Fetal Cocaine Exposure: Analysis of Vernix Caseosa

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Abstract

Preliminary data regarding the use of vernix caseosa (VC) as an alternative to other biological specimens for the determination of fetal cocaine exposure are presented. Advantages of VC analysis include its presence on all newborn babies, historical record of drug exposure, and ease of collection and storage. Fifteen samples of vernix caseosa—five from babies known to be cocaine-exposed because of a positive benzoylecgonine result from the urine and umbilical cord blood and ten from nonexposed neonates—were analyzed for the presence of cocaine and metabolites. VC samples from three of the five neonates known to be cocaine-exposed were positive for cocaine or its metabolites, the other two had little or no remaining specimen. The remaining ten were negative.

Introduction

The determination of fetal cocaine exposure is often carried out by the analysis of neonatal urine, meconium, or hair. However, all of these matrices have some disadvantages to their collection and analysis. Urine is difficult to collect and provides only a short historical record of drug use (2-3 days); meconium, while providing a longer historical record of maternal drug use (16-20 weeks), is not always available in large enough quantities for testing, particularly in premature babies; and hair also provides a longer detection window (8-10 weeks), but it is often not available, is difficult to collect, and requires very sensitive instrumentation for analysis (1-4).

Vernix caseosa (VC) is a white deposit that covers neonatal skin at birth. It is a mixture of sebum and desquamated cells that is produced by the fetus (5,6). It first appears between 20 and 24 weeks, and it thickly covers the fetus from 28 to 36 weeks gestation. Thereafter, its production lessens, and by 40 weeks it is found on the back and scalp and in skin creases. At 42 weeks, it is largely absent. VC prevents waterlogging and maceration of fetal skin by amniotic fluid. If the newborn is not bathed, the VC will dry up and flake off within hours.

We present for the first time a procedure for the analysis of vernix caseosa, and even though these are preliminary data, they suggest that VC may be a useful sample to collect and store for future analysis if neonatal problems occur.

Materials and Methods

Mixed-mode cation-exchange–hydrophobic solid-phase extraction columns were obtained from Jones Chromatography (Denver, CO). The derivatizing agent, N-methyl-N-(t-butyldimethylsilyl)-trifluoroacetamide containing 1% t-butyldimethylchlorosilane (MTBSTFA) was obtained from Regis Chemical (Morton Grove, IL). All chemicals were of ACS grade or better, and all solvents were HPLC grade or better.

In the fall of 1992, fifteen neonates born at San Francisco General Hospital were enrolled in this anonymous study, which was approved by the University of California, San Francisco Committee on Human Research. Vernix caseosa was collected by swabbing the skin of newborn babies with a sterile 2 x 2 cotton gauze. Umbilical cord blood was collected at birth into a sodium fluoride potassium oxalate-containing tube. The blood was centrifuged, and the plasma was removed and stored at -20°C until analysis by gas chromatography–mass spectrometry (GC–MS). The blood was centrifuged, and the plasma was removed and stored at -20°C until analysis by gas chromatography–mass spectrometry (GC–MS). Urine was squeezed from the diapers, acidified with sodium bisulfate, and stored at -20°C until analysis by GC–MS. Both the plasma and urine were analyzed at the Drug Dependency Research Laboratory, University of California, San Francisco. The limit of quantitation for cocaine and benzoylecgonine from plasma and urine was 5 ng/mL. The vernix samples were stored at -30°C for four years.

Extraction procedure

Sample preparation. VC samples were allowed to thaw. A section containing vernix (approximately 3 cm²) was cut from the gauze and placed into a test tube. The exact weight of the VC was unknown because of differences in water content of the gauze and thickness of coating. The assay is therefore a qualitative measure only. Deuterated cocaine, cocaethylene, and benzoylecgonine were added (500 ng) as internal standards for the assay. Methanol (5 mL) was added. The mixture was vor-
texted mixed and placed on a continuous shaker for 2 h. After squeezing the it to allow quantitative recovery of methanol, gauze was removed, and 0.1M phosphate buffer (pH 6, 12 mL) was added.

**Solid-phase extraction.** A mixed-mode solid-phase extraction column (200 mg/10mL, Isolute HCX, Jones Chromatography) was conditioned with methanol (3 mL), deionized water (3 mL), and 0.1M phosphate buffer adjusted to pH 3 (1 mL). The sample was added slowly to the column and drawn through under low vacuum. When the sample had passed completely through the column, the sorbent bed was washed with deionized water (3 mL), 0.1M hydrochloric acid (1 mL), and methanol (3 mL). The column was dried at full vacuum for 5 min. The isolates were eluted from the column with methylene chloride–isopropanol–ammonium hydroxide (78:20:2, v/v/v; 3 mL). The eluent was evaporated to dryness, reconstituted in butyronitrile (50 µL), transferred to an autosampler vial, and capped. Using a gas tight syringe, N-methyl-N-(7-butyl-dimethylsilyl)-trifluoroacetamide containing 1% MTBSTFA (20 µL) was added, and the mixture was heated for 20 min at 80°C. The extraction efficiency was determined by adding the drug to confirmed negative VC specimens, and it was greater than 80% for all drugs.

**Analytical conditions**

A Hewlett Packard (Naperville, IL) 5890 series II gas chromatograph coupled to a 5971A mass selective detector was used for the analysis of specimens. The column was a DB-5-MS (25 m x 0.2-mm i.d., 0.33-µm film thickness; J&W Scientific, Folsom, CA), and the injection volume was 3 µL. The injector was set at 270°C, and the detector was set at 310°C. The oven was programmed at 100°C for 1 min to 230°C at 30°C/min, to 240°C at 3°C/min, and finally to 310°C at 30°C/min, where it was held for 5.3 min. The total run time was 19.00 min.

The system was operated in selected ion monitoring and splitless injection modes to increase sensitivity and minimize interferences. The specific ions monitored for cocaine, benzoylecgonine, and the tertiary butyl dimethylsilyl derivatives of benzoylecgonine and meta-hydroxybenzoylecgonine were as follows: cocaine, 303, 182, 198; cocaine-d$_3$, 306, 185; benzoylecgonine, 317, 196, 212; benzoylecgonine-d$_3$, 320, 199; benzoyl-ecgonine, 403, 346, 282; benzoyl-ecgonine-d$_3$, 406, 285; meta-hydroxybenzoylecgonine, 533, 476, 282.

**Results and Discussion**

The results of the VC, plasma, and urine analysis are given in Table I. Only neonates who had a positive result are included. Cocaine or metabolites were not detected in the plasma, urine, or VC of ten neonates. Eight of the ten negative mothers had no history of using cocaine, and two had a history of cocaine use up to the second or early third trimester. Of the five neonates whose plasma tested positively for benzoylecgonine, three had cocaine or metabolites detected in their VC. Of the two plasma positive newborns with a corresponding negative VC result, one (subject 4) had no visible VC adhering to the gauze, and the other (subject 5) had a negligible amount of VC available for testing.

It is difficult to draw conclusions based on the small number of neonates in this study, but cocaine or its metabolites were found in the vernix of the plasma-positive neonates when the sample volume was adequate.

Vernix may offer a source of documentation of maternal cocaine use. It has some desirable properties compared with plasma, urine, hair, and meconium. It is present on the skin of all newborns, except those that are postterm. It can be collected noninvasively before the first bath and can therefore be used in anonymous studies without consent. It does not have the negative aesthetic properties associated with defecation (meconium). It is easier to collect than meconium because meconium diapers are often discarded. Vernix may offer a longer historical record of maternal drug use than urine, but probably not as long as meconium or hair. These preliminary data suggest that VC may be useful as an alternative specimen for the determination of drug exposure, especially when meconium and hair are unavailable. Ease of storage of cotton gauzes, as opposed to biological fluids and tissues, is an added advantage of VC analysis. In future research, colored gauze will be used so that the white VC contrasts against the background. This will allow easier collection and accurate weighing of the VC so quantitative results can be obtained.

**Conclusion**

Vernix covers the fetal skin and is bathed in amniotic fluid. The source of cocaine or its metabolites in VC is unknown. Possibilities include diffusion or adherence from amniotic fluid, secretion into the sebum as it is formed by the fetus, or desquamation of fetal epithelial cells.

One sample (subject 3) contained parent cocaine, benzoylecgonine, and meta-hydroxybenzoylecgonine (m-OH-BZE).
m-OH-BZE is a minor urinary cocaine metabolite in adults, to date not detected in maternal or fetal plasma samples, but found extensively in meconium (7,8). Para-hydroxy-benzoylcocone was not included because the work of Steele et al. (7) showed m-OH-BZE to be the major cocaine metabolite in meconium, and vernix is a constituent of meconium. The origin of metabolism to m-OH-BZE (mother or fetus) is as yet unidentified.

This is the first report on the use of vernix caseosa as a specimen for the determination of fetal drug exposure.

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References


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