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Identification of Unique Cocaine Metabolites and Smoking By-Products in Postmortem Blood and Urine Specimens

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ABSTRACT: Toxicological investigation of suspected cocaine-related deaths routinely involves the identification of cocaine (COC) and its metabolites including benzoylecgonine (BE) and ecgonine methyl ester (EME) in postmortem specimens. We utilized solid-phase extraction followed by gas chromatography/mass spectrometry for the qualitative and quantitative analysis of cocaine and eight cocaine-related analytes. These analytes included anhydroecgonine methyl ester (AEME), a unique product formed during cocaine smoking, and cocaethylene (CE), formed by transesterification of cocaine in the presence of ethanol. Thirteen pairs of postmortem heart blood and urine specimens were analyzed from cases of death due to acute cocaine intoxication, multiple drug intoxication, or other non-drug related causes. COC, EME, and BE were detected in all specimens. The range of concentrations in blood were: COC, 23-2088 ng/mL; BE, 215-9195 ng/mL; and EME, 220-7275 ng/mL. AEME was identified in 2 blood and 10 urine specimens, and CE was identified in 1 blood specimen and 4 urine specimens. The identification of AEME in the specimens indicated that "crack" cocaine had been smoked, and the presence of CE indicated co-administration of cocaine and ethanol. The presence of these unique cocaine analytes in postmortem specimens provides valuable information regarding the cause and manner of death.

KEYWORDS: forensic science, forensic toxicology, postmortem, cocaine, cocaine metabolites, smoking, anhydroecgonine methyl ester, cocaethylene

The toxicological investigation of suspected cocaine-related deaths typically involves the analysis of a combination of specimens including blood and urine for the presence of cocaine and its principal metabolites including benzoylecgonine (BE) and ecgonine methyl ester (EME). Usually cocaine and cocaethylene are the only analytes quantitated in blood. In recent years, other metabolites of cocaine have been identified such as norbenzoylecgonine (NBE), norcocaine (NCOC), and ecgonine (1,2). In 1979, Lowry et al. identified the presence of hydroxycocaine and anhydroecgonine methyl ester (AEME) in the bile of an individual who died as the result of a cocaine overdose (3). Identification of these analytes was based upon interpretation of mass spectral patterns because no analytical standards were available at that time. Further, in 1991, Jatlow et al. reported the presence of cocaethylene (CE) in seven postmortem blood samples from individuals who had co-ingested cocaine and ethanol (4).

Identification of analytes such as AEME or CE in biological specimens in death investigations may provide additional information for the pathologist in assigning the cause and manner of death. The presence of these analytes may corroborate eye witness or scene investigation information, or enable correct assessment of the relative toxicity of drugs to which an individual was exposed.

In this study, heart blood and urine specimens obtained from postmortem cases in which cocaine had been previously identified were extracted by solid-phase extraction (SPE) and analyzed by gas chromatography/mass spectrometry (GC/MS). The objective was to identify and quantitate the thermal degradation product of cocaine, AEME, the transesterification product of cocaine and ethanol, CE, and other cocaine metabolites including BE, EME, NBE, NCOC, norcocaethylene (NCE), and ecgonine ethyl ester (EEE). The origin of these cocaine-related analytes is illustrated in Fig. 1.

Materials and Methods

Standards and Reagents

Cocaine hydrochloride was obtained from Mallinckrodt (St. Louis, MO). Benzoylecgonine tetrahydrate, ecgonine methyl ester hydrochloride, benzoylnorecgonine, and norcocaine hydrochloride

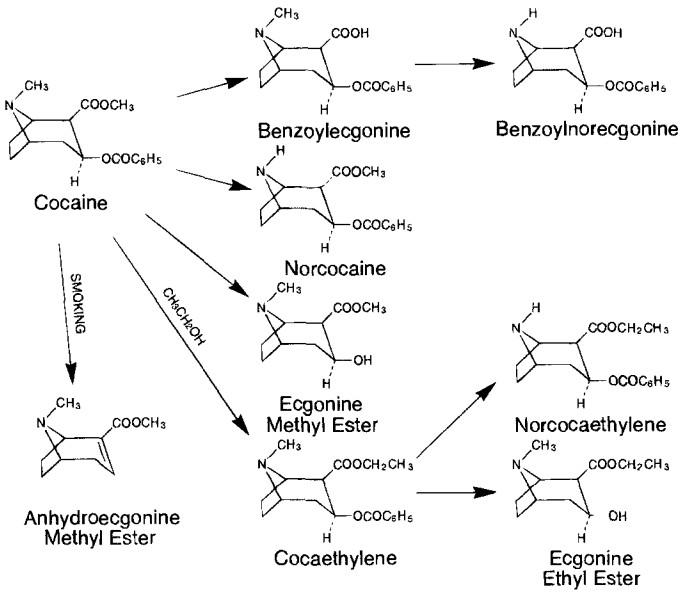

FIG. 1—*Origin of cocaine analytes.*

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were obtained from the Research Technology Branch, National Institute on Drug Abuse (Rockville, MD). Anhydroecgonine methyl ester and ecgonine ethyl ester were generous gifts from Dr. Andrew Allen, formerly of the Addiction Research Center, Intramural Research Program, National Institute on Drug Abuse (Baltimore, MD). Cocaethylene and norcocaethylene were also generous gifts from Ivy Carroll of the Research Triangle Institute (Research Triangle Park, NC). Deuterated internal standards, d_3-cocaine, d_3-benzoylecgonine tetrahydrate, and d_3-ecgonine methyl ester hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). d_3-Cocaethylene was purchased from Radian Corporation (Austin, TX).

All organic solvents were HPLC-grade and all chemicals were reagent grade (J.T. Baker, Phillipsburg, NJ). The SPE elution solvent, methylene chloride-isopropanol-ammonium hydroxide (80:20:2, v/v/v) was prepared daily. N,O-*bis*-(Trimethylsilyl)-trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) was purchased from Pierce (Rockford, IL).

Clean Screen® SPE columns (ZSDAU020) and 4-mL, 20-μm fritted reservoirs (RFV02F4P) were purchased from United Chemical Technologies (Horsham, PA).

Specimens

Postmortem heart blood and urine specimens were obtained from the Toxicology Laboratory of the Office of the Chief Medical Examiner in Baltimore, Maryland. Upon receipt in the Toxicology Laboratory, the blood specimens were mixed with approximately 200 mg of sodium fluoride and immediately placed into storage at −20°C. The urine specimens were stored untreated at 2–5°C for approximately 2 weeks and then transferred to a freezer and stored at −20°C.

Solid-Phase Extraction

The blood and urine specimens were extracted using SPE and analyzed by GC/MS according to a previously published procedure (5). Briefly, specimens were mixed with sodium acetate buffer and deuterated internal standards. After centrifugation, samples were filtered through fritted reservoirs prior to SPE. Extraction columns were conditioned sequentially with elution solvent, methanol, and deionized water. Sodium acetate buffer was added to the columns, followed by the buffered specimens. Vacuum was applied, and the columns were washed with deionized water, 0.1 M hydrochloric acid, and methanol before aspirating to dryness. Analytes were eluted from the column with elution solvent, and the eluates evaporated to dryness under nitrogen at 60°C. The samples were reconstituted in acetonitrile and BSTFA with 1% TMCS. The extracts were derivatized at 60°C.

Instrumentation

GC/MS analysis was performed on a Hewlett-Packard 5890A gas chromatograph with a 7673A automatic liquid sampler interfaced to a Hewlett-Packard 5970B mass selective detector operated in the selected ion monitoring mode. Analytes were identified based upon comparison of retention times and relative abundance of two confirming ions to the corresponding values of authentic standards. Quantitation was based upon ion peak area ratios of analyte to corresponding deuterated internal standard.

Standard curves were constructed in a concentration range of 6.25–1000 ng/mL. The limit of detection for cocaine, ecgonine methyl ester, and benzoylecgonine was 1 ng/mL, and for the remaining analytes, was between 3–6 ng/mL. The assay was linear over the concentration range of 6.25 to 1000 ng/mL. Specimens were diluted when necessary to ensure that the results fell within the linear range of the assay.

Controls were assayed in each run, including a 500 ng/mL cocaine control used to monitor the hydrolysis of cocaine during extraction and GC/MS analysis, and formation of AEME during GC injection. Hydrolysis of cocaine during the procedure was less than 10%, and no formation of AEME was detected at this concentration.

Results and Discussion

Blood and urine specimens from 13 cases were extracted by SPE and assayed by GC/MS for the presence of cocaine and 8 cocaine-related analytes. The results of these analyses are shown in Tables 1 and 2. The cases were chosen based upon the previous identification of cocaine by GC and GC/MS. The cause of death in these cases (listed in Table 1) was attributed to either acute cocaine intoxication, multiple drug intoxication, or a non-drug related cause.

COC, BE, and EME were present in all blood specimens. The blood COC concentrations ranged from 23-2088 ng/mL (mean = 278 ng/mL), the blood BE concentrations ranged from 215-9195 ng/mL (mean = 1824 ng/mL), and the blood EME concentrations ranged from 220-7275 ng/mL (mean = 1587 ng/mL). COC, BE, and EME were also present in all urine specimens in moderate to high concentrations.

AEME was present in 2 blood specimens at concentrations of 44 and 63 ng/mL and in 10 urine specimens at concentrations ranging from 41-4404 ng/mL. The identification of AEME in these subjects indicates recent use of smoked "crack" cocaine. In previous studies, Jacob et al. (6) and Jufer et al. (7) identified AEME in urine specimens obtained from subjects who smoked 100 mg and 42 mg of cocaine base, respectively. In addition, in a study of the pharmacokinetics and pharmacodynamics of smoked cocaine, AEME was not detected in plasma following doses of 10, 20, or 40 mg cocaine base (8). However, the mode of drug delivery was optimized to reduce pyrolysis of cocaine which may have resulted in non-detectable levels of AEME. In a study in which subjects smoked cocaine in a more realistic manner, Cone et al. (9) did not report the presence of AEME in blood following doses of 42 mg of cocaine base. In another study investigating the effects of passive inhalation of cocaine smoke, Cone et al. (10) reported the presence of AEME in the air and its deposition on the walls of a closed room in which 100 mg or 200 mg of crack cocaine was volatilized. However, the investigators failed to detect AEME in the blood of individuals sitting in the same room for 1 h during volatilization. Further, in a second experiment in this study (10), AEME was not detected in the urine of individuals exposed for 4 h to sidestream crack smoke during volatilization of 12.5–50 mg of cocaine base.

In this study, CE was detected in 1 blood specimen at a concentration of 36 ng/mL and 4 urine specimens at concentrations ranging from 47-357 ng/mL. EEE was detected in 4 blood specimens at concentrations ranging from 26-198 ng/mL and 7 urine specimens at concentrations ranging from 33-13506 ng/mL. NCE was present in 4 urine specimens at concentrations ranging from 38-944 ng/mL. The presence of cocaethylene and its metabolites is indicative of recent coadministration of cocaine and ethanol (4).

NCOC, a pharmacologically-active metabolite of cocaine, was

TABLE 1—*Cocaine, benzoylecgonine, and ecgonine methyl ester concentrations (ng/mL) in postmortem heart blood and urine specimens and the corresponding cause of death.*

Case Number	Cause of Death	Cocaine		Benzoylecgonine		Ecgonine Methyl Ester	
		Blood	Urine	Blood	Urine	Blood	Urine
1	Cocaine intoxication	74	8140	536	29560	612	9352
2	Cocaine intoxication	2088	114200	9195	7250	7275	126150
3	Gunshot wounds	34	12168	630	>300000	901	40380
4	Narcotic intoxication	139	129650	1645	>300000	673	11410
5	Gunshot wounds	36	13648	1450	962	279	27320
6	Cocaine intoxication	78	22335	1067	75550	429	43680
7	Drowning	66	10380	215	59250	1736	23845
8	Cocaine intoxication	66	48735	904	71650	556	34095
9	Cocaine and narcotic intoxication	614	53200	2718	245650	6010	84700
10	Gunshot wounds	138	56640	1736	1834	697	46980
11	Cocaine intoxication	91	1605	508	8402	370	3354
12	Cocaine and narcotic intoxication	172	391	2730	4511	872	831
13	Blunt injury	23	199225	370	33895	220	18510

TABLE 2—*AEME, and other cocaine metabolite concentrations (ng/mL) in postmortem heart blood and urine specimens.*

Case Number	AEME		CE		EEE		NCE		NCOC		NBE	
	Blood	Urine	Blood	Urine	Blood	Urine	Blood	Urine	Blood	Urine	Blood	Urine
1	44	118	0	58	70	79	0	64	0	0	0	2034
2	0	66	0	0	0	0	0	0	65	0	267	0
3	0	4404	0	0	42	13506	0	0	0	0	34	6730
4	0	396	0	357	0	480	0	0	264	350	0	0
5	0	2618	0	47	0	1644	0	38	0	0	0	3866
6	0	366	0	0	0	0	0	0	0	0	29	3298
7	0	102	0	0	0	0	0	0	23	0	0	35980
8	63	41	0	0	0	0	0	0	0	0	0	12460
9	0	129	0	0	0	657	0	944	0	0	91	22116
10	0	0	36	0	198	33	0	0	0	0	28	1718
11	0	0	0	0	0	0	0	0	0	0	0	78
12	0	0	0	0	0	0	0	0	0	0	107	115
13	0	982	0	139	26	2160	0	128	0	0	0	18780

Abbreviations: AEME = anhydroecgonine methyl ester; CE = cocaethylene; EEE = ecgonine ethyl ester; NCE = norcocaethylene; NCOC = norcocaine; NBE = norbenzoylecgonine.

detected in 3 blood specimens and 1 urine specimen at concentrations ranging from 23-264 ng/mL, and 350 ng/mL, respectively. The metabolism of NCOC by the cytochrome P450 systems leads to a hepatotoxic metabolite through the formation of a highly reactive free radical analyte (11). NBE was detected in 6 blood specimens at concentrations ranging from 28-267 ng/mL and 11 urine specimens at concentrations ranging from 78-35980 ng/mL.

In summary, COC and a number of cocaine-related analytes were detected in postmortem blood and urine specimens from subjects whose deaths were due to acute cocaine intoxication, multiple drug intoxication, or a non-drug related cause. There appeared to be no relationship between the COC concentration in blood and the cause of death. This supports the work of Karch (11) who suggests that there is no threshold concentration above which death due to COC intoxication can be assigned. The prevalence and concentration of AEME, CE, EEE, NCE, NCOC, and NBE in blood and urine had no apparent relationship to the blood concentrations of COC, BE, and EME. In addition, the pharmacologic significance and possible adverse effects are undetermined because little is known about the effects of these analytes. The presence of AEME is a marker for smoked "crack" cocaine, and the presence of CE indicates coadministration of cocaine and ethanol.

This is the first study in which minor cocaine metabolites have been measured in postmortem specimens. The relevance of these findings is unknown due to the small subject sample size. More investigations are needed to characterize fully the prevalence of these analytes in postmortem cocaine-related investigations, and to assess the relevance of these findings in assisting forensic investigators in determining the cause and manner of death.

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