The Effect of Hair Color on the Incorporation of Codeine into Human Hair

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Abstract

The influence of melanin on the binding of xenobiotics in hair will impact the interpretation of drug concentrations determined by hair testing. The purpose of this study was to determine if codeine, as a model compound of abused drugs, would be incorporated into black, brown, blond, or red hair as a function of melanin concentration. Such data would assist in the interpretation of codeine concentrations in hair and help elucidate the potential influence of hair color on incorporation of drugs. Male and female Caucasians with black (n = 6), brown (n = 12), blond (n = 8), or red hair (n = 6) and non-Caucasians with black hair (n = 12) aged 21–40 years were enrolled in the study. Each subject was administered oral codeine phosphate syrup in a dosage of 30 mg three times a day for five days. Twenty-four hours after the end of the treatment period, a 30-mg codeine dose was administered and the subject's plasma area under the concentration time curve (AUC) for codeine was determined. Codeine and melanin were measured in the first 3 cm of hair closest to the vertex region of the scalp prior to and 1, 4, 5, 6, and 7 weeks after dosing. The quantitative and qualitative melanin profiles were determined for each subject's hair to provide an objective measure of hair color. The plasma concentrations of codeine were measured to eliminate differences in the bioavailability and clearance of codeine as factors that might account for the differences in codeine hair concentrations. The subjects were asked not to cut their hair in the vertex region of the scalp or to use any form of chemical treatment on their hair, but otherwise normal hygienic measures were permitted. The mean (± SE) hair codeine concentrations 5 weeks after dosing were 1429 (± 249) pg/mg in black hair; 208 (± 17) pg/mg in brown hair; 99 (± 10) pg/mg in blond hair; and 69 (± 11) in red hair pg/mg. In black hair, codeine concentrations were 2564 (± 170) pg/mg for Asians and 865 (± 162) pg/mg for Caucasians. Similar concentration relationships were observed at weeks 4, 6, and 7. A strong relationship between the hair concentrations of codeine and melanin (R^2 = 0.73) was observed. Normalization of the codeine concentration with the melanin concentration reduced the hair color differences observed. These data demonstrate that the interpretation and reporting of hair test results for codeine are influenced by hair color. After this dosing protocol, the proposed federal guideline cutoff of 200 pg/mg of codeine would result in 100% of subjects with black hair and 50% of subjects with brown hair being reported as positive, and subjects with blond or red hair would be reported as negative. The incorporation of these drugs into hair should be studied carefully in humans to ensure the appropriate interpretation of drug concentrations.

Introduction

An effect of hair color on the interpretation of hair tests for drugs of abuse would affect the lives of workers, children, students, athletes, and persons in the civil and criminal justice systems. Studies in animals have shown that hair color influences the incorporation of drugs of abuse in the general pattern of black hair > brown hair > blond (yellow mouse) hair (1–3). A study in a small number of humans has shown that codeine concentration in hair is dependent on melanin content (4). It is then reasonable to hypothesize that hair color may affect the interpretation of hair testing in humans.

Hair testing is a rapidly growing technology for detecting the use of drugs of abuse. Several laboratories in the United States provide such tests (5). Their clients include Fortune 500 companies, large metropolitan police departments, Federal Reserve Banks, parole boards, and hundreds of high schools. One laboratory reported 750 corporate clients in 1997 and over 1700 clients in March of 2000. Child custody services commonly use hair tests to detect drugs of abuse (6–8). Collection kits in which a hair sample can be sent to a laboratory for analysis are marketed to parents through pharmacies and the Internet. General practitioners, Medical Review Officers, and other physicians are often asked by employers, parents, and the courts to

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interpret the results of hair tests for drugs of abuse.

Federal guidelines that include cutoff concentrations establishing the criteria for determining a positive from a negative hair sample have been proposed (9). In the United States, a hair sample is considered positive if the hair concentration of drug is greater than or equal to the cutoff concentration. It is possible that, even if identical drug doses are ingested, one person may incorporate more drug into his/her hair than another person and the determination of a positive from a negative hair test for that drug be influenced by hair color. Prior to promulgation of the widespread use of hair testing, a thorough understanding of the factors such as hair color that might influence the interpretation of results is necessary.

Urine drug tests are routinely used to detect the use of drugs of abuse. As an adjunct to urine tests, hair tests are noninvasive and have the potential to increase the window of detection of drug long after the drug has been eliminated from the rest of the body. Because of this unique sensitivity, it is critical to determine whether all subjects ingesting similar amounts of drugs of abuse have similar amounts in their hair, and if not, to understand why. An effect of hair color on the interpretation of hair tests for drugs of abuse will have profound social and cultural implications to thousands of persons undergoing hair tests annually. Understanding the scientific basis for such an effect will increase the credibility of a potentially useful test to detect the use of drugs of abuse.

To determine the role of hair color on drug incorporation, we administered identical doses of codeine to healthy human volunteers who had either red, blond, brown, or black hair. Black-haired subjects included Caucasians and non-Caucasians. The codeine concentrations were measured in hair collected for seven weeks after drug administration. The quantitative and qualitative melanin profiles were determined for each subject's hair to provide an objective measure of hair color. The plasma concentrations of codeine were measured to eliminate differences in the bioavailability and clearance of codeine as factors that might account for the differences in codeine hair concentrations.

Methods

Protocol

We studied 44 healthy male (n = 24) and female (n = 20) volunteers (ages 21–40 years) with black, brown, blond, or red hair. The subjects self-reported their race. The study was approved by the human studies committee of the University of Utah Health Sciences Center. All subjects gave written informed consent and were compensated for participation.

Each subject was given oral codeine phosphate syrup in a dosage of 30 mg three times a day for five days. Twenty-four hours after the end of the treatment period, a 30-mg codeine dose was administered, and the subject's plasma area under the concentration time curve (AUC) for codeine was determined. Blood for AUC determination was collected at 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 h after the single codeine dose. The subjects were asked not to cut their hair in the vertex region of the scalp or use any form of chemical treatment on their hair, but to use normal hygienic measures otherwise. Hair was collected by cutting approximately 50–100 hairs from the vertex region of the scalp immediately prior to dosing and at weeks 1, 4, 5, 6, and 7 after dosing. Hair was cut within 2 mm of the scalp. Hair was stored at –20°C in aluminum foil and oriented so that the scalp end could be identified. Prior to analysis, individual hair were aligned and the proximal (closest to scalp) 3 cm for each hair was identified, trimmed from the remainder of the hair, and used for analysis of codeine.

Analysis of codeine

Hair. After adding deuterated codeine ($^2H_3$-codeine, 7.5 ng/mg) as an internal standard to 20 mg of hair, the sample was solubilized with NaOH and extracted with n-butyl chloride/acetonitrile (4:1, v/v). An aliquot of the extract was analyzed on a high-performance liquid chromatograph–mass selective detector (1100 LC–MSD; Hewlett-Packard, Palo Alto, CA) in the electrospray (API-ES) positive ion mode using an ODS-AQ C18 S-5 column (50 m × 2.0-mm i.d., 120Å particle size, YMC, Inc., Wilmington, NC). The mobile phase was a mixture of water, 0.1% acetic acid, and methanol. The limit of quantitation (LOQ) of codeine in hair was 20 pg/mg hair (10). The coefficients of variation for intra-assay and interassay precision were less than 7% at codeine concentrations of 0.05, 0.3, and 5 ng/mg hair (11). At these same concentrations, the accuracy of the assay was within 10% of the target values with coefficients of variation of less than 7%.

Morphine, a metabolite of codeine, was not measured in this study because in a previous study with this dosage regimen, morphine was not detected in the hair (12). Hair was not washed in the laboratory prior to analysis for codeine. Previous research in our laboratory has shown that laboratory washing of hair can remove drug that has been incorporated into the hair via the circulation (13). It was unlikely that there would be external exposure to codeine from smoke or powder during the study time periods, and we did not want the potential removal of incorporated codeine to influence our results.

Plasma. Codeine quantitation in plasma (1 mL) was performed by GC–MS (EI, SIM mode, HP 5973) equipped with an HP-5MS column (30 m × 0.25-mm i.d., 0.25-µm film thickness) using deuterated codeine as an internal standard. Liquid–liquid extraction was performed with 2 mL buffer composed of a saturated solution of ammonium chloride in ammonium hydroxide (pH 9.5) and 3 mL chloroform/isopropanol (9:1, v/v). The sample was derivatized with 2,2,3,3,3-pentafluoro-1-propanol (PFP-OH, 70 µL, Regis Technology, Inc., Morton Grove, IL) and pentafluoropropionic anhydride (PFPA, 100 µL, Regis Technology, Inc.) for 30 min at 70°C. The LOQ, estimated by the signal-to-noise ratio method, was 2 ng/mL.

Analysis of melanin

Eumelanin. The degradation products pyrrole-2,3-dicarboxylic acid (PDCA) and pyrrole-2,3,5-tricarboxylic acid (PTCA) of eumelanin were measured by a modification of the method of Ito and Wakamatsu (14). Hair (5 mg) was cut into 1–2-mm pieces and degraded in 0.5M NaOH in 80 µL of 3% H_2O_2 that was heated in a boiling water bath for 20 min. Phthalic acid

(40 nmol) was added as an internal standard prior to degradation. Quantitation of PDCA, PTCA, and internal standard was carried out with a Waters 600E (Milford, MA) high-pressure liquid chromatography (HPLC) system with UV detection at 280 nm. Peak-height ratios of PDCA and PTCA to internal standard were compared to a standard curve made from pure PDCA and PTCA standards subjected to alkaline hydrogen peroxide degradation.

Pheomelanin. Hair (5 mg) was degraded with hydriodic acid and hypophosphorous acid in a 130°C oil bath for 16 h, and the degradation products 4-amino-3-hydroxyphenylalanine (AHP) and 3-aminotyrosine (3-AT) were measured by the method of Kölb et al. (15). Prior to degradation, an internal standard, L-α-methylDOPA (20 nmol), was added. The degradation products and internal standard were measured by HPLC with electrochemical detection (+400 mV relative to a silver–silver chloride reference electrode). The peak-height ratios of AHP and 3-AT to internal standard were compared to a standard curve made from pure AHP and 3-AT standards subjected to hydriodic acid degradation.

Statistical analysis

The data reported describe the total available study population. Means and standard errors of the mean (SE) are presented for different categories (i.e., hair color, race, and week of hair collection) and variables (i.e., codeine hair concentration, codeine AUC, eumelanin hair concentration, pheomelanin hair concentration, and total melanin hair concentration). Differences between group means were analyzed using fixed-factor, one-way analysis of variance (Microsoft Excel Office 98, Redmond, WA) for hair color and race. Comparisons among groups were considered statistically significant at $p \leq 0.05$. For each category, comparisons between groups (i.e., black hair vs. blond hair) were performed using an unpaired t-test for independent samples. Comparison of variances at the 0.025 level of significance was used to test for homogeneity of the variance. The assumptions of normality and homogeneity of variance were met for all comparisons.

Table I. Hair Color, Reported Race, and Area Under the Plasma Codeine Time Curve for Study Subjects

Hair Color	Race	*n*	Male	Female	Plasma Codeine AUC h-ng/mL*
Black	African American	2	2	0	127.7
	American Indian	1	1	0	296.4
	Hispanic	2	2	0	198.1
	Asian	5	1	4	299.5 ± 58.8†
	Caucasian	6	3	3	166.1 ± 14.6
	Total	**16**			
Brown	Caucasian	12	6	6	209.3 ± 17.1
Blond	Caucasian	8	6	2	177.9 ± 19.7
Red	Caucasian	6	2	4	250.5 ± 32.6

* Data are presented as mean ± SE. Plasma AUC was determined after a single 30-mg oral dose of codeine.
† Different from Caucasians with black hair ($p = 0.085$).

Results

The data from one African-American subject were dropped from the study because codeine was found in his predose hair (800 pg/mg), and he admitted taking codeine prior to the study. The data from one Asian subject were dropped because he did not complete the study. There were no significant differences ($p \leq 0.05$) in the codeine plasma AUC for any group of subjects (Table I). The difference between the AUC for Asian subjects and Caucasian subjects with black hair was not significant at the $p \leq 0.05$ level ($p = 0.085$). There were no significant differences in hair codeine concentrations or plasma AUC between male and female subjects in this study.

Codeine was not detected in the predose hair of 39 of 42 subjects; codeine was detected in the predose hair of three subjects, but it contributed less than 10% of the measured concentration of codeine during weeks 4, 5, 6, and 7. Codeine was detected in hair 1 week after codeine administration in 15 of 42 subjects; all subjects were positive for codeine 4 weeks after codeine administration. When all subjects were compared, those with black hair had a 7-fold greater codeine concentration than those with brown hair and a 14–15-fold greater codeine concentration than those with blond or red hair (Figure 1). Although there was a downward trend in the hair codeine

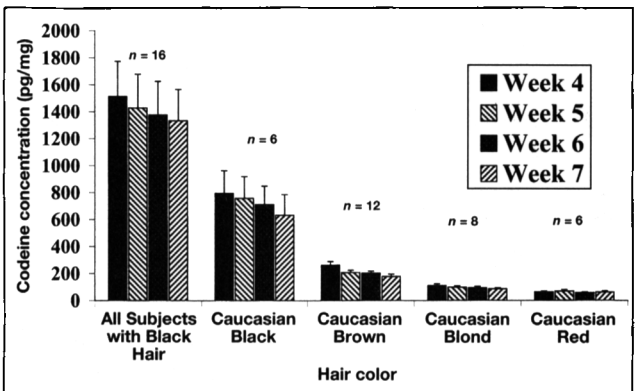

Figure 1. Mean codeine concentrations in the hair of all subjects with black hair and Caucasians with black, brown, blond, or red hair during weeks 4, 5, 6, and 7 after codeine administration. Error bars represent SE.

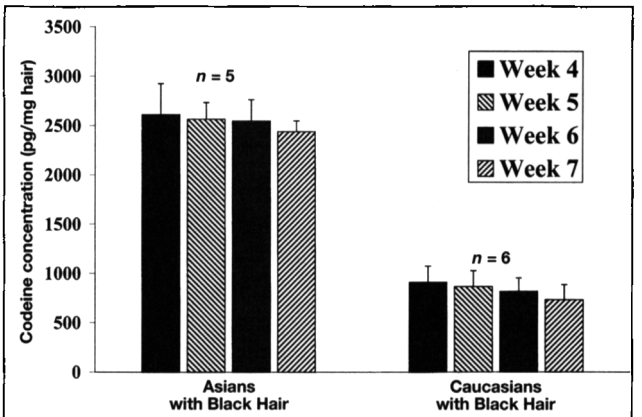

Figure 2. Mean codeine concentrations in the hair of Asian and Caucasian subjects during weeks 4, 5, 6, and 7 after codeine administration. Error bars represent SE.

concentrations between weeks 4 and 7 for all hair colors, there were no significant differences in the hair codeine concentrations for subjects with any hair color between weeks 4, 5, 6, or 7 after codeine administration. When the hair codeine concentrations of only Caucasian subjects were analyzed, there was still a 4–5-fold greater concentration in black hair than in brown hair and an 8–10-fold greater concentration in black hair than in blond or red hair.

Among subjects with black hair, a statistical analysis was possible only between Asian and Caucasian subjects because of the limited number of African-American, American-Indian, and Hispanic subjects. Asian subjects with black hair had a 2.5-fold greater hair codeine concentration than did Caucasian subjects with black hair (Figure 2).

The mean eumelanin, pheomelanin, and total melanin concentrations in the hair of each group of subjects are shown in Table II. The pigment in blond, brown, or black hair is composed of 95–99% eumelanin; pheomelanin contributes little to the total melanin content of blond, brown, or black hair. Asian black hair has a significantly greater eumelanin concentration than Caucasian black hair. Among Caucasians, there is a significantly lower total melanin concentration in subjects with brown, blond, or red hair than in subjects with black hair. The pheomelanin concentration is greatest in subjects with red hair. Among Caucasians with black, brown, blond, or red hair, there is an increasing contribution of pheomelanin to the total melanin concentration of 1.9%, 3.1%, 4.1%, and 36.6%, respectively.

Table II. Hair Color, Reported Race, and Melanin Concentrations in Study Subjects*

Hair Color	Race	*n*	Eumelanin (μg/mg)	Pheomelanin (μg/mg)	Total Melanin (μg/mg)
Black	African American	2	13.50	0.10	13.60
	American Indian	1	14.70	0.10	14.80
	Hispanic	2	10.00	0.10	10.10
	Asian	5	11.60 ± 1.83†	0.10 ± 0.01	11.69 ± 1.85†
	Caucasian	6	7.00 ± 0.90‡	0.14 ± 0.04	7.11 ± 0.89‡
Brown	Caucasian	12	3.50 ± 0.90§	0.11 ± 0.01	3.57 ± 0.28§
Blond	Caucasian	8	2.30 ± 0.1‖	0.11 ± 0.03	2.33 ± 0.10
Red	Caucasian	6	1.70 ± 0.30	0.96 ± 0.20**	2.62 ± 0.44

* Data are presented as mean ± SE.
† Significant differences at $p \leq 0.05$ were observed between Asians and all other groups.
‡ Significant differences at $p \leq 0.05$ were observed between Caucasians with black hair and Caucasians with brown, blond, or red hair.
§ Significant differences at $p \leq 0.05$ were observed between Caucasians with brown hair and Caucasians with blond or red hair.
‖ Significant differences at $p \leq 0.05$ were observed between Caucasians with blond hair or red hair.
** Significant differences at $p \leq 0.05$ were observed between Caucasians with red hair and all other groups.

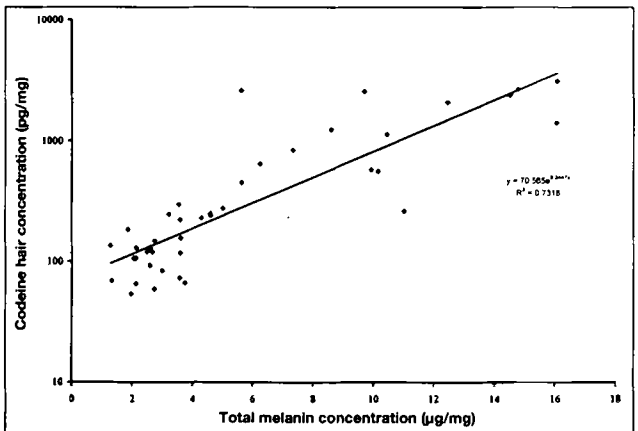

Figure 3. Correlation between codeine concentration in the hair of all subjects during week 5 after codeine administration and the total melanin concentration.

These data suggest a direct relationship between the melanin concentration and the concentration of codeine in hair. When analyzed, a positive relationship was observed ($R^2 = 0.73$, $p \leq 0.001$) for the comparison of the log of the week 5 hair codeine concentration for each subject and the total melanin concentration (Figure 3). The positive y-intercept suggests that even if hair contained no melanin, a small amount of codeine would still be incorporated.

Cutoff concentrations are frequently used by drug-testing laboratories to determine a positive from a negative specimen. A specimen drug concentration equal to or greater than the cutoff would be reported as positive. A specimen drug concentration less than the cutoff would be reported as negative. Figure 4 shows the percentage of our subjects that would be reported positive at various cutoff concentrations. Only at a cutoff of 50 pg/mg would all subjects be reported as positive. At a cutoff concentration of 500 pg/mg, only subjects with black hair would be positive and all subjects with brown, blond, or red hair would be negative. Even at a cutoff concentration of 3000 pg/mg, 6% of subjects with black hair would still be positive.

Discussion

These data clearly show that codeine is incorporated into black hair more readily than brown hair, blond hair, or red hair. Therefore, it is possible that hair color may affect the

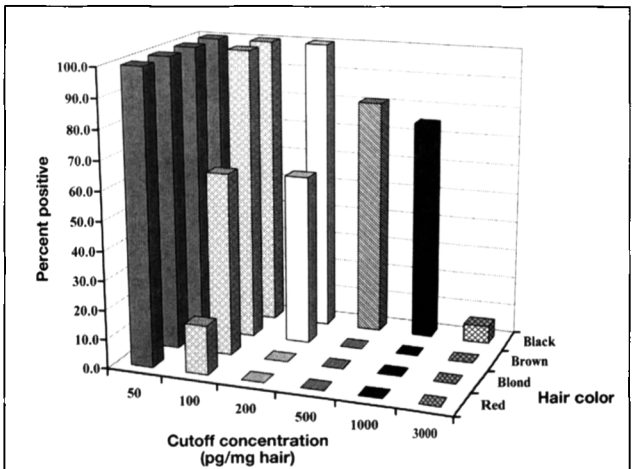

Figure 4. Percentage of subjects with various hair colors who would be reported as positive for codeine at different cutoff concentrations.

interpretation of hair codeine concentrations (at least after the doses used in this study). If the proposed federal guidelines are enacted, the suggested codeine cutoff of 200 pg/mg would result in 100% of subjects with black hair and 50% of subjects with brown hair being reported as positive, and no subjects with blond or red hair being reported as negative for codeine. Wennig (16) has proposed a cutoff of 500 pg/mg for opiates in hair. At this cutoff concentration, 100% of subjects with red, blond, or brown hair would be negative, whereas 80% of persons with black hair would still be positive. Raising the codeine cutoff to 1000 pg/mg or 3000 pg/mg would not totally eliminate the hair color effect for codeine.

The dose of codeine used in this study, although high, is likely to be prescribed for patients after surgery or trauma. Thus, it is possible that following a therapeutic dose of codeine, a person with black hair would be reported positive for the abuse of codeine based on a hair test; a person with red, blond, or brown hair would likely be reported negative. The dose proportionality in the hair concentration of codeine would lead to the conclusion that, even after a single codeine dose, persons with black hair could have a positive hair test for codeine (12,17).

Hair color results from melanin, which is synthesized in melanocytes within the hair bulb, being deposited into keratinocytes that mature into the hair shaft (18). Melanin is a polyanionic polymer consisting of eumelanin, which is responsible for black and brown hair color, and pheomelanin, which is responsible for red hair color. Eumelanin is a polymer composed primarily of subunits of 5,6-dihydroxyindole and 5,6-dihydroxindole-2-carboxylic acid. Pheomelanin is a polymer composed of benzothiazine subunits derived from cysteinyldopa (19). The quantity and type of melanins incorporated into hair from melanocytes are genetically controlled.

In careful analyses of hair testing results from commercial laboratories, several studies found no significant association between hair color or race and analyte detected in the hair (20–22). The most likely explanations for the difference between our study and these population studies are that in the population studies: (1) cocaine, rather than codeine, was the most common drug studied, (2) there is no accurate way of knowing the dose of drug ingested, and (3) there was no objective measure of hair color. The dose of codeine in our study was carefully controlled. This does not negate the importance of population studies, but does suggest that dose must be carefully considered when evaluating the factors that affect drug incorporation into hair.

Studies of limited scope have demonstrated an effect of hair pigment on drug incorporation (4,23,24), and one study has suggested that there is a racial bias in the interpretation of hair tests for cocaine (25). Kronstrand et al. (4), in a study of nine subjects with mostly light colored hair, have shown that the codeine concentration in hair is dependent on melanin content; they have reported similar findings for incorporation of selegiline metabolites in hair from 10 subjects (26). Cone and Joseph (27) have presented a strong case for the potential for a hair color effect on testing for drugs of abuse. The in vitro binding of codeine to hair has been shown to correlate well with its in vivo incorporation (3). The mechanism for codeine binding to melanin in hair is not completely understood. Melanin is polyanionic (28), whereas codeine, as a weak base, is cationic at physiological pH. Nakahara et al. (29) has demonstrated a correlation between drug basicity, melanin binding, and drug incorporation into rat hair. The importance of basicity is exemplified by experiments that show that the weak acid phenobarbital does not preferentially incorporate into pigmented hair (30). These findings lead to the hypothesis that a strong ionic bond forms between melanin and weak bases such as codeine and that this does not occur with weak acids such as phenobarbital. Many drugs of abuse, including opiates, cocaine, amphetamines, and phencyclidine, are weak bases, and their incorporation into hair would likely show a correlation with melanin concentration similar to what we have found for codeine.

We report a positive relationship between the codeine concentration and the total melanin concentration in hair. In non-Caucasian black hair the predominant pigment is eumelanin; pheomelanin comprises less than 1% (Asians = 0.8%) of the total melanin content. Caucasians with black hair have a total melanin content in hair that is significantly lower than non-Caucasians (Asians) and the percentage of the total melanin that is pheomelanin is greater (1.9%). The percentage of total melanin that is pheomelanin in Caucasians with brown, blond, or red hair is 3.1%, 4.1%, and 36.6%, respectively. Binding of drugs to eumelanin has been reported extensively (28,31), but there are few reports of drug binding to pheomelanin (32). We also found a positive correlation between the hair codeine concentration and the eumelanin concentration or the ratio of pheomelanin to eumelanin (data not shown). The precise role that pheomelanin plays in incorporating drugs of abuse into hair is unknown. In addition, there appear to be factors other than melanin that contribute to the incorporation of codeine into hair. The positive y-intercept in Figure 3 suggests that persons with no melanin would still incorporate a small amount of codeine into their hair. In previous studies conducted in animals with white hair that contains no measurable melanin, drugs of abuse, including codeine, were still incorporated (1,3). Thus, although melanin is a strong factor in determining the hair concentration of some drugs, it appears not to be the only factor.

Codeine was found in the hair of all subjects 4, 5, 6, and 7 weeks after oral administration of a therapeutic dose. Although there was a trend for the codeine concentrations to decrease from week 4 to week 7, there was no significant change in the hair codeine concentration during the course of the study. The binding of codeine to structural or chemical components of hair does not appear to be substantially disrupted by normal hygienic procedures. Chemical treatment (i.e., coloration) of the hair has been reported to affect drug concentrations (33); no subject in this study performed chemical treatment of his/her hair during the study period. The lower limit of quantitation of codeine in our assay was 20 pg/mg of hair. It is probable that after the administration of codeine in a therapeutic dose, persons with black hair would have detectable codeine in their hair for substantially longer than 7 weeks, long after there is no codeine in their plasma. This demonstrates the long window of detection of drugs in hair.

The observed difference in codeine hair concentrations be-

tween Asians and Caucasians with black hair is likely due to several factors. Asians in this study have a higher total melanin concentration in their hair than do Caucasians. Asians have a reported deficiency of CYP 2D6, which is responsible for the demethylation of codeine to morphine (34). This may contribute to the greater (although not statistically significant) plasma AUC in Asians and the significantly higher concentrations of codeine observed in Asian hair in our study. In addition, there might be inherent differences in the physical structure of Asian hair that facilitates the incorporation of codeine.

The correlation between the hair codeine and melanin concentrations suggests the possibility that the codeine concentration could be normalized for the hair color effect by expressing it as a function of the melanin concentration rather than by hair weight. Normalization reduced the difference between Caucasian subjects with black hair and subjects with brown hair from 4–5-fold to less than 2-fold; after normalization, there were no significant differences in the hair codeine concentration between subjects with brown, blond, or red hair. The normalized mean codeine concentrations in Caucasians with black, brown, blond, or red hair were 100.5, 60.5, 42.3, and 40.9 pg/μg melanin, respectively. Asians with black hair had a normalized mean codeine concentration of 251.8 pg/μg melanin. It is probable that the hair concentration of other drugs of abuse that are weak bases such as cocaine, morphine, amphetamines, and phencyclidine could also be normalized in this way.

These data clearly show that the interpretation of hair tests for codeine are influenced by the color of a person's hair. By normalizing the drug concentration with the measured melanin concentration it may be possible to substantially reduce this influence. Our data do not address the issue of whether the external deposition of drugs onto hair, such as by smoke, is also affected by hair pigmentation. Although our study involved only the incorporation of codeine into hair, it is probable, based on experiments in animals, that other drugs of abuse such as cocaine, phencyclidine, and amphetamines will follow a similar pattern. The incorporation of these drugs into hair should be studied carefully in humans to ensure the appropriate interpretation of drug concentrations.

Acknowledgments

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