Cocaine enhances myocarditis induced by encephalomyocarditis virus in murine model

JU-FENG WANG,1 JIELIN ZHANG,2 JIANG-YONG MIN,1 MATTHEW F. SULLIVAN,1 CLYDE S. CRUMPACKER,2 WALTER H. ABELMANN,1 AND JAMES P. MORGAN1

1The Charles A. Dana Research Institute and Harvard-Thorndike Laboratory, Cardiovascular Division, and 2Division of Infectious Diseases, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02215

Received 24 July 2001; accepted in final form 2 November 2001

Wang, Ju-Feng, Jielin Zhang, Jiang-Yong Min, Matthew F. Sullivan, Clyde S. Crumpacker, Walter H. Abelmann, and James P. Morgan. Cocaine enhances myocarditis induced by encephalomyocarditis virus in murine model. Am J Physiol Heart Circ Physiol 282: H956–H963, 2002.—This study was designed to investigate whether cocaine can exacerbate viral myocarditis and increase its incidence. Recent clinical evidence suggests that cocaine abuse increases the incidence of myocarditis. However, it has not been directly demonstrated that cocaine exposure enhances murine myocarditis. BALB/c mice were divided into eight groups: saline control, encephalomyocarditis virus (EMCV), 10 mg/kg cocaine (Coc-10), 30 mg/kg cocaine (Coc-30), 50 mg/kg cocaine (Coc-50), EMCV+Coc-10, EMCV+Coc-30, EMCV+Coc-50. After inoculation with EMCV, the mice were treated daily with either cocaine or saline for 90 days. Mice were euthanized at different days after EMCV inoculation. Mortality was recorded and myocarditis severity was evaluated. The mortality of the myocarditis mice treated with cocaine increased significantly, from 22% (EMCV) to 25.7% (Coc-10+EMCV), 41.4% (Coc-30+EMCV), and 51.4% (Coc-50+EMCV) (P < 0.05), respectively. The incidence and severity of inflammatory cell infiltration and myocardial lesions was higher in infected mice exposed to cocaine. Cocaine administered only before infection did not exacerbate myocarditis. Norepinephrine (NE) assay showed that cocaine exposure significantly increased myocardial NE concentration but this increase was partially inhibited in infected animals. Adrenalectomy abolished the effect of cocaine on mortality. Furthermore, propranolol, a β-blocker, significantly decreased the enhancing effects of cocaine on myocarditis mice. In conclusion, cocaine increases the severity and mortality of viral myocarditis in mice. Increased catecholamines may be a major factor responsible for this effect.

Both lymphocytic and eosinophilic myocarditis have been reported in cocaine abusers (9, 27). However, it has not yet been demonstrated experimentally that cocaine exposure enhances the susceptibility of animals to viral myocarditis.

Myocarditis is a multifaceted process involving viral infection, immune activation, and microvascular spasm (2, 10, 23). It is characterized by myocardial necrosis and inflammation in the acute stage, followed by necrosis, inflammation, myocardial fibrosis, calcification, and cardiac dilatation in the chronic stage. Most of the reported effects of the cardiotoxicity of cocaine are related to its two major pharmacological effects: increased sympathetic output (increased tissue catecholamine concentration) and reduction in sodium transport (local anesthetic effect) (11). Moreover, acute or chronic exposure of experimental animals to cocaine causes microvascular spasm and myocardial cell damage (8, 11, 17). Clinical studies concerning cocaine-related myocarditis, however, are based on autopsy data, and it is not known whether infectious agents are a possible primary or contributory factor. It is also unclear whether the adrenergic action of cocaine is involved in the myocarditic process.

The purpose of this study was to determine whether exposure to cocaine exacerbates the severity or course of myocarditis and whether it increases the pathological evolution of myocarditis toward cardiomyopathy. Furthermore, we attempted to identify possible mechanisms for this occurrence.

METHODS

Virus Preparation and Inoculation of Mice

The M variant of encephalomyocarditis virus (EMCV) (ATCC; Manassas, VA) was used in this study. Viral stock was prepared as described previously (14). Briefly, human amnion (FL) cell monolayers were infected with EMCV and harvested when cytopathic effects were completed. Viral titers were determined by plaque formation on a FL cell monolayer. The viral stock was stored at −70°C until use.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Mice were inoculated intraperitoneally with 140 plaque-forming units (0.1 ml) of EMCV diluted in Eagle’s minimum essential medium.

Experiment 1. A total of 350 4-wk-old BALB/c male mice were obtained from Charles River Laboratories (Wilmington, MA). The day of virus inoculation was defined as day 0. After viral inoculation, the mice were divided randomly into eight groups: saline control (n = 10), 10 mg/kg cocaine (Coc-10) (n = 20), 30 mg/kg cocaine (Coc-30) (n = 20), 50 mg/kg cocaine (Coc-50) (n = 20), EMCV control (70), EMCV+Coc-10 (n = 70), EMCV+Coc-30 (n = 70), and EMCV+Coc-50 (n = 70). Daily intraperitoneal injections of either cocaine or saline were started immediately after virus inoculation and continued for 90 days. Twenty-four hours after the last injection at 3, 7, 14, 21, 35, 60, or 90 days, four animals each of cocaine-treated infection and EMCV control groups were euthanized to determine pathological changes. In cocaine control groups, animals were euthanized only at 35, 60, and 90 days after injection because our previous experiment showed that there were no obvious pathological changes in myocardium treated with cocaine for 4 wk. These times were chosen to coincide with the acute phase (day 3), initial myocardial inflammation (day 7), peak inflammatory cell infiltration (day 14), beginning of fibrosis and dilated cardiomyopathy (day 35), and the chronic phase (day 60 or 90).

Experiment 2. A total of 120 mice were divided into four groups: saline control (n = 10), acute preexposure (5 days) (n = 30), subacute preexposure (15 days) (n = 40), and chronic preexposure (30 days) (n = 40). These mice were preexposed to cocaine (30 mg/kg ip) for 5, 15, and 30 days, respectively. The mice were then inoculated with EMCV. Cocaine exposure was terminated after virus inoculation. Myocardial histopathology was examined at day 7, 14, 21, and 35 in surviving animals.

Experiment 3 (norepinephrine assay). A total of 100 mice were divided into saline control (n = 8), cocaine control (n = 28), EMCV (n = 32), and EMCV+Coc-30 (n = 32) groups.

Daily intraperitoneal injections of either cocaine or saline were started immediately after virus inoculation and continued for 90 days. Mouse hearts were excised at day 7, 21, 60, and 90 after EMCV inoculation, and immediately put into liquid nitrogen. The samples were kept at −80°C until norepinephrine (NE) assay. The tissue was homogenized in 0.8 ml 0.4 M perchloric acid containing 0.15 M EDTA. The homogenate was centrifuged at 15,000 g for 15 min and the supernatant was used for NE assay. NE content was quantitated using norepinephrine-enzyme-linked immunosorbent assay kits (ICN Biomedicals; Aurora, OH).

Experiment 4. A total of 180 mice were anesthetized (ketamine + xylazine; 45 + 5 mg/kg ip) and underwent bilateral adrenalectomy (Adx). A small abdominal midline incision was made. The adrenal gland tissue was then removed. The animals were given drinking water after surgery that contained 1% saline combined with 5% sucrose, which significantly prolongs the survival of adrenalectomized animals (15). After 2-wk recovery from Adx (~38% animals died during the recovery period), animals were divided randomly into four groups: Adx (n = 20), Adx+ Coc-30 (n = 30), Adx+ EMCV (n = 30), and Adx + EMCV+Coc-30 (n = 30). Daily intraperitoneal injections of either cocaine or saline were started immediately after virus inoculation. Myocardial histopathology was examined at day 7, 14, 21, and 35 in surviving animals.

Experiment 5. A total of 200 mice were divided into five groups: 3 mg/kg propranolol (Prop) (n = 20), EMCV (n = 30), EMCV+Coc-30 (n = 50), EMCV+Prop (n = 50), and EMCV+Coc-30+Prop (n = 50). Daily intraperitoneal injections of either cocaine or saline were started immediately after virus inoculation. Myocardial histopathology was examined at day 7, 14, 21, and 35 in four animals each. All animal experiments were performed in accordance with National Institutes of Health guidelines. Protocols were approved by the Animal Care and Use Committees of Beth...
Israel Deaconess Medical Center and Harvard Medical School.

Histopathology

For the pathological studies, animals (n = 4–6) were selected randomly and at different days, without bias with regard to the physical status of the animals selected for death. Apical transverse sections of the left ventricle were fixed in 10% formalin and embedded in paraffin, cut into 3-μm-thick sections, and stained with hematoxylin and eosin. Several sections of each heart were scored blindly. For each myocardial sample (dead mice did not undergo necropsy), histological evidence of myocarditis and inflammation was classified in terms of the degree of cellular infiltration and myocardial cell necrosis and graded on a five-point scale ranging from 0 to 4+ (14).

Virus Detection in Murine Hearts

Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to determine the presence of virus in the murine heart. Mice from saline control, EMCV, and EMCV+Coc-30 groups were euthanized at day 7 after viral inoculation. The heart tissues were frozen in liquid nitrogen and kept at −80°C until virus detection. The frozen samples (5–10 mg) were homogenized in 500 μl of RNAzol B buffer. The total cellular RNA was extracted by following manufacturer instructions. The cDNA was synthesized as previously described (12). For PCR amplification, the following reagents were added to 50 μl of reaction volumes: 5 μl of cDNA, 25 mM MgCl₂, 2.5 mM dNTPs, 1 μM of each primer, and 1.25 U of Taq DNA polymerase. The EMCV-specific primers were synthesized from the published sequences (12), and α-tubulin primers were synthesized from sequences of mouse α-tubulin gene (5’ GCCCGGCAGTGTTCGTAGACCT; 5’ CAAGAAGCCCTGGAGACCTGTGC). The amplification conditions were as follows: 1) the sample was heated for 5 min at 95°C, 30 cycles of denaturation at 94°C for 0.5 min, 2) annealed at 55°C for 0.5 min, and 3) extended at 72°C for 1 min. Final extension was at 72°C for 7 min. The PCR products were examined on 2.5% agarose gel.

Statistical Analysis

Results are presented as means ± SE. Survival of mice was analyzed by the Kaplan-Meier methods. Comparison of...
within and between groups was performed using one-way analysis of variance. A $P$ value $<0.05$ was considered significant.

**RESULTS**

**Experiment 1**

Three days after virus inoculation, the mice appeared ill, and some developed coat ruffling, weakness, and irritability. RT-PCR results showed the presence of EMCV RNA in the mouse hearts 7 days after inoculation (Fig. 1). The mortality of the virus control group was 22%. Infection with EMCV produced pathological changes similar to those reported previously (14). Cocaine alone did not produce death in any of the three doses studied.

After exposure to cocaine, mortality of the infected mice significantly increased ($P < 0.05$ compared with the untreated group). The total survival rate (Fig. 2) was 78% for the virus group, 74.3% for EMCV+Coc-10, 58.6% for EMCV+Coc-30, and 48.6% for EMCV+Coc-50. As the dose of cocaine was increased, the mortality of infected mice increased significantly ($P < 0.05$). Moreover, in the EMCV+Coc-50 group, death occurred earlier than in the other groups.

On day 3, few scattered foci of myocyte necrosis associated with inflammatory cells were noted in infected mice. On day 7, 14, and 35, myocyte necrosis and accompanying inflammation had become extensive, confluent in some areas, and multifocal in others (Fig. 3). On day 60, there was obvious cavity dilatation and a decrease in wall thickness. In cocaine-treated groups, the pathological changes were exacerbated in both acute and subacute phases of myocarditis. During the chronic phase of myocarditis, fibroblastic and vascular proliferation was noted in regions of necrotic, focally calcified myocytes. On day 35 and later, the major histological findings were multifocal fibroblastic and vascular proliferation in the myocardium associated with necrotic calcified myocytes. Left ventricular dilatation was observed in the EMCV+Coc-30 group. On day 60, in addition to clear cavity dilatation, hearts showed a decrease in ventricular wall thickness. On day 90, the pathological changes were similar to day 60. These results showed that cocaine exposure exacerbated the course and severity of myocarditis. In the subacute phase, the histopathological scores for necrosis and inflammatory cell infiltration were significantly higher than those of the virus control. In the chronic phase, the scores for inflammatory cell infiltration and necrosis decreased in both groups; however, the percentage of mice with cavity dilatation increased (Fig. 4) ($P < 0.05$). There were no significant pathological findings in the myocardium of mice treated with cocaine alone.

**Experiment 2**

Acute (5 day), subacute (15 day), and chronic (30 day) preexposure to cocaine did not increase the mortality (Fig. 5) or exacerbate the severity of myocarditis. There were no marked differences in pathological changes between the preexposure and saline control mice (data not shown).

**Experiment 3**

Exposure to cocaine markedly increased NE concentrations in normal mouse hearts (Fig. 6). Over 100%
increase in the content of NE was seen after 7 days administration compared with saline controls \((P < 0.05)\). NE reached its peak concentration during the early period of cocaine administration. As administration of cocaine continued, the NE content decreased but was still significantly higher than in the saline controls. In the EMCV group without cocaine, NE concentration was significantly lower than in saline control animals \((P < 0.05)\). Cocaine exposure, however, significantly elevated the content of NE in EMCV mouse hearts \((P < 0.05\) compared with EMCV control).

**Experiment 4**

After adrenalectomy, the effects of cocaine on increasing mortality were abolished (Fig. 7). These data indicate that removal of the adrenal gland, a major source of catecholamines in mice, ameliorated the toxicity of cocaine in the EMCV group.

**Experiment 5**

Figure 8 shows that propranolol significantly decreased the mortality of myocarditis mice \((P < 0.05\) compared with EMCV control), and also attenuated the enhancing effect of cocaine on viral myocarditis \((P < 0.05\) compared with EMCV+Coc-30 group). The necrosis and inflammatory cell infiltration were less severe than in the EMCV group (data not shown). These data indicate that \(\beta\)-adrenergic blockade depressed the toxic effects of cocaine on myocarditis mice.

It should be noted that there was some variability in the survival of infected control mice from experiment to experiment. This variability may be a function of a decrease in the titration of virus in the frozen aliquots as the time spent in the freezer increased. Each experiment, however, had its own controls to normalize this variability.

**DISCUSSION**

This study provides direct evidence of the following: 1) exposure to cocaine significantly increased the mortality of viral myocarditis in mice and exacerbated the severity and time course of viral myocarditis, 2) acute or chronic preexposure to cocaine did not affect the mortality or degree of myocardial damage of viral myocarditis, 3) cocaine treatment significantly increased myocardial NE concentration, 4) adrenalectomy abolished the enhancing effects of cocaine on viral myocarditis, and 5) treatment with a \(\beta\)-blocker decreased the effects of cocaine on viral myocarditis mice.

![Fig. 5. Survival in myocarditis mice induced by virus (V) in experiment 2. Mice were preexposed to cocaine (30 mg/kg) for 5, 15, and 30 days, respectively. Mice were then inoculated with virus. 5-day, preexposure 5 days; 15-day, preexposure 15 days; 30-day, preexposure 30 days. Each group consisted of 30 mice. There was no significant effect of cocaine pretreatment.](image1)

![Fig. 6. Myocardial concentration of norepinephrine (NE). Mice were infected with virus and treated with cocaine 30 mg/kg. Cocaine significantly increased the concentration of NE both in normal and infected animals \((n = 6)\). In infected animals, NE levels remained significantly below saline control, whereas infected animals given cocaine exhibited increased levels of NE. \#\(P < 0.05\), compared with saline control; \*\(P < 0.05\), compared with infected group.](image2)
Present understanding of the pathology of myocarditis suggests that a three-step process occurs (10). The process begins with an acute phase 0–3 days after virus infection. There is an initial viral infection of genetically susceptible host myocytes that can lead to early pathological evidence of myocardial damage. After viral invasion of the myocardium, cell-mediated immunity activates the subacute phase of myocardial and endothelial damage. Finally, a chronic phase is characterized by a slow loss of myocytes and by myocardial fibrosis and progression to dilated cardiomyopathy.

Our data in experiment 1 indicate that exposure to cocaine did not exacerbate the acute phase of myocarditis. Histopathological data demonstrated that there were no differences in the degree of myocardial damage between EMCV and EMCV+Coc mice. However, cocaine had a potent effect on the second and third phases of myocarditis. During these periods, cocaine increased the mortality of viral myocarditis. Also, infiltration by inflammatory cells and myocardial necrosis were greater than in the EMCV control group.

In experiment 2, we asked whether the enhancing effects of cocaine were dependent on its administration simultaneous with and subsequent to viral inoculation. Our data indicate that neither acute nor chronic pre-exposure to cocaine exacerbated the time course and severity of myocarditis or increased its mortality. Although we pretreated the mice with cocaine for 5, 15, and 30 days, respectively, the mortality and degree of myocardial necrosis were not significantly different after virus infection. However, the cardiotoxicity of cocaine has been demonstrated after both acute and chronic administration (11). This suggests that the enhancing effect of cocaine on viral myocarditis depends on simultaneous and continuous exposure to cocaine.

It is well known that cocaine blocks the reuptake of catecholamines at the presynaptic level in the central and peripheral nervous systems and increases the release of catecholamines from both central and peripheral stores (11). Also, chronic administration of cocaine in the rat has been shown to significantly increase the concentration of NE in the left ventricle, and NE can cause myocyte necrosis with accompanying inflammatory infiltration (18, 24). Therefore, the cocaine effects observed might be secondary to increased myocardial catecholamines. Indeed, as found in experiment 3, cocaine exposure increased the cardiac concentration of catecholamines. Maintaining higher levels of catecholamines may enhance the cytotoxicity of the virus by inducing vessel spasm and myocardial ischemia, which may reduce a structural barrier to cellular penetration of the virus.

Virmani and co-workers (27) reported a 20% incidence of myocarditis in autopsies of 40 patients who had used cocaine. In addition, Isner and Chokshi (8) reported eosinophilic myocarditis associated with cocaine abuse. Eosinophilic myocarditis suggests a hypersensitivity myocarditis, which has been reported to
occur as an adverse reaction to various drugs (8, 17). Most recently, and when the present study was completed, Sepulveda and co-workers (21) reported that cocaine injection exacerbated myocarditis induced by Coxsackie virus group B and attributed this effect to the immunosuppressive effects of cocaine.

**Experiment 3** reviewed that NE levels in the myocardium of myocarditis mice were lower than in control mice (Fig. 6), consistent with observations that tissue stores of catecholamines are depleted in cardiac dysfunction and heart failure (3, 4). The NE concentration was significantly increased after exposure to cocaine, with or without viral infection. We conclude that elevation of catecholamines by administration of cocaine may be a significant cause of the exacerbation of its cardiotoxicity.

The adrenal gland is a major source of catecholamines in the mouse (5, 7, 11). When the adrenal glands were removed, the myocardial catecholamine stores decreased. Chiueh and Kopin (5) reported that cocaine caused a significant release of norepinephrine and epinephrine from the rat adrenal medulla. Removal of the adrenal gland, therefore, should decrease the catecholamines stores. In **experiment 4**, we found that when adrenal glands were removed bilaterally, the mortality and myocardial necrosis and inflammatory cell infiltration were not significantly different in cocaine-treated versus untreated myocarditis mice. These results indicate that the adrenal source of catecholamines may be necessary for cocaine to exacerbate the time course and severity of viral myocarditis in our animal model.

On the basis of these results, we conclude that increased levels of catecholamines may be a major mechanism of the cocaine effect. It should be noted, however, that adrenalectomy will also cause a decrease of steroids, which could affect the pathophysiology of myocarditis. Whether steroids modulate the effect of cocaine on myocarditis needs to be explored in further experiments.

Tominaga and co-workers (25) reported that the β-blocker carteol showed beneficial effects on the dilated cardiomyopathy induced by EMCV, but had no effect on the early stage of myocarditis. However, Rezkalla and co-workers (20), working with Coxsackie virus B3, found that metoprolol did not benefit infected mice, but increased the mortality. Generally, the suggested mechanisms of the effects of β-blockers on the heart include 1) decreased myocardial energy demand, 2) improved diastolic relaxation, 3) inhibition of sympathetically mediated vasoconstriction, and 4) protection of myocytes against the direct toxic effects of catecholamines. Increased tissue and serum concentration of catecholamines is one of the major effects of cocaine. Our data in **experiment 5** demonstrates that treatment with the β-blocker propranolol (Fig. 8) not only significantly decreased the effects of cocaine on mortality of myocarditis mice but also showed beneficial effects on the control myocarditis animals. Therefore, at least part of the ameliorating effects of β-blockade on the cocaine-treated infected mice may be attributed to its protective action on heart failure.

**Viral myocarditis** is a complicated pathological process involving multiple factors. Our previous study (28) demonstrated that tumor necrosis factor-α increased in mice after 7 or 14 days of cocaine treatment. Therefore, increased tumor necrosis factor-α levels may also be a possible contributing factor to the toxic effect of cocaine on the subacute phase of viral myocarditis.

Our hypothesis that catecholamines may exacerbate viral myocarditis is supported by evidence from several different sources. The most direct evidence arises from carefully controlled studies (16, 26) of murine myocarditis indicating that hyper-catecholaminergic states and sympathomimetic drugs can cause or significantly exacerbate myocarditis. Moreover, sympatholytic agents and states may ameliorate the manifestations of myocarditis and decrease mortality, although this effect is controversial (20, 25). In observations on effects of commonly abused substances such as nicotine, caffeine, and marijuana among the human immunodeficiency virus (HIV)-infected and general populations, only cocaine has been associated with an increased incidence of cases of myocarditis (8, 11, 19). This suggests that the unique sympathomimetic properties of cocaine, which are not shared with these other agents, may contribute to the cause of myocarditis. In the clinical arena, it has been accepted practice for many years to restrict the activities of patients with myocarditis, based on experimental studies that exercise exacerbates the disease (1).

The results of the present study may have implications for the drug abusing population infected with HIV. A growing amount of evidence indicates that cocaine abusers have an increased incidence of HIV infection and HIV-related myocarditis (6, 13). HIV associated myocarditis may be due to HIV itself or related to other viruses such as Coxsackie virus B or adenovirus, which are exacerbated due to the immune deficiency of HIV infection. Our data in an animal model may have implications for the management of viral myocarditis in humans. This study raises the possibility that treatment of HIV-infected patients with a β-blocker might not only prevent or ameliorate the development of myocarditis but also that of cardiomyopathy, which has been reported to reach 10–18% in some series (22). Our data also suggests that it is reasonable to caution patients with myocarditis against the use of cocaine or other sympathomimetic substances.

This work was supported by National Institutes of Health Grant R01 DA12774 (to J. P. Morgan).

**REFERENCES**


