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Original Article

Auranofin protects against cocaine-induced hepatic injury through induction of heme oxygenase-1

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ABSTRACT — Auranofin, a disease-modifying gold compound, has been empirically applying to the management of rheumatoid arthritis. We investigated a protective effect of auranofin against hepatic injury induced by cocaine. Cocaine (75 mg/kg) markedly increased serum alanine amino transferase (ALT) (4,130 IU/l) and aspartate amino transferase (AST) (1,730 IU/l) activities at 16 hr after treatment, and induced hepatic necrosis surrounding central veins in mice. Concurrently, overexpression of heme oxygenase-1 (HO-1), a rate-limiting enzyme for heme degradation and an oxidative stress marker, was identified at the edges of cocaine-mediated necrotic area. Auranofin (10 mg/ml, i.p.) significantly induced hepatic HO-1 protein in mice from 12 hr after treatment. Interestingly, pretreatment with auranofin resulted in the prevention of the increase of serum ALT and AST activities in a dose-dependent manner. On the other hand, although cocaine increased tumor necrosis factor α (TNFα) gene expression in mouse livers, cocaine-induced liver injury was observed in TNFα deficient mice as well as wild-type mice. Auranofin-inducded HO-1 gene expression was observed in human primary hepatocytes as well as mouse primary hepatocytes. The present findings suggest that auranofin is effective in preventing cocaine-induced hepatic injury, and HO-1 may contribute to protect aginst chemically-induced cytotoxicity.

Key words: Auranofin, Cocaine, Heme oxygenase-1, Hepatic injury, Tumor necrosis factor α, Mouse

INTRODUCTION

Auranofin, a gold compound, is used as a generic medicine of rheumatoid arthritis (RA) and its disease-modifying effects are empirical knowledge. RA is an autoimmune disease characterized by chronic inflammation of the synovial tissue in multiple joints (Brennan and McInnes, 2008; Okamoto *et al*., 2008). It is known that overexpression of various inflammatory cytokines, such as interleukin-1 (IL-1), IL-6 and tumor necrosis factor α (TNFα), is augmented in the joint of RA patients. It is reported *in vitro* that auranofin suppresses the release of IL-1β and TNFα from immune cells and NF-κB activation following TNFα release (Bondeson and Sundler, 1995; Jeon *et al*., 2000); moreover, the drug has an ability to induce heme oxygenase (HO) -1, an oxidative stress responsive protein (Kataoka *et al*., 2001). However, action of auranofin *in vivo* and effect against chemically-induced hepatic injury are obscure.

HO enzyme, a rate-limiting enzyme for heme degradation, breaks down heme into Fe^{2+}, carbon monoxide (CO) and biliverdin (Dulak and Jozkowicz, 2003). There are two major isozymes of HO-1 and -2. HO-1 isozyme is normally expressed at low levels in almost all tissues and sensitively induced by heme, heavy metals, nitric oxide, inflammatory cytokines and pathophysiological conditions (Exner *et al*., 2004; Li *et al*., 2007; Oguro and Yoshida, 2004). On the other hand, HO-2 is the consti-

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tutive isozyme including testis, liver, kidney and brain (McCoubrey *et al*., 1997). Several studies have reported that HO-1 protects against oxidative injury and tumor necrosis factor (TNF)-mediated apoptosis by regulating cellular redox state (Gozzelino *et al*., 2010). We also found that HO-1 plays an important role in the negative feedback regulation of the pro-inflammatory inducible nitric oxide synthase (iNOS) system (Ashino *et al*., 2008). The biological defense effect of HO-1 is provided by its enzymatic reaction products, CO and biliverdin which function as an anti-inflammatory agent and a radical scavenger, respectively (Dulak and Jozkowicz, 2003; Ryter and Choi, 2009).

Cocaine is a local anesthetic and well known to be one of major abuse drugs. It is known that cocaine mediates organ toxicities including injury to the heart and the liver (Aoki *et al*., 1997; Wang *et al*., 2001). The liver is more sensitive than other organs to cocaine toxicity (Wang *et al*., 2001). Cocaine is metabolized by the hydrolytic and/or the oxidative pathways. Hydrolysates of cocaine, which are ecgonine methy ester, benzoylecgonine and ecgonine, are inactive and nontoxic (Kloss *et al*., 1984). On the other hand, oxidative metabolites of cocaine, which are norcocaine, *N*-hydroxynorcocaine and norcocaine nitroxide, are believed to be responsible hepatotoxicity (Boelsterli and Goldlin, 1991; Bornheim, 1998). Two major mechanisms have been assumed to explain cocaine-induced hepatotoxicity. Firstly, oxidative metabolites of cocaine bind covalently to cellular proteins and/or lipids. Secondly, a redox cycling between *N*-hydroxynorcocaine and norcocaine nitroxide by cytochrome P450 (P450) results in production of reactive oxygen species (ROS) (Boelsterli and Goldlin, 1991; Goldlin and Boelsterli, 1991). These results lead to inactivation of enzymes and/or membrane damage followed by cell death.

It has been hypothesized that the binding of active metabolites to cellular macromolecules and the production ROS involved in organ toxicity caused by chemical compounds. Here we demonstrate the protective effect of auranofin against cocaine-induced hepatic injury associated with induction of HO-1, and show the overexpression of HO-1 surrounding central veins in the liver of mice treated with cocaine. These findings provide insight into auranofin as a protecting agent and HO-1 as a potential therapeutic target for organ injuries.

MATERIALS AND METHODS

Reagents

Cocaine hydrochloride was obtained from Takeda Pharmaceutical Company (Osaka, Japan). Auranofin and HO-1 antibody were obtained from Enzo Life Sciences (Plymouth Meeting, PA, USA). Deoxycytidine-5'-[α-^{32}P]-triphosphate (3,000 Ci/mmol) was from Japan Isotope Association (Tokyo, Japan). All other reagents used were of the highest grade commercially available.

Animals and Treatments

All animal experiments were carried out under the control of the Committee Regulation of Animal Care and Welfare of Showa University. Male BALB/c mice (8 weeks old) were purchased from Japan SLC (Shizuoka, Japan). The TNFα deficient $^{(-/-)}$ mice were established by Tagawa *et al.* (1997). BALB/c mice were mated with TNFα$^{(-/-)}$ mice. Wild-type (WT) and deficient mice progenies were selected by their respective gene expressions, and the lines for WT or deficient mice were established. All protocols used in this study were reviewed and approved by the Institutional Animal Care and Use Committee of Showa University (#25029). Cocaine was dissolved in saline and injected intraperitoneally at the doses indicated in the Figs. Auranofin was dissolved in corn oil containing 5% ethanol and injected intraperitoneally at the doses indicated in the Figs. Control groups were injected with respective vehicles in a volume similar to that of treated mice.

Cell culture

Mouse primary hepatocytes were separated from the livers of 8-weeks-old male BALB/c mice using the collagenase perfusion method. The mouse hepatocytes were plated in collagen-coated dish with Waymouth MB 752/1 medium containing 5 µl/ml SITE+3 Liquid Media Supplement. Human livers were provided from National Disease Research Interchange (Philadelphia, PA, USA) through Human and Animal Bridging Research Organization (Tokyo, Japan). The human hepatocytes were plated in collagen-coated dish with Williams' Medium E containing 10% fetal bovine serum. This study was approved by the Ethical Committee of Human and Animal Bridging Research Organization.

Measurement of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities

Serum samples were collected under etherization. Serum ALT and AST activities were determined by POP-TOOS method using the Transaminase CII-test WAKO kits (Wako Pure Chemical, Osaka, Japan).

Preparation of liver microsomal fraction

Livers were promptly removed from the body and

were perfused with ice-cold 0.9% NaCl solution. The livers were homogenized immediately with 4 volumes of 1.15% (w/v) KCl solution. The homogenates were centrifuged at 9,000 *g* for 20 min, and the resulting supernatants were further ultracentrifuged at 105,000 *g* for 60 min. The resulting microsomal pellet was suspended in 0.1 M sodium-potassium phosphate buffer (pH 7.4) containing 20% glycerol. Protein concentration was determined by the method of Lowry *et al.* (1951).

Western blot analysis

The relative level of HO-1 protein was detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting. Microsomal proteins (10 μg) were separated by SDS-PAGE (5% stacking gel, 10% separating gel) according to the method of Laemmli (1970) and transferred electrophoretically onto polyvinylidene difluoride membranes. Blots were immunolabeled with HO-1 antibody. Peroxidase-conjugated proteins were detected by chemiliminescence. The relative densities were analyzed using Scion image software (Version 1.62).

Immunohistochemical analysis

Livers were fixed in 4% paraformaldehyde in PBS overnight, and were embedded in paraffin. Paraffin-embedded tissue sections were stained with HO-1 antibody, followed by a peroxidase- and 2nd antibody-conjugated amino acid polymers (NICHIREI Biosciences, Tokyo, Japan). Staining was developed using the DAB Kit (Vector Laboratories, Burlingame, CA, USA). Images were captured by Biozero BZ-8000 microscope (Keyence, Osaka, Japan).

Northern blot analysis

Total RNA was isolated from the livers of mouse or the hepatocytes using the acid guanidine thiocyanate-phenol-chloroform extraction method. Total RNA (20 μg) was fractionated by electrophoresis on 1.1% agarose gel followed by transfer onto a nylon membrane. Northern blots were hybridized with ^{32}P-labeled cDNA for mouse HO-1 (1.1 kb, *Bam*HI fragment of pMp32cod), human HO-1 (1.0 kb, *Xho*I/*Xba*I fragment of pHHO-1) or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (0.5 kb, *Pst*I fragment of GD5). The hybridization levels were semi-quantitated with a bio-imaging analyzer (BAS3000, Fuji Photo Film Co., Tokyo, Japan).

Quantitative real-time polymerase chain reaction (PCR)

Total RNA was isolated from the liver using RNeasy® Mini Kit (QIAGEN, Hilden, Germany), and first-stranded cDNA was synthesized with a PrimeScript™ Master Mix (Takara Bio, Tokyo, Japan). The quantitative real-time PCR was performed according to the manufacturer's protocol using StepOne™ real-time PCR system (Applied Biosystems Japan, Tokyo, Japan) and QuantiTect SYBR Green RT-PCR (Qiagen). The respective PCR primers were prepared by QuantiTect Primer Assays (Qiagen). The mRNA levels were measured as the relative ratio to GAPDH mRNA.

Hepatic total P450 content

Hepatic total P450 content was determined from a carbon monoxide difference spectrum of dithionite-treated microsomes suspended in 0.1 M sodium-potassium phosphate buffer (pH 7.4) containing 20% glycerol and recorded with a MPS-2450 spectrophotometer (Shimadzu, Tokyo, Japan) using a difference extinction coefficient (450-490 nm) of 91 mM^{-1} cm^{-1} as described by Omura and Sato (1964).

Statistical analysis

All data are expressed as means ± S.E.M. The statistical analysis was performed at the analysis indicated in the figures. The accepted level of significance was set at $p < 0.05$.

RESULTS

Cocaine-induced hepatic injury in mice

Cocaine is known to induce hepatic injury (Wang *et al*., 2001; Aoki *et al*., 1997). Consequently, we first performed time-course experiments of cocaine treatment to characterize the increase pattern of serum ALT and AST activities, indicators of hepatic injury (Fig. 1A). Cocaine (75 mg/kg) markedly increased serum ALT (4,130 IU/l) and AST (1,730 IU/l) activities at 16 hr after treatment. We next observed histological changes of the liver 16 hr after cocaine treatment (Fig. 1B). The liver tissues from mice with high serum ALT and AST activities showed hepatic necrosis surrounding central veins.

Induction of HO-1 by cocaine in mouse livers

It is known that HO-1 is induced in injured organs (Bauer *et al*., 2000; Nakahira *et al*., 2003). Therefore, we examined time-course effects of cocaine on HO-1 induction in mouse livers (Fig. 2A). Cocaine (75 mg/kg) induced HO-1 protein at 12 hr after treatment, and reached to the peak level at 16 hr (400% of the controls) in mouse livers. To determine the location of cocaine-induced HO-1 expression, we examined an immunohistochemical analysis in cocaine-treated mouse livers. In the liver of control mice, positive staining of HO-1 was observed

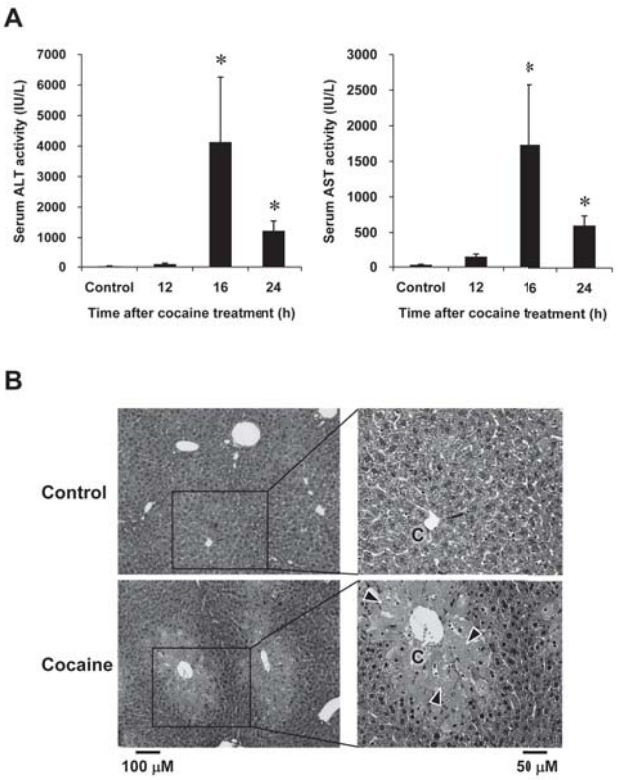

Fig. 1. Cocaine-mediated hepatic injury in mice. (A) Mice were treated intraperitoneally with cocaine (75 mg/kg), and their sera were obtained under etherization at the times indicated. Serum aminotransferase activities were determined by POP-TOOS method. Values represent the mean ± S.E.M. (*n = 3).* The significance of difference was assessed by *one-way ANOVA*, followed by the *Dunnett's test*. *, significantly different from the controls at $p < 0.05$. (B) Mice were treated intraperitoneally with cocaine (75 mg/kg), and their livers were excised at 16 hr after treatment. Areas of necrosis of hepatocytes were identified by hematoxylin/eosin staining. Arrowheads indicate necrotic hepatocytes. C denote central vein.

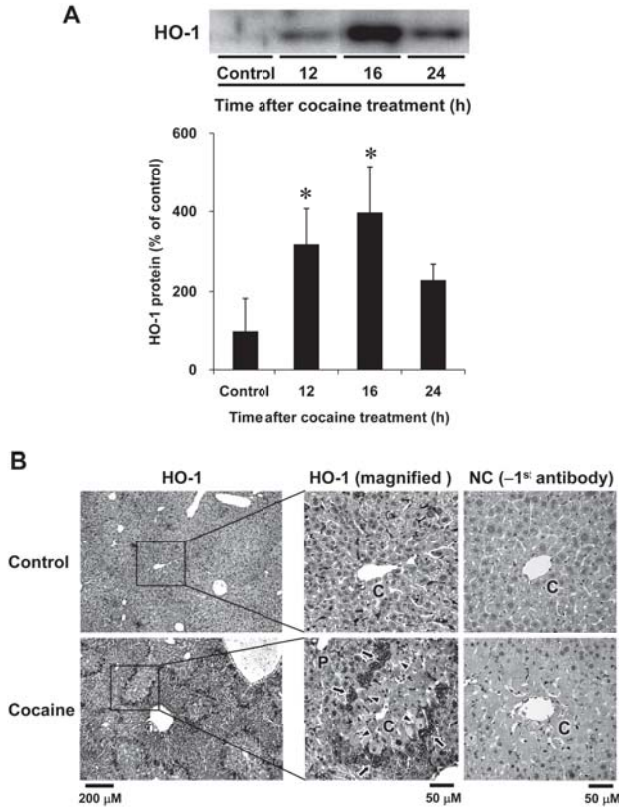

Fig. 2. Highly expression of HO-1 at the edges of cocaine-mediated necrotic hepatocytes. (A) Mice were treated intraperitoneally with cocaine (75 mg/kg), and their livers were excised at the times indicated. Microsomal protein was isolated from the livers, and Western blot analysis was performed using 10 μg microsomal protein. Values represent the mean ± S.E.M. (*n = 3).* The significance of difference was assessed by *one-way ANOVA,* followed by the *Dunnett's test*. *, significantly different from the controls at $p < 0.05$. (B) Mice were treated intraperitoneally with cocaine (75 mg/kg), and their livers were excised at 16 hr after treatment. Immunohistochemical analysis for vehicle-treated control liver or cocaine-treated injured liver stained with anti-HO-1 antibody. Arrows indicate positively stained hepatocytes, and arrowheads indicate necrotic hepatocytes. P and C denote portal tract and central vein, respectively.

only Kupffer cells (Supplemental Fig.) (Nakahira *et al*., 2003). In contrast, HO-1 protein expression was robustly increased in hepatocytes at the edges of cocaine-mediated necrotic area (Fig. 2B and Supplemental Fig.).

Time-dependent changes of hepatic HO-1 and P450 content in auranofin-treated mice

There are a few papers showing the induction of HO-1 by auranofin in some cell lines (Kim *et al*., 2010; Kataoka *et al*., 2001). However, effect of auranofin on HO-1 induction in mouse livers has not been determined. Accordingly, we investigated HO-1 induction by auranofin in mouse livers. Mice were injected with auranofin, and the mRNA levels and the protein levels of HO-1 were determined by Northern blot and Western blot analysis, respectively. Auranofin (10 mg/kg) induced hepatic HO-1 mRNA from 2 hr after treatment and reached a peak level at 4 hr (Fig. 3A). Likewise, the HO-1 protein reached 200% of control group at 24 hr after auranofin treatment

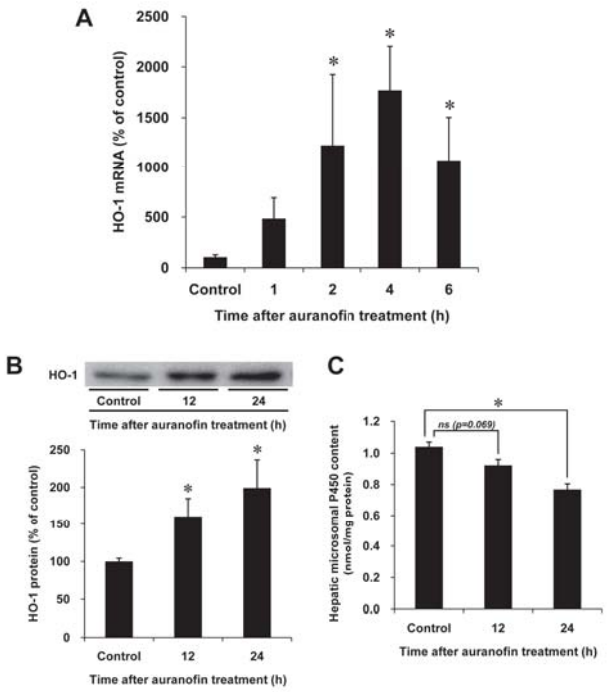

Fig. 3. Effect of auranofin on HO-1 expression and P450 content in mouse livers. Mice were treated intraperitoneally with auranofin (10 mg/kg), and their livers were excised at the times indicated. (A) Total RNA was isolated from the livers, and Northern blot was performed. Blots for HO-1 mRNA were semiquantified by normalizing with that for GAPDH mRNA. (B) Microsomal protein was isolated from the livers, and Western blot analysis was performed using 10 μg microsomal protein. (C) Microsomal protein was isolated from the livers, and total P450 content was assayed from the carbon monoxide difference spectrum of the reduced protein. Values represent the mean ± S.E.M. (*n = 3-4).* The significance of difference was assessed by *one-way ANOVA,* followed by the *Dunnett's test*. *, significantly different from the controls at $p < 0.05$. *ns*, indicating no significant difference.

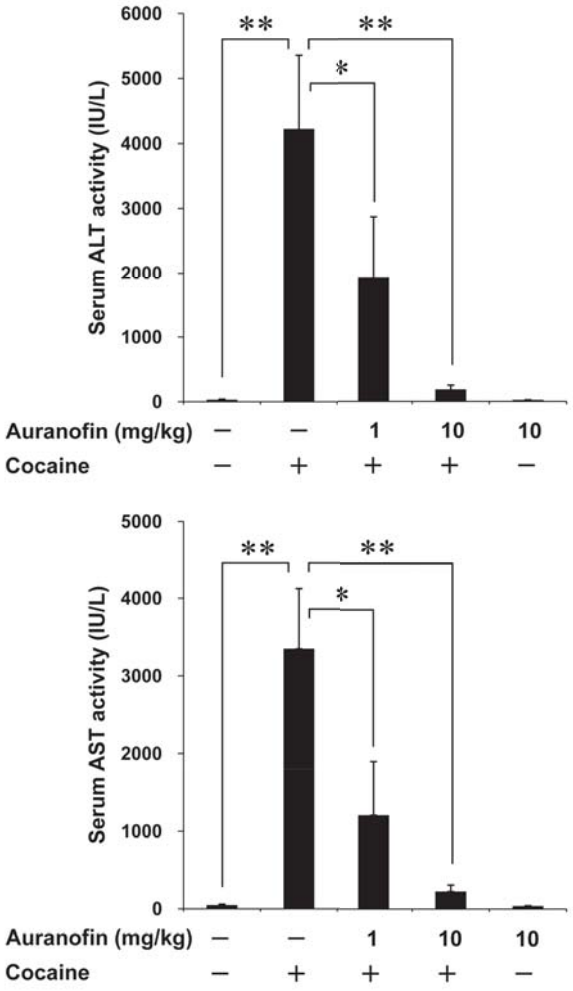

Fig. 4. Protection against cocaine-mediated hepatic injury by pretreatment with auranofin in mice. Mice were pretreated intraperitoneally with auranofin (1 mg/kg or 10 mg/kg). After 12 hr, the mice were treated intraperitoneally with cocaine (75 mg/kg), and their sera were obtained under etherization at 16 hr after cocaine treatment. Serum aminotransferase activities were determined by POP-TOOS method. Values represent the mean ± S.E.M. (*n = 3-7).* The significance of difference was assessed by *one-way ANOVA*, followed by the *Tukey-Kramer test* (**$p < 0.01$, *$p < 0.05$).

(Fig. 3B). HO enzyme breaks down heme. Therefore, we also examined effect of auranofin on heme-containing P450 enzyme content in mouse livers (Fig. 3C). Auranofin (10 mg/kg) decreased hepatic P450 content at 24 hr after treatment (74% of the controls), but no difference at 12 hr after treatment.

Change of cocaine-mediated increase of aminotransferase activities by pretreatment with auranofin in mice

Because auranofin induced HO-1 in mouse livers (Fig. 3), we next investigated a protective effect of auranofin against cocaine-mediated hepatic injury in mice (Fig. 4). Similarly to Fig. 1A, cocaine markedly increased serum ALT and AST activities approximately 4,230 IU/l and 3,350 IU/l, respectively. Pretreatment with 1 mg/kg or 10 mg/kg auranofin significantly inhibited cocaine-mediated increase of serum ALT activitiy 54% and 96%, respectively, and AST activity 64% and 93%, respectively.

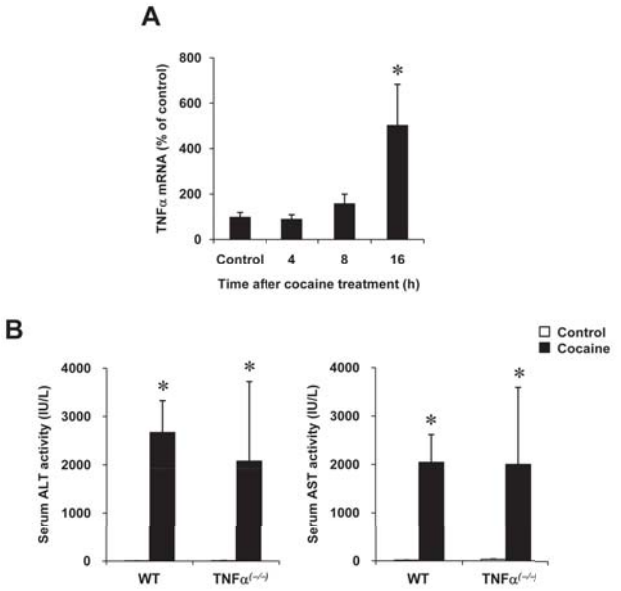

Fig. 5. Effect of cocaine on elevation of serum ALT and AST activities in TNFα$^{(-/-)}$ and WT mice. (A) Mice were treated intraperitoneally with cocainea (75 mg/kg), and their livers were excised at the times indicated. TNFα mRNA levels were determined by real-time PCR and semiquantified by normalizing with that for GAPDH mRNA. Values represent the mean ± S.E.M. (*n = 3-5*). The significance of difference was assessed by *one-way ANOVA*, followed by the *Dunnett test*. *, significantly different from the controls at $p < 0.05$. (B) Mice were treated intraperitoneally with cocaine (75 mg/kg), and their sera were obtained under etherization at 16 hr after treatment. Serum aminotransferase activities were determined by POP-TOOS method. Values represent the mean ± S.E.M. (*n = 4)*. The significance of difference was assessed by the Student's *t*-test. *, significantly different from the respective controls at $p < 0.05$.

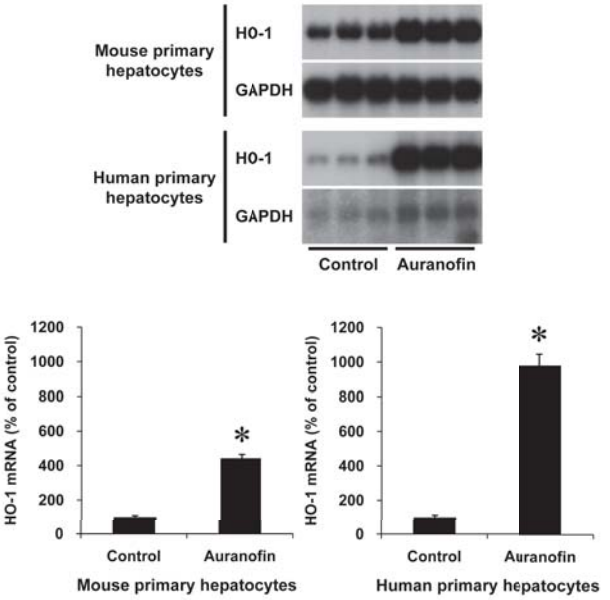

Fig. 6. Induction of HO-1 gene expression by auranofin in mouse and human primary hepatocytes. The respective hepatocytes were treated with anranofin (1 μM) and harvested at 4 hr after treatment. Total RNA was isolated from the hepatocytes, and Northern blot was performed. Blots for HO-1 mRNA were semiquantified by normalizing with that for GAPDH mRNA. Values represent the mean ± S.E.M. (*n = 3)*. The significance of difference was assessed by the *Student's t-test*. *, significantly different from the controls at $p < 0.05$.

TNFα is not involved in cocaine-induced liver injury in mice

It is known that TNFα is involved in development and exacerbation of liver injury (Schwabe and Brenner, 2006). Therefore, we examined effect of cocaine on TNFα gene expression in mouse livers (Fig. 5A). Cocaine (75 mg/kg) induced TNFα mRNA at 16 hr after treatment (504% of the controls). We next examined the effect of cocaine on elevation of serum ALT and AST activities in TNFα$^{(-/-)}$ and WT mice (Fig. 5B). Cocaine markedly increased serum ALT and AST activities at 16 hr after treatment in TNFα$^{(-/-)}$ mice as well as WT mice, while no statistical difference was seen between WT mice and TNFα$^{(-/-)}$ mice.

Effect of auranofin on HO-1 induction in mouse and human primary hepatocytes

HO-1 induction by auranofin in human hepatocytes has not been reported. Therefore, we examined the induction of HO-1 by auranofin using human and mouse primary hepatocytes (Fig. 6). Auranofin (1 μM) significantly induced HO-1 mRNA in mouse primary hepatocytes (440% of the controls) similarly to mouse livers *in vivo* (Fig. 3). Surprisingly, auranofin markedly induced HO-1 mRNA in human hepatocytes (980% of the controls).

DISCUSSION

In the present study, we dealt with the effect of auranofin on cocaine-induced hepatic injury. To our knowledge, this is the first report demonstrating that auranofin acts as a cytoprotective agent against chemically-induced hepatic injury. Furthermore, we showed that auranofin induced HO-1 in hepatocytes *in vivo* and *in vitro*. The present results provide a potential of auranofin

for cytoprotective agent and HO-1 as a therapeutic target against chemically-induced cytotoxicity.

We have shown in this study that HO-1 protein is robustly expressed in hepatocytes surrounding the necrotic area at 16 hr after cocaine treatment (Fig. 2B and Supplemental Fig.). Acetaminophen and carbon tetrachloride are well-known compounds to induce hepatic injury as well as cocaine, and also increase HO-1 in the liver (Bauer *et al*., 2000; Nakahira *et al*., 2003; Aleksunes *et al*., 2005). It is generally thought that hepatotoxicity caused by these three compounds is due to reactive intermediates, which are generated by hepatic P450. It is suggested that the reactive intermediates induce oxidative stress followed by HO-1 induction. Several studies have reported that HO-1 exerts protective effects against a variety of pathological conditions such as ischemia and reperfusion injury, endotoxin shock or chemically-induced hepatic injury (Devey *et al*., 2009; Zhang *et al*., 2004; Kamimoto *et al*., 2009; Nakahira *et al*., 2003). The cytoprotective effect of HO-1 on oxidative stress and pathological conditions depend on biliverdin, a radical acavenger, or CO, an anti-inflammatory gas, both of which are degradation products of heme catabolizing by HO-1 (Dulak and Jozkowicz, 2003; Ryter and Choi, 2009). These reports suggest that HO-1 induction occurs in order to protect hepatic cells from toxicity of cocaine.

Auranofin has been empirically applied to the management of RA; however, little has been reported on the effect of auranofin on other pathological conditions. Several studies have reported that auranofin induces HO-1 via Nrf2 system in immune cells such as THP-1 and U937 cells (Kataoka *et al*., 2001; Kim *et al*., 2010). However, HO-1 induction by auranofin *in vivo* had not been clarified. Hepatic HO-1 mRNA was increased and was reached a maximum level at 4 hr after auranofin treatment in mice (Fig. 3A). Consistent with a marked elevation in HO-1 mRNA, hepatic HO-1 protein was also induced from 12 hr after auranofin treatment (Fig. 3B). Our studies are the first to characterize HO-1 inducing effect of auranofin *in vivo*. We also demonstrated that cocaine-induced hepatic injury is blocked by pretreating mice with auranofin at 12 hr before cocaine treatment (Fig. 4). It is suggest that HO-1 induction by preloading with auranofin is important in prevention of reactive intermediates-induced oxidative stress and hepatotoxicity.

In the present study, cocaine significantly induced TNFα mRNA (Fig. 5A), suggesting that TNFα is derived from Kupffer cells. It has been reported that lipopolysaccharide, an immunostimulant, potentiated the hepatotoxicity of cocaine (Labib *et al*., 2002), and nitric oxide involved in cocaine-induced hepatic injury (Aoki *et al*., 1997). Auranofin was previously reported to inhibit transcriptional activation of TNFα gene by blocking IκB kinase followed by IκB degradation and NF-κB activation in macrophages (Bondeson and Sundler, 1995; Jeon *et al*., 2000, 2003). IκB/NF-κB pathway also plays a central role in the expression of iNOS gene (Pautz *et al*., 2010). We previously reported that HO-1 inhibits LPS-induced iNOS gene expression in mouse primary macrophages (Ashino *et al*., 2008). Other studies have shown that HO-1 protects endothelial cells from undergoing TNF-mediated apoptosis (Soares *et al*., 1998). These reports propose that auranofin inhibits TNFα expression, resulting in protection against cocaine-induced hepatic injury. However, cocaine-induced hepatic injury did not attenuate in TNFα$^{(-/-)}$ mice (Fig. 5B). These results suggest that the protective effect of auranofin against cocaine-induced acute hepatic injury is not involved in blocking production of TNFα.

We previously reported that TNFα is involved in HO-1 induction in the liver of mice treated with lipopolysaccharide (Oguro *et al*., 2002). The present study demonstrated that hepatic HO-1 mRNA is increased in hepatocytes of cocaine-induced hepatic injury in mice. These results assume that cocaine-induced TNFα increase HO-1 gene expression. HO-1 protein significantly increased from 12 hr, and reached a maximum level at 16 hr after cocaine treatment (Fig. 2A). In contrast, TNFα mRNA increased significantly at 16 hr after cocaine treatment (Fig. 5A). Furthermore, though HO-1 induction occurred exclusively in hepatocytes around the necrotic area (Fig. 2B and Supplemental Fig.), its induction was not observed around Kupffer cells, which release inflammatory cytokines (Supplemental Fig.). These results suggest that HO-1 induction by cocaine is not attributed to cocaine-induced TNFα.

Liver is an important organ in xenobiotic metabolism. In general, xenobiotics are polarized and excreted safely. However, in some cases, xenobiotics are metabolized by P450 reductively to reactive intermediates, thereby inducing hepatotoxicty. It is reported that hepatic injury caused by acetaminophen or carbon tetrachloride is involved in reactive intermediates (James *et al*., 2003; De Groot and Sies, 1989). It is assumed that the binding of oxidative metabolites of cocaine to cellular proteins and/or the norcocaine nitroxide radical-induced oxidative stress evoke hepatotoxicity (Boelsterli and Goldlin, 1991; Bornheim, 1998). Therefore, cocaine hepatotoxicity is affected by metabolizing activity of P450. HO enzyme breaks down heme, which is an important component of P450 enzymes. We demonstrated that auranofin shows no significant change in hepatic P450 holoenzyme content at

12 hr after treatment (Fig. 2C). It is suggested that pretreating mice with auranofin at 12 hr before cocaine treatment does not attenuate metabolism of cocaine by P450.

In summary, we showed the protective effect of auranofin against cocaine-induced hepatic injury, and the induction of HO-1 by auranofin in mouse livers. We conclude that HO-1 induction may play an important role in conferring protection on hepatocytes from oxidative damage caused by reactive intermediates. Furthermore, we demonstrated for the first time that auranofin potentially induced HO-1 in human primary hepatocytes (Fig. 6). This result suggests that auranofin is also effective to protect chemically-induced hepatic injury in human. Further studies are needed in order to elucidate the protective roles of auranofin-induced HO-1 against chemically-induced hepatic injury.

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