TECHNICAL NOTE

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Detection of Drugs in Human Hair Using Abbott ADx, with Confirmation by Gas Chromatography/Mass Spectrometry (GC/MS)

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ABSTRACT: The authors suggest use of the fluorescence polarization immunoassay (FPIA) technique in evaluation of chronic drug abuse using human hair.

Hair was decontaminated in 5 mL of ethanol for 15 min at 37°C and then incubated in 3 mL of 1*M* sodium hydroxide (NaOH) for 1 h at 100°C. Afterwards, the aliquots were neutralized and analyzed using Abbott ADx for a negative or positive response for the following drugs: benzodiazepines, barbiturates, antidepressants, opiates, cocaine, amphetamine, and cannabis.

All the positive samples were confirmed by gas chromatography/mass spectrometry (GC/MS). Only one false positive was detected (caused by interference of a phenothiazine with the antidepressants kit), clearly demonstrating the capability of ADx for toxicological screening of human hair.

KEYWORDS: toxicology, hair, abuse drugs, immunoassay, fluorescence polarization immunoassay

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. It is therefore possible to trace the drug intake of an addict over periods longer than six months, depending on the hair length. During testing of hair fragments, a drug addict is not able to hide the fact of drug abuse, even by deliberately abstaining for several days before sample collection, although this abstinence may produce a negative response in urine analysis.

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Until now, only two analytical methods have had sufficiently high sensitivity to have been successfully employed in this field: radioimmunoassay and gas chromatography, coupled with mass spectrometry, have been used for identifying and quantifying morphine, phencyclidine, phenobarbital, amphetamine, methamphetamine, methadone, cocaine, marijuana, digoxin, benzodiazepines, nicotine, and antidepressants [*1–8*].

The authors of this paper suggest use of the fluorescence polarization immunoassay (FPIA) on Abbott ADx for evaluation of chronic drug abuse using human hair.

Materials and Methods

Drug-free hair specimens were obtained from individuals working in the authors' laboratory. For drug screening, hair samples were collected from the subjects of 40 medical examiner's cases, including 18 postmortem cases.

Hair samples of 30 to 40 strands, weighing at least 50 mg, were cut as close as possible to the scalp on the back of the head. The hair was decontaminated by washing the specimen in 5 mL of ethanol for 15 min at 37°C. The protein matrix of the hair was destroyed by incubation in 3 mL of 1*M* sodium hydroxide (NaOH) for 1 h at 100°C. After neutralization with 3*M* hydrochloric acid (HCl) and centrifugation, the homogenate was half diluted with ADx buffer and directly analyzed for positive or negative response by fluorescence polarization immunoassay (FPIA), according to the manufacturer's recommendations for plasma or urine. The drugs analyzed included benzodiazepines, barbiturates, antidepressants, opiates, cocaine, cannabis, and amphetamine.

Positive results were confirmed by gas chromatography coupled with mass spectrometry, using a Perkin-Elmer chromatograph and an ion trap detector under standard conditions.

For chromatography, the hair samples (approximately 2 mL each) were extracted with 5 mL of chloroform/isopropanol/*n*-heptane (50:17:33, v/v), after alkalinization (1 mL of phosphate buffer, at 1 mol/L and pH 9.2) and the addition of SKF 525 A (10 μg/mL) as an internal standard. After evaporation to dryness, the residue was injected directly into the gas chromatograph (GC) column or, after derivatization, with trifluoroacetic anhydride.

Results and Discussion

The cutoff levels for FPIA were determined by analyzing eight hair specimens from proven nonusers of drugs. The results obtained are summarized in Table 1. When the manufacturer's cutoff values were used, barbiturates were positive in three nonabusers. Therefore, for toxicological screening using human hair, we used the proposed cutoff levels for all drugs, with the exception of barbiturates, for which the cutoff value was estimated to be 0.3 mg/L.

The problem that emerged from the evaluation of barbiturates in hair seems to be due to a matrix effect, since urine and plasma analyses exhibited no positive responses for nonusers of drugs.

Afterwards, hair samples collected from 40 medical examiner's cases were screened using Abbott ADx. In comparison with gas chromatography/mass spectrometry, (GC/MS, only three cases produced false positives, one with the antidepressants kit and two with the cannabis kit (Table 2). While the false positives with cannabis are not explained, we also found promethazine in the hair sample of the false antidepressant case. Promethazine is known to have a cross-reactivity of about 20% with the FPIA.

As all the hair specimens were analyzed by GC/MS, no false negative responses were observed. The drug quantifications are summarized in Table 3. The concentrations found were within the range of previously published values [*1–8*]. Eighteen of the specimens analyzed were obtained from fatal overdoses.

TABLE 1—*Results in drug-free hair by Abbott ADx.*

Drug	Response, mg/L	Positive or Negative	Cutoff Value, mg/L
Benzodiazepines	0.04–0.08	–	0.2
Barbiturates	0.15–0.29	+	0.2
Antidepressants	0.028–0.045	–	0.075
Opiates	<0.05	–	0.1
Cocaine	<0.02	–	0.3
Cannabis	0.016–0.023	–	0.025
Amphetamine	<0.05	–	0.15

TABLE 2—*Comparison of the hair analysis results for drug abusers by Abbott ADx and GC/MS.*

Drug	Cases Positive with FPIA	Cases Positive with GC/MS
Benzodiazepines	5	5
Barbiturates	5	5
Antidepressants	6	5
Opiates	14	14
Cocaine	2	2
Amphetamine	3	3
Cannabis	29	27

TABLE 3—*Concentrations measured by GC/MS.*

Drug	Number of Cases	Concentration, ng/mg hair
Benzodiazepines		
Diazepam	1	1.37
Desmethyldiazepam	2	1.04–1.47
Flunitrazepam	1	0.41
Nitrazepam	1	0.37
Barbiturates		
Secobarbital	2	21.6–58.9
Amobarbital	1	41.6
Phenobarbital	2	51.4–137.3
Antidepressants		
Amitriptyline	1	0.42
Nortriptyline	1	0.91
Clomipramine	2	0.37–0.79
Opiates		
Morphine	13	0.41–11.74
Codeine	1	4.21
Cocaine		
(Benzoylecgonine	2	1.21–3.41
Amphetamine	3	0.96–12.71
Cannabis		
(11-nor-Δ8-THC9-COOH)	27	0.4–2.7

Previously, in 1987, some authors [9], have proposed the detection of morphine in hair with FPIA. This was achieved using solid-phase extraction, followed by organic extraction and evaporation. This procedure appears to be inefficient, since the antibody used in FPIA can act directly in the homogenate of hair, as described in this report.

In hair analysis, the Abbott ADx method was sensitive, accurate, and rapid. Therefore, FPIA seems to be an excellent basis for toxicological screening in hair, but it must always be followed by a confirmation method since there is a strong possibility of obtaining false positives.

References

[1] Cone, E. J., "Testing Human Hair for Drugs of Abuse: I. Individual Dose and Time Profiles of Morphine and Codeine in Plasma, Saliva, Urine, and Beard Compared to Drug-Induced Effects on Pupils and Behavior," *Journal of Analytical Toxicology*, Vol. 14, No. 1, Jan. 1990, pp. 1–7.

[2] Baumgartner, A. M., Jones, P. F., and Black, C. T., "Detection of Phencyclidine in Hair," *Journal of Forensic Sciences*, Vol. 26, No. 3, July 1981, pp. 576–581.

[3] Smith, F. P. and Pomposini, D. A., "Detection of Phenobarbital in Bloodstains, Semen, Seminal Stains, Saliva, Saliva Stains, Perspiration Stains, and Hair," *Journal of Forensic Sciences*, Vol. 26, No. 3, July 1981, pp. 582–586.

[4] Suzuki, O., Hattori, H., and Asano, M., "Detection of Methamphetamine and Amphetamine in a Single Human Hair by GC/CIMS," *Journal of Forensic Sciences*, Vol. 29, No. 2, April 1984, pp. 611–617.

[5] Balabanova, S. and Wolf, H. U., "Methadone Concentrations in Human Hair of the Head, Axillary, and Pubic Hair," *Zeitschrift für Rechtsmedizin*, Vol. 102, 1989, pp. 293–296.

[6] Smith, F. P. and Liv, R. H., "Detection of Cocaine Metabolite in Perspiration Stain, Menstrual Bloodstain, and Hair," *Journal of Forensic Sciences*, Vol. 31, No. 4, Oct. 1986, pp. 1269–1273.

[7] Baumgartner, W. A., Hill, V. A., Bear, J. D., Lyon, J. W., Charuvastra, V. C., et al., "Detection of Drug Use by Analysis of Hair," *Journal of Nuclear Medicine, Supplement*, Vol. 29, No. 5, 1988, p. 980.

[8] Ishiyama, I., Nagai, T., and Toshida, S., "Detection of Basic Drugs (Methamphetamine, Antidepressants, and Nicotine) from Human Hair," *Journal of Forensic Sciences*, Vol. 28, No. 2, April 1983, pp. 380–385.

[9] Franceschin, A., Morosini, L., and Dell'Anna, L., "Detection of Morphine in Hair with the Abbott TDx," *Clinical Chemistry*, Vol. 33, No. 11, 1987, p. 2125.

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