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SHORT COMMUNICATION

Experimental study on external contamination of hair by synthetic cannabinoids and effect of hair treatment

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Abstract We recently reported a fatal case of poisoning by *N*-1-naphthalenyl-1-pentyl-1*H*-indole-3-carboxamide (NNEI). In this case, NNEI was detected from 13 sections of a 40 cm length of hair after washings. The concentrations of NNEI were generally similar among the hair segments. These results strongly suggested that externally contaminated NNEI could not be removed from the hair by washings. The aim of the present study is to demonstrate the adsorption and removal by washings for NNEI and [1-(5-fluoropentyl)-1*H*-indol-3-yl]-(4-methyl-1-naphthalenyl)-methanone (MAM-2201) using liquid chromatography–tandem mass spectrometry. NNEI and MAM-2201 were detected in black hair even after a single immersion in NNEI and MAM-2201 aqueous solutions, followed by washings. However, their adsorption seemed less pronounced for dyed or bleached hair. Therefore, hair analysis cannot be considered effective in testing of synthetic cannabinoid abuse, because the external synthetic cannabinoid(s) can be easily adsorbed to hair of a non-user by passive exposure.

Keywords MAM-2201 · NNEI · Synthetic cannabinoid · Hair analysis · External contamination · LC–MS–MS

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Introduction

In recent years, new classes of designer drugs such as synthetic cathinones and cannabinoids have appeared as recreational drugs of abuse in many countries. These compounds are of two types: herbal-type products that are sold as "legal herbs" or "incense" and liquid or powder-type products that are sold as "legal drug" or "aroma liquids" on the Internet [1–4]. Many cases of poisoning due to abuse of these drugs have occurred worldwide. Recently, we have reported cases of fatal poisoning due to the use of these compounds [5–7].

It has been well documented that drugs are deposited in hair from the bloodstream during hair growth, and the deposited drugs move in the distal direction according to hair growth. Therefore, forensic toxicologists can investigate the period of illegal drug use by analyzing segments of hair. Usually, cannabinoids and synthetic cannabinoids are consumed by smoking; therefore, the exclusion of external contamination of hair samples is a very important issue in forensic hair analysis. Several analytical methods for measuring trace amounts of cannabinoids and synthetic cannabinoids in hair samples from cases of drug abuse have been reported [8–10].

Recently, we encountered a fatal case of poisoning by NNEI (*N*-1-naphthalenyl-1-pentyl-1*H*-indole-3-carboxamide), and tried hair analysis for the segments to estimate the period of use of the synthetic cannabinoid [11]. In this case, hair strands of approximately 40 cm in length were divided into 13 sections after careful washings. NNEI was detected in all sections, and the distal segments showed higher concentrations of NNEI. However, the results were found to be qualitatively unreliable, because NNEI was not distributed in Japan until about two years ago.

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Hutter et al. [9] studied the presence of 22 different synthetic cannabinoids in human hair and demonstrated that they were concentrated in the hair of users; interestingly, they also reported that synthetic cannabinoids were detected in hair segments related to the time before the self-reported start of drug use. This also suggested that an additional route of synthetic cannabinoid incorporation into hair such as from sidestream smoke, may contribute to this phenomenon.

Therefore, the case of fatal poisoning in which we analyzed NNEI in the hair, together with the above report [9], led us to investigate the relationship between external contamination of human hair by synthetic cannabinoids and washings before their extraction. We first exposed some kinds of human hair to NNEI and MAM-2201 ([1-(5-fluoropentyl)-1*H*-indol-3-yl]-(4-methyl-1-naphthalenyl)-methanone) by immersing them in aqueous solutions containing NNEI or MAM-2201, and we subjected the contaminated hair samples to washings according to the method of Kim et al. [12].

Materials and methods

Chemicals and preparation of drug solutions

NNEI, MAM-2201, and MAM-2201-d_5 (500 μg/500 μl methanol solution) were purchased from Cayman Chemical Company (Ann Arbor, MI, USA); methanol (HPLC grade), and acetonitrile (HPLC grade) were from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade and were purchased from Wako Pure Chemical Industries (Osaka, Japan).

The MAM-2201 and NNEI stock solutions (1 mg/ml) were prepared in methanol. The MAM-2201-d_5 internal standard (IS, 500 μg/500 μl) was diluted in methanol to a final concentration of 1 μg/ml. MAM-2201 and NNEI used for hair immersion were diluted in water to a final concentration of 1.0 and 0.1 μg/ml, separately. All stock solutions and standard solutions were kept in amber glass vials at −30 °C until use. Blank hair samples were collected from a healthy volunteer after obtaining informed consent and were verified to be MAM-2201 or NNEI negative using the present method described below. The brown or blond colored hair was cosmetically dyed by the donor.

Hair treatment with MAM-2201 or NNEI solution

The hair was rinsed twice with 2 % sodium dodecyl sulfate aqueous solution and dried at room temperature. The hair was cut into 2–3 cm lengths, and approximately 100 strands were grouted using paraffin at both ends. In the single immersion group, hair strands were immersed in aqueous MAM-2201 or NNEI solution (1.0 and 0.1 μg/ml) and dried at room temperature. In the repeated immersion group, the hair strands were reimmersed after they had been completely dried.

Washings and extraction of hair samples

In this study, we adapted the previously described method [12] for washings and extraction of synthetic cannabinoids from hair samples. In brief, the contaminants were eliminated by washing the hair strands twice with 2 ml methanol, then twice with distilled water, then twice again with 2 ml methanol, and they were dried at room temperature. Both ends of dried hair strands capped with paraffin were cut off. Then the hair samples were cut into 1–2 mm pieces with scissors, placed in a 1.5 ml vial, and accurately weighed (approximately 10 mg hair). A 1-ml aliquot of methanol and 2 μl of IS solution were added to each vial. The mixture was gently stirred at 38 °C for 16 h for extraction of MAM-2201 or NNEI. The methanol extract was transferred into a new tube and dried under a nitrogen stream at 45 °C. The residue was dissolved in 100 μl of acetonitrile, and a 3-μl aliquot was injected into the LC–MS–MS system.

LC–MS–MS conditions

Chromatographic separation was performed on an Inert-Sustain® C_{18} HP 3 μm column (100 × 3 mm i.d.; GL Sciences, Tokyo, Japan) at 40 °C using the Agilent 1200 LC System (Agilent, Santa Clara, CA, USA). Gradient elution was used for chromatographic separation with the mobile phase A of 0.1 % acetic acid aqueous solution, and mobile phase B of acetonitrile. Linear gradient elution started from 80 % A/20 % B to 100 % B over 5 min. The 100 % B was held for 1 min. It was then returned to 80 % A/20 % B over 4 min for the next run. The flow rate was 0.6 ml/min, and the injection volume was 3 μl.

Electrospray ionization (ESI)–MS–MS detection was performed on an Agilent 6410 Triple Quad Tandem mass spectrometer in positive ionization mode with ions monitored in the selected reaction monitoring (SRM) mode (Agilent). The ESI source parameters were: high-purity drying gas (N_2) flow rate, 6 l/min; ion source temperature, 300 °C; capillary voltage, 4000 V; nebulizer, 15 psi; fragmentor and collision energies for MAM-2201 and IS, 170 and 25 V, respectively; fragmentor and collision energy for NNEI, 140 and 21 V, respectively; ion transitions for SRM, *m/z* 374.2 → 169.1 for MAM-2201, *m/z* 357.2 → 214.1 for NNEI, and *m/z* 379.2 → 169.1 for IS.

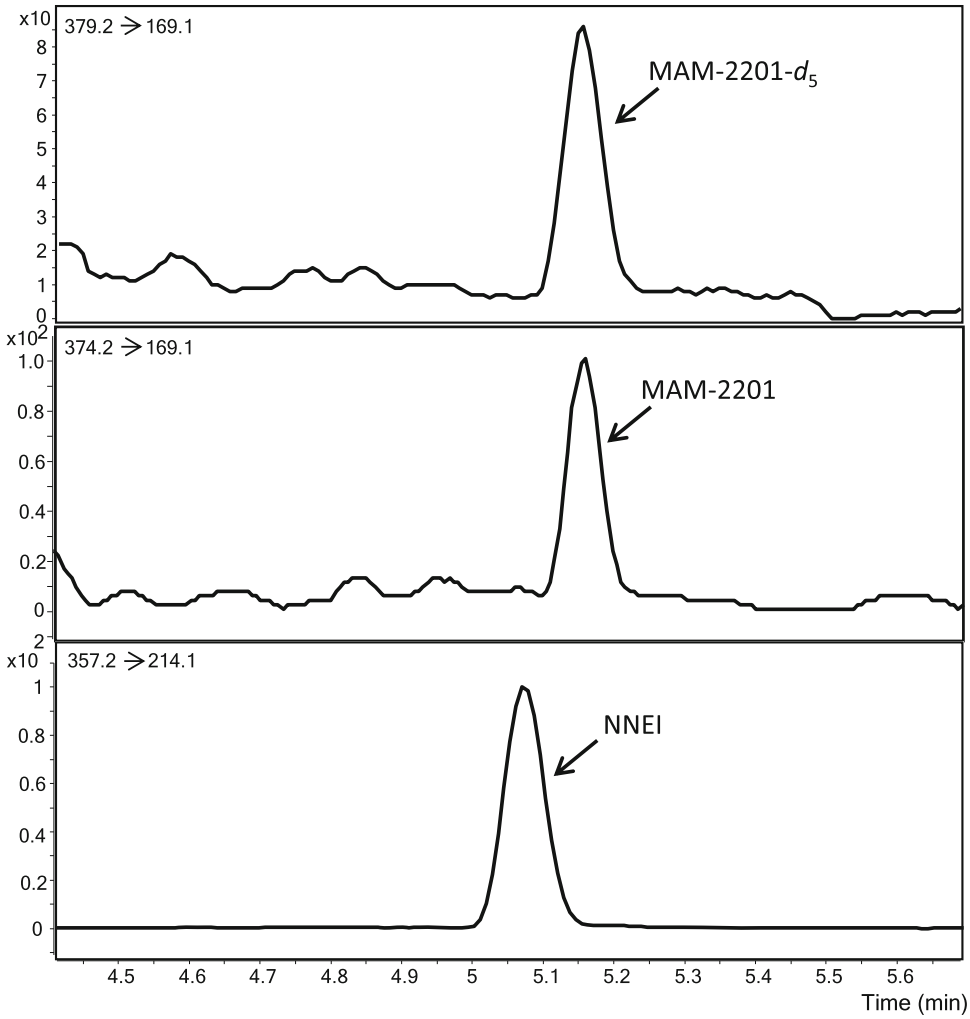

Fig. 1 Selected reaction monitoring chromatograms of a hair extract. The concentrations of MAM-2201, MAM-2201-d_5 (internal standard) and NNEI were 111, 2,000 and 115 pg/mg, respectively

Results and discussion

Figure 1 shows examples of SRM chromatograms of typical hair analysis of MAM-2201 and NNEI, which were analyzed after black hair strands were immersed once in each 1.0 μl/ml solution. Table 1 shows MAM-2201 and NNEI concentrations in hair after the samples were immersed in each solution and carefully washed . We also examined the effects of hair dyed to brown and blond colors because in our experience the hair of many synthetic cannabinoid users was dyed to brown or blond color.

Our data showed that both MAM-2201 and NNEI were easily adsorbed to hair for both black and dyed hair, and were not easily eliminated by a washing procedure (Table 1). MAM-2201 and NNEI were readily adsorbed to natural black hair, even with a single immersion. The difference in the concentration was more than double when immersed seven times. The extent of the adsorption was

Table 1 Concentrations of MAM-2201 and NNEI in hair samples with and without dying after immersion in aqueous solutions containing MAM-2201 or NNEI followed by washings

Sample (number of samples)	Liquid concentration (μg/ml) (the time of immersion)	Concentration range in hair (pg/mg)	
		MAM-2201	NNEI
Black hair without dyeing ($n = 4$ each)	1.0 (×7 times)	643–248[a]	566–116[e]
	1.0 (×3 times)	549–188[b]	457–89.2[f]
	1.0 (×1 time)	225–111[c]	115–77.8 [g]
	0.1 (×1 time)	45.9–n.d.[d]	32.4–n.d.[h]
Hair dyed brown and blond ($n = 6$ each)	1.0 (×7 times)	230–54.3[a]	244–64.2[e]
	1.0 (×3 times)	127–26.4[b]	116–46.1[f]
	1.0 (×1 time)	36.7–n.d.[c]	45.8–n.d.[g]
	0.1 (×1 time)	n.d.[d]	n.d.[h]

Statistically significant between each pair of groups: $P < 0.01$ for [a, b, e, f] and $P < 0.001$ for [c, g]

n.d. not detected

also dependent on the concentrations of MAM-2201 or NNEI in the aqueous solution for the immersion.

The data also showed that MAM-2201 and NNEI were adsorbed to natural black hair better than to dyed hair (Table 1). These substances were not detected in dyed hair that was immersed only once. The lower adsorption of the substances to the dyed hair is probably due to keratin damage. However, the detailed mechanism of the lower adsorption of the substances to the dyed hair is not clear at this time.

According to Romano et al. [13], even using the most sophisticated decontamination procedures, it is not possible to distinguish a drug-contaminated subject from an active user. Kintz [14] studied intact hair soaked in blood containing 7-aminoflunitrazepam; however, it could not be completely removed from soaked hair by washings. Therefore, the author concluded that the standard decontamination procedure is not able to remove completely external contamination in postmortem specimens. From the results of our study, although negative results (no detection) can exclude both chronic use and passive exposure to synthetic cannabinoid(s), positive results cannot be accurately interpreted as a sign of drug use. The phenomenon observed in a study of Hutter et al. [9] is likely caused by the sidestream smoke from synthetic cannabinoid(s). Our results obtained from the immersion of black hair in aqueous solution containing a synthetic cannabinoid support the idea that external contamination of hair can easily take place. Especially, the sweat of the synthetic cannabinoid abuser secreted from the scalp can contaminate the hair extensively; this may be also true for the sebum. These external contaminations mean that we are unable to identify the period of administration of the studied drugs by analysis of hair segments.

Conclusions

Previously, hair analysis was believed useful for the confirmation of drug use because of long time-scale windows. The easy adsorption of NNEI and MAM-2201 onto hair and difficulty of removal by washings were demonstrated in this study. Although NNEI and MAM-2201 were detected in black hair after a single immersion in NNEI and MAM-2201 solutions, their adsorption seemed less pronounced for dyed hair. Therefore, hair analysis cannot be considered effective in toxicological studies on synthetic cannabinoid abuse, but negative results are useful to deny the abuse. When hair analysis of synthetic cannabinoid(s) is carried out, the analysts should take into consideration the external contamination of hair by drugs of abuse on any occasion.

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