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Influence of Pigmentation on the Codeine Content of Hair Fibers in Guinea Pigs*

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ABSTRACT: Tortoise shell guinea pigs ($n = 7$) were administered codeine (1 mg/mL codeine-base) in their drinking water for 3 weeks. Black, reddish-brown and white hair was collected separately from each animal before and after treatment. The hair samples were analyzed by GC/MS. The experiment showed positive results for all hair fibers with large individual variability of drug incorporation. Low drug intake resulted in small differences of the drug content in hair fibers different in color, whereas in cases of high drug intake a strong influence of hair pigmentation on the analytical results was observed. The highest drug content was always found in black hair samples, non-pigmented hair showed the lowest drug concentrations and the drug content in reddish-brown fibers was less than in black hair samples from the same animal. From the results it was concluded, that eumelanins rather than pheomelanins are the decisive factor for codeine-melanin binding in hair and the amount of drug intake was suggested to determine the relevance of hair pigmentation on the analytical results.

KEYWORDS: forensic science, forensic toxicology, hair, hair analysis, drug-melanin binding, hair pigmentation, guinea pig

Pigmented tissues have been found to accumulate various chemically and pharmacologically unrelated compounds and to keep them bound for long periods (1). Occular toxicity caused by drug-induced retinal lesions, ototoxicity due to melanin binding drugs suspected for hearing loss and presbycusis as well as neurological disorders suggested to result from drug-induced lesions in pigmented nerve cells are well known in medical practice (2–5). The heterogeneity of substances with melanin affinity is enormous. Quite different categories such as neuroleptics e.g., haloperidol (6) or phenothiazine derivatives (7), tricyclic antidepressants e.g., imipramine (8), benzodiazepines e.g., flunitrazepam (9), β-agonists e.g., clenbuterol (10), amphetamines and alkaloids such as cocaine (11–13) are already known to accumulate in melanin containing tissue, hair being not an exception.

The wide range of hair color is produced by two major types of pigments. It is widely accepted that light colored hair contains predominantly pheomelanins in contrast to the eumelanin pigment usually present in dark hair. Both pigment groups are produced under genetic control in melanocytes in the hair root. The melanin granules are transferred to the surrounding matrix cells as they migrate into the keratinization zone. One of the most prominent features of melanin pigments is their existence as particles in nature. Synthetic melanins have proven to be valuable for studying basic physicochemical properties of melanins and characteristics of drug-melanin binding (14,15). However, it seems important to realize that extrapolation of findings in synthetic melanins to the particle nature of melanoproteins in hair requires caution. Animal studies may offer a more suitable model to study the influence of hair pigmentation on drug incorporation, because differentiation and keratinization processes and melanogenesis are very similar in mammals, although the final hair fibers differ in morphology. Most of the animal studies were performed to compare the drug content in white to black/brown hair fibers. For several drugs higher concentrations in pigmented than in non-pigmented hair have been reported (11,16–20). However, in hair analysis the situation is more complex due to the wide range of shades in hair color. The specific aim of the present study was to investigate the influence of eumelanins versus pheomelanins versus deficient melanin on the drug concentration in hair fibers. Tortoise shell guinea pigs were chosen as a model, for black (mainly eumelanin-), reddish-brown (mainly pheomelanin-containing hair) and white (melanin absent or deficient in the fibers) areas are present on the same animal avoiding differences in pharmacokinetics due to an animal-to-animal variability.

Material and Methods

Material

Codeine-d_3 was purchased from Radian Corp. (Austin, TX, USA). PBS-tabs and arylsulfatase (12 units/mL), β-glucuronidase (60 units/mL) were from Merck (Darmstadt, Germany). Pentafluoropropionic anhydride (PFPA) and 2,2,3,3,3-pentafluoro-1-propanol (PFPOH) were obtained from Aldrich Chemie (Steinheim, Germany).

Chromabond® C18 ec SPE columns (200 mg, 3 mL) were from Macherey & Nagel (Dueren, Germany). Reagents used were of analytical grade and purchased from Sigma, München, Germany, unless otherwise specified.

Instrumentation

The analyses were performed using a Hewlett-Packard 5971A mass selective detector operated in electron impact-selected ion monitoring (EI-SIM) mode equipped with a Hewlett Packard 5890 Series II gas chromatograph, a Hewlett-Packard 7673 auto sampler

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and Hewlett-Packard B.02.04 Chem Station software installed at an IBM-compatible 486 PC. Chromatographic separation was achieved on a HP-1 fused silica capillary column (12 m by 0.2 mm ID, 0.33 μm film thickness) from Hewlett-Packard (Waldbronn, Germany) using helium as the carrier gas (flow rate: 2 mL/min). Temperature program started at 70°C for 2 min, increased by 30°C/min to 200°C, by 10°C/min to 215°C, then by 30°C/min to 300°C. The final temperature was held for 10 min. The injector temperature was maintained at 260°C. The GC/MS-interface temperature was 280°C.

Drug Analysis

Hair samples (70–100 mg) were washed for three times with 10 mL methanol for 5 min. The washing solutions were evaporated under nitrogen at 60°C. The residues were dissolved in 1 mL TD_x-buffer. Screening the washing solutions for opiates was done by an immunoassay (TD_x-system, Abbott, Wiesbaden, Germany). The third wash gave negative results in all cases. The hair samples were pulverized in a ball mill (Retsch, Haan, Germany). Portions of hair powder (10–20 mg) were extracted by the standard procedure published by Moeller et al. (21). Briefly, to 10 mg of pulverized hair, 2 mL of acetate buffer pH 4.6, 300 ng of internal standard and 60 μL of arylsulfatase/β-glucuronidase were added. The mixture was incubated at 42°C for 1.5 h. After incubation, the mixture was neutralized with sodium bicarbonate and applied to the preconditioned Chromabond C18 ec SPE-column. The column was washed with 3 mL of distilled water, 3 mL of 5% sodium bicarbonate and again with 3 mL of distilled water. The analytes were eluted with acetone/dichloromethane (3:1, v:v). The organic phase was evaporated under nitrogen at 60°C. Derivatization was performed with 100 μL of PFPA and 75 μL of PFPOH at 60°C for 30 min. After evaporation under nitrogen at 60°C, the residue was dissolved in 100 μL ethyl acetate and 1 μL was injected in the splitless mode.

Experimental Design

Tortoise shell guinea pigs (n = 7; body weight: 305–430 g; single metabolic-cage housing) were administered codeine (1 mg codeine base/mL) in their drinking water for 3 weeks. The drinking bottles were equipped with a suction system. Water intake was monitored by weighing water bottles daily. Before drug administration the hair along the vertebral region from the head to the back had been removed by an electrical shaver and the shaved areas had been surrounded with a permanent marker. 7 days after drug administration had finished, the regrown hair fibers were collected from these sites by shaving. Black, reddish-brown, and white hair samples of each animal were collected separately. Hair analysis was performed for by GC/MS as described above. Blank samples, as well as spiked samples of different colored animal hair, were included. The tests were run in duplicate, values given are mean values. The national law on the care and use of laboratory animals was followed, the experiments were licensed by the government of Rheinland-Pfalz (177-07/941-12).

Results

The drinking behavior of the animals under investigation was characterized by a more or less highly frequent sucking. Therefore a low, but constant codeine level in the systemic circulation can be presumed during the experiment. All hair samples tested were positive for codeine showing wide variability of drug incorporation. The highest drug content was always found in black hair samples, non-pigmented hair showed the lowest drug concentrations, and the drug content of reddish-brown fibers was less than in black hair from the same animal (Fig. 1). Especially for pigmented fibers, there was an increase in codeine concentration with increasing amounts of codeine ingested. In cases of high drug intake a strong influence of hair pigmentation on the analytical results was observed (Table 1). However, low drug intake resulted in small differences of the drug content in hair fibers different in color. In three animals, which ingested total doses of codeine less than 400 mg/kg, the content of codeine in regrown hair fibers appeared less dependent on pigmentation. The concentration of codeine in white hair was always lower than 20 ng/mg, whereas the concentration in black fibers rose to concentrations greater than 70 ng/mg hair, when the oral intake had increased to doses higher than 50 mg/kg per day and total intake exceeded 1 g/kg. Because of the small number of animals investigated, statistical analysis of the data can not be valid and was omitted in this pilot study.

Discussion

The present study demonstrated an influence of hair pigmentation on the analytical results of codeine in guinea pig hair fibers. Drug content in hair was found to be dependent on the amount of drug incorporated as well as on the nature of hair pigments predominantly present in the particular hair sample. From the results it can be concluded that eumelanins rather than pheomelanins are the decisive factor for codeine-melanin interactions in hair, because drug concentration in reddish hair was always lower than in black hair fibers of the same individual. An additional result of the present study is the finding that hair components different from melanin granules must be sources for binding of codeine molecules in hair fibers due to the fact that non-pigmented hair showed some drug incorporation. This is in accordance with the biochemical concept of drug incorporation, which considers the cell membrane complex, the melanin granules, the intermediate filaments/intermediate filament associated proteins of keratinized hair to be the main drug localizations (22) and explains drug incorporation into non-pigmented hair. Our results do not support the hypothesis that the main localization of drug molecules within the hair shaft is the medulla (23), for guinea pig hair fibers regardless of color, as most animal fibers, are heavily medullated.

Nakahara and coworkers (24) have already pointed out that the structural and the physicochemical properties as well as the melanin affinity of a particular drug substance influence its incorporation into hair. Rollins reported on codeine incorporation into white and pigmented rat hair under high dosage. He mentioned preliminary animal experiments with phenobarbital that showed no difference in drug uptake into non-pigmented compared to pigmented hair (20). A high dose of phenobarbital certainly bears a high risk of lethal outcome and is unlikely to have been administered. Therefore, a low dosage as well as a low melanin affinity of phenobarbital compared to other drugs might have influenced the findings in the rat hair fibers. In our pilot study striking differences in the codeine content of different colored hair samples were not observed in animals with low dose codeine ingestion. However, under high drug intake obvious differences in codeine concentrations resulted in white, reddish-brown and black hair samples collected from a particular individual. Dose dependent drug-melanin binding and thus dose dependent influence of hair pigmentation on drug content in hair fibers was also observed for methadone (18), clenbuterol

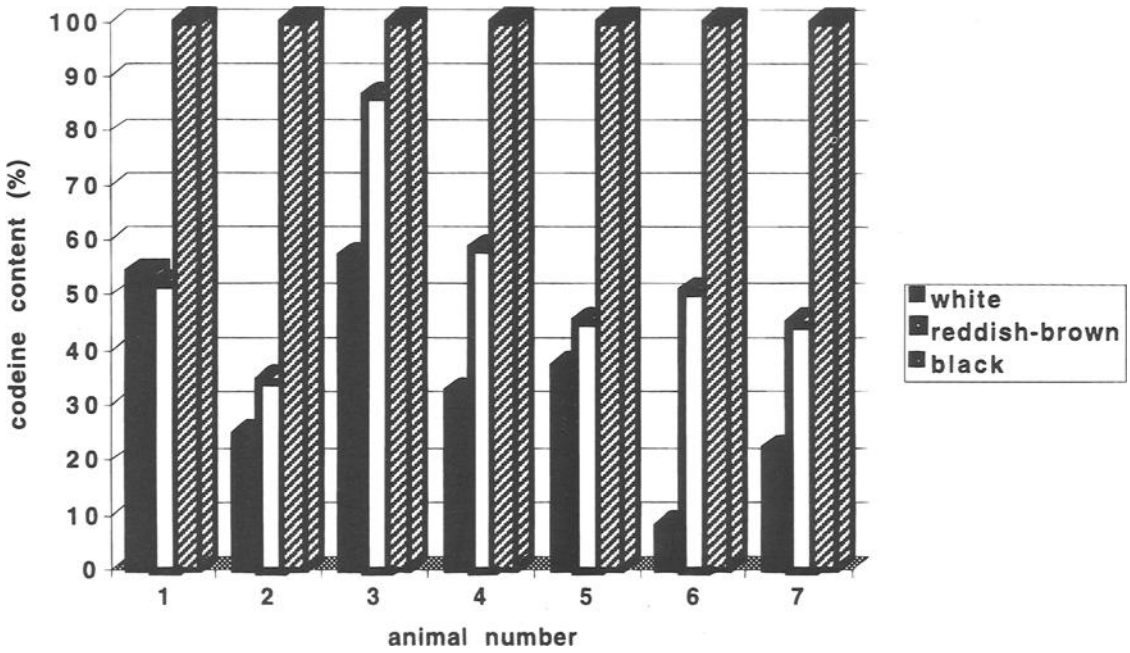

FIG. 1—*Comparison of the relative amount of codeine content (%) in different colored hair samples collected from tortoise shell guinea pigs. Amount of codeine in black hair fibers = 100 %.*

(25) and ofloxacin (26). Similar to our findings an increasing drug intake resulted in increasing differences of drug concentrations in white and black hair. In vitro studies with synthetic melanins and melanin granules revealed that drug binding to melanin showed distinct differences for various substances and even differences for the same substance due to heterogeneity of the binding sites of the melanins, but generally follows Langmuir adsorption theory (3,12,14,15,24). This is in accordance with our preliminary findings and also confirms that eumelanins rather than pheomelanins are the decisive factor for drug melanin binding in hair, because synthetic melanins and Sepia melanin, frequently used for in vitro binding studies, are pure eumelanins and large polyanions suggesting that ionic binding might be important. The molecular binding mechanisms are not known in detail. Beside ionic attractions, hydrophilic interactions are discussed and it is believed that a type of adsorption due to charge transfer reactions as well as electrostatic forces may occur (27,28).

For tortoise shell guinea pig hair fibers quantitative analysis of eumelanin and pheomelanin confirmed the high eumelanin content of black hair fibers (0.85% w:w) as well as the high pheomelanin content of reddish-brown hair samples (0.77% w:w) (29). However,

TABLE 1—*Codeine content of regrown hair fibers collected from different colored regions of tortoise shell guinea pigs.*

Tortoise Shell Guinea Pig Number	Total Dose of Codeine-Base Ingested mg/kg	Codeine Content of Different Colored Hair Fibers [ng/mg Hair]		
		white	reddish-brown	black
1	254	2,7	2,6	5,0
2	305	3,3	4,6	13,3
3	328	2,9	4,4	5,1
4	468	9,1	16,4	28,1
5	678	18,6	22,6	50,2
6	1164	6,1	38,4	76,2
7	1186	15,9	32,4	72,4

studies demonstrated that human hair, regardless of color, contains various portions of both eumelanins and pheomelanins. The ratios of these main groups of melanins show great inter- and intraindividual variability. The visual differentiation of hair color does not always reflect the predominant type of melanogenesis. In Caucasian hair "mixed melanogenesis," pheomelanogenesis and eumelanogenesis, is more frequent than pure pheo- or eumelanogenesis (30–33).

In addition, the suspectibility of eumelanins and pheomelanins towards chemical and physical agents seems crucial (34). Chedekel et al. (35) and other investigators have stressed that pheomelanins are degraded by irradiation with UV and visible light and a number of reports have already demonstrated that eumelanins also participate in oxidizing and reducing reactions (36,37). Melanin-associated drug molecules and thus drug concentration in hair fibers may also be affected. Only a few studies discuss drug stability in hair indicating that drug content may decline by time (38) or may be altered by cosmetic treatments (39–41), which mainly attack the hair pigment but also hair lipids and hair proteins.

In conclusion, it must be stressed that many questions concerning drug-melanin binding in hair remain unanswered. It would seem that research has just begun. In hair analysis it is important to clarify whether, for different drugs, the influence of pigmentation on the drug content in hair fibers, as seen in experimental animal studies demonstrating conditions of high drug intake, fast hair growth and in hair fibers of different morphology from human hair, is relevant or can be neglected for certain particular drugs.

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