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Cocaine-induced liver injury in mice is mediated by nitric oxide and reactive oxygen species

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Abstract

The modulating effects of nitric oxide (NO) and reactive oxygen species on cocaine-induced hepatotoxicity were examined by measuring plasma alanine aminotransferase activity and by carrying out histological studies. Liver injury was induced by a single injection of cocaine in adult male ICR mice. Pretreatment with aminoguanidine (an inhibitor of NO synthase), *N*-methyl-D-glucamine dithiocarbamate complex with iron ion (II) ($Fe^{2+}(MGD)_2$, a trapping reagent of NO) or deferoxamine complex with iron ion (III) (Fe^{3+}-deferoxamine, a scavenger of NO) produced a marked inhibition of the hepatotoxicity induced by cocaine. In addition, pretreatment with allopurinol (an inhibitor of xanthine oxidase) and 1,3-dimethylthiourea (a scavenger of hydroxyl radical) also produced a potent inhibition. These findings suggest that a hydroxyl radical produced by the reaction of NO and superoxide anion (O_2^-) via peroxynitrite may be involved in the pathogenesis of cocaine hepatotoxicity. © 1997 Elsevier Science B.V.

Keywords: Cocaine; Hepatotoxicity; Nitric oxide (NO); Reactive oxygen species; (Mouse)

1. Introduction

Cocaine is a local anesthetic and a sympathomimetic reagent. However, there is increasing clinical evidence that the liver is also a target of cocaine toxicity (Marks and Chapple, 1967; Perino et al., 1987; Kanel et al., 1990; Wanless et al., 1990). Of all experimental animals, male adult mice have the highest sensitivity to cocaine-induced hepatotoxicity. Two major pathways have been postulated to explain cocaine-induced hepatotoxicity. First, it has been suggested that the nitrosonium ion, generated during the oxidation of norcocaine, or other as yet unidentified reactive metabolites bind covalently to cellular macromolecules. Second, as a result of redox cycling between *N*-hydroxyl norcocaine and norcocaine nitroxide, superoxide anion (O_2^-) can be produced at the expense of NADPH. The reactive oxygen species formed is involved in cell membrane damage with or without lipid peroxidation (Göldlin and Boelsterli, 1991).

In this study, we investigated whether nitric oxide (NO) mediates cocaine-induced hepatotoxicity in adult male ICR mice and its relation to reactive oxygen species in order to assess the possible interaction between these radicals.

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2. Materials and methods

2.1. Animals

Male ICR mice (6 weeks old) were obtained from Clea Japan (Tokyo, Japan). Mice were housed five or six per cage on ALPHA-dri® (Shepherd Speciality Papers, Kalamazoo, MI, USA) in a room maintained at 23°C with a 12 h light–dark cycle. The mice had free access to standard rodent chow (F2, Sankyo laboratories, Tokyo, Japan) and water and were housed at our facilities for at least 1 week before use. The diet was withdrawn from mice at the beginning of the experiment or at the second administration of allpurinol.

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2.2. Reagents

Aminoguanidine, allopurinol, deferoxamine mesylate, pyrrolidine dithiocarbamate, lipopolysaccharide (from *Pseudomonas aeruginosa* Serotype 10), nitrate reductase (*Aspergillus* species), L-arginine and D-arginine were purchased from Sigma (St. Louis, MO, USA). *N*-Methyl-D-glucamine dithiocarbamate (MGD), superoxide dismutase (Cu, Zn–SOD; EC 1.15.1.1, 3000–4000 units/mg) and catalase (EC 1.11.1.6, 5,000–9,000 units/mg) were from Wako (Tokyo, Japan). 1,3-Dimethylthiourea was from Tokyo Chemical (Tokyo, Japan). Cocaine hydrochloride was obtained from Takeda (Osaka, Japan). All other reagents used in this study were of analytical grade.

2.3. Chemical treatments

Allopurinol was suspended in saline containing 0.5% carboxymethylcellulose sodium salt and MGD was dissolved in phosphate buffered saline pretreated with N_2 gas. All other drugs and chemicals were administered in saline. All compounds were administered by the i.p. route. The control mice received the vehicle at the same time the test mice received the test reagent. Because the sensitivity of mice to cocaine was not constant, the dose of cocaine had to be adjusted to achieve more than 80% survival 16–17 h after cocaine administration.

Treatment regimens consisted of: cocaine, 40–55 mg/kg; lipopolysaccharide, 2 mg/kg; aminoguanidine, 300 mg/kg 1 h before cocaine or lipopolysaccharide, or 15 mg/kg coadministration with cocaine and/or 1 g/kg of L- or D-arginine; $Fe^{2+}(MGD)_2$ (molar ratio of MGD: $FeSO_4$ $7H_2O = 5{:}1$), 326 mg/kg as MGD 15 min before cocaine (Lai and Komarov, 1994); 1,3-dimethylthiourea, 500 mg/kg 15 min before cocaine (Fox, 1984); pyrrolidine dithiocarbamate, 30 mg/kg 15 min before cocaine; allopurinol, 100 mg/kg 24 h and 1 h before cocaine (Neihörster et al., 1992); superoxide dismutase and catalase, 30 000 U/kg and 300 000 U/kg, respectively, 1 h before cocaine (Neihörster et al., 1992); deferoxamine, 0.51 mmol/kg, 15 min before cocaine (Oury et al., 1993); Fe^{3+}-deferoxamine (equimolar amounts of deferoxamine and $FeCl_3$, the concentration of Fe^{3+}-deferoxamine was determined spectrophotometrically by measuring absorbance at 425 nm ($\varepsilon = 2500\ M^{-1}\ cm^{-1}$)), 0.51 mmol/kg 15 min before cocaine administration (Oury et al., 1993).

2.4. Assays

Mice were bled by direct cardiac puncture under ether anesthesia 16–17 h after cocaine administration. The blood collected in a tube containing Na_2EDTA was centrifuged at 5000 rpm for 10 min. The plasma was stored at −50°C until assayed for alanine aminotransferase activity and total nitrite/nitrate (NO_2^-/NO_3^-) levels. Alanine aminotransferase activity was determined with the Transaminase CII-test WAKO (Wako, Osaka, Japan). The nitrite/nitrate levels were quantitated by the Griess assay after the reduction of nitrate to nitrite by nitrate reductase (Misko et al., 1993b). Liver tissues were obtained at the time of death, fixed in 10% formalin, embedded in paraffin, cut into 3–4-μm-thick slices and stained with hematoxylin and eosin for histopathologic examination.

2.5. Statistics

Student's *t*-tests were used for statistical analysis; all differences were considered significant when $P < 0.05$. Results were expressed as means ± S.E.M.

3. Results

3.1. Effects of single administration of cocaine

Liver damage caused by a single administration of cocaine (40–55 mg/kg, i.p.) was assessed by measurement of the plasma activity of the liver-specific enzyme, alanine aminotransferase, and by histological examination of liver sections. Plasma alanine aminotransferase activity began to rise significantly 4 h after cocaine administration and reached a maximum between 16 and 24 h. Liver sections from mice with high plasma alanine aminotransferase activity showed multiple centrilobular acidophilic necrosis (Fig. 1B).

3.2. Effects of aminoguaninidine and $Fe^{2+}(MGD)_2$ on cocaine-induced hepatotoxicity

Aminoguanidine and $Fe^{2+}(MGD)_2$ were used to deplete NO in vivo. Aminoguanidine is a selective inhibitor of inducible NO synthase and has few effects on the physiological functions of NO produced by constitutive NO synthase (Misko et al., 1993a). It was therefore considered to be suitable for use in vivo experiments. To confirm the effectiveness of aminoguanidine as a NO synthase inhibitor, mice were treated with aminoguanidine (300 mg/kg) 1 h before lipopolysaccharide (2 mg/kg). Plasma alanine aminotransferase activity and nitrite/nitrate levels were measured 16 h after lipopolysaccharide administration. A dose of lipopolysaccharide (2 mg/kg) resulted in elevated plasma nitrite/nitrate levels and no changes in alanine aminotransferase activity; it did not cause liver injury. Aminoguanidine had no effect in the absence of lipopolysaccharide, but when given with lipopolysaccharide, a significant suppression of nitrite/nitrate levels to control levels was seen. The plasma nitrite/nitrate levels of mice administered lipopolysaccharide and aminoguanidine + lipopolysaccharide were 161.6 ± 28.0 μM ($n = 4$) and 68.6 ± 11.2 μM ($n = 4$), respectively. Mice were then treated with the same dose of aminoguanidine (300 mg/kg)

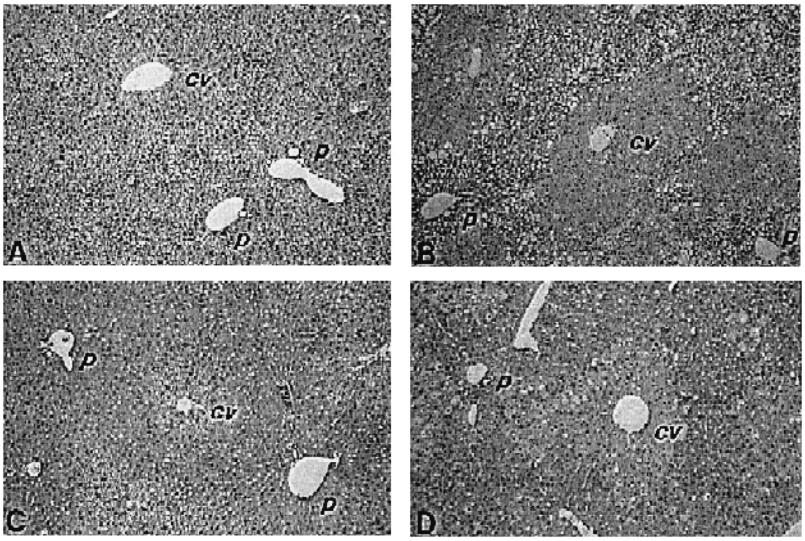

Fig. 1. Effects of aminoguanidine pretreatment on cocaine-induced hepatic necrosis (hematoxylin and eosin staining, magnification × 25). (A) Normal liver section. (B) Liver section from mice administered cocaine (50 mg/kg). (C) Liver section from mice administered aminoguanidine (300 mg/kg) 1 h before cocaine (55 mg/kg). (D) Liver section from mice simultaneously administered aminoguanidine (15 mg/kg) and cocaine (50 mg/kg). Livers were removed 16–17 h after cocaine administration for histopathologic examination. Plasma alanine aminotransferase activity (IU/l) was 4.5 (A), 2105.9 (B), 5.5 (C) and 252.0 (D). CV indicates central vein; P indicates portal vein.

1 h before cocaine (55 mg/kg), and plasma alanine aminotransferase activity and nitrite/nitrate levels were measured 16 h after cocaine administration. In contrast to lipopolysaccharide, cocaine elevated plasma alanine aminotransferase activity without changing the nitrite/nitrate levels and aminoguanidine pretreatment suppressed completely the cocaine-elevated alanine aminotransferase activity without having an effect on the nitrite/nitrate levels (Table 1). The liver section from mice administered aminoguanidine (300 mg/kg) and cocaine (55 mg/kg) also resembled that of the control mice (Fig. 1C).

Because it has been reported that the effect of a NO synthase inhibitor can be overcome in vivo by a large dose of L-arginine, a substrate of NO synthase (Billiar et al., 1990), L- or D-arginine was administered with aminoguanidine and cocaine to clarify that the protective effects of aminoguanidine on cocaine-induced hepatotoxicity were due to its ability to inhibit NO synthase. The minimum dose of aminoguanidine that suppressed the increase in alanine aminotransferase activity induced by simultaneous administration of cocaine (50 mg/kg) was 15 mg/kg. The liver section from mice administered aminoguanidine (15 mg/kg) and cocaine (50 mg/kg) simultaneously showed no necrosis, but vacuolization (ballooning degeneration) in the centrilobular region (Fig. 1D). As shown in Fig. 2, the

Table 1
Effects of aminoguanidine pretreatment on plasma alanine aminotransferase activity and nitrite/nitrate levels

Treatment (*n*)	Alanine aminotransferase activity (IU/l)	Nitrite /nitrate level (μM)
Saline (3)	24.1 ± 2.6	91.2 ± 18.0
Cocaine (5)	1364.0 ± 294.6 [a]	88.2 ± 16.9
Aminoguanidine + cocaine (5)	26.8 ± 2.2	68.6 ± 10.6
Aminoguanidine (3)	21.8 ± 2.0	54.1 ± 3.5

Mice were treated with aminoguanidine (300 mg/kg), a NO synthase inhibitor, 1 h before cocaine administration (55 mg/kg). Plasma alanine aminotransferase activity and nitrate/nitrate levels were determined 16 h after cocaine administration. Values are presented as means ± S.E.M. for 3 to 5 mice per group.
[a] $P < 0.05$ compared to hepatic injury control (cocaine).

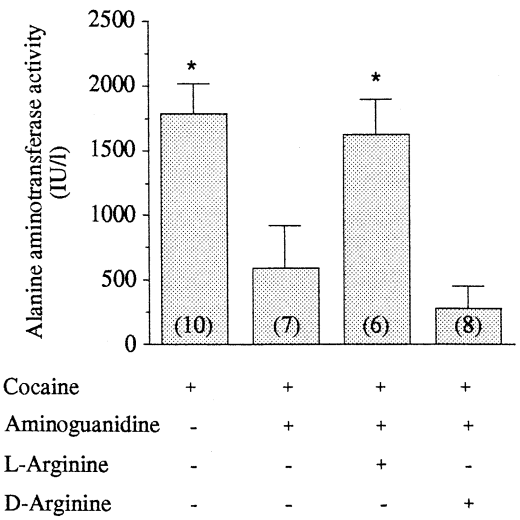

Fig. 2. Effects of L-arginine on aminoguanidine suppression of cocaine-induced hepatotoxicity. All mice received cocaine (50 mg/kg). With cocaine, some mice received simultaneously aminoguanidine (15 mg/kg), aminoguanidine + L-arginine (1 g/kg), or aminoguanidine + D-arginine (1 g/kg). Plasma alanine aminotransferase activity was determined 16–17 h after cocaine administration. Values are means ± S.E.M. for 6 to 10 mice per group. * $P < 0.05$ compared to aminoguanidine suppression of cocaine-induced hepatotoxicity (cocaine + aminoguanidine).

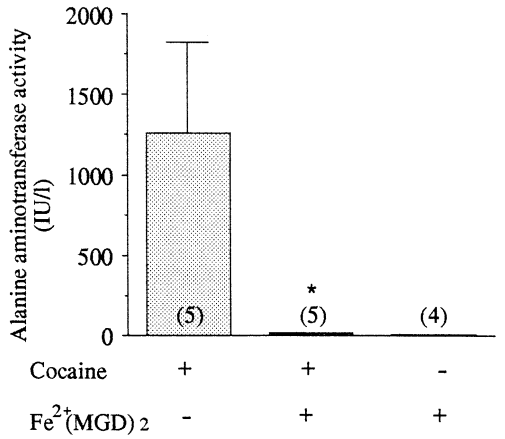

Fig. 3. Effects of *N*-methyl-D-glucamine dithiocarbamate (MGD) and reduced iron (Fe^{2+}) complex ($Fe^{2+}(MGD)_2$) on cocaine-induced hepatotoxicity. Mice were treated with $Fe^{2+}(MGD)_2$, 326 mg/kg as MGD, 15 min before cocaine administration (40 mg/kg). Plasma alanine aminotransferase activity was determined 16–17 h after cocaine administration. Values are means ± S.E.M. for 4 to 5 mice per group. * $P < 0.05$ compared to hepatic injury control (cocaine).

mice treated with L-arginine (1 g/kg) + aminoguanidine (15 mg/kg) + cocaine (50 mg/kg) had a higher alanine aminotransferase activity than the mice treated with aminoguanidine + cocaine or D-arginine (1 g/kg) + aminoguanidine + cocaine and comparable to that of mice treated with cocaine only.

The participation of NO in cocaine-induced hepatotoxicity was further confirmed by using a NO trapping reagent, the metal chelator complex consisting of *N*-methyl-D-glucamine dithiocarbamate (MGD) and reduced iron (Fe^{2+}). The complex, which is stable and water soluble, can trap NO and can be used in vivo for the NO spin-trapping technique to measure NO production in blood by electron spin resonance (ESR; Lai and Komarov, 1994). Treatment with $Fe^{2+}(MGD)_2$ 15 min before cocaine administration suppressed both the cocaine-elevated plasma alanine aminotransferase activity (Fig. 3) and the cocaine-induced hepatic necrosis to almost the control levels (Fig. 4A).

3.3. Effects of antioxidants on cocaine-induced hepatotoxicity

Mice were treated with allopurinol and superoxide dismutase + catalase to examine the role of O_2^- in cocaine-induced hepatotoxicity and with 1,3-dimethylthiourea, pyrrolidine dithiocarbamate, deferoxamine and Fe^{3+}-deferoxamine to examine the role of hydroxyl radicals (Table 2). Pretreatment with allopurinol, 1,3-dimethylthiourea and Fe^{3+}-deferoxamine resulted in an alanine aminotrans-ferase activity that was 12, 14 and 10% of that of mice only given cocaine, respectively. However, pretreatment with superoxide dismutase + catalase and deferoxamine could not protect mice against cocaine-induced hepatotoxicity. Furthermore, pyrrolidine dithiocarbamate enhanced the elevated alanine aminotransferase activity produced by cocaine to 180%. Livers from mice treated with Fe^{3+}-deferoxamine and allopurinol before cocaine were only slightly swollen at the centrilobular region (Fig. 4B and

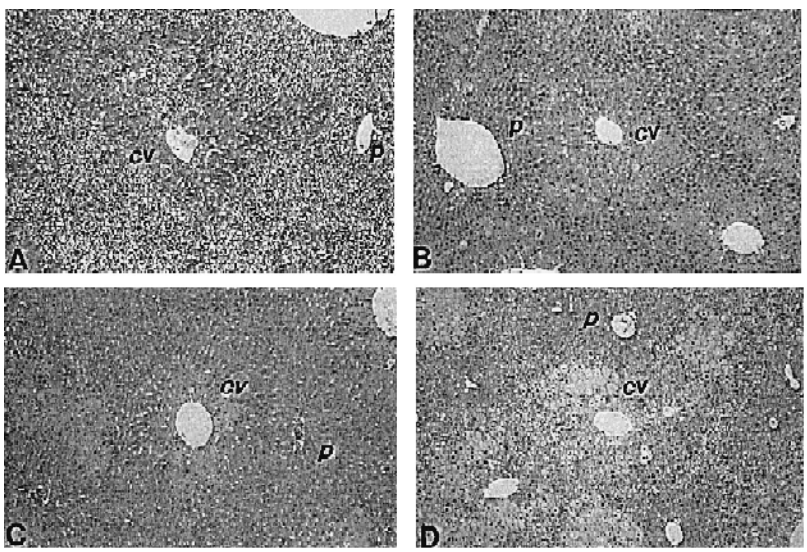

Fig. 4. Protective effects of $Fe^{2+}(MGD)_2$, Fe^{3+}-deferoxamine, allopurinol and 1,3-dimethylthiourea on cocaine-induced hepatic necrosis. (Hematoxylin and eosin staining, magnification × 25). (A) Liver section from mice treated with $Fe^{2+}(MGD)_2$ (326 mg/kg as MGD) 15 min before cocaine administration (40 mg/kg). (B) Liver section from mice treated with Fe^{3+}-deferoxamine (0.51 mmol/kg) 15 min after cocaine administration (C) Liver section from mice treated with allopurinol (100 mg/kg) 24 and 1 h before cocaine administration. (D) Liver section from mice treated with 1,3-dimethylthiourea (500 mg/kg) 15 min before cocaine administration. Livers were removed 16–17 h after cocaine administration for histopathologic examination. Plasma alanine aminotransferase activity (IU/l) was 13.7 (A), 259.1 (B), 179.1 (C) and 19.6 (D). CV indicates central vein; P indicates portal vein.

Table 2
Effects of antioxidants on cocaine-induced hepatotoxicity

Reagent	Alanine aminotransferase activity (IU/l)		
	cocaine (*n*)	reagent + cocaine (*n*)	reagent (*n*)
Deferoxamine	1968.5 ± 153.7 (9)	1539.0 ± 279.7 (9)	24.2 ± 7.8 (3)
Fe^{3+}-deferoxamine	1935.0 ± 171.3 (11)	224.4 ± 67.5 [a] (11)	46.5 ± 17.7 (7)
Allopurinol	1991.0 ± 234.7 (9)	251.0 ± 222.1 [a] (8)	3.6 ± 1.2 (4)
Superoxide dismutate + catalase	1238.6 ± 287.4 (8)	1495.2 ± 317.5 (8)	10.0 ± 1.4 (4)
1,3-Dimethylthiourea	927.9 ± 331.2 (8)	134.6 ± 89.6 [a] (8)	6.3 ± 2.1 (3)
Pyrrolidine dithiocarbamate	1079.9 ± 204.8 (6)	1954.4 ± 115.7 [a] (5)	4.9 ± 0.2 (3)

Mice were treated with antioxidants before cocaine administration (40 mg/kg). Deferoxamine (0.51 mmol/kg), Fe^{3+}-deferoxamine (0.51 mmol/kg), 1,3-dimethylthiourea (500 mg/kg) and pyrrolidine dithiocarbamate (30 mg/kg) were given 15 min before cocaine. Allopurinol (100 mg/kg) was administered 24 h and 1 h before cocaine. Superoxide dismutase (30 000 U/kg) + catalase (300 000 U/kg) was given 1 h before cocaine. Plasma alanine aminotransferase activity was determined 16–17 h after cocaine. Values are presented as means ± S.E.M. for 3 to 11 mice per group.
[a] $P < 0.05$ compared to hepatic injury control (cocaine).

C). Nevertheless, pretreatment with 1,3-dimethylthiourea resulted in modest vacuolization in the centrilobular and midzonal regions even in mice with low plasma alanine aminotransferase activity comparable to that of the controls (Fig. 4D).

4. Discussion

Single administration of cocaine is known to cause liver damage in male adult mice. Reactive oxygen species as well as unidentified metabolites of cocaine generated in the oxidative pathways have been postulated to play an important role in cocaine-induced hepatotoxicity. Boelsterli et al. (1993) demonstrated, by measuring luminol-amplified chemiluminescence, the production of cocaine-induced NADPH-dependent reactive oxygen species, mainly O_2^- and H_2O_2, in hepatic microsomal suspensions and in homogenates of cultured hepatocytes derived from phenobarbital-pretreated rats.

In this study we investigated the roles of NO and reactive oxygen species in cocaine-induced hepatotoxicity in male adult ICR mice by measuring plasma alanine aminotransferase activity as an index of hepatotoxicity and by carrying out histopathological examination. We found that the inhibition of NO production by aminoguanidine and trapping of NO by $Fe^{2+}(MGD)_2$ resulted in the suppression of cocaine-elevated plasma alanine aminotransferase activity and cocaine-induced hepatic necrosis almost to the control levels, i.e. mice were protected completely against cocaine-induced hepatotoxicity. Moreover, this protection by aminoguanidine was reversed by simultaneous administration of an excess of L-arginine to the mice. This result indicated that the protective effects of aminoguanidine against cocaine-induced hepatotoxicity were derived from its ability to inhibit NO synthase. However, no significant changes in plasma nitrite/nitrate levels after cocaine and/or aminoguanidine administration were observed. Taken together, these findings suggest that local NO production plays a harmful role in cocaine-induced hepatotoxicity, in contrast to the protective role of NO in a model of hepatic damage with *Corynebacterium parvum* and lipopolysaccharide (Billiar et al., 1990).

We further investigated the participation of reactive oxygen species in vivo. The reagents were selected in consideration of their representative properties: allopurinol, a xanthine oxidase inhibitor as an inhibitor of O_2^- production, superoxide dismutase + catalase as an eliminator of O_2^-, 1,3-dimethylthiourea and pyrrolidine dithiocarbamate as a hydroxyl radical scavenger, deferoxamine as an eliminator of ferric iron-dependent hydroxyl radical and Fe^{3+}-deferoxamine for comparison with deferoxamine. Allopurinol, 1,3-dimethylthiourea and Fe^{3+}-deferoxamine were effective in suppressing cocaine-induced hepatotoxicity, while superoxide dismutase + catalase and deferoxamine were ineffective and pyrrolidine dithiocarbamate exaggerated cocaine-induced hepatotoxicity.

Because allopurinol and its main metabolite, oxypurinol, are assumed to act as direct hydroxyl radical scavengers (Moorhouse et al., 1987) and as xanthine oxidase inhibitors, the treatment with allopurinol resulted in depletion of reactive oxygen species, then in protection of the mice against cocaine-induced hepatotoxicity. However, superoxide dismutase + catalase showed no protective effects against cocaine-induced hepatotoxicity. Superoxide dismutase, which is thought to be confined to the extracellular space, protected mice against hepatitis induced by lipopolysaccharide + D-galactosamine, an effect mediated by extracellular O_2^- (Neihörster et al., 1992). However, superoxide dismutase could not suppress phorbol myristate acetate-induced O_2^- production in hepatic macrophages from carbon tetrachloride-intoxicated rats, an effect mediated by intracellular O_2^- (Mochida et al., 1989). Moreover, gelatin-conjugated superoxide dismutase, which has a prolonged half-life, was effective in suppressing the development of collagen-induced arthritis, while superoxide dismutase, which has a short half-life, was ineffective (Kakimoto et al., 1993). Consequently, it was assumed that the O_2^- production responsible for cocaine-induced hepatotoxicity was intracellular and not extracellular, or the life

of superoxide dismutase was too short to protect against cocaine-induced hepatotoxicity.

1,3-Dimethylthiourea is known to act as a hydroxyl radical scavenger in the prevention of lung edema in vivo and in vitro, as a result of the great reactivity of its sulfhydryl group toward hydroxyl radicals (Fox, 1984). Pyrrolidine dithiocarbamate, which is a metal chelator and reactive oxygen species scavenger, is used in cell culture experiments as a selective and a potent inhibitor of nuclear factor kappa B (NF-κB) activation mediated by reactive oxygen species, especially hydroxyl radicals (Schreck et al., 1992). In in vivo experiments, diethyldithiocarbamate (another derivative of dithiocarbamate) was hepatotoxic because, as a potent copper chelator, it inhibited hepatic Cu, Zn-superoxide dismutase activity, thereby inhibiting O_2^- elimination (Ishiyama et al., 1990). Therefore, it is suggested that, given the deleterious effects of pyrrolidine dithiocarbamate on cocaine-induced hepatotoxicity, pyrrolidine dithiocarbamate acts in vivo as a copper chelator rather than as a reactive oxygen species scavenger in a similar manner to diethyldithiocarbamate.

The participation of hydroxyl radicals was suggested from the results of allopurinol and 1,3-dimethylthiourea. We tried to examine whether the hydroxyl radicals were produced in a transient metal ion-dependent Harber–Weiss reaction or Fenton reaction, as shown in reactions 1 and 2.

$$\text{(Harber Weiss reaction)}: O_2^- + Fe^{3+} \rightarrow O_2 + Fe^{2+},$$
$$H_2O_2 + Fe^{2+} \rightarrow {}^{\cdot}OH + OH^- + Fe^{3+} \quad (1)$$

$$\text{(Fenton reaction)}: H_2O_2 + Fe^{2+} \rightarrow {}^{\cdot}OH + OH^- + Fe^{3+} \quad (2)$$

Deferoxamine, an extracellular ferric ion chelator (Paller and Hedlund, 1994), can be used to determine the role of iron in the formation of hydroxyl radicals. The depletion of extracellular ferric ion by deferoxamine provided no protective effects against cocaine-induced hepatotoxicity. However, treatment with Fe^{3+}-deferoxamine almost completely protected mice against cocaine-induced hepatotoxicity. These results suggested that extracellular iron did not participate in the production of hydroxyl radicals. It has also been demonstrated that Fe^{3+}-deferoxamine as well as deferoxamine is capable of scavenging hydroxyl radicals and peroxynitrite (Hoe et al., 1982; Oury et al., 1993). Furthermore, in mice Fe^{3+}-deferoxamine was more protective against septic shock induced by *Corynebacterium parvum* + lipopolysaccharide than deferoxamine and its mechanism of protection was NO scavenging by the reduction of NO to N_2O (Kazmierski et al., 1996). Therefore, we hypothesized that it is the NO scavenging property of Fe^{3+}-deferoxamine that enables it to protect against cocaine-induced hepatotoxicity.

It is known that there is a transition metal-independent reaction to produce hydroxyl radicals. In this reaction, O_2^- reacts with NO to form peroxynitrite anion ($ONOO^-$), and the protonated peroxynitrite (peroxynitrous acid; HONOO) decomposes to produce hydroxyl radicals and nitrogen dioxide as shown in reaction 3.

$$O_2^- + NO \rightarrow ONOO^-, \quad ONOO^- + H^+ \rightarrow HONOO \rightarrow {}^{\cdot}OH + NO_2 \quad (3)$$

Because our results indicated that either NO depletion by aminoguanidine, $Fe^{2+}(MGD)_2$ and Fe^{3+}-deferoxamine, O_2^- depletion by allopurinol, or hydroxyl radical depletion by 1,3-dimethylthiourea protected mice against cocaine-induced hepatotoxicity, we concluded that hydroxy radicals produced from NO and intracellular O_2^- in accordance with reaction 3 may be responsible for cocaine-induced hepatotoxicity.

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