The Disposition of Cocaine and Opiate Analytes in Hair and Fingernails of Humans Following Cocaine and Codeine Administration

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Abstract

This study investigated the disposition patterns of cocaine and opiates into hair and fingernail specimens collected from 8 volunteers enrolled in a 10-week inpatient clinical study. All subjects were African-American males with a confirmed drug use history. Scalp hair and fingernail scrapings were collected weekly throughout the course of the study. Head hair was collected from the posterior vertex region, and fingernail scrapings were collected along the entire ventral surface of the nail plate. The specimens were introduced to successive decontamination washes including an isopropanol wash and three phosphate buffer washes. All decontamination washes were collected and analyzed. All specimens were enzymatically digested prior to being subjected to solid-phase extraction and derivatization. Analyses were performed using electron impact gas chromatography–mass spectrometry. Analytes investigated included eight cocaine analytes and five codeine analytes. The limit of quantitation for all analytes ranged from 0.1 to 0.5 ng/mg for both matrices. Cocaine was present at the highest concentrations of any analyte in both hair and nail. Benzoylecgonine and ecgonine methyl ester were the primary metabolites in both matrices and were typically less than 15% of cocaine concentrations. Codeine was the only opiate analyte identified in either hair or nail. The mean peak concentrations in hair after low dosing were half the concentration observed after high-dose administration. Generally, no clear relationship was evident between nail drug concentrations and dose. Decontamination washes removed less than 20% of the total drug present in hair, but removed most of the drug concentrations (60–100%) in nail. This investigation demonstrated that higher concentrations of drug were found in the subjects' hair than in their fingernails and that cocaine was found in both matrices at a greater concentration than codeine. Although both hair and nail have similar physical and chemical properties and may share common mechanisms of drug incorporation, this clinical study suggests that there are distinct differences in their disposition profiles.

Introduction

The use of keratinized matrices such as hair and nail to detect drugs has emerged as a complementary sensitive and specific technique to conventional blood and urinalysis. The use of hair specimens to identify long-term drug exposure has made it very useful in drug-testing applications. Despite controversies such as environmental contamination and color bias, hair analysis is being introduced into more laboratories worldwide. Hair analysis has transgressed the boundaries of being solely a research tool and is now analyzed in some commercial laboratories as a means to determine drug exposure. However, there remains a critical need for research to investigate the disposition of drugs in hair if its strengths and limitations are to become fully recognized. Furthermore, investigation of less-studied alternative matrices like nail is also warranted because this matrix can potentially offer advantages similar to hair.

Continued research may improve our understanding of the proposed processes of incorporation of drug analyte into keratinized matrices, the degree of each process's contribution, and the factors (e.g., chemical environment and physical and chemical properties of the drug) that promote or lessen one
process over the other. The internal and external incorporation routes of drug analyte into keratinized matrices include transport of drug analyte directly or indirectly from the blood supply to the hair follicle and nail bed; diffusion of drug into bodily secretions that can bathe the hair follicle and nail perionychium; exposure of hair fibers and nail surface to outward transdermal diffusion of drug from the skin; and exposure of keratinized matrices to the external environment, including drug residues, contaminated surfaces, or vaporized drug.

Human studies investigating drug disposition in hair have focused on various analytes including cocaine (COC) (1–4), digoxin (3), opioids (1), cannabinoids (3), haloperidol (5), and methamphetamine (6). Additional analytes, including amphetamines (6,7), phencyclidine (3), phenobarbital (8), methamphetamine (6). Additional analytes, including amiloride (9), and methaqualone (3), have been successfully detected in animal hair.

Dose-concentration relationships in hair reported for therapeutic drugs and drugs of abuse ranged from no apparent correlation to significant correlation. Therapeutic drugs deposited into human hair at a significantly correlated rate included haloperidol (5), morphine and codeine (1,10), and digoxin (3). Likewise, investigators have reported positive dose-concentration relationships in hair for abused drug analytes such as COC (4), benzoylcegonine (BE), and phencyclidine (3).

Conversely, other investigators reported no correlation between administered doses of drug and concentrations in hair. Puschemel et al. (11) used a combination of study designs to conclude that no correlation existed between administered doses of opiates and their concentrations found in hair. Kintz et al. (12) investigated the dose-concentration relationships of opioids in hair of subjects and reached similar conclusions.

Likewise, drugs of abuse and their metabolites including COC (13–15), amphetamines (16–18), and opiates (14) have also been successfully detected in nails. Although investigators have identified drugs of abuse in fingernails and toenails, dose–response studies in nails have been limited to the investigation of therapeutic drugs.

Uematsu et al. (5) reported that a significant correlation existed between haloperidol concentrations in nails of human subjects and daily administered dose. In addition, several studies have found a positive correlation for dose–response relationships of the antifungal drugs, terbinafine and itraconazole, in human nails (19–21).

Presently, there is no agreement on whether the time of drug use can be reliably inferred from analysis of keratinized matrices, or if the drug concentrations detected in these matrices accurately reflect the extent of drug use. Discrepancies among dose–response relationship studies suggest that drug disposition into hair varies from drug to drug and from individual to individual. Some drugs display a dose–response relationship, and others may not. Moreover, nonstandardized analytical methodology and study design may affect intralaboratory results of dose–response studies. Therefore, continued research must focus on these uncertainties when concluding if the extent of drug use can be estimated using hair and nail.

To our knowledge, there have been no reports of analysis of hair and nails from the same subject administered drugs of abuse in an inpatient study. This study used a controlled clinical environment to investigate the similarities and differences in the disposition of cocaine and opiate analytes into two keratinized matrices, hair and nail. It also compared paired results of hair and nail to determine the potential utility of nails because much less is known about drug incorporation into this matrix. Lastly, the study investigated decontamination wash procedures and quantitated the amount of drug removed during each wash step.

Materials and Methods

Standards, extraction materials, and reagents

All organic solvents were high-performance liquid chromatography grade, and chemicals were reagent grade. Anaerobic nutrient medium (Becton Dickinson-3978, Sparks, MD) and sodium chloride (J.T. Baker, Phillipsburg, NJ) were used to prepare anaerobic culture media. Bovine serum albumin (Fisher Scientific, Pittsburgh, PA) was used as a denaturing agent. The dosing pattern was designed to provide excretion data on the disposition of cocaine and opiate analytes into two keratinized matrices, hair and nail. All subjects had a history of drug use, which was confirmed by drug screening evaluations performed to verify each subject's health prior to admittance to the study. Medical and psychological evaluations were performed to verify each subject's health prior to study participation. All subjects provided informed consent and were compensated for their participation in the study.

The dosing pattern was designed to provide excretion data on
two distinctly different classes of drugs and involved administration of COC and COD on alternating days for six days. The study time line for dosing and specimen collection is illustrated in Figure 1. This design was chosen to simulate a short, multiple-drug use session such as might be typical of regular users who are “on a run”. The design also was chosen because it provided maximum safety to the subjects. Both hair and nail specimens were collected the first day of the study (day 0), and weekly collection continued for the remainder of the study on each Monday. Drugs were not administered during the first three weeks to allow for previously administered drug to be reduced or eliminated from the hair and nails (i.e., washout period). Beginning in week 4, subjects were administered a low dose of cocaine hydrochloride (75 mg/70 kg) by subcutaneous injections on Monday, Wednesday, and Friday. During the same period, codeine sulfate (120 mg/70 kg) was administered orally on Tuesday, Thursday, and the following Monday. Following low dosing, subjects were administered placebo doses subcutaneously and orally during weeks 6 and 7, observing the same daily schedule. Beginning in week 8, subjects were administered a high dose of cocaine hydrochloride (150 mg/70 kg) and codeine sulfate (120 mg/70 kg) following the same alternating schedule. Specimen collection was continued for two weeks after high dosing for follow-up of drug elimination.

Collection of specimens

Head hair was collected by the staff of the IRP, NIDA. Grooming clippers were employed to remove the first collection of scalp hair from subjects, collecting different regions of the scalp (temporal, frontal, nape, posterior vertex, and anterior vertex) separately. For the study reported herein, only hair from the posterior vertex region was analyzed. The remaining stubble was removed using a straight edge razor after collection. Hair from the initial collection was stored orally on Tuesday, Thursday, and the following Monday. Following low dosing, subjects were administered placebo doses subcutaneously and orally during weeks 6 and 7, observing the same daily schedule. Beginning in week 8, subjects were administered a high dose of cocaine hydrochloride (150 mg/70 kg) and codeine sulfate (120 mg/70 kg) following the same alternating schedule. Specimen collection was continued for two weeks after high dosing for follow-up of drug elimination.

Decontamination washes

A multistep procedure was employed to decontaminate the hair and nail surfaces. Briefly, a 15-min isopropanol wash (3.0 mL) was followed by three successive 30-min washes in 3.0 mL of a 100mM phosphate buffer (pH 6.0). All steps were performed at room temperature and specimens were agitated by stirring or placing in an oscillating water bath. Wash fractions were collected for further analysis. All four wash fractions for initial specimen collections (day 0) were analyzed separately, whereas the phosphate buffer wash fractions for the following weeks were combined prior to analysis.

Enzymatic digestion

Specimens were placed in 1.0 mL of digest solution containing 0.05 mg/mL Protease XI and 60 mg DTT dissolved in 50mM Tris buffer (pH 7.4). The final pH of the digest solution was approximately 7.2. Specimens were fortified with tritiated internal standards [BE-d3, COC-d3, CE-d3, EME-d3, 6-AM-d3, COD-d3, and MOR-d3] at a concentration of 50 ng/mg for minor analytes (CE-d3 and 6-AM-d3) or 100 ng/mg for all other deuterated analytes. Micro stir-bars (2-3 mm) were added, and the fritted filters were capped and placed in a 40°C water bath overnight (~16 h). The digestates were eluted from the fritted filters under vacuum force. The filters were rinsed twice (2.0 mL) with 100mM phosphate buffer (pH 6.0); rinsings were collected into the same tube as their respective digestate. The combined filtered digestates and rinses were centrifuged at 4000 rpm for 10 min prior to SPE.

SPE

The SPE cartridges were conditioned sequentially with elution solvent (1.0 mL), methanol (3.0 mL), deionized water (3.0 mL), and 100mM phosphate buffer (2.0 mL). Centrifuged digestates were loaded onto the cartridges, and the cartridges were washed sequentially with deionized water (2.0 mL), 10mM acetic acid buffer (2.0 mL), methanol (3.0 mL), and acetonitrile (1.0 mL) before aspirating to dryness under full-vacuum conditions. Analytes were recovered from the columns with elution solvent (4.0 mL), and the eluents were evaporated to dryness at 40°C under a stream of nitrogen. The residues were reconstituted in 20 µL of acetonitrile, vortex mixed, and transferred to
autosampler vials. BSTFA with 1% TMCS (20 μL) was added to each of the vials. Autosampler vials were capped, vortex mixed, and heated at 65°C for 30 min.

**Gas chromatographic–mass spectrometric (GC–MS) analysis**

Analyses were performed with a Hewlett-Packard 6890 series II GC and automatic liquid sampler interfaced with a Hewlett-Packard 5973 mass selective detector (MSD, Hewlett-Packard Co., Little Falls, DE). The GC was equipped with an HP-1 cross-linked 1% diphenyl, 99% dimethylpolysiloxane fused-silica capillary column (12 m × 0.2-mm i.d., 0.25-μm film thickness, Hewlett-Packard Co.). Automated injections (1 μL) were made in the splitless mode, and a 4-mm i.d. silanized borosilicate liner with a glass wool plug was used.

The injection port and transfer line temperatures were maintained at 250°C and 290°C, respectively. The oven temperature program was set at an initial temperature of 90°C (hold time, 0.5 min), and the temperature was ramped at 22.5°C/min to 225°C, and then at 17.5°C/min to a final temperature of 320°C (hold time, 4.0 min). The helium carrier gas was set at a constant flow of 1 mL/min, and the septum purge flow was set at 2.0 mL/min.

The MSD was operated in the selected ion monitoring mode. The following ions were monitored at a dwell time of 20 ms (quantitation ions are underlined): 152, 166, and 181 (AEME); 85, 99, and 274 (EME-d3); 82, 96, and 271 (EME); 83, 96, and 240 (EEE); 185, 275, and 306 (COC-d3); 182, 272, and 303 (COC); 199, 275, and 320 (CE-d3); 196, 272, and 317 (CE); 243, 259, and 364 (BE-d3); 240, 256, and 361 (BE); 140, 240, and 346 (NCOC); 140, 298, and 404 (NBE); 290, 343, and 402 (6-AM-d3); 287, 340, and 399 (6-AM); 181, 237, and 374 (COD-d3); 178, 234, 196 and 371 (COD); 239, 417, and 432 (MOR-d3); 236, 414, and 429 (MOR); 254, 292, and 429 (NCOD); and 254, 308, and 487 (NMOR). Quantitative analyses were performed by methods described previously by Garside et al. (13).

Calibration curves were constructed with a minimum of six calibrators prepared at a concentration range of 0.1–10.0 ng/mg using either 25 mg of prewashed drug-free hair or 15 mg of prewashed drug-free nails. The calibrators were fortified with standard solutions of AEME, EME, EEE, COC, CE, BE, NCOC, NBE, 6-AM, COD, MOR, NCOD, NMOR, and their respective trideuterated analogues. COC and 6-AM analytical controls (5 or 10 ng/mg) were included in all batches in order to monitor the hydrolysis during sample preparation and GC–MS analysis. Negative and positive controls (0.5 to 10 ng/mg), prepared in drug-free matrix, were also included in each run.

**Pharmacokinetic measurements and statistics**

Maximum concentration values (Cmax) were determined by visual inspection of a time versus concentration graph (Figures 2 and 3 and Tables I and II). In most cases after high-dose administration, the last collected specimen was treated as an “apparent” maximum concentration because completion of the study prevented confirmation of when drug concentrations actually began to decrease.

Cocaine and codeine concentrations in unwashed hair were analyzed by nonlinear regression analysis using WinNonlin™ software (Scientific Consulting Inc., Apex, NC). Model-independent methods were used to obtain partial area-under-the-curve (AUC) values in hair for cocaine and codeine. Partial AUC measures were only performed for low dosing intervals. In instances where the drug concentration did not return to baseline following low-dose administration and prior to high-dose administration (cocaine for subjects A, C, G, K, M, and N), the AUC was estimated by extrapolation of the line to baseline. Partial AUC high-dose measures could not be accurately determined because drug concentrations generally demonstrated an ascending phase of terminal drug elimination during the two-week collection period prior to completion of the study. AUC_{0-10} was determined by the following formula:

\[
\text{AUC}_{0-\text{t}} = \text{AUC}_{0-4} + \frac{C_t}{\lambda_z}
\]

Estimates for the elimination constant (\(\lambda_z\)) were obtained by linear regression analysis of drug concentrations in hair and nail following low and high doses of cocaine and codeine; (2) Cmax measurements in washed and unwashed hair analysis following low and high doses of cocaine and codeine; and (3) total drug detected (TDD) Cmax (TDD-Cmax) measurements in hair and nails following low and high doses of cocaine and codeine. The appropriateness of this test was based on the study’s small sample size (N = 8). The test evaluated the null hypothesis of no significant difference between variables with a chosen α level of 0.05.

**Results**

Hair and nail specimens were prepared for GC–MS analysis by subjecting hair and nail scrapings to a decontamination wash, overnight enzymatic digest, SPE, and derivatization. The limit of quantitation (LOQ) for analytes in hair and nail scrapings was defined as a signal-to-noise ratio ≥ 3 for the quantitative ion and acceptable ion ratios (+ 20%). In hair, the LOQs for cocaine analytes ranged from 0.1 to 0.3 ng/mg with CE, EME, and EEE having the highest LOQs. The LOQs of codeine analytes in hair ranged from 0.1 to 0.2 ng/mg. The LOQs for the analysis of nail scrapings ranged from 0.13 to 1.0 ng/mg with AEME and NBE having the highest LOQs. The calibration curves were linear over a concentration range of 0.1 to 10 ng/mg and the correlation coefficients of all standard curves were ≥ 0.990.

Hydrolysis controls were included in all batches. Observed hydrolysis was less than 5% for COC and less than 10% for 6-AM. Negative controls of drug-free hair and nail were monitored for contribution of matrix interference. COD was the only analyte demonstrating consistent interference for both qualitative ions (178 and 234). Consequently, ion ratios for another ion (196) were monitored to verify all COD signal responses. Positive controls ranging from 0.5 to 10 ng/mg were analyzed at the beginning, middle, and end of each run. Quan-
titative results for each analyte were only reported if positive controls were within 20% of the expected value.

Analysis of washed and unwashed specimens

By the end of the washout period (week 3), drug was eliminated from both hair and nail scrapings in a majority of the subject specimens; Subject K had cocaine concentrations (washed and unwashed) and Subject G had codeine concentrations (unwashed) near the LOQ. COC was the primary COC analyte in washed and unwashed hair and was detected in the first hair specimen collected within three days of completion of drug administration (week 5). Peak COC concentrations generally occurred in hair collected 2-3 weeks after dosing, and concentrations began to decrease in hair collected approximately four weeks after dosing. COD was the primary opiate analyte in washed and unwashed hair, and the timing profile followed a pattern similar to that of cocaine.

Other analytes present in hair included AEME, EME, CE, NCOC, BE, MOR, and 6-AM. These analytes were normally present in initially collected specimens (AEME, CE, MOR, and 6-AM) and/or the first collection directly following drug administration (EME, NCOC, and BE). The combined concentration of all other analytes present in a hair specimen was as much as 30% of the primary analyte concentration (i.e., COC or COD) but was generally less than 10% of the primary analyte concentration.

Dose-response relationships were determined by the evaluation of partial AUC and Cmax measurements for parent analytes. Partial AUC measurements for unwashed hair following the low-dose week ranged from 33.4 to 290 ng-day/mg for cocaine and 20.8 to 105 ng-day/mg for codeine. The means plus or minus standard deviations (SD) for unwashed hair AUC measurements were 92.6 ± 53.8 ng-day/mg for COC and 50.9 ± 27.8 ng-day/mg for COD. In one case (Subject G), an extra time point was required to determine the terminal elimination phase because the last day prior to high dosing was at peak drug concentrations following low-dose administration. Again, AUC measurements were not determined for the high-dose administration because of the limited collection period.

COC Cmax values (Figure 2 and Table I) for washed hair ranged from 1.7 to 15 ng/mg after low-dose administration and from 4.0 to 27 ng/mg after high-dose administration. Although the investigated subjects were from the same ethnic background and gender, there was considerable intrasubject

Figure 2. Concentration of drug analytes in washed and unwashed portions of hair from subjects enrolled in a 10-week in-patient clinical study. Subjects were given low and high doses of cocaine and codeine, and hair was collected weekly during study time. A, cocaine concentrations for Subjects A, C, F, and G. B, cocaine concentrations for Subjects H, K, M, and N. C, codeine concentrations for Subjects A, C, F, and G. D, codeine concentrations for Subjects H, K, M, and N.
variability with highest concentrations being more than 5 times greater than the lowest drug concentrations. Cmax values for unwashed hair were 5 to 50% higher. In one instance (Subject M), the cocaine Cmax value (27 ng/mg) for washed hair was higher than the unwashed specimen (24 ng/mg) following high-dose administration. Given the high concentration, the small difference could be explained by the nonhomogeneity of the sample matrix or analytical error. Peak codeine concentrations were 5 to 50% higher. In one instance (Subject M), the cocaine Cmax value (27 ng/mg) for washed hair was greater than the lowest drug concentrations. Cmax values were 8 to 55% higher. Figures 2A–2D illustrate the higher maximum drug concentration in unwashed hair analysis based on low-dose administration of cocaine (p = 0.0078) and time-concentration relationship of cocaine and codeine in washed versus unwashed hair specimens.

Wilcoxon sign rank sum test demonstrated a significantly higher maximum drug concentration in unwashed hair analysis based on low-dose administration of cocaine (p = 0.0078) and low- and high-dose administration of codeine (p = 0.0078 and p = 0.0156, respectively). However, the test showed no significant difference in Cmax between unwashed and washed specimens after high-dose administration of cocaine (p = 0.1484). The nonsignificant difference between these two analyses can be explained with subject M data discussed previously. Disregarding subject M data, the observed significance level decreased (p = 0.0156) and demonstrated a significant difference for unwashed and washed specimen analyses.

Generally, COC was only detected in nail specimens during the washout period prior to clinical drug administration (0 to 1.6 ng/mg). However, most of the cocaine was present in the decontamination washes. Data were not evaluated for unwashed nails because of the lack of specimen. Codeine was not present in nail scrapings and could only be detected in the decontamination washes. No clear relationship was evident between nail drug concentrations and dose. Presence of analytes other than the primary analytes in nail scrapings was rare. Other analytes present in nail scrapings included EME, CE, NCOC, and BE. These analytes were present in initially collected specimens (day 0) and/or the second collection directly following high-dose drug administration (EME and BE only). The combined concentration of all other analytes present in a nail specimen

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**Figure 3.** Total drug detected (combined washed + wash fractions) in hair versus fingernail scrapings after low- and high-dose administration of cocaine and codeine over a 10-week period. A, cocaine concentrations for Subjects A, C, F, and G. B, cocaine concentrations for Subjects H, K, M, and N. C, codeine concentrations for Subjects A, C, F, and G. D, codeine concentrations for Subjects H, K, M, and N.
Table I. Maximum Time and Concentration Measurements for Cocaine and Codeine Following Low- and High-Dose Administration in Washed Hair

<table>
<thead>
<tr>
<th>Subject</th>
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<th></th>
<th>Codeine</th>
<th></th>
<th></th>
</tr>
</thead>
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<td>Tmax</td>
<td>Cmax†</td>
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</tr>
<tr>
<td></td>
<td>(ng/mg)</td>
<td>(wks. after dose)</td>
<td>(ng/mg)</td>
<td>(wks. after dose)</td>
<td>(ng/mg)</td>
</tr>
<tr>
<td>A</td>
<td>6.6</td>
<td>2</td>
<td>9.9</td>
<td>3</td>
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</tr>
<tr>
<td>C</td>
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</tr>
<tr>
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* Abbreviations: Cmax, maximum concentration; Tmax, maximum time.
† These values are apparent Cmax values.

Table II. Total Drug Detected—Maximum Time and Concentration Measurements for Cocaine/Codeine Following Low/High-Dose Administration in Hair and Nail

<table>
<thead>
<tr>
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<th>Codeine</th>
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<td>TDD-Tmax</td>
<td>TDD-Cmax†</td>
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<td>(wks. after dose)</td>
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<tr>
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<tr>
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<tr>
<td>Mean ± SD</td>
<td>6.1 ± 4.0</td>
<td>12 ± 7.3</td>
<td>2.9 ± 1.6</td>
<td>5.1 ± 2.7</td>
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| Nails   |         |         |         |         |         |         |         |         |
| A       | 0.82    | 1       | 1.6     | 1       | 0.12    | 1       | 0.20    | 2       |
| C       | 0.25    | 1       | 0.73    | 2       | 0.12    | 1       | 0.19    | 2       |
| F       | 1.6     | 1       | 1.5     | 1       | n.c.o.  | n.t.o.  | 0.12    | 2       |
| G       | 0.64    | 1       | NS      | NS      | n.c.o.  | n.t.o.  | NS      | NS      |
| H       | 0.70    | 3       | 0.68    | 3       | 0.17    | 3       | 0.20    | 1       |
| K       | 0.75    | 3       | 0.62    | 1       | 0.31    | 3       | 0.19    | 1       |
| M       | 0.54    | 1       | 0.57    | 1       | n.c.o.  | n.t.o.  | n.c.o.  | n.t.o.  |
| N       | 1.5     | 1       | 2.7     | 1       | n.c.o.  | n.t.o.  | 0.12    | 2       |
| Mean ± SD | 0.85 ± 0.47 | 1.2 ± 0.79 | 0.18 ± 0.09 | 0.17 ± 0.04 |

* Abbreviations: TDD, total drug detected; Cmax, maximum concentration; Tmax, maximum time; n.c.o., no Cmax observed; n.t.o., no Tmax observed; NS, no specimen available for analysis; SD, standard deviation.
† These values are apparent Cmax values.
‡ Wash fractions lost during specimen preparation. This value is for washed specimen measurement only.
ranged from more than the primary analyte concentration (i.e., COC or COD) to approximately 40% of the primary analyte concentration.

The observed drug disposition profiles were different for these two keratinized matrices. Higher drug concentrations were observed in the washed hair specimen than the decontamination wash fractions, whereas nail specimens had lower drug concentrations than their decontamination washes. Less than 20% of drug in hair specimens was removed by solvent decontamination washes, and most of the drug concentration (≥ 60%) was removed by washes of the nail specimens.

Analysis of decontamination washes

All four decontamination wash fractions for the initially collected hair and nail specimens were analyzed separately. Generally, the first phosphate buffer wash fraction had the greatest concentration of drug analyte. The second phosphate buffer wash fraction or the isopropanol wash fraction had the second highest concentration, and the last phosphate buffer wash fraction had the lowest concentration of drug analytes. In most instances, COC and COD were the only analytes detected in the wash fractions. Occasionally, BE and 6-AM were detected in initially collected (washout) specimens at concentrations near the LOQ.

The percentage of drug removed by washing was calculated for hair using the following formula:

\[
\text{Drug removed by washing (\%) = \frac{[\text{combined wash fractions (ng/mg)/[unwashed specimen (ng/mg)] - [washed specimen (ng/mg)]}}{100}
\]

Generally, the percentage of drug removed by washing the hair was 30–50% of the total drug detected (combined drug concentration of wash fractions and washed specimen). In a few specimens, the percentage of drug removed by washing was greater than the drug concentration found in the hair itself. This observation usually occurred directly following drug administration. The combined wash fraction concentration was normally 5–30% of the washed hair specimen concentration.

The previously mentioned calculation could not be performed for nail specimens because unwashed specimens were not analyzed since the quantity of specimen was limited. However, drug concentrations in the wash fractions were greater than concentrations observed in the nail scrapings themselves. In many specimens, COC and opiate analytes were not detected in the nail scrapings, but were detected in the wash fractions. When cocaine and opiate analytes were detected in both nail scrapings and in wash fractions, the nail scraping concentrations were 5–40% of the combined concentration of the wash fractions.

Comparison of TDD (decontamination washes plus extract) from hair and nails

Drug concentrations in hair and nails collected from the same subject at the same time point were compared. Results for paired analysis were reported as “total drug detected” during analysis to allow an overall evaluation of the drug disposition into keratinized matrices. This calculation was necessary because disposition into these matrices appeared to behave differently using this methodology. The calculation for determining the total drug detected was as follows: total drug detected from specimen = washed specimen result (ng/mg) + combined wash fractions results (ng/mg).

Figures 3A–3D illustrate the time-concentration graphs of the TDD by hair and nail analysis following low and high dose administration of cocaine and codeine. To compare hair and fingernail analysis, maximum concentrations of TDD following high- and low-dose administration of cocaine and codeine were obtained and statistically evaluated. Table II summarizes maximum time and concentration results (TDD-Cmax and TDD-Tmax) for hair and nail analysis. The mean ± SD calculation for the TDD-Cmax of COC present in hair and nail specimens after low-dose administration were 6.1 ± 4.0 ng/mg and 0.85 ± 0.47 ng/mg, respectively. After high-dose administration, the mean TDD-Cmax values for cocaine present in hair and nail specimens were 12 ± 7.3 ng/mg and 1.2 ± 0.79 ng/mg, respectively. Likewise, the mean ± SD for the TDD-Cmax values of codeine were 2.9 ± 1.6 ng/mg (hair) and 0.18 ± 0.09 ng/mg (nail) following a low-dose regimen and 5.1 ± 2.7 ng/mg (hair) and 0.17 ± 0.04 ng/mg (nail) after a high-dose regimen. At low- and high-dose concentrations, the TDD-Cmax values for hair analysis were generally 5- to 30-times higher than those observed for nail analysis. Figure 4 illustrates the mean Cmax measurements for TDD from hair and nails after low- and high-dose administration.

The Wilcoxon Sign Rank sum test was used to compare the difference of the mean maximum concentration of TDD from hair and nail after high- and low-dose administration. This test determined that hair analysis showed a significantly higher amount of TDD than nail analysis. This analysis showed significance for both drug analytes at both dosing intervals. The p-values were as follows: (1) COC low dose p < 0.0078; (2) COC high dose p < 0.0156; (3) COD low dose p < 0.0078; and (4) COD high dose p < 0.0156.
Discussion

Previous research has demonstrated that analysis of hair and nails can be useful in detecting drug exposure. The ability of hair and nail analysis to detect drug exposure cannot be fully explored without information on dose-response relationships and the mechanisms for drug disposition into these matrices.

A brief understanding of the anatomy and physiology of hair and nail can help to explain some of the possible mechanisms of drug disposition into these keratinized matrices. It is most certain that drug disposition into these matrices is a complex, multifaceted process that is affected by both the physical and chemical components of hair and nail and the interaction of these components with the environment.

The anatomical structure of the hair consists primarily of the follicle, hair shaft, and surrounding dermal layers. There are approximately 100,000 follicles covering the human scalp (23). The hair shaft has an average diameter of 0.1 mm. Each shaft is imbedded 3 to 4 mm below the surface of the epidermal epithelium of the skin, growing outwardly from the hair follicle at an average rate of 0.25 cm/week (5,24,25). The shaft is composed of long, tightly compacted keratinized cells (100 μm) that form fibers through cross-linking bonds (26). These cells combine into macrofibrils to form the outer cuticle (when present) and the cortex, both of which surround the hollow central area, referred to as the medulla (27).

Modified horny cells of the epidermis, composed of a specialized keratin referred to as onychin, form the nail tissue. Essentially, growth occurs by the transformation of living, modified horny cells of the epidermis into layers of hardened, dead cells, forming a nail plate. Cells are incorporated into the nail plate both at the proximal germinal matrix and the underlying nail at approximately one third the growth rate of hair (5).

Three glands, including apocrine and eccrine sweat glands and sebaceous glands, secrete fluids that coat the skin, hair, and nail. Eccrine glands of the epidermis are found in close proximity to the hair follicle, whereas both the apocrine and the sebaceous glands have ducts opening directly into the follicle (1). The proximity of these glands to the nail plate is more remote. Sweat and sebum potentially introduce components such as water, lipids, inorganic salts, waste products, and drug to hair and nail (1,24).

Keratinized matrices and the immediate environment are highly vascularized. This rich blood supply surrounds the hair bulb, glands and the ventral floor of the nail bed allowing nutrients, waste products, and exogenous components to diffuse into these structures (1,28,29).

Drug disposition into keratinized matrices could occur through various mechanisms. The incorporation rate into these matrices has been demonstrated by several investigations to be both rapid and persistent. Cone (10) detected COD in human beard specimens within 24 h of administration. Another investigative group reported finding measurable concentrations of COC and COD in hair collected after 72 h following completion of a six-day dosing interval (1). Similarly, therapeutic drugs have been detected in the distal free edge of the nail bed within a week of administration. Proposed mechanisms of drug incorporation into nail given by these investigators included rapid diffusion from the skin under the nail plate, addition of newly formed matrix cells containing drug, longitudinal diffusion beginning at the proximal end, and transfer from sebum, stratum corneum, and sweat (19,20,30). As with nail, these incorporation routes have also been proposed with hair analysis. Thus, these studies suggest that interactions with blood supply, skin, bodily secretions, and the external environment lead to drug incorporation into keratinized matrices. However, the extent of contribution of each of these mechanisms and whether the keratinized matrices act the same or differently remain largely unknown.

To more completely evaluate the data of this study, the observed results are presented based on three different aspects. First, this paper presents overall timing profiles of drug disposition into hair and nails, considering when drug analytes first appeared and were no longer detected in the keratinized matrices. For this pharmacokinetic investigation, hair was observed in its washed and unwashed state to determine if washing influenced the results to a statistically significant level. Secondly, the decontamination washes were investigated to determine which washing procedure removed the greatest concentration of drug analyte and if both drug analytes were removed to a similar extent. Furthermore, the percentage of drug removed by washing was investigated for hair. Lastly, we combined wash fraction drug concentrations and the extract drug concentration to investigate the total sum of drug detected (total drug detected) in a given matrix at a given time point for each subject. This approach was necessary if the drug concentrations in the two matrices were to be compared because drug analytes were detected at greater concentrations in the hair extracts, but not the nail extracts when compared to the respective washes.

The time profiles for drug disposition into hair and nails are similar with drug concentrations appearing within three days after last drug administration, peaking within 2–3 weeks of drug administration, and decreasing approximately four weeks postadministration. Joseph et al. (1) also reported a similar timing profile when they investigated the drug concentration in hair obtained from a different region (anterior vertex) from five of eight subjects included in this study (posterior vertex).

Observed pharmacokinetic measurements including AUC and Cmax values demonstrated the high variability of drug analyze concentrations in keratinized matrices of different subjects. Information derived from AUC measurements for this study was limited. AUC measurements for high-dose administration could not be reliably determined because of inadequate collection time following drug administration. Collection of specimen only continued for two weeks after high-dose administration, and terminal drug elimination was still in its ascending phase. However, both AUC and Cmax measurements after low-dose administration and Cmax measurements after high-dose administration did demonstrate high intrasubject variability for cocaine and codeine disposition into hair. This variability shows the degree of difficulty in interpreting data for hair analysis because these measurements were obtained from subjects of the same race and gender under strictly controlled conditions.

This project also demonstrated that the observed drug disposition profiles were different for hair and nails. Less than 20% of drug in hair specimens was removed by decontamina-
Investigation of the decontamination washes demonstrated that the first phosphate buffer wash generally removed the highest concentration of cocaine and codeine analytes from both keratinized matrices. This was an important observation because the isopropanol decontamination wash was the first in the series of washing steps. Both alcohol and water-based decontamination washes have been employed in previous studies (2). In addition, Kidwell and Blank reported (4) similar findings when they used an ethanol wash followed by three successive phosphate buffer washes. Both hair from intravenous COC drug users and hair soaked in aqueous solutions of cocaine and a COC surrogate, p-bromobenzoyleconnine, were investigated. Moreover, the percentage of drug removed by washing the hair was 30-50% of the TDD, but the washing procedures removed the greatest concentration of drug from the nails and not hair. Practically all the drug analytes were contained in the nail decontamination washes, whereas only a fifth of the drug detected was found in the hair decontamination washes.

In order to evaluate drug disposition into the hair and nails of the same subject combined results of the washed specimens and their respective wash fractions were reported as “total drug detected” in hair or nails during analysis. This calculation was necessary because of insufficient quantities of nail specimens available for analysis (nails were only analyzed washed) and the different drug disposition profiles exhibited by hair and nails. Both hair and nail analyses demonstrated a dose-response relationship for cocaine based on the mean maximum drug concentration of TDD after low- and high-dosing levels. A dose-response relationship for codeine was also demonstrated by hair analysis at both dosing levels but only the low dosing level showed a relationship by nail analysis. This may be due to the low concentration of codeine observed in nail specimens. However, the mean cocaine and codeine concentrations in hair were 5-30 times higher than nail-drug concentrations.

There are at least two possible explanations for this observation. One explanation for this discrepancy may revolve around the method of specimen collection. The other explanation, a more theoretical one, involves the mechanisms by which the drug analytes are incorporated into hair versus nail.

Fingernail specimens were collected by scraping the dorsal surface of the nail plate to obtain shavings. This method of nail collection may have influenced the results for several reasons. First, the proposed mechanism of nail growth and incorporation of epidermal cells (i.e., keratinocytes) includes growth at the proximal end of the nail plate beginning at the nail fold and from the ventral side of the nail plate directly above the highly vascularized nail bed. Therefore, the nail specimen collected for this study may represent the most inaccessible region of drug-positive keratinocytes recently incorporated into the nail plate. Second, specimen collection may have been more affected by the nail growth rate. Nail grows at an average rate less than 0.1 cm/week. By completion of this study, nails would have grown a little over one-half of a centimeter, which would barely transfer the new growth at a position where the scraping for specimen collection occurred. Third, research has demonstrated that more mature melanocytes possessing melanin are mainly located towards the distal portion of the nail and the lower 2-4 layers of the matrix cells. Moreover, the number of melanocytes located in hair is five times that found in nail (31-33). Melanin has been proposed as a primary drug-binding site; this drug compartment was underrepresented by the method of specimen collection for this study.

Finally, this explanation is more plausible if one considers the postmortem nail data presented by Garside et al. (13). This study demonstrated that reasonably high COC concentrations were easily detected in distal, free-edge nail specimens using the same analytical technique of GC-MS analysis employed by this study. Although drug was removed by the methanolic wash procedure used during specimen preparation, the drug concentrations in the washes were always less than or equal to drug concentrations observed in the nail specimens themselves. At least by the methanolic wash procedure, the collection and analysis of the distal free edge of the nail does not appear to be as strongly influenced by decontamination washing.

Another explanation may be the extent of contribution of various mechanisms of drug deposition into these keratinized matrices and the potential difference in accessibility of the drug in the hair and nail matrix. Based on findings of this research, it appears that drug may incorporate more deeply into the inner matrix of hair in comparison to nails. If drug incorporates into the inner region of the hair shaft, largely through its vasculature route, this would prevent easy removal by decontamination washes. Conversely, decontamination wash procedures may more readily remove drug from nails because drug may be more surface-oriented or nail structure may be porous enough to allow a greater degree of drug removal by washing.

Although this study did not fully investigate the mechanism by which drugs are deposited into keratinized matrices, it did provide evidence of mechanistic contribution. This study supports drug diffusion from the vasculature into matrix cells of the hair and nail. However, results of this study suggest that drugs may absorb closer to the nail surface and can be easily removed by wash procedures, whereas drugs may penetrate further into the inner matrix of the hair, preventing easy removal. Drug was present in both hair and nail within three days of completion of drug administration. Joseph and co-workers (1, 22) reported that drug was found in plasma, sweat, sebum, and stratum corneum for up to 48 h following the same dosing scheme as this paper. Thus, this study strongly suggests transfer of drug from blood, skin, and bodily secretions such as sweat and sebum into hair and nail.

The study design employed in this investigation limited the degree of contribution from environmental contamination and bodily secretions that are proposed mechanisms of drug disposition. Because drug was administered by oral ingestion and subcutaneous injections, environmental contamination resulting from drug aerosols and vapors was not represented in this study. In addition, collection of hair by shaving the entire scalp each week limited the transfer of drug from sweat, sebum, and skin to hair. Instead of a hair shaft of several inches being available for deposition of drug-containing secretions, the hair collected for this study represents only one week's worth of drug secretions coating its surface. The role of this study limitation is not apparent because it is unknown to what extent
bodily secretions coat the hair near the surface of the scalp in comparison to hair of the distal shaft.

Reports investigating drug concentrations in hair and nail of the same subject have been limited. All of these reports are of small sample size. Table III summarizes results from these reports and the current study. Most of these investigations only report detection of a drug in these keratinized matrices and do not discuss dose-response relationships. Those investigating dose-response relationships only discuss therapeutic drugs. This is the first investigation to report dose-response relationships for drugs of abuse in paired hair and nail analysis. These reports differ in their observations as to whether drug concentrations are higher in hair or in nail. Reports investigating the same drug agree in their findings. In addition, these investigations demonstrate that both hair and nails may show a dose-response relationship, but the relationship appears to be stronger for hair than for nails.

The differences observed in just these few paired hair and nail studies are difficult to evaluate. Nevertheless, the limitations of these studies need to be considered when comparing their results. Because the data from paired hair and nail analyses are limited for dose-response relationship investigations, it is necessary to rely on investigations of hair alone to discuss the limitations and problems of these types of kinetic studies. Moreover, conclusions surmised from investigations into dose-response relationships in hair analysis remain contradictory because of differences associated with study design, analyte of interest, and analytical methodology.

Human studies vary in their method of determining dose-response relationships among investigators. Some investigators depend on self-reporting of drug use, whereas others control the dose administered. Moreover, conclusions made by investigators

<table>
<thead>
<tr>
<th>Investigators (reference #)</th>
<th>Drug concentrations</th>
<th>Method of analysis</th>
<th>Dose-response results</th>
<th>Basis of dose-response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suzuki et al. (17)</td>
<td>Amphetamines</td>
<td>Nail &gt; Hair (5 out of 7) Hair &gt; Nail (2 out of 7)</td>
<td>GC-MS-CI</td>
<td>Not investigated</td>
</tr>
<tr>
<td>Suzuki et al. (18)</td>
<td>Amphetamines</td>
<td>Nail &gt; Hair (6 out of 10) Hair &gt; Nail (4 out of 10)</td>
<td>GC-MS-EI</td>
<td>Not investigated</td>
</tr>
<tr>
<td>Uematsu et al. (5)</td>
<td>Haloperidol</td>
<td>Hair &gt; Nail (n = 20)</td>
<td>RIA</td>
<td>Yes: Hair b/o trough plasma conc. (SS) (r = 0.772) Yes: Hair b/o daily dose (r = 0.555) No: Nail b/o trough plasma conc. (SS) Yes: Nail b/o daily dose (r = 0.325)</td>
</tr>
<tr>
<td>Faergemann et al. (19)</td>
<td>Terbinafine</td>
<td>Hair &gt; Nail (n = 12)</td>
<td>HPLC</td>
<td>Yes: Hair followed typical time-concentration pharmacokinetic profile; [Hair] &gt; [Plasma] Yes: Nail followed typical time-concentration pharmacokinetic profile; [Nail] &gt; [Plasma]</td>
</tr>
<tr>
<td>Miller et al. (15)</td>
<td>Cocaine</td>
<td>Hair &gt; Nail</td>
<td>MS-MS</td>
<td>Not investigated</td>
</tr>
<tr>
<td>Cirimele et al. (16)</td>
<td>Amphetamines</td>
<td>Nail &gt; Hair (n = 1)</td>
<td>GC-MS-EI</td>
<td>Not investigated</td>
</tr>
<tr>
<td>This study</td>
<td>Cocaine (opioids)</td>
<td>Hair &gt; Nail (n = 8)</td>
<td>GC-MS-EI</td>
<td>Yes: Hair b/o TDD-Cmax. COC and COD at low and high dosing intervals Yes: Nail b/o TDD-Cmax. COC (low/high) and COD (low) No: Nail b/o TDD-Cmax. COD and at high dosing interval</td>
</tr>
</tbody>
</table>

* Abbreviations: A, amphetamine; MA, methamphetamine; MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxyamphetamine; COC, cocaine; BE, benzoylcegonine; COD, codeine; RIA, radioimmunoassay; HPLC, high-pressure liquid chromatography; TDD, total drug detected; Cmax, maximum concentration; SS, steady-state; b/o, based on; and NA, not applicable to this study.
based on results from similar study designs vary in their degree of conservativeness. Although controlled studies are more valid for determining dose-response relationships, they are also more cost prohibitive, and dosing schemes are limited by safety considerations. The human studies performed by Baumgartner et al. (3) were largely based on self-reported drug use. Digoxin was the only drug administered under controlled conditions. For these studies, a poor correlation between dose and self-reported cannabinoid concentrations in hair was observed, but the investigators attributed this to technical difficulties associated with the monoclonal antibody used by their radioimmunoassay. Nakahara et al. (6) also employed self-reports of patients admitted to a psychiatric hospital for methamphetamine abuse to determine the relationship between sectional analysis of hair by GC–MS analysis and drug histories. In 9 of 11 subjects, a positive correlation was demonstrated between methamphetamine concentrations in hair and self-reported drug histories. These authors did not discuss dose and concentration of drug in hair because of the uncertainty of the dose, unknown purity of the illicit drug, and the intra-individual variability of drug uptake from blood to hair. As demonstrated by these two studies alone, there is wide disparity with conclusions made by investigators using similar study designs.

Not only are drug disposition studies influenced by methodology and study design, different physiochemical properties of drug analytes can influence results of drug disposition and dose-response studies. For example, one animal study investigating the differences in acid-base nature and lipophilicity of phenobarbital (weak acid, highly lipophilic) and COD (weak base, less lipophilic) demonstrated different incorporation patterns for these two drugs (8). This study showed that COD was incorporated at higher concentrations in pigmented versus nonpigmented hair of the same animal, but phenobarbital concentrations were equal in both hair types. This study suggests that higher concentrations of drug in hair for drug incorporation studies could be influenced by physiochemical properties and may not be due to greater ingestion of drug alone.

In summary, this study demonstrated that drug can be detected in hair and nail by GC–MS analysis. Although the time profiles for drug disposition into hair and nails were similar, the difference in drug detection profiles for hair and nail was demonstrated by the greater drug removal by decontamination wash procedures for nail than hair. This difference may be attributed to influences of study design or differences in contributions of incorporation mechanisms (secretions vs. vasculature). By evaluating the maximum concentration of total drug detected from the matrix (i.e., combined concentrations of washed specimen plus wash fraction results), this study demonstrated that a dose-response relationship was apparent for cocaine and codeine in hair and nail. However, nail analysis failed to demonstrate a dose-response relationship for codeine at high-dose administration. Drug concentrations in hair were generally 5- to 30-times higher than nail concentrations.

Future study is needed to further determine similarities and differences of drug disposition into hair and nail. In addition, studies are needed to delineate the mechanisms that contribute to observed differences in timing profiles for drug disposition into these keratinized matrices of similar composition, vasculature, and inherent exposure to the environment.

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