Correlation of Saliva Cocaine Levels with Plasma Levels and with Pharmacologic Effects after Intravenous Cocaine Administration in Human Subjects

Edward J. Cone*, Karen Kumor, Loren K. Thompson, and Michael Sherer
National Institute on Drug Abuse, Addiction Research Center, P.O. Box 5180, Baltimore, Maryland 21224

Abstract

The behavioral and physiologic effects of single, intravenous bolus doses of cocaine in 5 male human subjects were correlated with cocaine levels in saliva and blood. All measures were performed under double-blind conditions. Two test doses of cocaine (15 mg and 40 mg) and one placebo test dose were administered to each subject in a random, cross-over design. Each test day was separated by a minimum of 48 h. Cocaine levels in saliva and blood significantly (p < 0.05) correlated with responses on self-rating scales for drug sensation (Feel Drug scale), psychotomimetic effects (LSD scale), and feelings of rush (Rush scale). Significant (p < 0.01) correlations also were obtained with cocaine biofluid levels and pulse rate. The close relationship observed between cocaine saliva levels and cocaine-induced behavior and physiologic effects presents the opportunity for development of a new noninvasive method for detection of current cocaine use.

Introduction

Rapid intravenous administration of the psychotropic alkaloid, cocaine, is thought to cause immediate stimulation of neural centers associated with reward mechanisms (1). This increase in central activity appears to be related to subject responses on self-rating scales that measure drug-induced changes in mood and feeling; dose-related elevations occur on both the Profile of Mood States (POMS) scale and selected scales of the Addiction Research Center Inventory (ARCI) questionnaire (2-4). After using cocaine, subjects generally report an initial rush lasting only 1-2 minutes followed by a prolonged state of euphoria lasting for 15-30 minutes. Concurrent with these changes are dose-related changes in heart rate and blood pressure (2-4). Physiological changes begin almost immediately and usually peak within 6-8 minutes. Changes in most subjective and physiological measures appear to parallel the descending phase of the plasma cocaine curve (5); however, some effects decline even more rapidly than cocaine plasma levels (5-7).

Despite the current level of understanding of cocaine's pharmacologic properties, many other effects of cocaine, such as the relationship of cocaine levels in saliva to plasma cocaine levels and to pharmacologic effects, are largely unknown. In the present study, single intravenous doses of cocaine were administered to human male subjects. Cocaine levels in saliva and blood and subjective and physiological effects were all measured under double-blind conditions. Correlations were made to define which measures were empirically related. The finding of significant correlations between levels of cocaine in saliva and plasma and between saliva cocaine and pharmacologic effects could offer a new noninvasive method for monitoring cocaine effects and current usage.

Subjects and Methods

Subjects

Five healthy male volunteer subjects with a history of intravenous cocaine abuse participated in the study. Informed consent was obtained and all procedures were approved by the Francis Scott Key Institutional Review Board. All subjects resided on a closed research unit of the Addiction Research Center, National Institute on Drug Abuse during the study. Their ages ranged from 26 to 38 years, and their weights ranged from 63.5 kg to 73.1 kg.

Procedure

Prior to their participation in the pharmacodynamic study, subjects were administered single, intravenous doses of 20 mg, 40 mg, and 60 mg of cocaine hydrochloride in ascending order to test for unusual sensitivity to cocaine. Each of these test doses was separated by a minimum of 24 h. Cardiac function was monitored with ECG during these initial cocaine trials and also later in the crossover study. After initial cocaine trials, each subject received three single intravenous bolus dose treatments administered in a double-blind, random, crossover design consisting of the following: placebo (saline), 15 mg of cocaine hydrochloride, and 40 mg of cocaine hydrochloride. The experimental protocol followed on test days is outlined in Table 1. Subjects were accommodated to this protocol during the ascending series of cocaine doses. In the morning of each test day and approximately 90 minutes before the test injection, an indwelling catheter was placed in each arm of the subject. The bolus test doses of cocaine and placebo (saline) were administered
manually over a 2-5 s period. Before and after test doses, blood samples were withdrawn from the catheter in the opposite arm. Mixed saliva samples were collected over a 2-4 minute period at equivalent times as blood. Physiologic measures and responses on self-report questionnaires were taken before and after test dose administration.

Cocaine analyses
Blood samples for cocaine determinations were collected in heparinized glass tubes containing 0.1 mL of saturated sodium fluoride solution to prevent cocaine hydrolysis. After centrifugation, the plasma was separated and frozen until time of analysis. Mixed saliva was collected in 50-mL polypropylene tubes containing 0.25 mL of saturated sodium fluoride. Saliva flow was stimulated during collection with a small piece of sour candy. Saliva samples were frozen until time of analysis. Cocaine content in plasma and saliva was determined by extraction followed by analysis with a gas chromatograph equipped with a nitrogen-sensitive detector (8). The sensitivity of the assay for cocaine was 5 ng/mL of biofluid.

Self-rating scales and physiologic measures
Before and after test dose administration, each subject completed self-rating scales and underwent physiologic measurements. The self-rating scales consisted of the following measures: subscales of the Addiction Research Center Inventory; i.e., MBG, PCAG, and LSD scales (9); Single Dose Questionnaire (10), which was modified to provide only subject ratings (Feel Drug scale) and observer ratings (Observer Signs scale and Observer Symptoms scale) (11); and Horizontal Interval Scales (HIS) (12). The HIS scales were designed to explore selected subjective sensations labeled good, bad, rush, tired, happy, energetic, sad, restless, anxious, loss of or miss rush, irritable, relaxed, do you feel that you are in danger?, do you feel that you were afraid of something?, and, do you feel that people are talking about you? Each scale was presented to the subject on a computer display as a horizontal line evenly numbered from 0 to 9. Subjects selected a numbered key pad to rate their feelings. A rating of 0 represented an absence of that sensation and a rating of 9 represented the maximum effect they could experience. These scales for measurement of individual subjective responses to cocaine are described in detail by Kumor et al. (12).

Data analyses
Pharmacokinetic analysis of cocaine levels in plasma and saliva was performed by computer fitting of the data with a software program (PCNONLIN) (13). The observed data were fitted to a one-compartment open model described by equation 1:

\[ C_f(t) = C_s e^{-kt} \]  

Eq. 1

where \( C_f(t) \) is observed cocaine biofluid level at time \( t \), \( C_s \) is cocaine biofluid concentration at zero time, and \( K \) is first-order elimination rate constant. The primary kinetic parameters, \( k \) and apparent volume of distribution \( (V_d) \), and the secondary parameters, area under the curve (AUC) and half-life \( (t_1/2) \), were obtained directly from the nonlinear estimation program. Other kinetic parameters, clearance \( (Cl) \) and \( C_s \) were calculated by standard methods (14).

Results from self-rating scales and physiologic measures were analyzed as difference measures from preinjection controls by two-way within-subjects analyses of variance. When a significant mean \( (p < 0.05) \) drug effect or drug \( \times \) time interaction was found, a post hoc analysis (Tukey's test) was conducted to determine which dose and time differed significantly.

Pearson product-moment correlations were calculated with combined mean data from both cocaine trials (15 mg and 40 mg doses). Correlations were made independent of time and dose among plasma cocaine (log concentration), saliva cocaine (log concentration), Feel Drug responses, and other self-rating scales and physiologic measures. Difference values from preco- caine controls were used in all correlation calculations. Correlations are reported only for measures in which a significant dose \( (p < 0.05) \) or significant drug \( \times \) time effect for cocaine vs. placebo was present. The correlations of the logarithm of the concentration of cocaine in biofluids with response were performed on the basis of equation 2:

\[ E = m \log C + c \]  

Eq. 2

where \( E \) is the intensity of effect, \( m \) is the slope of the effect vs. log biofluid concentration \( (C) \) and \( c \) is a constant (14). In cases where cocaine biofluids were not sampled simultaneously with other response variables, the biofluid levels were estimated by means of eq 1 employing individually determined pharmacokinetic parameters.

Pearson product-moment correlations also were calculated on individual subject data for each of the 5 subjects in the same manner as described for mean data analyses. The resulting correlations were then pooled to serve as a measure of the group response.

Results
Cocaine pharmacokinetics
Plasma levels of cocaine varied from 86 ng/mL to 273 ng/mL.
for the 5 subjects at 10 min after a 15-mg intravenous bolus dose of cocaine; at 5 h after cocaine, plasma levels had declined below assay sensitivity. After a 40-mg dose of cocaine, plasma levels varied from 204 ng/mL to 523 ng/mL at 10 min and had declined to an average of 6 ng/mL in 5 h. Saliva cocaine levels varied from 100 ng/mL to 520 ng/mL at 10 min after the 15-mg dose of cocaine and had declined to an average of 8 ng/mL in 5 h. After the 40 mg dose, saliva levels varied from 237 ng/mL to 1843 ng/mL at 10 min and had declined to an average of 29 ng/mL in 5 h. Semilog plots of mean concentrations of cocaine in biofluids after intravenous administration of 15 mg and 40 mg of cocaine hydrochloride are illustrated in Figures 1A and 1B, respectively.

Mean pharmacokinetic parameters for cocaine in saliva and plasma are shown in Table II. Overall, greater variability was obtained in the estimation of mean kinetic parameters for cocaine in saliva compared to plasma. An initial evaluation in saliva cocaine at 3 min after drug administration as a result of pH effects of saliva accounted for a substantial amount of this variability. The average half-life of cocaine in plasma of 34.9 min for both doses was comparable to that estimated for saliva (34.7 min), although individual half-life range was greater for saliva (7-81 min) than for plasma (11-41 min). The mean elimination rate constant (K) also was similar for plasma and saliva, whereas plasma volume of distribution (Vd) and plasma clearance (Cl) were approximately twofold greater than the corresponding estimates for saliva. Both the area under the curve (AUC) estimates and the extrapolated zero time cocaine concentrations (C0) increased proportionately with cocaine dose as expected.

Subjective effects
Elevated responses occurred immediately after cocaine administration on the MBG (euphoria), LSD (psychotomimetic effects), Feel Drug, Observer Signs, and Observer Liking scales and were near baseline within 60 min (Figure 2). A similar pattern was seen on self-rating scales for Good (HIS #1), Rush (HIS #3), Anxious (HIS #9), and Talking About You (HIS #15) (Figure 3). Of these measures, only the mean responses on the Feel Drug and LSD scales after 40 mg of cocaine were significantly elevated from placebo (p ≤ 0.05). In addition, as seen in Figures 2 and 3, peak responses were significantly elevated from placebo (p < 0.05) at the following times and cocaine doses: MBG, 3 min, 40 mg; Feel Drug, 3, 10, and 15 min, 40 mg; Feel Drug, 3 min, 15 mg; Good (HIS #1), 3 and 10 min, 40 mg; Good (HIS #1), 3, 10, and 15 min, 15 mg; Rush (HIS #3), 3 min, 40 mg. Peak responses on MBG, Feel Drug, and Rush (HIS #3) at 3 min after 40 mg of cocaine also were significantly different from the equivalent responses after 15 mg of cocaine.

![Figure 1](image-url)

**Figure 1.** Mean cocaine concentrations in saliva and plasma of 5 subjects after single-dose intravenous administration of 15 mg (A) and 40 mg (B) of cocaine hydrochloride.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dose (mg)</th>
<th>C0 (ng/mL)</th>
<th>t1/2 (min)</th>
<th>K (min^-1)</th>
<th>AUC (ng/mL-min)</th>
<th>Vd (L)</th>
<th>CI (nL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>15</td>
<td>191.1 ± 67.6</td>
<td>32.5 ± 5.0</td>
<td>0.0276 ± 0.0083</td>
<td>6568 ± 508</td>
<td>114.6 ± 22.8</td>
<td>2359 ± 200</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>470.4 ± 70.8</td>
<td>37.3 ± 1.8</td>
<td>0.0188 ± 0.0011</td>
<td>21838 ± 2970</td>
<td>111.2 ± 18.9</td>
<td>2042 ± 312</td>
</tr>
<tr>
<td>Saliva</td>
<td>15</td>
<td>564.0 ± 160.0</td>
<td>37.6 ± 13.3</td>
<td>0.0391 ± 0.0148</td>
<td>15855 ± 1868</td>
<td>56.1 ± 23.1</td>
<td>1024 ± 135</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>1907.5 ± 835.4</td>
<td>31.8 ± 12.2</td>
<td>0.0435 ± 0.0143</td>
<td>38299 ± 6454</td>
<td>58.9 ± 23.5</td>
<td>1210 ± 201</td>
</tr>
</tbody>
</table>

* Plasma and saliva data for 5 individual subjects were fitted to a one-compartment model described by C(t) = C0 * e^{-Kt}. Additional data were derived by standard methods (14) and are presented as follows: C0, extrapolated cocaine concentration at 0 min; t1/2, half-life; K, apparent first-order elimination rate constant; AUC, area under biofluid concentration curve; Vd, apparent volume of distribution; CI, clearance rate from biofluid. For all cases, kinetic parameters were calculated on the basis of individual data; each value represents mean data ± 1 standard error for five subjects. It may be noted that mean kinetic parameters are not directly related; for example, mean t1/2 is not equivalent to 0.693/K.
Elevations also occurred immediately after cocaine on the Energetic (HIS #6) scale and the Restless (HIS #8) scale but exhibited thereafter trends different from other scales. The Energetic (HIS #6) scale was initially elevated but decreased below placebo baseline 15 min after injection. Responses on the Restless (HIS #8) scale after cocaine remained elevated during a two-hour period after injection. Neither of these measures differed significantly from placebo ($p \leq 0.05$).

Reduction in responses on PCAG (sedation), Tired (HIS #4), and Loss of Rush (HIS #10) scales occurred early after cocaine
administration (3–60 min) with subsequent return to baseline response levels. Responses on the Irritable (HIS #11) scale were generally depressed over the entire test period after cocaine administration. None of the mean reduced responses differed significantly from placebo ($p \leq 0.05$).

**Physiologic effects**

Cocaine produced increases in respiration, pulse, and systolic and diastolic blood pressure at 3–15 min after injection; subsequent measures were similar to baseline or placebo control values (Figure 4). Only pulse measures after 40 mg of cocaine were significantly elevated over placebo measures (3, 10, and 15 min) and over equivalent measures after 15 mg of cocaine ($p \leq 0.05$).

**Pearson correlations of cocaine biofluid levels, Feel Drug responses, and other measures**

Cocaine concentrations in plasma correlated significantly with saliva cocaine and Feel Drug responses ($p \leq 0.01$, Table III). Each of these measures also correlated significantly with other measures after cocaine ($p \leq 0.05$), i.e., LSD, Rush (HIS #3), and pulse.

A more stringent test for correlational relationships among these variables was performed by analyses of each subject data set. Individual correlation coefficients were averaged to yield a group mean response. Significant correlations ($p \leq 0.05$) were again obtained among plasma cocaine, saliva cocaine, and Feel Drug measures obtained after cocaine administration. Each of these measures also significantly correlated with pulse ($p \leq 0.05$). Other group mean correlations are indicated in Table III.

**Discussion**

The intravenous administration of cocaine to human subjects in this study produced quickly fleeting behavioral changes identified by the subjects as an initial “rush” followed by a euphoric “high” which was more prolonged but disappeared in approximately 30 min. Other behavioral and physiological effects also returned to baseline within 60 min after cocaine administration. Similarly, peak saliva and plasma levels were detected at the onset of measures after drug administration and declined rapidly thereafter. This rapid disappearance of effects presents the addict with the immediate opportunity to regain a pleasurable state by taking more cocaine. Conversely, without more cocaine, chronic users exhibit symptoms of depression and anhedonic dysphoria (15). The short on–off time cycle of euphoria/dysphoria–cocaine craving undoubtedly has both neurochemical (16) and pharmacokinetic basis. Dackis and Gold (17) reviewed the effects of cocaine on endogenous reward systems and speculated that cocaine euphoria results from acute stimulation of dopamine neurotransmission, whereas the intense craving state and dysphoria result from depletion of synaptic dopamine. This central role of dopamine in the actions of cocaine has been well documented in animal self-administration studies (18–20).

The short cycle of cocaine stimulation and euphoria followed by dysphoria and craving also may be related to the bioavailability of cocaine to central effector sites. The concentration of drug at these effector sites is in turn presumably related to circulating plasma drug levels. The rapid metabolism of cocaine by plasma cholinesterase and liver quickly converts active drug into inactive polar metabolites (21) thereby accounting for cocaine’s short half-life. This rapid metabolic removal of cocaine from circulation also serves to terminate its pharmacological actions. The average half-life of the subjects in the present study was 34.9 minutes, a determination that is in close agreement with other kinetic studies of intravenous cocaine in human subjects (6, 22). Bioavailability of drug to effector sites also has been cited as one of the major functional parameters manipulated in animal self-administration studies; for example, it has been suggested that response rate for amphetamine regulates and maintains a constant blood level of parent drug (23). It can be noted that the pattern of self-administration in animals for stimulants is reminiscent of patterns of cocaine use by addicts on a “run” (24). It may be that the cocaine addict attempts to titrate blood levels (and thereby drug levels at effector sites) for the production of feelings of rush and euphoric high and the prevention of dysphoric effects and craving. The current observations of significant correlations of cocaine biofluid levels with behavioral and physiological effects support this thesis. Self-rating scales for feelings of drug (Feel Drug) and cocaine rush (Rush; HIS #3) showed significant positive correlations with plasma cocaine and saliva cocaine levels ($p \leq 0.05$).

The relationship between effect and cocaine biofluid level would not hold if the concentration of cocaine at the site of action in the CNS were not proportional to biofluid levels, that is, if they occurred in a physiological compartment which was not characterized by the pharmacokinetic analysis or if the measured effects were not a direct consequence of cocaine’s effects. Nonetheless, significant correlations between effects and cocaine biofluid concentration do not prove causation. Indeed,

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pearson correlation with plasma cocaine group mean</th>
<th>Pearson correlation with saliva cocaine group mean</th>
<th>Pearson correlation with Feel Drug group mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$p$</td>
<td>$p$</td>
<td>$p$</td>
</tr>
<tr>
<td>Plasma</td>
<td>-</td>
<td>0.89</td>
<td>0.75</td>
</tr>
<tr>
<td>Saliva</td>
<td>0.89</td>
<td>0.01*</td>
<td>-</td>
</tr>
<tr>
<td>Feel Drug</td>
<td>0.75</td>
<td>0.01*</td>
<td>0.74</td>
</tr>
<tr>
<td>LSD</td>
<td>0.84</td>
<td>0.01*</td>
<td>0.70</td>
</tr>
<tr>
<td>MBG</td>
<td>0.61</td>
<td>0.10</td>
<td>0.56</td>
</tr>
<tr>
<td>HIS #1 (Good)</td>
<td>0.43</td>
<td>NS</td>
<td>0.54</td>
</tr>
<tr>
<td>HIS #3 (Rush)</td>
<td>0.55</td>
<td>0.05</td>
<td>0.55</td>
</tr>
<tr>
<td>Pulse</td>
<td>0.81</td>
<td>0.01*</td>
<td>0.76</td>
</tr>
</tbody>
</table>

* Individual correlation data were pooled to yield a mean correlation which also tested significant ($p \leq 0.05$).
the rapid decline of euphoric and cardiovascular effects appears to precede the decline of plasma cocaine levels and has been the basis for suggestion of intervention of homeostatic reflex mechanisms or acute tolerance as additional factors to be considered in relating cocaine-biofluid levels to pharmacologic effects (5–7).

More immediately, the observation of significant correlations of saliva cocaine levels with plasma cocaine levels and almost identical correlations with behavioral and physiologic effects provide the opportunity for development of a new noninvasive method for chemical validation of recent cocaine use. In contrast, current procedures employed for testing for cocaine use involve screening for cocaine or benzoylecgonine in urine. A positive urine screen for cocaine use provides only recent historical evidence of cocaine use. Saliva is a readily available biofluid and its cocaine content clearly reflects cocaine blood levels after intravenous use. With administration of cocaine by other routes, that is, by smoking or intranasal use, saliva cocaine levels presumably would initially be elevated but should equilibrate rapidly with blood levels and thereafter reflect cocaine blood levels in a manner similar to intravenous use. The correlations observed in this study between cocaine saliva levels with plasma levels and with subjective and physiologic effects indicate that finding cocaine in saliva after intravenous use provide strong presumptive evidence that the subject is under the influence of cocaine.

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References

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