THE INCORPORATION OF COCAINE AND METABOLITES INTO HAIR: EFFECTS OF DOSE AND HAIR PIKATION

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ABSTRACT:

The relationship between xenobiotic concentrations in hair and the degree of systemic xenobiotic exposure is poorly defined. The purpose of this study was to evaluate the effect of dose, time, and pigmentation on the hair incorporation of cocaine (COC) and its metabolites, benzoylecgonine (BE), ecgonine methyl ester (EME), and norcocaine (NCOC). COC was administered by the i.p. route to male Long-Evans (LE) rats at three doses (5, 10, and 20 mg/kg) once daily for 5 days. Fourteen days after the initial injection, the hair was collected and analyzed by gas chromatography/mass spectrometry for the compounds of interest. COC, EME, and NCOC were preferentially incorporated into pigmented hair in a dose-dependent manner. None of the analytes were detected in nonpigmented hair. The plasma pharmacokinetic profile of each analyte was determined at each dose. After normalizing for the plasma concentrations, the incorporation of COC into pigmented hair was 2 orders of magnitude greater than BE. The time course of COC and metabolite distribution into hair was also investigated from 1 h to 14 days after a single dose. After COC disappears from plasma, there is a 3-day delay before maximal hair concentrations are reached in pigmented hair. In nonpigmented hair, concentrations of BE and COC did not exceed 0.25 ng/mg and were undetectable after 4 h and 2 days, respectively. This study demonstrates that the pigment-mediated differences in the incorporation of COC and its metabolites noted at 14 days after dosing are also evident a few hours after drug administration.

To use the analysis of hair as an adjunct or an alternative to urine or plasma for the detection of illicit drugs, detailed studies are needed to characterize and understand the factors that influence their incorporation into hair. The drug’s lipophilicity, ionization state, and affinity for binding to melanin and the hair pigmentation pattern have all been identified as factors that can influence the incorporation of drugs into hair. Studying a series of amphetamine congeners, Nakahara and Kikura (1994) concluded that substitutions that increased the drug’s lipophilicity also increased hair incorporation; substitutions that decreased the drug’s basicity also decreased hair incorporation. The nature of the hair itself may also determine the extent to which a drug is incorporated. Hair color and chemical treatment can play a role in the incorporation of drugs into hair (Gerstenberg et al., 1995; Green and Wilson, 1996; Gygi et al., 1996, 1997; Potsch and Skopp, 1996; Jurado et al., 1997). Nakahara et al. (1995) demonstrated a strong correlation between a drug’s affinity for melanin binding and its incorporation into hair. Codeine and phenylcyclidine (PCP1) incorporate into pigmented hair in significantly greater concentrations than into nonpigmented hair (Gygi et al., 1996; Slawson et al., 1996), whereas phenobarbital incorporates into pigmented and nonpigmented hair equally (Gygi et al., 1997). Applying permanent wave solution to the hair after drug incorporation has been demonstrated to reduce cocaine (COC) concentrations in human hair (Harkey and Henderson, 1989).

Nakahara and Kikura (1994) examined the incorporation of COC, benzoylecgonine (BE), and ecgonine methyl ester (EME) into the hair of Dark Agouti rats. They determined that COC was the major analyte detected in hair despite higher plasma concentrations of BE; the analyte ratio of COC:BE:EME was 20:2:1. After administering deuterrated metabolites to rats, they also demonstrated that BE present in hair came primarily from the hydrolysis of incorporated COC. Very little incorporated COC was converted to EME in the hair, and the hair EME concentrations were very low. They concluded that the incorporation of COC from plasma into hair was much greater than either BE or EME. Other researchers demonstrated in humans that, within the hair of a single individual, COC concentrations were higher in black hair than in gray hair (Reid et al., 1996).

In this study, three doses of cocaine hydrochloride were administered to a strain of rat (Long-Evans; LE) that has a black hood and white flanks. Both pigmented and nonpigmented hair were collected and analyzed for COC and its metabolites: BE, EME, and norcocaine (NCOC). With this research design, the roles of pigment, dose, and time have been examined for their effects on incorporation of cocaine and its metabolites into hair.

Materials and Methods

Chemicals and Reagents. Cocaine, cocaine-d6, benzoylecgonine, benzoylecgonine-d6, ecgonine methyl ester, ecgonine methyl ester-d6, norcocaine, and norcocaine-d6 were obtained from Radian Corp. (Austin, TX) for prepa-
ration of standard curves and quality control samples. Cocaine hydrochloride (for the dosing of animals), dithiothreitol, and protease VIII (13.5 units/mg; from *Bacillus licheniformis*) were purchased from Sigma Chemical Co. (St. Louis, MO). Sodium acetate was obtained from Fisher Scientific (Pittsburgh, PA), and Varian (Harbor City, CA) was the source for BondElut Certify solid-phase extraction columns. Methanol, isopropanol, and methylene chloride (HPLC-grade) were purchased from Burdick and Jackson (Muskegon, MI). Hexafluoroisopropanol was obtained from Aldrich Chemical Co. (Milwaukee, WI), and pentafluoropropionic anhydride was purchased from Regis Technologies, Inc. (Morton Grove, IL).

**Multiple-Dose Hair Experiments.** To eliminate the potential for external contamination of the hair from bedding or urine, the male LE rats (140–160 g) were housed individually in hanging wire mesh cages. The rats were provided with food and water ad libitum, a constant room temperature, and a 12-h light/dark cycle. Before dosing, a 1-inch square area of pigmented and nonpigmented hair was shaved from the dorsal region using an electric shaver. Cocaine hydrochloride dissolved in normal saline (5 mg/ml as free base) was administered by i.p. injection once per day for 5 days at either 5, 10, or 20 mg/kg (n = 8 per dose). Fourteen days and 28 days after the first dose, the same areas of pigmented and nonpigmented hair were again shaved to collect newly grown hair. All shaved hair was stored at −20°C until analysis by GC/MS.

**Single-Dose Time Course Experiment.** Male LE rats (140–160 g) were housed as previously described. Cocaine hydrochloride dissolved in normal saline (5 mg/ml as free base) was administered in a single i.p. injection (10 mg/kg; n = 4). Approximately 20 mg of hair was forcibly plucked from pigmented and nonpigmented areas at the following times postdose: 1, 2, 4, 6, and 24 h and 2, 3, 4, 6, 8, and 14 days. Samples were stored at −20°C until gas chromatography/mass spectrometry (GC/MS) analysis.

**Plasma Pharmacokinetic Experiments.** Male LE rats (140–160 g) were housed as described earlier, and cocaine in normalized saline (5 mg/ml as free base) was administered as a single i.p. dose at 5, 10, or 20 mg/kg (n = 3 per time point per dose). After chloral hydrate anesthesia (400 mg/kg, i.p.), the animals were exsanguinated via the abdominal aorta at the following time points postdose: 5, 15, 30, 45, 60, 90, 120, 360, and 480 min. Blood was collected in sodium fluoride/potassium oxalate tubes (gray-topped) and centrifuged, and the plasma was stored at −20°C until analysis.

**Isolation of Analytes from Hair.** Hair was solubilized and extracted using a modification of the method published by Harkey et al. (1991). Briefly, 20 mg of hair was aliquoted into 16 × 100-mm tubes, and deuterated internal standards were added. A multi-point standard curve was also prepared over the range of standard curves and quality control samples. Cocaine hydrochloride was administered by i.p. injection once per day for 5 days at either 5, 10, or 20 mg/kg (n = 8 per dose). Fourteen days and 28 days after the first dose, the same areas of pigmented and nonpigmented hair were again shaved to collect newly grown hair. All shaved hair was stored at −20°C until analysis by GC/MS.

**Isolation of Analytes from Plasma.** Plasma (400 µl) was fortified with 200 µl of a 1 ng/µl internal standard solution, and the pH was brought to 6.0 with 400 µl of 0.1 M potassium phosphate buffer. Fortified drug-free plasma was used to prepare standard curves over the range of 5 to 5000 ng/ml. Although the protease digest was not used on plasma, SPE and subsequent GC/MS analysis was performed as for the hair samples. The LLOD and LLOQ for the analytes in plasma were both 5 ng/ml, determined as described above for hair.

**GC/MS Conditions.** Extracts were analyzed by gas chromatography-positive-ion chemical ionization mass spectrometry on a Finnigan MAT 4500 mass spectrometer (methane/ammonia reagent gas). Chromatographic separation was achieved on a J&W Scientific DB-1 fused silica capillary column (30.0-m × 0.32-mm i.d., 1-µm film thickness) with helium as the carrier gas. The temperature program started at 120°C for 1 min and was increased to 300°C at a rate of 25°C/min. The source temperature was held at 130°C, and the electron multiplier operated at −1644 V. The transfer line was maintained at 180°C, and the injector temperature was 200°C. Extracts were injected in the splitless mode.

**Data Analysis.** The time versus plasma concentration AUCO–ω values were estimated using the trapezoidal rule. Incorporation ratios (ICR) were calculated by dividing the mean hair concentration by 5 times the plasma AUC after a single dose. Results are expressed as the mean ± S.E.M. Statistical comparisons of hair concentrations were performed with one-way ANOVA followed by Scheffe F test post hoc testing (Statview SE+ Graphics; Berkeley, CA). Differences were considered significant at P < .05.

**Results**

The concentrations of COC, BE, EME, and NCOC in the pigmented hair collected 14 days after cocaine hydrochloride was administered at doses of 5, 10, and 20 mg/kg/day and are displayed in Fig. 1. Hair samples collected before dosing and 28 days after the initiation of dosing were negative upon analysis (data not shown). COC and its metabolites were not measurable in nonpigmented hair (data not shown). Differences between the means of all analytes for all dose groups were significant (P < .05) when compared to each other, with the exception of the 10 and 20 mg/kg BE pigmented hair concentrations.

The plasma pharmacokinetic profiles of the analytes after a single

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**FIG. 1.** Concentrations of cocaine, ecgonine methyl ester, benzoylecgonine, and norcocaine in pigmented LE rat hair after cocaine administration.

Cocaine hydrochloride was administered i.p. at 5, 10, or 20 mg/kg (n = 8 per dose) for 5 days. Fourteen days after the initiation of dosing, new hair growth was collected from a previously shaved area of the animals back and analyzed for COC, EME, BE, and NCOC. Concentrations are the mean ± S.E.M. *P* < .05 versus respective 10 and 20 mg/kg/day groups; *P* < .05 versus the 20 mg/kg/day group.
i.p. dose of cocaine hydrochloride at 5, 10, and 20 mg/kg are shown in Fig. 2. The peak COC plasma concentrations were measured 5 min after the dose. All the analytes were eliminated from the plasma in less than 24 h.

The ICR is the hair concentration divided by the plasma AUC. The pigmented hair concentrations, plasma AUCs, and ICR are summarized in Table 1. The hair concentrations were the result of multiple doses of COC; therefore, ICRs were calculated using the single dose plasma AUC multiplied by 5. The incorporation of COC was an order of magnitude greater than that of EME and NCOC and exceeded the incorporation of BE by 2 orders of magnitude. The ICRs of each analyte were similar over the range of doses.

The time courses of COC, BE, EME, and NCOC in plucked pigmented and nonpigmented hair after a single 10 mg/kg i.p. dose are shown in Fig. 3. The hair was collected by plucking rather than by shaving (as in the multiple dose hair study). Using this technique, it is possible to measure COC and its metabolites associated with the hair root. If the hair had been shaved instead of plucked, the incorporation of COC and its metabolites would have been severely underestimated at early time points because most of the drug would be contained in hair that had not emerged above the skin. The pigmented hair concentrations of the analytes greatly exceeded the nonpigmented hair concentrations. Only COC and BE were detected in nonpigmented hair and then only at the early time points. In pigmented hair, COC demonstrated a 3-day delay until peak concentrations were reached and BE could not be measured in the hair after 1 day.

**Discussion**

The model used in these experiments is ideally suited for studying pigmentation effects upon the incorporation of xenobiotics into hair. The LE rat is predominately white (nonpigmented) with a black hood and stripe that extends dorsally from the head to the mid-thoracic area. Metabolic and pharmacokinetic differences that must be taken into consideration when comparing drug concentrations in hair from different strains of rat (e.g., nonpigmented Sprague-Dawley hair compared to Dark Agouti pigmented hair) are eliminated by using the LE model. No drug was found in hair collected before dosing or 28 days after dosing. Thus, we conclude that the administered drug at each dose was incorporated into the hair collected 14 days after dosing. The large difference between pigmented and nonpigmented hair concentrations of COC observed at all doses is attributed to the presence or absence of melanin. A quantitatively similar observation has been made for pigmented and nonpigmented hair concentrations of methadone, codeine, PCP, l-α-acetylmethadol, and stanozolol (Green and Wilson, 1996; Gygi et al., 1996; Hold et al., 1996; Slawson et al., 1996; Wilkins et al., 1997).

There are two types of melanin in hair: eumelanin and pheomelanin (Ortonne and Prota, 1993). The biosynthesis of the two melanin types within the melanocytes initiates and proceeds along a common pathway from tyrosine to the formation of dopaquinone. In the presence of cysteine, dopaquinone is eventually converted to benzothiazine derivatives that polymerize to become pheomelanin. Eumelanin arises from dopaquinone that is converted to dihydroxyindole and dihydroxyindole carboxylic acid subunits. These two subunits polymerize to form eumelanin (Furumura et al., 1996). Eumelanin is responsible for dark hair pigmentation (brown and black). Pheomelanin concentrations are greater in light hair (blonde and red) (Ortonne and Prota, 1993). Human hair typically contains combinations of the two melanin types in differing amounts that result in the wide range of hair colors (Jimbow et al., 1994). Slawson et al. (1998) demonstrated that the pigmented hair of LE rats is composed solely of eumelanin and that the amount of eumelanin is consistent from animal to animal. They also demonstrated that PCP incorporated less in blonde hair than black hair. Melanins occur in the hair as polyamic polymers with a large proportion of negatively charged carboxyl groups and α-semiquinones (Felix et al., 1978; Ito, 1986; Prota, 1992). Drugs that are cations at physiological pH appear to bind to these negatively charged sites in the melanin polymer through electrostatic forces. However, nonionic binding through Van der Waal’s forces and other cooperative binding mechanisms may also be involved and complicate the picture of xenobiotic incorporation and retention in hair (Larsson and Tjälve, 1979). Investigating the incorporation of codeine and phenobarbital into pigmented and nonpigmented hair, Gygi et al. (1997) concluded that the incorporation of weak bases was greatly affected by hair pigmentation, whereas the incorporation of weak acids was unaffected. Cocaine exhibits cationic properties under physiological con-
The plasma AUCs did not follow this pattern; in plasma, BE high dose, the plasma AUC of BE (137.82 mg/ml/min) into hair at a given plasma concentration. The disparity between COC followed the same qualitative pattern COC and 20 mg/kg) the concentrations of the analytes in pigmented hair into pigmented hair in a dose-related manner. At all three doses (5, 10, and 20 mg/kg) only time COC and its metabolites were detected in nonpigmented hair. Even if the drug were mobilized from the non-pigmented hair, the COC accumulates in the pigmented early times but it does not diffuse out of these cells as with the metabolites into the growing hair. Several considerations and should therefore display an affinity for melanin (see Fig. 4 for structures). Nakahara et al. (1995) have reported this affinity, and the data presented here confirm that this affinity is important in vivo in determining the disposition of cocaine into hair.

These data demonstrate that COC, EME, and NCOC incorporate into pigmented hair in a dose-related manner. At all three doses (5, 10, and 20 mg/kg) the concentrations of the analytes in pigmented hair followed the same qualitative pattern COC >> EME >> NCOC >> BE. The plasma AUCs did not follow this pattern; in plasma, BE >> COC >> EME >> NCOC. The ICR is the ratio of the hair concentration divided by the plasma concentration of an analyte at a given dose. The ICR is a measure of the ability of an analyte to be incorporated into hair at a given plasma concentration. The disparity between plasma and hair concentrations resulted in ICRs that clearly display a greater incorporation of COC and NCOC into pigmented hair. At the high dose, the plasma AUC of BE (137.82 mg/ml/min) was 3.14 and 18.09 times higher than those of COC and NCOC, respectively. However, the pigmented hair concentration of COC was 56.26 times greater and NCOC was 5.82 times greater than BE. As an explanation of the driving force for hair incorporation, the passive diffusion of xenobiotics into hair in equilibrium with their plasma concentrations is an inadequate and incomplete mechanism. In nonpigmented hair, the concentrations of the analytes were at or below the lower limit of quantitation for our assay (0.1 ng/mg of hair). Thus, an ICR could not be calculated for nonpigmented hair.

The selective incorporation of COC over BE (despite higher BE plasma concentrations) into pigmented hair indicates that factors other than diffusion from the plasma influence the incorporation of drug into hair. A pigmentation-based incorporation preference for COC has been demonstrated. The concentrations of COC in hair with different pigmentation patterns would be identical if diffusion from the plasma was the sole determinant of incorporation. These data confirm the work of Nakahara et al. (1995) that showed a high correlation between melanin affinity and incorporation into hair. Using an in vitro melanin incubation, the authors determined that COC’s affinity for melanin was 17-fold greater than that of BE (Nakahara et al., 1995).

Analyzing pigmented and nonpigmented hair collected 14 days after the initiation of dosing provides no information regarding the time course of incorporation and retention of xenobiotics in hair. It is possible that xenobiotics incorporate into the pigmented and nonpigmented hair follicular cells shortly after dosing and then gradually redistribute in equilibrium with falling plasma concentrations. In the time course of incorporation studies (Fig. 3), BE was detected in pigmented hair from 1 h to 1 day after a single dose of COC; however, the concentrations were very low (≤0.27 ng/mg over the period that it was detectable) and did not reflect the high plasma BE concentration (plasma Cmax at the 10 mg/kg dose was 662.4 ng/ml). It is possible that the BE measured in hair in our study represents the hydrolysis of COC as described by Nakahara and Kikura (1994). The only time COC and its metabolites were detected in nonpigmented hair was at the earliest time points. In nonpigmented hair, BE was only detectable from 1 to 4 h after dose, in low concentrations (≤0.18 ng/mg). Concentrations of COC in nonpigmented hair were also low (≤0.23 ng/mg) and returned to zero by 2 days after dose. These data also demonstrate that COC, and possibly BE, diffuse into the hair-forming cells of nonpigmented hair but they rapidly diffuse out of the cells as the plasma analyte concentration falls.

In the hair-forming cells of pigmented hair, COC is incorporated at early times but it does not diffuse out of these cells as with the nonpigmented hair. Rather, the COC accumulates in the pigmented hair over time. There is a delay in reaching maximal COC concentrations in pigmented hair. After a 10 mg/kg dose, COC pigmented hair concentrations did not reach their peak of 2.7 ng/mg for 3 days. COC is not present in the plasma by 6 h after this same dose. The COC that is detected in the hair between 6 h and 3 days after dosing must come from some site other than plasma. COC is not stored in nonpigmented hair. Even if the drug were mobilized from the non-pigmented hair into the plasma and then into pigmented hair, the amount of COC incorporated into nonpigmented hair is not sufficient to explain the increases in pigmented hair concentrations. It is possible that COC is sequestered into melanocytes (located among the follicular matrix cells and responsible for incorporating melanin into the forming hair shaft) and the delay in COC appearance is due to the time necessary to package and incorporate the melanin-bound COC into newly formed hair.

In conjunction with plasma diffusion, the possibility must also be considered that sebum, sweat, and skin cells in close proximity to the growing hair shaft can deposit drugs into the growing hair. Several studies have demonstrated the excretion of various drugs in the sweat and sebum of humans (Henderson and Wilson, 1973; Peck et al.,

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**TABLE 1**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Cocaine</th>
<th>Egonine Methyl Ester</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[Hair]</td>
<td>Plasma AUC</td>
</tr>
<tr>
<td></td>
<td>(ng/mg)</td>
<td>µg/ml/min</td>
</tr>
<tr>
<td>5</td>
<td>8.76 ± 0.73</td>
<td>14.05</td>
</tr>
<tr>
<td>10</td>
<td>14.46 ± 0.65</td>
<td>32.60</td>
</tr>
<tr>
<td>20</td>
<td>21.94 ± 1.03</td>
<td>43.94</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Benzylecgonine</th>
<th>Norcocaine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[Hair]</td>
<td>Plasma AUC</td>
</tr>
<tr>
<td></td>
<td>(ng/mg)</td>
<td>µg/ml/min</td>
</tr>
<tr>
<td>5</td>
<td>0.22 ± 0.02</td>
<td>29.48</td>
</tr>
<tr>
<td>10</td>
<td>0.32 ± 0.02</td>
<td>67.09</td>
</tr>
<tr>
<td>20</td>
<td>0.39 ± 0.03</td>
<td>137.82</td>
</tr>
</tbody>
</table>

* C Cocaine HCl was administered to rats once daily for 5 days by the i.p. route.
* b Hair was collected 14 days after initiating dosing protocol (n = 8/dose).
* c Value represents 5 times the plasma AUC0–8 h after single i.p. injection (n = 3/timepoint).
* d Incorporation ratio (ICR, unitless) is the hair concentration/plasma AUC0–8 multiplied by 5.
A single cocaine hydrochloride injection was administered i.p. (10 mg/kg; n = 4). Hair was forcibly plucked from both pigmented and nonpigmented dorsal regions and analyzed for COC, EME, BE, and NCOC. Concentrations are the mean ± S.E.M. A, pigmented hair; B, nonpigmented hair.

**Fig. 3.** Time course of concentrations of cocaine and its metabolites in plucked LE rat hair after a single dose.

A single cocaine hydrochloride injection was administered i.p. (10 mg/kg; n = 4). Hair was forcibly plucked from both pigmented and nonpigmented dorsal regions and analyzed for COC, EME, BE, and NCOC. Concentrations are the mean ± S.E.M. A, pigmented hair; B, nonpigmented hair.
Pigment and dose on cocaine hair incorporation

1987; Shah, 1987; Suzuki and Inoue, 1989; Faergemann et al., 1993; Burns, 1995). However, rats only have sweat glands on the soles of their feet, and the hair was plucked from an area inaccessible to the rat. Thus, the additional incorporation of COC probably did not come from sweat. The delay in the appearance of maximum COC concentrations may be explained by the deposition of drug in sebaceous glands, which gradually coat the hair with sebum until all the drug is deposited on the hair. However, if the drug was deposited from sebum, a matching increase would be seen in nonpigmented hair.

In this study, we demonstrated that COC is preferentially incorporated into pigmented hair and that COC hair concentrations are greater than BE despite higher plasma concentrations of BE. Our research also showed that COC hair concentrations increase during the first 3 days after a single dose. Further research is necessary to explain this COC incorporation delay phenomenon and may include immunohistochemical studies of skin slices from COC-dosed rats in an attempt to locate deposition sites in the dermis.

References


Fig. 4. The structures of cocaine and its metabolites.