Ethnic Hair Care Products May Increase False Positives in Hair Drug Testing

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Abstract

The question of why different races appear more susceptible to hair contamination by external drugs remains controversial. This research studied susceptibility of head hair to external cocaine and methamphetamine when hair products have been applied. Three different chemical classes of ethnic hair products were applied to Caucasian, Asian, and African hair. Some products increased the methamphetamine and cocaine concentrations in all hair types. A unique finding of this research is that certain ethnic hair products can replace moisture as a diffusion medium, thereby increasing the susceptibility to contamination over 100-fold compared to petroleum-based products.

Key Words: Cocaine, methamphetamine, false-positive, haircare products, hair

Introduction

Eliminating external drug contamination from hair test results remains a challenging task. To reduce false positives, hair testing must address: (1) removal of external drug contamination, (2) removal of contamination penetrating the hair due to moisture, (3) detection and quantification of unique metabolites that distinguish drug use from contamination, and (4) differential drug uptake due to bias from hair color, texture, damage, or cosmetic treatment. All decontamination procedures are based on the assumption that drugs deposited in hair from the external environment are loosely bound to either the surface of the hair or to the hair matrix, and therefore can be removed by decontamination procedures. There is no consensus about which decontamination procedure is optimum. For example, Martz decontaminated the hair using successive methanol extracts and tested each extract until the last extract showed that no further drugs had been removed [1]. Henderson *et al.* rinsed hair samples with a detergent followed by water then methanol prior to digestion and extraction [2]. Cone *et al.* rinsed hair samples with methanol and then extracted drugs by incubating with methanol solvent overnight [3,4,5]. Cone *et al.* have also studied more extensive decontamination procedures consisting of longer methanol washes [6]. Mangin and Kintz decontaminated hair using methylene chloride [7,8]. They concluded, based on negative results from the second wash, that one methylene chloride rinse was sufficient. Nakahara *et al.* washed their hair samples with 0.1% sodium dodecyl sulfate and water [9]. Moeller *et al.* used warm water followed by an acetone rinse [10].

Under dry conditions, contamination is more readily removed; not so when moist. Moisture swells hair and makes it permeable. In our early work, undamaged,

unbleached Asian hair was exposed to phencyclidine (PCP) in solution then allowed to dry[11]. PCP remained detectable in the hair after a number of decontamination washes in amounts that exceed hair from PCP users. Investigators replicated this, confirming that hair exposed to solutions as well as vapors of drugs cannot be completely decontaminated [12,13]. For example, Baumgartner and Hill showed that even nine normal hygienic washings were unable to remove all of the cocaine from hair exposed to crack cocaine smoke[14]. Welch *et al.* applied a quantity of powdered cocaine to hair and found that no procedure was successful in removing all external contamination[15]. Similarly, Cone *et al.* exposed hair to aqueous cocaine and reported that he could not wash all of the cocaine from the hair[16]. Henderson *et al.* reported that a single exposure of hair to cocaine in vapor form ("crack smoke") mimicked hair concentrations comparable to cocaine users in the upper range of amounts[5,17,18,19]. Using different hair types, Henderson *et al.* found that before decontamination, the concentrations of cocaine were relatively constant among hair specimens of diverse origin. After decontamination, concentrations varied substantially, suggesting that drugs incorporated from the external environment may be dependent on hair type.

Time and hygiene confound laboratory decontamination efforts to interpret results. Hair washing removes drug contamination on the outside and, to some extent, within the hair. Over time drugs decompose. Romano *et al.* transferred cocaine from hands by simply rubbing the scalp hair[20]. In the first week, hair tests identified contamination; however, over the next weeks of normal hygiene, cocaine in hair degraded increasingly to benzoylecgonine, causing test results that mimicked a cocaine user. Romano's results suggest that laboratory decontamination strategies work best for recently contaminated hair, but fail as post-contamination intervals lengthen. External contamination considerations have prompted the FBI to stop testing hair for drugs[21]. Some hair testing laboratories have consistently maintained they can distinguish contamination from use[22,23] while others have found that contamination cannot be completely removed, relying on drug metabolites and/or decomposition products to determine drug use [24,25].

Racial/ethnic/hair color/hair type bias is another unresolved issue in hair testing. Several controlled studies showed a difference in drug incorporation by race or hair color where known amounts of drugs were administered to human subjects[26,27]. Rollins *et al.*, administered ofloxacin and codeine to healthy volunteers[28,29]. They observed a trend of greater drug incorporation compared with hair color: black > brown > blond > red. They noted, "These data clearly demonstrate that, in a controlled study, the hair pigmentation pattern has a major effect on the incorporation of codeine and ofloxacin into human hair." Additional studies in several animal models showed that pigmented hair incorporated drugs preferentially over non-pigmented hair [30,31]. In limited human studies, black hair incorporated more drugs than unpigmented white hair on the same head[32]. A number of *in vitro* studies show that drugs bind to melanin [33,34,35,36,37,38]. Originally, we referred to the differential uptake of drugs as "hair type bias". Subsequently others expanded the term to "racial bias" then authored

research purporting to show that racial bias does not exist [39,40,41], a discrepancy which may result from the uncontrolled convenience samples studied and the frequent users from whom positive samples were obtained. In contrast, our previous studies showed that hair type bias is more pronounced near the cut-off concentrations and, more importantly, for hair externally contaminated with drugs [42]. Recently, in a study of police officers, a statistically significant difference between African and Caucasian cocaine positive hair samples was observed [43]. Cocaine was the only drug identified in these hair tests despite higher cannabis use in the general population, with cocaine and amphetamines less prevalent [44]. A sum of the positive data over an eight year period revealed 55/4222 (1.3%) African samples were positive vs. 30/10835 (0.28%) Caucasian samples, a 4.7 times greater cocaine positive rate in African hair.

The following research demonstrates that certain ethnic hair-care products enhance the uptake of drugs into hair, irrespective of hair type or color. It evaluates the mechanisms of incorporation of drugs and their metabolites from the environment. These results may indicate why some individuals are more susceptible to passive exposure than others who do not use these kinds of products.

2. Materials and Methods

2.1. Application of hair care products and external drug contamination

Three ethnic hair care products (“BB[®] Oil Moisturizer Hair Lotion with Castor Oil and Aloe”, “Isoplus[®] Styling Gel”, and “Blue Magic[®] Conditioner and Hair Dress”) were used to contaminate 200 mg hair aliquots by two methods.

2.1.1 First procedure: one gram each of BB[®] and Isoplus[®] or 0.5 g of Blue Magic[®]. The hair-care products were massaged separately onto cut head hair from three individuals (African, Asian, and Caucasian). Using disposable nitrile gloves each hair sample was rubbed into either 1 mg or 10 mg of cocaine and methamphetamine (calculated as the free base equivalents of cocaine hydrochloride and methamphetamine sulfate) to distribute the drugs uniformly. The drug-contaminated hair samples were incubated overnight at room temperature.

2.1.2 Second procedure: to achieve 100 µg contamination, drugs were serially diluted with the products before being applied to the hair, as above. Contaminated hair was incubated over the weekend.

Three types of control samples were analyzed under the same conditions. (1) Negative hair with no product, (2) Product-negative but drug positive hair rubbed with drug only, and (3) Product-positive but drug negative hair.

2.2 Decontamination of hair

Two procedures were employed to decontaminate hair. (1) Shampoo solution (200 µL of Nexxus[®] “Therappe”, 1 g/3 mL tap water) was added to the hair specimens in addition to 5 mL tap water, vortexed 10 sec repeatedly for 1-2 min., decanted, rinsed

three times with 5 mL tap water, vortexing each time, blotted, and allowed to air dry. (2) Before shampooing as described above, hair specimens were rinsed three times with 5 mL methylene chloride, vortexing 5 seconds each time, and pipetting off the rinses.

2.3 Extraction, derivatization, and analysis

A dental amalgamator equipped with disposable capsules and a single steel bead pulverized the decontaminated hair for 5-10 min. The resulting powder was extracted at room temperature for 1 hr. using 0.1 M HCl into which deuterated internal standards were added. The extract was centrifuged and filtered to remove particulates. The eluate from solid phase extraction was dried under nitrogen as previously described[45]; derivatized with 70 μ L 0.1% diisopropylethylamine in methylene chloride, 50 μ L propionic anhydride, and 20 μ L trifluoroethanol at 70 C for 30 min; cooled; dried under nitrogen; and reconstituted with 40 μ L ethyl acetate for CI-GC/MS analysis.

Results and Discussion

Several factors account for different incorporation rates: genetics, hair color, melanin content, and cosmetic treatment[23]. We have termed this bias “cultural bias” with the implication that cosmetic treatment may be the dominant factor. To cause a hair to test positive for drugs, first there must be the opportunity for contamination to occur. Then the drugs must penetrate the cuticle and enter the cortex. Water (or sweat) is important in swelling the cuticle, which facilitates diffusion of the drugs into the hair. Additionally, prior cosmetic treatment may damage the cuticle and reduce the requirement for water. Cosmetic treatments designed for straightened hair (hot combed or chemically straightened) to add shine[46] and prevent breaking[47] most often contain oil and humectants. Humectants, such as glycerol, serve as a replacement for water and are known to aid in drug transfer from keratin to an inert object[45]. Oils can absorb cocaine or methamphetamine from the environment, concentrating it on the hair. Oils and humectants provide the chemical mechanisms to facilitate drug contamination of hair. Hair washing every 1-2 weeks, due to the natural dryness and texture, is common with African hair [47].

To test the hypothesis that ethnic hair-care products would increase the uptake of external contamination, three classes of materials were selected: (1) those containing petroleum jelly as exemplified by Blue Magic[®] Conditioner and Hair Dress, (2) those containing oil plus a humectant (such as sorbitan sesquioleate and panthenol) as exemplified by BB[®] Oil Moisturizer Hair Lotion with Castor Oil and Aloe, and (3) those containing anionic polymers (Carbomer) as exemplified by Isoplus[®] Styling Gel. The latter two products have water listed as the first ingredient. In addition they contain hygroscopic materials. Products containing anionic polymers could enhance the uptake by remaining on the hair after washing and providing sites for ion exchange with the basic drugs similar to those sites thought to be present in hair[48].

Contamination procedures in this research were designed to simulate a speck of drugs in powder or smoke being attracted to cosmetically treated hair and then combed (or rubbed) through the hair later. Both could simulate a hair dresser applying a cosmetic product with hands contaminated with drug residue.

Two decontamination methods were tested. The first method used shampoo to simulate hair washing during personal hygiene. Cut hair was used and was handled in a tube. There was a concern that the shampoo solution would dissolve the drugs, swell the hair, and enhance the penetration – producing results consistent with exposure of hair to solutions of drugs rather than enhancement due to the cosmetic treatment. In normal hygiene, shampoo can be left on the hair for variable times (making a drug exposure solution) before rinsing. The second method (similar to Kintz *et al.* [8]) used methylene chloride to remove the hair care product and the loosely bound drug without swelling the hair[49]. Three methylene chloride washes were followed by shampoo and water. In preliminary experiments at high drug concentrations (10 mg), both methods produced hair with similar amounts of drugs. The second decontamination procedure was followed for all the data presented here.

Figure 1 shows the amounts of cocaine and amphetamine found in hair contaminated with 1 mg of drug (ca. 1/50th to 1/100th of a single dose of cocaine[50]) as the powder rubbed on the treated hair. Results of drug contamination at 10 mg were approximately equal to those at 1 mg. The 10 mg data set is not presented. Figure 2 shows the amounts of cocaine and methamphetamine from hair contaminated with 100 µg of drug/mL administered concurrently with the cosmetic product. Both procedures and contamination amounts produce drug levels that greatly exceed the proposed hair cut-off concentrations.

Figure 1 - Cocaine and amphetamine levels from hair contaminated with 1 mg of powdered drug rubbed on the cosmetically-treated and non-treated hair. The levels found greatly exceeded (up to 1000x) the SAMHSA proposed guidelines for a cut-off of 0.5 ng/mg (cocaine or methamphetamine) to report a hair sample positive[51].The SAMHSA guidelines were proposed in 2004 for U.S. Government regulated testing of hair but are not yet adopted. For “safety-net” testing (basically a second chance testing often of the same sample), where the cut-off level is lowered to near the LOQ of the testing technology (0.02 ng/mg), the levels found exceed the cocaine cut-off level by a factor of 25,000x (for cocaine)[52]. The error bars are from n=2. The blanks (product but no drug) have been subtracted from the data presented.

Figure 2 - Cocaine and amphetamine levels from hair contaminated with 100 µg of drug/mL mixed in the cosmetic product. The SAMHSA proposed cut-off of 0.5 ng/mg (cocaine or methamphetamine) is indicated by the line. The blanks (product but no drug) have been subtracted from the data presented. At this level all the petroleum jelly treated hair samples were below the cut-off for methamphetamine.

The relatively small amounts of drug used to contaminate these hair specimens produced contaminated hair that greatly exceeded the cut-off level to be considered positive. Forensic drug chemists, working under controlled laboratory safety conditions, have shown drug contamination in hair[25]. The hands of a cocaine or methamphetamine smoker or nasal insufflator could be less pristine. Hours later, studies have shown, these drugs remain and are easily transferred[53,54] even from the public areas of airports[55]. Since cocaine and methamphetamine abound in the environment[23], researchers have turned to metabolites to help distinguish use from

exposure, with the presumption that metabolites originate only from the metabolism of the individual being tested. Environmental contamination contains metabolites of cocaine and methamphetamine. While we observed neither amphetamine (from methamphetamine) nor benzoylecgonine (from cocaine) in our limited time exposures, “metabolites” are known to be generated inside the hair as chemical decomposition products over time [56]. Cocaethylene, once thought to form only in the liver when cocaine and ethanol are consumed simultaneously [57], is subject to reevaluation in light of studies including Gareri and co-workers concluding that fatty acid ethyl esters formed *in vitro* from hair care products [58], chemically comparable to transesterification of cocaine with alcohol in the hair care products producing cocaethylene *in vitro*.

The mechanisms by which a drug user’s hair incorporates drugs and metabolites include penetration from the subcutaneous follicular and external shaft zones. External moisture from sweat permeates and swells the hair’s diameter [59], carrying dissolved drugs and metabolites into the hair matrix where they bind, decompose, and are not easily removed.

Contamination arising from the sweat of another, for example a drug user, is especially problematic for the interpretation of hair testing results because sweat contains metabolites. Due to direct or secondary transfer (from a significant partner or barber/hair stylist), the presence of metabolites does not necessarily identify active drug use by the individual being tested. For sweat to contaminate hair efficiently, it would need to be transferred to the hair and the hair hydrated. Most prior exposure studies used low concentrations of drugs and limited the exposure to a few hours [48]. With sufficiently short exposure times and concentrations, drug may be incorporated, yet it may not be completely removed during decontamination. Nevertheless, the hair may be reported negative because it falls below a cut-off level [22]. Longer exposure times or higher concentrations produce externally contaminated hair that passes all laboratory criteria and would be considered hair from a drug user [27, 48]. Time, concentration, and temperature affect diffusion. With more drug, less time is necessary to achieve a specific level of drug incorporation. Conversely, lower drug concentrations require longer exposure times to reach the same drug level in the hair. Humectants, present in some hair-care products, effectively bathe contaminated hair in solutions of drugs for as long as the product is present, which can be weeks [47]. These hair-care products make contamination more efficient (and thus more probable), either for lower quantities of drugs or shorter contamination times because they supplement moisture from sweat or hygiene.

In our experiments, products with humectants caused more drug uptake compared with oil-only based products which do not have the capacity to hydrate hair. The enhancement is greater by orders of magnitude than the effects of hair damage or hair color, the focus of the majority of prior research. Ethnic hair care products are formulated with these materials due to the dryness of African hair [46]. However, any hair care products – those that hydrate the hair and bind drugs – should show this effect. Future research could explore individual classes of hair product components to confirm the

mechanism of drug enhancement-- concentration of the drug in the oil and hydration of the hair with humectants. Individuals using such products over lengthy exposure times between hair washing will increase the likelihood of a false positive hair test. The greater extent that African populations use these kinds of products may explain the disparity in hair positive rates in African populations compared to other populations.

Conclusions

Classes of ethnic hair care products containing water and humectants enhanced cocaine and methamphetamine uptakes by up to 100 fold over products that are exclusively oil based. Race or hair color made little difference in the enhancement. Less than 100 µg of drug/mL of product would be sufficient to make one appear to be a heavy drug user. The use of hair care products may be a major factor in the "cultural bias" observed with hair testing.

Acknowledgements

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