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Challenging declarations of abstinence by the determination of morphine in hair by radioimmunoassay

A. MARSH*† and M.B. EVANS‡

†*Pathology Department, The Bethlem Royal Hospital, Monks Orchard Road, Beckenham, Kent, UK*
‡*Division of Chemical Sciences, University of Hertfordshire, College Lane, Hatfield, Hertfordshire, UK*

Abstract: Morphine extracted from dated hair segments and analysed by adaptation of a specific solid-phase ^{125}I radioimmunoassay for the measurement of morphine in urine has provided long term histories of the heroin usage of individual patients. Results expressed as ng morphine/mg hair are compared with a pre-determined cut-off level of 0.3 ng morphine/mg hair, from a known drug-free population (n = 21). Morphine concentrations in hair samples from known heroin abusers are found to be above the cut-off limit (range 0.6–9.4 ng morphine/mg hair), and suggest a possible correlation between heroin intake and concentration of morphine in hair. Intra-assay relative standard deviation (RSD), at morphine levels of 9.27 and 1.12 ng morphine/mg hair are 2.4 and 5.5%, respectively, and acceptable recoveries from drug-free hair spiked with morphine are also achieved. The developed segmental hair analysis regime has been used successfully to challenge a self-declaration of heroin abstinence. Routine cumulative urine screening was unable to provide such evidence. The potential for hair drug analysis is discussed.

Keywords: *Hair; segmental analysis; morphine content; radioimmunoassay; drug abuse history; abstinence challenging.*

Introduction

The self reporting of intake by drug abusers is generally unreliable. Screening for drugs of abuse is commonly carried out on urine specimens using well-established methods. However, the analysis of urine specimens for opiates merely indicates recent drug usage, and unless certain guidelines are followed [1], specimen collection can be prone to problems of adulteration, substitution and pre-collection abstinence, resulting in misleading results. Typically morphine, a metabolite of heroin, is readily detectable in urine for 1–3 days post-ingestion.

The testing of human hair for drugs of abuse is a developing science, methods having been reported for morphine [2–8], methadone [9, 10], opiates [11, 12], cocaine [2, 13–17], amphetamine [18, 19] and other illicit substances.

During the process of keratinization, traces of drug and its metabolites may become embedded in the hair structure, remaining there throughout its lifetime [12]. Analysis of the hair, therefore, can provide information concerning the presence of drugs in the body fluids at the time of keratinization. The human body is covered by hairs of differing types, each being formed in individual follicles. The rate and patterns of hair growth can vary with race, sex, age and body site [20]. However, scalp hair in the posterior vertex region grows at a relatively constant rate of 1 cm month^{-1}. Hence drug concentration along the hair shaft could reflect the degree of drug exposure and the distance from the scalp a measure of the time elapsed since exposure [21]. Hair analysis offers the possibility of determining a long-term drug abuse history in circumstances where perhaps established methods are unable to do so.

A segmental hair analysis regime has been developed, based upon radioimmunoassay measurements, which can be used to monitor drug abuse. By taking segments along the lengths of strands of hair and determining the morphine extracted from each segment, a history of long-term heroin abuse can be obtained. The pattern of an individual's drug usage can be examined in terms of it being constant, increasing, decreasing or indeed abstinent.

In particular, the procedure has been used to

* Author to whom correspondence should be addressed.

challenge declarations of abstinence from a heroin abuser undergoing out-patient treatment. Routine cumulative urine screening was unable to provide a clinically satisfactory drug history due to erratic attendance at the clinic over the periods in question. The segmental hair analysis was able to overcome this problem. The results of this work are now reported.

Experimental

Apparatus

The radioactive counting was carried out using an LKB-Wallac 1260 multi-gamma II counter controlled by a RIACalc program operated on a Hermes PC110 (Pharmacia LKB Biotechnology, Finland), linked on-line to an Olivetti DM282 printer. The LKB-Wallac 1260 is a microcomputer controlled multidetector gamma counter, designed for the simultaneous counting of 12 samples of a gamma emitting isotope. It is compatible with standard 12 mm × 75 mm tubes used in the morphine assay, the protocol for which is installed in the computer prior to analysis.

Weighing procedures were performed by means of a Gallenkamp Mettler H20 balance (Fisons Instruments, UK) and mixing was aided by means of a vortex mixer.

Sample, calibrator and blank additions were performed using an Oxford monosampler micropipetting system (8000 series), whereas the addition of the radioactive tracer was carried out by means of a BCL 8000 repetitive pipette and syringe (Boehringer Manheim, UK).

Chemicals

The reagents were purchased from various sources. Hydrochloric acid (0.1 M), sodium hydroxide (1.0 M), phosphate buffer (Sørensen) [22] and dodecyl sulphate were obtained from BDH Chemicals (UK). The distilled water was as routinely used in the laboratory. Morphine sulphate was obtained from Macarthys (UK).

The hair morphine analysis was carried out by solid-phase radioimmunoassay using a Coat-a-Count system (DPC, Los Angeles, CA, USA). This consists of morphine antibody-coated tubes, ^{125}I-labelled morphine and morphine calibrators in morphine-free human urine. The method uses a solid-phase antiserum highly specific towards morphine, with minimal cross reactivity towards 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. The system is designed for the quantitative measurement of morphine in urine, but has been adapted for the analysis of hair morphine concentrations.

Subjects

Subject F attended out-patient clinic weekly during July 1989–January 1990. Routine urine screening showed the presence of methadone and benzodiazepine at each attendance and intermittent detection of morphine from November 1989. There was no clinic attendance between January and mid-April 1990, when re-attending the subject claimed heroin abstinence since January 1990. There were no urine records for the previous 14 weeks or any other medical contact to substantiate the abstinence claim.

Subjects W, S and N were known heroin abusers used as positive controls. Non-abusers, known to be drug free, were used as negative controls.

Hair collection

Full length hair samples were taken at scalp level, from the posterior vertex region, (50–100 hairs per sample). The aligned cut ends were tied off with cotton for future reference prior to separate storage. All the subjects consented to a hair sample being collected.

Hair preparation

The hair samples from subjects F, W, S and N were carefully measured and segmented from the scalp end (most recent growth). Randomly cut segments were taken from the drug-free subjects. All the segments were individually washed in 1% w/v sodium dodecyl sulphate solution, thoroughly rinsed in warm distilled water and left to air dry in a dust and drug-free environment. The weight of each dry segment was determined.

Hair morphine extraction

Each prepared sample was fully immersed in 0.1 M hydrochloric acid and incubated overnight at 55°C. Neutralization with equimolar amounts of 1 M sodium hydroxide was

followed by addition of 0.1 M phosphate buffer (pH 7.0), to the hair extract.

Hair morphine analysis

The hair extracts were analysed for morphine using solid-phase radioimmunoassay (RIA). Extracts from drug-free hair samples that had been spiked with known concentrations of morphine also were analysed for recovery and quality control purposes. The results obtained for each subject were ultimately expressed as ng morphine/mg hair and compared to a previously determined cut-off level based on drug-free hair sample analysis. Details of the experimental protocols have been published elsewhere [23].

Results and Discussion

Details of evaluation for specificity, accuracy and precision of the Coat-a-Count system have been published elsewhere [23]. The system, designed for the quantitative measurement of morphine in urine, was adapted to measure morphine in hair extracts. Dilutions of a morphine standard solution to represent 5, 10, 25, 100 and 250 ng ml^{-1} were prepared in hair extract from drug-free volunteers. Assay against the urine based standards gave results within 10% of the expected values at all levels. This indicated the hair extract to be a suitable matrix for morphine determination. The hair morphine results were calculated using a logit–log representation incorporating a smooth spline fitting algorithm and finally expressed in terms of ng morphine/mg hair.

Previous evaluation of a population of non-drug abusers has indicated that levels below 0.3 ng morphine/mg hair should be considered negative. This value was based on mean plus two standard deviations for the drug-free population (n = 21, mean = 0.12 ng morphine/mg hair and standard deviation of 0.085). The population contained both male and female volunteers, age range 16–40 years (mean 29 years), incorporating hair samples of varying colour and ethnic origin. The cut-off level found is similar to that described by other workers [2, 8, 24].

The extracts used for recovery and quality control were spiked at concentrations equivalent to 10, 100 and 250 ng ml^{-1} morphine. The relative standard deviations (RSDs), obtained were 11.9, 4.2 and 6.9%, respectively, with corresponding percentage recoveries of 88, 96 and 106%. The RSDs of duplicate assays at ng morphine/mg hair levels, mean values 9.27 and 1.12, were 2.4 and 5.5%, respectively, with corresponding standard deviations of 0.22 and 0.06 ng morphine/mg hair.

Morphine levels in segmented hair

The morphine concentration in the hair segments from known heroin abusers, subjects W, N and S, were found to vary between 0.6 and 9.4 ng morphine/mg hair. The morphine concentration in the hair segments from the subject, claiming periodic abstinence, F, varied between 0.06 and 0.63 ng morphine/mg hair. Random segments from non-drug abuser controls gave levels of 0.01–0.02 ng morphine/mg hair.

A striking feature of these results is the wide variation in the concentration of morphine detected in the known heroin abuse subjects and the subject under investigation for periods of claimed abstinence. At present the relationship between morphine levels detected in hair and drug intake is difficult to predict, although it has been reported that heavy dose users generally have higher concentrations in their hair [21]. The results obtained suggest this to be the case in the light of self-reportd heroin use from the subjects (Table 1).

The level detected in the non-abuser samples is unlikely to be due to heroin intake. It may be that the detectable levels resulted from

Table 1
Range of morphine concentrations detected in analysed subjects and reported heroin usage

Subject	Specimen	Morphine range in segments of hair (ng mg^{-1})	Reported heroin use*
W	Positive control	8.1–5.4	1–2 g day^{-1} decreasing to 0.25 g day^{-1}
N	Positive control	8.6–9.4	1 g day^{-1} constant
S	Positive control	0.6–1.7	Up to 0.25 g, 3 × weekly
F	Periodic claimed abstinence	0.06–0.63	0.25 g day^{-1}, 3.5 month abstinence claimed
No abuse	Negative control	0.01–0.02	Known drug-free

*Content of street heroin varies considerably and purity of 20% or more is infrequent.

environmental exposure or possibly codeine intake. The extensive pre-analytical wash procedure, low cross reactivity of the Coat-a-Count assay to codeine (<0.1%) and the fact the volunteers were known to be drug-free does not support this view. It is more likely that assay non-specific binding (NSB = 0.6%, counts min^{-1} = 505) and limits of analytical sensitivity (approximately 0.3 ng ml^{-1} equivalent to 0.01 ng morphine/mg hair, based on 30 mg hair weight used) are contributing factors.

Time relationship in hair

The concentration of morphine found in hair segments at different distances from the scalp are shown for subjects W, S, N and F (Fig. 1). The results of cumulative urine analysis for morphine where available for each subject during related segmental hair periods, are also depicted in this figure. This provided further indication of known periods of heroin abuse.

The three known heroin abuse subjects displayed hair morphine concentrations above the negative cut-off in all of the hair segments analysed. Subject W showed a higher morphine concentration in the hair for the 4–6 months segment, which gradually decreased towards the segment of newest hair growth. This is suggestive of a regular heroin user albeit perhaps using smaller doses in the most recent past. Morphine concentrations in the hair segments of subject N representing an 11 month history, indicated continuous heroin use at a relatively constant dose. The 9 month history of subject S showed a relatively constant level of morphine in each hair segment, suggesting perhaps a lower consumption of heroin, but on a continual basis.

The drug history of subject F, with respect to use of heroin, covered a period of some 15 months of hair growth. Attendance at the clinic with subsequent urine analysis had demonstrated intermittent periods of heroin abuse. Analysis of the hair segments representative of the period 7–15 months prior to hair sampling suggested that heroin at that time was not being abused (levels not above the 0.3 ng morphine/mg hair cut-off). However, more recent segments were suggestive of heroin use at a small dosage level.

The results of the hair morphine analysis from subject F, claiming periodic abstinence, are shown separately, along with the results from known non-abusers, to highlight particular dated segments during periods of known heroin abuse and claimed abstinence (Fig. 2). During the period from November 1989, cumulative urine analysis confirmed heroin

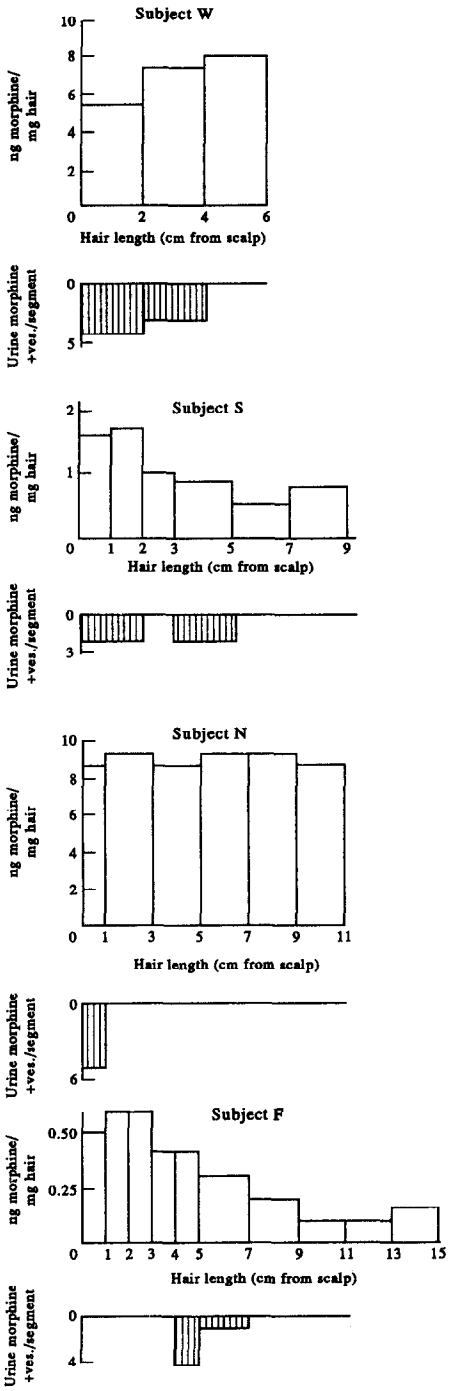

Figure 1
Concentrations of morphine found in hair segments at different distances from the scalp and where available the number of urine positives detected during cumulative urine screening. W, S and N — known heroin abusers, F — claimed periodic heroin abstinence.

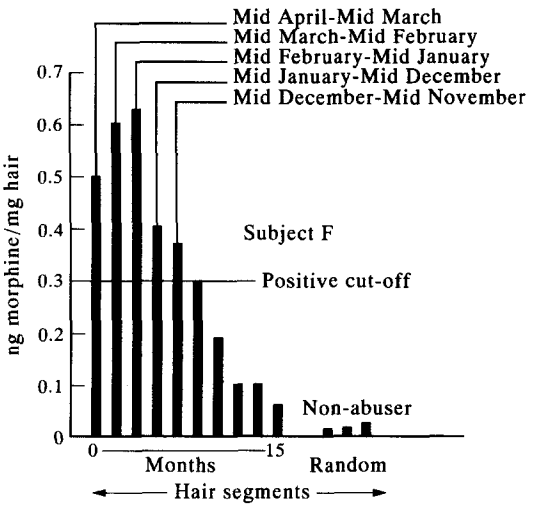

Figure 2
Detailed time relationship in hair sample of subject F, depicting concentrations of morphine found in hair segments at different distances from the scalp. Cut-off and random non-abuser levels also indicated.

was being abused by detection of morphine in the samples. However, from January until mid-April 1990 subject F claimed heroin abstinence. Pre-referral hair morphine levels suggested heroin had not been abused. During the periods of known heroin abuse the dated hair segments showed levels above the expected cut-off limit. However, during the 3.5 months of claimed abstinence, where no urine results were available, the morphine levels in the dated hair segments were even higher.

Research has shown it takes 7–8 days for morphine to appear in hair above the scalp [8]. Even allowing for variations in hair growth, absorption rates and individual time profiles, it was apparent that the hair segments which represented the second and third months post-claimed abstinence contained morphine levels up to 0.63 ng morphine/mg hair. This was over twice the negative cut-off limit.

The results obtained from the segmental hair analysis on subject F suggested that the self-report of heroin abstinence during January–April could be justifiably challenged. Subject F subsequently admitted that total abstinence was not true and that heroin had been used in small doses on an irregular basis.

The use of segmental hair analysis has provided an historical account of heroin abuse over a period of many months for the subject claiming abstinence and the known heroin abusers used as positive controls, during which results from urine screening were not always available. The application of the segmental regime to the known drug abusers with reference to self-reporting, has shown that it is possible to obtain information regarding individual patterns of drug usage in terms of being constant, increasing, decreasing or abstinent.

Some technical and pharmacological aspects of hair testing for drug abuse have still yet to be fully resolved. There is little documented evidence concerning drug concentration in hair collected from different body sites. Analysis of methadone in human head, axillary and pubic hair has shown that concentrations may vary at sample sites due to differing hair growth rates and blood circulation [9].

It is, therefore, desirable to collect hair specimens from a well defined site on the head whenever possible. The posterior vertex is the most widely accepted region, as hair growth there is less influenced by age, sex and growth cycles [21].

Interference with hair drug analysis due to racial bias is not apparent [13, 25], and evidence so far suggests that the effects of cosmetic treatments, such as shampooing, permanent waving, bleaching and dyeing do not reduce drug levels to a point at which a drug user would escape detection [6].

Extraction of the drug from the hair sample has been achieved by addition of acid or alkali, followed by incubation at raised temperature levels and organic solvent based procedures [21]. Any extraction procedure must ensure that analytes are not lost or altered during the process. Pronase, a non-specific peptide protein digester, has been used to dissolve the organic protein matrix of hair as a means of drug extraction [26]. Under optimum conditions, pronase contained in the hair digest may well react with the antibodies in the RIA causing their destruction. Excess active pronase can be inhibited using chemicals which in some instances are hazardous themselves. Approaches of this nature would necessitate the investigation of sufficiently mild conditions to ensure the protein antibodies of the RIA and the drug under investigation remain intact.

Hair analysis for drugs of abuse is a potentially exciting development, which could provide information in several areas (Table 2). Research into factors affecting the incorporation and retention of drugs in hair, standardization of methods and quality control development is required before its full potential can be

Table 2
Possible areas where hair analysis would be useful

- To determine historical drug abuse by months or years (not possible with single urine samples)
- Detection of previous drug use
- Assessment for rehabilitation programmes prior to entry (no previous drug record available)
- Assessment of rehabilitation programme success (retrospective analysis)
- Assessment of accuracy from self-report
- Prenatal exposure to drugs (mother and baby)
- Population studies
- Other uses (e.g. legal, personnel/employment)

assessed. Further work to this end is in progress.

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