Original Article

Effect of Cadmium on Lipid Peroxidation and on Some Antioxidants in the Liver, Kidneys and Testes of Rats Given Diet Containing Cadmium-polluted Radish Bulbs

Samir Haouem^{1*}, and Abdelhamid El Hani¹

¹ Département de Physiologie, Faculté de Médecine de Monastir, 5019 Monastir, Tunisie

Abstract: The aim of this study was to examine the effects of cadmium (Cd), incorporated in radish bulbs, on malondialdehyde and glutathione levels and on superoxide dismutase activity in the liver, kidneys and testes of male rats. The control animals were given diet containing ordinary radish bulbs for 4, 8 and 12 weeks, while contaminated animals were given diet containing Cd-polluted radish bulbs (1.1 mg Cd/g of diet) for the same periods as in the controls. At each time point, rats were euthanized and the liver, kidneys and testes were removed. The results indicated that the body weight gain of contaminated rats was identical to that of the control rats. Cd concentrations in the liver, kidneys and testes increased significantly and gradually from the 4th to 12th week of treatment. Malondialdehyde concentrations decreased significantly in the liver and increased significantly in the kidneys and testes after 12 weeks of treatment, while glutathione levels increased significantly in the liver, and decreased significantly in the kidneys and testes at the same time. No changes were observed in SOD activity in the liver, while in the kidneys and testes, this activity was increased after 12 weeks of treatment as compared with the control rats. (DOI: 10.1293/tox.2013-0025; *J Toxicol Pathol* 2013; 26: 359–364)

Key words: cadmium, liver, kidney, testes, oxidative stress, rat

Introduction

Living beings are evolving today in environments polluted by different types of pollution. Heavy metals are the most dangerous groups of anthropogenic environmental pollutants and are highly toxic and persist in the environment¹. Cadmium is one of the most toxic heavy metals. It is a nonessential trace element that is toxic to plants and animals². This metal is not always present in the environment in the metallic state but is often present as a mineral combined with other elements³. It is widely distributed in the earth's crust, where it exists at concentrations of about 0.1 to 0.2 mg/kg of soil associated with zinc and lead⁴.

Transfer of cadmium through the food chain seems to be one of the most dangerous roads⁵. This transfer occurs through the plants and crops grown in contaminated soil. Therefore, cadmium is a danger to human health due to consumption of plants that can absorb it intensely and concentrate it in their tissues, especially plants that are known for their tolerance to cadmium. Food consumption is the main source for environmental contamination for the nonsmok-

ing general population⁶.

This pollutant has a wide spectrum of distribution. Indeed, it accumulates in various organs, the kidneys, liver, testes, pancreas, thyroid, salivary glands, bone and central nervous system. However, it is mainly concentrated in the liver and kidneys (between 50 and 75% of the total)^{7,8}.

Previous studies have linked the toxic effects of this metal to oxidative stress, since it can alter the antioxidant defense system in several tissues in several animals, causing a decrease in glutathione levels and altered activity of antioxidant enzymes and a change in the structure of the cell membrane through a process of lipid peroxidation^{9,10}. Most of this data were derived from experiments on animals treated with cadmium salts. However, to our knowledge, the studies of the effect of cadmium incorporated naturally in plants are very rare in the literature, while these works are more indispensable and more aware of the origin of human exposure, since vegetables are the main source of nonoccupational exposure of humans to heavy metal¹¹.

So the purpose of this work was to study the accumulation of cadmium in the liver, kidneys and testes and its effect on lipid peroxidation and some antioxidant defense systems in male rats fed a diet containing cadmium incorporated into radish bulbs.

Received: 13 May 2013, Accepted: 8 July 2013

*Corresponding author: S Haouem (e-mail: samirzeineb@yahoo.fr)

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Materials and Methods

Diet preparation

Two lots of radish bulbs were used to prepare diets for rats. One lot consisted of ordinary radishes: control radish bulbs. The second lot consisted of radishes contaminated with cadmium (via irrigation with water contaminated with CdCl₂): contaminated radish bulbs. Before the two lots were incorporated into the diets, they were dried and ground into powders. Next, the control diet was prepared by mixing control radish bulb powder with granular flour at the rates of 5% and 95%, respectively, while the contaminated diet was prepared by mixing contaminated radish bulb powder with granular flour at the same rates as the control diet. Chemical analysis showed that the control diet contained 0.01 mg Cd/kg, while the contaminated diet contained 1.1 ± 0.1 mg Cd/kg.

Animals and treatment

Thirty-six male Wistar rats purchased from Siphath (Ben Arous, Tunisia) and weighing about 130 g were used in this study. Animals were housed individually in a room with the temperature ($22 \pm 2^\circ\text{C}$) and photoperiod (12-h light/dark cycle 07:00–19:00 h) controlled and allowed free access to the control diet. Five days later, the rats were separated into two groups: control and contaminated diet groups containing 18 rats each. Rats in the control group (control rats) continued to receive the control diet for a maximum period of 12 weeks, and rats in the contaminated group were given the contaminated diet for the same period as the control group. Diets and water were given ad libitum to rats. At the end of 4, 8 and 12 weeks of treatment, six rats of each group were weighed and then euthanized by exsanguinations by severing the brachial artery under anaesthesia. The liver, kidneys and testes were removed quickly from animals, washed in ice-cold physiological saline, weighed and stored at -80°C . Animals were maintained during the experimental period in accordance with guidelines for animals care of the Faculté de Médecine de Monastir, Tunisia.

Analytical procedure

Cd determination: Cadmium concentrations in the liver, kidneys and testes were determined as described in our previous study¹². Briefly, samples of the liver, kidneys and testes were dried to a constant weight. Then they were digested in concentric nitric acid. Once the digestion was complete, the samples were cooled at room temperature and brought to a constant volume (5 ml) by adding deionized water. For cadmium determination, an atomic absorption spectrophotometer (Perkin-Elmer AAnalyst 100) was used.

Malondialdehyde (MDA) determination: The concentration of MDA in the 10% homogenates of the liver, kidneys and testes (prepared in phosphate buffer, pH=7.4) was determined as thiobarbituric acid reactive substances (TBARS) according to Buege and Aust¹³.

Total glutathione (GSH) measurement: The concentration of GSH in the 10% homogenates of the liver, kidneys

and testes (prepared in phosphate buffer pH = 7.4) was determined according to Beutler¹⁴.

Superoxide dismutase (SOD) activity: To determine the activity of SOD, the 10% homogenates of the liver, kidneys and testes (prepared in phosphate buffer, pH=7.4) were centrifuged at 6500 rpm at 4°C for 45 min, and SOD activity was measured in the supernatant by the inhibition of nitro-blue tetrazolium (NBT) reduction due to O₂-generated by the xanthine/xanthine oxidase system¹⁵. One unit of SOD activity was defined as the amount of protein causing 50% inhibition of the NBT reduction rate.

Statistics

Data are expressed as means $\pm$ SD. The values were analyzed by the nonparametric Mann-Whitney U test. Differences at $P \leq 0.05$ were considered statistically significant.

Results

Cadmium daily intake

Based on the concentrations of cadmium in the control and contaminated diets and on the daily food consumption, which was approximately equal 120 g/kg/day, the daily intakes of cadmium were 1.2 $\mu\text{g/kg/day}$ and 132 $\mu\text{g/kg/day}$ for the control and contaminated groups, respectively.

Body weight gain

The results presented in Table 1 indicate that the body weight gain of contaminated rats was similar to that of the control rats during the experimental period.

Ratios of liver, kidney and testis weights to body weight

The results presented in Table 1 indicate that the ratios of the liver, kidney and testis weights to body weight of contaminated rats were similar to those of the controls rats during the entire experimental period.

Liver

In the control rats, Cd was detected in the liver at very low levels (on an average of 0.03 mg/g dry weight), whereas in contaminated rats, the Cd concentration increased significantly ($P \leq 0.01$) and gradually from the 4th (0.74 ± 0.18 mg/g dry weight) to the 12th (1.8 ± 0.23 mg/g dry weight) week of treatment (Table 2).

As shown in Table 2, the contents of MDA in the liver of rats of both groups were comparable after 4 and 8 weeks of treatment. By contrast, after 12 weeks of treatment, these contents were significantly lower ($P \leq 0.05$) in the treated rats compared with the corresponding controls.

The results in Table 2 indicate that the differences in the GSH concentrations in the liver between the contaminated rats and control rats after 4 and 8 weeks of treatment were not significant ($P \geq 0.05$). By contrast, after 12 weeks of treatment, these levels were significantly higher ($P \leq 0.05$) in contaminated rats compared with the corresponding controls.

Differences in SOD activity in the liver between the

Table 1. Body Weight Gain and Ratios of Liver, Kidney and Testis Weights to Body Weight of Control and Contaminated Rats

Groups		Period of treatment		
		4 weeks	8 weeks	12 weeks
Body weight gain (%)	Control	46 ± 10	83 ± 9	107 ± 22
	Contaminated	41 ± 11	85 ± 25	108 ± 22
Liver (%)	Control	4.06 ± 0.55	3.71 ± 0.37	3.28 ± 0.25
	Contaminated	4.34 ± 0.56	3.87 ± 0.14	3.28 ± 0.31
Kidney (%)	Control	0.85 ± 0.07	0.78 ± 0.02	0.71 ± 0.04
	Contaminated	0.85 ± 0.02	0.78 ± 0.05	0.71 ± 0.03
Testis (%)	Control	1.16 ± 0.05	0.94 ± 0.08	0.80 ± 0.09
	Contaminated	1.19 ± 0.05	1.01 ± 0.13	0.81 ± 0.04

Data are means ± SD.

Table 2. Cd Content and Changes in MDA and GSH Concentrations and SOD Activity in the Liver of Control and Contaminated Rats

Groups		Period of treatment		
		4 weeks	8 weeks	12 weeks
Cd (µg/g dry weight)	Control	0.02 ± 0.02	0.03 ± 0.02	0.04 ± 0.02
	Contaminated	0.74 ± 0.16**	1.11 ± 0.13**	1.80 ± 0.23**
MDA (nM/g fresh weight)	Control	76.0 ± 17.07	83.1 ± 18.9	86.3 ± 11.96
	Contaminated	67.1 ± 13.47	81.42 ± 16.68	52.15 ± 12.89*
GSH (mg/g fresh weight)	Control	1.38 ± 0.36	1.16 ± 0.41	0.81 ± 0.09
	Contaminated	1.24 ± 0.47	1.13 ± 0.24	1.29 ± 0.26*
SOD (U/g fresh weight)	Control	1180 ± 79.4	1160 ± 50.3	1452 ± 35.8
	Contaminated	1148 ± 179.3	1020 ± 129.1	1480 ± 61.1

Data are means ± SD; *P ≤ 0.05; **P ≤ 0.01.

Table 3. Cd Content and Changes in MDA and GSH Concentrations and SOD Activity in the Kidneys of Control and Contaminated Rats

Groups		Period of treatment		
		4 weeks	8 weeks	12 weeks
Cd (µg/g dry weight)	Control	nd	nd	nd
	Contaminated	0.31 ± 0.04**	0.77 ± 0.08**	1.67 ± 0.13**
MDA (nM/g fresh weight)	Control	93.08 ± 16.95	87.22 ± 11.05	98.75 ± 15.37
	Contaminated	106.58 ± 7.99	76.58 ± 15.7	135.17 ± 27.16*
GSH (mg/g fresh weight)	Control	0.55 ± 0.06	0.60 ± 0.04	1.04 ± 0.03
	Contaminated	0.58 ± 0.15	0.84 ± 0.22	0.77 ± 0.06*
SOD (U/g fresh weight)	Control	1837 ± 335.0	1650 ± 249.0	1538 ± 111.0
	Contaminated	1780 ± 296	1367 ± 219	2083 ± 337.0*

Data are means ± SD; *P ≤ 0.05; **P ≤ 0.01.

control rats and contaminated rats after 4, 8 and 12 weeks of treatment were not significant (Table 2).

Kidneys

In the control rats, Cd was not detected during the experimental period. In contrast, in the poisoned rats, the Cd concentration increased significantly ($P \leq 0.01$) and gradually from the 4th (0.31 ± 0.04 mg/g dry weight) to the 12th (1.67 ± 0.13 mg/g dry weight) week of treatment (Table 3).

MDA levels in the kidneys of contaminated rats were comparable to those in the kidneys of the control rats after 4 and 8 weeks of treatment. However, after 12 weeks of treatment, a significant increase ($P \leq 0.05$) in these levels was ob-

served in the kidneys of the treated rats compared with the control rats (Table 3).

As shown in Table 3, cadmium did not alter the concentrations of GSH in the kidneys of the treated rats compared with the control rats after 4 and 8 weeks of treatment. In contrast, after 12 weeks of treatment, we noted a significant decrease ($P \leq 0.05$) in GSH concentrations in the kidneys of contaminated rats compared with the corresponding controls.

As shown in Table 3, the SOD activity in the kidneys of rats of both groups was comparable after 4 and 8 weeks of treatment, whereas after 12 weeks of treatment, it showed a significant increase ($P \leq 0.05$) in contaminated rats compared

Table 4. Cd Content and Changes in MDA and GSH Concentrations and SOD Activity in the Testes of Control and Contaminated Rats

Groups		Period of treatment		
		4 weeks	8 weeks	12 weeks
Cd ($\mu\text{g/g}$ dry weight)	Control	nd	nd	nd
	Contaminated	$0.18 \pm 0.03^{**}$	$0.37 \pm 0.04^{**}$	$0.64 \pm 0.05^{**}$
MDA (nM/g fresh weight)	Control	81.11 ± 11.65	87.7 ± 27.99	72.8 ± 7.47
	Contaminated	79.13 ± 13.5	57.79 ± 29.14	$98.04 \pm 11.33^*$
GSH (mg/g fresh weight)	Control	0.92 ± 0.2	0.94 ± 0.11	1.03 ± 0.06
	Contaminated	1.31 ± 0.29	1.19 ± 0.2	$0.69 \pm 0.12^*$
SOD (U/g fresh weight)	Control	2369 ± 489	1872 ± 433	1915 ± 350
	Contaminated	2425 ± 377	2113 ± 451	$2609 \pm 338^*$

Data are means $\pm$ SD; * $P \leq 0.05$; ** $P \leq 0.01$.

with the corresponding controls.

Testes

In the control rats, Cd was not detected during the experimental period. In contrast, in poisoned rats, the Cd concentration increased significantly ($p \leq 0.01$) and gradually from the 4th (0.18 ± 0.03 mg/g dry weight) to the 12th (0.64 ± 0.05 mg/g dry weight) week of treatment (Table 4).

The results in Table 4 show that the levels of MDA in the testes of contaminated rats were comparable to those in the testes of the control rats after 4 and 8 weeks of treatment. By contrast, after 12 weeks of treatment, these levels were significantly higher ($P \leq 0.05$) in the treated rats compared with the corresponding controls.

The results in Table 4 indicated that the GSH concentrations in the testes were comparable between the two groups of rats after 4 and 8 weeks of treatment. By contrast, after 12 weeks of treatment, these concentrations were significantly lower ($P \leq 0.05$) in the treated rats compared with the corresponding controls.

The differences in SOD activity in the testis between the control and contaminated rats after 4 and 8 weeks of treatment were not significant, while after 12 weeks of treatment, a significant increase was noted in contaminated rats compared with the corresponding controls (Table 4).

Discussion

Our results showed no difference in body weight gain between contaminated and control rats, which reflects that feeding rats with diet containing Cd incorporated in radish bulbs at the rate of approximately 1 mg Cd/g dry weight of diet for up to 12 weeks (estimated achieved dosage: $132 \mu\text{g/kg/day}$) did not cause a retardation in the growth of rats. This may be explained by the low dose of Cd used, since the same result was obtained previously with a dose of 1 ppm of cadmium chloride (approximately 1.6 ppm of cadmium) added to drinking water and a period of up to 10 months¹⁶.

Many previous studies in several animal species showed that Cd, after chronic exposure, is accumulated in liver, kidney and testis^{17,18}. In agreement with these studies, our results showed that Cd was accumulated and detected

in these three organs from the fourth week of treatment. It is well known that the organs of animals do not accumulate cadmium in the same way¹⁷. The results of the present study showed that throughout the experimental period, the highest concentrations of Cd were observed in the liver, followed by the kidney and the testis. This is in agreement with some previous studies that have reported that cadmium is concentrated mainly in the liver^{19,20}. However it should be noted that the differences in the cadmium concentrations in the liver and kidney decrease as the duration of treatment increases. This can be explained by a gradual mobilization of cadmium from the liver to the kidney²¹.

The effect of Cd on the relative weights of the liver, kidneys and testes has been reported in several previous studies. Some studies have reported atrophy of the kidney, liver and testis^{4,22}, while others have reported enlargement of these organs under the effect of cadmium^{20,23,24}. The results presented in this study show that the Cd accumulated in these organs had no effect on the relative weights of the liver, kidneys and testes. This can be explained by the fact that the concentrations of Cd in these organs are not enough to produce a change in the weights of the liver, kidneys and testes.

Several previous studies have linked cadmium to oxidative stress^{9,10}. Therefore, the levels of MDA, an end product of lipid peroxidation, and some antioxidant defense systems were examined in the present study to find out whether cadmium incorporated into radish bulbs causes oxidative stress.

Our results showed a significant decrease in MDA levels in the liver of the contaminated rats compared with the control rats at the end of the study period. This excludes the possibility of an oxidative nature of this metal in the liver tissue. This confirms the work of Shibutani *et al.*²⁵ and Kawagoe *et al.*¹⁹ carried out respectively in rats and mice, which showed that cadmium decreases lipid peroxidation in the liver of these animals. In contrast, other studies have reported an increase in lipid peroxidation in the liver under the influence of Cd^{25,26}.

Along with the reduction of lipid peroxidation, we reported increased levels of GSH in this organ, while the activities of SOD were unchanged compared with the control

rats. Kawagoe *et al.*¹⁹ also showed that cadmium increases hepatic GSH levels. The increase in GSH levels may be a result of high transcriptional regulation of γ -glutamylcysteine synthetase, the enzyme that is responsible for the synthesis of GSH²⁷.

The inversely proportional relationship between lipid peroxidation and GSH content in the liver will let us suggest that the decrease in lipid peroxidation observed in this work in the liver is due to increased synthesis of GSH due to cadmium. Indeed, GSH is part of a nonenzymatic defense system. It is a central protective antioxidant. GSH is considered the first line of defense against oxidative damage and free radical generation²⁸. It can directly scavenge free radicals²⁹ or act as a substrate for glutathione peroxidase and glutathione S-transferase in the detoxification of hydrogen peroxide³⁰. The lack of effect of cadmium on the activity of SOD observed in our work can be explained by the short duration of the experiment or by low dose of cadmium used.

Unlike the liver, the kidneys and the testes of contaminated rats exhibited high levels of MDA compared with the control rats at the end of the experiment, which indicates an intensification of lipid peroxidation in these organs under the influence of Cd. This is in agreement with several previous studies^{31–33}. Parallel to this increase in MDA levels, we noted a decrease in GSH levels in the kidneys and testes of contaminated rats. This confirms the work of Koyutürk *et al.*³³ and El-Missiry and Shalaby³⁴. On the other hand, we reported an increase in SOD activity in these organs at the end of the study period. These results contradict those of several previous studies^{35,36}, which noted a decrease in the activity of this enzyme in the kidneys and testes of rats treated with Cd. The increase in SOD activity may be interpreted as a protective response against cadmium toxicity in the kidneys and testes. Indeed, it has been shown that the activity of antioxidant enzymes behaves in two different ways during oxidative stress. At the beginning of stress, this activity increases, while in the long term, it is reduced due to the massive production of free radicals. This reduction is the result of damage to the molecular machinery that is required to induce these enzymes^{37,38}.

The mechanism of induction of lipid peroxidation by cadmium is still poorly understood. As a transitional element, cadmium is unable to directly cause the formation of free radicals under physiological conditions³⁹. So it probably acts through indirect mechanisms⁴⁰. Based on the results of the present study, the increase in lipid peroxidation in the kidneys and testes might be attributable to the decrease in GSH levels.

In conclusion, the data of the present study suggest that cadmium incorporated in radish bulbs decreases hepatic lipid peroxidation but increases lipid peroxidation in the kidneys and testes of male rats.

Acknowledgments: This work was supported by research unit 04/UR/09-03 of the General Directorate Of Scientific and Technological Research of Tunisia.

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