TECHNICAL NOTE

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Detection of Cocaine and Its Metabolites in Breast Milk*

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ABSTRACT: A method was developed for measuring cocaine and its metabolites, benzoylecgonine, ecgonine methyl ester, norcocaine, ecgonine ethyl ester, cocaethylene, and *m*-hydroxybenzoylecgonine, in breast milk by gas chromatography/mass spectrometry. Limits of detection for this method ranged from 2.5 to 10 ng/mL, and limits of quantitation ranged from 5 to 50 ng/mL. For each of the compounds measured by this method, linear response was demonstrated to 750 ng/mL. Breast milk was collected from 11 mothers who admitted to drug use during pregnancy and ten drug-free volunteers serving as control subjects. Cocaine was detected in six of the specimens obtained from drug-exposed subjects, and in none of the drug-free control subjects. In breast milk specimens where cocaine and one or more of its metabolites were detected, the concentration of parent compound was greater than any of the metabolites. The highest cocaine concentration found was over 12 μg/mL. Breast-fed infants of cocaine abusing mothers may be exposed to significant amounts of drug orally.

KEYWORDS: forensic science, cocaine, cocaine metabolites, mass spectrometry, breast milk

Cocaine use during pregnancy exposes the fetus to potentially harmful concentrations of the drug, and is associated with low birth weight and antenatal morbidity (1–3). Postnatal opportunities for cocaine exposure also exist, including accidental ingestion, passive inhalation (4), and breast milk (5,6). In one report, a two-week-old baby developed tachycardia, tachypnea, hypertension, and irritability 3 h after being breast-fed (5). The mother was a cocaine user, and the infant's symptoms subsided 48 h after nursing was discontinued. Cocaine and benzoylecgonine were detected in the mother's breast milk for 36 h after her last reported use of the drug, and in the baby's urine for 60 h after nursing was discontinued. In another report, cocaine and cocaethylene were measured in maternal milk at concentrations estimated to be 20 times the concentration of drug in maternal blood (6). These results agree with studies of cocaine distribution in lactating rats (7). Cocaine concentrations in breast milk from cocaine using mothers can represent a health risk to a nursing baby.

We developed a method for measuring cocaine and several of its metabolites in breast milk by gas chromatography/mass spectrometry. The clinical application of the method was verified by measuring these compounds in breast milk obtained from women with a history of gestational cocaine use, and comparing the results with specimens collected from cocaine-free mothers.

Methods

Calibration and Controls—Calibration standards were prepared in baby formula (Carnation Good Start®, Nestlé S.A., Vevey, South Africa) by fortification with pure standards (Radian International LLC, Austin, TX) to produce concentrations between 25 and 750 ng/mL. A 500 ng/mL cocaine standard was included in each analytical batch to monitor spontaneous hydrolysis of the parent compound to benzoylecgonine and/or ecgonine methyl ester that might occur during extraction, derivatization, and analysis. Control samples (0, 200, and 600 ng/mL) were prepared by addition of pure standards (Radian International) to baby formula and were included in each analytical run.

Extraction and Derivatization—Tri-deuterated internal standards of cocaine, benzoylecgonine, ecgonine methyl ester, and cocaethylene were added to 1.0 mL of breast milk (final concentration 200 ng/mL), followed by 3.0 mL of 0.025 M phosphate buffer (pH 4). Cocaine and metabolites were extracted on a mixed bed solid-phase column (Clean Screen™, United Chemical Technologies, Bristol, PA) preconditioned with 3.0 mL methanol, 3.0 mL

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deionized water, and 2.0 mL 0.025 M phosphate buffer. After addition of specimens, the columns were dried for 30 s before washing with 2.0 mL deionized water, 2.0 mL 0.1 M HCl, and 6 mL methanol. Columns were dried again for 30 s, and analytes were eluted with an 80:20:2 mixture of methylene chloride:isopropanol:concentrated aqueous ammonium hydroxide. The eluates were evaporated to dryness at 40°C under nitrogen, and *tert*-butyl silyl derivatives were generated by adding 30 μL of methyl-(tert-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA, Pierce, Rockford, IL) and heating at 90°C for 60 min.

GC/MS Analysis—GC-MS analysis was performed on a Hewlett-Packard 5890 Series II Plus gas chromatograph equipped with a Hewlett-Packard 7673A automated sampler and interfaced with a Hewlett-Packard 5972A mass selective detector (Hewlett-Packard Corp., Little Falls, DE). Chromatography was performed on an HP-5MS (30 m × 0.25 mm ID) column with a 0.25 μm film thickness (Hewlett-Packard Corp.) using helium as carrier gas at a flow rate of 1 mL/min. The injection port temperature was 275°C and the detector temperature was maintained at 290°C. The oven temperature was set to 90°C for 1 min, increased to 220°C at 30°C/min, held for 0.5 min, then increased to 330°C at 20°C/min. The total analytical cycle time was 13 min per specimen.

The mass spectrometer was operated in the selected ion-monitoring (SIM) mode and calibrated using ratios of ion peak areas of target compounds to isotopically-labeled internal standards. Cocaine, benzoylecgonine, ecgonine methyl ester, and cocaethylene were measured relative to their corresponding trideuterated analogues. Norcocaine was measured relative to trideuterated cocaine, ecgonine ethyl ester was measured relative to trideuterated ecgonine methyl ester, and *m*-hydroxybenzoylecgonine was measured relative to trideuterated benzoylecgonine.

Method Performance—The limit of detection (LOD) was determined by integrating the signal response for blank specimens, multiplying the integrated area by three, and using the calibration slope to calculate the analyte concentration corresponding to a signal to noise ratio of three. The limit of quantitation (LOQ) was determined by measuring sequentially diluted standards and identifying the lowest concentration that was quantifiable within ±20% of the target concentration with acceptable ion ratios. Within-run precision was calculated from the results of ten repetitions. Between-run precision was calculated with ten measurements made at intervals ranging from seven days to one month. Analytical recoveries were determined by the difference between quantitative measurements made when internal standard was added before and after solid-phase extraction.

Clinical Specimens—The University of Florida Institutional Review Board approved the protocol. Subjects were recruited from women delivering in a regional tertiary care teaching hospital. Women who were less than 18 years of age, non-English speaking, or users of any illicit or abused drugs other than cocaine, marijuana, or alcohol were excluded from the study. After delivery, a detailed drug history was obtained from study subjects. Subjects were classified as cocaine users either by history or positive laboratory findings. The study sample included 11 cocaine users (all of whom admitted cocaine use) and 10 control subjects. Breast milk was collected from each mother as soon after delivery as possible. Milk was expressed into a sterile specimen container and stored frozen at −20°C until laboratory analysis.

Results

Analytical performance data for cocaine and its metabolites are summarized in Table 1. The minimum detectable concentrations using this technique were 2.5 ng/mL for cocaine, benzoylecgonine and ecgonine methyl ester, 5.0 ng/mL norcocaine and cocaethylene, 10 ng/mL of *m*-hydroxybenzoylecgonine, and 25 ng/mL of ecgonine ethyl ester. Detectable amounts of drug below the minimum quantifiable concentration, but meeting all identification criteria, were reported as "trace." Limits of quantitation ranged from 5 ng/mL, for benzoylecgonine and ecgonine methyl ester, to 50 ng/mL ecgonine ethyl ester. Extraction recoveries were poor for ecgonine ethyl ester, norcocaine, and *m*-hydroxybenzoylecgonine (41%, 49%, and 52%, respectively, for 100 ng/mL controls). Recoveries of all analytes were better at the higher concentration control (500 ng/mL). For benzoylecgonine, cocaethylene, cocaine, and norcocaine, coefficients of variation (within-run and between-run) were 11% or lower. Precision was poorest for ecgonine ethyl ester (CV = 28%, between-run for 100 ng/mL control).

Results of cocaine, benzoylecgonine, ecgonine methyl ester, and norcocaine measurements in six breast milk specimens collected from women admitting cocaine use are summarized in Table 2. None of the other metabolites was detected in any of the specimens. All of the control breast milk specimens, and five specimens from women, who admitted prenatal cocaine use, were negative for cocaine and its metabolites.

Discussion

Although clinicians have been concerned about the passage of cocaine in maternal breast milk, data upon which to make recommendations have been scarce. The Committee on Drugs of the American Academy of Pediatrics based its 1994 recommendation

TABLE 1—*Limit of detection (LOD), limit of quantitation (LOQ), recovery, and precision of cocaine and metabolites in breast milk.**

			Recovery (%)		Within Run Precision (% CV)		Between Run Precision (% CV)	
Analyte	LOD	LOQ	100	500	100	500	100	500
EME	2.5	5	63	71	16	11	19	19
EEE	25	50	41	53	23	22	28	19
NCOC	5	10	49	58	8	10	10	9
COC	2.5	10	100	108	9	8	9	8
CE	5	10	78	83	9	9	9	6
BE	2.5	5	82	89	11	10	7	11
MOHBE	10	25	52	64	8	16	14	18

*Concentrations noted in ng/mL. Abbreviations are as follows: ecgonine methyl ester = EME; ecgonine ethyl ester = EEE; norcocaine = NCOC; cocaine = COC; cocaethylene = CE; benzoylecgonine = BE; *m*-hydroxybenzoylecgonine = MOHBE.

TABLE 2—*Concentrations (ng/mL) of cocaine and its metabolites in breast milk from women admitting to cocaine use prior to delivery.*

Subject	Cocaine	Benzoylecgonine	Norcocaine	Ecgonine Methyl Ester
1	12 130	4 070	Trace	119
2	Trace	ND*	ND	ND
3	25	3.6	ND	Trace
4	15	11	ND	ND
5	Trace	ND	ND	Trace
6	Trace	Trace	ND	Trace

* ND: None detected.

against breast-feeding when maternal cocaine use is suspected on a single case report. It is widely accepted that breast-feeding is beneficial for infants, and therefore any recommendation against the practice should be based on clear evidence that potential risks outweigh the benefits. In this study, potentially harmful amounts of cocaine were detected in a breast milk specimen collected from a cocaine-abusing mother.

Cocaine is readily soluble in nonpolar solvents, so its distribution into lipid-rich breast milk is predictable. More polar metabolites of cocaine, such as benzoylecgonine and ecgonine methyl ester, may be more soluble in blood, and the results of this investigation support the disproportionate partitioning of cocaine, relative to its metabolites, into breast milk. The elimination half-life of cocaine in humans is approximately 1 h, so its distribution into breast milk must occur rapidly.

It has been suggested that regular use of cocaine by lactating mothers may result in significant exposure of nursing infants to the drug. The presence of 12.1 μg of cocaine per mL of breast milk from one of the subjects in this study supports this view. A 100-mL breast milk meal would deliver a 1.21 mg oral dose of cocaine. The oral bioavailability of cocaine is estimated at 60%, resulting in a 0.726 mg absorbed dose. If a 4-kg infant consumed that dose every 3 h, with a nominal clearance rate of 20 mL of blood per minute, the potential steady state blood concentration of cocaine could reach 200 ng/mL (using the equation: C_{ss} = FDose/Cl∗t, where C_{ss} = steady state blood concentration of cocaine, FDose = orally bioavailable dose, Cl = clearance, and t = dosing interval). This quantity is comparable to blood cocaine concentrations measured in adults after administration of a 1.5 mg/kg intranasal (8) or 16 mg intravenous dose (9).

Little is known about neonatal metabolism of orally ingested cocaine. There is some evidence that low birth weight infants exposed to cocaine *in utero* do not produce the hydrolytic products, benzoylecgonine and ecgonine methyl ester, to the same degree observed in adults (10), but this observation has not been confirmed. It is likely that concentrations of cocaine metabolites in breast milk are variable, depending on the temporal relationship between cocaine use and collection of the specimen, so it would be difficult to assess the degree to which metabolites found in neonatal blood or urine reflect neonatal, as opposed to maternal, cocaine metabolism.

One specimen (Subject 1) had cocaine, benzoylecgonine, and ecgonine methyl ester concentrations that vastly exceeded the other specimens measured in this study. Although this finding suggests more intense cocaine use by Subject 1, the patient history did not reveal this. The subject admitted to using cocaine five days prior to delivery, but did not disclose any additional historical information that would be consistent with the high cocaine concentration in her breast milk. However, several studies have demonstrated that maternal history is not a reliable indicator of prenatal cocaine exposure (11–13).

Breast milk may be an overlooked source of significant postnatal cocaine exposure in nursing infants. However, cocaine was detected in only 6 of 11 subjects who admitted to using the drug, so analysis of breast milk is not a sensitive method for revealing gestational cocaine exposure.

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