Cocaine and its Major Metabolites in Plasma and Urine Samples from Patients in an Urban Emergency Medicine Setting

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Abstract

In this retrospective study, we examined the levels of cocaine and its major metabolites in plasma and urine from 29 randomly selected emergency department patients (19 males and 10 females, aged 19 to 55) whose urine screened positive for benzoylecgonine using fluorescence polarization immunoassay. Levels of cocaine along with benzoylecgonine, ecgonine methyl ester, and norcocaine were quantitated in EDTA plasma and urine from each patient using gas chromatography–mass spectrometry with selected ion monitoring. Admission diagnosis and history were also obtained for each patient. In plasma, the levels were 16–130 ng/mL for cocaine (n = 3), 27–96 ng/mL for ecgonine methyl ester (n = 9), and 18–1390 ng/mL for benzoylecgonine (n = 22). Norcocaine was not detected in any of the plasma samples. In urine, the concentration ranges were 4–40,130 ng/mL for cocaine (n = 23), 36–660,500 ng/mL for ecgonine methyl ester (n = 27), and 9–2520 ng/mL for norcocaine (n = 9). All urine samples were positive for benzoylecgonine (106–3,361,000 ng/mL), and benzoylecgonine was the only metabolite present in two urine samples (at concentrations of 407 and 435 ng/mL). Two patients had plasma and urine samples positive for all analytes (except norcocaine in plasma). The patient with the highest urinary concentrations of cocaine (40,130 ng/mL), ecgonine methyl ester (660,500 ng/mL), benzoylecgonine (3,361,000 ng/mL), and norcocaine (2520 ng/mL) had a small quantity of benzoylecgonine (465 ng/mL) in plasma. No correlation was noted with patient history, admitting diagnosis or symptomatology, or plasma/urine levels of cocaine or any of its metabolites.

Introduction

Cocaine use has been associated with serious medical complications such as myocardial infarction, seizures, and stroke (1–4). In 1998, approximately 3.8 million Americans used cocaine with 1.7 million individuals ages 12 and older using it at least once per month compared to 5.7 million in 1987 (5). Of this 1.7 million, about 437,000 used crack cocaine (5). Despite the considerable decline in its use, cocaine is still of primary concern and continues to be one of the most commonly abused drugs mentioned by patients seen in emergency departments (6). In 1998, about 32% of the drug-related episodes seen in the emergency departments throughout the United States were due to cocaine (6). Of these 172,014 cocaine-related cases, 13,640 occurred in the Chicago area (6).

Cocaine and/or some of its metabolites have been studied in various body fluids with volunteers and addiction patients using controlled clinical trials (7–9) and in postmortem blood, vitreous humor, and urine (10–12). However, only a few studies have looked at cocaine and its major metabolites in hospitalized patients (13,14). This retrospective study was designed to look at the distribution and levels of cocaine and some of its major metabolites in plasma and urine from randomly selected patients who presented to an urban emergency department.

Materials and Methods

Subjects

Patients who presented to the emergency department at the University of Illinois at Chicago Medical Center during a six-week period and screened positive for urinary benzoylecgonine (cutoff of 300 ng/mL) were randomly selected for this study. The study group included 29 subjects (19 males and 10 females aged 19 to 55 years). Surplus EDTA plasma and urine obtained from samples collected within 1 h of patient presentation to the emergency department as part of routine testing were aliquoted and stored at –70°C prior to analysis by gas chromatography–mass spectrometry (GC–MS). History and admission diagnosis were obtained retrospectively. The protocol was approved by the Institutional Review Board at the University of Illinois at Chicago Medical Center.

Sample analysis

Urine samples were initially screened for the presence of

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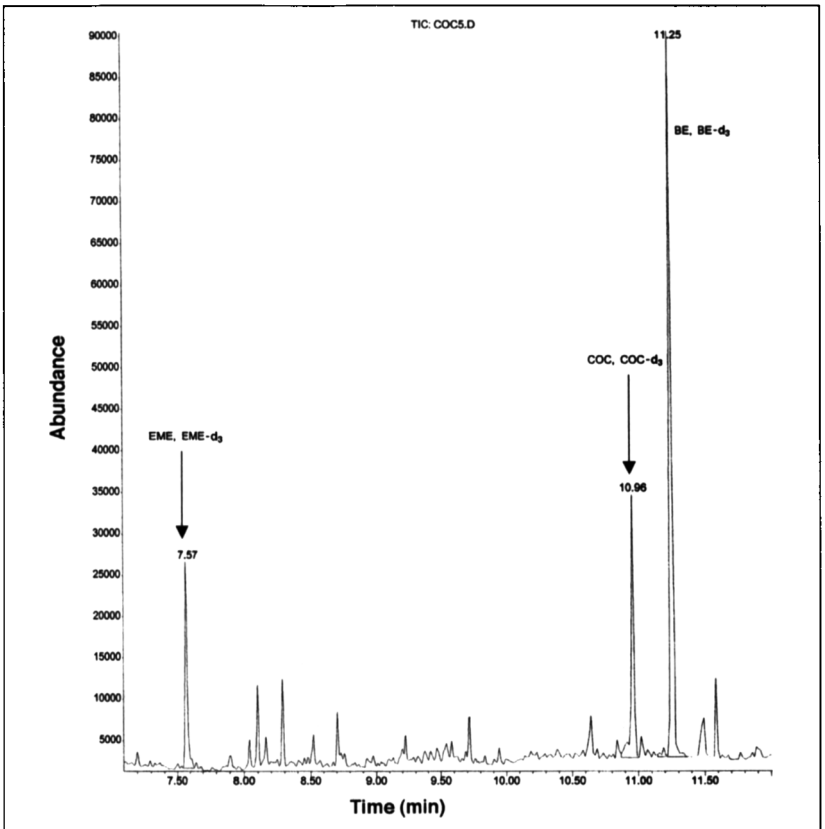

Figure 1. Total ion chromatogram of cocaine (COC), ecgonine methyl ester (EME), and benzoylecgonine (BE) in a plasma sample.

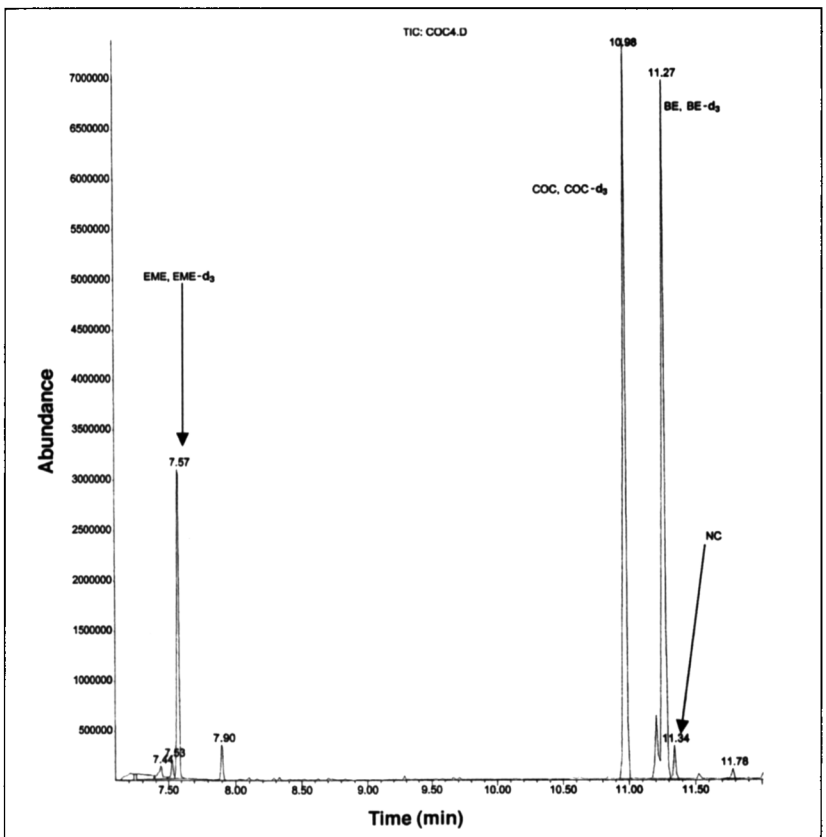

Figure 2. Total ion chromatogram of cocaine (COC), ecgonine methyl ester (EME), benzoylecgonine (BE), and norcocaine (NC) in a urine sample.

benzoylecgonine by fluorescent polarization immunoassay (FPIA) using the Abbott AxSYM® Immunoassay Analyzer (Abbott Laboratories, Abbott Park, IL).

Identification and determination of levels of cocaine and its metabolites was accomplished by GC–MS. For GC–MS studies, 40 µL of the appropriate deuterated standard (1 µg/mL) was added to 200 µL of each plasma or urine sample, standard, and control followed by the addition of 50 µL of perchloric acid (4.5N) to precipitate proteins. Following centrifugation the supernatant was transferred to 15-mL tubes, where 1 mL of 0.1M hydrochloric acid, 1 mL of 1.93M acetic acid, and 10 mL of deionized water were added sequentially. Each diluted sample was then added to an HCX 10-mL, 200-mg Isolute® solid-phase extraction column (International Sorbent Technologies, Lakewood, CO) that was preconditioned with methanol (3 mL), deionized water (3 mL), and 1.93M acetic acid (1 mL) and then passed through the column using positive pressure (< 5 mm Hg). Columns were then sequentially washed with deionized water (3 mL), 0.1M hydrochloric acid (1 mL), and methanol (3 mL). Columns were dried after each solvent addition. Cocaine and its metabolites were eluted from the column using 3 mL of a mixture of methylene chloride/isopropanol/ammonium hydroxide in a ratio of 78:20:2 (v/v). Samples were evaporated to dryness under air, reconstituted in 35 µL of acetonitrile, transferred to 100-µL conical inserts, and then placed in 0.1-mL autosampler vials, which were then crimped. Samples were derivatized by adding 70 µL of BSTFA containing 1% TMCS followed by incubation at 60°C for 30 min. For GC–MS analysis, 1 µL of derivatized sample was injected (splitless mode, injector at 250°C) onto a Hewlett-Packard 6890 series GC fitted with a HP-5MS capillary column (30 m × 250 µm × 0.25 µm, Hewlett-Packard, Palo Alto, CA), in conjunction with a Hewlett-Packard 5973 mass selective detector (ion source at 230°C; quadrupole at 150°C). The GC oven was heated at 70°C for 1 min, then increased to 300°C at 20°C/min, and maintained for 2 min. The following ions were monitored using the mass selective ion detector in the selected ion monitoring (SIM) mode: *m/z* 82, (96), 271, ecgonine methyl ester; *m/z* 85, (99), 274, ecgonine methyl ester-d_3; *m/z* (182), 272, 303, cocaine; *m/z* (185), 275, 306, cocaine-d_3; *m/z* 82, (240), 361, benzoylecgonine; *m/z* 85, (243), 364, benzoylecgonine-d_3; and *m/z* (140), 240, 346, norcocaine. The ions noted in parentheses were used for quantitation. The data were analyzed by Hewlett-Packard Chemstation® for Windows 95 software. For calculations, a standard curve ranging from 20 to 120 ng/mL was constructed.

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The limit of quantitation was based on the lowest corresponding standard (20 ng/mL). Samples that exceeded 120 ng/mL were diluted with deionized water and then reanalyzed.

Table I. Retention Times and Accuracy and Precision Data for GC–MS Analysis of Cocaine and Metabolites

Compound	Retention window (min)	Intraday target concentration (% target ± % CV) 25 ng/mL	Intraday target concentration (% target ± % CV) 70 ng/mL	Interday target concentration (% target ± % CV) 25 ng/mL	Interday target concentration (% target ± % CV) 70 ng/mL
EME	7.47–7.67	106.8 ± 4.0 (*n* = 3)	104.6 ± 6.5 (*n* = 3)	103.4 ± 6.9 (*n* = 25)	109.2 ± 8.7 (*n* = 18)
COC	10.86–11.06	98.8 ± 13.6 (*n* = 3)	98.0 ± 10.2 (*n* = 3)	105.2 ± 12.6 (*n* = 25)	96.2 ± 5.9 (*n* = 21)
BE	11.15–11.35	94.5 ± 15.2 (*n* = 3)	90.3 ± 8.2 (*n* = 3)	88.5 ± 9.6 (*n* = 16)	93.5 ± 6.6 (*n* = 18)
NC	11.24–11.44	92.6 ± 6.3 (*n* = 3)	105.7 ± 12.1 (*n* = 3)	92.4 ± 9.5 (*n* = 20)	100.3 ± 10.8 (*n* = 20)

* Abbreviations: EME, ecgonine methyl ester; COC, cocaine; BE, benzoylecgonine; and NC, norcocaine.

Results and Discussion

Table I contains the validation data and retention time windows for cocaine, norcocaine, ecgonine methyl ester, and benzoylecgonine. All were within acceptable limits (relative accuracy and coefficient of variation less than 20%). Standard curves for each respective compound were linear from 20 to 120 ng/mL (correlation coefficients were 0.995, 0.999, 0.997, and 0.997).

Figure 1 is a total ion chromatogram from a plasma sample that contained ecgonine methyl ester, cocaine, and benzoylecgonine. Figure 2 is a total ion chromatogram from a urine sample that contained norcocaine in addition to ecgonine methyl ester, cocaine, and benzoylecgonine. These chromatograms are representative of those typically seen with this method for plasma and urine samples.

Table II lists some of the demographic information along with the concentrations of cocaine and

Table II. Concentration of Cocaine and Metabolites in Plasma and Urine of Emergency Department Patients who Screened Positive for Urinary Benzoylecgonine

Subject	Age	Gender	Admitting diagnosis history	Heart rate (EKG)	Plasma EME*	Plasma COC	Plasma NC	Plasma BE	Urine EME	Urine COC	Urine NC	Urine BE
1	48	F	Chest pain/MI	sinus tach	ND	ND	ND	429	50,300	11,800	ND	160,000
2	49	M	Chest pain	sinus tach	81	130	ND	1390	25,000	6180	161	75,400
3	30	F	Contractions/labor	NSR	ND	ND	ND	278	323	16†	ND	3230
4	40	M	Depression/alcohol detox.	NSR	ND	ND	ND	ND	ND	ND	ND	407
5	39	M	Auditory hallucinations	sinus tach	96	16†	ND	374	29,500	25,200	910	31,800
6	52	M	Cellulitis	NSR	27	ND	ND	436	206	38	ND	1830
7	32	M	Depression	NSR	ND	ND	ND	324	5760	143	15†	57,200
8	29	F	Contractions/labor	(–)	ND	ND	ND	ND	36	4†	ND	106
9	39	M	Depression/hallucinations	sinus tach	65	ND	ND	964	95,300	5450	178	257,000
10	55	F	Chest pain	sinus tach	ND	ND	ND	ND	424	22	ND	1520
11	29	F	Contractions/labor	(–)	ND	ND	ND	174	3290	36	9†	14,000
12	42	F	Depression	NSR	50	18†	ND	636	32,200	6220	ND	83,100
13	46	M	Chest pain/MI	NSR	ND	ND	ND	27	295	24	ND	1770
14	46	M	Urosepsis	sinus tach	ND	ND	ND	ND	194	48	ND	343
15	19	F	Contractions/labor	(–)	ND	ND	ND	98	12,600	492	35	52,700
16	38	M	Depression/hallucinations	(–)	ND	ND	ND	18†	396	9†	ND	2140
17	39	M	Auditory hallucinations	sinus tach	57	ND	ND	732	261,000	16,500	229	484,000
18	44	M	Orbital fracture	(–)	ND	ND	ND	214	1150	76	ND	7710
19	32	M	Chest pain	NSR	ND	ND	ND	ND	486	6†	ND	2130
20	51	M	Chest pain	sinus tach	65	ND	ND	367	2720	563	ND	4160
21	36	M	Depression	(–)	ND	ND	ND	ND	126	7†	ND	525
22	42	M	Abdominal pain/cirrhosis	NSR	ND	ND	ND	458	1230	56	ND	8440
23	30	F	Depression	NSR	ND	ND	ND	466	661,000	40,100	2250	3,360,000
24	51	M	Chest pain	NSR	ND	ND	ND	156	280	ND	ND	1890
25	29	F	Contractions/labor	(–)	ND	ND	ND	19†	1120	ND	ND	6170
26	35	F	Chest pain/MI	sinus tach	ND	ND	ND	ND	ND	ND	ND	435
27	52	M	Depression/hallucinations	NSR	ND	ND	ND	836	735	ND	ND	40,200
28	32	M	Depression/hallucinations	(–)	41	ND	ND	286	8570	750	8†	20,200
29	39	M	Facial trauma/addiction	(–)	79	ND	ND	601	24,800	ND	ND	72,200

* Abbreviations: COC, cocaine; EME, ecgonine methyl ester; NC, norcocaine; BE, benzoylecgonine; ND, none detected; MI, myocardial infarction; sinus tach, sinus tachycardia; NSR, normal sinus rhythm; (–), EKG not performed.
† Below limit of quantitation.

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its metabolites that were detected in the plasma and urine samples of the patients seen in the emergency department. All 29 urine samples were found to have quantitatable amounts of benzoylecgonine, which showed 100% concordance between GC–MS and FPIA. The quantitation for benzoylecgonine ranged from 106 to 3,361,000 ng/mL. Twenty-seven (93.1%) urine samples were found to have detectable levels of ecgonine methyl ester, with values ranging from 36 to 660,500 ng/mL. This percentage is similar to Ramcharitar et al. (11) who reported 93% of the cases of cocaine use were positive for ecgonine methyl ester in postmortem urine. Parent compound (cocaine) was detected in 23 (79.3%) urine samples, ranging from 4 to 40,130 ng/mL, and norcocaine was detected in 9 (31.0%) urine samples, ranging from 8 to 2250 ng/mL. In plasma, benzoylecgonine was the most prevalent metabolite, detected in 22 (75.9%) samples, ranging from 18 to 1390 ng/mL. Nine (31.0%) plasma samples were found to contain detectable amounts of ecgonine methyl ester, ranging in concentration between 27 and 96 ng/mL. Only three (10.3%) plasma samples contained parent compound (cocaine) in concentrations of 16, 18, and 130 ng/mL. Norcocaine was not detected in any of the plasma samples. Although the analytical limit of quantitation for each component was 20 ng/mL, values less than 20 ng/mL were reported if qualifying ions were present.

History and diagnosis were obtained on each patient prior to admission to the hospital. The average age of the entire group was 39 years; the average age of male subjects was 42 years, and the average age of female subjects was 39 years. Of the 29 patients studied, 9 presented to the emergency department with sinus tachycardia and 7 complained of chest pain. Of those with chest pain, two of them had a confirmed myocardial infarction. Six patients, some of whom suffered from drug-induced depression, had hallucinations, but none experienced seizures. Regardless of patient history, diagnosis, or symptomatology, no distinct pattern was noted with the presence or levels of cocaine or any of its metabolites in plasma or urine. Because this was a retrospective study using precollected specimens, not all subjects were questioned as to their frequency of use (acute versus chronic), route of administration, or form or amount of drug used. These variables can affect the metabolism of cocaine (8,9,15–18), and thus it is not surprising that there was a broad range of plasma/urine levels of cocaine and its metabolites. Although only one patient had detectable levels of ethanol in the plasma and urine, measurement of cocaethylene would have been invaluable as well because it has been shown that it can be present in the absence of detectable levels of ethanol (15,19). Thus, a prospective clinical study with detailed patient history and measurement of other compounds, such as cocaethylene, which this study did not entail, is needed. Such information would be necessary if the intent of the study were to relate levels of cocaine and its metabolites to possible toxicity and patient outcome.

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