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Oxidative stress in rat liver during acute cadmium and ethanol intoxication

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Abstract: The aim of this study was to investigate the effects of binge drinking on the prooxidant/antioxidant system in rat liver in acute cadmium (Cd) intoxication. Male Wistar rats were used in the experiments. They were divided into the following groups: 1. control, 2. ethanol-treated group, in five subsequent doses of 2 g kg^{-1}, administered by an orogastric tube, 3. Cd-treated group in a single dose of 2.5 mg kg^{-1}, administered intraperitoneally, 4. group that received Cd 12 h after the last dose of ethanol. Blood and liver samples for determination of oxidative stress parameters, were collected 24 h after treatment. When administered in combination, ethanol and Cd induced a more pronounced increase in the serum and liver malondialdehyde levels than either of the substances alone (p<0.01). Liver manganese superoxide dismutase (MnSOD) activity was increased in both the ethanol- and Cd-treated groups (p<0.01), while liver copper/zinc superoxide dismutase (Cu/ZnSOD) activity was elevated in the Cd group only. However, when administered in combination, ethanol and Cd induced a more pronounced decrease in liver MnSOD and Cu/ /ZnSOD activity 24 h after treatment (p<0.01). Based on our study, it can be concluded that ethanol may act synergistically with Cd in inducing lipid peroxidation and reduction in liver SOD activity.

Keywords: ethanol; reactive oxygen species; cadmium; liver injury; rat.

INTRODUCTION

Cadmium is one of the most toxic substances in the environment due to its toxic effects on multiple organ systems and long elimination half-time.[1] The initial site of its accumulation is liver; hence, acute exposure to large Cd doses re-

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sults in liver injury. Morphologic changes in the liver that follow acute Cd exposition depend on the administered dose and on time when these changes are observed and may vary from dilation of the rough endoplasmic reticulum with loss of ribosomes to hepatocellular necrosis, becoming evident 10–12 h after exposure.[2] The mechanisms of acute Cd hepatotoxicity are not completely understood. Cd ions have a high affinity for thiol groups and form cadmium–thiol complexes. Interaction of Cd ions with thiol-containing molecules (glutathione, GSH, and metallothionein, MT) could protect cells and body from the toxicity of Cd. MT I and II are small proteins rich in cysteine thiols. Since thiol groups are involved in the function of many enzymes, structure proteins and receptors, the cadmium-thiol complexes possibly disturb many functions of cells. It is strongly believed that the toxic effects develop in two phases. Primary injury to hepatocytes is induced by Cd binding to sulfhydryl groups and their inactivation leads to mitochondrial dysfunction, mitochondrial permeability transition and oxidative stress.[2] Oxidative stress, at least partly, may develop as a result of GSH depletion.[3] An additional direct mechanism of acute Cd hepatotoxicity includes ischemia of hepatocytes due to Cd-induced direct injury of sinusoidal endothelial cells.[4–6] Secondary injury to the liver appears as a result of inflammation initiated by the activation of Kupffer cells. Infiltrating neutrophiles, macrophages, as well as resident cells (hepatocytes, endothelial cells, and stellate cells) synthesize and release various cytokines, chemokines and other proinflammatory mediators, thus aggravating initial injury caused directly by Cd.[2]

Ethanol, an active compound of alcoholic beverages, is a well-known hepatotoxin, when administered either acutely or chronically. Various mechanisms are involved in its hepatotoxicity, including direct damage by ethanol or its metabolite acetaldehyde, oxidative stress, release of endotoxin from gut lumen, induction of immune response and release of various cytokines and proinflammatory mediators from infiltrating leukocytes.[7–12] In addition, ethanol may induce hypoxia of hepatocytes, due to increased consumption of oxygen in ethanol metabolism.[13]

Interactions between ethanol and Cd are of great medical importance, since many people exposed to Cd are also prone to excessive alcohol consumption. Possible interactions between these hepatotoxins have been studied in various investigations, usually in models of chronic Cd intoxication.[14–17] It has been suggested that ethanol modifies Cd metabolism and its effect on the metabolism of other bioelements, including iron, zinc and copper.[14,18] On the other hand, precise data related to interactions between ethanol and Cd during acute intoxication are still lacking from the currently available literature. Since oxidative stress was suggested to be a possible mechanism of both Cd- and ethanol-induced liver injury, the aim of the present study was to investigate the effects of binge

drinking on the prooxidant/antioxidant system in rat liver in acute cadmium intoxication.

EXPERIMENTAL

Animals

The experiment was performed on adult male Wistar rats weighting 220–250 g, raised at the Military Medical Academy, Belgrade. The animals were kept under standard laboratory conditions (temperature 22±2 °C, relative humidity 50±10 %, 12/12 light–dark cycle with lights turned on at 9 am) and had free access to tap water and standard pelleted LM2 food (Veterinary Institute "Subotica", Subotica, Serbia). The diet, which had metabolizable energy of the least 11.5 MJ kg^{-1}, was composed of a maximum of 7 % cellulose, and a minimum of 19 % protein. On the day prior to the sacrifice, the rats were fasted overnight. The study was performed according to the Guidelines for Animal Study No. 282-12/2002 and was approved by the Ethic Committee of the Military Medical Academy for animal experiments.

All animals (n = 32) were randomly divided into the following groups: 1. control, saline--treated group (0.9 % NaCl) (n = 8); 2. ethanol-treated group (E; n = 8) in five subsequent doses of 2 g kg^{-1}, administered at 12 h intervals by the oral route (orogastric tube); 3. cadmium-treated group (Cd; n = 8) in a dose of 2.5 mg kg^{-1} intraperitonally (i.p.); 4. cadmium- and ethanol-treated group (CdE; n = 8). Ethanol was administered to the CdE group in five subsequent doses in the same manner as was employed for the E group. 12 h after the last dose of ethanol, the animals were treated with cadmium in a dose of 2.5 mg kg^{-1} i.p. The animals in the Cd and E group received saline instead of Cd and ethanol, respectively. For oral administration, ethanol was dissolved in distilled water in a 30 % v/v concentration. Cadmium was dissolved in saline (0.9 % NaCl) before intraperitonal administration.

Rats were sacrificed by cervical dislocation 24 h after cadmium administration (or saline for E and control group). Blood samples for determination of oxidative stress parameters were collected from the right side of the heart. For the same purpose, livers were excised and stored as described below.

Analysis

Liver samples for biochemical analysis were homogenized on ice (Ultrasonic homogenizer Sonopul), in cold buffered 0.25 M sucrose medium (Serva, Heidelberg, New York), 10 mM phosphate buffer (pH 7.0) and 1.0 mM ethylenediaminetetraacetic acid (EDTA) (Sigma, USA). The homogenates were centrifuged at 2000×g for 15 min at 4 °C (Eppendorf centrifuge 5804 R). The crude sediments were dissolved in the sucrose medium and centrifuged in the same manner. The supernatants were transferred into tubes and centrifuged at 3200×g for 30 min at 4 °C. The obtained sediments were dissolved in deionized water. After one hour of incubation, the samples were centrifuged at 3000×g for 15 min at 4 °C, and supernatants were stored at –70 °C. Proteins were determined by the Lowry method using bovine serum albumin as the standard.[19]

Lipid peroxidation analysis in the plasma and liver homogenates was measured as malondialdehyde (MDA) production, assayed in the thiobarbituric acid reaction as described by Girotti *et al*.[20] The results are expressed as μmol L^{-1} in the plasma or nmol mg^{-1} proteins in the liver homogenates.

The serum concentration of nitrates and nitrites (NO_x) as a measure of nitric oxide (NO) production was determined using Griess reagent. The nitrates were reduced to nitrites by incubating the serum sample with a nitrate reductase and the total amount of nitrite was then

determined by the Griess method. With nitrites, the Griess reagent forms a purple azo dye, which can be measured spectrophotometrically at 492 nm (Ultrospec 2000 spectrophotometer, Pharmacia Biotech).[21]

The thiol group of GSH reacts with DTNB (5,5'- dithiobis[2-nitrobenzoic acid], Ellman reagent) and produces yellow colored 5-mercapto-2-nitrobenzoic acid (TNB). The mixed disulfide, GSTNB that is concomitantly produced, is reduced by glutathione reductase to recycle the GSH and produce more TNB. The rate of TNB production is directly proportional to the concentration of GSH in the deproteinized sample. Measurement of the absorbance of TNB at 414 nm provides an accurate estimation of GSH in the sample and a GSH standard curve.[22]

Total content of sulfhydryl groups (–SH) in the plasma was measured spectrophotometrically at 412 nm in phosphate buffer (0.20 M + 2.0 mM EDTA, pH 9) using 5,5'--dithiobis[2-nitrobenzoic acid] (DTNB, 0.01 M, Sigma).[23]

Total superoxide dismutase (EC1.15.1.1.;SOD) activity in the liver was measured spectrophotometrically, as the inhibition of epinephrine auto-oxidation at 480 nm. After addition of 10 mM epinephrine (Sigma, USA), the analysis was performed in sodium carbonate buffer (50 mM, pH 10.2; Serva, Heidelberg, New York) containing 0.1 mM EDTA (Sigma, St. Louis, CA, USA). Samples for MnSOD were previously treated with 8 mM potassium cyanide (KCN) (Sigma, USA) and then analyzed as described.[24]

For all analyses, three probes were used.

Histological examination

For light microscopic evaluation, the liver tissues were fixed in 10 % neutral buffered formaldehyde and embedded in paraffin wax. Sections from paraffin blocks were cut at 5 mm, mounted on slides, stained with Masson Trichrome and examined by a Leica DFC280 light microscope.

Chemicals

All reagents and chemicals were of analytical grade or higher purity. Ethanol was purchased from Merck (Germany). Cadmium was obtained from Sigma (USA).

Statistical analysis

The results are expressed as means±*SD*. For testing the difference among groups, one--way analysis of variance with Fisher's post hoc test was used. The difference was considered statistically significant for $p<0.05$. Statistica 7.0 was used for the statistical analysis.

RESULTS

The obtained results showed that the liver MDA level was significantly higher in the groups that received ethanol (27.82±4.42 nmol mg^{-1} prot.) or cadmium (73.54±11.62 nmol mg^{-1} prot.) in comparison with the control group (14.19±3.23 nmol mg^{-1} prot.) 24 h after treatment ($p<0.05$ and $p<0.01$, respectively). Previous binge drinking was found to induce a more pronounced increase in the liver MDA level (86.62±22.09 nmol mg^{-1} prot.) than cadmium alone ($p<0.01$). The serum MDA concentration was significantly higher in all the treated animals. The highest MDA concentration (about 120 % higher than in control group) was measured in the serum of animals that were co-exposed to ethanol and cadmium (266.59±23.62 μmol L^{-1}).

The serum nitrates and nitrites (NO_x) concentration was significantly higher in the ethanol and in the cadmium-treated group (7.618±0.907 and 16.374±1.821 μmol L^{-1}, respectively) in comparison with the control group (121.65±6.82 μmol L^{-1}), as well as in the Cd-treated group compared to the ethanol group, 24 h after treatment (p<0.01). Moreover, administration of cadmium 12 h after the last dose of ethanol caused a significant rise in the serum NO_x concentration (16.079± ±2.546 μmol L^{-1}) in comparison with group that received ethanol alone (p<0.01) (Fig. 1). No significant difference in the NO_x concentration was detected between the Cd and CdE group (p>0.05).

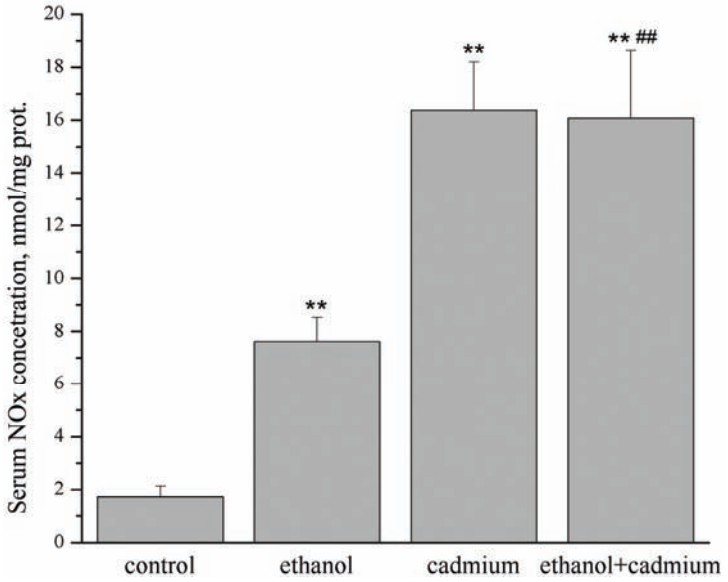

Fig. 1. Serum concentration of nitrites and nitrates (NO_x) in animals 24 h after treatment. For statistical evaluation, the one-way Anova with the Fisher *post hoc* test were used; **p<0.01 *vs.* the control group, $^{\#\#}p$<0.01 in comparison with the ethanol-group.

The liver GSH content was found to be significantly decreased in the ethanol-treated group (20.26±2.57 nmol mg^{-1} prot.) in comparison with the control group (29.41±4.21 nmol mg^{-1} prot.) (p<0.01). In contrast, a single dose of cadmium did not induce significant changes in the GSH level 24 h after administration (p>0.05). However, prior ethanol treatment induced a significant decrease in the liver GSH content (20.42±3.08 nmol mg^{-1} prot.) (p<0.01), but the extent of this decrease was not different from the decrease induced by ethanol alone (Fig. 2).

The plasma concentration of total sulfhydryl groups was significantly lower in ethanol-treated group (0.239±0.031 μmol L^{-1}) in comparison with the control animals (0.372±0.047 μmol L^{-1}) 24 h after ethanol administration (p<0.01). In

contrast, cadmium induced a significant increase in plasma concentration of total sulfhydryl groups (0.461±0.078 μmol L^{-1}) in the same time interval (p<0.05). When animals were exposed to ethanol prior to cadmium, a significant decrease in total sulfhydryl group concentration (0.225±0.020 μmol L^{-1}) was detected in comparison with animals that received cadmium alone (0.461±0.078 μmol L^{-1}) (p<0.01) (Fig. 3). However, no significant change was observed between E and CdE groups (p>0.05).

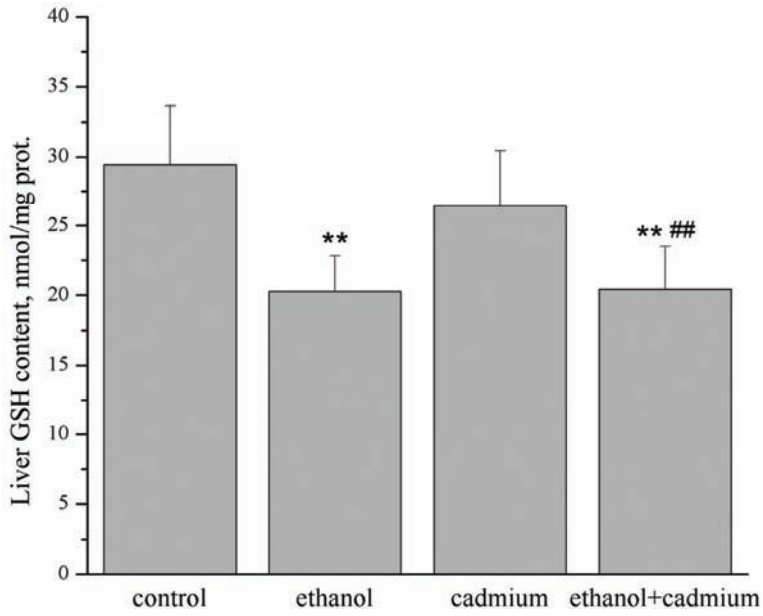

Fig. 2. The liver glutathione (GSH) level in the experimental animals. For statistical evaluation, the one-way Anova with the Fisher *post hoc* test were used. **p<0.01 *vs.* the control group, $^{\#\#}p$<0.01 in comparison with the Cd-group.

Total liver SOD activity was significantly higher 24 h after cadmium administration (2204.7±111.8 U mg^{-1} prot.) in comparison with the control group (570.5±27.3 U mg^{-1} prot.) (p<0.01). However, the total liver SOD activity was significantly lower in the CdE group (346.7±67.7 U mg^{-1} prot.) in comparison with Cd and E group (2204.7±111.8 and 563.2±29.6 U mg^{-1} prot., respectively) (p<0.01) (Fig. 4). No significant change was found in the total liver SOD activity in animals that received ethanol in comparison with control animals (p>0.05).

In addition to the total SOD activities, the activities of the hepatic isoforms of this enzyme were altered in a different manner in the various experimental groups. Cadmium was found to induce a significant increase of the activities of both SOD isoforms, but to a different extent. The Cu/Zn SOD activity was approximately fivefold higher in comparison with the control group (1931.9±111.2 and

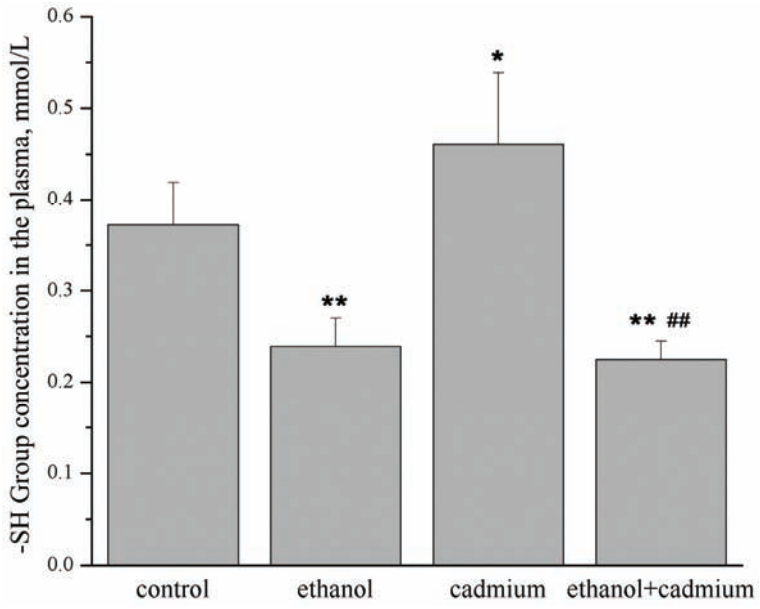

Fig. 3. Plasma sulfhydryl group (–SH) concentration in the experimental animals. For statistical evaluation, the one-way Anova with the Fisher *post hoc* test were used. *p<0.05, **p<0.01 *vs*. the control group, $^{\#\#}p$<0.01 in comparison with the Cd-group.

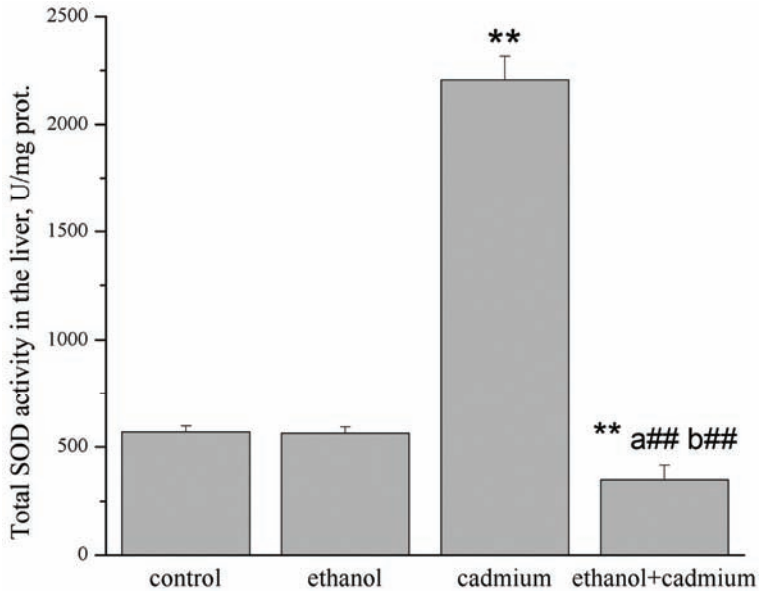

Fig. 4. Total liver superoxide dismutase activity (SOD) in the experimental animals. For statistical evaluation, the one-way Anova with the Fisher *post hoc* test were used. **p<0.01 *vs*. the control group, $^{\#\#}p$<0.01 in comparison with the ethanol- (a) or cadmium-treated group (b).

402.7±6.7 U mg^{-1} prot. in the Cd and control group, respectively) (p<0.01), while the rise in the MnSOD activity was only, approximately, 60 % in the Cd group (272.8±19.8 U mg^{-1} prot.) compared to the control group (167.8±30.9 U mg^{-1} prot.) (p<0.01). A similar rise in the MnSOD activity was measured in ethanol-treated animals (292.4±22.9 U mg^{-1} prot.). In contrast to cadmium, ethanol administration was followed by a significant decrease in the Cu/ZnSOD activity (270.8±40.8 U mg^{-1} prot.) (p<0.01) (Fig. 5). While the administration of either cadmium or ethanol caused a significant increase in the liver MnSOD activity; co-exposure of rats to these hepatotoxins induced a significant fall in its activity (86.2±19.3 U mg^{-1} prot.) in comparison with the control group (167.8± ±30.9 U mg^{-1} prot.) (p<0.01) (Fig. 6). In addition, the lowest Cu/ZnSOD activity was detected in the CdE group (260.5±73.6 U mg^{-1} protein), but its activity was not significantly different in comparison with the E group (p>0.05).

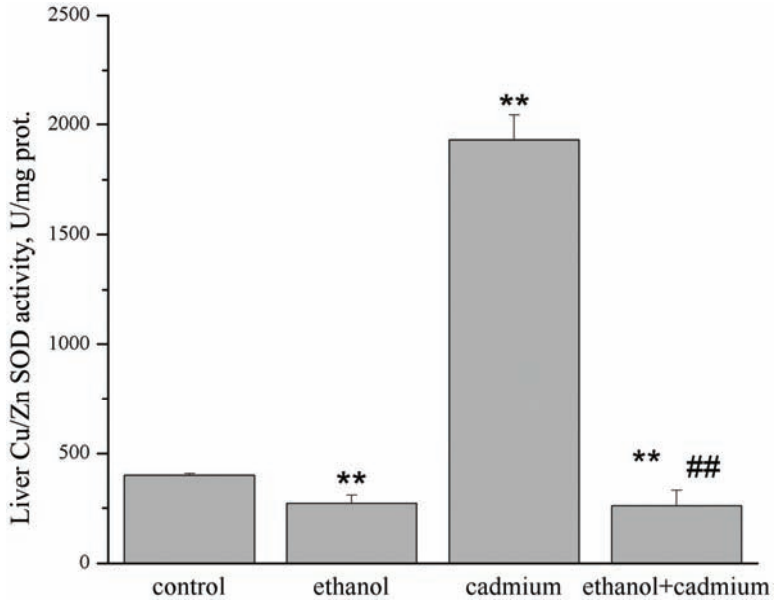

Fig. 5. Copper/zinc superoxide dismutase (Cu/ZnSOD) activity in the hepatocytes of treated animals. For statistical evaluation, the one-way Anova with the Fisher *post hoc* test were used. **p<0.01 *vs.* control group, $^{\#\#}p$<0.01 in comparison with the animals treated with cadmium alone.

Histological analysis

Histological examination confirmed liver damage in all groups that received cadmium or ethanol. Cadmium in a dose of 2.5 mg kg^{-1} caused vacuolar degeneration of hepatocytes with focal necrosis 24 h after administration. Ethanol was found to cause mild congestion with focal necrosis. Additionally, an apoptotic

body may be observed in the pericentral area. Kupffer cells were more numerous than in the control liver. In rats co-exposed to cadmium and ethanol, more extensive liver damage was observed in comparison with the ethanol- or cadmium-treated groups. A severe congestion with a prominent mononuclear infiltrate may be detected in the pericentral area. Vacuolar degeneration and necrosis were more pronounced in this group in comparison with the animals treated with cadmium (Fig. 7).

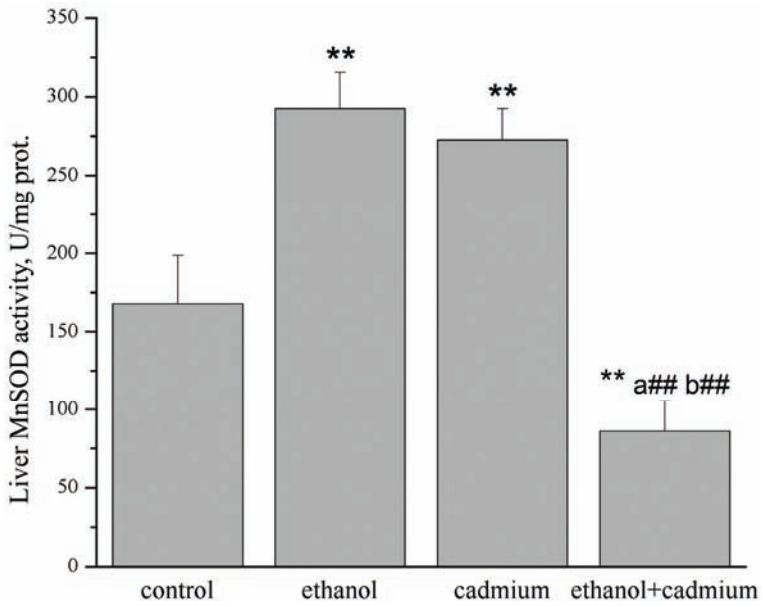

Fig. 6. Manganese superoxide dismutase (MnSOD) activity in the hepatocytes of treated animals. For statistical evaluation, the one-way Anova with the Fisher *post hoc* test were used. $^{**}p<0.01$ *vs*. control group, $^{\#\#}p<0.01$ in comparison with the animals treated with ethanol (a) or cadmium (b) alone.

DISCUSSION

Cd is a very toxic environmental pollutant that causes the production of reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide, hydroxyl radicals and NO,[1,25,26] thus impairing the balance between prooxidant and antioxidant systems.[27–29] In the present study, oxidative stress was found to be induced in the liver during acute co-exposure to ethanol and Cd. The results showed that ethanol and Cd increased the serum and liver MDA concentrations 24 h after administration. This indicates that lipid peroxidation is an important mechanism of acute Cd- and ethanol-induced hepatotoxicity. The role of lipid peroxidation in acute Cd intoxication was demonstrated in numerous studies using various doses and routes of its administration.[30–32] Cd in a dose of 2.5 mg

kg^{-1} body weight increased the MDA levels in the liver, kidney, and blood of rats 24 h after administration.[33,34] It was also observed that prior administration of antioxidants prevented Cd toxicity.[29,32,35–38] In accordance with the significant role of lipid peroxidation in the pathogenesis of acute Cd intoxication is also the fact that a rise in the concentrations of substances that react with thiobarbituric acid (TBARS) is less pronounced after previous α-tocopherol administration.[35] However, antioxidant substances may prevent Cd hepatotoxicity by various additional mechanisms, such as increasing metallothioneine (MT) and endothelial nitric oxide (eNOS) expression,[37] increasing the activities of antioxidant enzymes and improvement of the GSH level.[29,35,36]

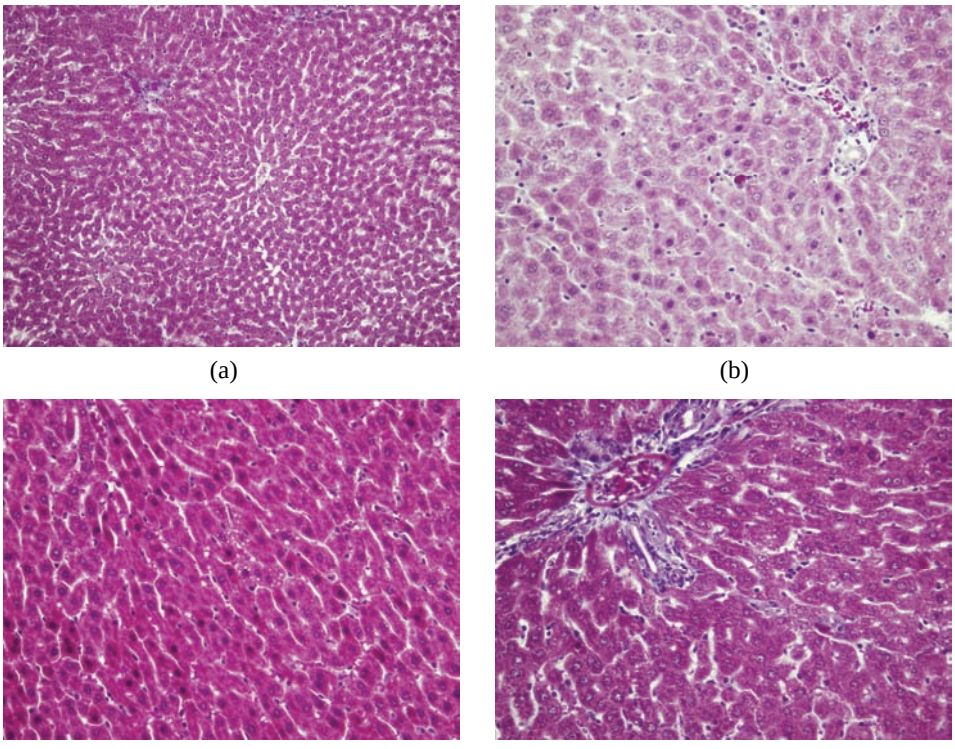

Fig. 7. a) Normal liver histology in the control group (Masson Trichrome X200); b) focal necrosis, increased number of Kupffer cells and an apoptotic body in the ethanol-treated group (Masson Trichrome X400); c) vacuolar degeneration in the pericentral area, necrosis and mild congestion in the cadmium-treated group (Masson Trichrome X400); d) severe vacuolar degeneration, necrosis and a mononuclear infiltrate with congestion in animals treated with cadmium and ethanol (Masson Trichrome X400).

In the present study, prior binge drinking was found to potentiate Cd-induced lipid peroxidation in the liver. This potentiation may lead to more exten-

sive vacuolar degeneration, inflammatory infiltration and necrosis of hepatocytes, observed after ethanol and Cd administration than after either of these hepatotoxins alone. Possible synergistic effects of ethanol and Cd are not surprising, since previous studies indicated that even acute ethanol intoxication causes lipid peroxidation in the liver, thus contributing to acute ethanol-induced liver injury.[39–41]

Serum NO_x, indicators of NO production, were increased in all groups treated with either ethanol or Cd, 24 h after administration. These findings suggest that reactive nitrogen species (RNS) are involved in acute ethanol- and Cd-induced liver injury. The role of NO in acute Cd liver damage is still not completely understood. In some studies, it was found that Cd administration at a dose of 30 μmol kg^{-1} in rats caused a twofold increase in the serum nitrate concentration 48 h after treatment.[42] However, administration of inducible nitric synthase (iNOS) inhibitors did not prevent Cd-induced liver injury; thus indicating that other sources, independent of iNOS, are responsible for the increased NO production.[43] A possible mechanism of NO-induced liver injury may be an increase in free cellular Cd level, due to the release of Cd from its association with MT. The mechanism by which NO mediates the release of Cd from MT appears to involve NO_x in liver injury. Nitrosation of intracellular GSH by NO_x is thought to play a key role in detoxication of this reactive intermediate. NO_x could nitrosate cysteine residues coordinated to Cd, resulting in the formation of *S*-nitrosothiol adducts and the release of Cd from MT. While such an occurrence would indeed result in the detoxification of NO_x, it would simultaneously "activate" Cd, posing a new toxicological threat to cells.[44]

Cd bound to MT was found to exert no hepatotoxic effects.[45,46] In contrast, Qu *et al.*[47] suggested that a liver-selective NO donor, which is metabolized by cytochrome P450 enzymes, protects against Cd hepatotoxicity. It was suggested that the protective effect of NO is mediated by potentiation of the Cd-induced increase in MT synthesis.[47] These discrepancies may be related to the dual effects of NO, depending on its amount. In low concentrations, NO exerts hepatoprotective effects, while in high concentrations, it reacts with superoxide radicals to form peroxynitrite as a potent oxidant.[48–50] Since the importance of NO as a mediator of liver injury depends on its amount and source,[49] its precise role in acute Cd-induced liver injury should be further investigated.

It has been reported that ethanol causes an increase in NO synthesis.[51] Alcohol administration increases inducible iNOS expression,[52] which, together with the alcohol-mediated increase in superoxide anion formation, could increase hepatic peroxynitrite formation. Peroxynitrite and/or peroxynitrite-generated reactive intermediates can nitrate proteins and can damage lipids and DNA.[53] The present results, in accordance with other studies,[54,55] show that ethanol induced a significant increase in the serum NO_x concentration, although its level was significantly lower than after Cd administration. This significant increase of NO_x in

the Cd-treated group could be explained by the more pronounced toxic effects of Cd compared to ethanol (Cd is a pollutant ranked eighth in the top 20 hazardous substances).[33] When administered prior to Cd, ethanol had no influence on the Cd-induced rise in NO_x generation. This indicates that binge drinking does not aggravate nitrosative stress induced by acute Cd exposition.

The obtained results showed that the liver GSH content was decreased in the ethanol-treated animals 24 h after its administration. Other studies indicated that GSH depletion plays an important role in ethanol-induced liver injury.[40,41,56,57] Among others, GSH depletion in the liver is recognized as a mechanism that may contribute to acute ethanol-induced oxidative injury within a few hours after intoxication. It was shown that silymarin and betaine exerted protective effects on binge-drinking mice by reducing GSH depletion.[41,57]

In the present study, Cd did not induce any change in the reduced GSH level in the liver 24 h after its administration. GSH is an intracellular non-enzymatic antioxidant that provides the first line of defense against oxidative injury. Similar to the present study, Siegers *et al.*[58] showed no change in the liver GSH level in acute Cd-hepatotoxicity. This indicates that GSH depletion is not a major mechanism of oxidative stress in acute Cd-induced liver injury. These results may be surprising, since the major direct Cd effect refers to its binding to sulfhydryl groups. However, Cd-induced inflammation in the liver is another important mechanism for Cd-induced oxidative stress. The activation of Kupffer cells is an important source for Cd-induced inflammatory mediators such as IL-1β, TNFα, IL-6, and IL-8, which in turn contribute to Cd-generated free radicals in the liver. Moreover, mitochondrion is an important target of Cd toxicity. It has been proposed that Cd initially binds to protein thiols in the mitochondrial membrane, affects mitochondrial permeability transition, inhibits the respiratory chain reaction and then generates ROS.[59] In addition, the lack of change in the liver GSH content could be explained by the simultaneous activation of adaptive mechanisms in hepatocytes. This adaptive response was also suggested by other studies that found an increase in GSH content within 24 h after acute Cd exposition.[60] However, this effect is dependent on the administered dose, and time and route of application.[35,60] Inside the cell, Cd elicits a number of reactions that may lead to stress adaptation and survival. These processes are mediated by signaling pathways that induce up-regulation of various protective molecules.[61] It was found that MT is relevant in Cd toxicity and survival.[62] Namely, the different localization and physiological functions of MTs, including the metal-free (apo-MT) and metal-bound (holo-MT) forms, in various organs and intracellular organelles affect the redox and energy status of cells. The double-edged role of MT in organ toxicity is emphasized by its involvement as a protein preventing (antioxidant, chelator) and mediating (carrier) of Cd toxicity.[62]

Kitamura and Hiramatsu[63] reviewed recent evidence for the involvement of ER stress signaling and the unfolded protein response (UPR) in Cd apoptosis *in vivo* and *in vitro*. Cd-induced cellular stress disturbs the proper folding of membranes and secreted proteins in the ER and triggers UPR, which determines whether damage control or death by apoptosis occurs. ROS may operate up- or downstream of the ER stress. The superoxide radical anion, but not H_2O_2, appears to trigger selectively activation of the pro-apoptotic branches of the UPR induced by Cd. In addition to the ER stress-UPR signaling, other pathways with broad anti-apoptotic potential are also activated by Cd and ROS, namely calcium and NF-κB signaling[63].

Additionally, Cuypers *et al*.[64] described the mechanisms and sources of ROS formation in the presence of Cd, which involved enhancement of ROS formation by the mitochondrial respiratory chain and induction of NADPH oxidases (NOX) enzymes. Induction of these enzymes play the key role of ROS in cellular adaptation to Cd. NADPH oxidases function as multi-component enzymes, and use electrons derived from intracellular NADPH to generate O_2^- from O_2. The NOX family of ROS-generating NADPH oxidases consists of seven members that participate in important cellular processes, related to signaling, cell proliferation and apoptosis. Thus, increased NOX4 gene expression probably may have led to increased NOX activity in mice kidneys following Cd exposure in this tissue. The exact role of NOX4 in Cd toxicity has not been described, but it may be linked to the production of free radicals for signal transduction to activate the antioxidative defense system or adaptive mechanisms.[64] NADPH-dependent oxidase could trigger signaling leading to protective measures in HepG2 cells.[65]

Generally, potentially all NOX expressing cells can be targeted by Cd and the influence of Cd on NADPH oxidase activity could result in signaling leading to the onset of cellular protection mechanisms or, alternatively, of cell death. Controlled levels of ROS production (*via* NADPH oxidase or other sources) are therefore necessary to ensure correct ROS levels for signaling or defense. Persistent NOX expression results from an amplification loop triggered by ROS and ROS-sensitive transcription factors, whereas a defense loop is mediated by ROS, which also trigger up-regulation of protective antioxidative mechanisms.[64]

The role of oxidative stress in chronic cadmium intoxication is well known. Chronic cadmium exposition inhibits the activity of antioxidant enzymes, including catalase, MnSOD, and Cu/ZnSOD.[28] Moreover, it is reported that chronic exposition to cadmium decreases the antioxidant capacity of hepatocytes, due to glutathione depletion and sulfhydryl groups inactivation in the liver.[35] It was shown that prior α-tocopherol administration reduced cadmium-induced GSH depletion in the liver.[35] On the other hand, adaptive mechanisms after chronic, low-dose exposure, including induction of MT, GSH, and cellular antioxidants, could diminish Cd-induced oxidative stress.[59] The increase in GSH content in

hepatocytes is probably due to the expression of gamma-glutamylcysteine synthetase, which catalyzes the key reaction of GSH biosynthesis.[66]

In the present study, co-exposure to ethanol and Cd was found to induce GSH depletion to the same extent as exposure to ethanol alone. This indicates that ethanol does not interact with Cd in inducing GSH depletion. Changes in liver GSH levels in these groups were accompanied by similar changes in the plasma –SH group concentration. Plasma proteins, as sources of –SH groups, reflect systemic toxic damages, which are caused by protein oxidation upon the action of Cd.

The results obtained in the present study showed that the total liver SOD activity was not altered in ethanol-treated animals 24 h after binge drinking, while Cd was found to increase its activity at the same time-point. However, the activities of its isoenzymes were significantly different in both groups. Ethanol was found to induce a significant increase in MnSOD with a simultaneous decrease in the Cu/ZnSOD activity. The increase in the MnSOD activity may be a result of an adaptive response of the hepatocytes to an increased production of ROS. Since mitochondria are the major source of ROS,[11] it is not surprising that activity of mitochondrial isoenzyme was increased after binge drinking. Similar results were obtained within 6 h after administration of a single dose of ethanol.[67] In contrast, Cd induced a significant increase in the activities of both SOD isoenzymes, with a more pronounced rise in the Cu/ZnSOD activity. The results of other studies related to the influence of Cd on SOD activity are contradictory. In accordance with the present study, Cd in a dose of 0.4 mg kg^{-1} was found to induce an adaptive increase in SOD activity.[34] In contrast, high doses of Cd (5 mg kg^{-1}) caused a decrease in both mitochondrial and cytosolic SOD activity,[68] probably because the antioxidative capacity of the hepatocytes was surmounted. *In vitro* investigations showed that MnSOD is more vulnerable to inhibitory effect of Cd than Cu/ZnSOD.[33] Possible mechanisms of greater sensitivity of MnSOD to Cd may be a substitution of Cd for manganese at active site of this enzyme or binding of Cd to another site at the active centre of the enzyme.[33]

When animals were co-exposed to ethanol and Cd, the activities of both SOD isoenzymes were decreased in comparison with the control group. Additionally, no adaptive response was observed 24 h after treatment. This finding, together with increased lipid peroxidation, indicates that prior binge drinking potentiates Cd-induced ROS production, thus causing decreased activities of these enzymes. This increased ROS generation could be responsible for suppression of the adaptive increase in MnSOD and Cu/ZnSOD activity.

CONCLUSIONS

According to this study, it can be concluded that oxidative stress plays an important role in liver injury caused by acute Cd intoxication and binge ethanol

drinking. However, its role in the interactions between these hepatotoxins remains incompletely understood. This study suggests that previous binge drinking may act synergistically with cadmium in inducing lipid peroxidation and consumption of SOD, especially its mitochondrial isoenzyme. Ethanol and cadmium, in contrast, were found to induce no synergistic effect on RNS production and glutathione depletion. Although the type of interactions between these agents are still unclear, increased lipid peroxidation and decreased SOD activity after co-exposure to ethanol and cadmium indicate that application of antioxidant therapy may be reasonable in the early phase of acute ethanol and cadmium intoxication.

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И З В О Д

ОКСИДАТИВНИ СТРЕС У ЈЕТРИ ПАЦОВА У ТОКУ АКУТНЕ ИНТОКСИКАЦИЈЕ КАДМИЈУМОМ И ЕТАНОЛОМ

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Циљ студије је био да се испита ефекат викенд пијанства на прооксидантни/антиоксидантни систем у јетри пацова у акутној интоксикацији кадмијумом (Cd). У експерименту су коришћени пацови, мужјаци Wistar соја подељени у следеће групе: 1. контрола, 2. животиње третиране етанолом (5 појединачних доза од по 2 g kg^{-1} путем орогастричне сонде), 3. животиње третиране Cd (појединачна доза од 2.5 mg kg^{-1} интраперитонеално), 4. животиње које су примиле Cd 12 сати после последње дозе етанола. За одређивање параметара оксидативног стреса узимани су узорци крви и јетре, 24 сата након третмана. Када су администрирани у комбинацији, етанол и кадмијум изазивају значајно повећање нивоа малондиалдехида (MDA) у серуму и јетри, него када су ове супстанце даване појединачно (p<0.01). Активност манган-супероксид-дисмутазе (MnSOD) је повећана у групама третираним етанолом и Cd (p<0.01), док је активност бакар/цинк-супероксид-дисмутазе (Cu/ /ZnSOD) повећана само у групи третираној Cd. Међутим, када је етанол администриран заједно са Cd дошло је до значајног смањења активности MnSOD и Cu/ZnSOD у јетри 24 сата после третмана (p<0.01). На основу нашег истраживања може се закључити да етанол може деловати синергистички са кадмијумом у настанку липидне пероксидације и смањењу активности SOD у јетри.

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