TECHNICAL NOTE

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Benzoylecgonine (Cocaine Metabolite) Detection in Hair Samples of Jail Detainees Using Radioimmunoassay (RIA) and Gas Chromatography/ Mass Spectrometry (GC/MS)

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ABSTRACT: Benzoylecgonine (BE) was detected in hair samples using nonproprietary extraction methodology and modifications of well-established radioimmunoassay (RIA) screening/quantitative gas chromatography/mass spectrometry (GC/MS) confirmation procedures. Samples collected anonymously from a population of 48 jail detainees weighed between 5.3 and 61.2 mg. All of the 22 hair samples which had RIA results indicating the presence of BE or immunologically similar substances above a cutoff amount of 1.25 ng/sample (50 ng/mL) were confirmed by GC/MS. Several varieties of hair color and texture were tested, although in each general category there were samples which contained BE as well as other samples which did not reveal detectable amounts of BE. The range of concentrations in 22 hair extracts that screened positive were 0.26 to 18 ng/mg hair as determined by GC/MS. In comparison with other reports of cocaine-related substances in hair, these data show consistent concentrations.

KEYWORDS: toxicology, hair, benzoylecgonine, radioimmunoassay, chemical analysis

Early studies of hair as a toxicological sample showed that evidence of the ingestion of the controlled substances phenobarbital and *d*-amphetamine was detectable in animal hair using ultraviolet spectrophotometry [*1*] and radioactive tracer analyses [*2*]. Later, the sensitivity of immunoassays permitted smaller quantities of drugs and drug metabolites to be detected from human hair samples [*3–14*]. More recently, gas chromatography/mass spectrometry (GC/MS) [*15–21*] and tandem MS/MS [*17–19*] have been used to confirm the presence of drugs and metabolites in hair.

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TABLE 1—*In hair samples (n = 48) of various colors; including black (BK), gray (GY), red (RD), and brown (BN); and textures; including straight, very (v.) curly, and curly; BE was detected in 22 using RIA screening and GC/MS confirmation.*

Sample No.	Color	Texture	Length, cm	Weight, mg	RIA BE Equiv./mL, ng	RIA BE/mg Hair, ng	GC/MS BE, ng	GC/MS BE/mg Hair, ng
01	BK	v. curly	1.3	8.0	<50	. . .		
02	BK/GY	straight	1.3	11.0	<50	. . .		
03	RD	straight	1.9	11.0	<50	. . .		
04	BN/RD	straight	1.9	8.2	<50	. . .		
05	BN/RD	straight	2.5	17.3	72	0.31	5.5	0.95
06	BK	v. curly	1.3	10.4	<50	. . .		
07	BK	curly	1.3	6.1	<50	. . .		
08	BN/blond	straight	1.3	34.5	74	0.16	3.1	0.27
09	BK	straight	2.5	30.1	<50	. . .		
10	BK/BN	straight	1.9	7.5	<50	. . .		
11	BK	v. curly	1.3	6.2	779	9.4	36.3	18
12	BN	straight	2.5	15.1	<50	. . .		
13	BK	straight	1.9	30.3	128	0.315	3.8	0.38
14	BN	straight	2.5	12.9	120	0.70	10.1	2.35
15	BK/GY	straight	0.8	29.6	<50	. . .		
16	BK	v. curly	0.8	8.7	146	1.3	11.1	3.8
17	BK	straight	2.5	10.1	<50	. . .		
18	BK	straight	2.5	31.0	774	1.9	76.2	7.37
19	BN/RD	straight	1.9	22.4	66	0.22	7.3	0.98
20	Blond	straight	1.3	7.8	250	2.4	12.1	4.6
21	BK	curly	1.9	6.5	<50	. . .		
22	BN	straight	1.3	6.5	<50	. . .		

23	BK	v. curly	0.8	5.3	362	5.1	16.3	9.2
24	BK	v. curly	0.8	10.2	<50	. . .		
25	BK	straight	1.7	8.4	557	5.0	36.3	13
26	BN	straight	2.5	18.0	<50	. . .		
27	BN	straight	1.9	22.8	86	0.28	3.5	0.46
28	BN/BK	straight	1.3	7.0	76	0.82	2.7	1.2
29	BK	v. curly	1.3	5.3	81	1.1	4.8	2.7
30	RD	straight	7.6	41.6	<50	. . .		
31	BN	straight	3.8	23.3	<50	. . .		
32	BN/blond	straight	7.6	28.6	<50	. . .		
33	BN/RD	straight	3.8	30.6	128	0.314	5.4	0.53
34	BK	curly	1.9	35.5	<50	. . .		
35	BN	straight	3.8	14.6	<50	. . .		
36	BK/GY	curly	1.9	55.5	<50	. . .		
37	BK	straight	3.8	47.0	1135	1.81	76.4	4.88
38	BN	straight	2.5	42.9	313	0.578	12.1	0.846
39	BK	v. curly	1.3	61.2	1412	1.73	97.6	4.78
40	BN	curly	2.5	14.3	<50	. . .		
41	RD/blond	straight	6.4	18.5	493	2.00	12.1	1.96
42	BK	straight	4.4	12.9	57	0.33	1.1	0.26
43	BN	straight	0.6	13.0	<50	. . .		
44	BK	curly	1.3	35.5	646	1.36	53.5	4.52
45	BN/RD	curly	2.5	16.7	<50	. . .		
46	BN	straight	2.5	14.9	177	35.6	9.3	1.87
47	BN	straight	2.5	7.6	<50	. . .		
48	Blond	straight	3.8	11.4	<50	. . .		
Neg cntl	RD/blond	curly	1.3	40.9	<50	. . .		

In this study, hair samples were anonymously collected from detainees at the Jefferson County Jail in Birmingham, Alabama, and were analyzed for the presence of benzoylecgonine (BE), the primary metabolite of cocaine. All samples were screened using radioimmunoassay (RIA). The presence of BE in each of the RIA-positive samples was confirmed by selected ion monitoring (SIM) GC/MS.

Material and Methods

Hair Extraction

The hair samples, weighing from 5.3 to 61.2 mg, were obtained anonymously from 48 detainees at the Jefferson County Jail and sealed in unmarked envelopes before they were analyzed later. No record was made or kept of the name or any other personal identification of the donors; and, therefore, no correlation of specific results with individuals was sought or obtained. Individual hair samples were prepared as previously described [*11*]. The ethanolic extract was filtered into three equal aliquots using pasteur pipets packed with glass microfiber filter paper and dried in 12 by 75-mm round-bottom glass culture tubes.

Immunoassay

RIA was performed by reconstituting one of the three aliquots from hair extract in 25 μL of RIA-negative control urine which was obtained from and verified as negative by the commercial RIA kit.[3] The RIA procedure was then followed according to the directions included in the manufacturer's instructions for urine testing. In addition, a standard concentration curve was prepared using dilutions of BE supplied in urine by the RIA kit manufacturer. The sample results were extrapolated from a logic plot generated from the standard curve data. Initially expressed in nanogram BE equivalents per millilitre (Table 1), the data were converted to nanogram BE equivalents per milligram hair by accounting for the 25-μL sample volume of known concentration standards, the hair sample weight, and the separation of the hair extract into three equal aliquots.

Electron-Impact GC/MS

GC/MS confirmation was performed using a Hewlett Packard Model 5890 gas chromatograph/Model 5970 mass spectrometer and modifications of well-established procedures [*22–24*] First, 150 ng of internal standard, d_3-benzoylecgonine[4] (D_3-BE), were added to the dried hair extract. The sample was then derivatized using 20 μL bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane[5] (TMS). The reaction was incubated at 65°C for 15 min. A sample (2 μL) of the resulting solution was injected into the GC/MS using the split mode, a DB-5 capillary column[6] (15 m, 0.25-mm inside diameter), selectively monitoring the following ions: 240, 243, 346, 349, 361, and 364. The injection port temperature was set at 250°C while the oven temperature remained at 230°C for the 5-min run. The retention time was previously determined using a retention time standard of known BE,[7] following the same derivatization procedure as for hair samples.

[3]Abuscreen® Radioimmunoassay for Cocaine Metabolite package insert, Roche Diagnostics Systems, Nutley, NJ 07110-1199, Dec. 1988.

[4]Obtained from Radian Corporation, 8501 Mo-Pac Blvd., P.O. Box 201088, Austin, TX 78720-1088.

[5]Obtained from Sigma Chemical Company, P.O. Box 14508, St. Louis, MO 63178.

[6]Obtained from J & W Scientific, 91 Blue Ravine Rd., Folsom, CA 95630.

[7]Obtained from Radian Corporation (see footnote 4).

Results and Discussion

The results reported in Table 1 show concentrations of 0.26 to 18 ng BE/mg hair in 22 of 48 samples.

For the purposes of this study, a RIA cutoff of 1.25 ng/5.3 to 61.2 mg hair extract aliquot (50 ng/mL by 25 μl/1 mL) was chosen, compared with 0.85 ng/10 mg hair proposed by Baumgartner [*20*]. (The total sample weight varied from 5.3 to 61.2 mg; the entire sample was extracted; a one-third aliquot was analyzed in each test.) This particular RIA exhibited high specificity for BE. Cocaine, which has been detected at high concentrations, up to ten times greater than BE in hair [*17,19,20*], was cross-reactive at only approximately 1% with the BE-specific antiserum in this RIA kit. Thus, even at the maximum of ten times the BE concentration, cocaine would have contributed only 10% of the RIA result. Because BE is the major metabolite of cocaine in humans, its presence in hair is significant as a check against cocaine from external contamination, as opposed to *in vivo* sources. All RIA results above 1.25 ng/sample were confirmed for BE by GC/MS.

BE quantitation results by GC/MS ranged from 0.26 to 18 ng BE/mg hair, as given in Table 1. In most instances, these concentrations exceeded RIA results. Cocaine-related substances, dried to the RIA reaction tube wall, most likely accounted for lower amounts detected. GC/MS derivatization provided chemical conditions (solvent and elevated temperatures) conducive to greater BE solubility and therefore to larger apparent concentrations. Forensic toxicologists may find the individual data points useful when conducting reproducibility studies. Consistent with an earlier report by Baumgartner et al. [*9*], factors such as hair color and degree of curl did not appear to interfere with the assay in that BE was both detected and not detected in samples from each general hair-type category.

The results in this report are consistent with those of other published accounts of concentrations of cocaine substances in hair. For example, Baumgartner et al. reported detecting a concentration range of 0.007 to 6.38 ng "benzoylecgonine equivalents"/mg hair [*9*] and, later, between 0.8 and 50 ng "cocaine metabolite"/mg hair [*20*] in RIA tests conducted on the hair from patients, probationers, and parolees [*20*]. Balabanova and co-workers [*16*] found 7.3 ng "cocaine"/mg hair from RIA tests on the postmortem hair sample of a cocaine user and 0.6 to 6.4 ng/mg hair from living cocaine addicts [*25*]. Somewhat higher concentrations were reported by Martz [*17*], who found 80 to 90 ng cocaine/mg hair using GC/MS. In a study on the use of hair to determine gestational cocaine exposure, Graham et al. found 0.032 to 29.089 ng "benzoylecgonine"/mg hair from adult cocaine users when a highly BE-specific RIA was used as the sole detection method. Most of these authors pointed out that the specificity of RIA can affect the reliability of quantitative results, particularly when an unknown, heterogenous sample such as a hair extract is analyzed. This underscores the usefulness of GC/MS for detection of drugs in hair.

Conclusions

The analysis of hair for drugs of abuse has recently been the subject of much discussion in order to determine its appropriateness as an analytical technique. Its advantages over the more widely used urinalysis methods, summarized elsewhere [*11,12,20,26*], warrant continued research and independent confirmation of methods and results.

In this study, screening tests show that nearly half of all hair samples from a jail population contained compounds that were immunologically reactive with cocaine metabolite (BE) antiserum in a commercially supplied RIA. A GC/MS procedure confirmed the presence of BE in each of the 22 hair samples on which confirmation was indicated. These data not only substantiate that BE can be detected and confirmed in hair, but

provide quantitative information that may be useful to forensic toxicologists interested in comparing the amounts of BE found in hair.

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References

[1] Goldblum, R. W., Goldbaum, L. R., and Piper, W. N., "Barbiturate Concentrations in the Skin and Hair of Guinea Pigs," *Journal of Investigative Dermatology*, Vol. 22, No. 2, Feb. 1954, pp. 121–128.

[2] Harrison, W. H., Gray, R. M. and Solomon, L. M., "Incorporation of D-Amphetamine into Pigmented Guinea-Pig Hair," *British Journal of Dermatology*, Vol. 91, 1974, pp. 415–418.

[3] Smith, F. P., *Detection of Drugs in Dried Bloodstains Using Radioimmunoassay*, doctoral dissertation, Department of Chemistry, University of Pittsburgh, 1978.

[4] Baumgartner, A. M., Jones, P. F., Baumgartner, W. A., and Black, C. T., "Radioimmunoassay of Hair for Determining Opiate-Abuse Histories," *Journal of Nuclear Medicine*, Vol. 20, No. 7, 1979, pp. 748–752.

[5] Klug, E., "Zur Morphinbestimmung in Kopfhaaren," *Zeitschrift für Rechtsmedizin*, Vol. 84, 1980, pp. 189–193.

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

[7] Valente, D., Cassini, M., Pigliapochi, M., and Vansetti, G., "Hair as the Sample in Accessing Morphine and Cocaine Addiction," *Clinical Chemistry*, Vol. 27, 1981, pp. 1952–1953.

[8] Arnold, W. and Puschel, K., "Experimental Studies on Hair as an Indicator of Past or Present Drug Use," *Journal of Forensic Sciences*, Vol. 21, No. 1, Jan. 1981, p. 83.

[9] Baumgartner, W. A., Black, C. T., Jones, P. F., and Blahd, W. H., "Radioimmunoassay of Cocaine in Hair: Concise Communication," *Journal of Nuclear Medicine*, Vol. 23, No. 9, 1982, pp. 790–792.

[10] Puschel, K., Thomasch, P., and Arnold, W., "Opiate Level in Hair," *Forensic Science International*, Vol. 21, 1983, pp. 181–186.

[11] Smith, F. P. and Liu, 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.

[12] Arnold, W., "RIA Analysis of Head Hair for Narcotics and Substitutes," *Journal of Clinical Chemistry and Clinical Biochemistry*, Vol. 24, No. 10, 1986, pp. 797–798.

[13] Offidani, C., Carnevale, A., and Chiarotti, M., "Drugs in Hair: A New Extraction Procedure," *Forensic Science International*, Vol. 41, 1989, pp. 35–39.

[14] Franchesin, A., Morosini, L., and Dell'Ana, L., "Detection of Morphine in Hair with the Abbott TDX," *Clinical Chemistry*, Vol. 33, 1987, p. 2125.

[15] Suzuki, O., Hattori, H., and Asano, M., "Detection of Methamphetamine and Amphetamine in a Single Human Hair by Gas Chromatography/Chemical Ionization Mass Spectrometry," *Journal of Forensic Sciences*, Vol. 29, No. 2, April 1984, pp. 611–617.

[16] Balabanova, S. and Homoki, J., "Determination of Cocaine in Human Hair by Gas Chromatography/Mass Spectrometry," *Zeitschrift für Rechtsmedizin*, Vol. 98, 1987, pp. 235–240.

[17] Martz, R. M., "The Identification of Cocaine in Hair by GC/MS and MS/MS," *Crime Laboratory Digest*, Vol. 15, Vol. 3, July 1988, pp. 67–73.

[18] Graham, K., Koren, G., Klein, J., Schneiderman, J., and Greenwald, M., "Determination of Gestational Cocaine Exposure by Hair Analysis," *Journal of the American Medical Association*, Vol. 262, No. 23, Dec. 1989, pp. 3328–3330.

[19] Kidwell, D. A., "Analysis of Drugs of Abuse in Hair by Tandem Mass Spectrometry," *Proceedings*, 36th American Society of Mass Spectrometry Conference on Mass Spectrometry and Allied Topics, San Francisco, 1989.

[20] Baumgartner, W. A., Hill, V. A., and Blahd, W. H., "Hair Analysis for Drugs of Abuse," *Journal of Forensic Sciences*, Vol. 34, No. 6, Nov. 1989, pp. 1433–1453.

[21] Nakahara, Y., Takahashi, K., Takeda, Y., Konuma, K., Fukui, S., and Tokui, T., "Hair Analysis for Drug Abuse: Part II. Hair Analysis for Monitoring of Methamphetamine Abuse by Isotope Dilution Gas Chromatography/Mass Spectrometry," *Forensic Science International*, Vol. 46, 1990, pp. 243–254.

[22] Gehrke, C. W. and Leimer, K., "Trimethylsilylation of Amino Acids, Derivatization and Chromatography," *Journal of Chromatography*, Vol. 57, 1971, pp. 219–238.

[23] Gleispach, H., "The Use of Different Silylating Agents for Structure Analysis of Steroids," *Journal of Chromatography*, Vol. 91, 1974, pp. 407–412.

[24] Mawhinney, T. P. and Madsen, M. A., "*N*-Methyl-*N*-(*t*-Butyldimethylsilyl)Trifluoroacetamide and Related *N*-*t*-Butyldimethylsilyl Amides as Protective Domors," *Journal of Organic Chemistry*, Vol. 47, 1982, pp. 3336–3339.

[25] Balabanova, S., Brunner, H., and Nowak, R., "Radioimmunological Determination of Cocaine in Human Hair," *Zeitschrift für Rechtsmedizin*, Vol. 98, 1987, pp. 229–234.

[26] Cone, E. J., "Basics of Drugs in Hair," presented at Hair Analysis Workshop, U.S. Department of Justice, National Institute of Justice, 9–10 Jan. 1989, Washington, DC.

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