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Short Communication

Analysis of meconium for cocaine in neonates

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ABSTRACT

A solid-phase extraction method was developed for the extraction of first-day meconium samples from premature infants of cocaine-dependent mothers. Extracts were analysed by high-performance liquid chromatography and gas chromatography–mass spectrometry for cocaine and its metabolites. Control stools showed no drug. Meconium from cocaine-dependent mothers showed cocaine in the range 0.1–0.78 μg/g. Benzoylecgonine, ecgonine and ecgonine methyl ester were not present in the samples, which suggests that the metabolism of cocaine in the premature neonate is limited.

INTRODUCTION

The practice of screening urine samples from infants of suspected drug abusers has been reported to result in a high percentage of false negatives [1]. This observation is thought to be a result of the low concentration of the drug that is present in the neonate. It has been our experience with several cases that the mother's urine has tested positive for cocaine by immunoassay techniques, whereas the child's urine has tested negative. Ostrea and co-workers [2,3] following studies in monkeys and humans, indicated that meconium (the first stool of the infant) showed a higher concentration of drugs of abuse than urine. They concluded that meconium is a more appropriate sample than urine for drug screening in the neonate. Meconium consists primarily of sloughed epithelial cells, and begins to form as early as the first trimester. It has not yet been established whether drugs will remain in the meconium throughout the pregnancy. If this is indeed the case, then meconium would represent a good indication of the mother's drug abuse history over the duration of the pregnancy, whereas the infant's urine is indicative only of drug exposure just prior to birth. We therefore examined meconium samples from infants of suspected cocaine-dependent mothers for cocaine and its major metabolites. The solid-phase extraction method reported here, followed by either high-performance liquid chromatographic (HPLC) or gas chromatographic–mass spectrometric (GC–MS) analysis, offers a rapid and efficient procedure for the examination of meconium for cocaine.

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EXPERIMENTAL

Materials

Day 1 meconium was collected from 34 premature infants born to mothers who were suspected of having abused cocaine. The stools were frozen at −20°C prior to analysis. All chemicals and solvents were obtained from Fisher Scientific (Springfield, NJ, USA) and were analytical grade or better. Drug standards were obtained from Sigma (St. Louis, MO, USA). Solid-phase extraction was performed on 1-ml Bond-Elut strong-cation-exchange (SCX) columns (Analytichem International, Harbor City, CA, USA) containing 100 mg of an alkyl-bonded sulfonylpropyl moiety.

Sample extraction

Samples were extracted in the following manner: meconium (0.5 g) was vortex-mixed with 2 ml of methanol for 1 min, then centrifuged at 450 *g* for 5 min. The supernatant was transferred to a stoppered tube and again vortex-mixed with 1 ml of 0.025 *M* potassium phosphate buffer (pH 3). A 1-ml portion of this extract was then applied to a 1-ml SCX column, which had previously been conditioned under vacuum on a Vac Elut manifold (Analytichem International) with methanol (2 ml), water (1 ml) and 0.25 *M* phosphate buffer (1 ml). After application of the sample, the column was air-dried for *ca.* 30 s and then washed with phosphate buffer (1 ml) and 0.1 *M* acetic acid. The column was again air-dried for 30 s before the adsorbed drugs were eluted with ammonical methanol (3%, 2 ml). The final extract was evaporated to dryness under nitrogen and the residue reconstituted in 50 μl of methanol. A 20-μl aliquot of the extract was used for HPLC analysis. The intra-sample accuracy and precision of the extraction procedure were determined by dividing a drug-free meconium sample into five aliquots (0.5 g) and spiking each with 1 μg/g of a mixture of cocaine and benzoylecgonine. Each sample was extracted and quantified by HPLC. This process was repeated ten times in order to determine inter-sample variation.

Sample derivatization

The remaining extract was evaporated to dryness under nitrogen and derivatized by the addition of 70 μl of pentafluoropropionic anhydride (PFPA) and 30 μl of 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP). The mixture was vortex-mixed for 10 s and then heated at 70°C for 10 min. After evaporation of the mixture to dryness under nitrogen, the residue was taken up in 20 μl of methanol, and 1 μl was used for GC–MS analysis [4].

High-performance liquid chromatography

Quantitative analysis was achieved by comparison of peak areas with those of unextracted standards. Each determination was taken as the mean of three replicate injections. The calibration graph was linear over the range 0.05–5 μg/ml. HPLC analysis was performed using a Perkin Elmer Series 3B pump (Norwalk, CT, USA) to deliver solvent at 1.5 ml/min to a Spherisorb 5-μm ODS column (25 cm × 4.5 mm I.D.) (Waters, Milford, MA, USA). A Waters 5-μm C_{18} Guard Pak (5 mm × 4.5 mm I.D.) precolumn was used to protect the analytical column.

The detector was a Spectra Physics Focus multiwavelength detector with an IBM Personal System/2 data system (Spectra Physics, Crown Point, IN, USA). The eluent was monitored at 230, 255 and 275 nm, and full spectra were recorded from 190 to 400 nm for each peak. The mobile phase was 0.025 *M* potassium phosphate buffer–acetonitrile (85:15) containing diethylamine (25 ml/l). The final pH was adjusted to 2.9 with concentrated orthophosphoric acid.

Gas chromatography–mass spectrometry

All GC–MS analyses were performed on a Finnigan Model 4510 mass specrometer coupled to a Finnigan 9610 gas chromatograph (Finnigan, San Jose, CA, USA). Samples were injected in the splitless mode, with the injection temperature maintained at 250°C. Separation was achieved on a 10 m × 0.22 mm I.D. DB-5 column (J & W, Deerfield, IL, USA), with a stationary phase of a 0.25-μm film of 5% phenyl-, 95% methylsilicone. Helium was used as carrier gas at a flow-rate of 2 ml/min. The GC oven was programmed from 150

to 260°C at 10°C/min, with the final temperature being held for 5 min. Full mass spectra were obtained in the range 50–500 a.m.u., with an electron energy of 70 eV and a scan time of 0.95 s.

RESULTS AND DISCUSSION

Both the HPLC and the GC–MS methods described above gave a good separation of cocaine from any endogenous materials and metabolites in drug-free meconium used as a control sample. Fig. 1a illustrates a typical HPLC profile of an extract of meconium spiked with 100 ng/g cocaine and benzoylecgonine. Fig. 1b is a chromatogram of drug-free meconium, and Fig. 1c is a typical HPLC profile of a cocaine-positive sample. HPLC resulted in a cocaine peak at 11.0 min and a benzoylecgonine peak at 6.5 min. GC–MS gave peaks at 2.1, 2.8 and 4.0 min for the derivatives of ecgonine, ecgonine methyl ester and benzoylecgonine, respectively, and a peak after 7.2 min for cocaine (Fig. 2).

Several different column-packing materials were evaluated for the extraction of cocaine from meconium, and the SCX columns were found to give the best recoveries. The 1-ml capacity columns used in this study were found to be the most efficient for samples containing not more than 5 μg/ml of the drug. The mean extraction recoveries for cocaine and benzoylecgonine from spiked meconium samples were determined as 98 and 100%, respectively. Similar recoveries were obtained over the concentration range from 30 ng/ml to 5 μg/ml. Intra-assay precision was within 1%, and inter-assay variability was ±2% for both drugs. Minimum detectable levels (signal-to-noise ratio >2) for cocaine and its metabolites were *ca.* 50 ng/ml by GC–MS and 30 ng/ml for cocaine and benzoylecgonine by HPLC.

The method allowed the identification of cocaine in the meconium of preterm infants born to cocaine abusers. Of the 34 samples examined, six were found to be positive. These results were compared with the findings of an independent hospital laboratory, which performed enzyme-multiplied immunoassay technique (EMIT) on the urines of the newborns and their mothers. Of the six positives identified by meconium analysis, two of the mothers showed the presence of cocaine in their urine. The offspring of one of these was also identified as cocaine positive by urine

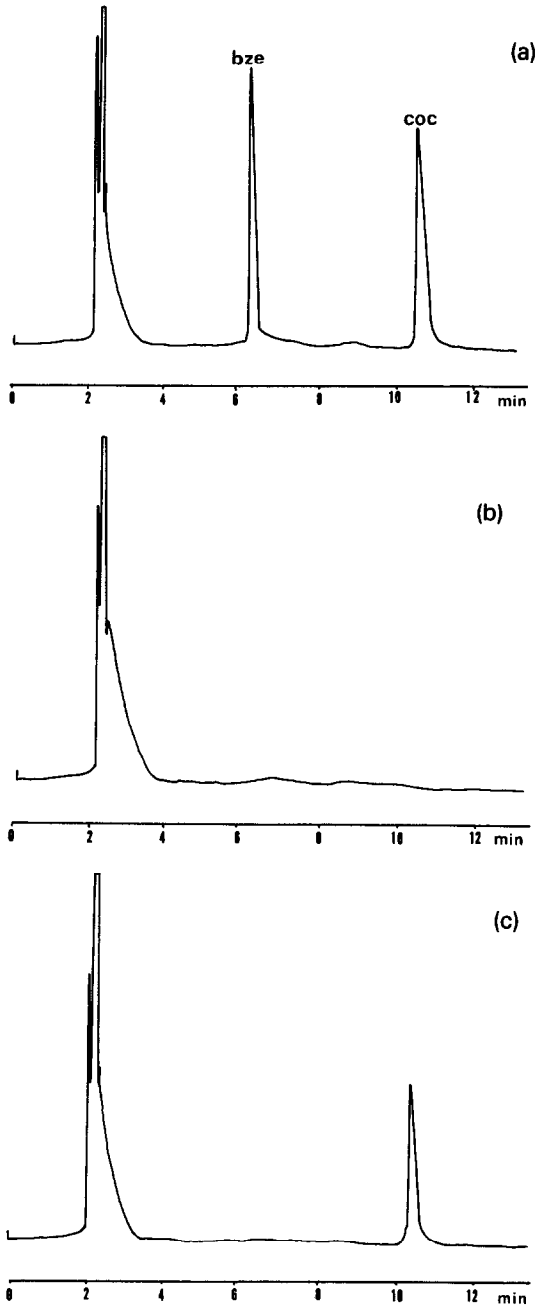

Fig. 1. HPLC of (a) an extract of meconium spiked with 100 ng/g cocaine (coc) and benzoylecgonine (bze), (b) drug-free meconium and (c) a meconium extract showing the presence of cocaine.

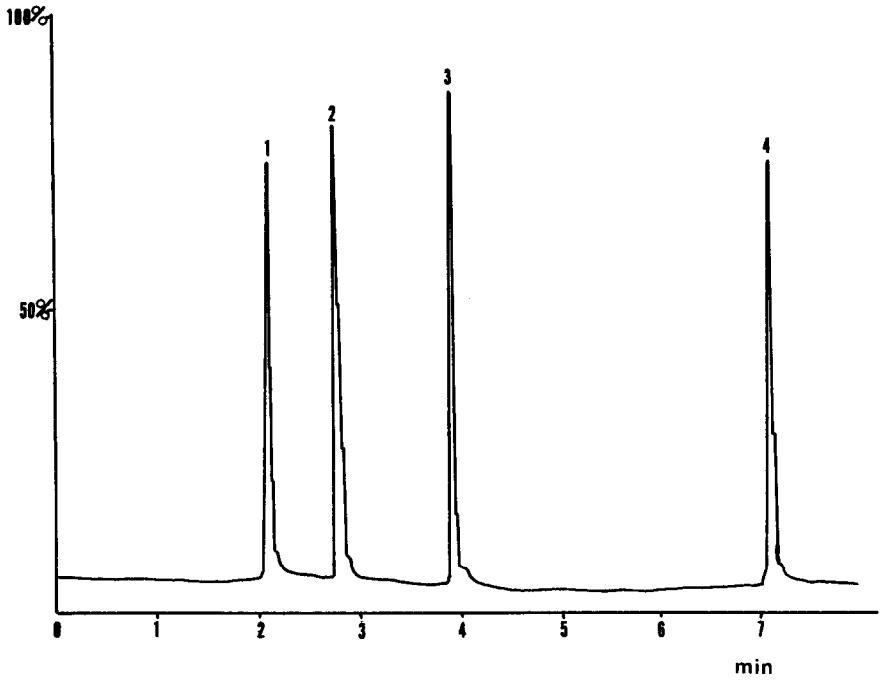

Fig. 2. Total ion chromatogram of a derivatized extract from meconium spiked with 100 ng/g ecgonine (1), ecgonine methyl ester (2), benzoylecgonine (3) and cocaine (4).

analysis. The urines of the other five babies that had cocaine in their meconium were negative.

The gestational ages of the six cocaine-positive babies in this study were between 29 and 33 weeks, and their birth weights were between 790 and 1400 g. Cocaine concentrations were in the range 0.1–0.78 μg/g of meconium, with a mean concentration of 0.31 μg/g. Benzoylecgonine, ecgonine and ecgonine methyl ester were not detected in any of the samples.

If present, the concentrations of these metabolites in meconium are below the level of detection of the techniques employed. These findings may also be indicative of the limited capability of the preterm neonate to metabolize cocaine. This may explain why, in some instances, the EMIT test for cocaine is negative. EMIT is far more sensitive in the detection of benzoylecgonine than the parent drug [5].

The solid-phase extraction method presented here offers a rapid procedure for the extraction of cocaine from meconium. The extract quality is sufficient to allow analysis for cocaine and its metabolites by both HPLC and GC–MS.

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