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Detection of Cocaine, Norcocaine, and Cocaethylene in the Meconium of Premature Neonates

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ABSTRACT: Our objective was to investigate the methodologic detection of cocaine abuse during pregnancy by determining the viability of meconium analysis for cocaine and its metabolites using chromatographic procedures as an alternative to urine testing using enzyme multiplied immunoassay technique.

Our design was as follows: meconium and urine were taken from 106 very low birthweight premature babies. Meconium analysis for cocaine and its metabolites using extraction and chromatographic analysis was compared with the criterion standard immunoassay testing for urine.

The work was carried out at The University of Chicago Hospital, Department of Pediatrics and the University of Illinois at Chicago, Department of Pharmacodynamics. Our patients were very low birthweight, premature babies (mean birthweight 1109 g; mean gestational age 29.1 weeks). Gender was evenly divided between male and female.

The outcome measures were as follows: two active metabolites, norcocaine and cocaethylene, were detected in the meconium, but not in the urine, of some of the neonates. Determination of cocaine exposure in the newborn influenced assignment of babies in research studies as well as psychosocial evaluation and subsequent treatment of the neonate.

Our results were: of the 106 meconium samples analyzed, 21 (19.8%) were positive for cocaine (n = 19, 0.24–0.78 mg/kg), norcocaine (n = 7, 0.10–0.56 mg/kg), cocaethylene (n = 1, 0.12 mg/kg) or combinations thereof. Benzoylecgonine was not detected in any of the samples. Of the urine samples analyzed by immunoassay, only 8 (7.5%) were positive for cocaine metabolites.

We conclude that meconium is a better sample than urine for determining cocaine exposure in utero. The presence of two neuroactive metabolites, norcocaine and cocaethylene, is reported, norcocaine for the first time. Immunoassay screening procedures for urinalysis are inadequate because false-negative results are obtained.

KEYWORDS: toxicology, cocaine, cocaethylene, norcocaine, meconium, HPLC, GC-MS

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Cocaine has been found in significant percentages of pregnant women and their newborn infants in large urban medical centers. In particular, low birthweight and low gestational age are associated with drug use during pregnancy [*1*]. The methods for determining exposure to cocaine vary. In early studies of substance-exposed infants, maternal interview was the means of identification of such infants. Frank et al. [*2*] demonstrated that more women were identified as cocaine users through the combination of maternal interview and maternal urine assay. More recently, the common practice in clinical settings when drug abuse during pregnancy is suspected is to perform urinalysis on both the maternal and neonatal urine. Analysis of the infant's meconium to detect in utero exposure to cocaine is thought to be of increasing utility. Meconium is a dark-green mass of water, cells, mucus and bile pigments that is formed in the fetal gut from swallowed amniotic fluid and sloughed gastrointestinal epithelial cells from 16 weeks to birth [*3*] and that is passed one, two, or three days after birth. Cocaine and metabolites are passed into the amniotic fluid from fetal urine, and meconium is thought to reflect these swallowed and/or sloughed stable metabolites. Analysis of meconium may therefore reflect in utero exposure to cocaine [*4*].

Analysis of urine in the clinical laboratory most commonly uses enzyme-multiplied immunoassay technique (EMIT) or radioimmunoassay (RIA), which predominantly test for benzoylecgonine, the major urinary metabolite of cocaine formed by chemical hydrolysis [*5*]. This method can detect cocaine only if it is present at a high concentration. Previously, Ostrea et al. [*4*] have reported the presence of cocaine and benzoylecgonine in meconium using RIA, but other metabolites—ecgonine, ecgonine methyl ester, norcocaine, etc.—have not been detected using this procedure. Abusada et al. [*6*] have reported the presence of ecgonine methyl ester, benzoylecgonine, cocaethylene and cocaine in the meconium of newborns using solid-phase extraction and gas chromatography-mass spectrometry.

We report the application of a high-performance liquid chromatographic method (HPLC) and confirmation by gas chromatography-mass spectrometry (GC-MS) for the identification of pharmacologically active metabolites, cocaethylene and previously undetected norcocaine, in the meconium of very low birthweight (VLBW) premature babies.

Experimental

Patient Population and Sample Collection

This protocol involving investigation of human subjects was approved by the Institutional Review Board of the University of Chicago. Waiver of informed consent was permitted by the IRB because of the nature of the collection of excreta. Reporting of results was protected from disclosure by certificate #DA-91-09 from the Department of Health and Human Resources.

Meconium was collected from 106 VLBW (< 1500 g) babies in an intensive care nursery as part of a large prospective clinical study [*8*]. The intensive care nursery is a level III perinatal center serving a large population which primarily includes the residents of South and Southwest portion of the Greater Chicago Area. The meconium was collected at random from among 178 such babies admitted during the period of June 1990 to February 1991. The stools were frozen and stored at −20°C prior to analysis. The analysis team was blind to the clinical status of the babies or their known drug exposure status. The range of gestational age of the babies was 25 to 33 weeks (mean 29.1. S.D. 2.1 weeks), and their birthweights ranged from 710 g to 1490 g (mean 1109, S.D. 238 g). Gender was evenly divided between male and female.

Drug Standards and Chemicals

Cocaine, benzoylecgonine, and bupivacaine were obtained from Sigma Chemical Co. (St. Louis, MO). Norcocaine and cocaethylene were obtained from the National Institute

of Drug Abuse (NIDA, Research Technology Branch, Division of Research). All chemicals used were analytical grade or better and all solvents HPLC grade (Fisher, Itasca, IL). Negative meconium stools were obtained and spiked with cocaine, benzoylecgonine, norcocaine and cocaethylene at three different concentrations (0.1, 0.5 and 1.0 mg/kg).

Extraction Procedure

Solid-phase extraction was performed on Clean-Screen™ DAU columns (Worldwide Monitoring, Horsham, PA), containing 0.2 g of a copolymer with dual activity (cation exchange and nonpolar interactions).

Meconium (0.5 g) was vortexed with methanol (2 mL), centrifuged and the supernatant was transferred to a tube containing phosphate buffer (3 mL; pH 3) and bupivacaine (0.1 mg/L; 0.02 mL) as an internal standard. The mixture was applied to a Clean Screen™ column that had been previously conditioned with methanol (2 × 3 mL), water (3 mL) and buffer (3 mL; pH 3). The sample was drawn through under vacuum and the column was air-dried. The sorbent was then washed with buffer (3 mL), 100 mM hydrochloric acid (3 mL) and methanol (3 mL). Finally, the isolates were eluted with chloroform:isopropanol : ammonium hydroxide (78:20:2, v/v) (3 × 1 mL). The eluent was evaporated to dryness without heating. The residue was reconstituted in methanol (0.1 mL) prior to analysis.

Chromatographic Procedures

High-Performance Liquid Chromatography (HPLC)—A Perkin Elmer Series 2 pump was used to deliver solvent at a rate of 1.5 mL/minute onto a C18 ODS μBondapak (300 mm × 3.9 mm i.d.) column (Waters, Milford, MA). A C18 guard column (Guard-Pak, Waters) and a Rheodyne injection system with a fixed loop (20 μL) were also incorporated into the system. A Spectra Physics Focus multiwavelength detector connected to an IBM Personal Data System 2 microprocessor was used to monitor the eluent from the column at 230, 255 and 275 nm and full spectra were recorded over the wavelength range 190 to 400 nm. The mobile phase consisted of 0.025 M potassium dihydrogen phosphate:acetonitrile: butylamine (500:125:12.5, v/v). The final pH was adjusted to 2.9 with 85% o-phosphoric acid.

Gas Chromatography-Mass Spectrometry (GC-MS)—All extracts that were positive using HPLC were confirmed using GC-MS. The remaining sample was evaporated to dryness and derivatized with pentafluoropropionic anhydride (PFPA) and 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) before electron impact ionization GC-MS analysis using a previously reported procedure [7].

Results

The extraction procedure was rapid (up to 10 samples could be extracted within 10 minutes), efficient and reproducible. Extracted standards gave the following percentage recoveries and coefficients of variation. Cocaine (100 ± 9.7%), norcocaine (86.1 ± 1.9%), cocaethylene (72.4 ± 8.6%) and benzoylecgonine (64.1 ± 10.6%). The extracts were sufficiently clean for direct injection onto the HPLC system without further cleanup stages. The HPLC analysis system was linear for spiked meconium samples over the concentration range 0.1–10 mg/kg. The minimum quantitation level of cocaine and its metabolites by HPLC was 0.1 mg/kg and the minimum detection level 0.03 mg/kg.

Of the 106 meconium samples analyzed, 21 (19.8%) were found to be positive for cocaine, norcocaine, cocaethylene or combinations thereof. Fourteen were found to be positive for cocaine; four for cocaine and norcocaine; two for norcocaine only; and one

for cocaine, norcocaine and cocaethylene (see Table 1). History available for the infant with cocaethylene in the meconium showed that the mother had a history of alcohol and cocaine abuse. None of the samples contained benzoylecgonine, the major urinary metabolite of cocaine in adults. In three cases, twins were sampled. In all the cases, the meconium of one of the twins tested positive and one negative. In Case 1, cocaine (0.78 mg/kg) and norcocaine (0.15 mg/kg) were detected. In Case 2, cocaine (0.32 mg/kg), norcocaine (0.10 mg/kg) and cocaethylene (0.12 mg/kg) were detected. In Case 3, a trace amount of cocaine only was detected.

Neonatal urine was concurrently tested using EMIT and only 8 (7.5%) positive results were obtained. In two cases both the meconium and urine were positive (1.9%); in 19 cases the meconium only was positive (17.9%); and in six cases the urine only was positive (5.7%).

Discussion

Usual immunoassay screening procedures designed to determine cocaine exposure in utero are most effective when benzoylecgonine is present. Our results in this VLBW population show that false negatives are obtained using the EMIT method since only eight positives out of a total of 106 samples (7.5%) were found compared to meconium analysis (19.8%). Previously, we reported a 34% incidence of cocaine exposure in this population of VLBW babies [*8*].

A positive result for urine may be due to recent exposure to cocaine. A positive result for meconium may be due to longer term or earlier exposure, since meconium accumulates throughout pregnancy after about 16 weeks. The presence of cocaine only in meconium may be due to limited chemical breakdown of cocaine by the fetus.

The detection of measurable quantities of norcocaine and cocaethylene, both of which are neuropharmacologically active, is more disturbing. Reports on fetal metabolism of cocaine are scarce and the reasons for the absence of benzoylecgonine and the presence of these metabolites are unclear. Cocaethylene, the ethyl homologue of cocaine, has been reported in the urine of cocaine users in the presence of ethanol. The transesterification of cocaine and ethanol to produce the neuroactive cocaethylene takes place in the liver. Further, cocaethylene has been shown to be greater than cocaine in potency and toxicity [*9*]. The presence of this metabolite in the meconium of neonates suggests that the fetus may be exposed to this neuroactive metabolite during gestation. Additionally, it is safe to assume that many women abusing cocaine are also likely to abuse alcohol to some extent. The fact that cocaethylene was confirmed in only one case out of 106 (0.94%) suggests that the amount of ethanol required to produce this metabolite in vivo is substantial.

We speculate that chemical breakdown and fetal metabolism is a function of gestational age and birthweight since we have failed to detect benzoylecgonine in this VLBW population while Ostrea et al. [*4*] has detected benzoylecgonine using immunoassay techniques in the meconium of term babies.

We propose that usual screening procedures such as immunoassay, which are intended only to determine the presence of benzoylecgonine and not parent cocaine or other metabolites, are inadequate in the detecting of neonatal cocaine exposure. As a result, incorrect conclusions

TABLE 1—*Meconium sample analysis in 106 very-low birthweight babies.*

Substance detected	No. of positive results	Concentration (mg/kg)
Cocaine	19 (17.9%)	0.24–0.78
Norcocaine	7 (6.6%)	0.10–0.56
Cocaethylene	1 (0.9%)	0.12

may be reached regarding the exposure of the neonate to cocaine in utero, which may influence both medical-social decision making and research exposure group assignment. We suggest that research studies investigating cocaine exposure in the neonate strongly consider using similar HPLC and GC-MS technique to determine prenatal cocaine exposure.

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