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Chapter VII

Route, Dose and Duration of Exposure to Cadmium-Relevance to Oxidative Stress Induction

Vesna Matović*, Danijela Đukić-Ćosić, Aleksandra Buha and Zorica Bulat
Department of Toxicology "Akademik Danilo Soldatović",
University of Belgrade-Faculty of Pharmacy, Belgrade, Serbia

Abstract

Cadmium (Cd) is a toxic metal currently ranked as seventh (out of 275) on ATSDR priority list of hazardous substances and it is considered as one of the most important occupational and environmental pollutants. This carcinogenic metal causes adverse effects in various tissues, particularly kidneys after prolonged exposure. However, the mechanisms of these toxic effects are still not completely understood. Literature data indicate different mechanisms of cadmium toxicity, oxidative stress being one of the most important. Experiments confirm that Cd, metal with no redox potential, can indirectly induce the production of reactive oxygen species (ROS) by affecting antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione S-transferase), nonenzymatic antioxidants (glutathione, total sulfhydryl groups, antioxidative vitamines), and Fenton metals, iron and copper.

This chapter summarizes our investigations on cadmium-induced oxidative stress conducted on three different animal species: rabbits, mice and rats. Application of a single oral dose of 50 mg Cd/kg b.w. in rabbits showed increased levels of lipid peroxidation in blood 4-6 hours after Cd intoxication indicating early development of oxidative stress in conditions of acute Cd poisoning. Experiments carried out on mice showed that single oral exposure to 20 mg Cd/kg b.w. resulted in intensive ROS production in liver while subacute oral dose of 10 mg Cd/kg b.w. induced more pronounced effect in kidneys, thus indicating different effect of Cd intoxication

* Corresponding author Email: vevodi@pharmacy.bg.ac.rs.

depending on duration of Cd exposure. Further experiments performed on rats provided strong evidence that Cd can induce oxidative stress in both blood and liver after oral (30 mg Cd/kg b.w.) and intraperitoneal (1.5 mg Cd/kg b.w.) acute intoxication, although intraperitoneal administration showed more pronounced negative effects.

Results of these studies demonstrate that oxidative stress is an important mechanism of Cd toxicity and that Cd ability to induce oxidative stress in different tissues depends on the route, dose and duration of exposure to this toxic metal.

Cadmium-Ubiquitous Toxic Metal of Nowadays

Over the last few decades, cadmium (Cd) has been recognized as one of the most important environmental and industrial pollutants and predictions are that the exposure to this metal will be even increased in future decades (Nawrot et al., 2008).

During the mining of ores and ore extraction processes, this naturally occurring element can contaminate environment (Casado et al., 2008). Occupational exposures to cadmium also include nickel-cadmium batteries industry, electroplating and paint pigments. Other anthropogenic sources such as application of phosphate fertilizers, sewage sludge to land, the disposal of wastewaters, and smelter emissions significantly contribute to soil contamination by this metal (ATSRD, 2012), thus increasing Cd uptake into crops and green leafy vegetables.

Food is the major source of Cd exposure for general population with average Cd intake via food varying from 8 to 25 μg/day (Järup and Åkesson, 2009). It was estimated that more than 80% of the food-cadmium intake comes from cereals and vegetables, although some kinds of food such as sea food (molluscs and crustaceans), as well as offal products such as liver and kidney from older animals contain high levels of Cd. However, in Cd contaminated areas such as those in the vicinity of cadmium emitting plants or mining districts, ground water and surface water as well as atmosphere may be substantially contaminated by Cd.

Tobacco smoking is another source of cadmium exposure since tobacco leaves can accumulate this toxic metal. One cigarette contains about 1-2 μg Cd and in heavy smokers cadmium uptake from inhaling cigarette smoke can even exceed the uptake from all other sources of Cd (Järup and Åkesson, 2009).

Discovered in 1817, the first reports on adverse effects of Cd were given by Friberg in the middle of last century indicating that damage to lungs and kidneys might be the earliest effects and "critical effects" occurring in occupational Cd exposure.

A few years later itai-itai disease confirmed adverse effect of Cd on bones (Nordberg, 2009). As recently reviewed by Matović et al. (2011), nowadays it is known that Cd can also cause toxic effects on liver, pancreas, reproductive organs, placenta, and hematopoietic, nervous, and cardiovascular systems, with pulmonary system being the target of Cd associated carcinogenesis. Thus, the International Agency for Research on Cancer and the US National Toxicology Program classified Cd as human carcinogen (IARC, 1993; NTP, 2011). However, recent investigations and epidemiological studies confirmed that even low levels of Cd due to its very long biological half-life (even 30 years in kidneys) may be associated with pre-diabetes and diabetes mellitus, myocardial infarction, osteotoxicity, and distruption of different endocrine functions (Matović et al., 2011; ATSDR, 2012). Recent studies have also

shown that Cd can alter thyroid function (Hammouda et al., 2008) and act as a metalloestrogen (Silva et al., 2012).

As a toxic metal with a wide range of organ toxicity, cadmium is expected to exhibit different mechanisms of toxicity. Majority of authors agree that the most important molecular mechanisms by which cadmium manifests its toxic effects and its carcinogenicity include changes in gene expression, inhibition of damaged DNA repair, inhibition of apoptosis, induction of oxidative stress, and interactions with bioelements (Joseph, 2009; Liu et al., 2009; Matović et al., 2010; Moulis, 2010).

Although over the last few decades substantial number of investigations has been conducted in order to precisely define mechanisms of Cd toxicity, further investigations are still necessary to provide additional information for understanding the complexity of Cd toxicity. However, it seems that induction of oxidative stress can have impact and important role in all other mechanisms of Cd toxicity.

Oxidative Stress in Cadmium-Induced Toxicity

Toxicity of metals, especially those with variable valence, is often associated with oxidative tissue damage. Although Cd is not a redox-active metal such as iron (Fe), copper (Cu) and chromium (Cr), it has been shown in *in vivo* and *in vitro* studies conducted in recent years that Cd can cause the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Indirect formation of ROS and RNS involving the superoxide radical ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$) and nitric oxide (NO) has been reported by many authors (Buha et al., 2012; Djukić-Ćosić et al., 2008; Liu et al., 2009; Matović et al., 2012; Yang et al., 2007; Waisberg et al., 2003). The concentration of ROS and RNS can be increased either by their overproduction or by an inability of antioxidant system to destroy them. Numerous experiments were performed in order to clarify Cd effects on antioxidants enzymes (superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), glutathione reductase, glutathione S-transferase) and nonenzymatic antioxidants (glutathione (GSH), total sulfhydryl groups (TSH), antioxidative vitamines) in various organs. These studies, although not coherent, indicate cadmium ability to interfere with components of antioxidant system. Another mechanism involved in the indirect role of cadmium in free radical generation is that cadmium can replace Fe and Cu in various cytoplasmic and membrane proteins (e.g. ferritin, apoferritin), and thus increase the amount of these metals in unbound ionic state that can induce oxidative stress via Fenton reactions (Casalino et al., 1997).

Since the role of oxidative stress in Cd toxicity still appears rather controversial, our researches were focused on this particular mechanism of Cd toxicity. In this chapter we will summarize the results of our experiments performed on three different animal species: rabbits, mice and rats. During these investigations different doses of Cd were administrated by different routes of exposure while duration of exposure to this toxic metal was either acute or subacute. Different parameters of oxidative stress were determined during these researches in order to get better insight into Cd mechanisms of oxidative stress induction. Concentrations of following reactive species were determined: $O_2^{\bullet-}$ and NO and total oxidative status (TOS) which besides $O_2^{\bullet-}$ levels include other reactive oxygen metabolites such as H_2O_2 and lipid hydroperoxides (LOOH). Furthermore, malondialdehyde (MDA) content as a measure of

lipid peroxidation and advanced oxidation protein products (AOPP) were determined. Among the parameters of antioxidative defense system, the activity of antioxidant enzyme SOD, as well as the content of nonenzymatic antioxidants TSH and GSH were determined.

Cadmium-Induced Oxidative Stress in Rabbits-Our Pioneer Investigation

Our investigations on Cd-induced oxidative stress started in 90s and were performed on rabbits *Oryctolagus cunniculus-Belgian hare* given single oral dose of 50 mg Cd/kg b.w. as aqueous solution of $CdCl_2$. Blood samples were taken from ear artery before intoxication and 1, 3, 4, 6, and 24 hours after the treatment. Levels of $O_2^{\bullet-}$ were determined as a rate of nitrobluetetrazolium (NBT) reduction according to Auclair and Voisin (1985), free MDA level was determined by the method proposed by Girotti et al. (1991), while stimulated MDA level was measured after stimulation with $FeSO_4$ by method given by Andreeva (1988). All parameters were determined in plasma. The results showed the increased generation of $O_2^{\bullet-}$ as a parameter of the earliest phase of oxidative stress. Significant elevation of $O_2^{\bullet-}$ was observed 4 hours after Cd administration, if compared with controls not treated with Cd. The increased level in both free and stimulated MDA developed also 4-6 hours after acute intoxication with Cd demonstrating an intensive lipid peroxidation and development of oxidative stress shortly after single treatment with Cd (Matović et al., 1998; Plamenac et al., 2002).

Cadmium-Induced Oxidative Stress in Mice-Influence of Exposure Duration

The aim of our further investigation was to evaluate the influence of duration of Cd exposure on parameters of oxidative stress in mice liver and kidneys as two target organs of Cd toxicity. The first part of this research was a time-course study carried out on 40 *Swiss albino* mice given a single oral dose of 20 mg Cd/kg b.w. by orogastric tube as aqueous solution of $CdCl_2$. The other one was performed on 16 *Swiss albino* mice orally treated with 10 mg Cd/kg b.w. every day for 14 days. In both acute and subacute intoxication MDA content, NO production, total SOD and Cu, Zn-SOD activity, as well as GSH and TSH content as parameters of oxidative stress were determined in liver and kidneys. Malondyaldehide content was determined by method of Girotti et al. (1991), NO production by modified Griess method given by Cortasa and Waklda (1990), SOD and Cu, Zn-SOD activity was determined by method proposed by Misra and Fridovich (1972) and GSH and TSH contents were determined by Ellman method (1959). These parameters were measured 4, 6, 12, 24, and 48h after single Cd treatment and on 7^{th} and 14^{th} day of subacute Cd intoxication (groups of 8 animals each). One group of eight animals was used as the control (untreated animals) and was sacrificed at time zero. Statistical analysis was performed by ANOVA followed by LSD post-hoc test and $P<0.05$ were considered as statistically significant.

Our results suggest development of early oxidative stress in liver of mice after acute intoxication with Cd, since significant increase of hepatic MDA was observed after 6, 12 and 24h (Djukić-Ćosić et al., 2008). These findings are in agreement with investigations performed by Eybl et al. (2004) who also found elevated MDA levels in liver of mice 24 hours after treatment with single subcutaneous dose of 33 μmol $CdCl_2$/kg b.w. The observed Cd-induced lipid peroxidation in liver is rather difficult to explain since Cd has no redox activity. However, since previous studies (Casalino et al., 1997) showed that Cd can cause redistribution of iron (Fe) and replacement of Fe in Fe-dependant enzymes and proteins, we extended our investigations on the effect of Cd on hepatic levels of this Fenton metal. Under the same experimental conditions statistically significant positive correlation between degree of Fe concentration and MDA in mouse liver was found across the time points for Fe (0, 4, 6, 12h) and for MDA (4, 6, 12, 24h) with ρ=0.44, P=0.026 (Djukić-Ćosić et al., 2008). These data confirm that Cd can indirectly induce production of ROS by its interaction with Fe, namely increasing the concentration of ionic Fe which as a Fenton metal stimulates free radicals production and oxidative damage of lipids. However, it should be pointed that acute Cd intoxication even caused reduction of hepatic MDA if compared with control levels at the end of experiment (48h). This finding could be explained by the development of delayed adaptive response of the organism to Cd exposure. When concerning kidneys, the effect of acute Cd intoxication on MDA levels was less pronounced and its statistically significant increase was observed only 12h after Cd treatment.

Production of RNS under the same experimental conditions was evaluated by determination of nitrate content in liver and kidneys. The only significance was observed in liver after 12h when nitrate levels were elevated when compared to control levels (Đukić-Ćosić et al., 2010; Đukić-Ćosić, 2011).

Numerous literature data indicate that Cd induces oxidative stress indirectly by affecting antioxidant defense system. Our results have shown that acute Cd intoxication induces a significant decrease of hepatic and renal total and Cu,Zn-SOD activity after 6h which lasted until the end of experiment if compared with controls. Decrease of Cu,Zn-SOD activity in mice kidneys was accompanied by reduced Cu and zinc (Zn) content in mice kidneys, but no significant alteration in Zn levels was observed in liver (Đukić-Ćosić et al., 2009; Đukić-Ćosić et al., 2012). These findings indicate possible replacement of Cu and Zn by Cd in kidneys, while reduced activity of this enzyme in liver could be the consequence of direct effect of high levels of Cd determined in this organ (Djukić-Ćosić et al., 2006; Đukić-Ćosić et al., 2012).

No changes in total SH levels were observed either in liver or in kidneys of mice exposed to acute Cd intoxication. However, single exposure to Cd induced significant alterations of GSH content in both investigated organs, with reduction of GSH observed in liver and its increase observed in kidneys. Hepatic GSH content was diminished if compared with controls after 4, 6 and 12h while statistically significant elevation of GSH level was obtained in kidneys after 12, 24 and 48h. In general, acute exposure to metals results in decrease of GSH levels as a consequence of metal-GSH complexes formation and/or GSH consumption by GPX which is elevated in oxidative stress. Similar observations were given by some other authors (El-Maraghy et al., 2001; Eybl et al., 2004). Predominant accumulation of Cd in liver if compared to kidneys could be also explanation for favorable inhibitory effect of Cd on hepatic GSH content during the first 12h of our experiment. Normal levels of hepatic GSH levels reached after 24 and 48h could be explained by *de novo* GSH synthesis and by

enhanced Cd elimination (Đukić-Ćosić, 2011). However, it is rather difficult to explain the effect of Cd on renal GSH content and it remains to be answered whether applied dose of Cd induces raised levels of GSH as a delayed activation of organism defense mechanisms (Djukić-Ćosić et al., 2007). The most pronounced effects of Cd acute intoxication on parameters of oxidative stress in mice liver are presented in Figure 1.

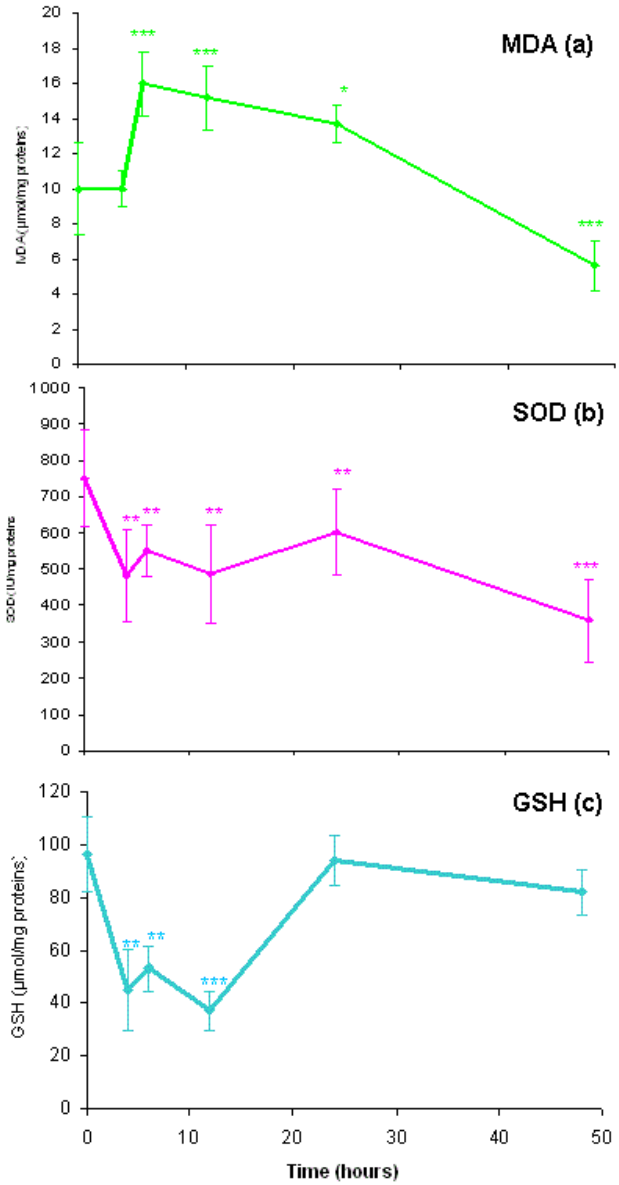

Figure 1. The effects of single oral dose of 20 mg Cd/kg b.w. on a) MDA content, b) SOD activity and c) GSH content in mice liver.

Values are presented as the means ± SD. Marked values differ significantly (ANOVA/LSD) from control group (zero time). *P<0.05; **P<0.01; ***P<0.001.

Results obtained for subacute Cd intoxication indicate less pronounced effect on parameters of oxidative stress in liver if compared to the results obtained for acute poisoning.

Levels of MDA and nitrates were even decreased after one and two weeks treatment with Cd. The observed decrease in MDA might be the result of different mechanisms. Boujelben et al. (2006) demonstrated that decreased lipid peroxidation in the liver after intoxication with 2.5 mg $CdCl_2$/day/kg b.w. for 10 days is a result of the activation of antioxidant defense mechanisms.

This mechanism was also proposed by Klaassen et al. (1999, 2009), Nordberg (1999) and Nordberg and Nordberg (2000) who showed that Cd induced synthesis of hepatic metallothioneins as efficient scavengers of Cd and free radicals.

Increased levels of GSH accompanied with elevated TSH content in hepathocytes observed in our study after one and two weeks (statistically significant after two weeks), which was also observed by El-Maraghy et al. (2001), could be the other possible explanation for protective mechanism against Cd-induced oxidative stress.

However, our opinion is that this phenomenon could be mainly attributed to depleted Fe content in liver during subacute exposure to Cd, since the correlation between MDA and Fe concentration was positive and statistically significant during the whole period of subacute experiment (ρ=0.69, P=0.003) (Djukić-Ćosić et al., 2008).

On the other hand, investigated Cu, Zn-SOD and total SOD in liver were in the levels of controls during the entire experiment. However, two weeks treatment with 10 mg Cd/kg b.w. resulted in oxidative and nitrosative stress in kidney of mice. The enhanced free radicals formation in kidneys was accompanied and can be explained by elevated levels of Fenton metals Fe and Cu, increased activity of SOD and diminished GSH and TSH levels (Đukić-Ćosić, 2011; Đukić-Ćosić et al., 2011). As suggested by Karmakar et al. (1998), the decrease in the GSH level might be a consequence of the increased activity of γ-glutamyl transpeptidase enzyme, an external cell-surface enzyme that can enhance the degradation of extracellular GSH.

Another possible reason for decreased GSH level may be attributed to increased levels of lipid oxidation products which could be associated with the less availability of NADPH required for glutathione reductase activity to transform oxidized glutathione (GSSG) to the reduced form (GSH) (Sarkar et al., 1995). The effects of subacute Cd intoxication on the levels of parameters of oxidative stress in kidneys of mice are summarized in Table 1.

Based on the presented results, it can be concluded that Cd treatment stimulates the production of the major mediators of oxidative damage, ROS and RNS, both in liver and kidneys. However, Cd behavior concerning its effect on oxidative stress was different in the conditions of acute and subacute poisoning. Mice exposed to single oral Cd treatment exerted more marked effects in liver than in kidneys reflected by the elevation of MDA levels and decreased activity of investigated antioxidant enzymes and GSH as a nonenzymatic component of antioxidative defense.

These effects were observed very shortly after Cd treatment (6h) suggesting that Cd exposure rapidly disrupts oxidative-antioxidative balance in liver through already suggested mechanisms. It should be emphasized once again that enhanced production of free radicals could be explained by Cd interactions with endogenous Fe since significant Fe elevation in liver was induced by Cd.

Table 1. The effect of two weeks Cd treatment (10 mg Cd/kg b.w.) on the MDA content, SOD activity, GSH and TSH content in mice kidneys

	Time (days)		
	control	7 days	14 days
MDA *(μmol/mg proteins)*	5.96 ± 0.48	5.91 ± 0.94	8.07 ± 1.55**
SOD *(IU/mg proteins)*	294.81 ± 38.37	258.81 ± 52.80	401.01 ± 76.36**
GSH *(μmol/mg proteins)*	11.62 ± 2.46	7.63 ± 2.15**	9.48 ± 2.98**
TSH *(mmol/mg proteins)*	80.65 ± 11.10	88.10 ± 19.39	67.10 ± 2.50

Values are expressed as mean±SD. Asterisk (*) denotes significant difference from the control group ($P<0.05$); double asterisk (**) denote significant difference from the control group ($P<0.01$) (one-way ANOVA plus LSD post-hoc test).

Conversely, following subacute Cd intoxication increased ROS and RNS levels were observed in kidneys, but not in liver. Indeed, kidneys are generally regarded as the critical organ of long-term exposure to Cd. The induction of metallothioneins which can sequester Cd is one of the plausible explanations for the amelioration of investigated parameters levels in liver after subacute intoxication.

The results of this investigation suggest that cadmium has significant effect on oxidative stress by generating ROS and RNS through depletion of endogenous radical scavengers and interactions with Fe, which in turn leads to various cell damages and significantly contributes to hepatotoxicity and nephrotoxicity of Cd. In addition, our results highlight the different effects of acute and subacute Cd intoxication on investigated parameters of oxidative stress in liver and kidneys suggesting dependency of these effects on duration of exposure to this toxic metal.

Cadmium-Induced Oxidative Stress in Rats-Influence of Route of Exposure

Our most recent study was aimed to determine how different routes of acute exposure to Cd affect parameters of oxidative stress in blood and liver of rats. Investigated routes of exposure were oral (*or*) and intraperitoneal *(i.p.)* exposure. These two particular routes of exposure were chosen based on two possible scenarios of human exposure to Cd: a) *or*-corresponding to exposure through food and water and b) *i.p.*- corresponding to parenteral exposure to Cd as a result of occupational Cd exposure via lungs and/or inhalation of tobacco smoke in general population.

The animals used in this study were randomized into three groups of eight rats each. For *or* treatment, aqueous solution of $CdCl_2$ was administrated in a single dose of 30 mg Cd/kg b.w. by orogastric tube. Intraperitoneally treated rats were injected an isotonic $CdCl_2$ solution in a single dose of 1.5 mg Cd/kg b.w. Control animals were unexposed through the whole experiment. Twenty four hours after intoxication animals were sacrificed under ether anesthesia; blood samples were collected from heart, and livers were excised. After separating plasma from blood and liver homogenization, following parameters of oxidative stress were

analyzed: $O_2^{\bullet-}$ and MDA concentrations, SOD activity, AOPP, TOS, TSH and GSH content. The rate of $O_2^{\bullet-}$ formation, MDA concentration, SOD activity in blood and liver and GSH and TSH groups content in liver homogenates were determined by the same methods used in our investigations on rabbits and rats, described above. Levels of AOPP as indicators of ROS-mediated protein damage were measured in plasma by method of Witko-Sarsat et al. (1991). Total oxidative status ($O_2^{\bullet-}$, H_2O_2 and LOOH levels) was assayed in plasma using the method described by Erel (2005). The results obtained in plasma were statistically analyzed by Kruskal-Wallis nonparametric test followed by post-hoc Conover test for pairwise comparison, while results obtained in liver were analyzed using ANOVA followed by post-hoc LSD test and $P<0.05$ were considered as statistically significant.

The effects of Cd treatment on investigated parameters of oxidative stress in plasma are represented in Table 2. Cadmium *or* and *i.p.* administration induced a significant increase in both $O_2^{\bullet-}$ and TOS plasma levels when compared to controls, suggesting Cd ability to induce increase in plasma levels of ROS. Investigations of other authors also indicated that exposure to Cd can elicit significant $O_2^{\bullet-}$ generation, as in a study conducted by Filho et al. (2000) who treated rats with a single intraperitoneal dose of 2 mg Cd/kg. In our investigation conducted on rabbits this increase was observed even 4h after Cd treatment, suggesting that this Cd effect can be regarded as an early marker of Cd induction of oxidative stress. Cadmium effect on $O_2^{\bullet-}$ generation can be explained by Cd adverse effect on SOD activity reflected by its decrease.

Table 2. The effect of Cd treatment on the SOD activity, $O_2^{\cdot-}$, TOS and MDA levels in plasma

Parameter		Control	Cd or	Cd ip
$O_2^{\cdot-}$ *(μmol red. NBT/min/L)*	Median	87.5	107*	284*,†
	Range	60-99	101-128	234-370
TOS *(μmol/L)*	Median	28.3	49*	68.3*,†
	Range	15.6-39.8	41.6-63.2	46-83.8
MDA *(μmol/L)*	Median	0.68	1.14*	1.82*,†
	Range	0.46-0.93	0.93-1.69	1.36-1.93
SOD *(IU)*	Median	137	130*	123*
	Range	131-139	127-134	119-133

Control group-non-treated animals, Cd_{or} group- rats intoxicated orally with 30 mg Cd/kg b.w.; $Cd_{i.p.}$ group-rats intraperitoneally injected a single dose of 1.5 mg Cd/kg b.w. The values are expressed as medians and ranges. Statistically significant differences (Kruskal-Wallis nonparametric test, Conover test for pairwise comparison) obtained for each parameter between groups are indicated by: *vs. control, †vs. Cd_{or}. $P<0.05$.

To our knowledge, levels of TOS with a reference to Cd intoxication have not been studied up to date. Obtained increase of TOS levels can also be the illustration of Cd ability to decrease the activity of enzymatic components of antioxidative protection. Gluthatione peroxidase plays an important role in LOOH reduction to corresponding alcohols and in the degradation of H_2O_2 to H_2O. Hence, increased levels of TOS observed in this study can be explained by adverse effect of Cd on GPX activity in plasma of treated rats, since some other authors announced decrease of GPX activity in liver in conditions of acute intraperitoneal intoxication (Liu et al., 2011) and subchronic Cd oral intoxication in serum, liver and kidney (Galazyn-Sidorczuk et al., 2012). Furthermore, significant increase in plasma levels of both $O_2^{\bullet-}$ and TOS in our study was much more marked in the group that received Cd intraperitoneally than in a group treated orally.

During oxidative stress, besides eliciting widespread damage to cells such as lipid peroxidation of polyunsaturated membrane lipids leading to MDA generation, ROS can also produce oxidative damage of proteins that can be marked by AOPP levels. Both *or* and *i.p.* Cd treatment resulted in a significant increase in MDA and AOPP when compared to controls, and these levels were significantly higher in the group treated intraperitoneally than in orally treated one. Similar results were acknowledged by some other authors. Thus, subcutaneous daily treatment of rats with 0.49 mg Cd/kg for 20 days and intraperitoneal daily treatment with 2 mg $CdCl_2$/kg during 10 days significantly increased MDA plasma levels (Nemmiche et al., 2007; Kanter et al., 2009). Although no literature data on the effect of Cd on AOPP levels are available, Gałazyn-Sidorczuk et al. (2009) investigated generated damage of proteins by investigating the concentration of protein-bound carbonyl groups in serum. In this experiment conducted on rats administration of Cd by drinking water containing 50 mg Cd/L induced significant increase of carbonyl serum levels after 12 weeks. In our acute studies on rabbits and rats, elevated plasma MDA and AOPP levels were obtained much earlier i.e. within 24 hours, probably as a result of different applied Cd doses.

As it was expected, Cd treatment decreased SOD activity in plasma if compared to the controls, but surprisingly no significant difference between SOD activity after *or* and *i.p.* administration of Cd was observed. Cadmium-incited decrease in SOD activity could be explained by previously confirmed antagonism between Cd and Zn (Bulat et al., 2008; Bulat et al., 2012; Jihen et al., 2011). Thus, Bauer et al. (1980) reported that Cd was able to occupy the site of Zn in the Cu, Zn-SOD isoenzyme, creating an inactive form of this enzyme. Furthermore, our recent investigation (Buha et al., 2012) proved positive correlation between blood Zn and SOD activity (ρ=0.339, P=0.035) which was then confirmed for both oral and intraperitoneal Cd treatment (ρ=0.894, P=0.041 and ρ=0.721, P=0.042, respectively). However, intraperitoneal Cd administration resulted in more pronounced negative effects on all other investigated parameters. This route-dependency observed for Cd effects on investigated parameters in plasma can be explained by significantly higher blood Cd levels obtained after *i.p.* Cd administration as shown in our recent study (Matović et al., 2012).

Parameters of oxidative stress in rats treated with Cd orally and intraperitoneally were investigated not only in plasma, but in liver homogenates as well since liver is regarded as one of the target organs of Cd acute toxicity. Obtained results are summarized in Figure 2. Similarly to our previously described study on mice, single oral Cd treatment produced early marked effects on oxidative-antioxidative balance in liver. Increase of $O_2^{\bullet-}$ and MDA levels was significant after both oral and intraperitoneal treatment which is in agreement with some other authors results. Significantly increased MDA content was found in liver of rats

sacrificed 24h after intraperitoneal treatment with single injection of 2.5 mg $CdCl_2$/kg b.w. (Casalino et al., 2002) and in liver of rats 16 hours after intraperitoneal treatment with 20 μmol Cd/kg b.w. when compared to controls (Liu et al., 2011). Besides MDA plasma levels, Nemmiche et al. (2007) investigated MDA levels in liver as well and found increased levels in Cd exposed group if compared with controls. Furthermore, in our study *i.p.* intake showed more articulate effect on these two parameters: levels of $O_2^{\bullet-}$ were almost 25% higher after *or* exposure and more than 50% higher after *i.p.* exposure when compared to controls, while MDA levels were 2.5 times higher in oral group and almost 3.5 times higher in intraperitoneal group than in control group. This route dependent effect on levels of both parameters can be contributed to significantly higher Cd content determined in liver after *i.p*. administration than after *or* administration (Matović et al., 2012). Indeed, correlation analysis showed strong positive correlations between Cd liver content and $O_2^{\bullet-}$ and MDA levels ($\rho = 0.703$, $P < 0.01$ and $\rho = 0.765$, $P < 0.01$, respectively).

Similarly to the results obtained in plasma of rats, SOD activity in liver was diminished with no statistically significant differences between SOD activities within groups treated orally or intraperitoneally. Decrease in liver activity of SOD shortly after Cd treatment was also observed in our study on mice, as well as in studies of other authors conducted on rats (Casalino et al., 2002; Yalin et al., 2005). As it has been previously proposed, interactions between Cd and bioelements Zn, Cu and Mn that are present in the SOD active center can be one of the explanations for the effect of Cd on SOD activity.

Besides enzymatic defense, we investigated TSH and GSH content in rats liver, as parameters of nonenzymatic defense system. Although considered to be important detoxifying factor against metal toxicity, TSH levels were significantly altered after Cd treatment, regardless the route of exposure. However, significant decrease of GSH, as one of the most important molecules containing SH groups, was observed after Cd *i.p.* treatment.

The explanation for different effect of *or* and *i.p*. Cd intoxication on GSH molecules content might be the assumption that GSH *de novo* synthesis could not overlap more pronounced reduction in GSH content after *i.p.* Cd administration when higher Cd levels in liver were determined (Matović et al., 2012). This is in accordance with our results obtained on mice and results of other authors (Đukić-Ćosić et al., 2007; El-Maraghy et al., 2001; Eybl et al., 2004).

The results of this study show significant decrease in SOD activity, which is not route dependent, but is accompanied with concurrent decline in GSH content after *i.p.* administration. This phenomenon can provide possible explanation for more profound lipid peroxidation observed in liver of intaperitoneally treated rats.

According to the results of our study performed on rats, it can be concluded that Cd induces oxidative stress in a route dependent manner. Elevated ROS levels were observed in both plasma and liver of orally and intraperitoneally treated rats. Further consequences related to ROS elevation, such as increased MDA and AOPP levels were also detected. Intraperitoneal which corresponds to parenteral exposure to Cd resulted in more serious damage of lipids and proteins than *or* exposure. This phenomenon can be attributed to higher Cd body burden observed after *i.p.* intoxication. Since Cd is not a redox-active metal, Cd-induced generation of ROS happens through depletion of enzymatic and non-enzymatic components of antioxidant defense system. Decrease in investigated enzymatic component of antioxidant defense (SOD activity) can be mainly attributed to displacement of metal co-factors from active site of SOD and surprisingly was not route-dependent.

On the other hand, decrease of GSH content as one of the important non-enzymatic component of antioxidant defense system was much more expressed after *i.p.* exposure. These observations suggest that non-enzymatic antioxidant system is more sensitive and more affected by higher Cd concentrations in the organism than enzymatic and/or can be ameliorated less effectively.

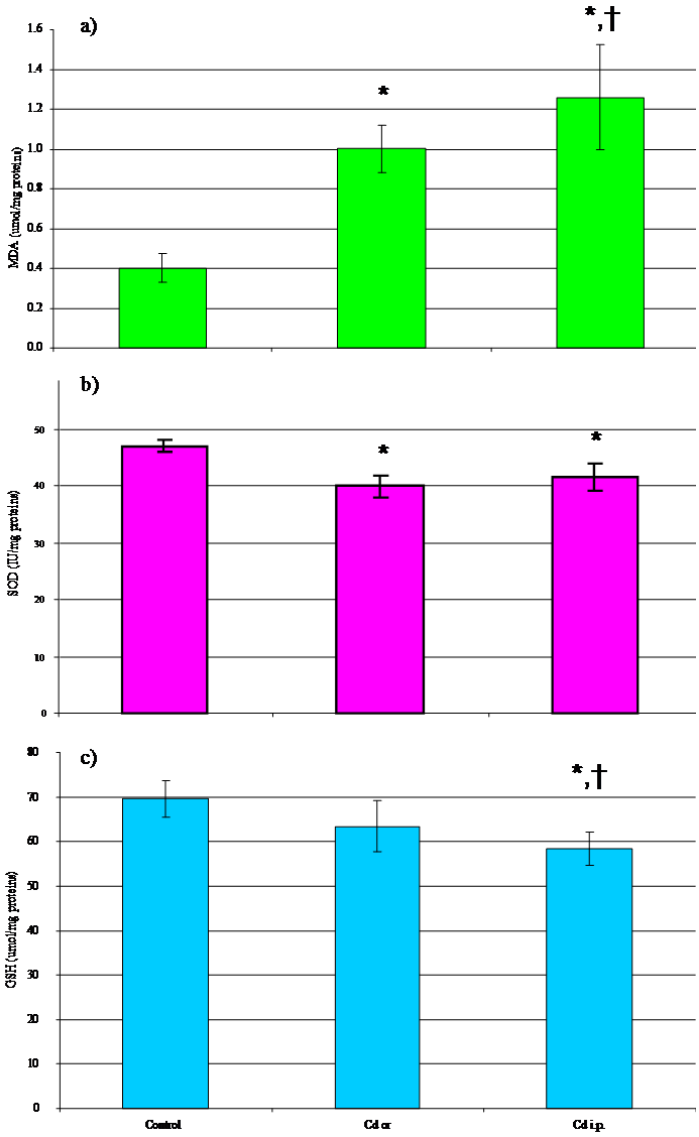

Figure 2. The effects of Cd treatment on a) MDA content, b) SOD activity and c) GSH content in liver of rats.

Control group-non-treated animals, Cd_{or} group-rats intoxicated orally with 30 mg Cd/kg b.w.; $Cd_{i.p.}$ group-rats intraperitoneally injected a single dose of 1.5 mg Cd/kg b.w. The values represent mean ± SD; n=8 animals. Statistically significant differences (ANOVA, LSD test) obtained for each parameter between groups are indicated by: *vs. control, †vs. Cd_{or}. $P<0.001$.

Conclusion

Our studies conducted on three different animal species with different dose levels of Cd provide substantial body of evidence that confirms oxidative stress as one of the mechanisms of Cd toxicity which can lead to serious and profound cell damages. Based on these results, lipid peroxidation can be regarded as early and sensitive consequence of acute Cd exposure since elevated MDA levels were obtained in all species within 24 hours of Cd administration. Duration of exposure was proved to be the major determinant for the critical organ of Cd toxicity, suggesting liver as an organ in which Cd rapidly exerts adverse effects after acute intoxication and kidneys as the critical organ of prolonged exposure to Cd. The route of exposure to Cd was also shown to play a crucial role in the oxidative potency of this toxic metal, demonstrating *i.p.* Cd administration as a route of exposure that has more pronounced adverse effects on majority of investigated parameters of oxidative stress in rat plasma and liver than the *or* one.

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