Evaluation of the effects of cadmium on rat liver

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Abstract

Cadmium is one of the most toxic pollutants in environment. Cadmium accumulation in blood affects the renal cortex and causes renal failure. In this study, we aimed to evaluate the effects of cadmium on rat liver tissue. Eighteen male albino rats aged ten weeks old were used in the study. 15 ppm of cadmium was administered to rats via consumption water daily. At the end of the 30th study day, the animals were killed under ether anesthesia. After the liver tissue samples were taken, histopathological and biochemical examinations were performed. Histopathologic changes have included vacuolar and granular degenerations in hepatocytes, heterochromatic nucleuses and sinusoidal and portal widenings. Central vein diameters were normal in cadmium exposed group. Whereas, there was statistically significant difference between two groups by means of sinusoidal ($p < 0.001$) and portal triad diameters ($p < 0.01$). Malondialdehyde (MDA) is an indicator of lipid peroxidation. In this study, MDA was used as a marker of oxidative stress-induced liver impairment in cadmium exposed rats. Superoxide dismutase (SOD) and catalase (CAT) activities were also measured to evaluate the changes in antioxidative system in liver tissues. Current findings showed that MDA levels were increased and SOD and CAT activities were decreased in cadmium exposed group compared to control group. The difference between two groups was statistically significant (*p* values: MDA, $p < 0.01$; CAT, $p < 0.01$ and SOD, $p < 0.05$). In conclusion, these findings suggest the role of oxidative mechanisms in cadmium-induced liver tissue damage. (Mol Cell Biochem **284**: 81–85, 2006)

Key words: cadmium, histopathologic changes, liver, oxidative stress

Introduction

Cadmium is an inorganic toxicant of great environmental and occupational concern, which was classified as a human carcinogen in 1993. Cadmium is one of the most toxic pollutants in environment [1, 2]. When cadmium accumulates in blood, it affects the renal cortex and causes renal failure [3, 4]. It was reported that there have been some degeneration findings in aortic endothelial tissues of the rats exposed to cadmium toxicity [5]. Cadmium can also lead to some cardiovascular problems under certain industrial conditions [6, 7]. Liver and kidney damage due to acute or chronic cadmium exposure has been well characterized. While hepatocytes and endothelial cells of the liver sinusoids are supposed to be the primary cellular targets in liver, ultrastructural changes may vary depending upon the exposure type and duration following administration [8]. There are some suggested mechanisms for cadmium toxicity. In cellular level, cadmium depletes glutathione and protein-bound sulfhydryl groups, resulting in enhanced production of reactive oxygen species such as superoxide ion, hydroxyl radicals, and hydrogen peroxide. These reactive oxygen species lead to increased lipid peroxidation, enhanced excretion of urinary lipid metabolites, modulation of intracellular oxidized states, DNA and cell membrane damages, altered gene expression and apoptosis. Enhanced production of nuclear factor-kappa B and protein kinase C

activation occur [9]. Oxidative stress appears to play a major role in chronic Cd-induced hepatic and renal toxicity, as the toxicity inhibits antioxidative defense system components. It has also been observed that antioxidant administration prevents Cd toxicity [10]. The mechanism of cadmium-mediated acute hepatotoxicity has been the subject of numerous investigations and sufficient evidence has emerged to reveal reasonable mechanisms for the toxic process, although some unexplained aspects still persist. Acute hepatotoxicity involves two pathways: one for the initial injury produced by direct effects of cadmium and the other for the subsequent injury produced by inflammation. Primary injury appears to be caused by the binding of Cd 2+ to sulfhydryl groups on critical molecules in mitochondria. Thiol group inactivation causes oxidative stress, mitochondrial permeability transition and mitochondrial dysfunction. Although cadmium may directly destruct hepatocytes, there are compelling reasons to believe that hepatocellular injury is produced *in vivo* as the result of ischemia caused by damage to endothelial cells. Secondary injury from acute cadmium exposure is assumed to originate from the activation of Kupffer cells and a cascade of events involving several types of liver cells and a large number of inflammatory and cytotoxic mediators [11]. In one study, it has been reported that age-associated changes in Kupffer cell function and neutrophil infiltration are important determinants of cadmium-induced hepatotoxicity in rats [12]. Kupffer cell activation and neutrophil infiltration are important events in the toxic process and the involvement of proinflammatory cytokines and chemokines has also been implicated [11]. Also, some cytokines like TNF-alpha have been implicated in cadmium's toxic effect on liver. TNF-alpha released from non-parenchymal cells and other associated cytokines are responsible for certain manifestations observed with cadmium-induced hepatotoxicity [8]. However, we could not able to find any study indicating objective findings about cadmium induced liver damage in the literature. Thus, by using some objective criteria we aimed to find out whether the liver tissue damage seen in experimental settings is due to cadmium toxicity. Our findings have been supported by the biochemical evidence.

Subjects and methods

Eighteen male albino rats aged ten weeks old were used in this study. Standard rat chow and tap water was provided ad libitum. The cadmium group was given consumption water containing 15 ppm/day cadmium chloride for 30 days [13]. At the end of 30th day, animals were killed under ether anesthesia. The tissues were fixed with 10% neutral formaline solution. After routine histological laboratory procedures, tissues were blocked in paraffine. Fifteen sections were taken from each block by systematical randomized sampling method.

Sections were stained with hematoxyline-eosine and evaluated under Olympus BX-40 microscope. Ocular micrometer was used to measure the sinusoidal diameters, central vein diameters, portal triad wideness and hepatocyte degeneration. Non-parametric Mann–Whitney U Test was used in statistical analysis. Furthermore, biological parameters have also been measured. Malondialdehyde (MDA) level was estimated by Draper-Hadley's double heating method [14]. In this measurement, the principle method was spectrophotometric color measurement, which has been generated by thiobarbituric acid (TBA) and MDA interaction. For this purpose, 2.5 mL of 100g L-1 trichloroacetic acid solution was added to 0.5 mL homogenate in each centrifuge tube and tubes were placed in a boiling water bath for 15 min. After cooling in tap water, the tubes were centrifuged at $1000 \times g$ for 10 min and 2 mL of the supernatant was added to 1 mL of 6.7g L-1 TBA solution in a test tube and tube was placed in a boiling water bath for 15 min. The solution was then cooled in tap water and its absorbance was measured via spectrophotometer (Shimadzu UV-1601, Japan) at 532 nm. MDA concentration was calculated by the absorbance coefficient of MDA–TBA complex (absorbance co-efficient. $= 1.56 \times 105 \, cm^{-1} \, M^{-1}$). This is expressed as nanomoles per gram ($nM \, g^{-1}$) wet tissue. Total (Cu-Zn and Mn) SOD activity was determined [15]. The principle method is based on the inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine/xanthine oxidase system as a superoxide generator. SOD activity was assessed in the ethanol phase of the supernatant, after adding 1.0 mL of ethanol/chloroform mixture (5/3, v/v) to the identical sample volume and centrifuging them. One SOD unit is defined as the enzyme amount causing 50% inhibition in NBT reduction rate. Activity was expressed as units per gram protein. CAT activity was determined according to Aebi's method [16]. This method was based on rate constant determination (k) or H_2O_2 decomposition rate at 240 nm. Results were expressed as k (s^{-1}) per gram protein in liver tissue. Liver protein content was measured Lowry *et al.* with bovine serum albumin as the standard [17]. Data were presented as means ± standard deviation (S.D). SPSS 9.0 for Windows (SPSS Inc. Chicago, IL, USA) software was used in statistical analysis. Differences between groups were calculated by two-way ANOVA and Bonferroni's *t*-statistics. *P*-values under 0.05 were considered as statistically significant.

Results

Liver tissue sections taken from control group were examined under light microscopy and they have been found in normal range (Fig. 1). In cadmium exposed group, vacuolar and granular degenerations in hepatocytes, heterochromatic nucleuses and sinusoidal and portal widenings were observed in liver tissue (Figs. 2 and 3). However, central vein diameters
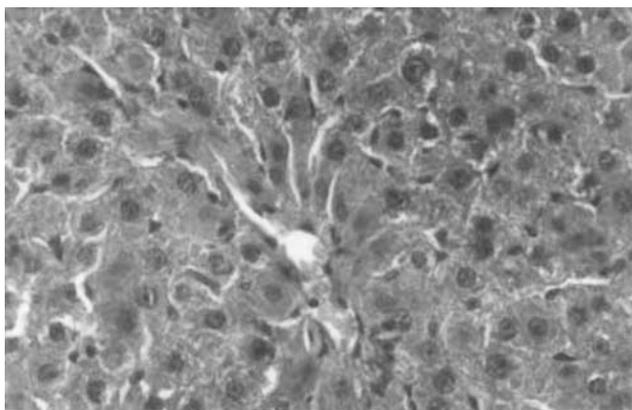
Fig. 1. Normal liver tissue taken from the control group (Hematoxyline – Eosin, 40×)
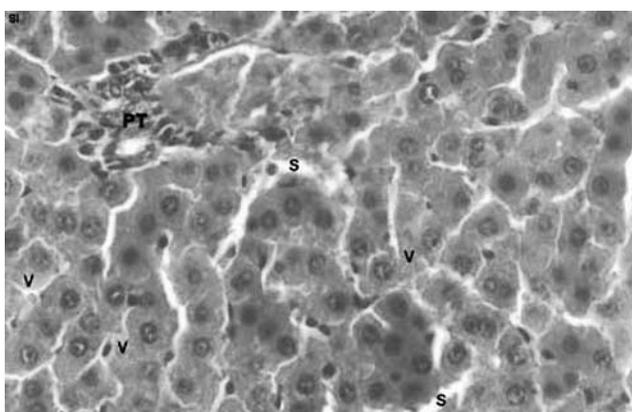

Fig. 2. Liver tissue from cadmium exposed group. V – Vacuolar and granular degeneration sites, S – Sinusoidal dilatations and PT – Portal triad widening. (Hematoxyline – Eosin, 40×)
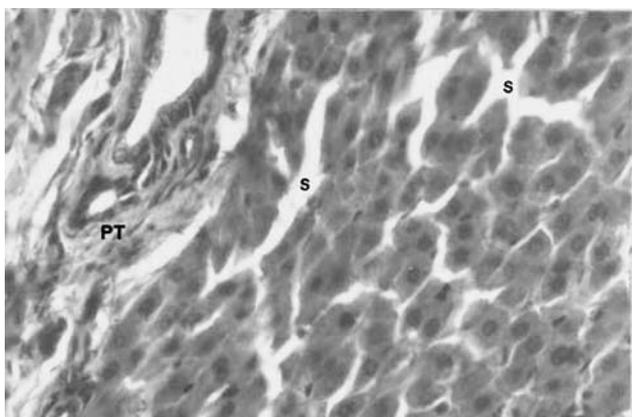

Fig. 3. Liver tissue from cadmium exposed group. S – Sinusoidal dilatations and PT – Portal triad widening (Hematoxyline – Eosin, 40×).

Table 1. Liver histopathological parameters in control and cadmium groups

	Sinusoidal diameter (μ)	Central vein diameter (μ)	Portal triad diameter (μ)	Hepatocyte degeneration (in 100 cells)
Control group ($n = 9$)	4.3 ± 0.57	33.5 ± 6.3	76.6 ± 5.5	8.4 ± 1.8
Cadmium group ($n = 9$)	8.7 ± 0.44	38.7 ± 5.9	95.6 ± 17.5	26.6 ± 2.3
P values	0.0001	0.09	0.007	0.0001

Table 2. Activities of SOD, CAT and MDA levels in liver homogenates of control and cadmium groups

	SOD (U/mg protein)	CAT (k/g protein)	MDA (nmol/g protein)
Control group ($n = 9$)	0.176 ± 0.012	3.575 ± 0.176	5.003 ± 0.547
Cadmium group ($n = 9$)	0.151 ± 0.019	2.330 ± 0.185	7.127 ± 0.523
P values	0.042	0.004	0.004

were also normal in cadmium exposed group. There was statistically significant difference between the two groups by means of sinusoidal ($p < 0.001$) and portal triad diameters ($p < 0.01$). There was no difference in central vein diameters ($p > 0.05$) between two groups. Hepatic degeneration was evaluated by counting 100 cells under $100 \times$ magnifications. After cell count, chi – square test was performed for statistical analysis and was found meaningful ($p < 0.0001$) (Table 1). The mean values in control and Cadmium groups were 4.3 ± 0.57 and 8.7 ± 0.44 microns (μ) for sinusoidal diameter, 33.5 ± 6.3 and 38.7 ± 5.9 microns (μ) for central vein diameters, 76.6 ± 5.5 and 95.6 ± 17.5 microns (μ) for portal triad diameters, respectively. The mean MDA, SOD and CAT values in two groups are shown in Table 2. The mean MDA levels in control and Cadmium groups as U nmol g protein^{-1} were 5.003 ± 0.547 and 7.127 ± 0.523, respectively. The mean SOD values in control and Cadmium groups as U mg protein^{-1} were 0.176 ± 0.012 and 0.151 ± 0.019, respectively. The mean CAT values in control and Cadmium groups as k g protein^{-1} were 3.575 ± 0.176 and 2.330 ± 0.185, respectively. The results showed that MDA level was increased and SOD and CAT activities were decreased significantly in cadmium exposed group compared to control group (p values: MDA, $p < 0.01$; CAT, $p < 0.01$ and SOD, $p < 0.05$).

Discussion

We have found statistically significant difference between two groups by means of liver tissue damage. We have

used the method by Gokcimen *et al.* [18] to evaluate the cadmium-induced liver tissue damage. In this study, granular and vacuolar degenerations were prominent in hepatocytes of cadmium group. Hoffman *et al.* [19] have found single parenchymal cell necrosis, deterioration of rough endoplasmic reticulum, proliferation of smooth endoplasmic reticulum, autophagocytosis and mitochondrial degenerative changes in their study. Also, Wlostowski *et al.* [20] have reported that dietary cadmium-induced liver degeneration. In cadmium exposed group, sinusoidal diameters were significantly wider than the control group. Rikans and Yamano [11] have concluded that hepatocellular injury was produced *in vivo* as a result of ischemia caused by damage to endothelial cells. They have also postulated that secondary injury from cadmium exposure was thought to occur from the activation of Kupffer cells and a cascade of events involving several types of liver cells and a large number of inflammatory and cytotoxic mediators. Thus, the observed sinusoidal diIatations may be related to this mechanism. In one previous study, it has been reported that there has been endothelial dysfunction in aortic rings of cadmium exposed rats. [5]. Also, Bilgen *et al.* have found similiar results regarding this issue [13]. Wada *et al.* [21] have reported that cadmium ion directly interacts with ET receptor and inhibits the binding of endothelin, which is a potent vasoconstrictive peptide produced by endothelial cells of blood vessels. In one study, it has been demonstrated that higher concentrations of cadmium inhibits portal vein contractions elicited by adrenegic stimuli [22]. This result suggests that portal vein pressure does not increase in cadmium-induced hepatotoxicicty. We haven't observed any dilatation in central veins. This is probably because central veins are originated from the protal vein. We have also observed that mean portal triad diameter has significantly increased in cadmium exposed group. As portal triad includes the interlobular artery and vein, this widening can be due to the dilatation mechanism told above. Current study has shown that exposure to cadmium had significant effects on rat liver, suggesting that reactive oxygen species (ROS) were generated under the experimental conditions employed. A significant increase was observed in MDA level in the cadmium exposed group. The change in activities of antioxidant enzymes and MDA levels in current study was regarded as an indicator of increased ROS production during the exposure period and may reflect the pathological process of the exposure. The main ROS that have to be considered are superoxide anion (O_2^-), which is predominantly generated by the mitochondria; hydrogen peroxide (H_2O_2) produced from O_2 by superoxide dismutase (SOD) action and peroxynitrite ($ONOO^-$), generated by the reaction of O_2^- with nitric oxide (NO). SOD is a specific antioxidant enzyme which dismutates O_2^- and forms H_2O_2 as a result, which is scavenged by peroxisomal CAT or GSH-Px [23, 24]. It protects the cells against toxic effect of superoxide radicals [25]. Three SODs,

copper/zinc SOD (cytosolic SOD), manganese SOD (mitochondrial SOD) and extracellular SOD (ECSOD), are major antioxidant enzymes based on cellular distribution and localisation. Of the three isoforms, ECSOD may be the most important in blood vessels, accounting for up to 70% of the total activity of SOD in blood vessels [26].

The distribution of extracellular SOD in the vessel walls of liver tissue seems ideal for detoxifying superoxide anions produced in sera of cadmium exposed rats. Extremely rapid interconversion of ROS (O_2^- H_2O_2) within the cell can make it difficult to identify the originating species. The mitochondrial respiratory chain is the major site for the generation of superoxide radicals (O_2^-) [27]. Cyclooxygenase-2 (COX-2) activation and neutrophil infiltration seem to represent other resources of ROS under our experimental conditions [28, 29]. It is possible that cadmium affects the mitochondrial membranes to produce large amounts of oxygen radicals that results in extreme use of SOD in cadmium exposed rat liver. In this study, decreased CAT activity of liver tissue in cadmium exposed rats also suggests an over expression of this enzyme related to increased oxidative stress due to H_2O_2 *in vivo*. These continuously produced ROS are scavenged by SOD and CAT. Under some circumstances, these endogenous antioxidative defenses are likely to be perturbed as a result of overproduction in oxygen radicals, inactivation of detoxification systems, consumption of antioxidants and failure to adequately replenish antioxidants in tissue. In numerous studies, it has been demonstrated that ROS are directly involved in oxidative damage of cellular macromolecules such as lipids, proteins and nucleic acids in tissues.

In our study, the association between increase in MDA level and decrease in SOD and CAT activities indicates that there is oxidant-induced tissue damage in liver. Erdogan *et al.* [30] have reported that cadmium has increased the plasma malondialdehyde (MDA) level as an indicator of lipid peroxidation and lowered the activity of blood superoxide dismutase (SOD) level. As a result, we concluded that cadmium has an important toxic effect on liver tissue. Our study has indicated that cadmium exhibits its toxicity by increasing lipid peroxidation.

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