The effect of melatonin on cadmium-induced renal injury in chronically exposed rats

Melatoninin kronik olarak kadmiyum verilen sıçanlarda böbrek hasarı üzerine etkileri

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Abstract

Objective: This study was designed to investigate the effects of melatonin as an antioxidant, in prevention and treatment of cadmium (Cd)-induced renal toxicity in rats.

Materials and methods: Fifty adult male Sprague-Dawley rats weighing 340 to 370 g were used. Renal toxicity was induced with the use of 200 μg/ml of cadmium chloride in tap water. Ten rats were assigned to receive only tap water as the control group for three months and seven days. Two study groups were designed. To investigate the ability of melatonin to prevent Cd damage (Study 1), 10 rats received Cd and 10 rats received Cd plus 0.02% co-treatment melatonin in tap water for three months. To test whether melatonin would reverse CD-induced damage to the kidney (Study 2), 10 rats received Cd in tap water for three months+7 days, and 10 rats received Cd for three months followed by administration of high-dose (0.08%) melatonin in tap water for seven days.

Results: Cadmium exposure significantly increased the kidney malondialdehyde (MDA) levels, as a marker of lipid peroxidation, decreased the kidney superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities, increased Cd concentrations in the renal cortex, but did not change glomerular filtration rate (GFR) and fractional excretion of sodium (FE-Na). In Study 1, melatonin decreased MDA levels, increased SOD, CAT and GSH-Px activities. In Study 2, melatonin decreased the kidney MDA levels, increased the SOD activity, but did not change the CAT and GSH-Px activities. Kidney accumulation of Cd did not change in both melatonin-treated groups. Histopathologically, Cd intake affected proximal tubules of the nephron more than the glomerular parts. Melatonin did not change these alterations.

Conclusion: Our findings suggest that Cd-induced renal toxicity is related with oxidative stress and exogenously administrated melatonin might reduce the toxic effects of Cd on kidney without any reduction in tissue Cd burden.

Key words: Antioxidants; cadmium/toxicity; glutathione; kidney/drug effects; lipid peroxidation/drug effects; malondialdehyde; melatonin; oxidative stress/drug effects; rats.

Özet

Amaç: Bu çalışmada melatoninin antioksidan olarak sıçanlarda kadmiyumla oluşturulan renal toksisitenin önlenmesi ve tedavisi üzerindeki etkileri araştırıldı.

Gereç ve yöntem: Çalışmada, ağırlıkları 340-370 gr arasında değişen 50 adet Sprague-Dawley cinsi erişkin erkek sıçan kullanıldı. Renal toksisite 200 μg/ml kadmiyum kloridin içme suyuna eklenmesiyle oluşturuldu. On adet sıçana, üç ay yedi gün süreyle sadece içme suyu verildi (kontrol). Geri kalan sıçanlardan iki çalışma grubu oluşturuldu. Melatoninin kadmiyuma bağlı hasarı önleyip önlemediğini araştırmak için (Çalışma 1), 10 sıçana üç ay süreyle içme suyunda kadmiyum verilirken, 10 sıçana kadmiyumla birlikte %0.02 melatonin hasarın önlenmesi amacıyla verildi. Melatoninin böbrekteki kadmiyuma bağlı hasarı geri döndürüp döndürmediğini araştırmak için (Çalışma 2), 10 sıçana üç ay yedi gün süreyle içme suyunda kadmiyum verilirken, 10 sıçana, üç aylık kadmiyum verilmesi ardından yedi gün daha yüksek dozda (%0.02) melatonin verildi.

Bulgular: Kadmiyum, lipid peroksidasyonunun göstergesi olan böbrek malondialdehit (MDA) düzeylerini anlamlı derecede yükseltti; süperoksit dismutaz (SOD), katalaz (CAT) ve glutatyon peroksidaz (GSH-Px) aktivitelerini düşürdü; renal kortekste kadmiyum konsantrasyonunu artırdı; glomerüler filtrasyon hızı (GFR) ve fraksiyonel sodyum salınımında (FE-Na) ise değişiklik olmadı. Çalışma 1'de, melatoninle MDA düzeyleri düşüş, SOD, CAT ve GSH-Px aktiviteleri artış gösterdi. Çalışma 2'de melatoninle MDA düzeyleri düşüş, SOD aktivitesi artış gösterirken, CAT ve GSH-Px aktivitelerinde değişiklik olmadı. Melatonin iki grupta da böbrekteki kadmiyum birikiminde değişiklik oluşturmadı. Histopatolojik incelemede, kadmiyumun glomerüler alanlardan ziyade nefrondaki proksimal tübülleri etkilediği gözlendi. Melatonin bu değişikliklere etkili olmadı.

Sonuç: Bulgularımız, kadmiyumun yol açtığı renal toksisitenin oksidatif stresle ilişkili olduğunu ve melatonin uygulamasının, dokulardaki kadmiyum birikiminde düşüş olmaksızın böbrekteki toksik etkileri azaltabileceğini göstermektedir.

Anahtar sözcükler: Antioksidan; kadmiyum/toksisite; glutatyon; böbrek/ilaç etkisi; lipid peroksidasyonu/ilaç etkisi; malondialdehid; melatonin; oksidatif stress/ilaç etkisi; sıçan.

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Cadmium (Cd) has been widely used in industry as an anticorrosive agent for steel, iron, brass, and other alloys, and as a stabilizer for paints, pigments, nickel-Cd batteries, and plastics.[1] However, Cd-contaminated topsoil is considered to be the most likely mechanism for the greatest human exposure through uptake into edible plants and tobacco.[2] Cadmium mainly accumulates in the kidney and liver. Its extremely long biological half-life (10-30 years) in humans makes it a cumulative toxin.[3,4] It is known that chronic exposure to Cd can induce severe nephropathy in humans[4] and animals.[5-8] Renal injury is believed to be caused by Cd-metallothionein which is originally produced in the liver, released into the circulation, taken up by the renal proximal tubular epithelial cells, and degraded to liberate toxic Cd ions.[9,10] Cadmium has been demonstrated to stimulate free radical production, resulting in oxidative deterioration of lipids, proteins, and DNA, and initiating various pathological conditions in humans and animals. The generation of the reactive oxygen species plays an important role in the pathogenesis of Cd-induced renal damage.[11]

Melatonin is a potent free radical scavenger and antioxidant which is known to be highly effective in protecting against oxidative damage caused by a variety of toxins.[12-17] It also enhances the antioxidant potential of the cell by stimulating the synthesis of antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and glutathione reductase, and by augmenting glutathione (GSH) levels.[18,19]

In the present study, we investigated whether chronic Cd toxicity involved oxidative stress, and analyzed renal malondialdehyde (MDA), GSH levels and SOD, catalase (CAT), and GSH-Px activities. One part of this study investigated the ability of melatonin to prevent Cd damage while another part tested whether melatonin would reverse Cd-induced damage to the kidney.

Materials and methods

Animals

Fifty adult male Sprague-Dawley rats weighing 340 to 370 g were obtained from animal facility of Trakya University School of Medicine. They were kept in a windowless room at a constant temperature of 22±2 °C with 12 hours of light and dark cycles and fed with standard rat chow. All experimental protocols were approved by the Animal Care Ethics Committee of Trakya University School of Medicine.

Chemicals

Cadmium chloride ($CdCl_2$) was obtained from Fluka (Taufkirchen, Germany), and melatonin was obtained from Sigma (St. Louis, MO, USA). Other chemicals used for analytical grade were obtained from Sigma and Merck (Darmstad, Germany). Melatonin was dissolved freshly in pure ethanol and then diluted in saline (0.09% NaCl w/v) to give a final concentration of less than 0.05% in drinking water.

Chronic intoxication with cadmium and prevention and treatment by melatonin

The study groups and experimental design are shown in Table 1. To induce renal toxicity, 200 µg/ml of cadmium chloride ($CdCl_2$) was used in tap water for three months. We designed two study groups. One part of the study investigated the ability of melatonin to prevent Cd damage (Study 1) and the other part tested whether melatonin would reverse Cd-induced damage to the kidney (Study 2). Ten rats were assigned to receive only tap water as the control group for three months and seven days. The remaining rats were divided into two study groups each consisting of two subgroups. In Study 1, one group of rats (n=10) received Cd-1 in tap water for three months, and the other group (n=10) received Cd plus 0.02% co-treatment melatonin (CoM) in tap water for three months. In Study 2, one group of rats (n=10) received Cd-2 in tap water for three months+7 days, and the other group (n=10) received

Table 1. Study groups and experimental design

Groups		Experimental design
Control (n=10)		Tap water for 3 months + 7 days
Study 1	Cd-1 (n=10)	200 µg/ml $CdCl_2$ in tap water for 3 months (Designed as the control of Cd+CoM group)
	Cd+CoM (n=10)	200 µg/ml $CdCl_2$ and low-dose melatonin (0.02%) in tap water for 3 months.
Study 2	Cd-2 (n=10)	200 µg/ml $CdCl_2$ in tap water for 3 months + only tap water for 7 days (Designed as the control of Cd+M group)
	Cd+M (n=10)	200 µg/ml $CdCl_2$ in tap water for 3 months + high-dose melatonin (0.08%) in tap water for 7 days

Cd for three months followed by administration of high-dose (0.08%) melatonin (Cd+M) in tap water for seven days.

To collect 24-hour urine samples to analyze glomerular filtration rate (GFR) and fractional excretion of sodium (FE-Na), the animals were housed individually in metabolic cages. At the time of sacrificing, the rats were anesthetized with 10 mg/kg of xylazine and 50 mg/kg of ketamine. Blood samples were collected by cardiac puncture and bilateral kidneys were removed. For histopathologic analyses, a portion of the right kidney was cut longitudinally and fixed in 10% formalin solution. The rest of the renal tissues were stored at -85 °C. Blood samples were centrifuged (1500 g for 10 min at 4°C) immediately and the plasma samples were also stored at -85 °C until assayed. Afterwards, tissue MDA levels, an end product of lipid peroxidation, GSH levels, a key antioxidant, and SOD, CAT and GSH-Px activities were measured in these samples.

MDA and GSH assays

The kidney tissue samples were homogenized with 150 mM of ice-cold KCl for the determination of MDA and GSH levels. Homogenates were centrifuged at 2600 g for 10 min at 4°C. The MDA concentration in the renal tissue was assayed in the form of thiobarbituric acid reacting substances according to the method described by Ohkawa et al.[20] Two hundred microliters of supernatant were added to 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% acetic acid (pH 3.5), 1.5 ml of 0.8% thiobarbituric acid, and 0.6 ml of distilled water. This mixture was heated to 95°C for 60 minutes. After cooling with tap water, 1.0 ml of distilled water and 5.0 ml of the mixture of n-butanol-pyridine (15:1, v/v) was added, the mixture was vigorously shaken and centrifuged at 2600 g for 10 min at 25°C. The absorbance of the organic layer was read at 532 nm. Malondialdehyde was quantified using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} and expressed as nanomoles MDA per milligram tissue.

The level of GSH was determined according to the Ellman method.[21] The concentration of GSH was monitored spectrophotometrically at 412 nm. The results were expressed as μmol/g tissue.

Determination of enzyme activities

All enzyme activities were determined after homogenization of the renal tissue with phosphate buffered saline at pH 7.4. The total (Cu-Zn and Mn) SOD activity was determined according to the method of Sun et al.[22] This method is based on the inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine-xanthine oxidase system as a superoxide generator. One unit of SOD was defined as the amount of the enzyme causing 50% inhibition in the NBT reduction rate. The specific activity was expressed in units per milligram of protein. The CAT activity was measured as described by Aebi.[23] It was based on the determination of the rate constant (k; /s) of hydrogen peroxide decomposition rate at 240 nm. The results were expressed as the rate constant per milligram homogenate protein. The GSH-Px activity was measured as described by Lawrence and Burk[24] by monitoring the oxidation of reduced NADPH (Nicotinamide adenine dinucleotide phosphate) at 340 nm. Enzyme units were defined as the number of μmol NADPH oxidized/min and calculated using the extinction coefficient of NADPH at 340 nm (6.22×10^3 M^{-1} cm^{-1}). Results were reported as units/mg protein. The protein content of tissue samples was determined by the method described by Lowry et al.[25]

Cadmium measurement in kidney tissue

Kidney samples of about 0.5 g wet weight were placed in a polyethylene vessel with 2.0 ml of concentrated HNO_3. After 24 hours of sample digestion at room temperature, 1 ml of concentrated $HCOl_4$ was added. Cadmium analyses of kidney tissues were carried out by electrothermal atomic absorption spectrometry using a Varian GTA 120 SPEKTRA AA instrument (Varian Associates Inc., PaloAlto, CA, USA).

Blood and urine urea and creatinine assay

Blood samples obtained directly from the heart were centrifuged to separate serum and analyzed to assess renal function by measuring serum and urine concentrations of urea and creatinine with a Synchron LX20 analyser (Beckman, USA).

Glomerular filtration rate and fractional excretion of sodium

Glomerular filtration rate was calculated using the formula UV/P, where U refers to creatinine concentration in urine, V refers to urine volume/min, and P to serum creatinine. Fractional excretion of sodium was calculated using the following formula: [(urine Na x serum Cr)/(urine Cr x serum Na)] x 100%.

Histopathological analysis

A portion of each kidney tissue was fixed in 10% formalin solution, dehydrated in ethanol and xylene, and embedded in paraffin. Tissues were then sectioned at 5 μm, stained with hematoxylin and eosin (H&E)

Table 2. Malondialdehyde, glutathione levels and enzyme activities (superoxide dismutase, catalase, and glutathione peroxidase), and glomerular filtration rate, fractional excretion of sodium and kidney tissue cadmium levels in the two study groups

		Study 1			Study 2		
	Control	Cd-1	*p*	Cd+CoM	Cd-2	*p*	Cd+M
Malondialdehyde (nmol/mg protein)	0.09±0.02	0.41±0.15[a]	0.000	0.12±0.04	0.47±0.11[a]	0.000	0.21±0.12
Glutathione (nmol/mg protein)	2.52±0.26	1.99±0.31[b]	N S	2.48±0.26	1.90±0.36[b]	N S	2.20±1.19
Superoxide dismutase (U/mg protein)	8.40±0.82	6.30±0.59[a]	0.000	7.76±0.49	6.16±0.42[a]	0.000	8.21±0.53
Catalase (U/mg protein)	0.80±0.11	0.65±0.13[a]	0.001	0.92±0.13	0.76±0.12[a]	N S	0.85±0.13
Glutathione peroxidase (U/mg protein)	1.99±0.33	1.60±0.33[a]	0.015	2.00±0.13	1.81±0.14[a]	N S	1.73±0.12
Glomerular filtration rate	2.2±0.46	2.31±0.32[a]	N S	2.53±0.83	2.77±0.7[a]	N S	2.27±0.51
Fractional excretion of sodium	0.10±0.07	0.12±0.07[a]	N S	0.11±0.05	0.17±0.07[a]	0.007	0.05±0.03
Tissue cadmium levels (μg/g)	0.16±0.01	1.27±0.32[b]	N S	1.10±0.37	1.46±0.45[a]	N S	1.75±0.51

a: p<0.0001; b: Not significant (NS).

and examined by the same pathologist, who did not know about the study groups, under a light microscope (Nikon Eclipse E600W). The slides were then subjected to periodic acid-Schiff (PAS) reaction in order to show the basement membranes, brush border of the tubules, vessels, and Bowman's capsule.

Statistical analysis

All statistical analyses were performed using Minitab Release 13 (Reference number: wcp 133100197). All data were expressed as mean ± standard deviation (SD). Distribution of the groups was found normal in one-sample Kolmogorov-Smirnov test. Analysis of variance (ANOVA) was used to determine whether differences between means were significant. A post hoc comparison (Scheffe's procedure) made after the ANOVA resulted in a significant F test. Values of $p<0.05$ were considered statistically significant.

Results

Study 1

Following Cd exposure renal MDA levels increased. Compared to the Cd-1 group, the kidney MDA levels were significantly lower in the Cd+CoM group ($p<0001$). There was no significant difference in GSH levels between the control and Cd-1 groups. Additional treatment with melatonin did not change the kidney GSH level. The kidney SOD, CAT, and GSH-Px activities were found to be significantly lower in the Cd-1 group than the control group. A marked increase in these enzyme activities were found in the Cd+CoM group (Table 2). Cadmium intake and co-treatment with melatonin did not change the GFR and Fe-Na levels. Cadmium concentrations in the renal cortex increased in the Cd-1 group compared to the control group. Co-treatment with melatonin did not change the kidney tissue Cd level (Table 3).

Study 2

Cadmium exposure significantly increased the kidney MDA levels. Treatment with melatonin after Cd toxication significantly decreased the elevated MDA levels. Cadmium intake did not change the kidney GSH level. In addition, melatonin treatment did not affect the kidney GSH level. The kidney SOD, CAT, and GSH-Px activities were found to be significantly lower in the Cd-2 group than the control group. While a marked increase in SOD activity was found in the Cd+M group compared to the Cd-2 group, melatonin treatment did not change the kidney CAT and GSH-Px activity (Table 2). Chronic Cd exposure did not affect GFR and Fe-Na. While melatonin treatment did not change the GFR, FE-Na was higher in the Cd-2 group compared to the Cd+M group. Cadmium concentrations in the renal cortex increased in the Cd-2 group compared to the control group. However, treatment with melatonin did not change the kidney tissue Cd level (Table 2).

Histopathological results

Cd-1 and Cd-2 groups showed similar changes representing nephrotoxic effects of Cd exposure. Cadmium intake affected proximal tubules of the nephron more than the glomerular parts. Hydropic degeneration of epithelia and acidophilic changes of renal tubules, interstitial mononuclear cell infiltration, and desquamation of the epithelium into tubular lumen (tubular cell apoptosis) were noted (Fig. 1). A diffuse PAS positive reaction was observed in the renal glomeruli, basement membranes of the tubules, walls of the blood vessels and brush borders of the tubules in the control rats. However, most of the proximal tubules in the groups exposed to Cd revealed a lack of regular

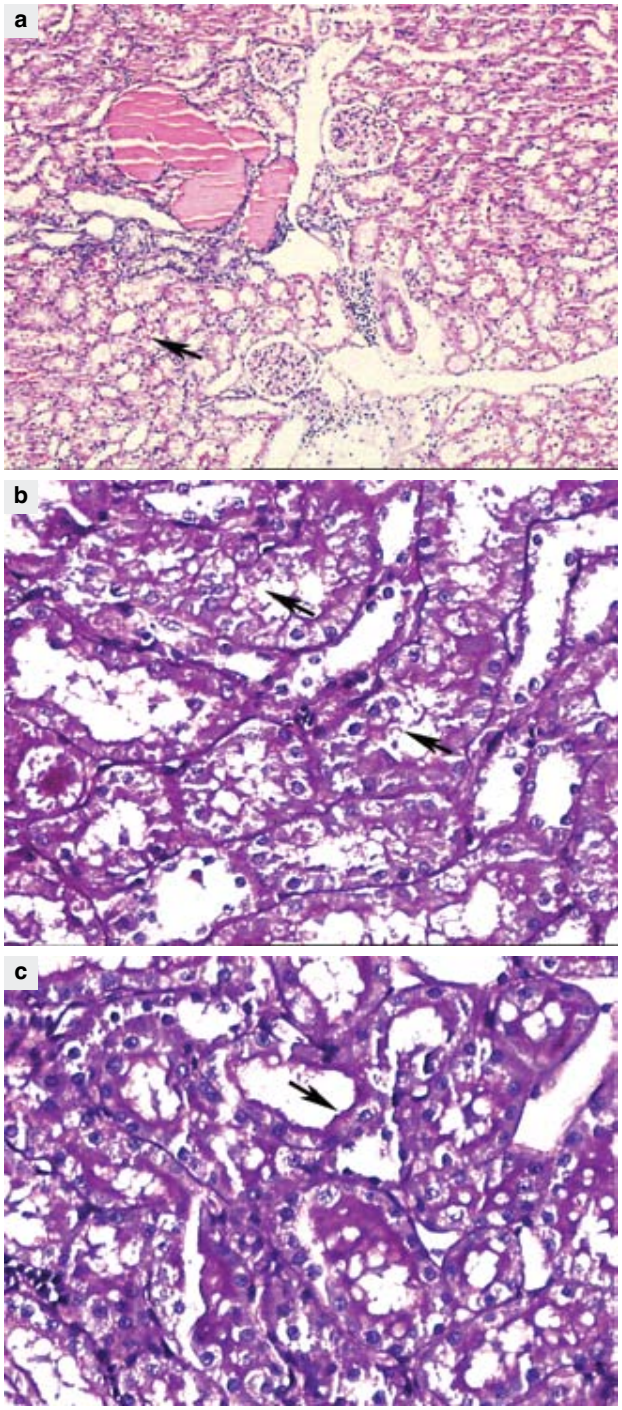

Figure 1 **(a)** Interstitial mononuclear cell infiltration, tubular thyroidization, and vacuolar degeneration of tubular epithelia (arrow) in rat kidney after cadmium (200 μg/ml) administration in the Cd-1 group (H&E x 50). **(b)** Lack of brush border, prominent vacuolar degeneration, desquamation of the cells (arrows) in the Cd-1 group (PAS stain x 200). **(c)** Regular brush border (arrow) of the tubules in the control rats (PAS stain x 200).

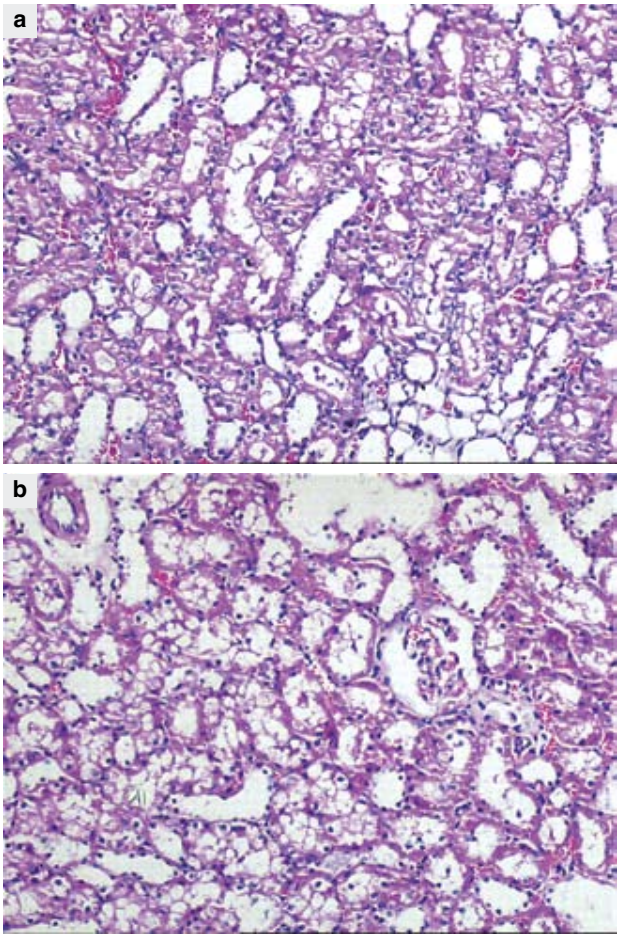

Figure 2 **(a)** Note the persistence of vacuolar degeneration of the epithelia with decreased intensity and congestion in the melatonin-co-treated rats (H&E x 100). **(b)** The same morphological findings in the melatonin-treated rats (H&E x 100).

brush border with PAS stain. Glomerular changes were not marked. Slight glomerular swelling was seen in some of the rats. Although Cd intake showed a clear difference with the tubular function compared to the control group, Cd+CoM and Cd+M groups did not show prominent differences from the groups exposed to Cd. Histopathological changes were similar, but slight with decreased intensity (Fig. 2).

Discussion

Our study differs from previous studies analyzing the toxicity of Cd in the kidney, in that it includes the examination of the oxidative stress, glomerular function, and histopathologic changes, and also the measurement of kidney tissue Cd level in the same study. Besides, the effect of melatonin was examined both by giving a low dose with Cd for a long time (co-treatment) to test whether melatonin would prevent

Cd-induced damage and giving a high dose of it for a short time after toxication with Cd (treatment) to test whether melatonin would reverse the effect of Cd.

The most important finding in this study is that Cd-induced nephrotoxicity is related to oxidative stress and both giving a low dose of melatonin with Cd for a long time and giving a high dose of it for a short time as a treatment modality after toxication result in pronounced improvement in the oxidative damage, but melatonin achieves this by its direct antioxidant effect and by increasing the antioxidant enzyme activities without changing the kidney tissue Cd level (Table 2).

Using tissue levels of MDA in the present study, it was shown that lipid peroxidation in kidney tissue significantly increased in Cd-1 and Cd-2 groups. Co-treatment with melatonin along with Cd for three months significantly decreased the elevated kidney MDA levels. Treatment with melatonin also significantly decreased the elevated MDA levels. Since melatonin is incorporated into cell membranes, as is Cd, it is possible that melatonin reduces lipid peroxidation by lowering the uptake of Cd and thereby reducing the resulting breakdown of lipids.[17,26] However, in the present study, although the Cd levels of all the groups were similar in the kidney tissues except for the control group, lipid peroxidation decreased only in the Cd+CoM and Cd+M groups. Based on these findings, it is thought that melatonin prevents lipid peroxidation directly by itself or by increasing antioxidant enzyme activities, not by decreasing the Cd level in the kidney tissue.

Another part of this study, which was published previously, showed that N-acetylcysteine (NAC) prevented and ameliorated kidney damage induced by Cd.[27] Some other researchers used different agents to prevent Cd-induced renal injury and found similar results. Shaikh et al.[5] found significant increases in Cd-induced lipid peroxidation in the kidney and reported that co-treatment with NAC protected against nephrotoxicity. The same authors also reported the beneficial effect of Minophagen (a mixture of glycyrrhizin, glycine, and cysteine) in chronic Cd-induced nephrotoxicity in rats. It was also proposed that selenium and vitamin E administration might prevent Cd toxicity caused by lipid peroxidation.[28-30]

Glutathione provides major protection in oxidative injury by participating in the cellular system of defense against oxidative damage.[31] Since Cd is well-documented as an intracellular GSH depleter in some organs,[32] the stimulatory effect of melatonin on GSH homeostasis may in part account for its protective actions against oxidative stress. As well as recycling of GSH in the cell, it also influences the de novo synthesis of GSH, thus has an important role in the maintenance of this crucial antioxidant.[33] In the present study, although the GSH levels did not differ significantly, the GSH levels were found to be decreased in the kidney in the Cd-1 and Cd-2 groups compared to the control group. In the Cd+CoM and Cd+M groups, kidney GSH levels were increased compared to their control groups. Some authors reported an increase in tissue GSH levels in rats after oral[30,34] and parenteral[35] administration of Cd. Singhal et al.[36] reported that depletion of GSH enhanced the toxicity of Cd and elevation of tissue GSH levels protected against acute Cd toxicity. In our study, melatonin acted as an antioxidant and this action was probably independent of GSH. These results are in discordance with the literature. It is thought that this may be due to several differences in the designs of other studies such as Cd concentration, exposure time, and the way of exposure. Further studies are needed to substantiate these conclusions.

Kidney tissue contains some antioxidant enzymes such as SOD, CAT, and GSH-Px to protect itself from the hazardous effects of oxidative attack. Following a long period of Cd exposure, SOD activity was found to be strongly reduced in the rat kidney. In addition, Cd also reduced the SOD enzyme when it was given in vitro.[37] It has been proposed that the enhancement of lipid peroxidation by Cd in rats is a consequence of decreased CAT activity. However, some authors reported that high levels of lipid peroxides were found in rat tissues following Cd exposure, but that SOD activities increased[38] and no significant change in the CAT activity occurred.[38,39] SOD and CAT are also influenced by melatonin. Melatonin is known to increase tissue mRNA levels of SOD,[40] and stimulates CAT activity.[16] In the present study, it was found that the kidney SOD and CAT activities were significantly lower in the Cd-1 and Cd-2 groups than the control group. Co-treatment and treatment with melatonin significantly increased the SOD activity. Although the kidney CAT activity was found to be higher in the Cd+CoM group, there was no significant difference between the Cd+M and Cd-2 groups. It was determined that while SOD activity increased in both low-dose long-term Cd+CoM group and high-dose short-term Cd+M group, CAT activity increased just in the low-dose short-term Cd+CoM group. On the basis of this finding, it can be stated that melatonin is not dependent on period and dose in increas-

ing the SOD activity, but it is dependent on period in increasing the CAT activity. In order to support this conclusion, further studies are needed, in which high doses of melatonin is used for a long period.

From another aspect, since giving a low dose of melatonin with Cd prevents antioxidant damage just from the beginning, it might also have prevented the decrease in CAT. For this reason, CAT activity can be found higher in the Cd+CoM group than the Cd+M group. However, since the rats were exposed to oxidative damage for three months in the Cd+M group, giving melatonin for seven days, even in a high dose, might not have been enough to cure the damage.

Glutathione peroxidase is an important enzyme which reduces hydroperoxides and lipid peroxides. The activities of GSH-Px are suppressed by Cd at different steps of toxin metabolism as well as other antioxidant enzymes.[41] In this study, GSH-Px activity was significantly decreased in the kidney of rats receiving Cd (Cd-1 and Cd-2 groups). Although melatonin treatment did not change the tissue GSH-Px level, co-treatment with melatonin significantly increased the GSH-Px activity. In other words, the reduction of GSH-Px activity in the renal tissue of rats exposed to Cd was prevented by co-treatment with melatonin, but could not be treated with melatonin after toxication with Cd. Since the findings on GSH-Px were similar to those of CAT, the same comments for CAT can also be made on GSH-Px. For this reason, based upon the findings on CAT and GSH-Px, it can be stated that decrease in antioxidant enzyme level as a result of exposure to Cd for a long time cannot be cured by a short-term melatonin treatment.

Up to date, there have been many studies about the histopathological changes of Cd nephrotoxicity in rats. However, most of them are concerned with the nephrotoxic effects of Cd.[42-45] Tubular dysfunction including degeneration and desquamation of tubular cells accompanying with interstitial nephritis are the main alterations of Cd nephrotoxicity which are consistent with the results of this study. A few studies have investigated the effects of melatonin on Cd burden. Although the studies indicated that melatonin treatment alleviated histopathological alterations of the kidney induced by Cd exposure,[46,47] our results did not show distinct amelioration in Cd nephrotoxicity. A slight decrease in the intensity of interstitial nephritis and tubular dysfunction were observed in the Cd+CoM and Cd+M groups.

The GFR and FE-Na levels did not change in the Cd-1 and Cd-2 groups. Although a change was not determined between the groups in terms of GFR and FE-Na levels, which is a biochemical reflection of nephrotoxicity caused by Cd, it was observed that there was a tubular toxic effect histopathologically, and increased oxidative damage.

In the present study, Cd content of the kidney tissue was also examined. Chwelatiuk et al.[47] showed that orally administered melatonin together with Cd reduced tissue accumulation of this metal. They concluded that the reduction of renal Cd accumulation by melatonin was probably responsible for the prevention of Cd-induced injury to the kidney. However, the same authors showed in another study that melatonin co-treatment brought about a significant increase in renal Cd concentrations as compared to those in the Cd alone group in the bank voles.[48] In the study of Eybl et al.[49] pre-treatment with melatonin did not affect Cd distribution in the tissues of Cd-intoxicated mice. Similar to this study, we observed that Cd concentrations increased in the renal cortex in both Cd-1 and Cd-2 groups compared to the control group. Co-treatment and treatment with melatonin did not change the kidney accumulation of this metal.

In our study, although GFR and FE-Na as indicators of renal damage were not affected by long-term Cd exposure, histopathological examination and biochemical analysis of lipid peroxidation and kidney antioxidant enzyme activities revealed that Cd caused renal damage. Based on these findings, oxidative stress seems to be a primary mechanism of chronic Cd-induced renal toxicity and we propose that exogenously administrated melatonin might reduce the toxic effects of Cd (either by its antioxidant effect directly or by increasing the activities of antioxidant enzymes) in the kidney without any reduction in tissue Cd burden.

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