Pharmacokinetics of Cocaine and Metabolites Following Intragastric Administration to Ten-Day-Old Rat Pups

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Received September 17, 1997; accepted December 15, 1997

KEY WORDS: cocaine; prenatal cocaine exposure; intragastric administration; absorption kinetics; rat pups model.

INTRODUCTION

Fetal cocaine exposure is a major problem resulting from the illicit use of cocaine by pregnant women. Studies examining the prevalence of cocaine use during pregnancy estimate usage ranges from 5–17% (1). Although no definitive syndrome has been defined, prenatal cocaine exposure is associated with decreased birth weight and size, brain injury and congenital anomalies (2).

In order to study the effects of cocaine on the fetus, appropriate animal models are required. Ideal animal models produce cocaine concentrations that approximate those seen in the human fetus. Unfortunately, there is extremely limited information on the blood or plasma cocaine concentrations that occur during fetal exposure. Three cases were found providing quantitative fetal cocaine concentrations (3–5). In two of these studies cocaine blood levels were determined from a midterm fetus whose mother either died (4), or who aborted her fetus (5). Fetal blood cocaine concentrations in these cases were 1.5 μg/ml and 3.0 μg/ml, respectively. In the third report nine infants who were either stillborn or who died within two days of birth showed a mean blood cocaine concentration of 0.6 μg/ml (range of 0 – 4.2 μg/ml) and all showed blood concentrations of cocaine metabolite (6). Based on these limited studies, animal models investigating the consequences of developmental cocaine exposure will need to produce comparable levels of blood cocaine and metabolite concentrations in order to provide useful information.

The primary aim of this study was to define an animal model for postnatal cocaine administration producing plasma cocaine concentrations similar to those of the human fetus. Ten day old rat pups were chosen for this experiment in order to correlate with exposures during the brain growth spurt period (7). The oral route of administration was used in order to minimize trauma to the pup resulting from necrosis accompanying sub-cutaneous administration. Treatment of neonatal rat pups with cocaine may adversely affect nutritional status via drug-induced alterations of growth and/or drug-induced decreases in suckling. To compensate for these changes the vehicles used to administer cocaine orally can include nutritional replacements. Since nutritional composition of the treatment vehicle may affect pharmacokinetic parameters, three different vehicles were used to compare the effect of vehicle composition on pharmacokinetic parameters. Sustacal® and Intralipid®-II were selected for use as vehicles in order to evaluate the effects of different nutritional composition on cocaine absorption kinetics. In addition, we have previously demonstrated alterations in drug toxicity following administration in similar nutritional vehicles (8).

METHODS

Cocaine hydrochloride was obtained from Mallinckrodt, Inc. (St. Louis, MO 63105) and benzoylecgonine, benzoylnorecgonine, and norcocaine hydrochloride were obtained from Research Biochemical International (Natick, MA 01760). All other reagents were from the Sigma Chemical Co. (St. Louis, MO). HPLC grade solvents were from Fisher Scientific Co. (Fairlawn, NJ 07410).

The HPLC system consisted of a Model 510 solvent delivery system and a Model 486 ultraviolet absorbance detector (Waters Corp., Milford, MA), a Model SIL-9A autoinjector (Shimadzu Scientific Instruments, Columbia, MD 21046), a prepacked 25 cm x 4.6 mm i.d. 5μm base-deactivated C-18 Alltima® column (Alltech Associates, Deerfield, IL 60015) and guard column. Mobile phase consisted of potassium phosphate buffer (10mM, pH 3) and acetonitrile (80:20, v/v). Tetrabutylammonium hydrogen sulfate, an ion-pairing agent, was added to the mobile phase to achieve a final concentration of 2×10^{-4} M. Column temperature was maintained at 37°C and mobile phase flow rate at 0.8 ml/min. Detector output was recorded at 233 nm.

Cocaine (COC), benzoylecgonine (BEC), benzoylnorecgonine (BNE), and norcocaine (NOR) were dissolved in methanol to give stock solutions of 0.1 mg/ml and were stored at -70°C until use. The internal standard, bupivacaine (BUP), was dissolved in water to yield a stock solution of 80 μg/ml. The borate buffer (pH 9) consisted of boric acid (1M), potassium chloride (1M) and sodium carbonate (1M), 31.5:31.5:37(v/v/v); diethylamine was added to yield a final concentration of 0.5%(v/v).

Aliquots of the cocaine and metabolite stock solutions were added to 15 ml silanized borosilicate glass conical tubes and the organic solvent evaporated to dryness under nitrogen. Drug-free rat pup plasma (100 μL) was added and vortex-mixed for 30 seconds to give plasma standards ranging from 25 to 5000 ng/mL. To each tube, 10 μl of the bupivacaine stock solution and 10 μl of the saturated sodium fluoride solution were then added. Next, 200 μl of borate buffer was added followed by 2 ml of the chloroform: ethanol mixture. The contents of each tube were vortex-mixed at low speed for 40 seconds, allowed to stand for 1 minute, and vortex-mixed again for 40 seconds. Tubes were centrifuged at 2000 x g (5°C) for 5 minutes. The upper aqueous layer was aspirated and discarded. The lower organic layer was transferred to a clean, silanized

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0724-8741/98/0300-0488$15.00/0

tube and evaporated to dryness at 50°C under nitrogen. The samples were reconstituted in 100 μl of mobile phase and vortex-mixed for 30 seconds. Hexane (500 μl) was added to the reconstituted samples and vortex-mixed for 30 seconds followed by centrifugation at 2000 x g for 5 minutes. The upper hexane layer was aspirated and discarded. The lower aqueous phase was filtered through a 0.2 μm PVDF syringe filter (Whatman Inc., Clifton, NJ 07014) and 40 μl was injected onto the column.

The absolute recovery of COC, BEC, BNE, and NOR was calculated by dividing the slope of the calibration standards in plasma by the slope of the standards in mobile phase (mobile phase standards were not extracted). Peak height ratios (i.e. COC/BUP) were used for quantitative computations. Calibration curves were calculated by least squares linear regression analysis using a commercial software package (Graph Pad Prism®, Graph Pad Software, Inc., San Diego, CA 92121). Accuracy and precision were determined by replicate analysis of five known concentrations equally divided over the calibration curve. Inter- and intra-day accuracy (%RE) was expressed as percentage deviation from the theoretical (spiked) value. The lower limit of quantitation was defined as the concentration of the lowest standard in the analytical run which was quantitated with a definite level of certainty (relative standard deviation, <15%, and %RE<15).

Twenty-four litters of Sprague-Dawley (Sasco, Inc., Omaha, NE) rat pups were housed in a temperature and humidity controlled environment, with free access to food (Agway 3000) and water, and were on a 12 hour light/dark cycle. On postnatal day (PN) 1 all pups born on the same day were culled and randomly reassigned to dams in litters of 10 pups each.

On PN 10, the pups were separated from the dams and randomly assigned to dose/vehicle/time-course group.

Intragastric intubation was used to administer COC to the animals (8). Each group of ten pups received one of six intubation solutions of COC (25 mg/kg or 50 mg/kg) in different intubation vehicles. The vehicles were either Sustacal® (Mead Johnson & Co., Evansville, IN 47721) at a 20% (v/v) dilution in water (ST) as previously described (9,10); Intralipid®- II (a 20% w/v soybean oil emulsion; Kabi Pharmacia Inc., Clayton, NC 25720) used without dilution (IL); or water (H).

All usage of animals was approved by the Institutional Animal Care and Use Committee following detailed review according to the "Principles of Laboratory Animal Care (NIH publication #85-23, revised 1985)".

Blood samples were collected at 5, 15, 30, 45, 60, 90, 120, 180, 240, and 360 minutes following COC administration. One pup was used per time point, and groups of ten pups provided one dose x vehicle time-course curve. Four replicates of each dose x vehicle time-course were completed for each dose-vehicle combination.

Rat pups were anesthetized with carbon dioxide gas and 500–1000 μl of blood was collected by cardiac puncture using heparin-rinsed syringes (heparin solution = 162 units/ml). Immediately after sample collection, the animals were euthanized by cervical dislocation. The blood was placed in a microcentrifuge tube containing 0.1 grams of sodium fluoride, vortex-mixed, and then centrifuged at 10,000 x g (5°C) for 5 minutes. Plasma was separated and stored at -70°C until HPLC analysis.

Plasma COC, BEC, BNE, and NOR concentration-time profiles were fitted to select polyexponential equations using a nonlinear least squares fitting program (WinNonlin, Scientific Consulting, Inc., Cary, NC), and pharmacokinetic parameters (AUC, Cmax, Tmax, ka, ke, CL/F and Vss/F) for each dose x vehicle combination were calculated from computer-generated coefficients and exponents via compartmental analysis (11).

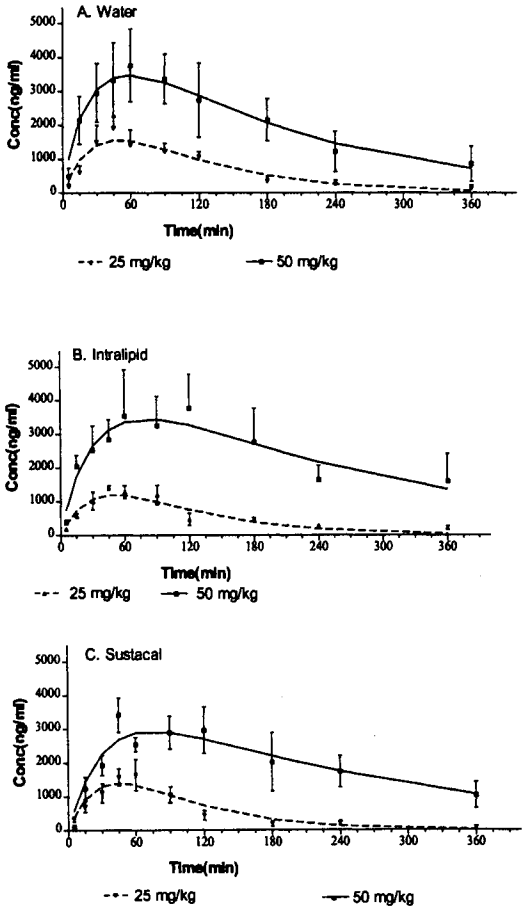

Fig. 1. Plasma cocaine concentrations following intragastric intubation to PN10 pups at two doses in three different vehicles. Data shown are the observed data points and the fitted curve for each dosage and vehicle combination. **A** cocaine administered in water, **B** cocaine administered in Intralipid-II®, and **C** cocaine administered in Sustacal®.

Two factor analysis of variance (ANOVA) was conducted with dose and vehicle serving as the two factors. The finding of a significant main effect and the absence of significant interactions allowed one factor ANOVA. Significant group differences were identified with Tukey's post-hoc analysis at the $p < 0.05$ level. Statistical analysis was performed using commercial software, Statgraphics Plus (Manugistics, Inc., Rockville, MD 20852).

RESULTS

Retention times for BNE, BEC, COC, NOR, and BUP were 6.6, 7.4, 9.0, 10.2, and 14.7 minutes, respectively. Absolute recovery(mean±s.d.) for COC, NOR, BEC, and BNE was 90.8±5.0, 89.9±3.8, 84.4±6.6, and 65.0±5.1%, respectively. The method proved to be linear over the entire concentration range; linear regression analysis of calibration curves produced correlation coefficients > 0.999. Intra-day accuracy (2–

Table 1. Cocaine Pharmacokinetic Parameters[a]

Vehicle dose	Cmax (μg/ml)*	Tmax (min)	Cl/F (L/min)*	V/F (L/kg)	ka (min^{-1})	ke*(min^{-1})
W25	1.58 ± 0.65	52.2 ± 12.5	2.22 ± 0.99	0.159 ± 0.071	0.028 ± 0.013	0.015 ± 0.004
IL25	1.24 ± 0.31	51.5 ± 7.1	2.84 ± 1.63	0.173 ± 0.059	0.025 ± 0.003	0.016 ± 0.005
ST25	1.42 ± 0.17	46.1 ± 9.3	2.99 ± 1.11	0.132 ± 0.025	0.023 ± 0.005	0.022 ± 0.005
W50	3.76 ± 1.74	65.1 ± 18.4	1.44 ± 0.84	0.151 ± 0.093	0.042 ± 0.046	0.010 ± 0.006
IL50	3.55 ± 1.81	70.8 ± 14.2	1.14 ± 0.70	0.276 ± 0.223	0.045 ± 0.039	0.005 ± 0.002
ST50	3.56 ± 0.46	85.4 ± 33.1	1.34 ± 1.26	0.152 ± 0.046	0.021 ± 0.010	0.010 ± 0.009

[a] Maximum concentration (Cmax); time to maximum concentration (Tmax); apparent clearance (Cl/F); volume of distribution / bioavailability (V/F), absorption (ka) and elimination (ke) rate constants for cocaine calculated from the four experimental trial. Values are mean ± standard deviation for each vehicle and dose combination. W = water, ST = 20% (w/v) Sustacal®; IL = Intralipid-II®.* = $p < 0.05$ significant differences between doses, regardless of vehicle.

17%RSD) and precision (-20 to 26%RE) and inter-day accuracy (0.28 to 25 %RSD) and precision (-2 to 11%RE) were within acceptable limits. The limit of quantitation (LOQ) for COC was set at 25 ng/mL while LOQ for the metabolites was restricted to 50 ng/mL.

Concentration-time profiles were best fit to a one compartment open model with first order input (absorption) and first order elimination. Figure 1 shows the time course data for COC. Results obtained for the various pharmacokinetic parameters are presented in Table 1. As expected, *Cmax* demonstrated a significant effect of dose ($F_{(1,23)} = 22.03$, $p < 0.002$) but not vehicle ($F_{(2,23)} = 0.25$, $p < 0.78$). No dose x vehicle interactions were identified ($F_{(2,23)} = 0.13$, $p < 0.88$). In addition, significant effects of dose (but not vehicle) were identified on CL/F ($F_{(1,23)} = 8.884$, $p < .008$) and ke ($F_{(1,23)} = 15.881$, $p < 0.001$). None of the other kinetic parameters showed significant dose, vehicle, or interactions.

Figure 2 shows the data for the three primary metabolites of COC. Each of these metabolites was detectable by the 15 minute post-administration time point.

DISCUSSION

This study demonstrates that cocaine produces significant and relevant plasma concentrations of the parent drug and its primary metabolites following intragastric intubation. Following a dose of 25 mg/kg by the intragastric route, the maximum plasma concentrations of cocaine ranged between 1.4 and 1.9 μg/ml. These values are at least twice as high following the administration of a 50 mg/kg dose. In addition, significant plasma concentrations of the primary cocaine metabolites BEC, BNE, and NOC were detected.

This study also demonstrated that the intragastric intubation model for COC administration to postnatal rat pups produces plasma concentrations similar to those observed in the human fetus (3–5). In addition, the present study provides an analysis of pharmacokinetic parameters for plasma cocaine following intragastric intubation using two different nutrient vehicles compared to water. Intubation vehicle considerations are important since the pharmacological actions of cocaine include an ability to disrupt animal behavior including the ability of the animal to suckle. The possibility of nutritional compromise must be addressed so that viable investigations into the mechanisms of cocaine's alterations of brain development can be conducted. Data from the present study demonstrate no signifi-

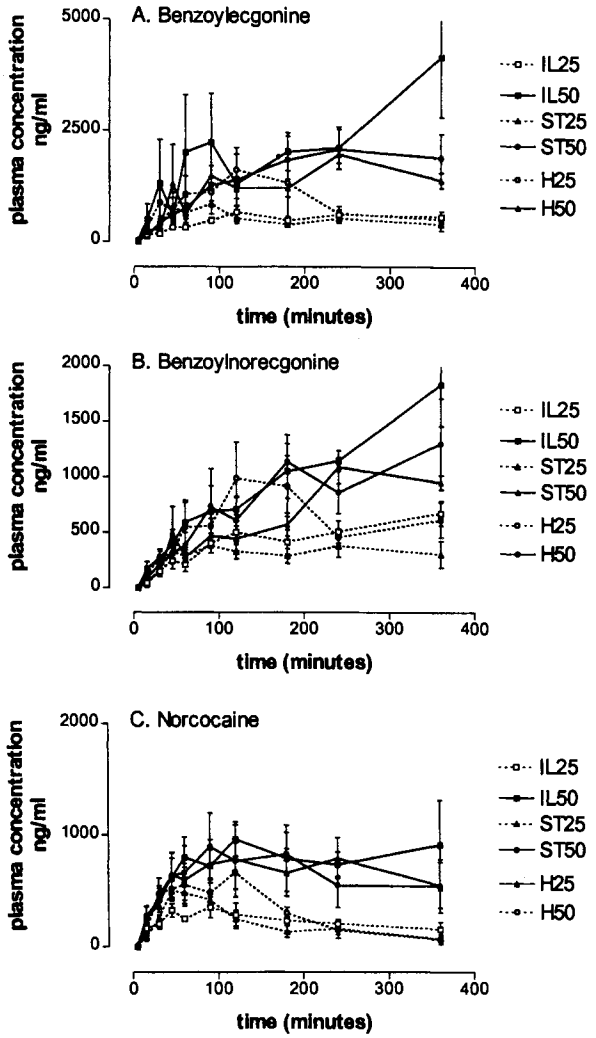

Fig. 2. Plasma concentration-time profiles for the three primary metabolites of cocaine following administration of the parent compound at two doses in three different vehicles. **A** benzoylecgonine; **B** benzoylnorecgonine; and **C** norcocaine.

cant effect of the intubation vehicles on the pharmacokinetics of cocaine. This is important since it may be desirable to deliver cocaine using a multiple dosing paradigm with a high calorie nutrient vehicle similar to the Intralipid-II® vehicle of the present study.

The present study identifies a dose-dependency of the ke and CL/F for cocaine in the PN10 rat pup. The ke and CL/F for the 25 mg/kg dose were significantly greater than for the 50 mg/kg dose. This finding is not unique, other studies have identified dose dependent or non-linear kinetics for cocaine following intravenous administration in humans (12, 13) pregnant guinea pigs (14) and following intragastric intubation in adult rats (15).

Since esterase activity is low in neonatal rats, the most likely cause of the observed dose dependency is partial saturation of one or more of the observed metabolic pathways. Exogenous metabolite was not administered in our study thus, it is impossible to identify the responsible pathway. In a situation of partial saturation of an enzymatic pathway, apparent oral clearance will vary inversely with plasma drug concentration (and therefore with dose). In theory, this situation can cause a saturable first pass effect, with a resulting dependence of extent of absorption, on dose since the fraction drug escaping the first pass effect will be dose dependent. This would allow the higher dose to escape initial hepatic metabolism and enter the systemic circulation to a greater degree (13).

Finally, the choice of intubation vehicle did not have a statistically significant impact on cocaine pharmacokinetics at either dosage. This is an important factor for multiple dosing studies since caloric replacement is necessity and the presence of such intubation vehicles may alter the bioavailability of the drug of interest. Additionally, the ability to administer cocaine by intragastric intubation using a variety of nutritional vehicles allows for the design of studies to evaluate the role of neonatal nutritional factors on the developmental teratogenic effects of the drug.

ACKNOWLEDGMENTS

The College of Pharmacy, University of Arkansas for Medical Sciences, provided support for this research.

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