RADIOIMMUNOASSAY

Radioimmunoassay of Cocaine in Hair: Concise Communication

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Cocaine was detected in hair of all of 13 patients from a drug-abuse clinic who acknowledged having used the drug in varying amounts during the last 6 mo. A correlation was observed between the amount of drug used and the quantity trapped in the interior of hair grown during the 6-mo period. In contrast to hair analysis, urinalysis by thin-layer chromatography was negative in all cases, indicating that cocaine had not been used by the patients within 48–72 hr before the urine collection. Hair analysis thus appears to be far superior to urinalysis for establishing histories of drug use.

J Nucl Med 23: 790–792, 1982

Previous work from our laboratories has demonstrated that drugs of abuse, such as morphine, heroin, and phencyclidine, accumulate in detectible quantities in hair of drug users (*1*,*2*). Since drugs deposited in deep-lying hair structures during biosynthesis of the fiber cannot be removed by repeated washings with shampoo, hair analysis can provide valuable long-term information on prior drug use. This provides a major advantage over blood and urine analysis, since most illicit drugs can be detected in these fluids for only 2–3 days after last use. In the present study we have extended our investigation of hair analysis to cocaine, using hair samples from patients with known drug histories.

MATERIALS AND METHODS

Sample collection and preparation. Hair samples were obtained from admitted cocaine users at the Long Beach Drug and Alcohol Rehabilitation Center. Histories of drug abuse were obtained from patient interviews at the time of hair sampling. Urine samples were also collected at this time and were analyzed for cocaine by an independent laboratory using a standard thin-layer chromatography procedure (*3*).

To distinguish between cocaine loosely adhering to hair surface from that trapped in the interior of hair during biosynthesis of the fiber, we washed all samples extensively with shampoo solution before extraction with solvent. Hair samples were prepared using sections cut near the root, but not including the root. Hair was cut into 1-in. sections before washing since this facilitated separation from shampoo solution and, unlike the finely cut-up hair used in our previous studies, these longer lengths are not entrapped in foam. Hair samples (10–20 mg) were vigorously shaken for 10 min in a shampoo* solution (200 μl shampoo in 10 ml of water at 60°C). After shampooing, hair was rinsed with ten 10-ml volumes of distilled water to ensure removal of residual traces of shampoo (which was found to interfere with the subsequent RIA procedure) and dried for 10–20 min

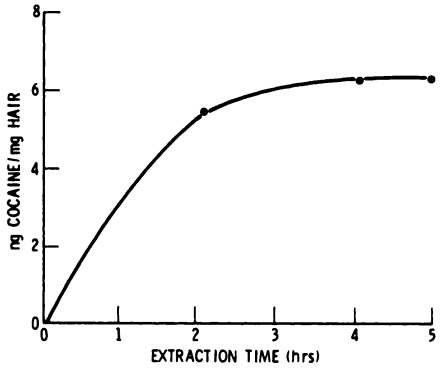

FIG. 1. Total amount of cocaine (benzoylecgonine equivalents) extracted from washed hair as function of extraction time in boiling ethanol.

Received Dec. 21, 1981; revision accepted April 16, 1982.

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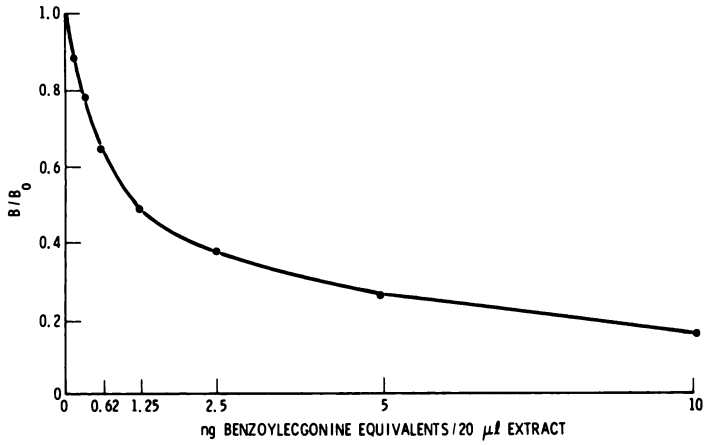

FIG. 2. Radioimmunoassay calibration curve for cocaine expressed in benzoylecgonine equivalents (cocaine metabolite). B_0 = counts/min at zero benzoylecgonine concentration; B = counts/min at other benzoylecgonine concentrations.

under vacuum at 60°C.

Cocaine extracted after 2, 4, and 5 hr was measured. After refluxing for 4 hr in ethanol, cocaine was extracted from shampooed hair cut into smaller pieces (~0.5-cm sections). The extract was transferred to a 5-ml polystyrene test tube and evaporated. Crushing the hair to a fine powder with a mortar and pestle before extraction did not increase the amount of extracted cocaine, suggesting that accessibility is not a factor in the extraction of cocaine from intact hair. The less tedious method of using cut instead of ground hair was adopted for all subsequent analyses.

Radioimmunoassay. For analysis, the dried extracted material was redissolved in 200 μl of ethanol at 60°. Radioimmunoassays (RIA) were performed with 20 μl of the redissolved material. Control experiments verified that no losses of cocaine occurred through adsorption on the glass boiling vessel or during evaporation and storage in polystyrene or glass test tubes. In cases of very low cocaine concentrations, the dried hair extract was taken up in 20 μl of ethanol, achieving thereby a tenfold increase in detection limit.

Extracted cocaine or its metabolites expressed as benzoylecgonine equivalents was measured with a commercial RIA kit.† By adding a 20-μl aliquot of the hair extract to cocaine-free urine (100 μl), we adhered as closely as possible to the test procedure described in the manufacturer's test manual. The cocaine metabolite benzoylecgonine, and not cocaine, was used for preparation of the standard curve. The relative reactivity (RR) of cocaine in the radioimmunoassay was 1.390, RR being defined as the mean μg/liter benzoylecgonine equivalent

TABLE 1. SUMMARY OF ANALYSES OF HAIR SAMPLES FOR COCAINE

Hair sample	Sample appearance	Cocaine intake/ 6 mo (g)	Hair weight (mg)	Cocaine* in sample (ng)	ng Cocaine* / g hair	Urinalysis† for cocaine
1a	Straight, dark brown, unwashed	<1	21.3	6.1	285	–
1b	Washed	<1	18.3	0.6	33	–
2a	Curly, black, unwashed	1	41.7	74.7	1790	–
2b	Washed	1	38.7	40.7	1050	–
3	Straight, brown	1	24.7	5.8	234	–
4	Straight, black	0.5	14.0	1.8	130	–
5	Straight, brown	20	20.0	78.3	3910	–
6	Curly, black	2	10.8	2.5	230	–
7	Straight, dark blonde	3	7.4	1.0	152	–
8	Curly, black	10	9.4	60.0	6380	–
9	Straight, dark brown	2	24.8	51.4	2070	–
10	Straight, dark brown	30	17.0	71.3	4190	–
11	Straight, brown	0.25	19.2	0.13	6.9	–
12	Straight, brown	0.25	21.0	0.15	7.4	–
13	Straight, brown	0.25	23.0	0.4	17	–

† Urinalysis reported as positive (+) or negative (–).
* Benzoylecgonine equivalents.

to 1 μg/liter of cocaine.

RESULTS AND DISCUSSION

Figure 1 shows that the extraction of cocaine or cocaine metabolite is effectively completed after refluxing for 4 hr in ethanol. We do not know, however, whether this represents the total cocaine or metabolite trapped in deep lying hair structures, or only the fraction accessible to boiling ethanol.

A standard RIA curve (Fig. 2) of good sensitivity and reproducibility was obtained. However, this was the case only if the ethanol extract per tube was kept below 20 μl. With larger quantities of ethanol, the sensitivity and reproducibility of the assay was adversely affected. The precision of B/B_0 at zero concentration was 1.0 ± 0.05 (2 s.d.), resulting in a detection limit of approximately 0.08 benzoylecgonine/20 μl extract.

The results in Table 1 show that cocaine (benzoylecgonine equivalents) was detected in all hair samples of the 13 admitted cocaine users. No false-positive values were obtained with five negative controls. In contrast to hair analysis, the TLC analysis of urine specimens gave negative results for all 13 patients.

Note that the low detection rate with TLC is not due to the lower sensitivity of TLC relative to RIA, but to the disappearance of cocaine and cocaine metabolites from urine within 48–72 hr after last drug use (*4*).

As in our previous studies (*1*,*2*), the amount of drug used during the previous 6 mo correlated with the amount of cocaine extracted from hair (Fig. 3). The 6-mo period was chosen because this corresponded approximately to the length of hair used in the analysis (5–6 cm.). It must be remembered, however, that our estimates of drug intake are subject to such uncertainties as the veracity or accuracy of the users' estimates and the purity of the drug used. Also, retention of cocaine in hair may be affected by cosmetic treatments (perming, dyeing, bleaching) or environmental effects (oxygen, uv light). In view of these uncertainties, the correlation shown in Fig. 3 is surprisingly good.

In two instances where sufficient hair sample was available, we analyzed both washed and unwashed hair (samples 1 and 2, Table 1) and found that a considerable fraction of the cocaine could be removed by washing with shampoo. It appears likely that this material is derived from the hair surface, where it may have been deposited by perspiration, sebum, or direct contact.

In general our study shows that RIA analysis of hair is far more effective than TLC analysis of urine in detecting sporadic cocaine users. Hair analysis also uniquely provides the potential for establishing an historical record of a person's drug use. If hair retains cocaine essentially for the duration of the hair sample, considerable information on the history of drug use can be expected from analysis of hair sections cut at different lengths from the root. Thus, hair analysis could be extremely useful in monitoring drug abuse in patients, parolees, prisoners, and armed forces personnel.

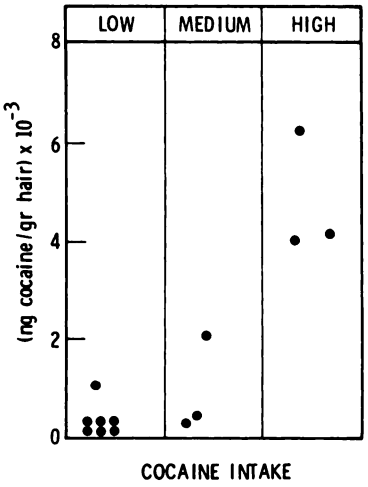

FIG. 3. Correlation of cocaine use with benzoylecgonine equivalents in washed hair. Cocaine use per 6 mo: low, 0–1 g; medium, 2–3 g; high, 10–30 g.

FOOTNOTES

* Prell shampoo.

† Abuscreen Cocaine Metabolite Test Kit, Roche Diagnostics, Hoffman-LaRoche, Nutley, NJ.

ACKNOWLEDGMENTS

The authors acknowledge the cooperation of the patients and staff of the Long Beach Drug and Alcohol Rehabilitation Center; the excellent technical assistance of Charles Porter, B.S., and Sunne Genova; and the helpful suggestions of Dr. Martin Mach and Virginia Hill.

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