Effect of Ketamine on Cocaine-Induced Immunotoxicity in Rats

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The abuse of cocaine (COC) with ketamine (KET) is currently popular among young drug abusers and has been associated with increased risk of human immunodeficiency virus (HIV) infection. The effect of subacute exposure to COC and KET alone and in combination on the immune system was assessed in adult male Sprague-Dawley (SD) rats. To simulate the route and mode of human exposure, rats were treated with COC alone (5 mg/kg, IV), KET alone (100 mg/kg, PO) or KET followed immediately by COC (same doses and routes of administration) once-a-day for 7 consecutive days. Rats were sacrificed 30 minutes following the last treatment. Total circulating leukocyte and lymphocyte counts were decreased with relative neutrophilia, whereas immunoglobulin M (IgM) antibody response to sheep erythrocytes (SRBCs) was increased in animals treated with COC. Moreover, treatment with COC alone increased serum interleukin-10 (IL-10) concentration; however, it did not affect serum interferon gamma (INF-γ) concentration. Spleen histology showed hyperplasia of white pulp whereas thymus gland demonstrated mild cortical degeneration. On the other hand, KET treatment did not produce any significant change of any of these parameters. However, when coadministered with COC, significant reduction of bodyweight, spleen/bodyweight, and thymus/bodyweight ratios with degeneration of splenic white pulp and thymic cortex occurred. Moreover, the primary immunoglobulin response to SRBC and serum IL-10 concentration were decreased without significant change in serum IFN-γ or circulating leukocytic counts. COC caused a significant increase in serum corticosterone concentration that KET effectively prevented. On the other hand, a significant increase in plasma and tissue concentrations of norcocaine (NC) resulted following KET and COC administration in combination. Daily SKF-525A pretreatment at a dose of 30 mg/kg, IP, for 7 days 1 hour prior to KET and COC in combination effectively reversed the effects of this combination on body weight, organ/bodyweight ratios, histopathology, and serum IgM and IL-10 concentrations without affecting leukocytic counts. On the other hand, SKF-525A pretreatment did not change the immunomodulatory effects of COC compared to non-pretreated animals. The results suggest that COC-induced immunomodulation most likely occurred through neuroendocrinal mechanisms. On the other hand, enhanced oxidative metabolism of COC in the presence of KET-induced immunosuppression.

Keywords Cocaine, Corticosterone, Ketamine, Norcocaine, SKF-525A, Th1/Th2

Recently, illicit drugs abused by young adults have changed dramatically with extensive use of so-called “designer or club drugs,” such as 3,4-methylenedioxymethamphetamine (MDMA, ecstasy), ketamine (KET, special K), and gamma hydroxybutyrate (GHB, liquid ecstasy). They are usually used in conjunction with alcohol, marijuana, and cocaine (COC) at “raves or circuit parties” (Rome 2001).

The combination of COC and KET (CK) is currently popular among party goers (Dillmann 1998; Jansen and Darracott-Cankovic 2001). Use of these drugs recently has been correlated with human immunodeficiency virus (HIV) risk in homosexual men as they may facilitate unsafe sexual behaviors by decreasing both anxiety and self-observation, heightening sensory perceptions, euphoria, exaggerating emotional expressiveness, and facilitating communication through social disinhibition (Gorman and Carroll 2000; Mattison et al. 2001). However, no studies have been conducted to determine the consequences of COC and KET coabuse on immune response.

Use of COC alone has been associated with high risk of HIV infection among intravenous COC users (Chaisson et al. 1989; Anthony et al. 1991). Additionally, COC addicts have serum concentrations of antiplatelet immunoglobulin elevated twofold over normal (Borradori et al. 1990); this may explain the high incidence of thrombocytopenic purpura in these subjects (Burdas and Martin 1991). Recently research has revealed that COC exerts a biphasic effect on immune function via indirect mechanisms (Bagasra and Forman 1989; Kump et al. 1998). At high doses (40–60 mg/kg, intraperitoneal [IP]), and with...
esterase inhibitors or cytochrome P-450 inducers, COC causes suppression through the production of reactive intermediates formed through P-450 oxidative metabolism (Holsapple 1995; Jeong et al. 1995a, 1995b, 1996; Kump et al. 1996; Matulka et al. 1996). However, at lower doses (20 to 30 mg/kg, IP), COC potentiates immunoglobulin M (IgM) antibody response to sheep red blood cells (SRBCs), an effect that is mediated through the production of corticosterone via stimulated hypothalamic-pituitary-adrenal (HPA) axis (Stanulis et al. 1997b). Additionally, it has been shown that a T-helper (Th) cell population of lymphocytes is the target for COC effect (Stanulis et al. 1997a; Kump et al. 1998), with COC causing a shift in Th1/Th2 balance in favor of a Th2 response (Stanulis et al. 1997a; Kump et al. 1998).

KET in anesthetic doses has variable effects on the immune system. Markovic and Murusko (1990) reported that KET inhibits natural killer (NK) cell cytotoxicity in mice, but they concluded that the inhibition was due to the state of general anesthesia rather than to the pharmacological properties of the anesthetic. On the other hand, Nishina et al. (1998) studied the inhibitory effects of KET on human neutrophil (polymorphonuclear neutrophil, PMN) functions. At clinically relevant plasma KET concentrations, KET did not impair chemotaxis or reactive oxygen species (ROS) production, but impaired PMN phagocytic activity. However at higher concentrations (10 times clinically relevant plasma concentrations), there was a significant reduction in the chemotaxis, phagocytosis, and ROS production by PMN.

KET appears to suppress the production of the proinflammatory cytokines: tumor necrosis factor alpha (TNF-α) in adult mice (Takenaka et al. 1994), TNF-α and interferon gamma (IFN-γ) in in vitro rat heart cell culture (Hill, Anderson, and Lyden 1998), plasma TNF-α in a rat model (Mastronardi, Yu, and McCann 2001), and interleukin-6 (IL-6) in humans (Royblat et al. 1998). On the other hand, Takaono and colleagues (2002) recently reported that KET did not affect the production of either IL-6 or IL-10 in healthy human volunteers.

KET has been shown to be a cytochrome P-450 inducer (Livingston and Waterman 1978; Marietta et al. 1977). This laboratory has demonstrated that COC administration in KET-pretreated rats was associated with production of plasma norcocaine (NC) (Rofael and Abdel-Rahman 2002a). In addition, Torres, Rivier, and Weiss (1994) reported that KET completely reversed the potent stimulatory effects of COC on adrenocorticotropic hormone (ACTH) secretion, an intermediate step in corticosterone secretion. The antagonism of COC-induced ACTH secretion appears to be related to the antagonist properties of KET on N-methyl-D-aspatate (NMDA) receptors. These receptors have been found to play a significant role in activation of the HPA axis (Damianopoulos and Carey 1995). Therefore, KET may affect the pharmacokinetics and pharmacodynamics of COC.

The objective of this study was to investigate the impact of concurrently administered KET and COC on the immune system in adult rats. Possible mechanisms of COC-mediated immunomodulation and the impact of KET coexposure were investigated. Previously, we investigated the effect of daily exposure for the first 21 days of life to this drug combination on the immune functions in postnatal rats. Due to immaturity of the metabolic enzymes involved in oxidative COC metabolism during the postnatal period, it was not possible to assess the role of NC in the immunomodulatory effect of COC (Rofael, Turkall, and Abdel-Rahman 2003). However, with mature COC-metabolizing enzymes in the adult model used in these studies, the role of metabolism in mediating immunotoxicity of COC was further elucidated by using SKF-525A (CYP 450 inhibitor) as a pretreatment.

**MATERIALS AND METHODS**

**Animals**

Male Sprague-Dawley (SD) rats (225 to 250 g) were obtained from Taconic Farms (Germantown, NY) and quarantined for 1 week prior to use. The rats were housed three per cage for ad libitum to water and Purina Laboratory Chow. The handling of all animals was maintained in accordance with the National Institute of Health (NIH) Guidelines for the Care and Use of Laboratory Animals. The use of animals for this experiment was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Medicine and Dentistry of New Jersey, Newark, NJ.

**Drugs**

COC hydrochloride was provided by the National Institute on Drug Abuse (NIDA), Bethesda, MD. Racemic KET hydrochloride was purchased from Abbott Laboratories (North Chicago, IL). The drugs were dissolved in an appropriate volume of physiological saline prior to administration. SKF-525A and alanine aminotransferase (ALT) reagent kit (ALT 59-UV) were purchased from Sigma Chemical (St. Louis, MO). High-performance liquid chromatography (HPLC) grade solvents were purchased from Aldrich Chemical (Milwaukee, WI).

**Experimental Design**

Adult male SD rats (5/group) were randomly assigned to one of four treatment groups:

- **Group 1**: Saline control.
- **Group 2**: COC hydrochloride (5 mg/kg, intravenous [IV]).
- **Group 3**: KET hydrochloride (100 mg/kg, per os [PO]).
- **Group 4**: KET hydrochloride (100 mg/kg, PO) followed immediately by COC hydrochloride (5 mg/kg, IV).
COC hydrochloride (10 mg/ml) or KET hydrochloride (50 mg/ml) was prepared fresh daily in saline. COC hydrochloride was slowly administered (over 3 minutes) into the lateral tail vein while the animals were restrained utilizing a commercially available Plexiglas device designed for this purpose. Control rats were similarly treated with saline. This regimen was administered once a day for 7 consecutive days. On the 7th day of treatment, rats were euthanized by decapitation at 30 minutes following the last drug administration. Blood, liver, thymus, and spleen were obtained and immediately placed on ice. The dosage of the drugs and duration of exposure were chosen on the basis of previous reports (Di Francesco et al. 1994; Jeong et al. 1995b; Kump et al. 1996; Rafael and Abdel-Rahman 2002a).

In a separate study, adult male rats were pretreated daily for 7 consecutive days with SKF-525A (30 mg/kg, IP) followed 1 hour later by saline, COC alone, KET alone, or KET combined with COC as described above. Rats were euthanized by decapitation 30 minutes after the last treatment. The SKF-525A dose used was selected based on previous reports demonstrating the drug’s effectiveness in blocking the production of ROS due to COC metabolism (Devi and Chan 1996; Labib, Turkall, and Abdel-Rahman 2002).

**Leukocyte Quantitation**

Heparinized blood collected at the time of sacrifice was diluted 1:20 with Turk’s solution (glacial acetic acid, 1 ml; gentian violet, 1% aqueous, 1 ml; distilled water to 100 ml) to lyse red blood cells. Total leukocyte, lymphocyte, and PMN counts were performed using a hemocytometer.

**Measurement of the Primary IgM Antibody Against SRBCs**

Six days prior to the completion of the treatment schedule, rats were immunized via injection of the lateral tail vein with an optimal concentration of SRBCs (i.e., 0.5 ml of 4 x 10⁸ SRBCs/ml sterile saline). SRBCs obtained from a single animal source were purchased from Colorado Serum Company (Denver, CO), stored in Alsever’s solution, and washed three times in sterile saline before use. The same sheep source of SRBCs was used for all experiments. Enzyme-linked immunosorbent assay (ELISA) of serum anti-SRBC IgM was performed as previously detailed by Temple et al. (1993, 1995). Plates were read at λ = 405 nm on a microplate reader Sigma Diagnostics (Englewood, NJ), blanked against wells that had not been exposed to test serum. Mean absorbance at 405 nm (Abs₄₀⁵) was calculated for the duplicate wells of each serum dilution and plotted on the y-axis against five consecutive numbers (1, 2, 3, 4, and 5 on the x-axis). These numbers represent log of serial, two-fold dilutions of serum. A linear equation for the curve was calculated and the Abs₄₀⁵ at the midpoint (3 on the x-axis) determined (OD₅₀) (Robinson et al. 1997).

All ELISA procedures were optimized prior to titration of test sera. This included determination of optimal SRBC membrane protein and secondary antibody concentrations, incubation times, and peak day for collection of serum (i.e., day 6 after immunization).

**Measurement of Serum IFN-γ and IL-10**

To investigate the effect of COC exposure on the Th1/Th2 cell function and the effect of KET coadministration on this response, immunoassay kits (R&D systems, Minneapolis, MN) were used to determine serum concentration of IFN-γ and IL-10 as an indicator of Th1 and Th2 cell function, respectively (Stanulis et al. 1997b; Vandebril et al. 2000).

**Radioimmunoassay Determination of Serum Corticosterone Concentrations**

To address the role of the neuroendocrinal mechanism in COC-induced immunotoxicity and the effect of KET coadministration on this possible mechanism, serum corticosterone concentration following COC, KET, or their combination was measured. The concentration of serum corticosterone 30 minutes after the last treatment was determined by radioimmunoassay kit.

**TABLE 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Spleen/body weight (x 10⁻³)</th>
<th>Thymus/body weight (x 10⁻³)</th>
<th>Liver/body weight (x 10⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>281.2 ± 6.9a</td>
<td>3.2 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td>3.7 ± 0.1</td>
</tr>
<tr>
<td>KET</td>
<td>256.0 ± 7.7c</td>
<td>2.9 ± 0.1</td>
<td>2.2 ± 0.1</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td>COC</td>
<td>264.8 ± 4.1c</td>
<td>3.2 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>3.5 ± 0.2</td>
</tr>
<tr>
<td>COC/KET</td>
<td>218.0 ± 5.7b</td>
<td>2.3 ± 0.1</td>
<td>1.0 ± 0.1b</td>
<td>5.1 ± 0.1</td>
</tr>
</tbody>
</table>

*Note. Rats treated for 7 days with saline, cocaine alone (5 mg/kg, IV), ketamine alone (100 mg/kg, PO), or ketamine followed by cocaine (same doses and routes of administration) and sacrificed 30 minutes after the last treatment.

aValues represent the mean ± SEM for five animals per group. Statistical significance was determined by ANOVA followed by Tukey-Kramer HSD test at (p < .05).

bSignificantly different from control.

cSignificantly different from ketamine and cocaine in combination.
utilizing a radioactive-labeled antigen hormone $^{125}$I-antibody complex (Diagnostic Products, Los Angeles, CA). Peak serum corticosterone level is reached 30 minutes following IV cocaine administration in rats (Torres and Rivier 1992). All experiments were performed with a staggered pattern of dosing allowing serum collection between 11 AM and 1 PM. Serum samples were stored at $-20^\circ$C until analysis.

Analytical Methodology

To characterize the role of KET on the metabolic profile of COC and its impact on immune function, COC, its metabolites (benzoylecgonine [BE] and NC) and KET were quantified in tissues and plasma by reversed-phase HPLC utilizing a method developed in this laboratory, with minimum detectable concentration of 5 ng/ml for COC and 10 ng/ml for BE, NC and KET (Rofael and Abdel-Rahman 2002b).

TABLE 2
Effect of cocaine and/or ketamine exposure on white blood cell count in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leukocytes/ml ($\times 10^6$)</th>
<th>Lymphocytes/ml ($\times 10^6$)</th>
<th>Lymphocytes/leukocytes ($\times 10^2$)</th>
<th>Neutrophils/ml ($\times 10^6$)</th>
<th>Neutrophils/leukocytes ($\times 10^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>8.5 ± 0.6 $^a$</td>
<td>7.2 ± 0.6</td>
<td>84.4 ± 1.2</td>
<td>1.2 ± 0.1</td>
<td>14.4 ± 1.1</td>
</tr>
<tr>
<td>KET</td>
<td>8.2 ± 0.4 $^b$</td>
<td>6.9 ± 0.5 $^c$</td>
<td>84.7 ± 1.5</td>
<td>1.1 ± 0.1</td>
<td>14.0 ± 1.4</td>
</tr>
<tr>
<td>COC</td>
<td>4.2 ± 0.3 $^b$</td>
<td>3.2 ± 0.3 $^b$</td>
<td>74.6 ± 1.9</td>
<td>1.0 ± 0.1</td>
<td>23.0 ± 2.0</td>
</tr>
<tr>
<td>COC/KET</td>
<td>6.8 ± 0.3 $^c$</td>
<td>5.6 ± 0.3 $^c$</td>
<td>82.4 ± 0.8 $^c$</td>
<td>1.1 ± 0.04</td>
<td>16.1 ± 0.8 $^c$</td>
</tr>
</tbody>
</table>

Note. Rats treated for 7 days with saline, cocaine alone (5 mg/kg, IV), ketamine alone (100 mg/kg, PO), or ketamine followed by cocaine (same doses and routes of administration) and sacrificed 30 minutes after the last treatment.

$^a$Values represent the mean ± SEM for five animals per group. Statistical significance was determined by ANOVA followed by Tukey-Kramer HSD test at ($p < .05$).

$^b$Significantly different from control.

$^c$Significantly different from cocaine alone.

Serum Alamine Aminotransferase Assay

The activity of serum ALT was determined using a Sigma Diagnostics ALT test kit (Sigma).

Histopathology

Liver, spleen, and thymus were collected at the time of sacrifice, rinsed in phosphate-buffered saline (pH 7.5), fixed in phosphate-buffered formalin, sliced, embedded in paraffin, and stained by hematoxylin and eosin (H&E) for light microscopy examination.

Statistical Analysis

Results are expressed as mean ± SEM. Multiple comparisons were performed by analysis of variance (ANOVA) and followed by the Tukey-Kramer honestly significant difference (HSD) test. Statistical analysis between two groups was performed by the Student’s independent $t$ test. In all analyses, the level of significance was set to $p < .05$. Statistical analysis was performed

TABLE 3
Effect of cocaine and/or ketamine exposure on serum anti-SRBC IgM and cytokines concentrations in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>OD$_{50}$ ± SEM</th>
<th>IL-10 (pg/ml)</th>
<th>IFN-γ (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>8.3 ± 0.2 $^a$</td>
<td>38.7 ± 1.6</td>
<td>42.9 ± 2.0</td>
</tr>
<tr>
<td>KET</td>
<td>8.3 ± 0.1</td>
<td>34.9 ± 1.6</td>
<td>36.8 ± 2.5</td>
</tr>
<tr>
<td>COC</td>
<td>9.0 ± 0.03 $^{b,c}$</td>
<td>67.6 ± 6.7 $^{b,c}$</td>
<td>37.4 ± 2.5</td>
</tr>
<tr>
<td>COC/KET</td>
<td>6.9 ± 0.2 $^{b,c,d}$</td>
<td>10.1 ± 2.3 $^{b,c,d}$</td>
<td>34.7 ± 3.0</td>
</tr>
</tbody>
</table>

Note. Rats treated for 7 days with saline, cocaine alone (5 mg/kg, IV), ketamine alone (100 mg/kg, PO), or ketamine followed by cocaine (same doses and routes of administration) and sacrificed 30 minutes after the last treatment.

$^a$Values represent the mean ± SEM for five animals per group. Statistical significance was determined by ANOVA followed by Tukey-Kramer HSD test at ($p < .05$).

$^b$Significantly different from control.

$^c$Significantly different from ketamine alone.

$^d$Significantly different from cocaine alone.

TABLE 4
Effect of administration of cocaine and/or ketamine on serum corticosterone concentration in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Corticosterone concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>218.2 ± 25.0 $^a$</td>
</tr>
<tr>
<td>KET</td>
<td>214.2 ± 9.9 $^c$</td>
</tr>
<tr>
<td>COC</td>
<td>639.2 ± 29.6 $^b$</td>
</tr>
<tr>
<td>COC/KET</td>
<td>250.2 ± 19.3 $^c$</td>
</tr>
</tbody>
</table>

Note. Rats treated for 7 days with saline, cocaine alone (5 mg/kg, IV), ketamine alone (100 mg/kg, PO), or ketamine, followed by cocaine (same doses and routes of administration) and sacrificed 30 minutes after the last treatment.

$^a$Values represent the mean ± SEM for five animals per group. Statistical significance was determined by ANOVA followed by Tukey-Kramer HSD test at ($p < .05$).

$^b$Significantly different from control.

$^c$Significantly different from cocaine alone.
TABLE 5
Plasma and tissue distribution of cocaine, its metabolites, and ketamine in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Analyte</th>
<th>Plasma (ng/ml)</th>
<th>Liver (µg/g)</th>
<th>Spleen (µg/g)</th>
<th>Thymus (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COC</td>
<td>COC</td>
<td>240.6 ± 12ab</td>
<td>0.4 ± 0.02</td>
<td>3.7 ± 0.3</td>
<td>0.4 ± 0.01</td>
</tr>
<tr>
<td>COC + KET</td>
<td>KET</td>
<td>106.4 ± 5.6b</td>
<td>0.1 ± 0.01b</td>
<td>1.7 ± 0.1b</td>
<td>0.2 ± 0.02b</td>
</tr>
<tr>
<td>COC</td>
<td>BE</td>
<td>1233.6 ± 46.4</td>
<td>15.1 ± 1.5</td>
<td>3.3 ± 0.3</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>COC + KET</td>
<td>NC</td>
<td>6153.2 ± 122.5b</td>
<td>22.9 ± 2.0b</td>
<td>5.1 ± 0.2b</td>
<td>4.7 ± 0.3b</td>
</tr>
<tr>
<td>COC</td>
<td>NC</td>
<td>68.4 ± 14.2</td>
<td>2.1 ± 0.2</td>
<td>0.1 ± 0.01</td>
<td>0.1 ± 0.02</td>
</tr>
<tr>
<td>COC + KET</td>
<td>NC</td>
<td>215.4 ± 28.5b</td>
<td>4.1 ± 0.1b</td>
<td>0.8 ± 0.01b</td>
<td>0.8 ± 0.04b</td>
</tr>
<tr>
<td>KET</td>
<td>KET</td>
<td>409.8 ± 72.0</td>
<td>1.9 ± 0.3</td>
<td>5.9 ± 1.1</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>KET + COC</td>
<td>COC</td>
<td>373.1 ± 64.6</td>
<td>1.9 ± 0.3</td>
<td>6.6 ± 0.6</td>
<td>2.1 ± 0.4</td>
</tr>
</tbody>
</table>

Note. Male rats were treated with cocaine (5 mg/kg, IV), ketamine (100 mg/kg, PO), or ketamine followed by cocaine (same doses and routes of administration) for 7 days.

*Values are expressed as means ± SEM (N = 5). Animals were sacrificed 30 minutes following the last treatment. Tissue homogenates and plasma samples were analyzed by HPLC for quantification of cocaine, benzoylecgonine, norcocaine, and ketamine.

aSignificantly different from treatment with cocaine in the absence of ketamine using Student’s independent t test (p < .05).

RESULTS

Effect of COC and/or KET Administration on Growth Rate and Lymphoid Organ/Body Weight Ratios

The effect of COC and KET alone or in combination, administered daily for 7 consecutive days on body and organ weights, is shown in Table 1. Concurrent COC and KET exposure caused significant wasting, reduction of spleen and thymus weight/body weight ratios, with significant increase in liver weight/body weight ratio versus the other treatment groups.

Effects on White Blood Cell Counts

The effect of COC and KET on total and differential leukocytic counts is presented in Table 2. Daily COC injections for 7 days significantly decreased the absolute number of total circulating leukocytes and the number of lymphocytes compared to the other treatment groups. Although the absolute number of PMNs per milliliter was not significantly different from the control group, the ratio of PMNs to leukocytes was significantly higher for the COC group compared to the other treatment groups. KET treatment did not affect the absolute nor the relative white blood cell counts; however, when combined with COC, it reversed the decrease in leukocytic counts produced by COC.

Quantification of the Primary IgM Antibody Response to SRBC and Serum Cytokines by ELISA

The effects of daily COC and/or KET exposure for 7 days on serum anti-SRBC IgM and cytokines concentrations are presented in Table 3. A significant increase in the quantity of serum anti-SRBC IgM was observed in COC-treated rats compared to the other treatment groups. KET treatment did not affect the concentration of the primary immune response to SRBCs in rats; however, when administered with COC, it markedly changed the COC activity profile and resulted in a significant suppression of serum IgM concentration versus the other treatments. Similarly, serum IL-10 concentration, a Th2-related cytokine, was significantly higher following treatment with COC compared to the other treatment groups, whereas, following COC and KET in combination, serum IL-10 concentration reduced in a statistically significant manner versus the other treatment groups. On the other hand, the level of the Th1-related cytokine, IFN-γ, was unaffected by any of the treatment regimens.

TABLE 6
Effect of administration of cocaine and/or ketamine on serum alanine aminotransferase (ALT) activity in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALT activity (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>27.2 ± 3.8a</td>
</tr>
<tr>
<td>KET</td>
<td>31.5 ± 4.9b</td>
</tr>
<tr>
<td>COC</td>
<td>55.2 ± 6.8c</td>
</tr>
<tr>
<td>COC/KET</td>
<td>132.3 ± 13.9bc</td>
</tr>
</tbody>
</table>

Note. Rats treated for 7 days with saline, cocaine alone (5 mg/kg, IV), ketamine alone (100 mg/kg, PO), or ketamine followed by cocaine (same doses and routes of administration) and sacrificed 30 minutes after the last treatment.

*Values represent the mean ± SEM for five animals per group. Statistical significance was determined by ANOVA followed by Tukey-Kramer HSD test at (p < .05).

aSignificantly different from saline control.

bSignificantly different from ketamine and cocaine in combination.
significant rise of 2.9-fold in serum corticosterone concentration compared to the other treatment groups (Table 4). Some stress may be involved in the handling and dosing procedure for all the treatment groups. However, COC treatment alone is responsible for an elevation of corticosterone concentration. KET treatment did not produce any effect on serum hormone concentration relative to control. However, when combined with COC, KET significantly abolished the stimulatory effect of COC on serum corticosterone concentration, rendering it slightly higher, but not significantly different, from that of controls.

**FIGURE 1**

Histological examination of liver following cocaine and/or ketamine administration in rats. Livers sections were obtained 30 minutes after the last treatment and were sliced, fixed, and stained with hematoxylin-eosin as described in Materials and Methods (magnification × 250). (A) Liver section from control rats. Livers appeared normal with central vein (CV) and portal tract (PT). (B) Liver section from rats treated with oral ketamine (100 mg/kg) for 7 consecutive days. Liver shows areas of apparent fatty change (arrow). (C) Liver section from rats administered cocaine IV for 7 consecutive days (5 mg/kg). Liver appeared normal except for the presence of infiltrating inflammatory cells and congestion (arrow). (D) Liver sections from rats administered oral ketamine followed by IV cocaine (100/5 mg/kg) daily for 7 days. Note centrilobular necrosis (arrow).
Analytical Findings

The simultaneous presence of KET with COC significantly increased the rate of COC metabolism as manifested by significantly decreased plasma and tissue concentration of COC, and corresponding significant increases in BE and NC (Table 5). Furthermore, the relative increase in concentration of NC in the presence of KET was highest in spleen and thymus (eightfold each), followed by plasma (threelfold), then liver (twofold) compared

**FIGURE 2**
Histological comparison of the paraffin sections of spleens following cocaine and or/ketamine administration in adult male rats. Spleen sections were obtained 30 minutes after the last treatment and were sliced, fixed, and stained with hematoxylin-eosin as described in Materials and Methods (magnification × 50). (A) Normal spleen section from rats administered saline for 7 consecutive days shows germinal centers (G) separated from red pulp (R) by marginal zones (M). (B) Spleen section from rats treated with daily oral ketamine for 7 days (100 mg/kg). Spleen appeared normal with no apparent changes versus control. (C) Spleen section from rats administered cocaine (5 mg/kg, IV) once a day for 7 consecutive days. Note prominent germinal centers and marginal zones. (D) Spleen section from rats treated with ketamine (100 mg/kg, PO) followed immediately by IV cocaine administration (5 mg/kg) given once daily for 7 consecutive days. Note small and reduced numbers of germinal centers and depletion of the marginal zones.
to COC treatment alone. On the other hand, KET concentrations were not significantly altered by COC coadministration.

**Effects on Liver Cell Integrity**

Seven days of concurrent, daily administration of COC and KET significantly increased serum ALT concentrations versus the other treatment groups; ALT was increased by 4.9-fold compared to saline control (Table 6). Although ALT activity following COC administration was twofold higher, it was not significantly different from saline control. KET alone did not change serum ALT activity versus saline control.

![Histopathology of thymuses following cocaine and/or ketamine administration in rats.](image)

**FIGURE 3**

Histopathology of thymuses following cocaine and/or ketamine administration in rats. Adult male rats were administered either saline (A), ketamine (100 mg/kg, PO) (B), cocaine (5 mg/kg, IV) (C), or ketamine followed by cocaine (same doses and routes of administration) (D) as described in Material and Methods. Thymus sections were obtained 30 minutes after the last treatment and were sliced, fixed, and stained with hematoxylin-eosin as described in Materials and Methods (magnification ×50). Note normal architecture of the thymus gland cortex (C) and medulla (M) following saline and ketamine treatments, slight cortical lymphocytic depletion following cocaine treatment, and massive cortical lymphocyte depletion and inverse pattern of lymphocyte density in the cortex and medullary areas following ketamine and cocaine combination treatment.
Histopathological Findings

The livers of rats treated with KET alone showed apparent fatty changes (Figure 1B). Livers of rats treated with 5 mg COC/kg for 7 consecutive days did not reveal any significant microscopic changes versus control (Figure 1A) except for the presence of some leukocyte infiltration and congestion (Figure 1C). On the other hand, livers of rats receiving COC and KET in combination appeared pale and mottled. Microscopically, they showed signs of centrilobular to midzonal hepatic cellular necrosis, with sparing of a few hepatocytes immediately adjacent to the central vein. The hepatocytes appeared varied in size, some enlarged and some partially disintegrated. (Figure 1D).

The white pulp of adult rat spleen consists of two zones: germinal centers, indicative of antigenic challenge surrounded by marginal zones, which separate the white pulp from the red pulp (Figure 2A). Spleens of rats treated with COC manifested expanded white pulp; the germinal centers are prominent together with hyperplasia of the marginal zones (Figure 2C). Spleens of rats treated with KET alone did not show any significant change versus saline control rats (Figure 2B). However, COC and KET in combination resulted in small and reduced
numbers of germinal centers and depletion of marginal zones (Figure 2D).

Thymus gland is composed of two layers; the outer cortical layer, which is densely populated with lymphocytes, whereas the inner medullary core is formed mainly of reticular epithelium (Figure 3A). COC treatment produced slight cortical lymphocytic depletion (Figure 3C). Administration of KET alone did not affect the cellular architecture of the gland (Figure 3B); however, when combined with cocaine, the cortex showed severe lymphocyte depletion and reversed pattern of lymphocyte density, in which a higher density is observed in the medulla (Figure 3D).

Effects of SKF-525A Pretreatment

Pretreatment of rats with SKF-525 A effectively inhibited the effect of COC and KET in combination on bodyweight (Figure 4A), spleen/bodyweight ratio (Figure 4B), thymus/bodyweight ratio (Figure 4C), and liver/bodyweight ratio (Figure 4D); SKF-525A pretreatment values were not significantly different from saline control. On the other hand, SKF-525A did not alter the total nor differential leukocytic counts versus nonpretreated groups (data not shown). Meanwhile, SKF-525A pretreatment significantly reversed the inhibitory effects of COC and KET in combination on serum anti-SRBC IgM (Figure 5) and serum IL-10 concentration (Figure 6), their concentrations approached that of saline control.

Investigating the effects of SKF-525A pretreatment alone on serum corticosterone concentration revealed the absence of any significant difference versus the corresponding nonpretreated groups (data not shown). However, HPLC analysis of COC, its metabolites (BE and NC), and KET in plasma and tissues showed that SKF-525A pretreatment significantly increased the metabolism of COC to BE, whereas NC was not detected (Table 7). KET concentrations also significantly increased in plasma and tissues following SKF-525A pretreatment. Moreover, SKF-525A pretreatment significantly reversed the increase in ALT activity seen following COC treatment alone or COC in combination with KET. The ALT activity in all treatment groups was not significantly different from saline control (Figure 7).

Histopathologically, the liver section from rats pretreated with SKF-525A prior to COC alone or COC and KET demonstrated no necrosis (data not shown); no significant changes

FIGURE 5
Effect of SKF-525A pretreatment on the primary IgM antibody response to sheep erythrocytes in rats exposed to cocaine and/or ketamine. For 7 consecutive days, rats (N = 5/group) were pretreated with SKF-525A (30 mg/kg, IP) or saline, 1 hour prior treatment with to cocaine (5 mg/kg, IV), ketamine (100 mg/kg, PO), or ketamine followed by cocaine (100/5 mg/kg). Rats were immunized with IV 0.5 ml of 4.0 × 10⁸ SRBC/ml saline on day 2 (6 days) prior to sacrifice. Rats were sacrificed 30 minutes after the last treatment. Serum IgM was determined by ELISA. Data are expressed as mean OD₅₀ ± SEM. Statistical significance was determined by ANOVA followed by Tukey-Kramer HSD test at p < .05. a Statistical difference versus columns 1, 3. b Statistical difference versus columns 1, 3, 5. c Statistical difference versus column 7.

FIGURE 6
Effect of SKF-525A pretreatment on serum IL-10 concentration in rats administered cocaine and/or ketamine. Values represent mean serum IL-10 measured by ELISA for five male rats/group ± SEM. For 7 consecutive days, rats were pretreated with SKF-525A (30 mg/kg, IP) or saline, 1 hour prior to treatment with cocaine (5 mg/kg) alone, ketamine (100 mg/kg) alone, or ketamine followed by cocaine (same doses and route of administration) and were sacrificed 30 minutes following the last treatment. Statistical significance was determined by ANOVA followed by Tukey-Kramer HSD test at p < .05. a Statistical difference versus columns 1, 3. b Statistical difference versus columns 1, 3, 5. c Statistical difference versus column 7.
were observed versus the saline controls. Moreover, pretreatment with SKF-525A prior to COC and KET effectively reversed the splenic and thymic histopathological changes (Figures 8D and 9D, respectively). However, SKF-525A pretreatment prior to COC administration did not change the splenic or thymic histopathologic findings versus non-pretreated groups (Figures 8C and 9C, respectively).

**DISCUSSION**

In this model, the COC and KET were administered once a day for 7 days reflecting a typical pattern of binge COC and KET use (Jansen and Darracot-Cankovic 2001; Stanulis et al. 1997b). The results of this study revealed that COC alone induced leukopenia primarily due to lymphocytopenia with relative neutrophilia, splenic white pulp hyperplasia, mild thymic cortical atrophy, increased production of anti- SrBC IgM and Th2 cytokine (IL-10) without any significant change in Th1 cytokine (IFN-γ), body weight, or lymphoid organ/body weight ratios. These results are consistent with previous investigations that have demonstrated that COC produces leukopenia (Ou, Shen, and Luo 1989; Di Francesco et al. 1994), but it is not immune suppressive. Further, the results are consistent with COC enhancement of the T-dependent immune response (Havas et al. 1987; Bagasra and Forman 1989; Stanulis et al. 1997b) and its selective effect on Th2 cells (Stanulis et al. 1997a).

These immunomodulatory effects of COC are most likely due to an increase in corticosterone production. Corticosterone is known to induce lymphocytopenia by causing cells to redistribute to secondary lymphoid organs such as spleen for antigen processing and eventual production of antibodies against antigens (Berny 1974; Mashaly et al. 1998). Moreover, physiological concentrations of corticosterone have been reported to enhance antibody production in mice (Moynihan et al. 1994; Stanulis et al. 1997a), rats (Fleshner et al. 2001), and humans (Tuchinda, Newcomb, and Devald 1972). Furthermore, previous investigators have demonstrated that corticosterone promotes the Th2 cytokine response (Blotta, DeKruyff, and Umetsu 1997; Daynes and Araneo 1989; DeKruyff, Fang, and Umetsu 1998; Ramirez et al. 1996; Stanulis et al. 1997a). Additionally, adrenal corticoids may cause thymus regression (Dorfman and Dorfman, 1961).

On the other hand, the contribution of oxidative metabolism does not appear to explain the observed immunological effects in rats treated with COC alone. Only a small amount of NC was detected in thymic and spleen tissues. The small amount formed in spleen is consistent with the limited metabolic capacity of splenocytes (Yang et al. 1986). However, splenocyte...
suppression is only exhibited with a high concentration of NC (Jeong et al. 1995b). Further, inhibition of oxidative metabolism by SKF-525A pretreatment had no effect on the splenic or thymic histopathological changes produced by COC alone.

Although KET alone did not induce any significant change on the immune parameters studied, when administered with COC in combination, it markedly changed the immunotoxic profile of COC. Rats treated with COC and KET in combination showed marked growth retardation, significant reduction of spleen and thymus weight/body weight ratios, thymic and splenic atrophy, and hepatotoxicity. The immunologic parameters showed significant reduction in serum anti-SRBC IgM and serum IL-10 as well as the reversal of effects of COC on leukocytic counts. Moreover, tissue and plasma NC concentration significantly increased whereas serum corticosterone concentration significantly decreased when KET was coadministered with COC. Administration of SKF-525A (30 mg/kg, IP) 1 hour before COC and KET in combination completely reversed the effect of this combination on body weight, organ/body weight ratios, organ histopathology, antibody response, IL-10 concentration, and plasma and tissue NC concentration without affecting the leukocytic counts and serum corticosterone concentration.

The effect of KET in this drug interaction can be explained, in part, by indirect effect on corticosterone secretion. KET significantly prevented the stimulatory effect of COC on serum corticosterone concentration presumably via blockade of NMDA receptors. This effect is in agreement with Torres, Rivier, and Weiss (1994) and Damianopoulous and Carey (1995) who reported that NMDA receptor stimulation is essential for corticosterone release induced by COC in treatment in rats. Reduction of serum corticosterone concentrations by KET correlates with reversal of COC-induced effects on leukocytic counts, serum IgM, and IL-10 concentrations.

Although corticosterone concentrations in the combination group nearly approached those of saline controls, the result was not lack of immunostimulation, but was immunosuppression. However, in addition to suppressing corticosterone release, KET also has effects on oxidative metabolism. Previously, we have demonstrated that COC administration in KET-pretreated rats was associated with production of plasma NC (Rofael and Abdel-Rahman 2002a). Oxidized COC metabolites may produce hepatotoxicity and immunotoxicity through generation of ROS (Devi and Chen 1996; Holsapple et al. 1993; Labib, Turkall, and Abdel-Rahman 2002). Moreover, free radicals also have been detected during KET metabolism (Reinke et al. 1998). ROS have been reported to be responsible for the immunosuppressant effects of COC on the primary antibody response (Jeong et al. 1995a, 1996; Kump et al. 1996; Matulka et al. 1996). On the other hand, neither COC or BE caused suppression of the primary antibody response to SRBCs (Jeong et al. 1995b). The immunosuppressive effects of ROS on serum anti-SRBC IgM appear to be mediated through direct inhibition of Th2 cells, as there was a significant reduction of serum IL-10 without change in IFN-γ. This is consistent with the results of Malmberg et al. (2001) who reported that IL-10 production is profoundly influenced by ROS compared to IFN-γ. Moreover, ROS have been shown to cause atrophy and depletion of thymic cortex and splenic pulp (Cha et al. 2000; Jeong et al. 1996; Kump et al. 1996). The effect of pretreatment with SKF-525A (cytochrome P-450 inhibitor) in antagonizing the hepatotoxicity and immunotoxicity of COC/KET suggest the importance of ROS generation in mediating the hepatotoxic and immunosuppressive effects of this combination.

The results of this study are in agreement with the findings of other investigators who documented that COC alone is an immunostimulant (Havas et al. 1987; Bagasra and Forman 1989; Stanulis et al. 1997b), but is an immunosuppressant when combined with agents that modify its metabolism, i.e., inhibitors of esterase pathway or inducers of P-450 pathway (Jeong et al. 1995a, 1996; Kump et al. 1996; Matulka et al. 1996). Immunosuppression could ultimately contribute to a greater susceptibility to infections and tumors.
FIGURE 8

Effect of SKF-525A pretreatment on spleens of male rats administered cocaine and/or ketamine. Spleen sections from rats pretreated daily for 7 consecutive days with SKF-525A (30 mg/kg, IP) 1 hour before treatment with saline (A), oral ketamine (100 mg/kg) (B), IV cocaine (5 mg/kg) (C), and ketamine in combination with cocaine (same doses and routes of administrations) (D). Spleen sections were obtained 30 minutes after the last treatment and were sliced, fixed, and stained with hematoxylin-eosin as described in Materials and Methods. Spleen in sections A, B, and, D appeared normal with no apparent changes versus control. In section C, prominent hyperplasia of germinal centers and marginal zones were observed compared to controls.

To summarize, the results of this investigation indicate that COC alone can cause an enhancement of the T-dependent antibody response, and that effect most probably is mediated by release of corticosterone. KET did not show any immunotoxic reactions in this model. However, when combined with COC, KET suppressed the immune response in rats through inhibition of corticosterone release and induction of the cytochrome P-450 pathway, resulting in generation of ROS.
Effect of SKF-525A pretreatment on thymuses of male rats administered cocaine and/or ketamine. Thymus sections from rats pretreated daily for 7 consecutive days with SKF-525A (30 mg/kg, IP) 1 hour before treatment with saline (A), oral ketamine (100 mg/kg) (B), IV cocaine (5 mg/kg) (C), and ketamine in combination with cocaine (same doses and routes of administrations) (D). Thymus sections were obtained 30 minutes after the last treatment and were sliced, fixed, and stained with hematoxylin-eosin as described in Materials and Methods. Thymus in sections A, B, and D appeared normal with no apparent changes versus control. In section C, cortical lymphocytic depletion was observed compared to controls.

REFERENCES


