Drug Testing in Sweat

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Introduction

Given the limitations of self-reports of drug use, testing for drugs of abuse is important for most treatment programs, both for monitoring the progress of the patient and for assessing the effectiveness of particular interventions in controlled clinical trials.

For many years, analysts have detected the presence of drugs in biological materials using blood and urine. In recent years, advances in sensitive analytical techniques have enabled the analysis of drugs in unconventional biological samples such as sweat. Researchers have known since 1911 [1] that drugs are excreted by the body in sweat but until recently no one has developed a practical solution to the problem of capturing sweat before testing. Occlusive bandages consisting of one to three layers of filter paper or pieces of cotton, gauze or towel were proposed to collect sweat. Significant advances have been made during the past years to develop a sweat patch technology, which was recently developed by Sudormed ™ and marketed by Pharmchem ™ Laboratories under the trade-market name Pharm-Chek ™. The sweat patch acts as a specimen container for non-volatile and liquid components of sweat, including drugs of abuse. Sweat components are collected on a special absorbent pad, located in the center of the patch. Non-volatile substances from the environment cannot penetrate the transparent film, a semi-permeable membrane over the pad that allows oxygen, water and carbon dioxide to pass through the patch leaving the skin underneath healthy. Over a period of several days, sweat saturates the pad and drugs present will be retained.

To date, only a few applications of the sweat patch have been described [2-4]. In these studies, the authors concluded that sweat testing appears to offer the advantage of being a relatively non-invasive means of obtaining a cumulative estimate of drug exposure over a period of several days. The aim of this report is to compare the usefulness of urine and sweat for the monitoring and managing patients in a substitution maintenance program.

Material and methods

Chemicals

Methanol and acetonitrile were HPLC grade (Merck, Darmstadt, Germany). Other chemicals were analytical grade and were provided by Merck. All

drugs and deuterated internal standards were purchased from Radian (Austin, TX).

Specimens

Twenty four heroin addicts participated in the study. All the subjects were verbally informed about the procedure and gave a verbal informed consent agreement. The sweat patches were applied to the outer portion of the upper arm or back. The selected skin site for patch placement was gently cleaned with a 70 % isopropanol swab before application. In all cases, the patch was applied on a Monday morning and removed 5 days later, on Friday, by pulling an edge of the adhesive backing, taking care not to touch the absorbent pad. After removal of the patch, the pad was stored in plastic tubes at - 20 ° C. At the same time, on day 0 and day 5, a urine specimen was collected, which was frozen until analysis.

Sweat patches were generously provided by Pharmchem Laboratories (Menlo Park, USA).

Analysis of sweat patches

The target drugs were extracted from the absorbent pad in 5 ml methanol in presence of 100 ng of the following deuterated internal standards: morphine-d_3, codeine-d_3, 6-monoacetylmorphine-d_3, heroin-d_9, cocaine-d_3, benzoylecgonine-d_3, ecgonine methylester-d_3, delta-9-tetrahydrocannabinol-d_3, nordiazepam-d_5, oxazepam-d_5, amphetamine-d_5, methamphetamine-d_5, methylenedioxyamphetamine-d_5, methylenedioxyethylamphetamine-d_5, methylenedioxymethamphetamine-d_5, methadone-d_3, and EDDP-d_3. The tubes were shaken for 30 min on an orbital shaker at 200 rpm. Then the methanol was evaporated to dryness.The drugs were derivatized and then analyzed by GC/MS.

A 1.5 μl portion of the derived extract was injected through the column (HP 5-MS capillary column, 30 m x 0.25 mm i.d.) of a Hewlett Packard 5890 gas chromatograph coupled with a Hewlett Packard 5989B Engine. The injector temperature was 260 °C and splitless injection was employed with a split-valve off-time of 0.75 min. The flow of helium through the column was 1 ml/min. The temperature of the column was programmed to rise from an initial value of 60 C° kept 1 min, to 290°C at 30 °C/min and kept at 290 °C for the final 6 min. Benzodiazepines were detected using negative chemical ionization, while other drugs were detected by electron impact.

Analytical parameters were determined in the previous studies [5, 6]. Standard curves were constructed by addition of drug analytes and deuterated standards to drug-free absorbent pads. The assays were linear in the range tested. The limits of detection for the target compounds were in the range 0.01 to 2.0 ng/patch with a minimal signal-to-noise ratio of 3. The extraction recoveries

were higher than 83 % for all the drugs. Within-run and between-run coefficients of variation were less than 16 % for all the drugs.

Urines samples were screened using fluorescent polarization immunoassay (FPIA) conducted on an Abbott ADx and confirmation of the positives was performed with standards GC/MS procedures [5, 7, 8]. Specimens were considered positive using the positive cut-off proposed by the manufacturer.

Results and discussion

The subjects wore the patch with minimal discomfort for the five days. Nevertheless, a few individuals developed a slight skin irritation after exposure to the sun. It was possible for each subject to continue his normal hygiene practices. Nobody accidentally abraded the patch.

Although less common than urine collection, no one refused to wear the patch. It was generally necessary to explain the nature of the study for 10 minutes before applying the patch, which was rather considered as a curiousity by the subject than a device for control.

Twenty four subjects were recruited for this study. Sweat patches and urine specimens were tested for opiates (heroin, 6-monoacetylmorphine, morphine, codeine), methadone and EDDP, cocaine (cocaine, benzoylecgonine, ecgonine methylester), cannabis (delta9-tetrahydrocannabinol), benzodiazepines (nordiazepam, oxazepam), and amphetamines (amphetamine, methamphetamine, methylenedioxyamphetamine, methylenedioxymethamphetamine, methylenedioxyethylamphetamine). Results of the sweat patch analysis are presented in table 1.

The patches were positive for opiates in 12 cases. Heroin and/or 6-monoacetylmorphine were identified in 8 cases (67 %) while codeine exposure was determined in 4 cases (33 %). Heroin, when detected, was always present in lower concentrations than 6-monoacetylmorphine. In some cases, Cone *et al* [2] reported higher heroin concentrations. The unique finding of heroin in sweat is of particular interest to document drug exposure of the subject. Its detection leads to a therapeutically useful change in the balance of power between patients and clinical staff, particularly when presenting to the addict the result of the analysis and the mention "heroin" on the sheet of paper.

As it is the case for hair testing [9], 6-monoacetylmorphine appears to be the major analyte in sweat after heroin intake. Therefore, care is necessary to prevent the conversion of 6-monoacetylmorphine to morphine. As heroin street samples generally contain codeine, this is also detected in sweat in cases of heroin abuse. Morphine is a metabolite of codeine and is detected in urine and blood when codeine is used. This is also the case when sweat is tested and the

Table 1.Results of the sweat patch analyses. Concentrations in ng/patch.

Subject	HER	6-MAM	MOR	COD	THC	NOR	OXA	Miscellanous
1	---	117	42	36	38	10	3	---
2	157	1835	113	189	---	---	---	---
3	---	---	40	4018	18	44	3	---
4	---	---	---	---	---	---	---	---
5	---	---	---	---	---	15	2	---
6	---	---	---	---	---	---	---	---
7	---	---	---	---	---	---	---	324 (COC) 58 (BZE) 89 (EME)
8	---	---	---	---	---	---	15	---
9	87	958	81	56	---	---	---	---
10	---	---	---	67	---	2	---	---
11	---	---	---	---	27	---	---	---
12	---	---	---	---	4	---	---	---
13	---	328	145	10	14	---	---	---
14	---	60	29	40	9	---	---	---
15	175	2386	271	139	14	4	---	---
16	37	108	165	85	---	---	---	---
17	---	---	110	1812	---	---	---	---
18	---	---	---	---	---	---	---	---
19	---	---	21	206	6	---	---	121(MDEA) 22(MDA)
20	---	431	181	89	11	31	4	---
21	---	---	---	---	6	17	---	1327 (MET) 84 (EDDP)
22	---	---	---	---	8	13	---	1041 (MET) 37 (EDDP)
23	---	---	---	---	---	11	---	681 (MET) 58 (EDDP)
24	---	---	---	---	---	8	---	859 (MET) 42 (EDDP)

HER: heroin, 6-MAM: 6-monoacetylmorphine, MOR: morphine, COD: codeine, COC: cocaine, BZE: benzoylecgonine, EME: ecgonine methylester, NOR: nordiazepam, OXA: oxazepam, THC: delta9-tetrahydrocannabinol, MDA: methylenedioxyamphetamine, MDEA: methylenedioxyethylamphetamine, MET: methadone, EDDP: methadone primary metabolite.

amount of morphine was about 0-10 % the amount of codeine, which appears consistent with a previous report [5].

Cocaine and metabolites were detected in only one case, indicating that cocaine use by heroin addicts is unfrequent in France, which is consistent with epidemiological studies. Cocaine was the major analyte excreted in sweat. Smaller amounts of ecgonine methyl ester and benzoylecgonine were present. Contrary to hair [7], ecgonine methyl ester was in greater amount than benzoylecgonine, which was also observed in a previous study [2]. Delta-9-tetrahydrocannabinol, the active ingredient of cannabis, was detected in 11 cases, with concentrations ranging from 4 to 38 ng/patch.

As demonstrated previously [6], benzodiazepine concentrations are low in sweat. Excretion in sweat appears to be maximal with basic drugs having high partition coefficients and pKa values close to the value of sweat pH near 5.0 [10]. The sweat patch is operating as an ion trap for the group of drugs that are weak bases with pKa-values around 8.0 [3]. The presence of low nordiazepam concentrations is consistent with a pKa-value of 3.5 for the drug. Oxazepam, which is more polar, will probably not diffuse across skin membranes as well as the parent drug. Methadone and EDDP tested positive in all the four subjects receiving the drug for substitution.

Finally, the identification of methylenedioxyethylamphetamine (121 ng/patch) and its metabolite methylenedioxyamphetamine (22 ng/mg) represents the first report of stimulant excretion in sweat. Again, the parent drug, more apolar, was present in greater amount compared to the metabolite.

Urine specimens were collected at the beginning and at the end of the patch wear. All the urinalyses were performed by GC/MS to identify the target compounds. When tested positive for heroin exposure (presence of heroin or 6-monoacetylmorphine) by the sweat patch, 6-monoacetylmorphine was always found in one or both corresponding urine samples. Similar findings were observed in case of codeine exposure where only morphine and codeine were simultaneously identified in urine and sweat. Therefore, sweat can be accurately used to differentiate heroin and codeine abuse, based on the presence of heroin and/or 6-monoacetylmorphine. Testing sweat for heroin or 6-monoacetylmorphine leads the toxicologist to use GC/MS and not immunoassay, although the latter procedure can find applications in drug screening. For the other drugs, urine findings and sweat findings were also in agreement.

No subject was urine positive and sweat negative. Also, no subject was sweat positive and urine (based on the sum of the two specimens) negative. However, if only one urine test was performed, in 6 cases it would have been possible to observe sweat positive and urine negative subjects. These cases involved opiate exposure in 3 subjects using heroin and 2 subjects using codeine

and the only positive for cocaine. Sweat tested positive in 13 cases for opiates and cocaine. The first specimens of urine, collected on day 0 tested positive in 10 cases for opiates. The second specimens of urine, collected on day 5 tested positive in 9 cases for opiates and 1 case for cocaine. Sweat testing was able to show drug exposure after urine collection (the first urine specimen) and after drug has been eliminated from the body (the second urine specimen) when total metabolization had occured. Therefore, the sweat patch appears to be more effective than urine in detecting the use of opiates and cocaine. The same observations were presented by Fogerson et al (11). Sweat testing appears to be as effective as two urine tests performed each week to detect a single drug exposure. These findings can be observed with drugs having urinary detection times of 2-3 days, depending on immunoassay cut-offs. Compounds with longer elimination half-life, like benzodiazepines or cannabinoids were positive in all the urine samples. In such cases, one would observe sweat positive and first urine specimen negative only when the drug is taken for the first time during the time of patch application.

Conclusions

In conclusion, sweat analysis may be a useful adjunct to conventional drug testing. Specimens of sweat can be more easily obtained with less embarrassment than urine specimens. As analytical procedures generally involve GC/MS to document heroin exposure, the routine analysis of sweat is not accessible to most laboratories, but from a clinical point of view, the generated data are extremely helpful. This new technology may find useful applications in the treatment and monitoring of substance abusers.

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