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Opiate Concentrations in Human Head, Axillary, and Pubic Hair

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ABSTRACT: The concentrations of morphine and codeine were investigated in hair from the head, axillary and pubic regions obtained from 12 fatal heroin cases. Hair preparation involves a decontamination procedure in dichloromethane at 37°C for 15 min, solubilization in sodium hydroxyde at 100°C for 5 min, neutralization with hydrochloric acid and centrifugation. After extraction in chloroform-isopropanol-*n*-heptane (50:17:33; v/v) at pH 9.2, drugs were derivatized with BSTFA + 1% TMCS and separated on a 12-m BP-5 capillary column. Quantification was done by GC/MS using selected ion monitoring. The highest morphine concentrations were found in pubic hair (0.80 to 41.34 ng/mg), followed by hair of the head (0.62 to 27.10 ng/mg), and axillary hair (0.40 to 24.20 ng/mg). Codeine was also detected in all samples, and the codeine-to-morphine ratios ranged from 0.069 to 0.273. The differences observed in drug concentration in the 3 types of hair are discussed in the light of the existing literature.

KEYWORDS: toxicology, opiates, hair, analysis, drug testing

Although it has been a long time since the evidence first appeared in the literature, only recently has particular attention been devoted to the use of hair as a sample for detection of illicit drugs.

For example, morphine can be easily detected in biological fluids only within a few days of heroin intake, and the morphine levels determined are strongly influenced by the dose and the time of the last injection. In contrast, hair appears to be a particularly interesting substrate for the investigation of chronic drug abuse. The drug passes from the circulating fluids into the hair and remains firmly bound there. For identifying and quantifying opiates in human hair, several analytical methods have been successfully employed, including fluorescence polarization immunoassay [*1*, *2*], radioimmunoassay [*3–7*], liquid chromatography [*8*] and gas chromatography coupled to mass spectrometry [*9–12*].

However, data on the occurrence of the drugs in axillary and pubic hair are not yet available.

In this study, we investigated the presence of morphine and codeine in hair of the head, axillary and pubic regions obtained from 12 fatal heroin overdose cases.

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Materials and Methods

Materials for Examination

Simultaneously collected hair samples from the head, axillae and pubes were obtained from 12 male subjects, aged 19 to 34 years, deceased from fatal heroin overdose.

Hair samples, weighing at least 30 mg, were cut as close as possible to the skin (from the posterior vertex in case of hair of the head). In cases of long hair, only 6 cm of the proximal end (near the root) were analyzed. The hair was decontaminated by washing the specimen one time in 5 mL dichloromethane for 15 min at 37°C. This procedure was enough to remove external contamination. In the fact, the GC/MS analysis of a second wash remained negative, although the former was positive.

Sample Extraction

The protein matrix of the hair was destroyed by incubation in 1 mL of 1 M sodium hydroxide solution for 5 min at 100°C. After neutralization with 1 mL of 1 M hydrochloric acid and centrifugation, the homogenate was extracted with 10 mL of chloroform/isopropanol/*n*-heptane (50:17:33; v/v) under alkaline conditions (1 mL of phosphate buffer at 1 mol/L and pH 9.2) with levallorphan (10 mg/L) as an internal standard. After agitation and centrifugation, the organic phase was purified by an additional acid extraction (5 mL of 0.2 M hydrochloric acid) and aqueous layer was re-extracted with 2 mL phosphate buffer, 0.5 mL concentrated ammonia solution, and 5 mL chloroform. After agitation and centrifugation, the organic phase was taken off and evaporated to dryness at 45°C in a Speed Vac Concentrator. N,O,-bis(trimethylsily)trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) (40 μL) was added to the dry extract, which was sealed and heated at 70°C for 20 min. A 4 μL portion of the derivatized extract was injected into the GC column [*13*]. Blood, urine and liver samples were analyzed using the same procedure, previously described [*13*].

GC/MS Method

The GC system consisted of a Perkin Elmer (8500) chromatograph with an Ion Trap Detector (ITD), operated at 70 eV with an ion source temperature of 210°C. The electron multiplier voltage was set at 1350 V.

The flow of carrier gas (helium, purity grade N55) through the column (BP-5 capillary column, SGE, 5% phenyl-95% methyl siloxane, 12 m × 0.22 mm i.d.) was 1.8 mL/min. The column oven temperature was programmed to rise from an initial temperature of 60°C to 280°C at 30°C/min and kept at 280°C for the final 3 min. Splitless injection with a split valve off-time of 1 min was employed. The ions monitored for levallorphan, codeine, morphine, and 6-monoacetylmorphine along with their respective retention times were as follows: levallorphan, m/z 355, 9.24 min; codeine, m/z 371, 9.58 min; morphine, m/z 429, 10.22 min; 6-monoacetylmorphine, m/z 399, 10.37 min. The assay had a > 75% extraction efficiency for all components; the limit of detection was approximately 0.1 ng/mg with a signal/noise > 10 for each analyte. Calibration curves were prepared with homogenates of hair of drug-free controlled subjects, collected among laboratory personnel. Quantification was done by plotting peak area ratios (drug/IS) against the concentration of standards to produce standard curves and by comparing the results for the case samples with the curves. The linearity of the method was established with 6 single standards of a hair mixture of codeine and morphine (0.1, 0.5, 1.0, 5.0, 10.0 and 50.0 ng/mg). The correlation coefficients were 0.997 and 0.996 for codeine and morphine, respectively. The interday precision (expressed as the relative standard deviation at 1.0 and 10.0 ng/mg) was in the range 4.7 to 9.2% for both drugs.

Results and Discussion

In the 12 cases, fatal heroin overdose was clearly confirmed by the presence of 6-monoacetylmorphine in the urine (Table 1) which is generally considered to be specific of heroin exposure [*12*,*13*]. Morphine and codeine were detected in all hair samples. In each case the highest opiate concentrations were found in pubic hair, followed by hair of the head and axillary hair. The concentrations measured are reported Table 2. The morphine and codeine values found in the different kinds of hair of each subject are presented in Fig. 1. Concentrations measured in hair of the head were in the range of previous reports [*1*–*12*]. No correlation between blood and hair concentrations could be established. This is not surprising, since the blood concentration represents a measure at the present moment, and hair concentration, chronic accumulation. Nevertheless, the presence of drugs in hair was particularly interesting for the medical examiner as it was possible for him to rule the cause of death to be an overdose occurring in a chronic drug abuse situation. These findings were also suitable for criminal inquiries.

A particular problem in the detection of opiates is evaluating whether morphine has resulted from heroin or morphine consumption or from a misuse of medication containing codeine. Small amounts of morphine (about 5 to 10%) are produced from codeine by metabolic demethylation, but almost all illegally sold heroin contains acetylcodeine as an impurity of opium which is quickly deacetylated to codeine after intake. In both cases, codeine and morphine co-exist. It is generally admitted that if the morphine level is clearly higher than the codeine level in the examined hair sample, heroin or morphine abuse is highly probable [*10*]. In the 12 reported cases, codeine to morphine ratios in hair of the head ranged from 0.069 to 0.273.

Recently, the differentiation of heroin users from individuals exposed to other sources of morphine alkaloids, was achieved by identifying directly heroin and 6-monoacetylmorphine [*12*]. These findings are particularly important for justice applications. Since a strong alkaline procedure for homogenization was used, no mono-acetylmorphine or heroin were identified, probably due to hydrolysis.

The axillary and pubic hair grows at a rate of up to 0.37 mm per day [*14*] and measures between 1 and 60 mm in length. In order to compare concentrations, we chose to analyze only 6 cm of the head hair. The head hair grows 0.39 to 0.44 mm per day [*15*] and, if left uncut, can grow more than 100 cm.

TABLE 1—*Levels of morphine, codeine, 6-monoacetylmorphine in blood, urine, and liver in the 12 fatal heroin overdoses.*

Sample	Morphine (mg/L or mg/kg)	Codeine (mg/L or mg/kg)	6-monoacetylcodeine mg/L
Blood	0.06–0.81	0.01–0.24	ND
Urine	0.04–13.21	0.01–3.44	0.11–2.47
Liver	0.08–1.37	0.02–0.56	ND

ND: not detected.

TABLE 2—*Opiate concentrations in hair of the head, axillary and pubic regions.*

Sample	Morphine (ng/mg)	Codeine (ng/mg)
Head	0.62–27.10	0.15–1.87
Axillae	0.40–24.20	0.12–1.56
Pubis	0.80–41.34	0.22–2.34

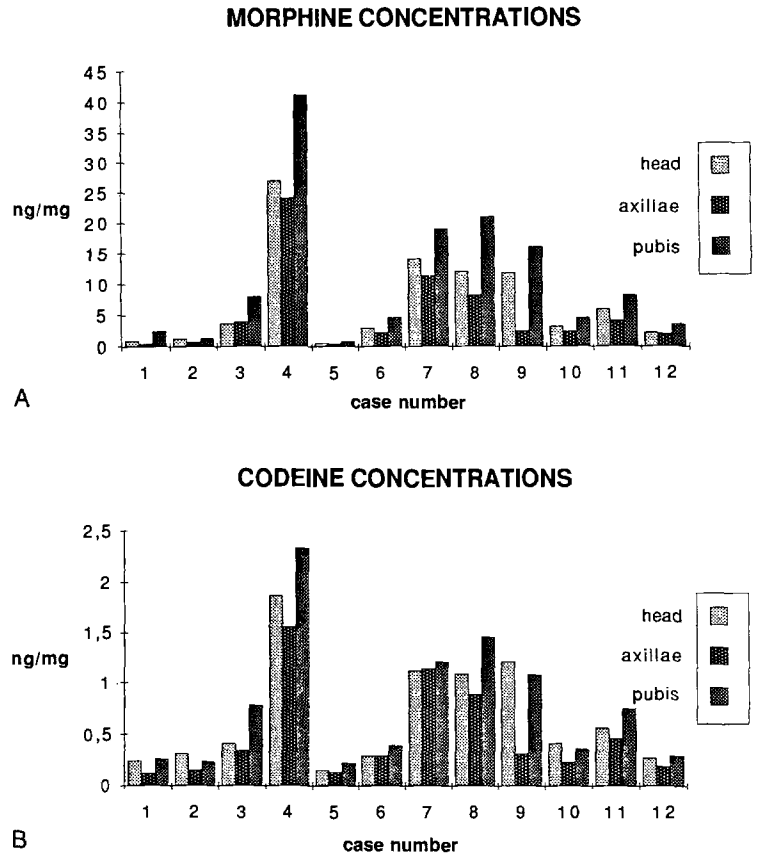

FIG. 1—*Opiate concentrations in hair of the head, axillary and pubic regions; A: morphine; B: codeine.*

In the literature, comparison of the concentrations in different kinds of hair provides controversial results: for methadone, cocaine, phenobarbital and morphine the highest values were found in the axillary hair, followed by the pubic hair and the head hair [*16, 17*] and for cotinine, in the pubic hair, the axillary hair and the hair of the head [*18*]. Our findings are not in accordance with these results. The significant differences of the drug concentrations in axillary hair and head hair were probably the result of a better blood circulation in the axillae, a greater number of apocrine glands, and a reabsorption into the skin of the drug secreted by perspiration [*16,17*]. To the best of our knowledge, it is well admitted that drugs are present in the sweat [*19*] but their reabsorption and their transfer in the axillary hair are hypothesis to be investigated.

In our experience the difference between the opiate concentrations found in the hair of the head, axillary and pubic region could be explained by different growth rates of the hair. Pubic hair, with a low growth rate, may be able to concentrate drugs more than hair from the head. Moreover, it is possible that morphine secreted in perspiration and sweat may be incorporated in hair, or on the other hand, eliminated by absorption onto clothing. Similarly high levels of drug present in urine may contaminate pubic hair. (Note—it was demonstrated that after incubation during four days at room temperature

in a highly concentrated solution of cocaine and methadone, 200 mg/mL that no drug was found in drug laced hair samples [*20*]). The decontamination procedure is absolutely necessary and replicate wash procedures were shown sufficient to remove external contamination.

In conclusion, our results suggest that the concentration of drugs in the hair of the head collected from the posterior vertex provides a good historical record of drug exposure. In comparison with hair from the head, concentrations of drug can be overestimated by using pubic hair and under evaluated by using axillary hair. Although we offer some explanation, these are intuitive and not based on extensive research. Other explanations may be equally valid.

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