

Effect of Single Dose Cadmium Chloride Administration on Oxidative Stress in Male and Female Rats

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Abstract: This study was carried out to determine the effect of single dose cadmium chloride ($CdCl_2$) administration on the possible development of oxidative stress by measuring malondialdehyde (MDA) levels in the liver, kidney and blood serum of male and female rats. For this purpose 80 animals (14-16 weeks old) were initially divided into two 2 groups according to sex, each containing 40 rats. Then both were divided into 5 equal treatment groups [0 (control) 0.5, 1, 2 and 4 mg/kg $CdCl_2$ body weight by subcutaneous injection, respectively]. The blood serum and tissues of the liver and kidney were collected after 24 h of treatment from anesthetized rats. MDA levels of the samples were determined by high performance liquid chromatography. Oxidative stress was evident in both sexes following 24 h exposure to all doses of $CdCl_2$ compared to the controls. Liver MDA levels of male rats were higher than those of female ones. Although MDA levels in the serum and kidney of male rats were higher than those in females, these alterations were not statistically significant. In conclusion, these results show that cadmium causes early oxidative stress as demonstrated by increase in MDA levels in the blood serum and liver and kidney tissues. In addition, the reason for the higher MDA level of in the liver of male rats compared with females could be due to differences in p450 dependent NADPH and higher levels of metallothionein in female rats.

Key Words: Cadmium, malondialdehyde, oxidative stress, male and female rat

Erkek ve Dişi Ratlarda Tek Doz Kadmiyum Klorür Uygulamasının Oksidatif Stres Üzerine Etkisi

Özet: Bu araştırma, erkek ve dişi ratların karaciğer, böbrek ve kan serumlarında malondialdehit (MDA)'in ölçülmesi ile tayin edilen oluşması muhtemel oksidatif stres üzerine, tek doz kadmiyum klorür ($CdCl_2$) uygulamasının etkisini belirlemek amacıyla yapıldı. Bu amaçla toplam 80 hayvan (14-16 haftalık), her grupta 40 rat olacak şekilde dişi ve erkek olarak iki gruba ayrıldı. Daha sonra her iki grup eşit sayıda 5 uygulama grubuna bölündü [Sırasıyla 0 (kontrol), 0,5, 1,0, 2,0 ve 4,0 mg/kg dozunda deri altı enjeksiyon olarak $CdCl_2$]. Uygulamadan 24 saat sonra anestezi edilen ratlardan kan serumu ile karaciğer ve böbrek dokusu örnekleri toplandı. Örneklerdeki MDA düzeyleri High Performance Liquid Chromatography (HPLC) ile tayin edildi. Kontrol değerleri ile karşılaştırıldığında, hem erkek hem de dişi ratlarda $CdCl_2$ 'ün tüm dozlarında 24 saat sonra oksidatif stresin belirgin olduğu gözlemlendi. Erkek ratların karaciğer MDA düzeyleri dişilerden daha yüksek bulundu. Böbrek ve serum MDA düzeyleri erkeklerde dişilere göre yüksek olmasına rağmen, istatistik olarak önemli değildi. Sonuç olarak; araştırmadan elde edilen bulgular, kadmiyumun kan serumu, karaciğer ve böbrek dokularında MDA düzeylerinin artmasına, dolayısıyla erken oksidatif strese neden olabileceğini gösterebilir. Ayrıca, erkek ratların karaciğer MDA düzeyinin dişilerden daha yüksek olmasının sebebi, sitokrom p-450'ye bağlı NADPH farklılığından ve dişilerde metallothionein düzeyinin daha fazla olmasından kaynaklanabilir.

Anahtar Sözcükler: Kadmiyum, malondialdehit, oksidatif stres, erkek ve dişi rat

Introduction

Cadmium, a non-essential element, enters human and animal bodies via different industrial products, environmental pollution and different contaminated foods (1,2). When cadmium enters the body, it reaches the liver

within the first 6 h and binds to metallothionein, which is a protein with a low molecular weight (6000-10,000 Da), and which is rich in cystein (3,4). The Cd-metallothionein complex generated in the liver was reported to be mainly distributed to the kidney and other tissues and hence it causes damage in these tissues (5,6).

Free radical levels are important for the growth and development of all cells. Free radical species affect all important components of cells such as lipids, proteins, carbohydrates and nucleic acids (7). One of the most important effects of free radicals is oxidation of polyunsaturated fatty acids (PUFAs). In particular hydroxyl (OH[•]), peroxy (RO[•]) and alkoxy (ROO[•]) radicals play important roles in the oxidation of PUFAs (8). As a result of free radical attack, lipids are oxidized and hence membranes are damaged (9). Malondialdehyde (MDA), a well-known secondary product of lipid peroxidation after exposure to reactive oxygen species and free radicals, may be used as an indicator of cell membrane injury (10).

It has been reported that administration of cadmium via different routes causes increased lipid peroxidation in membranes of erythrocytes and tissues such as the liver, kidney, brain and testes where MDA is used as an indicator of oxidative damage (11,12).

Intake of cadmium results in consumption of glutathione and protein binding sulfhydryl groups and subsequently the levels of free radicals such as hydrogen peroxide, hydroxide and superoxide are increased. Increased lipid peroxidation results in changes in intracellular stability, DNA damage and apoptosis (12). Bagchi et al. (13) reported that the levels of glutathione peroxidase (GSH-Px) were increased whereas a reduction was observed in the activity of glutathione reductase in experimental cadmium-induced toxicity.

As demonstrated by some researchers (14-17) cadmium toxicity may also alter according to sex differences. In the present study we aimed to determine the effect of cadmium administration at different doses on the levels of MDA, which is an end product of lipid peroxidation, in the serum, liver and kidney of male and female rats.

Materials and Methods

Wistar rats (200-220 g) 14-16 weeks old were used in this study. Rat chow and water were given ad libitum. The rats were housed under temperature controlled (22-25 °C) conditions with a 12:12 light:dark cycle. Eighty rats were initially divided into 2 equal groups according to sex and then 5 groups (control and 4 groups to be administered different doses of cadmium) of each sex were randomly chosen to have 8 rats each.

CdCl₂ was given via subcutaneous (s.c.) injection at doses of 0.5, 1.0, 2.0 and 4.0 mg/kg body weight. The animals were anesthetized by ether 24 h after the injection and blood samples were taken from their hearts into tubes and the sera were separated. Whole tissues of the liver and kidneys were obtained by dissection and were immediately weighted. All tissues were kept at -20 °C until analyzed.

For determination of MDA levels, blood samples were centrifuged at 3000 g for 5 min at 4 °C and the sera were separated. For the preparation of samples, to an aliquot of 0.1 ml of rat serum was added 0.1 ml of 0.5M HClO₄ and 0.8 ml of distilled water. Addition of acid was necessary to precipitate proteins and release the MDA bound to the amino groups of proteins, and other amino compounds. Then the samples were centrifuged at 4500 g for 5 min and used for HPLC analysis. Some 20 µl of supernatant was taken and injected into the HPLC. HPLC separations were accomplished at room temperature with a Cecil liquid chromatography system (Series 1100) consisting of a sample injection valve (Cotati 7125) with a 20 µl sample loop, a UV spectrophotometric detector (Cecil 68174), an integrator (HP 3395) and a Supelcosil C18 (5 µm particle and 80 Å pore size) column (250 x 4.6 ID) with a mixture of 30 mmol KH₂PO₄ and methanol (65%-35%, H₃PO₄ by pH 4), and the mobile phase at a 1.5 ml min⁻¹ flow rate (18,19).

Tissue MDA levels were determined after homogenization as described for serum MDA levels (18,19).

Data for parameters were grouped and expressed as mean ± pooled standard errors of means. Differences between the means of male and female rats of each treatment group were identified by Student's t test. The means obtained from the control group and from the groups administered 0.5, 1.0, 2 and 4 mg/kg CdCl₂ were subjected to analysis of variance. If appropriate (P < 0.05), post hoc analyses were carried out using Duncan's multiple range tests for multiple comparisons. Statements of statistical significance are based on P < 0.05 (SPSS for Windows, Ver, 10; SPPS Inc., Chicago, IL., USA).

Results

MDA levels in the liver, kidney and serum of animals that received different doses of cadmium and the controls

are presented in the Table and Figures 1-3. After the administration of 0.5 mg/kg cadmium, MDA levels in the liver increased significantly in both sexes (male: $0.61 \pm 0.05 \mu\text{mol/g}$; female: $0.49 \pm 0.06 \mu\text{mol/g}$) compared to the controls (Table, Figure 1). There was a dose-dependent increase in MDA levels in the liver tissues and they were higher in the male rats than in the female ones after doses of 0.5, 1.0, 2.0 and 4.0 mg/kg of CdCl_2 (Table, Figure 1).

Kidney MDA levels also increased in a dose-dependent manner after the administration of cadmium and reached 0.31 ± 0.04 and $0.29 \pm 0.03 \mu\text{mol/g}$ with an increase in male and female rats, respectively, compared to the controls (male: $0.12 \pm 0.03 \mu\text{mol/g}$, female: $0.11 \pm 0.02 \mu\text{mol/g}$; Table, Figure 2).

Serum MDA levels in the control male and female rats were 2.02 ± 0.38 and $1.90 \pm 0.44 \mu\text{mol}/100 \text{ ml}$ and there was a dose-dependent increase in the groups given cadmium (Table, Figure 3). Although there were

differences in MDA levels in the kidney and serum of rats given cadmium, they did not differ significantly between male and female rats ($P > 0.05$) (Table, Figures 2 and 3).

Discussion

Functional deficiencies may develop in the organelles due to lipid peroxidation caused by free oxygen radicals. Cellular death and increased synthesis of collagen follow increased fragility of lysosomes and changes in microsomal enzymes. These lipid peroxidation products easily create reactive carbon compounds, the most important of which is MDA. Determining the level of MDA is usually the most practical and reliable method for detecting and screening for oxidative stress (20).

Cadmium has been reported to cause damage in vital organs such as the liver, kidney, testes and brain by several researchers (2,21). In acute cadmium intake, induction of lipid peroxidation has been reported to be

Table. Levels of MDA in the liver, kidney and serum after 24 h administration of different doses of CdCl_2 in male and female rats.

Dose of CdCl_2	Liver MDA ($\mu\text{mol/g}$)		Kidney MDA ($\mu\text{mol/g}$)		Serum MDA ($\mu\text{mol}/100 \text{ ml}$)	
	Male	Female	Male	Female	Male	Female
0	0.21 ± 0.03^a	0.20 ± 0.02^a	0.12 ± 0.03^a	0.11 ± 0.02^a	2.02 ± 0.38^a	1.90 ± 0.44^a
0.5 mg/kg	0.61 ± 0.05^b	$0.49 \pm 0.06^{b*}$	0.16 ± 0.02^b	0.15 ± 0.02^b	2.87 ± 0.67^b	2.65 ± 0.58^b
1 mg/kg	0.76 ± 0.05^c	$0.60 \pm 0.05^{c*}$	0.20 ± 0.03^c	0.19 ± 0.02^c	3.48 ± 0.47^c	3.43 ± 0.40^c
2 mg/kg	0.93 ± 0.07^d	$0.74 \pm 0.06^{d*}$	0.26 ± 0.03^d	0.25 ± 0.03^d	4.62 ± 0.57^d	4.48 ± 0.60^d
4 mg/kg	1.05 ± 0.08^e	$0.97 \pm 0.08^{e*}$	0.31 ± 0.04^e	0.29 ± 0.03^e	6.60 ± 0.71^e	6.41 ± 0.70^e

a, b, c, d, e. Values within columns with no common superscripts are significantly different ($P < 0.05$), according to Duncan's multiple range tests.

* Values are significantly different from the same parameters of male rats ($P < 0.05$), according to Student's t tests.

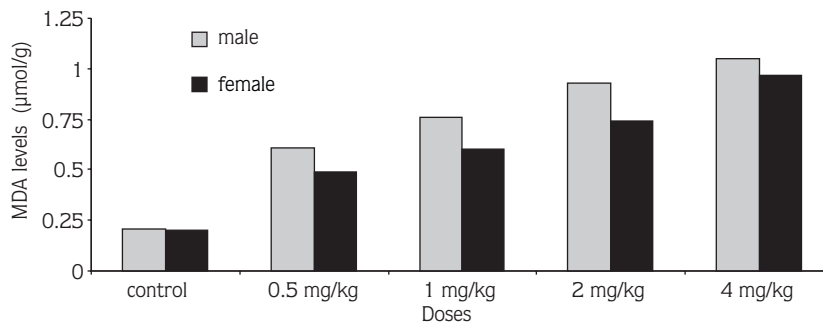


Figure 1. Levels of MDA in the liver after administration of different doses of CdCl_2 in male and female rats.

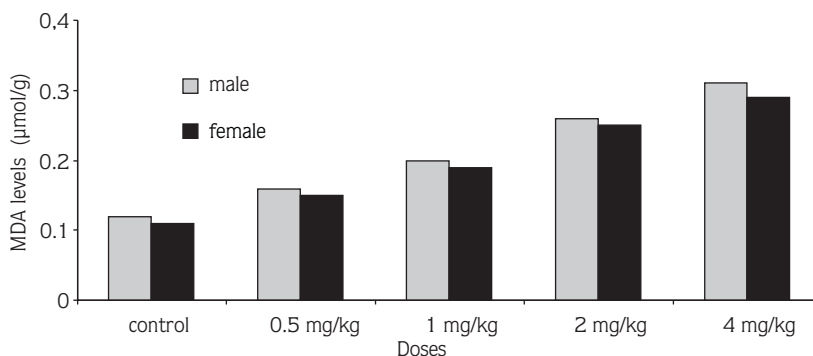


Figure 2. MDA levels in the kidney after administration different doses of CdCl₂ in male and female rats.

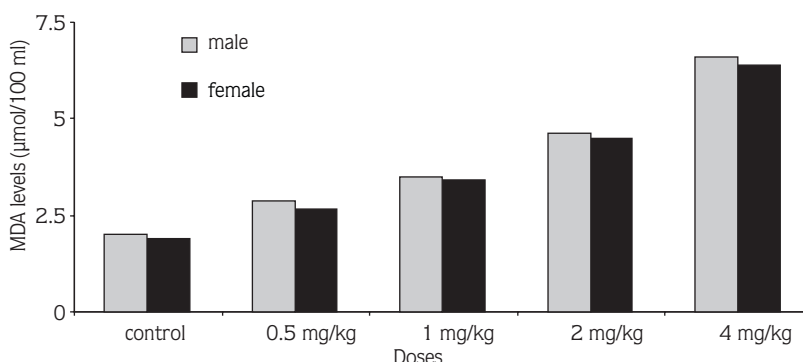


Figure 3. MDA levels in serum after administration of different doses of CdCl₂ in male and female rats.

the reason for cadmium toxication and has hence been demonstrated to cause generation of free radicals in early cadmium intoxication (22,23). We demonstrated in the present study that when single doses of different concentrations of cadmium are administered the dose-dependent increase in MDA levels is in agreement with this knowledge.

NADPH is involved in the catalyzing effect of cytochrome P-450 in oxidative stress and in the conversion of molecular oxygen to H₂O₂. Subsequently lipid peroxides are formed from H₂O₂. There are reports indicating that cytochrome P-450 and NADPH show differences between species and sexes (14-16).

Andersen and Andersen (24) have reported that following the administration of 25 µmol/kg CdCl₂ lipid peroxidation levels in the liver were higher in male mice than in female ones, but at toxic doses of CdCl₂ gender based differences were not present. When rats received 1.5 mg/kg CdCl₂ for 2 days, liver MDA levels were higher

in male than in female rats (14). Sato and Nagai (25) have reported that NADPH dependent lipid peroxidation levels in the liver of male rats were higher than in female ones. In the present study liver MDA levels in male rats that received 0.5, 1.0, 2.0 and 4.0 mg/kg CdCl₂ were significantly higher than in female ones (P < 0.05) (Table, Figure 1). On the other hand, although MDA levels in the kidney and serum were higher in all animals that received CdCl₂, there were no statistically significant differences between the MDA levels of male and female animals. Our results are similar to the findings of other reports (14,24,25). We think that the higher MDA levels in the liver of male rats compared to female ones might be due to differences in the levels of cytochrome p-450 dependent NADPH.

Metallothionein, which plays roles in cadmium toxicity, transportation and detoxification, is reported to be increased by β-estradiol and progesterone administration (26). On the other hand, Shiraishi et al.

(27) reported that pretreatment with progesterone did not change cadmium toxicity in male rats. Following subcutaneous cadmium administration to male and female rats, liver metallothionein levels in female rats were reported to be higher than those in male ones (28). Blazka et al. (17) demonstrated that after the administration of 10 µmol/kg cadmium to young rats, cadmium levels in the liver of male rats were higher, whereas metallothionein levels in mature female rats were 2 times higher than those in mature male rats. Previously we reported that the administration of single dose cadmium with estrogen and progesterone administration resulted in differential distribution in the tissues of mice (29).

Considering the basal metabolism of female rats, estrogen and progesterone levels are very high compared to those in male rats. Therefore, metallothionein levels would be expected to be higher in females than in males. Increased MDA levels in male rats that received cadmium compared to female ones were also related to metallothionein levels.

In conclusion, liver MDA levels of male rats that received 0.5, 1.0 2.0 and 4.0 mg/kg cadmium were significantly higher than those of female ones. These results might be related to higher levels of metallothionein in female rats and faster lipid peroxidation due to NADPH in male rats.

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