TECHNICAL NOTE

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The Routine Analysis of Breast Milk for Drugs of Abuse in a Clinical Toxicology Laboratory

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ABSTRACT: Drug screening of breast milk in a clinical toxicology laboratory is reported. Findings from three cases include cocaine, ethylbenzoylecgonine (cocaethylene), ethanol, oxycodone, codeine, and nicotine. We believe this to be the first report of ethylbenzoylecgonine in human breast milk. One other specimen submitted for analysis was screened with negative results. Screening and confirmation procedures adapted for use with breast milk are described. Finally, the potential for cocaine intoxication from mother to baby is discussed. Estimates of infant blood cocaine concentration are given which may increase awareness of the need to monitor milk and blood cocaine concentrations in the infant when the situation warrants.

KEYWORDS: toxicology, breast milk, drug analysis, cocaine, ethylbenzoylecgonine, cocaethylene, ethanol, nicotine, codeine, caffeine, pharmacokinetics, infant intoxication, breast feeding

We report the use of breast milk specimens in the clinical toxicology laboratory for the screening and confirmation of drugs of abuse. Recently, physicians have submitted breast milk specimens to our laboratory when drug transfer through milk was a potential problem. Recent review articles [*1*,*2*] are available that provide pharmacokinetic data for the passage of many therapeutic drugs into milk. Much less information is available for drugs of abuse, although it is acknowledged that cocaine blood concentrations in the breast-fed infant may become high enough to result in deleterious effects [*1*]. The milk specimen from Case 1 of this report contained cocaine, ethylbenzoylecgonine and ethanol. Infant cocaine intoxication (hypertension, tachycardia, sweating, mydriasis and apnea) from breast milk has been documented [*3*]. The toxic cocaine metabolite associated

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with ethanol use, ethylbenzoylecgonine or cocaethylene, has recently received attention [*4–6*]. We believe this to be the first report of ethylbenzoylecgonine in human breast milk.

Our procedures using breast milk as a specimen involve EMIT drug screening and confirmations by liquid/liquid extraction with GC/FID and GC/MS analysis. Milk specimens are treated similarly to blood/plasma specimens and incorporated in the normal blood screening and confirmation batch procedures. Solid-phase extraction of breast milk has also recently been reported [*7*].

Materials and Methods

Specimen Collection

A minimum of 10 mL (20 mL preferred) of each specimen was collected in a polypropylene tube for transport and storage in the lab. Storage was at 2 to 8°C or at −20°C if not processed within four hours.

EMIT Screening

The methods used were based on a previously published procedure [*8*] modified for milk specimens. Unknown milk samples (0.5 mL) were processed with drug spiked human milk (low calibrator and positive control) and negative milk control. Analysis was performed on the Cobas-Bio (Roche) analyzer for cocaine metabolites, barbiturates, opiates, THC metabolites, benzodiazepines, methadone, methaqualone, phencyclidine and propoxyphene.

Confirmation of Alkaline Drugs by Gas Chromatography with Flame Ionization Detection (GC/FID) and Mass Spectrometry (GC/MS)

The methods used were based on a previously published procedure [*9*] modified for milk specimens. Unknown milk samples (4.0 mL) were processed with positive (drug spiked) and negative (blank) human milk as controls. The positive control contained cocaine (500 ng/mL), ecgonine methyl ester (500 ng/mL), methamphetamine (500 ng/mL), meperidine (100 ng/mL), desipramine (500 ng/mL), diazepam (200 ng/mL) and codeine (100 ng/mL). Mepivicaine (500 ng) was added to each sample as an internal standard.

Case Reports

Table 1 summarizes the breast milk screening and confirmation findings from four case reports.

Case 1

History

The mother (26 years old, G3P2) was admitted after spontaneous rupture of membranes and in premature labor. She had a history of both ethanol and cocaine use. Ten days prior to delivery she tested positive for cocaine metabolites in the urine and for chlamydia. She refused tocolytics and had an uncomplicated vaginal delivery of a 2015 g male baby with an estimated gestational age (EGA) of 32 weeks. The baby had Apgar scores of 3_1 and 8_5 and was admitted to the Neonatal Intensive Care Unit (NICU) with

TABLE 1—*Results of four cases of drug screening (EMIT and GC volatiles) with alkaline drug confirmation (GC/FID and GC/MS) in breast milk.*

Case #	EMIT	GC Volatiles	GC/FID	GC/MS
1	Cocaine Opiate	Ethanol	Cocaine Ethylbenzoylecgonine Nicotine	Cocaine Ethylbenzoylecgonine Nicotine Erythromycin
2	Opiate	Negative	Oxycodone	Not Done
3	Opiate	Negative	Codeine Nicotine Caffeine	Not Done
4	Negative	Negative	Negative	Not Done

respiratory distress. Cord blood pH was 7.32. Metabolic screen revealed a blood phenylalanine of 2.31 (0.4 to 1.82) and tyrosine of 0.58 (0.63 to 2.28), which is consistent with phenylketonuria (PKU). The baby also had an auditory brainstem response (ABR) which was interpreted as "significant hearing loss, cannot rule out neuropathy." The baby's hospital stay was further complicated by hyperbilirubinemia which was treated with phototherapy.

The mother returned to the hospital for a dilatation and curettage (D&C) on postpartum day 13 and an order was written for a breast milk drug screen. The infant was still hospitalized at this time. When the breast milk was found to be positive for cocaine and ethylbenzoylecgonine (Fig. 1) a urine drug screen was ordered on the mother. The urine was collected 30 h after the breast milk was collected. Urine results were positive for EMIT with confirmation (TLC and GC/FID) for cocaine metabolites (ecgonine methyl ester and benzoylecgonine but no cocaine), THC-COOH, benzodiazepines and nicotine/cotinine.

Case 2

History

The mother (19 years old, primigravida) had premature labor with spontaneous rupture of membranes more than 38 h prior to delivery. Prenatal screening revealed cultures positive for gonococcus. Delivery was by emergency caesarean section secondary to fetal distress. Presentation was breech. The baby was given Surfactant. A male infant (1110 g) was born at an EGA of 29 weeks and admitted to NICU. He had Apgar scores of 1_1, 5_5, and 7_{10} and a cord pH of 7.36. Urine drug screen (EMIT) on the baby was negative. The mother's breast milk was collected on the sixth postpartum day.

Case 3

History

The mother (35 years old, G10P4146) had a prenatal history of poor fetal growth and oligohydramnios. She went into premature labor with subsequent fetal distress and emergency caesarean section. An 1185 g female infant was born with Apgar scores of 2_1 and 7_5. EGA was 36 weeks. The baby was admitted to the NICU and the following abnormalities were subsequently documented: a two vessel cord, Trisomy 21, and patent ductus arteriosus. An infant urine drug screen was ordered on postpartum day two and

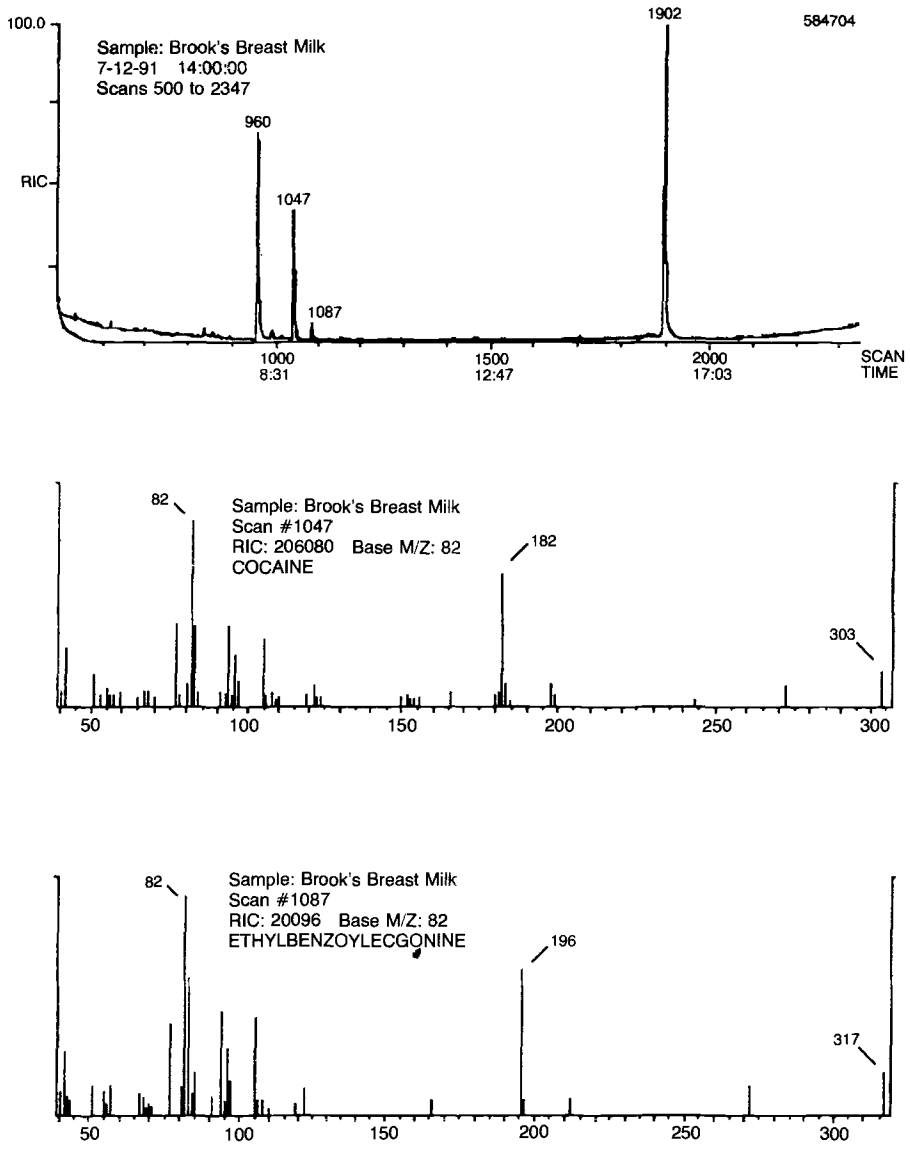

Fig. 1- *GC/MS chromatogram of the breast milk alkaline extract from case 1, showing cocaine (scan# 1047) and ethylbenzoylecgonine (scan# 1087). Mass spectra of these peak are shown. Erythromycin (scan# 1902) and the internal standard, mepivicaine (scan# 960), are also seen on the chromatogram.*

revealed caffeine only. A breast milk was collected at home and brought to the clinic by the mother when the infant was three weeks old and still hospitalized.

Case 4

History

The mother (21 years old, G3P0020) presented to labor and delivery with contractions and intact membranes and an uneventful delivery followed. The prenatal history was significant for a maternal urinary tract infection, trichomonas infection and drugs of abuse. One day before delivery a urine drug screen revealed cocaine and ecgonine methyl ester (confirmed by GC/FID), THC-COOH (confirmed by TLC) and nicotine (confirmed by GC/FID).

A 3385 g male baby was born at 40 $^{3}/_{7}$th weeks EGA. The baby's Apgar scores were 1_1, 3_5 and 5_{10} and arterial cord blood pH was 7.28. There was some meconium staining of the amniotic fluid. The baby was admitted to the NICU with respiratory distress and presumptive meconium aspiration. The baby was lethargic and had decreased reflexes. A urine drug screen on the baby on the second hospital day was positive by EMIT for cocaine (benzoylecgonine confirmed by TLC). The mother was discharged on the second postpartum day. Samples of the mother's breast milk were collected on the 4th postpartum day.

Discussion

The mother in Case 1 returned to the hospital for a Dilatation and Curettage (D&C) on postpartum day thirteen and her breast milk was screened at that time. She had a history of drug abuse during her pregnancy which may have contributed to the infant's problems, especially the auditory brainstem response and possible neuropathy. The infant was observed to be agitated day after day, which prompted the breast milk screen. The mother was reported to be noncompliant to any instructions and so it was thought, to explain the infant's behavior, she was breast-feeding during her daily visits to the hospital. Urine or blood from the baby was not screened for drugs at any time. This may be because there were other problems (phenylketonuria, a possible neuropathy and hyperbilirubinemia) that were more pressing.

Cocaine (COC, 9.00 min.) and ethylbenzoylecgonine (EBE, 9.25 min) were identified (Fig. 1) in the milk for Case 1. Unfortunately, these peaks were not quantitated at the time using quantitation calibrators. It is not known if benzoylecgonine (BE) and ecgonine methyl ester (EME) were present since this general confirmation scheme was not designed to detect these cocaine metabolites. A solid-phase extraction with a subsequent derivatization step could have been used to detect these metabolites. Even so, derivitization for specific metabolites is not practical in a general confirmation scheme. A separate aliquot should have been saved specifically for a quantitation of COC and metabolites by solid-phase extraction and derivatization.

The large peak (16.2 min) in the confirmation procedure for case 1 had a base peak of m/z 158 (scanned m/z 40-800) and was not found in the negative breast milk. This spectra did not match any entry in the on-line toxicology library of the Finnigan Incos™ data system, but a reasonably good match to erythromycin was found in the Handbook of Mass Spectra of Drugs [*10*]. A qualitative standard of erythromycin was injected and similar mass spectra were obtained. Erythromycin is a basic drug and is known to be excreted in breast milk [*1*]. Ten days prior to delivery the patient had chlamydia and had been prescribed erythromycin.

In Case 2 the woman underwent an emergency caesarean section. She was given

Percocet (oxycodone), Demerol, Phenergan and Vistaril that day and the following day. On the sixth postpartum day breast milk was collected. The EMIT screened this milk specimen positive for opiates. The alkaline extraction and GC/FID analysis confirmed the opiate screen as oxycodone.

In Case 3 an emergency caesarean section was performed. Three weeks after her delivery her breast milk was tested. The EMIT screened this milk specimen positive for opiates. The alkaline extraction and GC/FID analysis confirmed the opiate screen as codeine. It is likely that she received codeine for pain during her recovery at home.

In Case 4 the mother had a history of drug abuse. One day before delivery a urine screen was positive for cocaine, THC-COOH and nicotine. Also, the infant's urine was screened positive for cocaine metabolites 24 h post-delivery. The infant had likely received cocaine *in utero* with excretion of cocaine metabolites in the early postpartum period. The breast milk specimen was clear of cocaine and metabolites when tested six days postpartum. Either the cocaine was eliminated from the milk during this time or it was never present in the milk.

It is generally accepted that virtually any pharmaceutical compound will in part be excreted into breast milk [*1,11*]. This generally results in only minute doses of the drug delivered to the infant. Cocaine, however, may present a different situation. The combined effect of very high blood concentrations during cocaine abuse and a large milk to blood partition coefficient (m/b) may lead to toxic infant blood concentrations. Adult blood concentrations from a typical street "high" can range from 0.25 to 5.0 μg/mL. Fatal blood concentrations can range from 1 to 12 μg/mL [*12*]. The cocaine milk to blood partition coefficient (m/b) in humans has not been established, but in rats it has been measured at 7.8 [*13*]. Infant cocaine intoxication may occur frequently since breast feeding and cocaine abuse are common activities. The following estimates of infant blood cocaine concentration show a clear indication for screening breast milk, maternal and infant urine or infant serum for cocaine and metabolites in the breast-fed infant when maternal abuse is suspected.

Detailed models for predicting drug transfer are available that take into account such physicochemical variables as plasma protein binding, milk protein binding and drug partitioning into milk fat [*14,15*]. The cocaine m/b for human milk can be estimated (Eq. 1) based on the pKa of cocaine ($pK_a = 8.6$) and the pH difference between the maternal blood ($pH_b = 7.4$) and milk ($pH_m = 6.1$). Equation 1 is derived from the Henderson-Hasselbach equation for a weak base and reflects the effect of "ion trapping." The unionized form of cocaine freely passes between blood and milk and is in equilibrium. The ionized form of cocaine will be trapped in the more acidic milk and will concentrate there.

$$\text{Total cocaine in milk } (m) \text{ or blood } (b) = [U] + [I] = [U]^*(1 + 10^{pKa-pH}) \quad (1)$$

where

$[I]$ = ionized cocaine concentration and $[U]$ = unionized cocaine concentration.

$$pH = pKa + \log([U] / [I]) \text{ Henderson-Hasselbach Equation (weak base)}$$

$$\text{Using Eq. 1 the m/b} = [U]^*(1 + 10^{pKa-pH_m})/[U]^*(1 + 10^{pKa-pH_b})$$

$$m/b = 1 + 10^{8.6-6.1}/1 + 10^{8.6-7.4} \approx 10^{1.3} = 20$$

The concentration of cocaine in milk could be twenty times that of the mother's blood. This estimate does not consider the lipophilic nature of cocaine and could therefore be

TABLE 2—*Theoretical cocaine concentrations in breast milk and infant blood that could result from differing maternal blood concentrations and differing milk to blood partition coefficients (m/b). Milk volume (100 mL), dosing interval (180 min) and infant systemic clearance (23 mL/min) are kept constant. Maternal blood cocaine concentrations used are from a typical "high" and range from 0.25–5.0 μg/ml. Fatal blood concentrations can range from 1–12 μg/mL.*

Maternal plasma cocaine conc. (μg/mL)	Partition coefficient (m/b)	Cocaine dose (μg) in 100 mL of milk	Estimated average plasma cocaine conc. at steady-state in the infant (μg/mL)
0.25	10	250	0.060
1.0	10	1000	0.242
5.0	10	5000	1.208
0.25	20	500	0.121
1.0	20	2000	0.483
5.0	20	10 000	2.415
0.25	30	750	0.181
1.0	30	3000	0.725
5.0	30	15 000	3.623

an underestimate. Cocaine concentrations in breast milk could reach extremely high levels in a person who regularly uses large amounts of cocaine. The amount of cocaine to the infant at one feeding could be considered a single bolus dose. This single dose could then be calculated using the volume of milk ingested (approximately 100 mL per feeding) multiplied by the milk cocaine concentration. This dose of cocaine would then be available for absorption in the GI tract [*13,16,17*] and enter the bloodstream. Cocaine oral bioavailability in the infant may be similar to that of an adult.

There are other factors including the number of feedings and the rate of cocaine elimination in the establishment of steady-state concentrations in the infant. A mother breast feeds her newborn 6 to 12 times in a 24 h period. If the mother has a cocaine habit the newborn is likely to receive multiple dosages. Also, if cocaine clearance is smaller in the infant than in the adult, as it is with many drugs due to immature metabolic pathways, higher blood concentrations in the infant could be reached. Steady-state blood cocaine concentrations in the infant could lead to severe cocaine dependency and possibly affect neuronal development. The average steady-state blood concentration can be estimated in Equation 2 using the single dose cocaine systemic clearance (Cl_s), where τ is the dosing interval [*18*]:

$$\overline{C}_{ss} = \text{Dose}/Cl_s * \tau \quad (2)$$

Equation 2 assumes that cocaine is fully absorbed from the GI tract, follows a one-compartment pharmacokinetic model and is distributed instantaneously. The cocaine Cl_s is listed as 10–30 mL/min/kg (dose-dependent) [*12*]. If a smaller Cl_s value (5 mL/min/kg or 23 mL/min for a 4.55 kg infant) is chosen to account for the infant's immature metabolic pathways, estimates of average steady-state infant blood cocaine concentration can be made at various maternal concentrations and at various m/b's (Table 2).

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