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Short Communication

An investigation of the effect of washing upon the morphine content of hair measured by a radioimmunoassay technique

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Introduction

The spectroscopic detection of trace levels of toxic elements in human hair is a well established analytical procedure. However, the biological significance of the results obtained has been the subject of some controversy [1, 2], because it has proved to be virtually impossible to distinguish between trace metals incorporated into the hair via nutritional routes from those embedded through external contamination. Thus the analysis of hair as an indicator of the physiological effects of nutritional changes needs to be viewed with some caution.

In recent years the use of the hair matrix as a means of evaluating the problems of drug abuse has attracted much attention. In particular methods have been reported for the assay of morphine [3–6], methadone [7, 8], opiates [9, 10], cocaine [3, 11–13] amphetamines [14] and other illicit substances in the context of clinical and forensic investigation.

Protocols for the pretreatment of hair samples prior to analysis usually include a thorough wash procedure [3, 11, 12, 15, 16]. A variety of washing procedures have been described, that appear to be characteristic of the drug being analysed and the method of analysis employed. However, there appears to be very little information concerning factors governing the choice of the method of washing. In fact there is some doubt as to whether washing prior to assay is absolutely necessary. This lack of consensus or uniformity in the washing procedures has led to the use of shampoo [3], non-biological detergents such as dodecyl sulphate, in differing concentrations [12, 13] and organic solvents such as methanol [14], ethanol [7], acetone [4] and diethyl ether with dilute hydrochloric acid [5]. Typically a standard statement, "the hair was washed thoroughly prior to analysis", is included in the analytical method protocol for the removal of hair contaminants [3, 11, 12, 15, 16]. Due to the uncertainty regarding the requirements of pretreating hair samples prior to analysis, a systematic study has been performed in which the effects of various washing protocols on hair morphine levels have been determined. Hair samples were obtained from known heroin abusers and known non-abuser volunteers and the morphine levels determined by radioimmunoassay. The results obtained are now presented and their significance discussed.

Experimental

Apparatus

The radioactive counting was performed by means of an LKB-Wallac 1260 Multigamma II counter controlled by a RIACalc program operated on a Hermes PC110 (Pharmacia LKB Biotechnology, Finland), online to an Olivetti printer. The LKB-Wallac 1260 Multigamma II is a microcomputer controlled multi-detector gamma counter designed for simultaneously

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counting 12 samples of a gamma emitting isotope. It is compatible with standard 12 × 75 mm tubes used in the morphine assay, the protocol of which is installed onto the computer prior to analysis. Mixing procedures were carried out with the aid of a vortex mixer.

Chemicals

The reagents were purchased from several sources. Ethyl alcohol (Absolute AR) was obtained from James Burrough Ltd (Essex), acetone (Analar) and dodecyl sulphate (concentration used 1%) were obtained from BDH Chemicals (Poole, Dorset), and mild shampoo (concentration used 2%) was obtained from Safeway Foodstores (Aylesford, Kent). The distilled water used during the investigation was as routinely used in the laboratory. Hydrocholoric acid (0.1 M), sodium hydroxide (1.0 M) and phosphate buffer (Sørensen) [17] were prepared from reagents obtained from BDH Chemicals.

The analysis for hair morphine was carried out by solid-phase radioimmunoassay using a Coat-a-Count system (DPC, Los Angeles, CA, USA), consisting of morphine antibody-coated tubes, ^{125}I-labelled morphine and morphine calibrators in morphine-free human urine. The adopted method uses a solid-phase antiserum highly specific for morphine, with minimal cross reactivity to morphine-3- or 6-glucuronides (<0.17%), codeine (<0.10%), dihydrocodeine (<0.06%) and other opioids such as methadone. Significant cross reactions occur with normorphine (9.6%), a minor metabolite of morphine and nalorphine (27%), a narcotic antagonist (information sheets supplied by DPC, Los Angeles, CA, USA). The Coat-a-Count solid-phase ^{125}I radioimmunoassay is designed for the quantitative measurement of morphine in urine, adapted for the analysis of hair morphine concentrations.

Sample collection

Hair samples were taken, as full length strands, from known heroin abusers and non-abuser volunteers. The hair was cut as close as possible to the scalp, tying the cut end firmly with cotton for future reference. Each sample was stored in separate plastic bags until required for use.

Sample preparation

Each hair sample was divided into five full length sub-samples, which were subjected to different wash protocols prior to analysis, as follows:

(i) unwashed;
(ii) ethyl alcohol then distilled water, 10 ml each × 3;
(iii) acetone–distilled water (50:50, v/v), 10 ml each × 3;
(iv) shampoo (2% in warm distilled water), 10 ml × 3, followed by warm distilled water rinse, 10 ml × 10;
(v) dodecyl sulphate (1% in warm distilled water), 10 ml × 3, followed by warm distilled water rinse 10 ml × 10.

All washed samples were appropriately labelled and left to air dry, free from dust. The weight of each sample (mg), was determined prior to extraction.

Extraction

Each sample was fully immersed in 1 ml of 0.1 M hydrochloric acid and incubated overnight at 55°C. Neutralization with 1.0 M sodium hydroxide was followed by buffering to the required pH for analysis using 0.1 M phosphate buffer (pH 7.0). The adopted procedure was in the ratio 0.5 ml sample extract–0.05 ml 1.0 M sodium hydroxide–0.45 ml 0.1 M phosphate buffer.

Sample analysis

The prepared extracts were analysed for morphine content by radioimmunoassay (Coat-a-Count; DPC, Los Angeles, CA, USA), summarized in the following way.

Fifty microlitres of calibrators, controls and hair sample extracts were incubated in antibody coated tubes with 1 ml of ^{125}I-labelled morphine tracer for 1 h at room temperature. The tubes were decanted thoroughly and the radioactivity counted. A calibration curve was constructed to cover a range from zero to 500 ng ml^{-1} using morphine calibrators in morphine-free human urine. The radioactivity, measured as counts per minute, obtained from hair and quality control samples, was converted into ng ml^{-1} values from the computed calibration curve.

Results and Discussion

The results were calculated using a logit–log representation incorporating a smooth spline fitting algorithm, and following conversion, expressed as ng morphine/mg hair levels. Sample measurements were carried out in

duplicate using the radioimmunoassay method for morphine, which had previously been evaluated for specificity. The accuracy of the morphine radioimmunoassay was determined using a range of morphine standards prepared in blank urine. Over the range 0–250 ng ml^{-1} the observed values were between 97 and 107% of the actual concentrations.

Interassay relative standard deviations (RSD) at morphine levels of 3.0, 25.0 and 300.0 ng ml^{-1} were 9.5, 5.9 and 5.8%, respectively with corresponding standard deviations of 0.33, 1.4 and 16.4 at the same morphine levels (n = 5).

Intra-assay RSD at morphine levels of 3.0 and 300.0 ng ml^{-1} were 11.8 and 2.6%, respectively with corresponding standard deviations of 0.39 and 7.4 at the same morphine levels (n = 4). These were comparable to the stated performance characteristics of the system. (Technical information sheet supplied by Coat-a-Count; DPC, Los Angeles, CA, USA.)

The hair samples from the non-abuser group indicated the differences in hair morphine levels between non-abuser (detected range for all wash processes 0.010–0.080 ng morphine/mg hair) and known heroin abuse group (detected range for all wash processes 1.20–16.3 ng morphine/mg hair) to be analytically significant (note, previous experimental data have shown that levels up to 0.3 ng morphine/mg hair need to be neglected, A. Marsh, unpublished results).

The hair morphine levels (ng morphine/mg hair) detected in the heroin-abuser group following pre-analytical washing protocols are shown in Table 1. The hair sample from subject B and subject C were in fact from the same subject, but not the same analytical assay, so providing reproducibility data in respect of the extraction of morphine and its analysis by radioimmunoassay on a 'real' analytical sample. Comparison of the morphine levels (ng morphine/mg hair) detected in subjects B and C are shown in Table 2, and depict acceptable reproducibilities based on previous data and suggested performance characteristics stated by the kit manufacturer. The RSDs obtained (3.1–10.3%) show analytical reproducibility on all the pre-analytical wash protocols used.

All the results in this heroin-abuser group show a decrease in hair morphine levels on inclusion of a pre-analytical wash procedure, although it is not possible on statistical grounds to propose one wash method being better than another with respect to the analytical methodology. However, on the basis of the results obtained shown in Table 2, it could be proposed that particular care should be taken in the case of using shampoo to ensure thorough rinsing. The presence of detergent is known to cause interference in radioimmunoassay procedures which could explain the high RSD obtained (10.3%).

In the subjects studied, subject A showed a mean reduction of 27% in hair morphine content, subject B a mean reduction of 20%,

Table 1
Morphine levels in hair samples of known abusers

Washing procedure	Subject A ng morphine/mg hair	Subject B ng morphine/mg hair	Subject C ng morphine/mg hair	Subject D ng morphine/mg hair
None	16.3	9.2	8.8	1.8
Ethanol–water	11.8	7.2	8.0	1.6
Acetone–water	10.2	7.7	7.3	1.4
Shampoo–water	11.5	7.3	6.3	1.3
Dodecyl sulphate–water	12.9	6.9	7.0	1.2

Table 2
Reproducibility of morphine levels in a hair sample from a known abuser

Washing procedure	Subject B ng morphine/ mg hair	Subject C ng morphine/ mg hair	Mean value ng morphine/ mg hair	Standard deviation ng morphine/mg hair	RSD (%)
None	9.2	8.8	9.0	0.28	3.1
Ethanol–water	7.2	8.0	7.6	0.56	7.4
Acetone–water	7.7	7.3	7.5	0.28	3.1
Shampoo–water	7.3	6.3	6.8	0.70	10.3
Dodecyl sulphate–water	6.9	7.0	6.95	0.22	3.2

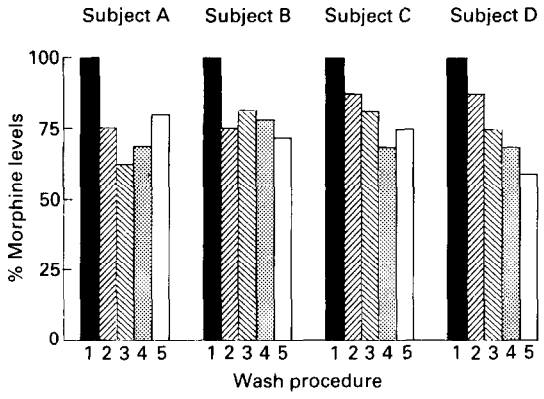

Figure 1
Per cent morphine levels detected using pre-analytical wash protocols, in known abusers. 1, no wash; 2, ethanol–water; 3, acetone–water; 4, shampoo–water; and 5, dodecyl sulphate–water.

subject C a mean reduction of 19% and subject D a mean reduction of 22% when pre-analytical hair washing was employed (Fig. 1).

The analytical significance of reductions of this order could affect the use of hair drug analysis in some areas to which it is perhaps best suited, such as provision of a 'true' historical record of drug abuse and claims of abstinence both from clinical and forensic viewpoints [18]. The omission of a pre-analytical wash procedure could lead to incorrect interpretation of results in cases of borderline levels and passive exposure to drug environments.

It was not possible to determine the significance of employing a wash procedure in the known non-abuser because all the results obtained were below the previously stated limit of 0.3 ng morphine/mg hair. This was not unexpected as the subjects were known non-abusers, not exposed to drug environments and who had not taken anything that may lead to false positive results, such as codeine, morphine containing medication or excessive consumption of poppy seed containing foodstuffs.

The results from the known abuser group suggest that drug molecules can be loosely bound to the hair structure, probably derived from external sources. Hair may be contaminated with drugs as a result of environmental exposure or adsorption onto the hair from perspiration, sebum, drug handling and other sources. This potential external contamination is perhaps particularly relevant with drugs such as cannabis, cocaine and heroin that are smoked. The importance of a pre-analytical wash to remove externally bound drug in hair phencyclidine analysis has been demonstrated [19].

It is possible that some internally bound drug may be removed if pre-analytical washing is carried out in certain organic solvents and acids, or if the hair is damaged through natural or chemical means. Contact of the hair specimen for any length of time, particularly with organic solvents and acids, may cause the hair structure to swell to an extent where leaching of drug from within the hair matrix could occur. Marigo *et al.* [20] have reported levels of up to 66% of the total morphine found in the hair can be removed by washing with diethyl ether and dilute hydrochloric acid.

On the present evidence it would seem to be prudent in hair drug testing, to employ a pre-analytical washing procedure to remove any material derived from external sources, which could lead to spurious results.

In conclusion it is apparent that pre-analytical washing is an important necessity in hair drug analysis in order to remove any material derived from external sources, which could lead to analytical contamination and possible misinterpretation of the results. The wash times in the present study were relatively short, so any dissolution occurring would be expected not to be excessive and likely to be confined to sources of external contamination. The physical and chemical properties of drugs are not condusive to their permanent embedding into hair structures through external sources [15].

Although drug molecules are not generally present in the environment, a simple pre-analytical wash can ensure that errors due to external sources are prevented. Although the present work was restricted to the determination of morphine in hair, similar considerations would be expected to apply to the assay of other substances incorporated into the hair matrix. However, it is apparent that more work has to be done to investigate the effect of washing time and procedure in order to ascertain the presence of loosely bound drug on the surface of the hair and establish some degree of uniformity in washing procedures used in the analysis of drugs in hair, including morphine.

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