Differential Patterns of Cocaine-Induced Organ Toxicity in Murine Heart versus Liver

JU-FENG WANG,* XIANGLIN REN,† JOSEPH DEANGELIS,* JIANGYONG MIN,* YUFENG ZHANG,* THOMAS G. HAMPTON,* IVO AMENDE,* AND JAMES P. MORGAN*,[1]

*The Charles A. Dana Research Institute and Harvard-Thorndike Laboratory, *Cardiovascular Division, and †Division of Experimental Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02215*

To determine cocaine's toxicity in different organs, BALB/c mice were intraperitoneally injected daily for 15 days with either saline or cocaine: 10 mg/kg, 30 mg/kg, or 60 mg/kg. Cardiac function, hepatic pathophysiology, heart and liver apoptosis, and tumor necrosis factor (TNF-α) levels were analyzed. After administration of cocaine, cardiac function decreased. Inflammatory cell infiltration and eosinophilic contraction bands were visible in the hearts of mice treated with 60mg/kg cocaine. Moreover, histopathology demonstrated that cocaine caused hepatic necrosis. TdT-mediated dUTP nick end-labeling (TUNEL) staining and DNA ladder analysis indicated that cocaine caused apoptosis in both the heart and liver. Moreover, immunoassay showed that TNF-α levels significantly increased in the heart and liver with cocaine administration. However, our RT-PCR study showed that there was no significant difference in either the heart or liver in the levels of mRNA for TNF-α between cocaine-treated and saline control mice. The present study demonstrated that cocaine is toxic to multiple organs, and at low dose can induce hepatic damage without gross pathological injury to the heart. The results suggest that the liver is more sensitive than the heart to cocaine toxicity, and induction of apoptosis or TNF-α elevation may be a common mechanism responsible for cocaines toxicity.

[E.B.M. 2001, Vol 226:52–60]

Cocaine abuse presents a significant health hazard in the United States. In recent decades, many investigators have studied the specific organ toxicity caused by cocaine including injury to the central nervous system (CNS), the cardiovascular system, the neuromuscular system, and the liver. Potentially life-threatening toxicity to the cardiovascular system may manifest as myocardial ischemia and infarction, cardiac arrhythmia, myocarditis, or dilated cardiomyopathy (1–3). However, in addition to cardiotoxicity, cocaine can produce serious hepatotoxicity (4–6). Theoretically, cocaine has two primary pharmacological properties that produce toxicity in the cardiovascular system: inhibition of presynaptic catecholamine reuptake and a local anesthetic effect (1). However, with regard to hepatotoxicity, numerous studies have demonstrated that there are two additional mechanisms involved. One possible mechanism by which cocaine could elicit hepatotoxicity is through the formation of a metabolite that is capable of alkylating cellular macromolecules (5, 6); a second mechanism involves the mediation of cellular damage by reactive oxygen species as cocaine is metabolized to norcocaine nitroxide, a free radical formed in the liver (7, 8). Those studies suggested that the mechanisms of cocaine's toxicity might be different between various organs, such as heart and liver. However, the mechanisms underlying the toxicity of cocaine remains incompletely understood, especially whether there is a common mechanism accounting for cocaine's toxicity in heart and liver or other organs, and which organ may be more sensitive to cocaine's adverse effects.

In recent studies, apoptosis has been implicated as a fundamental pathogenic mechanism in a variety of human diseases. Excessive programmed cell death may cause cellular atrophy and organ failure. In addition, several studies demonstrated that apoptotic cell death plays a critical role in a variety of cardiovascular and hepatic diseases (9–14). Furthermore, clinical and laboratory evidence indicate that tumor necrosis factor (TNF) is an important mediator of cardiovascular and hepatic diseases (15, 16). Recent studies suggested that myocardial TNF is an autocrine contributor to myocardial dysfunction and cardiomyocyte death and may induce apoptosis (15). We therefore hypothesized that cocaine abuse may initiate apoptosis or stimulate the production of TNF-α and, consequently, programmed cell death, or TNF-α induction may be a common mechanism responsible for cocaine's toxicity in the heart and liver.

Accordingly, the purpose of the present study was to investigate the effects of cocaine on myocardium and liver.

Partial support of this study was provided by NIH RO1 DA11762–01.

[1] To whom requests for reprints should be addressed at the Cardiovascular Division, Beth Israel Deaconess Medical Center, Harvard Medical School, 330 Brookline Avenue, Boston, MA 02215. E-mail:jmorgan@caregroup.harvard.edu

Received May 9, 2000.
Accepted August 22, 2000.

0037-9727/01/2261-0000$15.00/0

We first defined the dose range of cocaine in the BALB/c mouse, then observed the cardiac and liver changes after exposure to cocaine.

Materials and Methods

Animals. Male BALB/c mice weighing 20–25 g were divided into four groups: 10 mg/kg, 30 mg/kg, 60 mg/kg of cocaine, and saline control. The mice were treated daily with cocaine intraperitoneally (ip) for 15 days. Twenty-four hours after the last dose, mice were anesthetized with ketamine and xylazine (45 mg/kg + 5 mg/kg, ip), then cardiac function was measured and blood was collected through the inferior vena cava to prepare serum. At the end of the experiment, mouse hearts and livers were excised and embedded in tissue-freezing medium (Triangle Biomedical Sciences, Durham, NC) and frozen in liquid nitrogen. Then, the hearts and livers were cryostat-sectioned to 5-μm thickness. Those sections were kept at 80°C until histopathologic examination or TNF-α assay.

All animal experiments were performed in accordance with the National Institutes of Health guidelines. Protocols were approved by the Animal Care and Use Committee of Beth Israel Deaconess Medical Center and Harvard Medical School.

Measurements of Cardiac Function. Mice were placed on a thermally controlled operating plank in a supine position. A 4–0 silk suture was placed behind the front lower incisors and pulled taut to slightly extend the neck. The right carotid artery was cannulated with a 1.4F Millar MIKRO-TIP transducer (model SPR-671, Millar Instruments, Houston, TX). To assess myocardial performance, the tip of the transducer was carefully advanced from the right carotid artery through the ascending aorta and into the left ventricle. After 10 min of equilibration, cardiac function was measured. At the end of each experiment, the Millar transducer was withdrawn immediately from the carotid artery and zeroed in 37°C saline. The pressure signals from the Millar transducer were amplified with a Gould amplifier model 2107–4490–00 (Cleveland, OH). Analog signals were digitized using a Data Translation Series (Model DI-220) analog-digital converter and then analyzed and stored on a Windaq data-acquisition system (Data Instruments Inc., Akron, OH). Raw data for the pressure signals were acquired at a sampling rate of 2500 samples/sec/channel, and calculations of the first derivative of the ventricular pressure wave (dP/dt) were made by the Windaq program. Left ventricular systolic pressure (LVP), and left ventricular end-diastolic pressure (LVEDP) were all measured directly from the pressure waveforms.

Assay for Serum Alanine Aminotransferase (ALT) and Alkaline Phosphatase (AP) Level. ALT and AP activities were measured with commercially available diagnostic kits purchased from Sigma Company (St. Louis, MO). Detection of ALT and AP activity was performed on an ultraviolet/visible scanning spectrophotometer (Beckman DU-640, Beckman Instrument, Inc., Fullerton, CA). ALT activity was expressed as Sigma-Frank Units/ml. AP activity was expressed as U/l.

Histopathology. Sections of hearts and livers were fixed for 10 min in methanol, and then stained with hematoxylin for 1min. The slides were immersed in 70% acid-alcohol for ≈2 sec and then rinsed with water. The sections were placed in 0.02% ammonium solution for 5 min and then stained with eosin. The myocardial and hepatic pathology was characterized by light microscopy.

***In Situ* Cell Death Detection.** The sections of heart and liver tissue were fixed with 4% paraformaldehyde for 20 min at room temperature and then washed for 30 min with phosphate-buffer solution (PBS). The sections were incubated in permeabilization solution for 2 min on ice, then rinsed twice with PBS. A 50-μl TdT-mediated dUTP nick end-labeling (TUNEL) reaction mixture (*In situ* cell death detection kit, Boehringer Mannheim, Indianapolis, IN) was added to the samples. The sections were then incubated in the dark in a humidified chamber for 60 min at 37°C. PBS was used to rinse the sections three times. Then, the sections were stained with Hoechst 33258 (0.05 μg/ml) for 15 min at room temperature and rinsed twice with PBS. The sections were analyzed by a fluorescent microscope (Axioplan 2, Zeiss and Kodak DCS 460c Digital Camera, Eastman Kodak Co., Rochester, NY). The number of TUNEL-positive cell nuclei in each section was counted with a Documentation & Analysis System, AlphaImager 2000 (Alpha Innotech Corporation, CA). The total numbers of cell nuclei were counted in the same sections and area as TUNEL-staining with Hoechst 33258 stain. Apoptosis was expressed as a percentage of the TUNEL-positive nuclei versus the number of Hoechst 33258 positive nuclei.

DNA Isolation and Electrophoresis. Each heart and liver was removed and rinsed quickly in ice-cold PBS. The tissues were ground with a mortar and pestle in liquid nitrogen and digested overnight at 37°C in 1 ml lysis buffer containing 10 m*M* Tris [pH 8.0], 100 m*M* NaCl, 25 m*M* EDTA, 0.5% SDS, and 1.0 mg/ml proteinase K (Sigma Chemical Co., St Louis, MO). Protein was removed by precipitation in NaCl at a final concentration of 1.2 *M* followed by centrifugation at 10,000*g* for 30 min. After extraction of the supernatant with phenol-chloroform, genomic DNA was precipitated by isopropanol and resuspended in 10 mM Tris (pH 8.0) and 1.0 m*M* EDTA. After treatment with RNase A for 30 min at 37°C, equal quantities of each sample (10 μg) were subjected to electrophoresis on 1.8% agarose gels containing 0.5 μg/ml ethidium bromide. One kb DNA was used as a marker (Gibco BRL,Gaithersburg, MD).

TNF-α Assay. *ELISA immunoassay.* The hearts and livers were homogenized with PBS buffer. The homogenate was centrifuged at 13,000*g* for 30 min at 4°C. The supernatant was taken to measure TNF-α concentration using a TNF-α immunoassay kit (R & D System, Minneapolis, MN).

RT-PCR. Reverse transcriptase polymerase chain reaction (RT-PCR) was used to measure the mRNA of TNF-α. Total RNA (3 μg) extracted from hearts and livers with Tri Reagent (Sigma), was reverse transcribed with 100 U of Superscript II in 20 μl reaction volume. First, total RNA was mixed with 0.5 μg Oligo$(dT)_{12-18}$ primers. The mixture was heated to 70°C for 10 min and quickly chilled on ice. Then, 100 U of Superscript II, 40 U of RNasin RNase inhibitor, 0.75 m*M* of dNTPs, and 10 m*M* DTT were added. The reaction was carried out in a buffer containing 50 m*M* Tris-HCl, pH 8.3, 75 m*M* KCl, and 3 m*M* $MgCl_2$ at 42°C, for 60 min. The reverse transcriptase was then inactivated by heating at 70°C for 10 min. Three μl of single-stranded cDNA were amplified for 30 cycles (94°C for 30 sec, 55°C for 30 sec, and 72°C for 90 sec) in a solution containing 0.2m*M* dNTPs, 0.5 μ*M* of TNF-α primers (forward primer 157–177: 5′-ATGAGCACA GAAAGCATGATC-3′, reverse primer 864–844: 5′-TCACAGAGCAATGACTC-CAAA-3′), 1.5 m*M* $MgCl_2$, 10 m*M* Tris-HCl, 50 m*M* KCl, and 1 U of Tag DNA polymerase (Sigma). Amplification of the glyceraldehyde-3-phosphate dehydrogenase (G3PDH) gene was used as an internal control.

Data Analysis. All data are presented as mean ± SEM. A one-way analysis of variance (ANOVA) was performed to assess significant differences between the groups. A *P*-value of less than 0.05 was considered to be significant.

Results

Effects of Cocaine on Cardiac Function. To evaluate cardiac function in cocaine-treated mice, ventricular variables were measured in all groups. Figure 1 reveals the effects of cocaine on LVP, LVEDP, and dP/dt. After cocaine injection, LVP decreased from 106.4 ± 3.9 mmHg in saline control to 93.2 ± 2.8, 89.5 ± 4.2, and 86.9 ± 7.6 mmHg, respectively, in the 10-, 30-, and 60-mg/kg cocaine groups ($P < 0.05$ compared with saline control). Furthermore, after exposure to cocaine,+dP/dt decreased ≈12.4%–18.3% among the mice receiving three doses of cocaine. Also, ventricular diastolic function was depressed after administration of cocaine.

Biochemical Analysis. Figure 2 summarizes the effects of cocaine on liver toxicity based on serum ALT and AP activities. Serum ALT and AP activities in mice injected

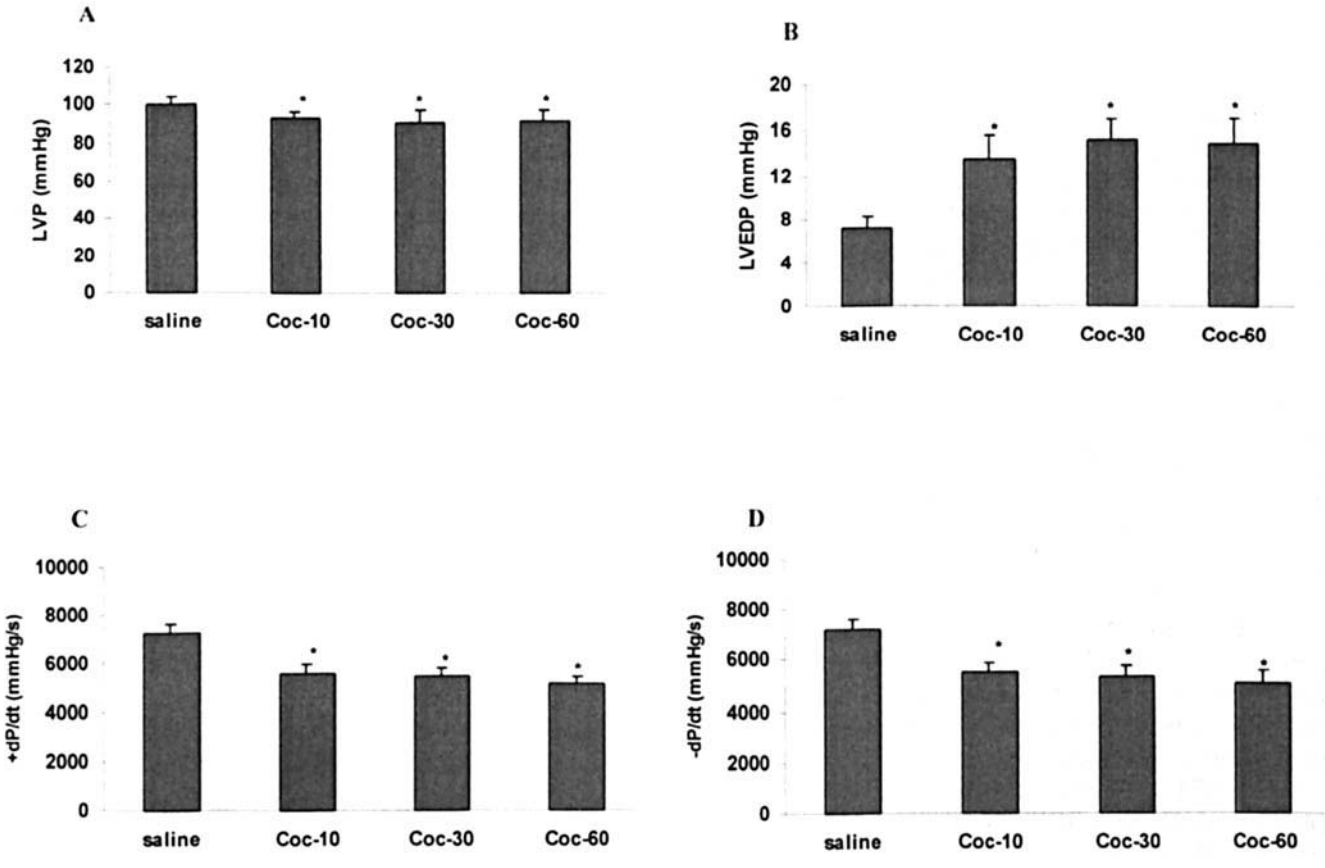

Figure 1. Effects of cocaine on cardiac function. Mice were continuously treated with cocaine for 15 days. Left ventricular function was monitored. Data are expressed as means ± SEM. $n = 13$, $^*P < 0.05$ compared with saline control. (A) left ventricular pressure (LVP); (B) left ventricular end-diastolic pressure (LVEDP); (C) +dP/dt; (D) –dP/dt. Coc-10: cocaine 10 mg/kg; Coc-30: cocaine 30 mg/kg; Coc-60: cocaine 60 mg/kg.

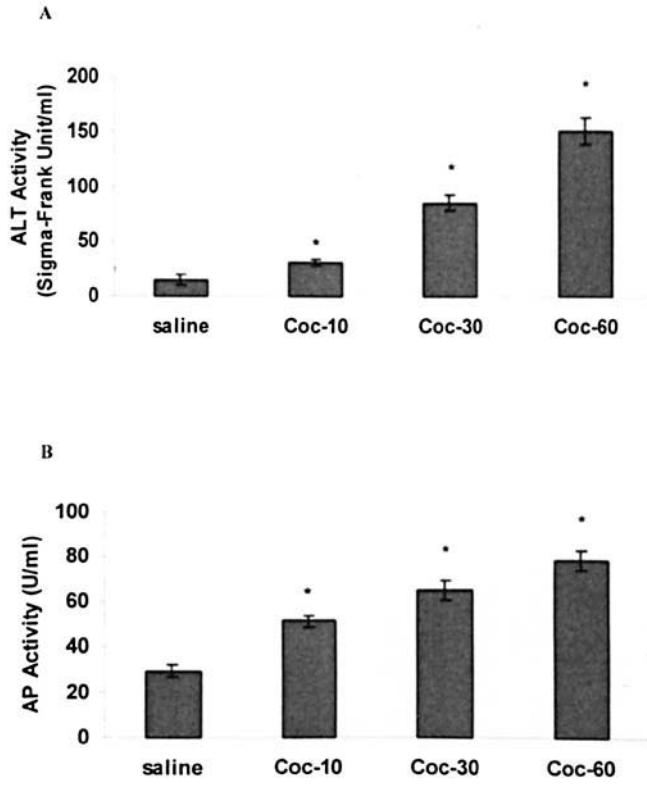

Figure 2. (A) Serum alanine aminotransferase (ALT) activities and (B) serum alkaline phosphatase (AP) activities in mice treated with cocaine. Data are expressed as means ± SEM, $n = 9$. $^*P < 0.05$ compared with saline control. Coc-10: cocaine 10 mg/kg; Coc-30: cocaine 30 mg/kg; Coc-60: cocaine 60 mg/kg.

with 10 mg/kg cocaine were significantly different from the saline controls ($P < 0.05$). Serum ALT and AP levels were highest in mice injected with 60 mg/kg cocaine. ALT activity was 8-fold higher than in the saline control ($P < 0.01$). Although serum ALT and AP levels were higher in the 60-mg/kg cocaine group than in the 30-mg/kg group, those results were not significantly different.

Histopathology. Light microscopic findings (Fig. 3) showed induction of inflammatory cell infiltration in hearts from mice treated with 60 mg/kg cocaine. Also, eosinophilic contraction bands were visible in myocardium from mice treated with 60 mg/kg of cocaine. Figure 4 shows the pathological changes in the cocaine-treated mouse livers. Light microscopic examination of livers from mice given 10 mg/kg cocaine revealed increased eosinophilia of centrilobular hepatocytes in a few lobules. Subcapsular lobules contained swollen hepatocytes. Also, the disorganization of the trabecular arrangements of hepatocytes was obvious. At the dose of 30 mg/kg, there was increased eosinophilia of the centrilobular hepatocytes of many lobules, and many subcapsular lobules contained centrilobular foci of hepatocellular necrosis accompanied by accumulation of inflammatory cells present in clusters. Furthermore, swollen hepatocytes and coagulation necrosis were observed. Mice administered 60 mg/kg cocaine had distinctly increased eosinophilia of the centrilobular hepatocytes, and the hepatocytes were severely pale, swollen, and finely granular. Massive necrosis of whole lobules of the liver was seen in some areas. Hepatocyte necrosis was present in multiple zones, surrounded by neutrophils, lymphocytes, plasma cells, and cellular debris.

Cocaine-Induced Apoptosis. In cardiac sections, apoptosis was evaluated by TUNEL staining and DNA laddering. TUNEL staining of histologic sections from the hearts subjected to cocaine treatment revealed nuclear fluorescence that identified apoptotic cells. In Figs. 5A & 5B saline control sections are shown. There are no TUNEL-positive nuclei, but Hoechst 33258-positive nuclei are present in saline control mice. TUNEL-positive nuclei were observed only in the hearts of animals treated with 60 mg/kg

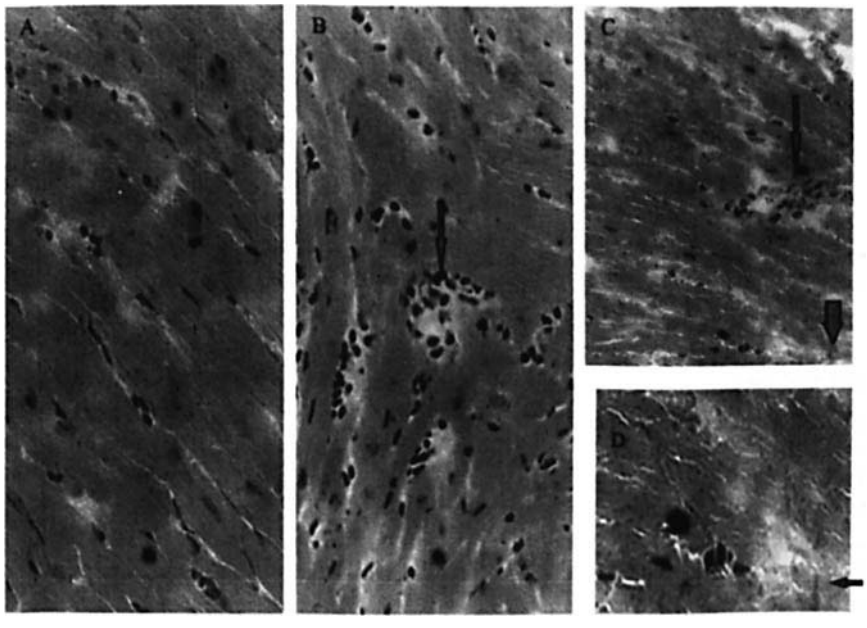

Figure 3. Sections from mouse hearts treated with cocaine (60 mg/kg, ip) for 15 days. (A) Saline control heart; (B and C) cocaine-treated hearts with inflammatory cell infiltration (arrow); (D) enlarged area of C (short arrow) showing eosinophilic contraction band (narrow arrow) (hematoxylin-eosin stain, × 100).

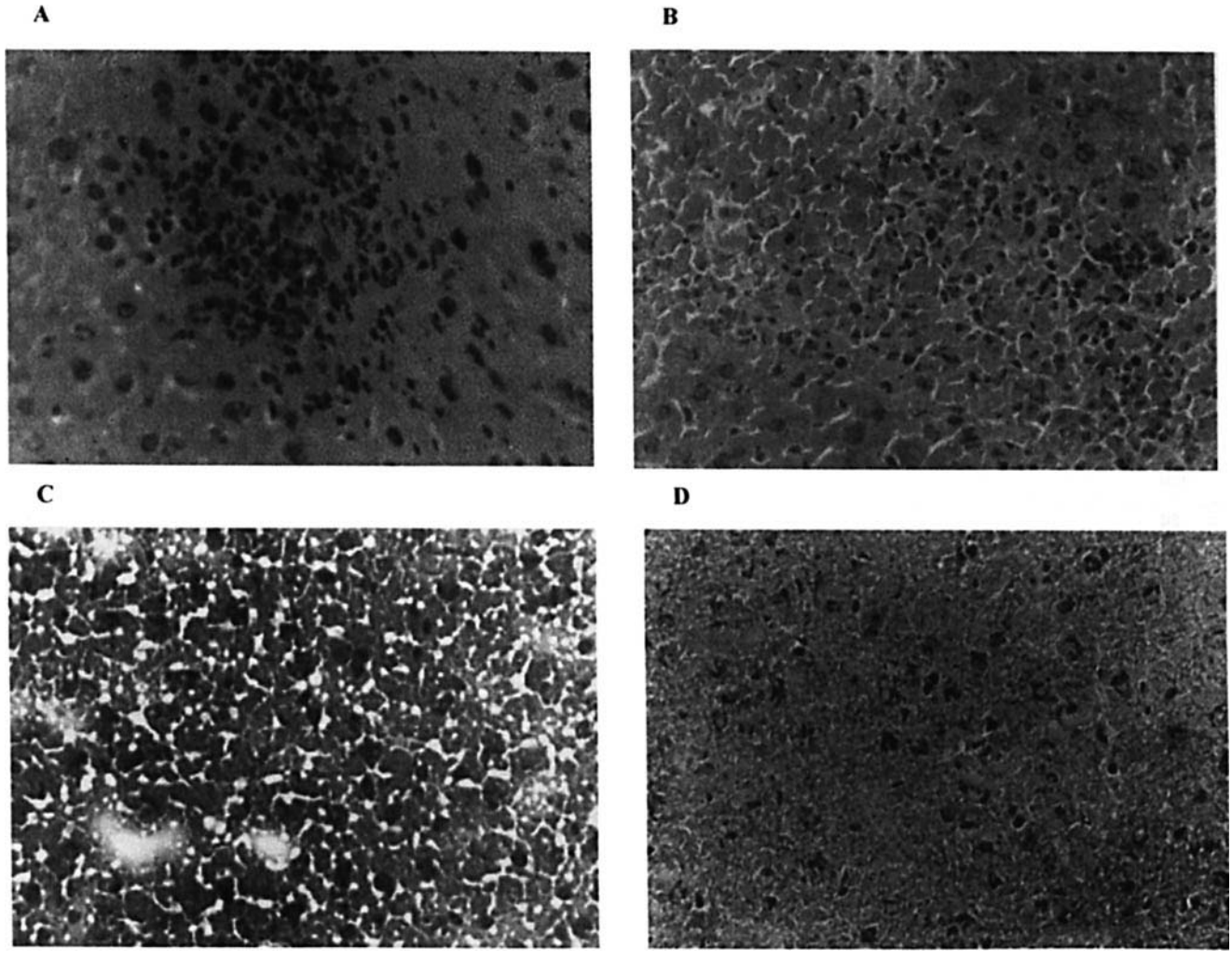

Figure 4. Sections from mouse livers treated with cocaine (10, 30, and 60 mg/kg, ip) for 15 days. Liver sections stained with hematoxylin-eosin show (A) inflammatory cell infiltration, (B) eosinophilia of the centrilobular hepatocytes and swollen cells, (C) coagulation necrosis, and (D) cellular debris (original magnification × 400).

cocaine (Figs. 5C & 5D). In addition, TUNEL-positive nuclei were observed in the livers of mice that had been exposed to cocaine (Figs. 5E & 5F). The incidence of TUNEL and Hoechst 33825 staining nuclei was 5.1 ± 1.3% in 10 mg/kg, and 8.2 ± 1.7 and 11.3 ± 3.2%, respectively, in 30- and 60-mg/kg groups. There was a significant dose-response relation (Fig. 6).

Data from experiments examining qualitative DNA damage using agarose gel electrophoresis are shown in Figure 7. Using the 1-kb DNA ladders as a marker (Lane 9), DNA ladders were clearly visible and indicative of apoptotic internucleosomal DNA fragmentation in cocaine-treated mouse hearts and livers. Lanes 1 and 5 (saline controls) remained totally unfragmented.

TNF-α Level. Figure 8 shows that after short-term treatment with cocaine, TNF-α concentrations in the heart (Fig. 8A) increased significantly ($P < 0.05$ compared with the saline control). Moreover, in the liver, the content of TNF-α was increased from 621.8 ± 73 ng/g in the saline control to 867.2 ± 147 ng/g, 940.8 ± 61 ng/g, and 1032.8 ± 69 ng/g, respectively, in the 10-, 30-, and 60-mg/kg cocaine groups (Fig.8B). The results indicate that cocaine induced the production of TNF-α. Figure 9 shows the result of RT-PCR; the mRNA density of TNF-α was not significantly different among the cocaine- and saline-treated groups in either the mouse heart or liver. These results indicate that cocaine did not affect the mNRA for TNF-α.

Discussion

Cocaine is a naturally occurring alkaloid that may produce toxicity in multiple organs. In the present study, we found that short-term administration of cocaine caused heart and liver injury. Our results may be summarized as follows: (i) cocaine caused multiorgan damage, and the liver is more sensitive to the drug's toxic effect than the heart; (ii) cocaine showed concentration-related hepatotoxicity but not cardiotoxicity; (iii) cocaine initiated apoptosis and induced TNF-α production in both the heart and liver.

Previous experiments have demonstrated that myocardial function was inhibited by acute or chronic administra-

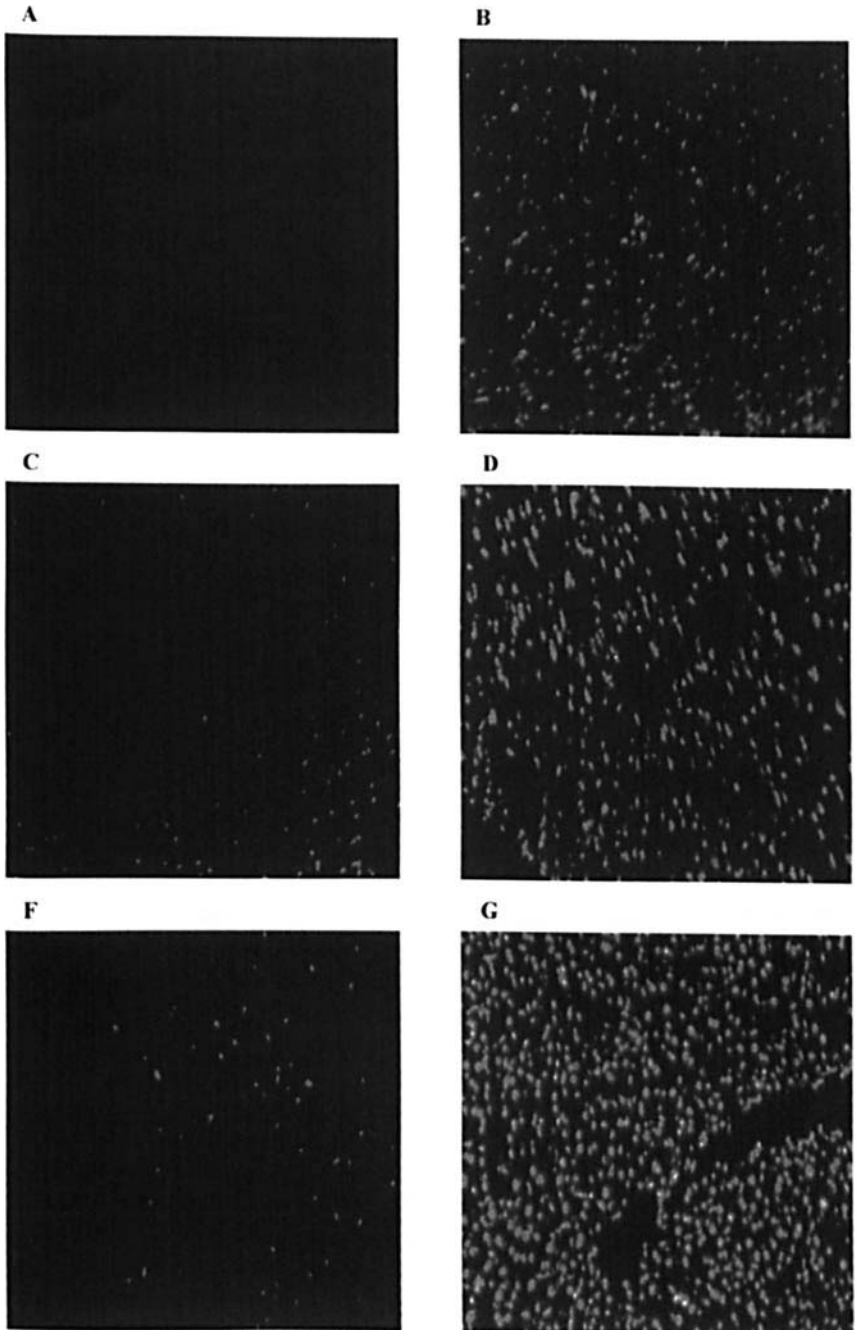

Figure 5. Sections from mouse hearts and livers treated with cocaine for 15 days. Apoptotic cells were stained by TUNEL, and other cells were stained by Hoechst 33258. (A) TUNEL fluorescence in saline control heart; (B) same section and region as Panel A, displaying Hoechst fluorescence; (C and D) TUNEL and Hoechst fluorescence in same section and area in cocaine-treated (60 mg/kg) heart (×100). (F and G) TUNEL and Hoechst 33258 fluorescence in the same liver section and area in cocaine-treated (60 mg/kg) liver (×100).

tion of cocaine (13). Moreover, Stambler and Shannon (17) demonstrated that acute intravenous cocaine caused a biphasic effect on myocardial and left ventricular function with a transient depression followed by significant sustained increase in left ventricular contractility. In our study, impairment of ventricular function was observed in the three dose cocaine groups. Ventricular pressure was reduced by 12.4%, 15.2%, and 18.3%, respectively, in the 10-, 30-, and 60-mg/kg cocaine groups, all significantly different from saline control. However, there was no significant dose-response relationship. These results were similar to the study of Bernerds *et al.* (18) reported in chronic cocaine-treated sheep. These investigators used an osmotic pump to deliver cocaine continuously, 2.4 mg/kg and 4.8 mg/kg, to sheep for 18 days and observed that MAP and HR were not significantly different between these two dose groups. In addition, Arbeille *et al.* (19) found that systolic, diastolic, and MAP were not significantly different between long-term 70- and 140-mg cocaine-treated groups of ewes. Both human and animal studies have demonstrated reduced responsiveness to adrenergic stimulation after long-term exposure to cocaine. This reduced responsiveness is presumed to result from catecholamine receptor catabolism or uncoupling (18). It has also been reported that prolonged exposure of animals *in vivo* to cocaine results in the elevation of catecholamines and consequently desensitization of β-adre-

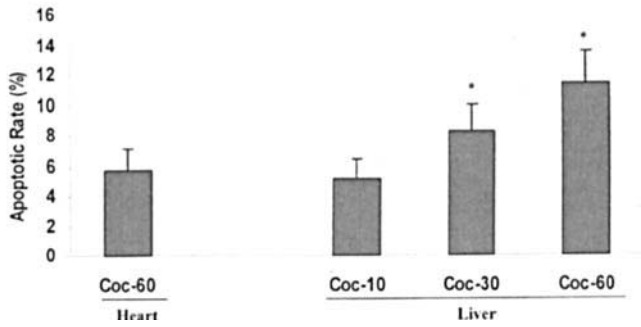

Figure 6. Apoptosis rate in cocaine-treated mouse hearts and livers. Data are expressed as means ± SEM, $n = 9$. *$P < 0.05$ compared with cocaine 10 mg/kg group. Coc-10: cocaine 10 mg/kg; Coc-30: cocaine 30 mg/kg; Coc-60: cocaine 60 mg/kg.

noceptors (18). Moreover, Wilkerson *et al.* (20, 21) found that at doses over 10mg/kg, cocaine caused a prolonged depression of myocardial contractile force in the dog model, and that large doses may alter cocaines elimination kinetics resulting in much higher, more prolonged plasma levels. These factors may explain why cocaine did not show an apparent dose-response relationship in our model.

Some investigators have examined the effects of cocaine on intracardiomyocytic alterations using electron microscopy. Knuepfer *et al.* (2) demonstrated that cocaine caused myocardial ultrastructural alteration in rat hearts, including focally dilated sarcoplasmic reticulum and myofibril derangement, early signs of mitochondrial alterations, and foci of myocardial fibrosis. In the present study, we were unable to identify by light microscopy significant

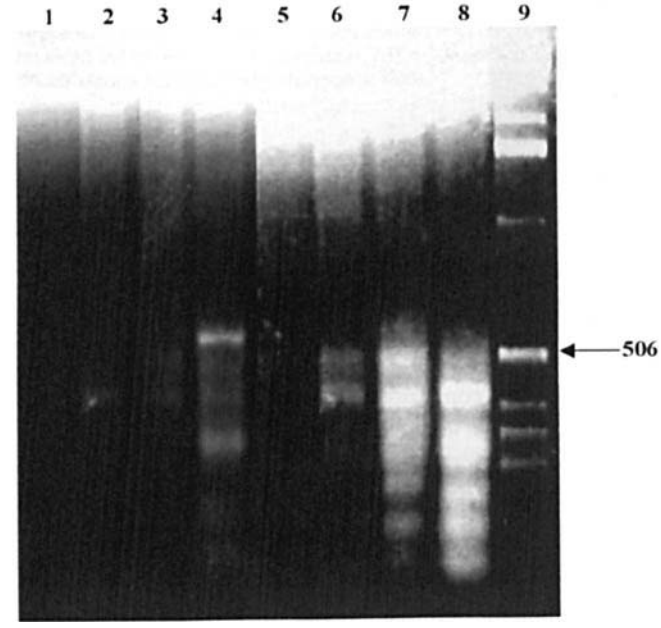

Figure 7. Agarose gel electrophoresis of heart and liver DNA from cocaine-treated mice. DNA ladders are evident at all doses of cocaine. (Lane 1) Saline control (heart); (Lane 2) 10 mg/kg (heart); (Lane 3) 30 mg/mg (heart); (Lane 4) 60 mg/kg (heart); (Lane 5) saline control (liver); (Lane 6) 10 mg/kg (liver); (Lane 7) 30 mg/mg(liver); (Lane 8) 60 mg/kg (liver); (Lane 9) an indicating molecular weight of 560.

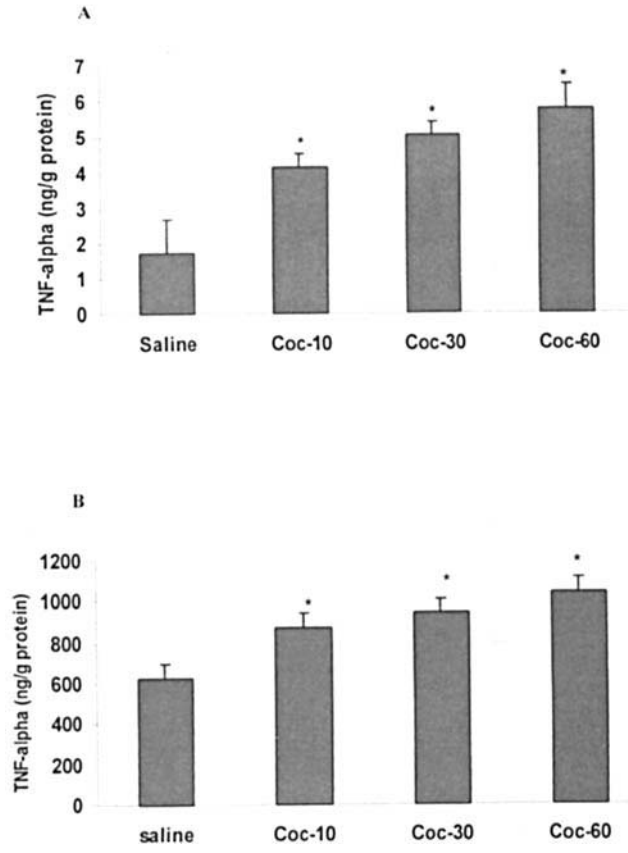

Figure 8. TNF-α concentration in mice treated with cocaine for 15 days. (A) Content of TNF-α in the hearts; (B) content of TNF-α in the liver. Data are expressed as mean±SEM, n=8, P<0.05 compared with saline control. Coc-10: cocaine 10mg/kg; Coc-30: cocaine 30mg/kg; Coc-60: cocaine 60mg/kg.

gross changes in morphology when examining cardiac tissue from mice. However, we observed some inflammatory cell infiltration and eosinophilic contraction bands without myocyte necrosis but only in the 60-mg/kg cocaine group. Generally, inflammatory cell infiltration and myocyte necrosis are considered two separate manifestations of cardiotoxicity (3, 22). We interpreted the appearance of inflammatory cells to be evidence of a hypersensitivity response of the heart to cocaine.

In our study, the eosinophilic contraction bands were visible in the 60-mg/kg cocaine group. In general, contraction band necrosis results from a complex series of interactions that are probably initiated by elevated levels of nor-

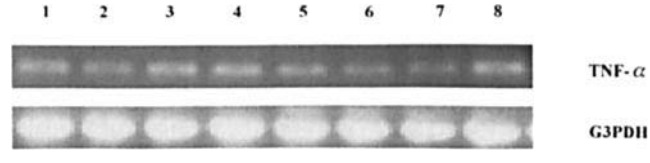

Figure 9. RT-PCR results of mRNA of TNF-α in cocaine-treated mouse hearts and livers. Total RNA was extracted from frozen hearts and livers. (Lane 1) Saline control (liver); (Lane 2) 10mg/kg (liver); (Lane 3) 30mg/kg (liver); (Lane 4) 60 mg/kg (liver); (Lane 5) saline control (heart); (Lane 6) 10 mg/kg (heart); (Lane 7) 30 mg/kg (heart); and (Lane 8) 60 mg/kg (heart).

epinephrine. If local concentrations of catecholamines are sufficiently great, myofilaments may ratchet past each other and end up as an amorphous mass incapable of contracting again (3). Moreover, Turizzo *et al.* (23) reported that chronic administration of cocaine in the rat has been shown to markedly increase the concentration of norepinephrine in the left ventricle. Based on these findings, we suggest that the high dose of cocaine may increase catecholamine levels in the myocardium sufficiently to produce the eosinophilic contraction bands.

In liver, it is well established that cocaine causes hepatic cell death by necrosis after both acute and chronic administration (4, 7, 8, 13). The present study clearly shows that cocaine is hepatotoxic, resulting in liver necrosis. Necrogenic changes were observed at all three doses of cocaine in the present study. Although cocaine hepatotoxicity has been observed previously, the precise mechanisms by which it occurs remain unclear. The irreversible binding of reactive cocaine metabolites to cellular macromolecules and the generation of reactive oxygen species (ROS) during metabolism are two possible mechanisms related to cocaine's hepatic toxicity (8). Some reports have suggested that the severity of cocaine-induced hepatotoxicity was dependent upon the extent of cocaine oxidation by the cytochrome P-450 system (6, 24, 25). Approximately 10% of cocaine undergoes N-demethylation to norcocaine in hepatocytes by the cytochrome P-450 mixed-function oxidase system. Norcocaine then undergoes further enzymatic breakdown to the known hepatotoxins, N-hydroxynorcocaine and norcocaine nitroxide (8, 26). The remaining cocaine is subject to metabolism by nonspecific pseudocholinesterases in the blood serum. Thompson *et al.* (6) demonstrated that these esterase metabolites of cocaine, benzoylecgonine and ecgonine methyl ester, are not hepatotoxic. Therefore, we concluded that cocaine causes hepatoctoxicity *via* different pathways from those in the heart. In the heart, the toxicity of cocaine appears to be related to catecholamine and local anesthetic effects. In contrast, in the liver, the toxicity is linked to metabolism of cocaine by cytochrome P450 (6, 24, 25). Results of the present study indicate that cocaine exposure causes cardiac and hepatic damage simultaneously. However, in the heart only 60 mg/kg cocaine induced inflammatory cell infiltration and eosinophilic contraction bands. Moreover, myocardial apoptosis only appeared in the 60-mg/kg cocaine-treated mice. In contrast, our data demonstrated that three doses of cocaine 10, 30, and 60 mg/kg caused significant hepatic pathological damage and apoptosis. Based on these results, we conclude that the liver is more sensitive to cocaine's toxicity compared with the heart in the BALB/c mouse.

It is well known that apoptosis is a common biological mechanism for removing unwanted cells and is considered an important regulator of cell number and function (10). Excessive apoptosis of the myocardial conduction system may lead to complete heart block and fatal arrhythmia associated with absence of the AV node, sinus node, and internodal pathways (11). The progressive loss of cardiomyocytes is considered to play a major role in cardiovascular disease (10). Moreover, apoptosis plays an important role in hepatic damage caused by various pathophysiology stimuli (14). It has been established that cocaine abuse may cause or exacerbate the development of each of these cardiovascular abnormalities and produce hepatic injury.

Several studies (4–7) have demonstrated that the predominant type of liver cell death observed in cocaine-treated animals is necrosis accompanied by massive inflammatory cell infiltration. However, Wu *et al.* (27) reported that after 1 week of administration, cocaine caused thymocyte apoptosis. The percentages of apoptotic cells were 13.4% and 2.5%, respectively, in the 50-mg/kg and 25-mg/kg cocaine groups. However, apoptosis was un-detectable in their 10-mg/kg cocaine group. Also, Cascales *et al.* (13) found only 60 mg/kg cocaine induced programmed death of hepatocytes after a single dose. In our experiments, DNA laddering and TUNEL-staining show that cocaine treatment also induces hepatocyte apoptosis. Therefore, we believe that programmed cell death may be an important factor contributing to the myocardial injury and hepatic damage associated with cocaine abuse.

TNF-α is a proinflammatory cytokine that has been implicated in the pathogenesis of cardiovascular disease, including acute myocardial infarction, chronic heart failure, atherosclerosis, viral myocarditis, and sepsis-associated cardiac dysfunction (15, 28, 29). Also, TNF-α mediates myocardial cell apoptosis *via* the TNF-α receptor I or nitric oxide and *Bcl*-x (28, 30, 31). Shen *et al.* (32) demonstrated that cocaine inhibited the release of cytokines, including TNF-α. However, several *in vivo* studies showed that the mouse splenocytes were stimulated to increase the secretion of TNF-α (33). In the present study, we found that the three doses of cocaine induced the production of TNF-α in both mouse hearts and livers. However, RT-PCR results indicated that cocaine intake did not affect mRNA of TNF-α among the three-dose groups studied in either heart or liver. This increase of TNF-α may be due to the effect of cocaine on the post-transcriptional levels of TNF-α.

The heart and liver are TNF-producing organs. Both macrophages and cardiac myocytes or hepatic cells can synthesize TNF. Once TNF gene transcription occurs, TNF mRNA is translated into the 26-kDa TNF precursor in the cytoplasm (28).Myristoylation in the cytoplasm facilitates membrane insertion/association, where it is cleaved by TNF-α-converting enzyme. The mature 17-kDa TNF is then released into the extracellular space. Effects at any of these steps may modulate TNF-α levels. However, the pathway through which cocaine acts to regulate the production of TNF-α is unclear. Further investigations are required to determine whether cocaine acts directly on post-transcriptional regulation or indirectly on other pathways. It is also important to determine whether the apoptosis in myocardium and liver is secondary to the increase of TNF-α induced by administration of cocaine or whether cocaine

simultaneously triggers apoptosis and elevates the TNF-α level.

In conclusion, cocaine exposure causes multiple organ damage, including cardiac and hepatic injury. Liver is more sensitive to cocaine's toxicity compared to the heart, and the histology is different, at least in our mouse model. Finally, we propose that induction of TNF-α with consequent apoptosis may be a common mechanism for cocaine's toxicity both in heart and liver.

1. Kloner RA, Hale S, Alker K, Rezkalla S. The effects of acute and chronic cocaine use on the heart. Circulation **85:**407–419, 1992.
2. Knuepfer MM, Branch CA, Gan Q, Fischer VW. Cocaine-induced myocardial ultrastructural alterations and cardiac output responses in rats. Exp Mol Pathol **59:**155–168, 1993.
3. Karch SB, Billingham ME. The pathology and etiology of cocaine-induced heart disease. Arch Pathol Lab Med **112:**225–230, 1988.
4. Roth L, Harrison RD, James BC, Tobin T, Roberts SM. Cocaine hepatotoxicity: Influence of hepatic enzyme inducing and inhibiting agents on the site of necrosis. Hepatology **15:**934–940, 1992.
5. Boyer CS, Perterson DR. Hepatic biochemical changes as a result of acute cocaine administration in the mouse. Hepatology **14:**1209–1216, 1991.
6. Thompson ML, Shuster L, Shaw K. Cocaine-induced hepatic necrosis in mice: The role of cocaine metabolism. Biochem Pharmacol **28:**2385–2395, 1979.
7. Aoki K, Ohmori M, Takimoto M, Ota H, Yoshida T. Cocaine-induced liver injury in mice is mediated by nitric oxide and reactive oxygen species. Eur J Pharmacol **336:**43–49, 1997.
8. Devi BG, Chan AWK. Cocaine-induced peroxidative stress in rat liver: Antioxidant enzymes and mitochondria. J Pharmacol Exp Ther **279:**359–366, 1996.
9. Veinot JP, Gattinger DA, Fliss H. Early apoptosis in human myocardial infarcts. Hum Pathol **28:**485–492, 1997.
10. Fliss H, Gattinger D. Apoptosis in ischemic and reperfused rat myocardium. Circ Res **79:**949–956, 1996.
11. James TN, Martin E, St. Willis PW, Lohr TO. Apoptosis as a possible cause of gradual development of complete heart block and fatal arrhythmias associated with absence of the AV node, sinus node, and internodal pathways. Circulation **93:**1424–1438, 1996.
12. Ray SD, Mumaw VR, Raje RR, Fariss MW. Protection of acetaminophen-induced hepatocellular apoptosis and necrosis by cholesteryl hemisuccinate pretreatment. J Pharmacol Exp Ther **279:**1470–1483, 1996.
13. Cascales M, Alvarez A, Gasco P, Fernandez-Simon L, Sanz N, Bosca L. Cocaine-induced liver injury in mice elicits specific changes in DNA ploidy and induces programmed death of hepatocytes. Hepatology **20:**992–1001, 1994.
14. Habeebu SSM, Liu L, Klaassen CD. Cadmium-induced apoptosis in mouse liver. Toxicol Appl Pharmacol **149:**203–209, 1998.
15. Kumar A, Thota V, Dee L, Olson J, Uretz E, Parrillo JE. Tumor necrosis factor-α and interleukin-1β are responsible for *in vitro* myocardial cell depression induced by human septic shock serum. J Exp Med **183:**949–958, 1996.
16. Wang JH, Redmond HP, William R, Watson G, Bouchier-Hayes D. Role of lipopolysaccharide and tumor necrosis factor-α in induction of hepatocyte necrosis. Am J Physiol **269:**G297–G304, 1995.
17. Stambler BS, Komamura K, Ihara T, Shannon RP. Acute intravenous cocaine causes transient depression followed by enhanced left ventricular function in conscious dogs. Circulation **87:**1687–1697, 1993.
18. Bermards CM, Cullen BF, Powers KM. Effect of chronic cocaine exposure on the hemodynamic response to vasopressors in sheep. J Trauma **43:**680–686, 1997.
19. Arbeille P, Maulik D, Salihagic A, Locatelli A, Lansac J, Platt JD. Effect of long-term cocaine administration to pregnant ewes on fetal hemodynamics, oxygenation, and growth. Obstet Gynecol **90:**795–802, 1997.
20. Wilkerson RD. Cardiovascular effects of cocaine in conscious dogs: Importance of fully functional autonomic and central nervous system. J Pharmacol Exp Ther **246:**466–471, 1988.
21. Wilkerson RD, Temesy-Armos PN, Fraker TD. Pharmacokinetics and time-action profile of cocaine in dogs. In: Thadani P, Ed. Cardiovascular Toxicity of Cocaine: Underlying Mechanisms. Bethesda, MD: National Institute on Drug Abuse, pp28–39, 1991.
22. Virmani R, Robinowitz M, Smialek JE, Smyth DF. Cardiovascular effects of cocaine: An autopsy study of 40 patients. Am Heart J **115:**1068–1076, 1988.
23. Turizzo V, Rubio MC. Effects of cocaine on several adrenergic system parameters. Gen Pharmacol **16:**71–76, 1985.
24. Pellinen P, Honkakosh P, Stenback F, Niemitz M, Alhava E, Pelkonen O, Lang MA, Pasanen M. Cocaine N-demethylation and its metabolism-related hepatotoxicity can be prevented by P_{450} 3A inhibitors. Eur J Pharmacol **270:**35–48, 1994.
25. Dellinen P, Stenback F, Kojo A, Honkakoski P, Gelboin HV, Pasanen M. Regenerative changes in hepatic morphology and enhanced expression of *CYP*2B10 and *CYP*3A during daily administration of cocaine. Hepatology **23:**515–523, 1996.
26. Ndikum-Moffor FM, Schoeb TR, Roberts SM. Liver toxicity from norcocaine nitroxide, an N-oxidative metabolite of cocaine. J Pharmacol Exp Ther **284:**413–419, 1998.
27. Wu YB, Shen ML, Gu GG, Anderson KM, Ou DW. The effects of cocaine injections on mouse thymocyte population. Proc Soc Exp Biol Med **214:**173–179, 1997.
28. Meldrum DR. Tumor necrosis factor in the heart. Am J Physiol **274:**R577–R595, 1998.
29. Munger MA, Johson B, Amber IJ, Callahan KS, Gilbert E. Circulating concentrations of proinflammatory cytokines in mild or moderate heart failure secondary to ischemic or idopathic dilated cardiomyopathy. Am J Cardiol **77:**723–727, 1996.
30. Ing DJ, Zang J, Dzau VJ, Webster KA, Bishopric NH. Modulation of cytokine-induced cardiac myocyte apoptosis by nitric oxide, *Bak*, and *Bcl*-x. Circ Res **84:**21–33, 1999.
31. Krown KA, Page MT, Nguyen C, Zechner D, Gutierrez V, Comstock KJ, Glembotski CC, Quintana PJE, Sabbadini RA. Tumor necrosis factor-α–induced apoptosis in cardiac myocytes: Involvement of the sphingolipid signaling cascade in cardiac cell death. J Clin Invest **98:**2854–2865, 1996.
32. Shen HM, Kennedy JL, Ou DW. Inhibition of cytokine release by cocaine. Int J Immunopharmacol **16:**295–300, 1994.
33. Wang Y, Huang DS, Watson RR. *In vivo* and *in vitro* cocaine modulation on production of cytokines in C57BL/6 mice. Life Sci **54:**401–411, 1994.