Cocaine and Metabolite Concentrations in Plasma During Repeated Oral Administration: Development of a Human Laboratory Model of Chronic Cocaine Use

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Abstract

Long-term use of cocaine may produce neurophysiological and metabolic alterations that differ from acute drug use. A laboratory model that was capable of evaluating the effects of chronic cocaine administration in human subjects was needed. Chronic oral administration of cocaine was considered a feasible route because of the ease of administration, control of dosing patterns, and possible reduction in medical risks compared with the intravenous and smoked routes. This clinical study was conducted to evaluate chronically administered oral cocaine as a means of studying cocaine addiction and withdrawal in humans. Cocaine-abusing volunteers were given multiple doses of oral cocaine each day in up to 16 daily sessions (including three placebo sessions). In each daily session, volunteers received five equal doses separated by hourly intervals. Across sessions, the dose was increased from an initial dose of 100 mg (500 mg/day) to 400 mg (2000 mg/day) in the last session. The dose for each consecutive cocaine session was increased by 25 mg/dose/session (125-mg total increase per session). Twelve subjects were enrolled in the study; however, three subjects dropped out prior to completion of at least three sessions. Two subjects completed all 13 cocaine sessions. The remaining seven subjects completed from 3 to 11 sessions; their participation was terminated early for safety and behavioral reasons. Plasma was collected during all sessions and analyzed for cocaine and metabolites by solid-phase extraction followed by gas chromatography–mass spectrometry. Oral cocaine administration resulted in peak plasma concentrations of cocaine approximately 1 h after administration. Accumulation of cocaine was evident between hourly doses and there was evidence of dose-proportional increases in area under the curve (AUC) measures across sessions. A variety of cocaine metabolites was measured in plasma including benzoylecgonine, ecgonine methyl ester, norcocaine, benzoylepseudococaine, and p- and m-hydroxy metabolites of cocaine and benzoylecgonine. During chronic oral dosing, there appeared to be a trend for AUC ratios (AUCmetabolite/AUCcocaine) of benzoylecgonine and ecgonine methyl ester to decrease and norcocaine to increase, indicating the possibility of dose-, time-, or route-dependent changes in the absorption and/or metabolism of cocaine. Overall, this study demonstrated that chronic oral dosing of cocaine produced dose-related increases in plasma cocaine concentration, and this model could be useful for studying the effects of chronic cocaine use in human subjects.

Introduction

Although the number of cocaine abusers in the United States has stabilized since its peak in 1992, cocaine abuse and dependence continues to pose a significant public health problem. Preliminary data for the 1996 National Household Survey on Drug Abuse estimate that there are 1.7 million current cocaine users, compared with only 216,000 current heroin users (1). It is estimated that at least 10% of individuals who engage in recreational cocaine use progress to problematic heavy use (2). Despite the substantial research effort directed toward the evaluation and development of both behavioral and pharmacological therapies, there has been limited progress in developing effective treatments for cocaine dependence.

Numerous laboratory models have been developed to examine different aspects of cocaine abuse and dependence in human subjects and also to serve as an adjunctive approach to large-scale clinical trials for the development of pharmacotherapies. Cocaine administration to human subjects has been demonstrated to be tolerated safely under controlled medical conditions when low-to-moderate acute doses of cocaine are given by different routes of administration. Human laboratory models have been designed to study the direct effects of cocaine, cocaine self-administration, and cocaine-induced craving, and pharmacological-interaction studies have examined the ability of numerous putative pharmacotherapies to modulate these dependent measures. Few studies have sought to examine the pharmacodynamic or pharmacokinetic effects of repeated cocaine dosing, although this pattern of cocaine use more accurately reflects the manner in which cocaine is used...
Cocaine is extensively metabolized by both enzymatic and nonenzymatic pathways after acute administration. The primary metabolites include benzoylecgonine (BZE), eegonine methyl ester (EME) and norcocaine (NCOC), although a number of other metabolites have been reported. A metabolic scheme for cocaine is illustrated in Figure 1. BZE is the primary metabolite detected in plasma following acute cocaine administration by the intranasal, intravenous, and smoked routes. The area under the curve (AUC) for BZE in plasma has been reported to range from one- to sevenfold greater than the AUC for cocaine following intranasal, intravenous, and smoked administration (8). BZE is formed from the cleavage of the methyl ester bond of cocaine, and this has been reported to occur through a non-enzymatic pathway, particularly at alkaline pH (9,10). More recently, an enzymatic pathway for the production of BZE has been identified. The enzyme responsible for this conversion has been identified as a human liver carboxylesterase, hCE-1 (11). This enzyme has also been implicated in the formation of other metabolites including cocaethylene when cocaine and ethanol are present as well as during the metabolism of heroin to 6-acetylmorphine and morphine (12,13).

EME is produced by the enzymatic hydrolysis of the benzoyl ester of cocaine. Typically, EME is detected only at low concentrations in plasma following cocaine administration by the intranasal, intravenous, and smoked routes (8). The enzymes responsible for the conversion of cocaine to EME are plasma butyrylcholinesterase and the newly identified human liver carboxylesterase, hCE-1 (11). This enzyme has also been implicated in the formation of other metabolites including cocaethylene when cocaine and ethanol are present as well as during the metabolism of heroin to 6-acetylmorphine and morphine (12,13).

NCOC is a particularly important cocaine metabolite because it has been recognized as a precursor of hepatotoxic metabolites (16). The human cytochrome P-450 3A4 (CYP3A4) has been reported to produce NCOC (17). CYP3A4 is induced by drugs such as rifampacin, barbiturates, and dexamethasone and inhibited by drugs such as gestodene and troleandomycin (18). Other cocaine metabolites have also been identified including benzoylecgonine (BNE), and the m- and p-hydroxy metabolites of cocaine and BZE (m- and p-HOCOC; m- and p-HOBCZE). Both m- and p-HOCOC have been reported to be formed by hepatic microsomes from mice, rats and guinea pigs (19). In addition, p-HOCOC has been shown to have comparable pharmacological activity to cocaine when administered as a 20-mg/kg intraperitoneal dose to mice (19).

Concerns arise that chronic administration of a drug may produce metabolic alterations that result in a different pattern of metabolism compared with acute drug use. Phenytoin is a well-known example of a drug that exhibits
dose-dependent metabolism. Phenytoin is metabolized by an enzymatic pathway that displays capacity-limited kinetics, resulting in a disproportionate increase in plasma concentration following multiple doses (20). Recently, it has been suggested that dose- and/or time-dependent metabolism may be observed following chronic cocaine administration (21).

This study examined the profile of cocaine and metabolites in plasma following chronic oral cocaine administration. A range of pharmacodynamic data was concurrently collected but will be reported elsewhere. Plasma specimens collected during each session were analyzed by gas chromatography–mass spectrometry (GC–MS) for cocaine and metabolites. Area-under-the-curve (AUC) measures were compared across sessions for evidence of accumulation and for changes in the metabolic profile over time with chronic dosing. In the evaluation of our model for chronic cocaine administration, an important goal was to determine if any metabolic alterations occurred as a result of chronic dosing because such alterations could result in potentially toxic cocaine and/or metabolite concentrations.

### Methods

#### Chemicals and materials

Drug standards were obtained from the following sources: cocaine hydrochloride from Mallinckrodt (St. Louis, MO); BZE tetrahydrate, NCOC, BNE, m-HOCOC, p-HOCOC, m-HOBZE, and p-HOBZE from Research Biochemicals International (Natick, MA); and EME HCl, $[^3]$H$_3$-cocaine HCl, $[^3]$H$_3$-BZE tetrahydrate and $[^3]$H$_3$-EME HCl from Sigma Chemical Co. (St. Louis, MO). Methanol, methylene chloride, 2-propanol, and acetonitrile were high-performance liquid chromatographic grade, and all other chemicals were reagent grade. N,O-bis (Trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) was purchased from Pierce Chemical Co. (Rockford, IL). Clean Screen® solid-phase extraction columns (ZSDAU020) were purchased from United Chemical Technologies (Bristol, PA).

#### Human research subjects

Subjects were healthy male and female cocaine users who reported a cocaine use history of at least 6 months in duration. Inclusion criteria included self-reported use of smoked or intravenous cocaine at least two times per week for the 6 weeks prior to admission, and recent cocaine use was confirmed by urinalysis prior to participation in the study. The protocol was approved by the Johns Hopkins Bayview Medical Center Institutional Review Board. Subjects were determined to be free from significant medical or psychiatric disturbance by physical examination, history, routine laboratory chemistries, and psychiatric assessment, including a structured interview. A summary of subject characteristics is shown in Table I.

#### Clinical protocol

Subjects resided on the closed clinical ward of the Johns Hopkins University Behavioral Pharmacology Research Unit (Baltimore, MD) for approximately 4–5 weeks. Upon admission, a 5–7-day washout phase allowed for the elimination of illicit cocaine used prior to admission. After the washout phase, multiple oral cocaine dosing sessions commenced. Each subject participated in up to 16 daily (Monday–Friday) multiple dosing sessions. During each session, five equal doses of oral cocaine hydrochloride or placebo were administered at 1-h intervals, beginning at 9:00 a.m. Subjects were instructed to complete breakfast 2 h prior to the start of the session in order to decrease variability in gastrointestinal drug absorption. Cocaine was administered in ascending doses in increments of

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### Table I. Subject Characteristics

<table>
<thead>
<tr>
<th>Subject I.D.</th>
<th>Age (years)/ gender</th>
<th>Weight range (kg) (admission-discharge)</th>
<th># Sessions completed (of 13)/ highest dose received (mg)</th>
<th>Lifetime cocaine usage (months)</th>
<th>Preferred route/ secondary route</th>
<th>Drug-use history</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>38 / M</td>
<td>75.8-87.1</td>
<td>13 / 2000</td>
<td>6</td>
<td>IN / IV</td>
<td>CA</td>
</tr>
<tr>
<td>B</td>
<td>42 / M</td>
<td>89.4-91.6</td>
<td>8 / 1375</td>
<td>36</td>
<td>IV / SM</td>
<td>CA / OP</td>
</tr>
<tr>
<td>C</td>
<td>34 / M</td>
<td>83.5-99.8</td>
<td>13 / 2000</td>
<td>180</td>
<td>SM / IV</td>
<td>CA</td>
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<tr>
<td>D</td>
<td>33 / M</td>
<td>70.3-74.4</td>
<td>9 / 1500</td>
<td>60</td>
<td>IV / SM</td>
<td>CA</td>
</tr>
<tr>
<td>E</td>
<td>34 / M</td>
<td>64.4-74.4</td>
<td>11 / 1750</td>
<td>24</td>
<td>SM / IV / IN</td>
<td>CA / OP</td>
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<tr>
<td>G</td>
<td>34 / M</td>
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<td>3 / 750</td>
<td>98</td>
<td>SM / none</td>
<td>CA</td>
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<tr>
<td>H</td>
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<td>5 / 1000</td>
<td>36</td>
<td>SM / none</td>
<td>CA</td>
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<tr>
<td>J</td>
<td>33 / M</td>
<td>68.9-74.8</td>
<td>7 / 1250</td>
<td>10</td>
<td>IV / none</td>
<td>OP</td>
</tr>
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</table>

* All subjects were African-American.
* Abbreviations: M, male; F, female; IN, intranasal; IV, intravenous; SM, smoked; CA, cannabis; OP, opiates.

### Table II. A Sample Dosing Schedule for a Subject Indicating Individual Doses, Total Dose, and Number of Plasma Specimens Collected for Each Dosing Session

<table>
<thead>
<tr>
<th>Session</th>
<th>Dose (mg)</th>
<th>Total session dose (mg)</th>
<th># Plasma specimens collected</th>
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<td>500</td>
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<tr>
<td>2</td>
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<td>625</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
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<td>150</td>
<td>750</td>
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<tr>
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<td>1250</td>
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<td>0</td>
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</tr>
<tr>
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<td>275</td>
<td>1375</td>
<td>12</td>
</tr>
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<td>0</td>
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</tr>
<tr>
<td>16</td>
<td>400</td>
<td>2000</td>
<td>12</td>
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</table>
25 mg/dose across successive sessions, resulting in a total increase of 125 mg per session. Three placebo sessions were included during different weeks of the study and placed randomly into the cocaine dosing schedule to serve as a control condition for changes in status that could occur as a function of time of day or result from the repeated test procedures. Participation was terminated if designated cardiovascular safety parameters were exceeded (1. if heart rate > 130 or blood pressure > 165/100 within 4 min preceding a dose; 2. if heart rate did not fall below 110 during the period between doses; 3. if heart rate exceeded [220 - subject age] x 0.85 at any time; or 4. if blood pressure exceeded 180/120 for 4 or more min). A sample dosing schedule is illustrated in Table II. Plasma specimens were collected during each session based on either a 4-point or 12-point schedule. Following completion of the multiple dosing sessions, no further cocaine was administered to the subjects. Subjects remained on the ward for an additional week.

**Specimen collection and analysis**

Because of limitations on the number and volume of blood specimens that could be collected safely during the study period, two plasma collection schedules were employed. The collection timeline for each schedule is illustrated in Figure 2. The four-point plasma collection schedule was generally employed when cocaine was dosed in the range of 125 mg (× 5 daily) to 250 mg (× 5 daily). The 12-point plasma collection schedule was used in all other sessions.

Blood specimens (4 mL) were collected in heparinized vacutainer tubes containing 2% (w/v) sodium fluoride and acetic acid. Plasma was separated and immediately frozen at −30°C until time of analysis. Plasma specimens were analyzed for cocaine and metabolites by a modified procedure published previously (22). Briefly, plasma specimens were mixed with internal standard solution and acidified with sodium acetate buffer (2M, pH 4.0). The specimen mixture was centrifuged (3000 rpm for 10 min) and the supernatant extracted by solid-phase extraction. Cocaine analytes were eluted with freshly prepared elution solvent (methylene chloride/2-propanol/ammonium hydroxide, 80:20:2, v/v/v). The eluent was evaporated under nitrogen in a 40°C water bath and reconstituted in 20 µL acetonitrile. The samples were then transferred to autosampler vials and combined with 20 µL of derivatizing reagent (BSTFA with 1% TMCS). The vials were sealed and incubated at 80°C for 30 min.

Duplicate matrix-matched calibration curves for each analyte were processed with each batch of specimens. Curves were constructed across the concentration range of 3.1 to 1000 ng/mL for cocaine, BE, and EME and across 1.5 to 500 ng/mL for BNE, NCOC, m-HOCOC, p-HOBZE, p-HOCOC, and p-HOBZE. The limit of detection for all analytes was approximately 1 ng/mL. Control samples containing all analytes at concentrations of 50 and 250 ng/mL (100 and 500 ng/mL for cocaine, BZE, and EME) were processed in duplicate with each run. Accuracy of control measurements was within 20% for all analytes, and coefficients of variation ranged from 2.5 to 9.2%.

Cocaine and metabolites were analyzed with a Hewlett-Packard (Wilmington, DE) 5971 mass selective detector interfaced to a Hewlett-Packard 5890A GC with an autosampler (HP7673A). A 1-µL aliquot of the derivatized sample was injected in the splitless mode onto an HP-1 fused-silica capillary column (12 m × 0.2-mm i.d., 0.33-µm film thickness). The MS was operated in the selected ion monitoring mode.

**Pharmacokinetic and statistical analyses**

AUC was calculated by the trapezoidal rule. Linear regression was performed with Delta Graph (SPSS, Chicago, IL). Confidence intervals for regression lines were generated with Microsoft Excel®. The Wilcoxon Signed-Ranks Test was employed to test for significant differences between the metabolite/cocaine ratios across ascending doses of oral cocaine.

**Results**

**Subject participation**

Twelve subjects (10 males; 2 females) provided informed consent and participated in this
study. Three subjects (2 male; 1 female) were discharged before the third session because of lack of venous access (1 subject), paranoia (1 subject, unrelated to the study drug), and unwillingness to continue (1 subject). Data from these subjects were not included in this report. Two subjects completed all 13 active drug sessions. Seven subjects completed 3–11 active sessions; their participation was terminated for one or more of the following reasons: cardiovascular safety parameters exceeded \( N = 6 \), sensory hallucination \( N = 1 \), or vomiting \( N = 1 \).

Cocaine plasma concentrations during daily dosing sessions

Cocaine was detected in plasma within 30–60 min after the first dose administered during the multiple oral cocaine dosing sessions. Peak plasma cocaine concentrations occurred within 3–6 h after the first drug administration with a mean peak time of 4.6 ± 0.13 h (time of last drug administration was at 4 h). The maximum concentrations of cocaine achieved at doses from 1250 mg (5 × 250 mg) to 2000 mg (5 × 400 mg) ranged from 653 to 1899 ng/mL across subjects.

The accumulation of cocaine in plasma over repeated dosing within single sessions is illustrated in Figure 3. Plasma cocaine concentration increased across doses, resulting in concentrations greater than 1000 ng/mL at the highest doses. By 24 h after the first dose, cocaine concentrations had usually declined below 10 ng/mL.

Metabolite plasma concentrations during daily dosing sessions

A variety of cocaine metabolites were identified in plasma following multiple doses of oral cocaine including BZE, EME, BNE, NCOC, \( m \)-HOCOC, \( m \)-HOBZE, \( p \)-HOCOC, and \( p \)-HOBZE. A representative plasma concentration-time profile for these metabolites is illustrated in Figure 4. BZE and EME were typically detected within 30–60 min after cocaine dosing, whereas the minor metabolites were detected within 1–2 h after dosing. Across subjects, the relative peak concentrations of individual metabolites were as follows: BZE > EME > BNE = \( p \)-HOBZE > NCOC > \( p \)-HOCOC > \( m \)-HOBZE > \( m \)-HOCOC.

The time course for EME, NCOC and \( m \)- and \( p \)-HOCOC closely paralleled that observed for cocaine, with peak concentrations occurring approximately 5 h after the first dose. The plasma concentration-time profile for BNE and \( m \)- and \( p \)-HOBZE was similar to that for BZE, with peak concentrations occurring from 5 to 8 h after the first dose. At 24 h after the first dose, BZE, EME, BNE, \( p \)-HOBZE, and \( m \)-HOBZE generally were the only metabolites detected in plasma.

Cocaine AUC values across doses

The individual and mean AUC determinations across doses are illustrated for cocaine in Figure 5. In Figures 5A and 5C, the 4- and 12-point cocaine AUCs are shown for individual subjects. Whereas the 4-point AUC calculation produced larger area values than the 12-point AUC calculation (note the different y-axis scales), both area measurements indicated that AUC increased in a dose-related fashion for all subjects. The mean data, displayed in Figures 5B and 5D, also reflected this dose-related increase. The correlation coefficients of the regression lines for the mean 4-point and 12-point AUC across doses were 0.865 and 0.881, respectively. Inspection of the 95% confidence intervals for the y-intercepts of both regression lines indicated that neither intercept was significantly different from zero. However, these regression lines must be interpreted with caution because only two subjects were included at the two highest doses, which may not provide an accurate representation for a larger sample.

Metabolite AUC values across doses

The AUC determinations across doses are illustrated for BZE, EME, BNE, and NCOC in Figure 6. The calculations of metabolite AUC used only data obtained from sessions during which the 12-point plasma collection schedule was employed. This limitation was necessary because the 4-point collection schedule provided an inadequate representation of the time course for cocaine metabolites in plasma. A
linear relationship was observed between metabolite AUC and oral cocaine dose for each metabolite, with correlation coefficients ranging from 0.877 to 0.986. The positive slope of each of the regression lines indicated a dose-related increase in metabolite AUC values. The 95% confidence intervals were calculated for the y-intercept of each regression line. Inspection of these data indicated that only the intercept of the regression line for BZE AUC versus dose was significantly different from zero. The 95% confidence range for the BZE intercept was 4133–19917.

Metabolite/cocaine AUC ratios across doses

The mean AUC ratios for metabolite/cocaine versus time are shown in Figure 7. The BZE/COC and EME/COC ratios always exceeded unity, whereas the BNE/COC and NCOC/COC ratios were always less than unity. Both the BZE/COC and EME/COC ratios appeared to decrease as a function of increasing dose, as indicated by the negative slopes of the regression lines. The BNE/COC and NCOC/COC ratios appeared to be stable across doses, with the slope of the regression lines being almost zero. However, the Wilcoxon Signed-Ranks Test indicated no significant differences between the first dose and the last dose for all metabolite/cocaine AUC ratios.

Discussion

The present study examined the profile of cocaine metabolism in humans following repeated oral dosing with cocaine. The profile observed was substantially different from that reported after acute administration of parenteral cocaine. Firstly, the primary metabolites, BZE and EME, were detected at substantially higher concentrations than reported following parenteral administration. The ratio of BZE to cocaine (AUCBZE/AUCCOC) was larger than that reported after intranasal, intravenous, and smoked administration of cocaine (8). Moreover, EME concentrations were greater than or equal to concentrations of cocaine. Secondly, BZE and EME are usually the only metabolites detected in plasma following parenteral cocaine administration and EME generally is found at low concentrations. In the present study, a number of other cocaine metabolites were detected at concentrations exceeding 50 ng/mL, including BNE, NCOC, m- and p-HOBZE, and m- and p-HOCOC.

The mean time to peak plasma cocaine concentrations generally occurred after the fourth...
or fifth oral dose of cocaine (4.6 ± 0.13 h; \(N = 34\)). Similarly, the mean times to peak plasma concentration for EME and NCOC were 5.4 ± 0.11 h (\(N = 34\)) and 4.9 ± 0.12 h (\(N = 34\)), respectively. The peak concentration of BZE in plasma occurred after the fifth dose of the session (mean time to peak concentration: 6.5 ± 0.16 h; \(N = 34\)), and BZE remained detectable for at least 24 h. NCOC and \(m\)- and \(p\)-HOCOC concentrations appear to mimic the time course of cocaine in plasma, whereas other metabolites followed the time course observed for BZE.

Previous studies in humans have reported that EME is detected only in trace amounts following parenteral administration of single, low doses of cocaine (8). This production of EME is typically attributed to butyrylcholinesterase activity in plasma and liver and liver hCE-2 (12–14). The detection of higher concentrations of EME in plasma following chronic oral administration (up to 3073 ng/mL) could have been due to changes arising in dose- and time-dependent metabolic processes from repeated dosing and/or to the oral route. EME may have been produced prehepatically under the present chronic oral dosing conditions. It is possible that cocaine may have been enzymatically hydrolyzed to EME in the gut or during passage through the gut wall. Cholinesterase activity has been identified in the ileum and various other tissues (23). The cholinesterase activity present in the ileum is determined by the same structural gene as plasma and liver butyrylcholinesterase and is therefore capable of metabolizing cocaine when administered by the oral route (23). In addition, the production of EME may also have occurred as a result of first-pass metabolism in the liver.

BZE, like EME, was detected in concentrations greater than expected based upon data from studies of parenteral cocaine administration (8). This may be attributable to the formation of BZE in the gastrointestinal tract. It is well established that BZE can be formed chemically, especially at a pH greater than neutrality (9,10). The pH of the small intestine ranges from 6.6 to 7.5, conditions that are suitable for the chemical production of BZE (20). Also, it is possible that an enzymatic first-pass effect in the gut may have contributed to the formation of BZE. However, the ability of enzymes in the gut to metabolize COC to BZE has not been assessed.

The detection of substantial amounts of other cocaine metabolites after chronic oral cocaine administration may be the result of prehepatic and hepatic production of minor metabolites. The cocaine doses administered orally (up to

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**Figure 6.** Mean metabolite AUC values across session doses for BZE (A), EME (B), BNE (C), and NCOC (D). The number of subjects represented at each dose are indicated in A and identical for B, C, and D. Error bars represent standard error of the mean. The equations shown on each graph were obtained using standard linear regression.
2000 mg/session) were much greater than parenteral doses administered in previous studies. The large doses can partially explain the detection of minor metabolites in plasma, by increasing the amount of metabolite formed during and after absorption. In addition, prehepatic metabolism may have contributed to their formation. The presence of the enzyme CYP3A4 has been detected in the stomach and small intestine (24). It is possible that this enzyme contributed to the formation of NCOC (17). The presence of CYP3A4 in the stomach and small intestine also appears to be involved in the metabolism of other drugs such as triazolam after oral administration (24).

Linear kinetic processes were supported by the present data for COC, EME, NCOC, and BNE because their AUC values increased in a linear dose-proportional manner \( (r^2 = 0.88-0.99) \) with intercepts passing through zero. In contrast, the AUC values for BZE versus dose were linear, whereas the intercept for this function was significantly greater than zero. A limitation of the regression lines calculated for metabolite AUC versus dose is that only two subjects were included at the two highest doses, which may not provide an accurate representation for a larger sample. The observed elevation of the intercept for BZE may have been the result of two independent metabolic processes, one non-saturable and the other saturable, both leading to the formation of BZE. The high pH of the gut could have produced substantial amounts of BZE prior to absorption. In addition, the high concentrations of cocaine entering the liver could have initially caused saturation of the hCE-1 enzyme followed by a return to linear enzyme kinetics. The combination of a non-saturable process followed by a saturable process may account for the increased abundance of BZE and its elevated intercept.

There were no statistical differences observed between the AUC ratios of metabolite and cocaine between the first and last doses. However, because of the small number of subjects who completed the higher doses \( (N = 2) \), the statistical inferences that can be made from this comparison are limited. The metabolite/cocaine AUC ratio for BNE appeared to remain stable over ascending doses of oral cocaine, indicating that the formation of this metabolite was not altered as a function of time or dose after repeated oral cocaine administration. A slight, but nonsignificant increase appeared to occur for the NCOC/COC ratio over time. In addition, the AUC ratios for BZE and EME appeared to decrease over the chronic dosing sessions, particularly if one compares the first session to the later

**Figure 7.** Mean $\text{AUC}_{\text{metabolite}}/\text{AUC}_{\text{cocaine}}$ ratios across session doses for BZE (A), EME (B), BNE (C), and NCOC (D). The number of subjects represented at each dose are indicated in A and also apply to B, C, and D. All AUC values were calculated using data from the 12-point sessions. The error bars represent standard error of the mean. The equations shown on each graph were obtained using standard linear regression.
sessions. In the case of EME, the ratio decreased only slightly over the sessions. This suggests that if a metabolic pathway(s) for EME was altered, this change contributed only a small portion of the EME produced. It is possible that the production of EME by plasma cholinesterase may have been decreased after repeated oral cocaine administration as a result of enzyme saturation. Such a phenomenon has been suggested to occur after administration of large doses of cocaine or as a result of repeated cocaine administration typical of “bingeing” (25).

The AUC ratio of BZE also appeared to decrease across sessions, and this was more notable than for EME. This pattern suggested a saturable metabolic process or that metabolism may be inhibited after chronic administration. This decrease in the AUC ratio most likely occurred as a result of saturation of the hCE-1 enzyme, thereby allowing somewhat greater amounts of cocaine to survive. If saturation of hCE-1 occurred, “shunting” of cocaine into other metabolic pathways could have occurred, leading to greater abundances of other metabolites. Such an interpretation is consistent with the slight increase in the NCOC/COC ratios over time.

Conclusion

The present study characterized the metabolic profile of cocaine after repeated administrations of both low and high oral dose exposure to cocaine (up to 2000 mg within a session). The data indicated that repeated oral cocaine administration can be used to study the effects of chronic cocaine use in human subjects. This study was conducted to identify doses of oral cocaine that could be safely administered on a chronic basis. Although only two subjects completed the entire study (receiving the highest dose of 2 g/day), eight subjects received at least 1000 mg in a single session without exceeding the safety parameters, which clearly demonstrates that oral cocaine could be administered repeatedly with minimal risk. The model used in the present study was capable of producing dose-related increases in plasma cocaine concentrations while minimizing the risks associated with parenteral cocaine administration. The unique metabolic profile observed after repeated oral cocaine administration appeared to be a result of prehepatic and hepatic pathways. The metabolic pathways responsible for the production of BNE did not appear to undergo any dose- or time-related alterations, whereas the enzymatic production of EME and BZE appeared to shift downward. This pattern appeared to be accompanied by a slight increase in the production of NCOC over time. These data suggest that cocaine’s metabolic profile may be altered by a number of factors, including the route of administration, the frequency of dosing, and the total dose. It is unclear the extent to which each of these factors contributed to the unique pattern of metabolism observed in this study because few previous studies have examined the pharmacokinetic profile of cocaine following repeated administration, despite the fact that closely spaced repeated dosing more accurately mimics the typical pattern of illicit cocaine abuse. The present study provides new information suggesting that repeated dosing with oral cocaine may be a useful strategy for examining the consequences of repeated cocaine use in humans that minimizes the risks associated with more rapid routes of administration.

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