Disposition of Cocaine and Norcocaine in Blood and Tissues of B6C3F1 Mice

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Abstract

The biodisposition of cocaine and norcocaine in blood and tissues of immunological importance in B6C3F1 mice following exposure to cocaine or cocaine plus an organophosphate esterase inhibitor, diazinon, is presented. Analysis of specimens was by gas chromatography-mass spectrometry. Results from these studies indicate that pretreatment with diazinon significantly increases cocaine and norcocaine concentrations in the blood, spleen, thymus, and liver. Following acute exposure to cocaine-diazinon, cocaine was found in the spleen and thymus up to 1 hour after exposure. Norcocaine was not detected at this time. Following 7-day exposure to cocaine-diazinon, both cocaine and norcocaine were found in liver, blood, and spleen up to 1 hour after the last exposure; however, only cocaine was detected in the thymus at 1 hour. Cocaine and norcocaine were not detected in any tissues 24 hours after the last exposure.

Introduction

The urinary excretion and cumulative toxicity of cocaine have been extensively studied in animals and in humans, whereas relatively few studies have sought to characterize the biodistribution of cocaine or metabolites of cocaine. Woods et al. (1) studied disposition in the dog and rabbit after administering subcutaneous and intravenous (iv) injections of 20 mg/kg cocaine. Two hours after iv injection, cocaine concentrations were determined to be highest in kidney and spleen, followed by brain, then pancreas, fat, liver, heart, and skeletal muscle. The lowest concentrations were found in the plasma. Cocaine values in the kidney and spleen were approximately 30% higher than those in the brain and nearly threefold higher than those in the liver.

In 1976, Nayak et al. (2) conducted a study of the disposition and biotransformation of cocaine in Wistar rats. Following an acute subcutaneous dose of 20 mg/kg [3H]cocaine, tissues were analyzed at time points from 30 minutes to 24 hours post-injection using a liquid scintillation spectrometer. At 30 minutes post-injection, cocaine concentrations were highest in the spleen (4.34 µg/g wet tissue), followed by heart and brain, 3.67 and 2.24 µg/g, respectively. At 2 hours post-injection, concentrations were as follows: kidney (5.02 µg/g) > lung (4.32 µg/g) > spleen (3.88 µg/g) > fat (3.23 µg/g) > testes (2.99 µg/g) > brain (2.96 µg/g) > intestine (2.87 µg/g) > muscle (1.34 µg/g) > heart (1.25 µg/g) > liver (0.93 µg/g) > plasma (0.45 µg/g). By 12 hours post-injection, values in all tissues were low or barely detectable. A slightly different profile was seen in rats chronically exposed to cocaine (20 mg/kg twice a day for 21 days). At 30 minutes post-injection, concentrations were highest in the spleen, followed by lung and fat. At 2 hours, fat and lung contained the highest concentrations, then kidney and spleen. At 24 hours, cocaine was still detectable, and fat concentrations were nearly threefold higher than kidney and spleen (2).

Although biotransformation and elimination of cocaine in humans have been extensively studied, the disposition of cocaine in humans was not characterized until 1985 when Poklis et al. (3) conducted a case study of a fatal cocaine overdose following intravenous injection. Analysis of cocaine by thin-layer chromatography and gas chromatography using a nitrogen detector resulted in the following tissue concentrations in descending order: kidney = spleen > brain > heart > skeletal muscle > lung > blood > liver > adipose. Concentrations of cocaine in the spleen were found to be three- to fourfold higher than those in blood; these findings are similar to the relative tissue distribution of cocaine reported by Woods et al. (1) as discussed previously. In several other prior studies of cocaine fatalities (4–6), concentrations in blood, kidney, and liver were reported, and these, too, supported the results obtained by Poklis. Together these studies clearly demonstrate that kidney and spleen are the major organs of deposition following iv injection. Liver concentrations appear to be low in relation to other tissues; this may be due to the fact that the liver is the major site of cocaine biotransformation.

At present, studies of cocaine biodisposition have not been conducted in the mouse; yet the mouse is a primary experimental animal for the investigation of cocaine hepatotoxicity and for immunotoxicological studies. As part of an investiga-
tion of the effects of cocaine on immunocompetence, we determined cocaine concentrations in blood and tissues of immunological importance in B6C3F1 mice. As intermediates of norcocaine biotransformation are responsible for cocaine hepatotoxicity in the mice, it was suggested that norcocaine may play a role in the possible suppression of cellular immunological response. Therefore, in order to enhance norcocaine formation from cocaine, we also determined if pretreatment with the esterase inhibitor, diazinon, would significantly increase the disposition of cocaine and norcocaine in tissues.

Materials and Methods

Animals
Female virus-free B6C3F1 mice, 5–7 weeks of age, were purchased from the National Cancer Institute (Bethesda, MD). Upon arrival, the mice were randomized and housed, four per cage, in plastic cages containing sawdust bedding. They were quarantined for 1 week and were not used for experimentation until body weights reached 17–20 g. Mice were given food (Purina Certified Laboratory Chow) and water ad libitum. Animal holding rooms were maintained at 21–24°C and 40–60% relative humidity with a 12-h light–dark schedule.

Drugs and chemicals
Cocaine hydrochloride and norcocaine were provided by the National Institute on Drug Abuse. Diazinon was purchased from Chem Service Co. (West Chester, PA). Potassium phosphate dibasic and n-butylchloride were purchased from Fisher Scientific (Pittsburgh, PA).

Standards
Stock solutions of cocaine (1.0 mg/mL), benzoylecgonine (BE; 1.0 mg/mL), ecgonine methyl ester (EME; 1.0 mg/mL), and norcocaine (NC; 1.0 mg/mL), as well as internal standard stock solutions of cocaine-d<sub>3</sub> (100 pg/mL), were purchased from Radian Corporation (Austin, TX). Working standards of cocaine and NC were made by diluting 1 mL of the stock standard solutions to 100 mL with methanol. These were stored in dark glass bottles in the freezer. According to specifications provided by Radian Corp., these solutions are stable for 1 year.

Dosing regimen
Diazinon stock solutions of 1.5–2 mg/mL were prepared in corn oil in advance, and cocaine stock solutions of 1–3 mg/mL were prepared fresh daily in saline. Mice were pretreated with 0.2 mL corn oil or diazinon (15 mg/kg) via intraperitoneal (ip) injection, and 30 min later, saline or 10, 20, or 30 mg/kg cocaine was administered intraperitoneally in a 0.2-mL volume. This regimen was administered once a day for 7 consecutive days. For acute studies, mice received a single pretreatment of 15 mg/kg diazinon and a single-treatment injection of 30 mg/kg cocaine.

Preparation of tissues and serum for GC–MS analysis
Following either the 7-day or the acute-dosing regimen, mice were anesthetized with CO<sub>2</sub>, and blood was drawn via car-diac puncture with a 1.0-cm<sup>3</sup> syringe (Becton–Dickinson) and placed in a heparin-coated Vacutainer test tube to prevent coagulation (Becton–Dickinson). Mice were then sacrificed by cervical dislocation. Liver, spleen, and thymus were removed aseptically, weighed, and placed in 75- x 12-mm glass Falcon test tubes containing sodium fluoride (1%, w/v) to prevent further drug metabolism. All tissues and blood samples were frozen quickly in a SoLo freezer at –70°C for 30 min, then they were removed and placed in a freezer at –20°C until analyzed.

Liquid–liquid extraction of cocaine and norcocaine for GC–MS analysis
Tissue samples (approximate weight: blood, 1.0 mL; liver, 1.2 g; spleen, 0.20 g; and thymus, 35 mg) were thawed, and homogenates were prepared in 3 mL of 50% potassium phosphate dibasic (pH 11) with a tissue sonicator. Two hundred microliters of cocaine-d<sub>3</sub> internal standard (4 µg/mL) was added to each blood and tissue sample. The following controls were included in each analytical batch: a blank sample (containing no internal standard or working standards); a negative sample (containing only internal standard); calibrators; and a nonextracted sample (containing internal standard and calibrators). Sodium chloride (0.5 g) was added to each sample to reduce emulsion formation, followed by the addition of 4 mL n-butyl chloride. The tubes were capped, inverted, and rotated for 10 min. Tubes were then centrifuged for 10 min at 2500 rpm. After centrifugation, the organic layer was carefully pipetted to a clean tube. The organic extract was then evaporated to dryness using a Savant evaporator with a setting for medium heat, 40°C, for 45 min. Tubes were allowed to cool to room temperature, and then the extract was reconstituted with 50 µL ethyl acetate and transferred with a pipette to 200-µL injection vials for analysis.

Instrumentation and conditions for GC–MS analysis
Cocaine and NC identification and quantitation were performed by use of a Hewlett-Packard (HP) 5890 gas chromatograph (GC) (Palo Alto, CA) with a 5971A mass selective detector (MSD). An HP Chem Station was used to integrate and process data. A 7673 automatic liquid sampler was used to inject samples. The analytical column was an HP-1 capillary column (12 m x 0.2-mm i.d, 0.33-µm film thickness).

The following chromatographic parameters were employed: The injection port was operated in splitless mode at 250°C with a split glass liner (50:1 split-ratio) and the purge valve on for 0.75 min. The oven temperature program was as follows: initial temperature, 130°C (0.1 min), to 165°C at 10°C/min, to 222°C at 35°C/min, to 231°C at 3°C/min, and to 280°C at 35°C/min. The helium flow rate was 1–1.5 mL/min, and the transfer line was 280°C. Data acquisition was performed in positive electron-impact mode with an ion source temperature of 172°C, an analyzer temperature of 180°C, an electron energy of 1000 V relative to the daily autotune voltage, and an emission current of –70 µA. The mass spectrometer was tuned on a daily basis using perfluorotributylamine and the HP Autotune program.

The reference sample elution times were as follows: 7.6 min for cocaine and cocaine-d<sub>3</sub> and 7.5 min for norcocaine. Non-
extracted standards containing BE and EME were analyzed and found not to interfere with cocaine and NC determinations. Quantitation of fragments was carried out in selective ion monitoring mode on the GC-MSD by monitoring mass fragment ions of m/z 303, 182, and 82 for cocaine; m/z 306, 185, and 85 for cocaine-d$_3$; and m/z 289, 168, and 136 for norcocaine. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated by analyzing four samples of each tissue containing internal standard only. The threshold was set to detect any peak in the retention time window, and the mean peak abundance and standard deviation were calculated. The LOD was determined as the mean value ±3 standard deviations, and the LOQ was determined as the mean value ±10 standard deviations. The LODs for cocaine and norcocaine were approximately 1.0 ng/mg, and the LOQs were approximately 3.0 ng/mg for all tissues.

Statistics
The mean plus or minus the standard error was determined for each treatment group, and post hoc analysis by Dunnett's two-tailed t test was performed to determine significant differences between treatment groups and control groups. A value of p<0.05 was considered significant for all statistical tests.

Results
Biodisposition of cocaine and norcocaine following acute exposure to cocaine–diazinon
In a preliminary study to determine the effect of diazinon on blood levels of cocaine, mice were pretreated with 15 mg/kg diazinon prior to a single ip administration of 30 mg/kg cocaine. Analysis of blood 15 min after exposure revealed that cocaine concentrations in mice pretreated with diazinon were elevated 280% greater than those in mice receiving cocaine alone (Table I). Thirty minutes after exposure, cocaine concentrations in the diazinon pretreatment group were 245% greater than those in mice receiving cocaine alone. However, by 30 min post-dose, concentrations in both groups had dropped to approximately one-third of their original values at 15 min.

Cocaine and norcocaine concentrations were determined in the spleen and thymus after acute exposure. In addition to 15- and 30-min time points, tissues were analyzed at 1, 2, and 24 h after acute exposure to cocaine (30 mg/kg)–diazinon (15 mg/kg). Fifteen minutes after exposure to cocaine–diazinon, cocaine concentrations in the spleen were approximately threefold greater than those in mice receiving cocaine alone (Table I). By 30 min, spleen values had declined but were still fivefold greater in diazinon-pretreated mice than in the cocaine group. At 1 h, cocaine was not detected in mice receiving cocaine alone but was still detectable in the cocaine–diazinon treatment group. The same trends were observed in the thymus (Table I); however, cocaine concentrations were markedly lower than those in blood and spleen. The highest concentrations in all tissues were observed in the specimens collected 15 min after exposure to cocaine–diazinon. The blood concentration was the highest, approximately 7300 ng/mL. The highest concentration in the spleen was approximately 2100 ng/mg, whereas the highest thymus value was only 350 ng/mg. Cocaine was not detected in these tissues at time points later than 1 h after exposure. Norcocaine was not detected in blood or tissues at any time.

Biodisposition of cocaine and norcocaine following subchronic exposure to cocaine–diazinon
Following 7-day exposure to cocaine (30 mg/kg/day)–diazinon (15 mg/kg/day), cocaine and norcocaine were determined in blood, liver, spleen, and thymus 1 h and 24 h following the last exposure. A summary of this data is presented in Table II. In the blood, cocaine was detected at 1 h post-exposure in the mice receiving cocaine alone. However, in agreement with the results following acute exposure (Table I), cocaine concentrations were markedly elevated in mice receiving cocaine–diazinon. In the liver, spleen, and thymus, cocaine was detected at the 1-h time point only in mice receiving the diazinon pretreatment. Cocaine concentrations were highest in the liver (7700 ng/mg), followed by blood (465 ng/mL), then spleen (190 ng/mg), then thymus (7 ng/mg). Norcocaine was detected in the blood, liver, and spleen of mice pretreated with diazinon but was not detected in the thymus. Norcocaine concentrations were highest in the liver (approxi-

| Table I. Cocaine Concentrations Following Acute Exposure to Cocaine–Diazinon* |
|-----------------|-----------------|-----------------|-----------------|
| Tissue          | 15 min post-injection | 30 min post-injection | 60 min post-injection |
|                 | cocaine alone     | cocaine-diazinon  | cocaine alone     | cocaine-diazinon  | cocaine alone     | cocaine-diazinon  |
| Blood           | 2559 ± 627        | 7190 ± 1054      | 862 ± 223        | 2118 ± 602       | ND              | ND               |
| Spleen          | 665 ± 44          | 2064 ± 193       | 117 ± 59         | 632 ± 86         | ND              | ND               |
| Thymus          | 165 ± 40          | 355 ± 40         | 71 ± 37          | 147 ± 75         | ND              | 65 ± 10          |

* Mice (n=4) were administered intraperitoneal (ip) diazinon (15 mg/kg) or corn oil 30 min prior to a single ip injection of 30 mg/kg cocaine. Blood was removed either 15 or 30 min after cocaine exposure, and other tissues were removed either 15, 30, or 60 min or 2 or 24 h after cocaine exposure. Cocaine was not detected in the tissues at the 2- or 24-h time points.
* Blood values represent the mean (ng/mL) ± standard error (SE). Spleen and thymus values represent the mean (ng/mg wet tissue) ± SE.
* ND = None detected.
Table II. Cocaine and Norcocaine Concentrations 1 Hour Post-injection Following 7-Day Exposure to Cocaine-Diazinon

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cocaine concentration (ng/mg or ng/mL)</th>
<th>Norcocaine concentration (ng/mg or ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cocaine alone</td>
<td>cocaine-diazinon</td>
</tr>
<tr>
<td>Blood</td>
<td>25 ± 2.2</td>
<td>465 ± 274</td>
</tr>
<tr>
<td>Liver</td>
<td>ND</td>
<td>7648 ± 2175</td>
</tr>
<tr>
<td>Spleen</td>
<td>ND</td>
<td>186 ± 90</td>
</tr>
<tr>
<td>Thymus</td>
<td>ND</td>
<td>7 ± 3</td>
</tr>
</tbody>
</table>

† Mice (n=4) were administered intraperitoneal (ip) diazinon (15 mg/kg) or corn oil 30 min prior to an ip injection of 30 mg/kg cocaine once a day for seven consecutive days. Tissues were removed either 1 or 24 h after the last exposure to cocaine. No cocaine or norcocaine was detected in the tissues at the 24-h time point.

‡ Blood values represent the mean (ng/mL) ± standard error (SE). Liver, spleen, and thymus values represent the mean (ng/mg wet tissue) ± SE.

† ND = None detected.

Mice (n=4) were administered intraperitoneal (ip) diazinon (15 mg/kg) or corn oil 30 min prior to an ip injection of 30 mg/kg cocaine once a day for seven consecutive days. Tissues were removed either 1 or 24 h after the last exposure to cocaine. No cocaine or norcocaine was detected in the tissues at the 24-h time point. Blood values represent the mean (ng/mL) ± standard error (SE). Liver, spleen, and thymus values represent the mean (ng/mg wet tissue) ± SE.

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References


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