

The Effect of Long-Term Supplemental Dietary Cadmium on Lipid Peroxidation and the Antioxidant System in the Liver and Kidneys of Rabbits

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Abstract: We investigated the effects of chronic exposure to dietary cadmium (Cd) on the levels of lipid peroxidation (LPO) and reduced glutathione (GSH) associated with activities of glutathione peroxidase (GSH-Px) and the catalase (CAT) enzyme in the liver and kidneys of rabbits. Twenty 10-month-old clinically healthy New Zealand white rabbits (10/group) of 3545 ± 250 g average body weight were used. The control group was allowed to take food and tap water *ad libitum*, while the experimental group was given tap water containing 200 ppm $CdCl_2/L$ for 28 weeks. Lipid peroxidation contents increased significantly in the liver ($p < 0.001$) and kidneys ($p < 0.005$) of Cd-treated rabbits. Glutathione levels decreased significantly in both the liver ($p < 0.001$) and the kidneys ($p < 0.005$) of the experimental group. Cadmium treatment caused a significant decrease in the activities of GSH-Px and CAT in the liver ($p < 0.001$, $p < 0.01$) and kidneys ($p < 0.005$, $p < 0.1$) of the rabbits. These results indicated that a Cd-induced increase in LPO was associated with reductions in GSH-Px and CAT enzyme activities, and in GSH levels in the liver and kidneys of rabbits.

Key Words: Cadmium, Lipid peroxidation, Glutathione peroxidase, Catalase, Glutathione, Rabbit

Tavşanlarda Diyete Uzun Süreli Kadmiyum İlavesinin Karaciğer ve Böbrekte Lipid Peroksidasyonu ve Antioksidan Sistem Üzerine Etkisi

Özet: Bu çalışmada, içme sularına $CdCl_2$ ilave edilen tavşanların karaciğer ve böbreklerinde glutatyon peroksidaz (GSH-Px) ve katalaz (CAT) enzim aktiviteleri ile birlikte redükte glutatyon (GSH) ve lipid peroksidasyonu (LPO) düzeylerinin belirlenmesi amaçlandı. Çalışmada ortalama ağırlıkları 3545 ± 250 g olan, klinik olarak sağlıklı, 10 aylık 20 adet (her grupta 10 adet) Yeni Zelanda beyaz tavşanı kullanıldı. Deneme grubuna 200 ppm $CdCl_2/L$ içeren çeşme suyu ve kontrol grubuna ise çeşme suyu ve yem 28 hafta süresince *ad libitum* olarak verildi. Kadmiyum verilen tavşanların karaciğer ve böbreklerinde LPO düzeylerinde sırasıyla önemli ($p < 0.001$, $p < 0.005$) artış gözlenirken; GSH düzeylerinde ise anlamlı ($p < 0.001$, $p < 0.005$) düşüş saptandı. Deneme grubunun hem karaciğer ($p < 0.001$, $p < 0.01$) hem de böbreklerinde ($p < 0.005$, $p < 0.1$) GSH-Px ve CAT enzim aktivitelerinde önemli düşüş tespit edildi. Bu sonuçlar, Cd'un tavşanların karaciğer ve böbreklerinde LPO miktarını arttırmakla birlikte, GSH-Px ve CAT enzim aktiviteleri ve GSH düzeylerinde azalmaya neden olduğunu göstermektedir.

Anahtar Sözcükler: Kadmiyum, Lipit peroksidasyon, Glutatyon peroksidaz, Katalaz, Glutatyon, Tavşan

Introduction

In recent years, cadmium (Cd) has been recognized as one of the most toxic environmental and industrial pollutants due to its ability to induce severe alterations in various organs and tissues following either acute or chronic exposure (1). Consequently, extensive studies have been carried out to identify the mechanisms of Cd toxicity (2,3). Although information regarding the primary intracellular sequences of Cd toxicity is still lacking, it is now well established that liver and kidneys represent the major target of Cd toxicity (1-3).

Among the various effects induced by Cd in biological systems, the oxidative destruction of membrane polyunsaturated fatty acids, a phenomenon termed lipid peroxidation (LPO), has been observed in numerous tissues both in vitro and in vivo (4,5), despite the apparent incapacity of Cd to directly generate free radicals under physiological conditions (6). The Cd-induced increase in LPO is associated with reductions in glutathione peroxidase (GSH-Px) (5) and catalase (CAT) activities (7), and with reduced glutathione (GSH) levels (8). Oxygen radical scavengers such as GSH-Px, CAT, and

GSH are protective against Cd-induced oxidative damage in laboratory animals (9). Recently, there have been several reports extolling the protective effect of GSH-Px (10,11) and CAT (7) in preventing peroxidative injury induced by a wide variety of toxic agents. Reduced GSH is the most important nonprotein thiol in living systems (12). Furthermore, reduced GSH is a potent factor in controlling lipid peroxidation (8,13). Cd is known to have a high affinity for thiol and sulphhydryl groups (14,15).

Therefore, the aim of this study was to examine the effect of chronic exposure to dietary Cd on GSH-Px and CAT activities, and to examine its effect on reduced GSH levels in the liver and kidneys of rabbits. The level of peroxidation products (LPO) in tissues in the presence of Cd treatment was also investigated.

Materials and Methods

Animals and Treatment

The experiment was carried out on twenty 10-month-old clinically healthy New Zealand white rabbits weighing 3545 ± 250 g, divided into control and experimental groups of 10 animals each. They were kept in a temperature-controlled room (24 °C) in individual cages and given a basic diet (Table 1) and tap water *ad libitum*. Tap water containing 200 ppm CdCl₂ was given to the experimental group for 28 weeks.

Table 1. Composition of diet (%).

Ingredients	%
Barley	30
Wheat	18.5
Corn	11
Bran	17.02
Soybean	20
Fish meal	0.8
Salt	0.08
Marble dust	0.08
Metionin	0.02
Vitamin mix. ¹	1.25
Mineral mix. ²	1.25

¹Vitamin mix. (ppm): Tocopherol 30,000; vitamin D₃ 2,400,000 IU; Vitamin K₃ 2500; Vitamin B₁ 3000; Vitamin B₂ 7000; Vitamin B₆ 4000; Vitamin B₁₂ 15; Ascorbic acid 50,000; Nicotin amid 40,000; Folic acid 1000; Choline Chloride 125,000; Calcium D-Pantothenat 8,000; Retinol 12,000,000 IU; D-Biotin 45.

²Mineral mix. (ppm): Mn 80,000 ; Fe 80,000; Zn 60,000; Cu 8,000; I 500; Co 200; Se 150; Antioxidant 10,000.

Tissue Preparation

After 28 weeks of exposure both groups were sacrificed under ether anesthesia and their livers and kidneys were immediately excised. The tissues were weighed, rinsed with ice-cold deionized water, cut into small pieces, then dried with filter paper and homogenized using the appropriate buffer depending upon the variable to be measured. The homogenates were centrifuged at 600 g for 10 min and recentrifuged at 13,000 g for 20 min at 4 °C to obtain a postnuclear homogenate and postmitochondrial supernatant fractions.

Analytical Procedures

Lipid peroxidation in tissues was measured by the thiobarbituric acid reacting substance (TBARS) method of Placer et al. (16) and was expressed in terms of the malondialdehyde (MDA) content, which served as the standard of 1,1,3,3-tetraethoxypropane (Sigma). Samples assayed for MDA contained 1.0 mM butylated hydroxytoluene (BHT) (Sigma) in order to prevent artefactual LPO during the boiling step. Values were expressed as MDA equivalents in nmol/g tissue. The reduced GSH levels of the tissue homogenates were measured spectrophotometrically using Ellman's reagent (17). Glutathione peroxidase activity was expressed in the presence of GSH and cumene hydroperoxide substrates using an end-point direct assay (18). The activity was expressed as loss of reduced GSH/min. Glutathione peroxidase activity was expressed in units (1 unit is the enzyme quantity that oxidizes 1 μmol GSH/min in the above system at 25 °C). Enzymatic determination of CAT in tissues was performed according to the method of Aebi (19) in which the decomposition of H₂O₂ was followed spectrophotometrically at 240 nm. The difference in absorbance (ΔA_{240})/unit time was a measure of CAT. Triton X-100 was used in the preparation of the homogenate at a final concentration of 1%. The enzyme activity was expressed as k/mg prot. (1 μmol hydrogen peroxide loss/min at 25 °C).

The activity of the enzymes and GSH were calculated to 1 g protein content of the 10 000 g supernatant fraction, which was determined by Folin-phenol reagent with bovine serum albumin as the standard (20).

Statistical analysis

The results were expressed as mean values \pm SD. The significances between mean values were determined

according to the Mann-Whitney U test evaluated by the SPSS (version 6.0) computer program. The 0.05 level was chosen as the minimum statistical significance throughout.

Results

Lipid peroxidation in Cd-treated rabbits, as measured by TBARS assay (MDA), increased to 40% and 17% above the control levels in the liver and kidneys, respectively ($p < 0.001$, $p < 0.005$) (Table 2, Figure 1).

Reduced GSH contents were significantly decreased in the liver and the whole kidney homogenates of Cd-treated rabbits (respectively, $p < 0.001$, $p < 0.005$) (Table 2, Figure 2).

In the kidney of Cd-treated rabbits, GSH-Px was about 19% lower than in control animals and measured 44.66 ± 0.47 U/g prot., dry w ($p < 0.005$) (Table 2, Figure 3). Again, the results presented in Table 2 clearly show that dietary intake of Cd induces a significant reduction in liver GSH-Px activity. Expressed as a

percentage, GSH-Px activity decreased 1.14-fold (12%) and measured 74.72 ± 2.12 U/g prot., dry w ($p < 0.001$).

Catalase activity had significantly decreased ($p < 0.01$) in the liver by the end of the experiment as a consequence of the Cd treatment (Table 2, Figure 4). The activity of the same enzyme had also significantly decreased ($p < 0.1$) in the kidneys of experimental animals.

Catalase activity in the liver and kidneys of rabbits exposed to Cd amounted to 7.16 ± 0.12 and 8.14 ± 0.08 k/mg prot., respectively.

Discussion

Oxygen radicals and other activated oxygen species are common products of cellular metabolism. They have been reported to be responsible for considerable tissue damage (21,22). It has been shown that reactive oxygen species are also formed by some heavy metals including Cd (23). Recently, Cd-induced increases in superoxide anion radical production (24) and lipid peroxidation (5)

	MDA(nmol/g) (dry w)	GSH(nmol/g) (dry w)	GSH-Px(U/mg prot.) (dry w)	CAT(k/mg prot.) (dry w)
Control				
Liver	2.69 ± 0.29	2.23 ± 0.08	85.24 ± 0.73	7.73 ± 0.38
Kidney	5.48 ± 3.04	2.18 ± 0.04	55.25 ± 0.39	8.47 ± 0.55
Cd-treated				
Liver	4.48 ± 0.36 d	1.04 ± 0.04 d	74.72 ± 2.12 d	7.16 ± 0.12 b
Kidney	6.63 ± 0.46 c	1.05 ± 0.05 d	44.66 ± 0.47 c	8.14 ± 0.08 a

a $p < 0.1$, b $p < 0.01$, c $p < 0.005$, d $p < 0.001$

Table 2. Activity of antioxidant enzymes and levels of GSH with tissue peroxidation products (MDA) in the liver and kidneys of Cd-treated rabbits. Values are mean \pm SD of control (n = 10) and Cd-treated (n = 10) rabbits.

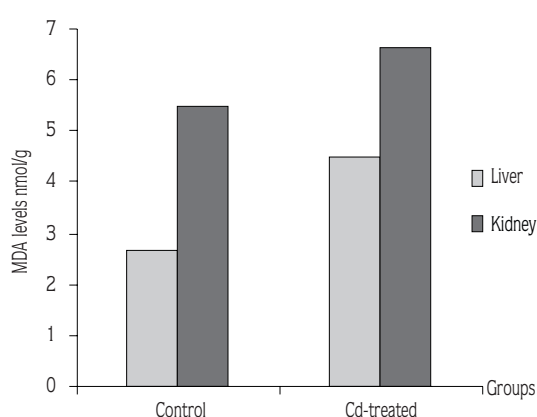


Figure 1. MDA levels in liver and kidneys of control and Cd-treated rabbits.

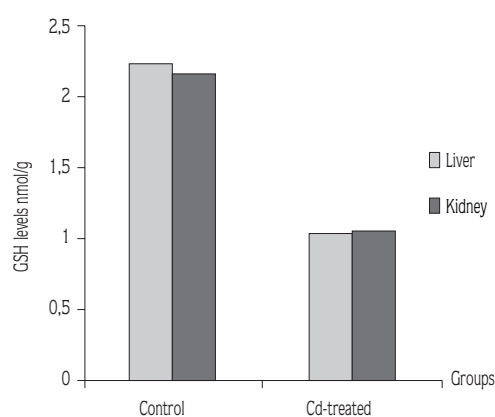


Figure 2. GSH levels in liver and kidneys of control and Cd-treated rabbits.

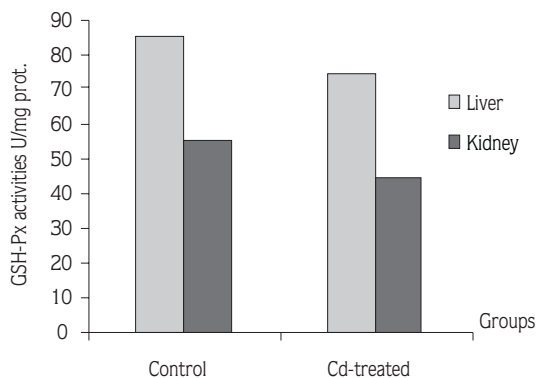


Figure 3. GSH-Px activities in liver and kidneys of control and Cd-treated rabbits.

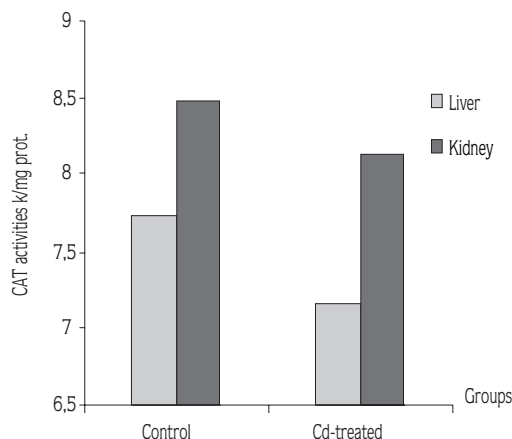


Figure 4. CAT activities in liver and kidneys of control and Cd-treated rabbits.

and nonenzymatic scavengers have been reported (25,26). However, the mechanisms responsible for their formation are not well understood.

The data presented in this paper show that chronic dietary intake of Cd induces peroxidative injury in the liver and kidneys of rabbits. Cd-induced peroxidative injury in these organs is mediated via lipid peroxidation, reflected by significant reductions in GSH-Px and CAT activities, and in reduced GSH levels (Table 2).

In rabbits treated with Cd for 28 weeks the level of LPO in the liver and kidneys respectively, estimated according to the detected amount of MDA, was about 40% and 17% higher than in control animals (Table 2, Figure 1). Thus, it appears that a period of 28 weeks is sufficient for the development of oxidative damage in tissues by enhancing peroxidation of membrane lipids. These findings are consistent with research on rats (27).

Cadmium has a strong affinity for sulphhydryl groups. Furthermore, Cd depletes GSH and protein-bound sulphhydryl groups, resulting in enhanced production of reactive oxygen species such as superoxide, hydroxyl radicals and hydrogen peroxide (15). It has been reported that the hepatic toxicity of Cd may be due to its binding to intracellular sulphhydryl groups and that intracellular GSH levels may provide protection against Cd cytotoxicity in the liver (28). According to our results, the GSH levels in the liver and kidneys of Cd-treated rabbits were significantly lower than in controls (Table 2, Figure 2). Similarly, some authors (8,28) have shown that chronic exposure to Cd decreases the level of GSH in the liver and

kidneys of rats. Contrary to these results, Kamiyama et al. (29) reported an increase in GSH levels in both the liver and the kidneys of Cd-treated rats. However, it must be noted here that Kamiyama et al. (29) administered Cd intraperitoneally (0.228 mg Cd/kg, 3 d/w) for one year. Increased GSH levels in the liver and kidneys of rats may be a consequence of that.

The antioxidant enzymes and other antioxidants provide the cells with protection against oxidative damage (30). Glutathione peroxidase activity in the liver and kidneys was about 1.12-fold and 1.23-fold lower in Cd-treated rabbits. The liver and kidneys of Cd-treated rabbits showed peroxidative tissue damage, with Cd-induced reductions in the activity of the GSH-Px. This result concurs with that of Jamall and Smith (5) and Congiu et al. (31), who reported decreased GSH-Px activity in the liver and kidneys of Cd-treated rats.

Data presented in Table 2 show that the CAT activity decreased significantly in the liver and kidneys of the experimental group in comparison with the control group. Catalase activity was about 8% and 4% lower in the liver and kidneys of rabbits exposed to Cd compared with the controls. Similarly, Jamall and Sprowls (32) reported a reduction in CAT activity in Cd-treated rats. The reduction in CAT activity, along with a decrease in GSH-Px activity in rats fed Cd may reflect decreased absorption of the essential element required for the activity of these enzymes. These data confirm that GSH-Px may be more important than CAT in preventing peroxidative injury to the liver and kidneys.

In conclusion, the results of this study indicated that chronic dietary intake of Cd induces oxidative damage in the liver and kidneys by enhancing peroxidation of membrane lipids and altering the oxidant systems of the

cells. Cadmium also caused reductions in GSH-Px and CAT enzyme activities and GSH levels in the liver and kidneys of rabbits.

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