SHORT COMMUNICATION

Hair analysis and self-report of methamphetamine use by methamphetamine dependent individuals

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ABSTRACT

The questions of whether the dose of drug that is consumed corresponds to drug concentration levels in hair and how results of hair analyses can be interpreted are still debated. The aim of this study was to investigate (1) whether there is a correlation between doses of Methamphetamine (MA) use and MA concentration levels in hair and (2) whether results of hair analyses can be used to estimate dose, frequency, and patterns of MA use. In this study, segmental hair analysis was performed through consecutive 1 cm as well as 1–4 cm (=3 cm) segmental hair lengths. MA dependent individuals (n=9) provided information on doses (0.25–4 g/day) of MA use as well as the frequency of MA use. The concentrations of MA and its metabolite amphetamine (AP) in hair were determined using gas chromatography/mass spectrometry (GC/MS). One-way analysis of variance (ANOVA) test was performed to evaluate whether MA and AP concentrations in consecutive 1 cm length segmental hair were consistent with the history of MA use. The cumulative doses of MA use calculated from the daily dose and the frequency during 1–4 months were well correlated to the concentrations of MA and AP in 1–4 cm segmental hair length (correlation coefficient, r=0.87 for MA and r=0.77 for AP). The results from this study show the patterns and histories of MA use from MA dependent individuals and could assist in the interpretation of hair results in forensic toxicology as well as in rehabilitation and treatment programs.

Keywords:
Methamphetamine
Hair analysis
Drug history
Segmental analysis
GC/MS

1. Introduction

Examination of drug concentration levels in hair is a useful method for studying the history of drug addiction. In addition, segmental hair analysis is capable of clarifying the time course of drug use. Researchers have attempted to elucidate the history of drug use using segmental hair analysis. Nakahara et al. postulated that hair analysis, especially segmental analysis, may be useful in determining past drug history [1,2]. Pichini et al. reported the suitability of segmental hair analysis of Methylenedioxymethamphetamine (MDMA) to monitor past chronic exposure to the drug in ecstasy consumers [3].

The correlation between the dose of drug use and the concentration of the drug in hair still remains controversial. Forensic scientists commonly encounter the problems and doubts in application of hair testing and data interpretations. There have been many studies about relationship between drug histories and drug concentration in hair related to cocaine and heroine [4–10]. These studies reported either a strong or a weak association between drug dose and the hair analysis. Most likely, weak relations and interindividual differences are due to a substantive individual bio-variability, variability of hair-growth cycles, hair color, cosmetic treatment and unknown purity of compounds [5,11,12].

Methamphetamine (MA) is a sympathomimetic amine whose abuse has become a serious problem in Asian countries and the United States. However, MA is not among the compounds frequently studied in correlation studies and the number of MA subjects in existing studies is rather small [13]. Despite the prevalence of MA use, there is little data on the concentrations of MA and AP in hair in the United States and the mode of administration in published studies is not specifically explained [14,15]. To our knowledge, there have been few studies of MA concentration levels in human hair following controlled administration of MA. Most studies rely on self-reports of MA use [14,16] or conducted animal studies [1,16] to investigate the relationship between the dose of MA consumed and MA concentrations in hair.

Fearing legal consequences and embarrassment of admitted MA use, most suspects tend to deny or, to underreport MA consumption. However, the self-reported histories of MA use from subjects who voluntarily participate in and consent to our research design in an alcohol and drug treatment program should be more accurate because disclosure of information has no consequences regarding...
forensic or legal issues and participants of our study were requested to record their drug consumption in unit of day, week and month as accurate as possible.

In this study, the relationships between self-reported data given by MA dependent individuals and MA concentrations in hair were evaluated. Hair was cut into 1 cm length segments to investigate whether MA concentrations in hair were consistent with pattern and history of MA use in each subject and was cut into 1–4 cm segments to evaluate the relationship between dose and frequency of MA use and MA concentrations in hair.

2. Materials and methods

2.1. Subjects

This study was designed on the basis of the ongoing studies at the University of California, San Diego. The University of California, San Diego Institutional Review Board approved this study. Stimulant-dependent subjects had voluntarily entered the 28-day inpatient Alcohol and drug treatment program (ADTP) at the San Diego Veterans Affairs Medical Center. Subjects were all veterans and fulfilled criteria for current substance dependence on Methamphetamine according to DSM IV (diagnostic and statistical manual of mental disorders) criteria. Only those subjects without a lifetime history of independent disorders of Axis I (major affective, psychotic, or anxiety disorders) and Axis II (antisocial personality disorder), as assessed by the structured clinical interview for DSM-IV diagnosis [17], were asked to participate in the study. The subject protocols followed the procedures of Paulus et al. [18,19] and Wittmann et al. [20]. MA dependent individuals who could submit hair samples were selected. It took about 2 years to collect 9 hair specimens to satisfy both MA dependence criteria and minimum hair length and volume criteria because veterans in San Diego typically have very short hair. No other substance use disorder was present. 7 male and 2 female MA dependent individuals aged 31–52 years (mean 40.3; SD 8.4) participated. Each subject gave informed consent before participating in the study and completed a questionnaire and provided hair samples for analysis (which was cut by the investigator). The questionnaire inquired primarily about their patterns of MA use by day, week and month as accurate as possible although information about the use of other drugs as well as alcohol and tobacco was also obtained.

2.2. Reagents and standards

Methanol, ethyl acetate, and hydrochloric acid were analytical grade. MA HCl, AP sulfate, MA-d5, and AP-d5 were purchased from Radian International LLC (Austin, TX, USA), trifluoroacetic anhydride from Sigma–Aldrich (St. Louis, MO, USA), and 2-fritted reservoir (3 ml) from Varian (Habor City, CA, USA). Working standards of MA and AP (1 μg/ml) and of the internal standard AP-d5 and MA-d5 (1 μg/ml) were prepared in methanol and stored at −20 °C in the dark until use.

2.3. Hair collection protocol and hair analysis

Hair specimens from 9 MA dependent individuals, a lock of hair (about 50 mg) each, were collected from the posterior vertex of the head and cut as close to the scalp as possible. Hair was placed in secured paper, aligned from proximal to distal direction and stored in the dark at room temperature until analysis. Hair length was from 6 to 20 cm (mean 9.4; median 8; SD 4.2). The first segmenting pattern was several segments measuring 1 cm each in order to cover individual months (the growth rate of hair is on average 1 cm/month [21,22]). Hair analysis of a total of 66 segments (1 cm in length) was conducted for the purpose of examining the consistency between the self-reported dose of MA use and the concentrations of MA in hair over the corresponding time period in each subject. The second segmenting pattern was one segment measuring 1–4 cm to cover the recent 1–4 months. A proximal 1–4 cm length (3 cm length) segment was analyzed to evaluate the relationship between MA consumptions during 3 months before the last MA use and MA hair concentrations. At the time of collecting hair samples, nine subjects had been abstinent from MA for 14–39 days (mean 24.3; SD 9.6), and the concentrations of MA and AP in 0–1 cm segments as the days of abstinence were thus excluded for statistical analysis. Drug-free hair was obtained from healthy volunteers.

The hair analysis procedure has previously been described [23–25]. Approximately 10 mg of hair was weighed, washed and cut into small pieces of less than 1 mm. The hair was incubated for 20 h in 1 ml methanol containing 1% hydrochloric acid in the presence of 50 ng of each of the following internal standards: MA-d5 and AP-d5. Hair extracts were evaporated under reduced pressure. Thirty microliters of ethylacetate and 30 μl of TFAA were added to the residue, and the mixture was incubated at 65 °C for 30 min. Excess TFAA was removed under a stream of dry nitrogen for 4 min and reconstituted with ethanol (40 μl) prior to GC/MS analysis.

2.4. GC/MS analysis

The procedure has previously been described [23–25]. A 1 μl portion of the derivatized extract was analyzed by GC/MS. The GC/MS system consisted of a Hewlett Packard 7683 series injector, HP 6890N series GC system (Wilmington, DE, USA), and HP 5975 inert XL mass selective detector. The column used (Agilent Technologies, Foster, CA, USA) was a fused silica capillary column (HP-5 MS capillary column, 30.0 m × 250 μm × 0.25 μm). The injector was operated in the splitless mode, the injection volume was 1 μl and the injector temperature was 250 °C. The ionization energy was 70 eV and the transfer line temperature was 280 °C. Initial oven temperature was 100 °C, maintained for 1 min, increasing at 20 °C/min–270 °C and maintained at this temperature for 10 min. The GC/MS was operated in selective ion monitoring (SIM). The quantification of MA and AP was based on peak area ratios. The mz/lz of TFAA-derivatized MA, AP, MA-d5 (internal standard), and AP-d5 (internal standard) was as follows: MA, mz 154, 118, 91; AP, mz 140, 118, 91; MA-d5, mz 158, 122; AP-d5, mz 144, 122 (the underlined ions were used for quantification). For MA, acceptable range for the ratio of mz/lz 154–118 was 3.0 ± 20% and for AP, acceptable range for the ratio of mz/lz 140–118 was 1.0 ± 20%.

2.5. Method validation of hair analysis

For hair analysis, method validation was carried out by establishing linearity, limit of detection (LOD), intra- and inter-assay accuracy and precision, and percentage recovery. Six sets of calibrators with MA at concentrations between 0.25 and 50 ng/mg and with AP at concentrations between 0.25 and 20 ng/mg were prepared, respectively, using 10 mg of blank hair. The LOD was estimated from extracted samples spiked with decreasing concentrations of the compounds, where the response of qualifying ions was equivalent to three times the background noise. Drug-free hair samples (n = 6) were spiked with 4, 8 and 16 ng/mg MA and AP to assess the intra-assay accuracy and precision. For the inter-assay accuracy and precision, drug-free hair samples (n = 3) were spiked with 4, 8, and 16 ng/mg MA and AP, and examined in series on six consecutive days. Percentage recovery was determined by adding 25, 50 and 100 ng of standards to 10 mg of pulverized drug-free hair samples, corresponding to 2.5, 5 and 10 ng/mg hair and peak areas
Table 1: Validation data of MA and AP in hair.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentration targeted (ng/mg)</th>
<th>MAa in hair</th>
<th>APa in hair</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD (ng/mg)b</td>
<td>0.1</td>
<td>0.125</td>
<td></td>
</tr>
<tr>
<td>Accuracy [%]c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-assay (n=6)</td>
<td>4</td>
<td>−1.15</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.97</td>
<td>1.49</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>−5.18</td>
<td>−0.67</td>
</tr>
<tr>
<td>Inter-assay (n=18)</td>
<td>4</td>
<td>−2.25</td>
<td>−4.19</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>−3.21</td>
<td>−0.83</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>0.72</td>
<td>−4.14</td>
</tr>
<tr>
<td>Precision [%]d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-assay (n=6)</td>
<td>4</td>
<td>1.15</td>
<td>5.03</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>3.86</td>
<td>1.30</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>1.35</td>
<td>3.43</td>
</tr>
<tr>
<td>Inter-assay (n=18)</td>
<td>4</td>
<td>0.11</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>0.07</td>
<td>0.11</td>
</tr>
<tr>
<td>Recovery [%]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=3)</td>
<td>2.5</td>
<td>106</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>97</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>95</td>
<td>104</td>
</tr>
</tbody>
</table>

a MA = methamphetamine, AP = amphetamine.
b Limit of detection.
c Calculated as [(mean calculated concentration – nominal concentration)/nominal concentration] × 100 (% bias).
d The coefficient of variance (% CV); SD/mean × 100%.

of MA and AP were compared with those of methanolic standards and derivatization.

2.6. Statistical analyses

Data analysis was performed by analysis of variance (ANOVA) test and linear regression analysis. Analysis of variance (ANOVA) was used for investigating difference between drugs measured in 1 cm length segmental hair across time in each subject. Continuous variables such as ‘MA concentrations’, ‘AP concentrations’ and ‘the AP/MA ratios’ were used and two or three data points per segment were obtained. For subject no. 3, ANOVA was not performed because one value per segment for AP, MA and the AP/MA ratios was obtained. P-values under 5% were accepted as indicating significance (two-tailed).

Linear regression analysis was used for investigating relationship between hair concentration and cumulative doses during recent 1–4 months with MA, AP and the AP/MA ratios as the dependent variables. Pearson’s correlation coefficient r was used as an index of association. Statistical analyses were performed using Minitab 15.1.3 statistical software.

3. Results

3.1. Method validation for MA and AP in hair

Table 1 shows the results of method validation for MA and AP in hair. The calibration curves of MA and AP in hair were linear in the concentration range of 0.25–50 ng/mg and 0.25–20 ng/mg and R² was 0.9951 and 0.9961, respectively.

3.2. Characteristics of MA dependent individuals and the history of MA use

The characteristics of 9 MA dependent subjects and the history of MA use are given in Tables 2 and 3. Seven male and two female MA dependent individuals, aged 31–52 years (mean 40.3; SD 8.4) participated (Table 2). Their education ranged from 10 to 15 years (mean 13; SD 1.6). MA was used mainly by smoking but one subject of both smoked and snorted MA and another snorted MA. The age of first MA use ranged from 17 to 47 years (mean 29.3; SD 8.5) and the period of MA use ranged 0.3–23 years (mean 10.7; SD 10.5). Five among the nine subjects used both cocaine and MA, which is a popular phenomenon in San Diego [26,27].

The reported dose of MA use ranged from 0.25 to 4 g/day (mean 1.2; median 0.9; SD 1.2) (Table 3). Seven Caucasian subjects were daily MA users, one Caucasian subject used MA 3–4 times per week (non-daily use), and one Hispanic subject used MA 5–6 times per week (non-daily use). There were some cigarette smokers who smoked 4 cigarettes a day with MA and one pipe smoker who used his pipe 40 times a day to inhale MA. Subject no. 6 had 30–40 hits of use during one day.

3.3. Consistency between history of MA use and MA concentrations in hair in each subject (1 cm length segments)

Hair specimen was cut into 5–11 segments from the root side, with 1 cm length each subsequently according to hair length and drug histories. The concentrations of MA and AP and the distribution of the metabolite to parent drug ratios (AP/MA) found in hair segments are presented in Figs. S1–S9. The MA and AP concentrations in 66 segmental hair ranged from 0.38 to 53.15 ng/mg (mean 6.10; median 2.12; SD 9.56) and from 0.22 to 3.59 ng/mg (mean 0.95; median 0.75; SD 0.68), respectively. The AP/MA ratios ranged from 0.03 to 0.84 (mean 0.25; median 0.20; SD 0.16). The statistical results of each subject and supplementary figures (Figs. S1–S9) are presented in supplementary data.

3.4. Relationships between MA dose, frequency of use, and MA concentrations in hair (1–4 cm length segment)

Hair specimen was cut into 1–4 cm length segments (3 cm in length) from the root side. We investigated relationships between MA dose, frequency of use, and MA concentrations in hair during 1–4 months. The concentrations of MA and AP in hair segments according to cumulative doses of MA during 1–4 months are shown in Fig. 1. The concentrations of MA and AP in 1–4 cm segmental hair ranged from 0.39 to 35.23 ng/mg (mean 7.49; median 2.63; SD 11.66) and from 0.45 to 2.76 ng/mg (mean 1.16; median 0.81; SD 0.87), respectively. Because the doses of MA use and frequency in subject no. 6 were different during the last four months, total doses were calculated by one-month units each and then summed up. The cumulative doses of MA use calculated from daily dose and the frequency during 1–4 months were well correlated to the concentrations of MA and AP in 1–4 cm length segmental hair (Pearson correlation coefficient, r = 0.87, p < 0.05 for MA and r = 0.78, p < 0.05 for AP).
### Table 2
The characteristics of MA dependent individuals that provided hair samples (n = 9).

<table>
<thead>
<tr>
<th>No. 1</th>
<th>No. 2</th>
<th>No. 3</th>
<th>No. 4</th>
<th>No. 5</th>
<th>No. 6</th>
<th>No. 7</th>
<th>No. 8</th>
<th>No. 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>50</td>
<td>35</td>
<td>44</td>
<td>39</td>
<td>48</td>
<td>31</td>
<td>32</td>
<td>52</td>
</tr>
<tr>
<td>Sex</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Veteran</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Race</td>
<td>White</td>
<td>White</td>
<td>White</td>
<td>White</td>
<td>White</td>
<td>Hispanic</td>
<td>White</td>
<td>White</td>
</tr>
<tr>
<td>Education (years)</td>
<td>10</td>
<td>14</td>
<td>12</td>
<td>12</td>
<td>14</td>
<td>14</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Use</td>
<td>Smoking</td>
<td>Smoking</td>
<td>Snorting</td>
<td>Smoking</td>
<td>Smoking</td>
<td>Snorting</td>
<td>Smoking</td>
<td>Smoking</td>
</tr>
<tr>
<td>Age of first use (years)</td>
<td>27</td>
<td>34</td>
<td>22</td>
<td>17</td>
<td>47</td>
<td>25</td>
<td>47</td>
<td>34</td>
</tr>
<tr>
<td>Period of MA use (years)</td>
<td>23</td>
<td>0.8</td>
<td>21.6</td>
<td>22</td>
<td>0.3</td>
<td>5.3</td>
<td>1.7</td>
<td>20</td>
</tr>
<tr>
<td>Hair color</td>
<td>Blond</td>
<td>Blond</td>
<td>Brown</td>
<td>Dark brown</td>
<td>Brown</td>
<td>Brown</td>
<td>Dyed</td>
<td>BrownBlack</td>
</tr>
<tr>
<td>Hair treatment</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Other drugs</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Cocaine</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* High school diploma = 12 years.

b Perm, dyeing, shading or bleaching.

### 4. Discussion

#### 4.1. Consistency between history of MA use and MA concentrations in hair in each subject (1 cm length segment)

There have been many publications on the analysis and application of MA and homologues in hair. MA has been detected in hair under various extraction methods such as LLE [2,28–30], SPE [31–33] or SPME [34] and derivatization such as TFAA [23–25,35,36], HFBA [31,37] or PFPA [30] and detection methods such as GC/MS [1,2,30–37], HPLC [38,39] and LC-MS/MS [28,36,39,40]. However, the relationship between 1 cm-segmental hair analysis results and drug consumption habits has rarely been reported in the literature [1,34,37].

In this study, hair analysis of a total of 66 segments of 1 cm length was conducted for the purpose of examining the consistency between the reported dose of MA and the concentration of MA and AP in hair over the corresponding time periods in each subject on the basis that scalp hair grows at the rate of about 1 cm/month [21,22]. The MA concentrations in 66 segmental hairs were consistent with the results of other reports [1,16], and within the range of previously published concentrations of our studies [23,35]. The results of the hair analysis clearly indicated repeated exposure to MA. Subject no. 3 had both gray and brown hair. As shown in Fig. 3, Table 3

#### Table 3
The dose and frequency of MA use by day, week and month.

<table>
<thead>
<tr>
<th>The days of abstinence (0–1 cm)</th>
<th>24</th>
<th>21</th>
<th>31</th>
<th>38</th>
<th>15</th>
<th>16</th>
<th>39</th>
<th>14</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Last use-1 month ago (1–2 cm)</td>
<td>1</td>
<td>1–2</td>
<td>0.5</td>
<td>1</td>
<td>3–4</td>
<td>0.5</td>
<td>0.5–0.75</td>
<td>0.25–0.3</td>
<td>0.5–0.75</td>
</tr>
<tr>
<td>1–2 months ago (2–3 cm)</td>
<td>4</td>
<td>4</td>
<td>4–5</td>
<td>3</td>
<td>2</td>
<td>40*</td>
<td>4</td>
<td>6–8</td>
<td>5</td>
</tr>
<tr>
<td>2–3 months ago (3–4 cm)</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7–6</td>
<td>7</td>
<td>3–4</td>
<td>3–4</td>
</tr>
<tr>
<td>3–4 months ago (4–5 cm)</td>
<td>1</td>
<td>1–2</td>
<td>0.5</td>
<td>1</td>
<td>3–4</td>
<td>0.25</td>
<td>0.5–0.75</td>
<td>0.25–0.3</td>
<td>0.5–0.75</td>
</tr>
<tr>
<td>4–5 months ago (5–6 cm)</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>4</td>
<td>5–6</td>
<td>7</td>
<td>3–4</td>
</tr>
<tr>
<td>5–6 months ago (6–7 cm)</td>
<td>1</td>
<td>0.5–1</td>
<td>0.5</td>
<td>1</td>
<td>sober</td>
<td>0.25</td>
<td>0.5–0.75</td>
<td>0.25–0.3</td>
<td>0.5–0.75</td>
</tr>
<tr>
<td>6–7 months ago (7–8 cm)</td>
<td>4</td>
<td>2–3</td>
<td>4–5</td>
<td>3</td>
<td>20*</td>
<td>4</td>
<td>6–8</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>7–8 months ago (8–9 cm)</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>3–4</td>
<td>3–4</td>
</tr>
<tr>
<td>8–9 months ago</td>
<td>1</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
<td>sober</td>
<td>sober</td>
<td>0.25</td>
<td>sober</td>
<td>0.5–0.75</td>
</tr>
</tbody>
</table>

* g/day (dose).

b Times/day (frequency): cigarette smokers, times; pipe smoker, hits*.

c Day/week (frequency).
the concentration of MA and AP in brown hair was higher than that in gray hair as suggested by some studies [41,42]. Several studies have shown that the melanin content in hair plays a role for some weak basic drugs such as codeine, cocaine or AP [43–46].

The highest concentrations of MA and AP were detected in subject no. 5. He used the highest dose of MA among subjects. The concentrations of MA and AP in 4 distal segments (4–5, 5–6, 6–7 and 7–8 cm) of subject no. 5 were extremely high although he reported that he did not use MA during this period of time. The high concentrations in 4 distal hair segments may origin from a later use if MA was not really taken in the corresponding time [47].

Although 7 subjects (nos. 1–5, 6 and 8) had been abstinent from MA for about one month, MA and AP were detected in all 0–1 cm segments. There is little information on the decline and disappearance of MA concentration in hair after the discontinuance of intake. For cocaine, it takes another few months to completely disappear from the hair segments closest to the root [11]. Through hair analysis, cocaine was detected for 2–6 months after a single 25–35 mg dose of cocaine administered intravenously [48]. But, Nakahara et al. reported that during the time when subjects were in the hospital for 1–2 months before hair collection, MA was not detected and their results were definitely different with the results of this study [2].

Subsequent segmental hair analysis for subject nos. 7 and 9 was below the detection limit for AP in all segments although they used MA more than three times a week and MA was detected. This study suggests that both MA and AP could be detected in hair at a dose in the range of approximately 0.5–1 g daily by smoking for regular users in terms of threshold of detection for MA.

Fig. 2. The relationships between hair concentration and cumulative doses calculated from daily dose and frequency (1–4 cm segments). *Cumulative doses (g) during 3 months × dose (g/day) × frequency (times/week) × 4 (4 weeks) × 3 (3 months).

7 had her hair cosmically treated 2 days before collecting hair samples. The drug concentrations in hair have limitations resulting from cosmetic treatment of hair such as permanent waving, coloring or bleaching [4,33,49–51]. Nadulski and Pragst reported a similar subject with a red dyed hair sample and there was a decline in drug concentrations due to repeated aggressive hair cosmetics [52].

After statistical analysis, MA and AP concentrations and the AP/MA ratios in hair segments of subject nos. 1, 4 and 5 and the AP/MA ratios in hair segments of subject no. 8 as well as MA concentrations in hair segments of subject no. 9 were similar because subjects used MA at the same dose and frequency continuously during the designated time period. Because histories of MA use (dose and frequency) in subject nos. 1 and 4 were similar, the concentrations of MA and AP, the AP/MA ratios and statistical analysis results were all similar. MA concentrations in hair segments of subject no. 2 and MA and AP concentrations in hair segments of subject no. 6 were different because subjects used MA with different dosage and frequency during the designated time. In other words, the distribution of MA in the hair was consistent with history of MA use through 1 cm length segmental hair analysis. However, AP concentrations and the AP/MA ratios in segments of subject no. 2, the AP/MA ratios in hair segments of subject no. 6, MA concentrations in hair segments of subject no. 7 and MA and AP concentrations in hair segments of subject no. 8 did not correspond well to history of MA use. We assume that subject no. 8 had memory problems reporting MA use or he used unknown purities of MA. Subject nos. 2 and 6 reported that they used MA several times a day and increased the dose and frequency of MA use. The increase of drug dose and frequency is a typical phenomenon among drug users. In their cases, an interruption or a change of the dose and frequency was not clearly seen in the hair segments. Those results were consistent with the results of Rothe et al. [47]. Some authors showed an increase in drug concentration from proximal-to-distal orientation even if no decrease of the consumption amount or frequency in the time before sampling was reported. This can be explained by a more frequent drug use in the past and diffusion via sweat and sebum [13,53]. However, in this study, a systematically increasing or decreasing drug concentration level was not found.

As explained in our previously published paper [24], the ratio of AP/MA can be considered as a useful tool to discriminate high drug consumption and recent drug intake and to understand MA metabolism. As for the AP/MA ratios, the concentrations of AP were lower than those of MA in all hair segments and the range of AP/MA ratios varied widely. As the concentrations of MA in the hair increased, the levels of AP also generally increased [14]. However, the levels of AP varied widely relative to those of MA. Lee et al. reported the AP/MA ratios ranged from 0.01 to 1.04 (mean = 0.11, median = 0.08) and discussed variation of the levels of AP was likely due to variations in the purity of MA, metabolic variability and hair color [35]. In this study, segmental hair analysis proved to be a useful diagnostic tool in determining the histories of MA use, providing reliable data about MA concentrations in hair although the consistency between history of MA use and MA concentrations in hair were not found in all segments of 9 subjects. The analysis of 1 cm sections of hair play an important role in the evaluation of patterns or habits of MA use, as suggested by Rossi et al., in which hair samples were cut into segments of 2 cm length, to obtain information about the variation in cocaine and heroin abuse over periods of approximately 2 months [7].

Fig. 3. Distribution of the metabolite to parent drug ratios (AP/MA) in 9 subjects (1–4 cm segments).

4.2. Relationships between MA dose, frequency and MA concentrations in hair (1–4 cm length segment)

The correlation between reported drug dose and drug concentration in hair still remains controversial. Ursitti et al. reported that
there was a statistically significant correlation between reported dose used and hair concentrations of cocaine [5]. Similarly, Welp et al. revealed a good agreement between the reported dose of cocaine, heroin and/or methadone used and the concentration of these drugs or their metabolites in hair [54]. Moreover, Rothe et al. reported that despite inter-individual differences an increase of the total concentration of MDA, MDMA and MDE in the proximal segments with increasing frequency of ecstasy use was obtained [47]. Ledgerwood et al. showed an agreement between self-reports and confirmation drug hair testing for marijuana, cocaine, opiates and methamphetamine [15]. Nakahara reported that results of sectional analysis and histories of MA use coincided except for two cases [1]. On the other hand, Tassiopoulos et al. found an increasing opiate level in hair that was associated with a less likelihood of reporting cocaine use [9]. Musshoff et al. reported that for methadone, cocaine, cannabinoids and amphetamines, no correlation between dose and hair concentration of the drug or metabolites was found [13].

In this study, the concentrations of MA and AP in 1–4 cm length segmental hair were well correlated to the cumulative MA dose calculated from daily dose and the frequency during 1–4 months despite the small number of subjects as shown in Fig. 1. However, the correlation coefficient is difficult to interpret in a straight forward manner because of an insufficient sample size. More MA dependent individuals who smoke MA at a dose of about 2 g would be needed to evaluate our correlation coefficient more accurately. And, the highest concentrations of MA and AP in subject no. 5 had a significant impact on the correlation coefficient. The inclusion of the concentrations of MA and AP in hair of subject no. 5 changed the slope significantly and the correlation coefficient indicated a strong relationship. Moreover, MA and AP concentrations in hair of subject nos. 6 and 8 were much higher than other subjects who used more doses of MA. It can be attributed to hair color, hair thickness and unknown purity of MA. The thick brown and black hair of subject nos. 6 and 8 would have stronger MA and AP concentrations in comparison to blond and light brown hair. Some authors elucidated the influence of hair color on drug concentrations in hair [41,42,55,56] and other studies suggested that the color of hair or melanin content may be the major determinant of drug binding [57–59]. Nevertheless, the concentrations of MA in hair increased with increasing dose following the general pharmacological principle of the greater concentration consumed, the greater concentration excreted [44]. Further results could be extrapolated to the present study where the range of concentrations of 0.39–35.23 ng/mg was suggestive of daily MA consumption and MA dose (0.5–4 g/day) for MA smokers, and the concentrations above those levels seemed to be linked with dose above 4 g or intravenous injection route. Caution is given extrapolating the present data to light or occasional users of MA.

Smoking MA is the most popular method throughout the United States [26]. When MA is smoked, the user tends to consume more of it than by other methods. When smoking is compared with intravenous injection, the dose of MA use in this study was 3–60 times higher than that of youn et al.’s paper [60]. They reported an average dose per week in 119 MA users was 0.41 g/week and their mode of intake was intravenous injection (typical in Korean MA users). MA users often claim that smoking is safer than injection because of less loss of control over MA intake and less occurrence of psychosis, although there is no evidence that smoking use is actually a safer form of MA use than injection [61]. However, there are strong indications that MA dependence is related to the route of administration [61,62].

The AP/MA ratios in 1–4 cm length segmental hair were negatively correlated with the concentrations of MA and AP in hair. As the concentrations of MA in hair increased, the AP/MA ratios definitely decreased. AP concentrations increased with the increase in MA concentrations as might be expected, but concentrations of MA were much higher than those of AP. Those results are consistent with our previously published results [23,35].

The present study has limitations. First, this study included a relatively low number of participants. The data needs to be confirmed in a much larger sample. Second, some variability was expected through sample collection and cutting. Cutting as close as possible to the scalp during sample collection and cutting into 1 cm segment could be inaccurate. Moreover, self-reported histories of MA use may be inaccurate because subjects may forget, underreport consumption or may not know the purity of MA. But subjects participated voluntarily in this study and were requested to record history of MA use as accurate as possible and wrote down the dose and frequency in detail by units of one day, one week, and one month without fearing legal consequences. Those histories were thus considered as reliable data.

5. Conclusions

Despite the inter-individual differences and some limitations, the present study provided useful information on relationships between self-reported history of MA use and concentrations of MA in hair. The results can be summarized: for short periods of time drug self-reports and hair testing concentrations agree. Correlation of self-reported MA use and hair testing could be used as a guide in estimating dose of MA use in the field of forensic toxicology as well as for rehabilitation and treatment programs. We investigated what dose of MA or frequency of use is required to produce detectable amounts of MA and AP in hair. A dose in the range of approximately 0.5–1 g daily for SMK smokers was required to produce detectable amounts of both MA and AP in hair.

The small sample size analyzed can at least give a preliminary idea of the trend of MA use among MA dependent individuals. And, segmental hair analysis is a useful tool in determining histories of MA use, providing reliable information about MA concentrations in hair.

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Appendix A. Supplementary data


References


