Effect of Cocaine on Plasma Constituents Involved in Endogenous Thrombosis

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A. Statement of study and purpose rationale

With an estimated 25 million Americans having used cocaine, and 5 million Americans using cocaine regularly, research efforts are being directed towards the possible mechanisms by which cocaine may adversely affect the cardiovascular system. [1,14]

Cocaine use has been associated with sudden cardiac death and myocardial ischernia. Its effect of myocardial oxygen supply and demand is well-established. Cocaine blocks the presynaptic reuptake of the neurotransmitters, epinephrine, norepinephrine, dopamine, and serotonin, resulting in an excess of these substances at postsynaptic receptor sites. The resultant adrenergic stimulation increases heart rate, blood pressure, and left ventricular contractility and, therefore, myocardial oxygen demand. Cocaine also causes coronary arterial vasoconstriction and, subsequently, decreased myocardial oxygen supply. [2,9]

Additional pathophysiological bases for cocaine-induced myocardial infarction may include endothelial dysfunction, accelerated atherosclerosis, and thrombogenesis - especially involving platelet-rich coronary thrombi in otherwise normal vessels. More than 1/3 of patients who have sustained cocaine-related myocardial infarction and, subsequently, have undergone either angiographic or pathological examination did not have coronary artery stenosis. [10]

Few recent studies have addressed the association between cocaine ingestion and endogenous thrombosis. The mechanisms by which cocaine promotes thrombus formation have not been well-defined. One study found that cocaine (in concentrations similar to those found clinically) when added to individual platelets in whole blood from drug-naive donors intermittently induced platelet activation *in* vitro. [7] In contrast, another study published during the same year suggested that cocaine and its carrier has a direct inhibitory effect on the activation and aggregation of human platelets *in* vitro. [5]

David Hillis and colleagues at the University of Texas South Western Medical Center reported on cocaine's effect on plasma constituents involved in endogenous hemostasis. Their methodology involved procuring blood samples from drug-naive patients undergoing cardiac catheterization before and 15 minutes after the administration of intranasal saline or cocaine, and then measuring the plasma concentrations of fibrinogen, plasminogen, and lipoprotein(a), as well as tissue plasminogen activator activity and plasminogen activator inhibitor (PAI-1) activity. They found that intranasal cocaine administration was associated with an increase only in plasma PAI-1 activity and that this may be "important in recreational users of cocaine who experience vascular thrombosis." [11]

Similarly, Kugelmass *et al.* established that individual circulating platelets are activated by intravenous cocaine in vivo. Here, the cocaine was administered intravenously to conscious, chronically instrumented dogs and the expression of P-selectin (an antigen found on the external cell membrane of platelets only after stimulation) in whole blood arterial samples was found to be elevated. [8] Notably, Kugelmass found a delayed increase in the detection of activated platelets after treatment with IV cocaine, and postulated that this may have been due to adhesion and subsequent detachment of activated platelets or, alternatively, due to activation by cocaine metabolites rather than the drug itself.

Examining the extent of platelet P-selectin expression in humans with cocaine use was the next logical study. Rinder *et al.* found that long-term cocaine use in some subjects was intermittently associated with high basal levels of P-selectin, or equivalently, circulating activated platelets. Interestingly, this study also looked at the effects of intravenous cocaine and its metabolites (at concentrations that produce the cocaine "high" in long-term users) on platelet Pselectin expression *in vitro* , and found no increase in platelet activation or aggregation, either directly or in concert with platelet agonists. [13]

The main hypothesis of this pilot study is that smoked cocaine substantially increases platelet activation within the first two hours after inhalation. In the controlled cocaine administration setting available to this study it is appropriate to posit a pharmacokinetic model in which P-selectin, as a sensitive biomarker of platelet activation, and perhaps some plasma constituents of endogenous thrombosis, rise from baseline levels in the first 15-30 minutes following cocaine administration, and retreat toward baseline levels following the cessation of cocaine ingestion.

We will examine the temporal relationship between the bioavailability of plasma constituents involved in endogenous hemostasis: fibrinogen, plasminogen, von Willebrand factor, tissue plasminogen activator (tPA) activity, and plasminogen activator inhibitor (PAI-1) activity as well as P-selectin (platelet activation) and cocaine and its metabolites levels. This study will look at the effects of increasing doses of smoked cocaine on the aforementioned plasma constituents in long-term cocaine users who are not presently using other drugs (especially opiates and alcohol).

B. Description of study design and statistical analysis

This is a pilot study designed in three parts. Proposed sample size is N=8.

All volunteers in the substance abuse investigations involving smoked cocaine, who also agree to participate in this study, will have blood samples (see below) drawn from an indwelling catheter at the beginning of their substance abuse protocol. These baseline measurements will constitute Part I. The volunteers who then proceed to abstain from cocaine use for 3 days, in concordance with the abstinence substance abuse protocol, and while under strict supervision as inpatients on Harkness 10, will have the above blood samples repeated a second time at the conclusion of the abstinence period from cocaine use. These abstinence measurements will constitute Part II. The volunteers at this phase of the substance abuse investigations involving smoked cocaine receive either 12.5, 25 or 50 mg of the drug. The doses are repeated every 14 minutes - a frequency that approximates that of street use - up to a total of 6 doses. In Part III of our study, the measurements taken in Parts I and II will be repeated at several time points during the drug administration phase: 18 minutes, 35 minutes, 60 minutes, and 90 minutes, as well as 2 hours following completion of the drug administration.

The following blood samples will be drawn (a total of 15 ml or 1 tablespoon of blood) during each of the three parts of the study:

- 7 ml in 1 iced, acidified, citrate (blue-top) tube in order to measure plasma levels of plasminogen, fibrinogen, von Willebrand factor, tPA activity, and PA-1 activity.
- 4 ml in 1 iced, acidified, citrate (blue-top) tube in order to measure whole blood levels of P-selectin.
- 4 ml in 1 (grey-top) tube containing sodium flouride (to inhibit plasma cholinesterase activity) in order to measure plasma levels of cocaine and its major metabolites benzoylecgonine and ecgonine methyl ester.

Note: Dr. Fischman and colleagues compared the concentration of cocaine and its major metabolite benzoylecgonine in plasma of humans following intravenous administration and smoking of cocaine, and found that the average half-life of cocaine by either route after two successive doses (spaced 14 minutes apart) was between 38 and 39 minutes. Between 30 and 40 minutes after the first dose, the concentrations of cocaine and benzoylecgonine were found to be equivalent, regardless of the route of administration and quantity of drug administered. The peak concentration of cocaine and benzoylecgonine in those subjects who received 2 doses of 25 mg of smoked drug occurred at 18 minutes and 60 minutes, respectively. The peak concentration of cocaine and benzolyecgonine in those subjects who received 2 doses of 50 mg of smoked drug occurred at 18 minutes and 90 minutes, respectively. [4]

C. Statistical Analysis (Devised by Donald J. McMahon, Director of Clinical Research Information Systems):

Levels of P-selectin, fibrinogen, plasminogen, von Willebrand factor, tPA, and PAI-1 will be graphically plotted against time, with each subject's data aligned to the time of cocaine administration. Serum cocaine levels and levels of major metabolites, benzoylecgonine and ecgonine methyl ester, will be plotted on the same graph to discover the temporal relationship between the bioavailability of these compounds and platelet activation.

A repeated measures variance model will be employed to establish the statistical reliability of the temporal change in P-selectin levels from baseline to 2-hours after the last cocaine administration. With 8 subjects as the proposed sample size, we will have 80% power to detect a 1.5 standardized difference in the within-subject effect of time in the repeated measure model. Secondary models will be systematically compared with the primary model to determine the magnitude and direction of influence of other variables of the time course of platelet activation as the other variables are entered as continuous covariates. Other variables include: pre-washout baseline P-selectin levels, baseline thrombogenic factors, serum cocaine levels, and the serum levels of major cocaine metabolites.

D. Description of study procedures

Under the auspices of the New York State Psychiatric Institute and Columbia University Department of Psychiatry, and as part of ongoing research involving the behavioral observation and testing of subjects with substance abuse histories, cocaine will be administered by the research nurse under the supervision of a physician. Physiological effects (heart rate, blood pressure) will be monitored. Criteria for administering cocaine, stopping a session, or treating a cocaine-induced problem are outlined in the appended protocol.

Part of the substance abuse studies involves the drawing of whole blood samples to measure cocaine, benzoylecgonine and prolactin levels. Our study will involve the collection of additional whole blood samples taken at the same time as these above draws as well as during separate time points (See Section B). These determinations will impose no further risk or pain on the patients.

E. Study Drugs

Cocaine is found in the leaves of *Erthrooxylon coca,* trees indigenous to Peru and Bolivia, and is classified chemically as a benzoylmethylecgonine (an ester of benzoic acid and a nitrogencontaining base). As already mentioned, cocaine blocks the reuptake of catecholamines at adrenergic nerve endings and, hence, potentiates the responses of sympathetically innervated organs to norepinephrine, sympathetic nerve stimulation, and, to a lesser degree, epinephrine. Additionally, cocaine blocks the initiation and conduction of the nerve impulse following local application.

The half-life of cocaine in the plasma after oral, nasal, or intravenous administration has been reported to be 38 to 39 minutes. [4] Inhaled cocaine in doses of 12.5, 25, and 50 mg will be used in this study.

Cocaine stimulates the CNS generally. This is manifested by a feeling of well-being and euphoria (sometimes dysphoria) which is often accompanied by garrulousness, restlessness, and excitement. As the dose is increased, cocaine can produce toxic effects including cardiac arrhythmias, myocardial ischemia or infarction, myocarditis, high-output congestive heart failure, dilated cardiomyopathy, cerebrovascular spasm with transient neural ischemia or infarct of the brain or spinal cord, intracerebral hemorrhage, aortic dissection, rhabdomyolysis with acute renal and hepatic failure, disseminated intravascular coagulation, convulsions, hyperpyrexia, and respiratory depression. [3]

F. Medical devices

Not applicable.

G. Study questionaires

Not applicable.

H. Description of study subjects and method of recruitment

All research volunteers will already be enrolled in one of several substance abuse investigations under the direction of Drs. Marian Fischman and Richard Foltin of the New York State Psychiatric Institute and Columbia University Department of Psychiatry.

The participants will be 21-45 years of age, and will have passed extensive medical and psychiatric assessment. In addition, an extensive battery of laboratory tests will have been performed including urinalysis, chest radiograph, 12-lead ECG, cardiac stress test, and blood chemistry. Those subjects with abnormal results will not be accepted into and study, and will be referred for treatment.

Cocaine use history is ascertained through several structured telephone and personal interviews with a research nurse, psychologist, and psychiatrist. Dependence on drugs other that cocaine, requesting drug treatment, on parole or probation, or previously convicted of a crime serve as exclusion criteria. Additionally, pregnant women are not accepted for participation.

I. Confidentiality of study data

All study data, subject identification, and records shall remain confidential. Subjects will be identified by protocol ID numbers and all study data will be stored in a secure location accessible only to the investigators. No subject will be identified in any publication unless a release is signed.

J. Location of study

This study will employ the adult inpatient facilities (Harkness 10) affiliated with the Irving Center for Clinical Research at CPMC.

K. Risks and benefits

a. Risks

The major risk of research participation is related to drug administration. Dr. Marian Fischman and colleagues of the New York State Psychiatric Institute have tested more than 300 volunteers during a 7 1/2 year period with intravenous and inhaled cocaine in the doses to be used in this study, and have recorded no adverse effects either immediately after administration or after 2 weeks of study participation. The risk of an idiosyncratic sensitivity to cocaine is considered unlikely since all research subjects are tested for pseudocholinesterase activity prior to acceptance, and every subject has a substantial cocaine use history prior to acceptance in the study.

All of the research subjects participating in the substance abuse studies at CPMC are monitored 24 hours/day during the entire protocol. Emergency medical equipment is available in the research laboratory, which is located in a hospital where a full medical emergency back-up is always available.

b. Benefits

Patients enrolled in the substance abuse investigations conducted by the New York State Psychiatric Institute receive financial reimbursement. Prior to acceptance in these studies, all subjects undergo medical and psychiatric tests. Many of the people volunteering for the substance abuse protocols have had limited contact with the medical profession, and have not had a physical examination in several years. Moreover, all subjects will be offered the option of obtaining help to abstain from drug taking. Subjects requesting treatment prior to the start of the study will be referred to treatment and will not be accepted into the study. Treatment referral is again offered at the conclusion of the study.

L. Alternative therapies

This study involves no experimental therapies.

M. Compensation and costs to subjects

Patients enrolled in the substance abuse investigations conducted by the New York State Psychiatric Institute and Columbia University Department of Psychiatry receive financial reimbursement. There is no additional compensation for participation in this study.

N. Minors and research subjects

This study will not include minors.

O. Radiation or radioactive substances

Not applicable.

P. References

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