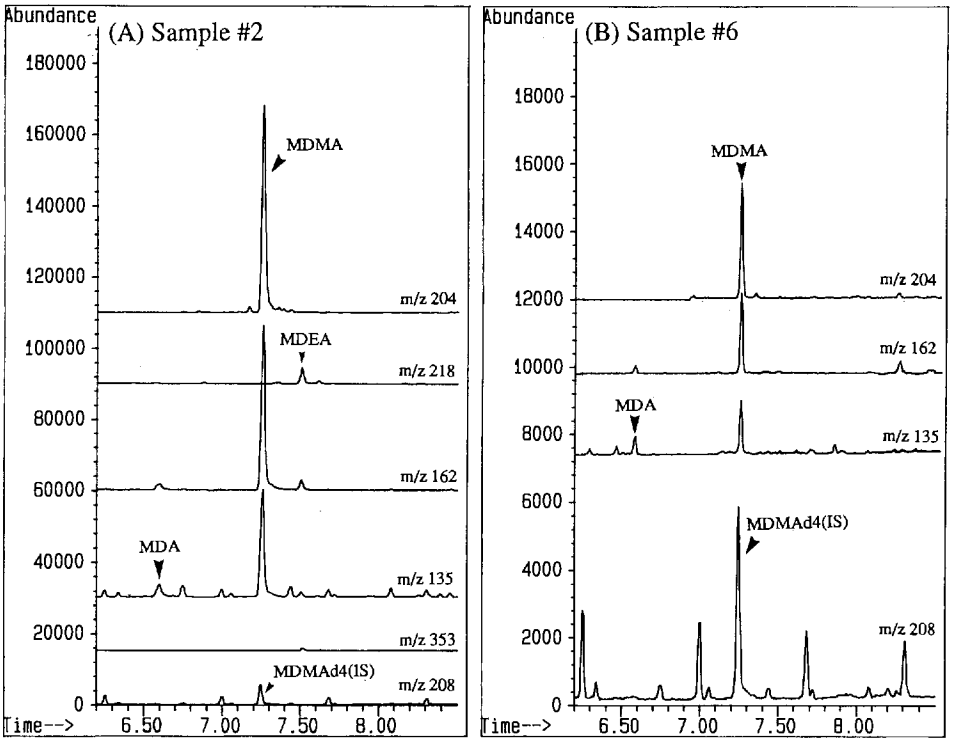


Fig. 7. GC/MS-SIM chromatograms of PFP-derivatized extracts from MDMA abusers' scalp hair; (A) sample 2 and (B) sample 6.

3.5. Detection of drugs from MDMA abuser's scalp hair

In the animal experiments, it was demonstrated that methylenedioxyamphetamines were well incorporated into hair. In order to apply our results from the

Table 1
Drug concentrations in seven MDMA abuser's hair samples

Samples	Hair color	Drug concentrations in hair (ng/mg)		
		MDMA	MDA	Other drugs
1	Dark brown	0.31	TR[a]	
2	Black	12.51	0.79	MDEA 0.74
3	Light brown	0.48	0.18	
4-W	Bleached hair	1.05	TR[a]	
4-B	Black	3.88	0.38	
5	Dark brown	0.15	TR[a]	MDEA 0.70
6	Light brown	1.14	0.13	
7	Brown	1.41	1.25	

[a] < 0.1 ng/mg

animal experiments to human cases, the scalp hair samples of seven MDMA abusers were analyzed. Fig. 7 shows the SIM chromatograms of the PFP-derivatized extracts from two samples of the MDMA abusers' hair (samples 2 and 6). MDMA and its metabolite, MDA, were simultaneously detected in all abuser's hair extracts. The concentrations of MDMA were 0.15–12.51 ng/mg, while those of MDA were trace levels (< 0.1)–1.25 ng/mg (Table 1). MDEA was also detected in samples 2 and 5 at the concentrations of 0.74 and 0.70 ng/mg, respectively. However, no 4-hydroxy-3-methoxy metabolites were found in the human hair samples in spite of the fact that HMMA has been reported as one of the major metabolites in human urine [8]. In regard to the disposition of MDMA and its related compounds into hair, there was a similar tendency between animals and humans.

References

[1] Y. Nakahara, K. Takahashi and R. Kikura, Hair analysis for drugs of abuse X. Effect of physico-chemical properties of drugs on the incorporation rates into hair. *Biol. Pharm. Bull.*, 18(9) (1995) 1223–1227.

[2] Y. Nakahara and R. Kikura, Hair analysis for drugs of abuse XIII. Effect of structural factors on incorporation of drugs into hair: the incorporation rates of amphetamine analogs. *Arch. Toxicol.*, 70 (1996) 841–849.

[3] A. Poklid, M.A. Macwell and W.K. Drake, Fatal intoxication from 3,4-methylenedioxyamphetamine. *J. Forensic Sci.*, 24 (1979) 70–75.

[4] G.P. Dowling, E.T. McDonough III and R.O. Bost, 'Eve and Ecstasy' A report of five deaths associated with the use of MDEA and MDMA. *J. Am. Med. Assoc.*, 257(12) (1987) 1615–1617.

[5] J.A. Henry, K.J. Jeffreys and S. Dawling, Toxicity and deaths from 3,4-methylenedioxymethamphetamine('ecstasy'). *Lancet*, 340 (1992) 384–387.

[6] A.R.W. Forrest, J.H. Galloway, I.D. Marsh, G.A. Strachan and J.C. Clark, A fatal overdose with 3,4-methylenedioxyamphetamine derivatives. *Forensic Sci. Int.*, 64 (1994) 57–59.

[7] H.K. Lin and R.L. Foltz, In vivo and in vitro metabolism of 3,4-(methylenedioxy)methamphetamine in the rat: identification of metabolites using an ion trap detection. *Chem. Res. Toxicol.*, 1 (1988) 370–378.

[8] H.K. Lin and R.L. Foltz, Identification of metabolites of 3,4-(methylenedioxy)-methamphetamine in human urine. *Chem. Res. Toxicol.*, 2 (1989) 142–143.

[9] M.R. Moeller, H.H. Maurer and M. Roesler, MDMA in blood, urine and hair. A forensic case. *Proc. of the 30th TIAFT Meeting*, (1989) 347–361.

[10] P. Kintz, V. Cirimele, A. Tracqui and P. Mangin, Simultaneous determination of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine in human hair by gas chromatography-mass spectrometry. *J. Chromatogr. B.*, 670 (1995) 162–166.

[11] G. Skopp, R. Aderjan and J. Koster, Hair analysis in the diagnosis of toxic hepatitis after drug abuse with ecstasy. *Dtsch. Med. Wochenchr.*, 120 (1995) 1165–1168.

[12] F.A. Ramirez and A.W. Weston, The reduction of phenolic β-nitrostyrenes by lithium aluminium hydride. *J. Am. Chem. Soc.*, 72 (1950) 2781–2782.

[13] R.L. Fitzgerald, R.V. Blanke, R.A. Glennon, M.Y. Yousif, J.A. Rosecrans and A. Poklis, Determination of 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine enantiomers in whole blood. *J. Chromatogr.*, 490 (1989) 59–69.

[14] U. Braun, A.T. Shulgin and G. Braun, Centrally active N-substituted analogs of 3,4-methylenedioxyphenylisopropylamine (3,4-methylenedioxyamphetamine). *J. Pharm. Sci.*, 69(2) (1980) 192–195.

[15] Y. Nakahara, K. Takahashi, M. Shimamine and Y. Takeda, Hair analysis for drug abuse I. Determination of methamphetamine and amphetamine in hair by stable isotope dilution gas chromatography/mass spectrometry method. *J. Forensic Sci.*, 36 (1991) 70–78.

[16] Y. Nakahara, T. Ochiai and R. Kikura, Hair analysis for drugs of abuse V. The facility in incorporation of cocaine into hair over its major metabolites, benzoylecgonine and ecgonine methyl ester. *Arch. Toxicol.*, 66 (1992) 446–449.

[17] Y. Nakahara, K. Takahashi, M. Shimamine and A. Saitoh, Hair analysis for drugs of abuse IV. Determination of total morphine and confirmation of 6-acetylmorphine in monkey and human hair by GC/MS. *Arch. Toxicol.*, 66 (1992) 669–674.

[18] Y. Nakahara and R. Kikura, Hair analysis for drugs of abuse VII. The incorporation rates of cocaine, benzoylecgonine and ecgonine methyl ester into rat hair and hydrolysis of cocaine in rat hair. *Arch. Toxicol.*, 68 (1994) 54–59.

[19] Y. Nakahara, R. Kikura and K. Takahashi, Hair analysis for drugs of abuse VIII. Effective extraction and determination of 6-acetylmorphine and morphine in hair with trifluoroacetic acid-methanol for the confirmation of retrospective heroin use by gas chromatography-mass spectrometry. *J. Chromatogr. B.*, 657 (1994) 93–101.

[20] R. Kikura and Y. Nakahara, Hair analysis for drugs of abuse IX. Comparison of deprenyl use and methamphetamine use by hair analysis. *Biol. Pharm. Bull.*, 18 (1995) 267–272.

[21] R. Kikura and Y. Nakahara, Hair analysis for drugs of abuse XI. Disposition of benzphetamine and its metabolites into hair and comparison of benzphetamine use and methamphetamine use by hair analysis. *Biol. Pharm. Bull.*, 18(12) (1995)1694–1699