Cocaine Metabolite Kinetics in the Newborn*

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Abstract

The study goal was to determine the half-life elimination of cocaine and benzoylecgonine (BZE) in the newborn. Three 0.3-mL blood samples were collected during the first day of life. Urine was collected once daily. Cocaine and BZE concentrations were determined by gas chromatography–mass spectrometry. An extraction method was developed for measuring low concentrations of cocaine and BZE in small (0.1 mL) blood samples. Cocaine had a half-life of 11.6 h in one subject. The half-life of BZE during the first day of life, based on blood data in 13 subjects, was 16 h (95% confidence interval [CI], 12.8 to 21.4 h). The half-life of BZE during the first week of life, based on urine data in 16 subjects, was 11.2 h (95% CI, 10.1 to 11.8 h). The novel extraction method for small blood sample volumes should be applicable to other basic drugs.

Introduction

The cocaine epidemic continues in the U.S., and thousands of neonates are born with cocaine and/or its metabolite, benzoylecgonine (BZE), in their bodies each year (1). It is unknown how rapidly neonates eliminate cocaine or BZE. The goal of this study was to determine the elimination half-life of cocaine and BZE in neonates. Cocaine is hydrolyzed to BZE by liver carboxylesterases (2) and to ecgonine methyl ester by plasma pseudocholinesterase (3). Liver carboxylesterases are important in drug metabolism and hydrolyze many prodrugs to their active form (4,5). BZE is the major metabolite of cocaine and is the compound identified on commercial/hospital urine toxicology screens to indicate exposure to cocaine (6). In adults, cocaine has a half-life of 30 (7) to 90 min (8) and metabolism is its major route of elimination (9). In adults, BZE has a mean half-life of 7.5 h, and the route of elimination is renal (8). Data on the neonatal elimination of cocaine and BZE will add to our understanding of fetal and neonatal capabilities for drug metabolism and elimination.

Clinical pharmacology studies in neonates are hampered by the large volume of blood required for multiple plasma drug assays (10). A second goal of this study was the development of a sensitive assay for basic drugs in small volumes of whole blood. This study presents a novel extraction method for gas chromatography–mass spectrometry (GC–MS) that allows analysis of a very small volume of whole blood (0.1 mL). The extraction was developed for cocaine and its major metabolite, BZE, but it should be applicable to other basic drugs as well.

Methods

Study site and subjects

San Francisco General Hospital (SFGH) is the county hospital serving an ethnically diverse population that is predominantly of lower socioeconomic status and uninsured or government insured. Neonates were enrolled if all of the following criteria were met: 1. at least one maternal risk factor for illicit drug use was present (no or very poor prenatal care, obstetrical suspicion [e.g., abruptio or intrauterine growth retardation], history of drug use in the mother or father, history of prostitution, history of incarceration in the mother or father, family violence); 2. a research study sample of umbilical-cord blood was collected at birth; 3. birth weight was over 2000 g; 4. neonate was not admitted to the neonatal intensive care unit; 5. consent was given by the mother. Participants received an article of baby clothing and a small stuffed animal. The study was approved by the University of California, San Francisco Committee on Human Research.

Neonatal sample collection

An umbilical-cord blood research sample was collected at birth, and three timed and dated 300-µL blood samples were collected during the first day of life. A minimum of 4 h separated each blood sample. A timed and dated neonatal urine sample was collected once daily until discharge. This study was designed to have a minimal clinical impact on the study subjects and not to interfere with their care. To satisfy this requirement, the volume of study blood samples was very small (300 µL), the research urine samples were collected into a diaper and not a urine bag, and the research urine sample was not collected until a 30-mL hospital
toxicology urine screen sample was obtained. Thus, the first research urine sample was usually not collected until after 24 h of life. These design restraints were used to satisfy the human subjects protocol to minimize the impact of the study upon the newborn and to obtain the cooperation of nursery personnel.

The umbilical cord was double-clamped and cut. The placental side of the umbilical cord was briefly unclamped, and blood was drained into a 60-mL syringe, then transferred into a vactuator tube containing sodium fluoride and potassium oxalate and refrigerated until centrifugation. Plasma was separated and frozen (-25°C) until analysis was performed on 1 mL of plasma. Each 300-μL sample was collected into a tube containing potassium oxalate and sodium fluoride as preservatives. The sample was frozen (-25°C). Analysis was performed on 100 μL of whole blood. Urine was collected into diapers that were free of meconium. The sample was squeezed from the diaper, acidified with sodium bisulfate, and frozen at −25°C until analysis.

Sample Analysis

Quantitative determination of cocaine and BZE in whole blood

The extraction procedure has not been previously published. It is a substantial modification of a previously published method (11) that was modified to optimize sensitivity for determination of low levels of cocaine and benzoylecgonine in small blood samples. To 50 ng each of the internal standards, cocaine-(N-methyl-d₃) and BZE-(N-methyl-d₃) dissolved in 100 μL of 0.01M H₂SO₄ in silanized 16 × 100-mm culture tubes, was added 100 μL whole-blood samples, aqueous standards, or spiked whole-blood controls. The tubes were vortex mixed briefly, and 2 mL of 90:10 toluene/t-amyl alcohol and 0.5 mL of 2M potassium carbonate were added. The tubes were capped and vortex mixed for 4 min and centrifuged in a refrigerated centrifuge for 5 min. The tubes were placed in a dry ice/acetone bath to freeze the aqueous layers, and the organic layers were decanted into silanized 13 × 100-mm culture tubes containing 0.5 mL 0.5N sulfuric acid. The tubes were vortex mixed for 1 min and then centrifuged in the cold for 5 min. The aqueous layers were frozen in a dry ice/acetone bath, and the organic layers were poured off and discarded. The aqueous layers were washed with 2 mL of 90:10 toluene/t-amyl alcohol, vortex mixed for 1 min, then centrifuged in the cold for 5 min, after which the aqueous layers were frozen in a dry ice/acetone bath. After the organic layers were poured off and discarded, 150 μL of diethyl ether/n-butyl acetate and 0.5 mL of 2M potassium carbonate were added to each tube. The tubes were vortex mixed for 4 min, centrifuged in the cold for 5 min, and then placed in a dry ice/acetone bath to freeze the aqueous layers. The organic layers were decanted into silanized 13 × 100-mm culture tubes containing 0.5 mL of 0.5N sulfuric acid. The tubes were vortex mixed for 1 min and then centrifuged in the cold for 5 min. The aqueous layers were frozen in a dry ice/acetone bath, and the organic layers were poured off and discarded. The aqueous layers were washed with 2 mL of 90:10 toluene/t-amyl alcohol, vortex mixed for 1 min, then centrifuged in the cold for 5 min, after which the aqueous layers were frozen in a dry ice/acetone bath. After the organic layers were poured off and discarded, 150 μL of diethyl ether/n-butyl acetate and 0.5 mL of 2M potassium carbonate were added to each tube. The tubes were vortex mixed for 4 min, centrifuged in the cold for 5 min, and then placed in a dry ice/acetone bath to freeze the aqueous layers. The organic layers were poured into silanized autosampler microvial inserts. A gentle stream of nitrogen was used to evaporate the ether and concentrate the extracts to a volume of about 20 μL. The vials were capped, and 3 μL of each sample was injected into the GC–MS for cocaine determination.

The A tubes were thawed, and 5 mL of 40:60 methylene chloride/2-propanol and 0.5 mL of 1M potassium carbonate buffer, pH 8.5, which had been saturated with sodium chloride, were added to each tube. The tubes were vortex mixed for 4 min and then centrifuged in the cold for 5 min. The tubes were placed in a dry ice/acetone bath to a depth such that only the aqueous layers were below the surface of the acetone. The tubes were removed and allowed to stand at room temperature for 1 min to make sure that only the aqueous layers were frozen, after which the organic layers were decanted into silanized 13 × 100-mm culture tubes. A gentle stream of nitrogen was used to evaporate the extracts to dryness while the tubes were heated in a warm water bath.

Benzoylecgonine and internal standard were converted to the butyl ester derivatives for GC–MS analysis. To each tube was added 100 μL of oxalyl chloride (carried out in a fume hood); the tubes were mixed by vortex mixing and rolling by hand to make sure that the BZE-containing residue on the sides of the tubes came in contact with oxalyl chloride. The tubes were allowed to stand for 10 min, and 1 mL of n-butyl alcohol was added to each tube. This was done slowly because the vigorous reaction with oxalyl chloride releases HCl gas and causes foaming. The tubes were vortex mixed briefly and then heated at 90°C in a heating block for 1 h. After the tubes were cooled, 2 mL of 90:10 toluene/t-amyl alcohol and 1 mL of 2M potassium carbonate were added. The tubes were vortex mixed for 1 min, centrifuged in the cold for 5 min, and then placed in a dry ice/acetone bath to freeze the aqueous layers. The organic layers were decanted into silanized 13 × 100-mm culture tubes containing 0.5 mL of 0.5N sulfuric acid. The tubes were vortex mixed for 1 min and then centrifuged in the cold for 5 min. The aqueous layers were frozen in a dry ice/acetone bath, and the organic layers were poured off and discarded. The aqueous layers were washed with 2 mL of 90:10 toluene/t-amyl alcohol, vortex mixed for 1 min, then centrifuged in the cold for 5 min, after which the aqueous layers were frozen in a dry ice/acetone bath. After the organic layers were poured off and discarded, 150 μL of diethyl ether/n-butyl acetate and 0.5 mL of 2M potassium carbonate were added to each tube. The tubes were vortex mixed for 4 min, centrifuged in the cold for 5 min, and then placed in a dry ice/acetone bath to freeze the aqueous layers. The organic layers were poured into silanized autosampler microvial inserts. A gentle stream of nitrogen was used to evaporate the ether and concentrate the extracts to a volume of about 20 μL. The vials were capped, and 3 μL of each sample was injected into the GC–MS for benzoylecgonine determination.

GC–MS analyses were carried out on a fused-silica, cross-linked methyl silicone capillary column (HP-1, 25 μm x 0.2-mm i.d.). Initial oven temperature was 150°C (3-min hold) followed by a 26°C/min ramp to 265°C (5.5-min hold), and a 70°C/min ramp to 300°C (2-min hold). The analyses were carried out in the selected ion monitoring mode (SIM), monitoring the molecular ions of the analytes and internal standards, m/z 303, 306, 343, and 348 for cocaine, cocaine-d₃, benzoylecgonine butyl ester, and BZE-d₃ butyl ester, respectively. Quantitation was achieved with standard curves of concentration versus peak-height ratios of analyte/internal standard constructed using linear regression. Nine standards spanning the concentration range of 5 to 2000 ng/mL were used. The limits of quantitation were 5 ng/mL for cocaine and BZE.
Quantitative determination of cocaine and BZE in umbilical-cord plasma and in urine

Concentrations of cocaine and BZE were determined by a modification of the method of Jacob et al. (11). The modification used deuterium-labeled analogues, cocaine-d$_3$ and BZE-d$_3$, as internal standards and quantitation using GC–MS with selected ion monitoring of the molecular ions of the analytes and internal standards. The GC–MS method was the same procedure used for whole blood. The analyses of cord plasma and urine were identical, except that a 1-mL sample volume was used for plasma, and a 0.1-mL sample volume was used for urine.

Data and statistical analysis

The half-lives were calculated by linear least squares of the log concentration versus time (LLS) (12) or non-linear mixed effect model (NONMEM) (13). If the data set was of an adequate size for NONMEM, then data from subjects with two or more data points in the elimination phase were pooled, the data were fit to an exponential function, and a population half-life was calculated. If the data set was too small for NONMEM, then subjects with three or more data points during the elimination phase had an exponential function fit to the data, using LLS. If the fit was statistically significant, the half-life was calculated only from statistically significant data.

Results

Thirty-six neonates were enrolled, 18 had umbilical-cord plasma samples positive for BZE, and nine of these were also positive for cocaine. The BZE-positive subjects had the following characteristics: multiple risk factors for maternal substance abuse, 89%; lack of prenatal care, 61%; mean birth weight, 2920 g (standard deviation [SD] ± 505); mean head circumference, 33.2 cm (SD ± 1.4); and African-American race, 72%. The cocaine umbilical-cord plasma concentration ranged from 5 to 88 ng/mL with a mean of 39 ng/mL (SD ± 32), and the BZE concentration ranged from 74 to 3880 ng/mL with a mean of 843 ng/mL (SD ± 1030). For this cohort of neonates, individual umbilical-cord blood concentrations, first urine sample concentrations, and hospital toxicology urine screen results have been previously reported (14).

Among the nine subjects with cocaine-positive cord plasma, four had cocaine-positive postpartum blood samples, but most concentrations in the whole blood samples were near the levels of quantitation and/or only one or two samples per subject were positive. This rendered the data inadequate to determine the half-life of cocaine by either LLS or NONMEM methods. The mean time of the first urine collection was 28 h of life (SD, 18 h). Seven subjects had cocaine detectable in their first urine sample, but only one subject had three positive urine samples and generated a statistically significant log linear straight line ($p = 0.04$, LLS). The half-life for that newborn was 11.6 h.

Three subjects had two and ten subjects had three BZE-positive blood samples. The data from these 13 subjects were pooled, and the population half-life was estimated to be 16 h with a 95% confidence interval of 12.8 to 21.4 h (NONMEM). The blood data span the first 24 h of life. The blood levels and times for these 13 subjects are presented graphically in Figure 1. Sixteen subjects had two to five BZE-positive urine samples spanning the first 6 days of life. Their data were pooled, and, based on urine data, the

![Figure 1. Blood benzoylecgonine concentration versus time from birth.](https://academic.oup.com/jat/article-abstract/23/1/24/830007)

![Figure 2. Urine benzoylecgonine concentration versus time from birth.](https://academic.oup.com/jat/article-abstract/23/1/24/830007)
population half-life was estimated to be 11.2 h with a 95% confidence interval of 10.1 to 11.8 h (NONMEM). The urine levels and times for these 16 subjects are presented graphically in Figure 2.

Discussion

This is the first report of the elimination kinetics of cocaine and its major metabolite, BZE, in newborns. Based on urine concentrations, one subject had a half-life of 11.6 h for cocaine. Nine subjects had cocaine-positive umbilical-cord plasma, yet the elimination half-life could only be determined in one subject. The low levels of cocaine in the blood samples were not due to cocaine metabolism occurring in the collection tubes after the samples were collected. The blood collection tubes contained sodium fluoride and potassium oxalate, which deactivate plasma cholinesterases and acidify the blood, preventing enzymatic metabolism and spontaneous hydrolysis of cocaine (3). The low levels of cocaine in the postpartum blood samples and the rapid disappearance of cocaine from the urine in almost all subjects suggest that, for most newborns in this study, the elimination half-life of cocaine was more rapid than 11.6 h and that this single measured half-life is not the norm. The half-life of 11.6 h is nearly 20 times longer than the cocaine half-life reported in adults, which ranges from 30 (7) to 90 (8) min. Whether this half-life is anomalous or real needs to be confirmed. A study design involving frequent timed and dated urine samples collected during the first 24 h of life should answer this question. If this subject represents a subset of newborns with slow elimination of cocaine, it may indicate that some newborns have the potential for cocaine to persist in their bodies for 24 h after birth. The clinical significance of this is unknown, but transient signs of cardiovascular and cerebrovascular cocaine toxicity have been reported in newborns on the first day of life (15,16).

Metabolism is the major route of cocaine elimination (9). Hepatic microsomal carboxylesterases metabolize cocaine to BZE and, in the presence of ethanol, also metabolize cocaine to cocaethylene (2). Liver carboxylesterases are induced by phenobarbital and are important in the hydrolysis of many drugs, prodrugs, and steroid hormone esters (aspirin, procaine, chloramphenicol, meperidine, hydrocortisone, and methylprednisolone) (4). There are at least two nonspecific human hepatic carboxylesterase isoenzymes (2), and one of them has been shown to have catalytic activity for cocaine and may be the same human carboxylesterase that has recently been shown to have catalytic activity for many substrates (5). The specificity of the human cocaine hydrolyzing hepatic carboxylesterase for other substrates remains to be determined. An understanding of the elimination of cocaine in the newborn may add to our understanding of the elimination of other drugs in the newborn.

The half-lives of BZE reported here are 1.5 to 2.1 times longer than average adult values (8). The major route of BZE elimination is renal (9). Neonates have a reduced glomerular filtration rate and renal tubular secretory capacity compared with adults (17). The longer half-life for BZE may reflect this intrinsic decrease in renal function (17) in the neonate.

BZE was detectable above 300 ng/mL, the threshold of a typical hospital or commercial toxicology screen, in all neonates during the first day of life (6). Thereafter, there was a steady decline in the number of neonates with urine levels above the 300-ng/mL threshold. By 96 h of life, the urine levels of all neonates had fallen below the threshold of a commercial screen. Even the neonate with the highest urine BZE levels detected (41,000 ng/mL at 27 h) had its urine level fall to 170 ng/mL by 102 h postpartum. The ability to detect a BZE-positive urine by tests employing a 300-ng/mL threshold is excellent during the first day of life, but it lessens substantially thereafter.

The volume of sample material required for the extraction procedure for assays often impedes, at a practical level, clinical pharmacologic studies in the very young (10). But in pediatrics, it is ill neonates and infants who fill the largest number of intensive-care beds. This is the population where clinical pharmacologic data are most scant and there is likely to be the largest deviation from adult values. This study presents a method for determining the concentration of cocaine and BZE in 0.1 mL of whole blood. An extraction procedure that provides a clean extract in a small volume of solvent, thereby maximizing sensitivity, has been developed. The extraction procedure was developed for cocaine and BZE, but it is applicable to other basic drugs. It should be valuable for clinical pharmacologic studies in young humans and in small animals. The combination of very small volumes and statistical models like NONMEM that can pool a few data points from each subject should facilitate clinical pharmacologic studies in the very young.

In summary, cocaine had a prolonged half-life in one neonate (11.6 h). Whether this is the case for neonates in general needs to be examined by another study. The major metabolite of cocaine, BZE, has an average half-life of 11.2 h in the neonate. This is longer than the value in adults and is consistent with the lower GFR and renal tubular secretory ability reported in newborns (17). Our study presents a novel method for determining the concentration of cocaine and BZE in very small volumes, and the method should be applicable to other basic drugs. Finally, our study demonstrates the utility of the statistical modeling program, NONMEM, when applied to a relatively small sample population. Small sample volumes and the use of NONMEM should facilitate clinical pharmacologic studies in the very young.

Acknowledgments

We thank the nursing staff of SFGH Labor and Delivery for their cooperation and dedication to study completion. We also thank Neal Benowitz, M.D., and Donna Ferriero, M.D., for their contributions and Kaye Welch for editorial assistance.

References


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Manuscript received October 17, 1997; revision received May 1, 1998.