Experimental Study / *Deneysel Çalışma*

Trace Elements in a Rat Model of Cadmium Toxicity: the Effects of Taurine, Melatonin and N-Acetylcysteine

Sıçan Kadmiyum Toksisitesi Modelinde Eser Elementler: Taurin, Melatonin ve N-Asetilsisteinin Etkileri

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Objectives: This study was undertaken to investigate copper, zinc, iron, and selenium in a rat model of cadmium toxicity and effects of antioxidant substances such as taurine, melatonin and N-acetylcysteine.

Materials and Methods: Ninety male Sprague Dawley rats were divided into nine groups. Group 1 received tap water comprising the controls; the remaining eight groups received 200 μg/ml cadmium chloride ($CdCl_2$) for three months. Group 2 had $CdCl_2$. Groups 3, 4, and 5 were administered taurine, melatonin and N-acetylcystein for three months together with $CdCl_2$. Groups 6, 7, 8, and 9 had $CdCl_2$ for three months and then only water as the second control or antioxidants for seven days. Cadmium, copper, zinc, iron, and selenium levels of heart and brain were measured by atomic absorption spectrophotometer.

Results: Cadmium accumulated in significant amounts in brain and heart tissues when compared with controls. $CdCl_2$ levels in Group 1 and Group 2 were 2.56±0.77 and 27.2±5.82 in the heart, 46.16±14.81 and 300.34±58.19 in the brain, respectively ($p<0.001$). We found that melatonin was more effective in brain tissue ($p<0.05$) whereas N-acetylcysteine was more effective in heart tissue ($p<0.001$) against cadmium accumulation.

Conclusion: We suggest that taurine, melatonin and N-acetylcysteine have some protective effects in brain and heart tissues against cadmium accumulation. Furthermore, trace element levels were restorated in different degrees after taurine, melatonin and N-acetylcysteine administration.

Key words: Trace elements; cadmium; taurin; melatonin; N-acetylcystein, rat.

Amaç: Bu çalışma kadmiyum toksisitesi oluşturulan sıçan modelinde bakır, çinko, demir ve selenyum düzeylerini araştırmak ve taurin, melatonin ve N-asetilsisteinin etkilerini belirlemek amacıyla yapılmıştır.

Gereçler ve Yöntemler: Doksan erkek Sprague Dawley sıçan dokuz gruba ayrıldı. Hayvanlara serbestçe ulaşabilecekleri pellet yem ve su verildi. Grup 1'e çeşme suyu verildi ve kontrol olarak kullanıldı, diğer sekiz grup üç ay süreyle 200 μg/ml kadmiyum klorid ($CdCl_2$) aldı. Grup 2, $CdCl_2$, Grup 3, 4, ve 5 $CdCl_2$ ile birlikte sırasıyla taurin, melatonin ve N-asetilsistein aldı. Grup 6, 7, 8, ve 9 üç ay süreyle $CdCl_2$ ve sonra yedi gün süreyle kontrol olarak sadece su veya antioksidan aldı. Kalp ve beyinde kadmiyum, bakır, çinko, demir, ve selenyum düzeyleri atomik absorpsiyon spektrofotometresi ile ölçüldü.

Bulgular: Kontrollerle karşılaştırıldığında kadmiyum beyin ve kalpte anlamlı düzeylerde birikim gösterdi. Grup 1 ve Grup 2 $CdCl_2$ düzeyleri sırasıyla kalpte 2.56±0.77 ve 27.2±5.82, beyinde ise 46.16±14.81 ve 300.34±58.19 idi ($p<0.001$). Kadmiyum birikimine karşı koruyucu olan en etkili maddenin beyin dokusunda melatonin ($p<0.05$), kalpte ise N-asetilsistein ($p<0.001$) olduğunu bulduk.

Sonuç: Bu bulgular kadmiyumun beyin ve kalpte birikimine karşı taurin, melatonin ve N-asetilsisteinin bazı koruyucu etkileri olduğunu düşündürdü. Ayrıca, eser element düzeylerinin taurin, melatonin veya N-asetilsistein uygulaması ile farklı düzeylerde olsa da kontrol değerlerine yaklaştığı gözlendi.

Anahtar sözcükler: Eser elementler; kadmiyum birikimi; taurin; melatonin; N-asetilsistein.

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The main sources of cadmium exposure are specific professional atmosphere air, diet, drinking water, soil and tobacco use. Due to accumulation of cadmium in organism and low excretion rates, its half-life ranges between 10 to 30 years in healthy individuals. Cadmium shows similar effects like heavy metals such as lead and mercury which have high neurotoxic effects. It especially functions through inhibiting superoxide dismutase and other enzymes of oxidative stress and increasing lipid peroxidation.[1] The basis of cadmium toxicity lies in the substitution of cadmium with other metal ions (especially Zn, Cu, and Ca) in metalloenzymes. Eventually, cellular enzymatic systems are negatively affected. It shows high affinity to SH-group containing biological structures such as protein, enzyme and nucleic acids. Cadmium shows its effects via interacting micro and macro elements required for Zn, Cu, Fe and Se. Brain, heart, lung, testes, kidney, and liver are target organs for cadmium toxicity.[2] Unlike other heavy metals, cadmium per se cannot lead to free radical formation. But, it was reported that cadmium may give rise to superoxide, hydrogen peroxide, hydroxil and nitric oxide radicals indirectly.[3,4] Furthermore, cadmium was reported to harm other antioxidant enzymes, cause alterations in thiol proteins, inhibit energy metabolism, and change DNA structure and membrane function.[3,4]

Copper ions contribute to both cellular ROS production and defensive mechanisms against ROS during resting and exercising.[5] Interaction of intracellular copper ions with GSH and thiols such as oxygen results in superoxide and hydrogen peroxide formation.[6] Zinc is a redox inert metal and it is not used in redox reactions. Acute effect of zinc as an antioxidant was first explained in the 80's with two distinct mechanisms: *i)* protection of sulfhydryl groups of proteins and enzymes against free radical attacks, *ii)* parturition of OH from H_2O_2 with protecting antagonism of active transition metals such as iron, copper, and zinc.[7]

It has long been known that cadmium decreases intestinal absorption of iron. In cadmium-exposed animals, iron levels in liver and other organs decrease. Recently, several studies reported that iron deficiency may increase cadmium absorption and accumulation in different organs.[8] Selenium is found in active site of the enzyme glutathione peroxidase and it is required for catalytic activity. One of the major roles of this trace element is its cofactor function for this key antioxidant enzyme and it also contributes to catalytic activation and conformational changes.[6]

Pineal gland product melatonin (N-acetyl-5-methoxytryptamine) is an effective hormone in removing free radicals and reactive oxygen species. Melatonin also prevents membrane fluidity resulting from lipid peroxidation.[9-11] N-acetylcysteine (NAC) is a thiol compund which, besides increasing activity of glutathione S-transferase, has important role in scavenging free radicals.[12-14] Taurine shows chemical similarities with acetylcysteine and conjugates with bile acids in liver. It is used in heavy metal intoxication.[15,16]

In this study, we aimed to investigate the effects of taurine, melatonin and NAC against cadmium accumulation in the brain and heart in rats. We also assessed selenium, iron, copper, and zinc levels in conditions of cadmium exposured for three months and the effects of taurine, melatonin and NAC on these trace elements.

MATERIALS AND METHODS

Study Design

After obtaining ethical approval, nine study groups were formed by using a total of 90 adult male Sprague-Dawley rats (340-370 g) that were allowed free access to food and water. Group 1 (n=10) served as home-cage control. Groups 2, 3, 4, 5, 6, 7, 8, and 9 were given cadmium chloride ($CdCl_2$) 200 μg/ml (Fluka, US) in drinking water for three months. Then, animals in Group 2 were sacrificed immediately at the end of three months. Group 3, 4, and 5 had 1.0% taurine, 0.02% melatonin, and 0.5% NAC respectively for three months together with $CdCl_2$. These groups were also sacrificed at the end of three months. Animals in Group 6, 7, 8, and 9 had drinking water without $CdCl_2$, 4.0% taurine, 0.08% melatonin, and 2% N-acetylcystein respectively for seven days following three-month $CdCl_2$ exposure. Then, these last four groups were sacrificed for tissue sampling. One animal in Group 7 and two animals in Group 8 were died during cadmium exposure stage. Animals were humanely killed by decapitation under general anesthesia using intramuscular xylazine 10 mg/kg (Rompun, Bayer Turkey) and ketamine 50 mg/kg (Ketalar, Eczacıbaşı, Turkey) and then cardiac and brain tissue were taken. All tissue samples were stored at –18 ºC until trace element assessments.

Spectrophotometric Measurements

The tissue samples were weighed and transferred into metal–free glass tubes for digestion. Tissues were kept at –18 ºC until use. The samples were first digested with 2 mL of concentrated nitric acid at 100 ºC in the furnace for 1 h and 2 mL of perchloric acid (60%) was added to the cooled materials. The materials were then completely digested at 120 ºC until the materials diminished to the half of the original total volume. Digested materials were diluted with deionized water to 10 mL. The last dilutions of the samples were mixed on a shaker for 15 min just before measurement. Se, Fe, Cu, Zn and Cd levels of the whole brain and heart tissues were measured by graphite furnace – flame atomic absorption spectrophotometer (Shimadzu AA- 6800, Japan). Results were expressed as μg/g and ng/g wet weight.[17] In order to prevent interference, 10 ppm palladium-nitrate modifier was used in graphite furnace measurements.

Table 1. Effects of taurine, melatonin and N-acetylcysteine on selenium, iron, copper, and zinc levels during and after cadmium toxicity in the brain and heart

		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
			3-month Cadmium + simultaneous admin. of				3-month Cadmium after 7-day admin. of			
Trace elements		Control	Water	TAU (1%)	MEL (0.02%)	NAC (0.5%)	Water	TAU (4%)	MEL (0.08%)	NAC (2%)
Brain	Cadmium, ng/g	2.56±0.77	46.16±14.81 A***	42.83±10.72	30.24±11.00 C*	42.25±12.92	16.07±3.81 H***	10.32±4.23	8.07±4.24	11.85±4.68
	Selenium, μg/g	0.54±0.12	0.65±0.07	0.86±0.12 B***	0.77±0.60	1.01±0.11 D***,G***	0.16±0.06 H***	0.59±0.07 İ***	0.45±0.12 K***	0.55±0.13 L***
	Iron, μg/g	19.34±3.31	19.16±1.49	19.99±1.29	19.72±1.02	21.50±3.44	13.86±0.79 H***	15.04±3.50	18.26±2.02 K*	14.86±2.00
	Copper, μg/g	4.02±0.24	3.63±0.22	3.51±0.26	3.33±0.48	3.17±0.48	3.74±0.46	3.55±1.12	3.50±0.39	3.68±0.32
	Zinc, μg/g	17.68±1.15	18.62±0.93	18.80±1.36	20.35±1.54	18.63±0.77	18.07±0.89	19.14±2.84 M*	16.26±2.76	15.09±1.56 L*,N***
Heart	Cadmium, ng/g	27.20±5.82	300.34±58.19 A***	107.08±28.45 B***	87.89±19.52 C***	50.98±15.50 D***,F*	172.23±42.33 H***	51.15±18.24 I***	64.76±19.94 K***	41.12±8.34 L***
	Selenium, μg/g	0.57±0.07	0.52±0.20	0.69±0.12 B*	0.73±0.12 C*	0.58±0.13	0.37±0.06	0.43±0.10	0.43±0.09	0.52±0.04
	Iron, μg/g	110.66±8.73	108.93±7.84	70.43±5.27 B***	122.77±11.46 C*,E***	77.73±12.01 D***,G***	105.43±10.87	109.49±6.05	108.88±7.63	111.50±9.44
	Copper, μg/g	3.20±3.34	3.20±0.45	3.40±0.59	3.88±0.79	3.85±0.70	3.76±0.25	3.64±0.26	3.69±0.40	3.65±0.27
	Zinc, μg/g	24.17±3.94	38.57±13.36 A*	24.70±1.87 B*	29.04±7.18 C*	26.13±5.70 D*	26.84±7.58 H*	26.60±8.69	22.50±2.28	39.49±17.90

Data are given as mean±SD. *p<0.05, **p<0.01, ***p<0.001. Group Comparisons: A: 1 and 2; B: 2 and 3; C: 2 and 4; D: 2 and 5; E: 3 and 4; F: 3 and 5; G: 4 and 5; H: 2 and 6; I: 6 and 7; K: 6 and 8; L: 6 and 9; M: 7 and 8; N: 7 and 9; O: 8 and 9. Abbreviations: MEL: melatonin; TAU: taurine; NAC: N-acetylcysteine.

Statistical Analysis

Inter-group comparisons were made by using Kruskal-Wallis test and post-hoc Mann-Whitney U tests. P<0.05 was accepted as statistically significant. All data were expressed as mean ± standard deviation.

RESULTS

Trace element levels of brain and cardiac tissues of all study groups are given in Table 1. In the first part of the study, although the antioxidant substances (namely, taurine, melatonin, and NAC) were given simultaneously with cadmium for three months, cadmium levels in brain and heart tissues of Groups 2, 3, 4, and 5 were significantly higher when compared to Group 1 home-cage controls (p<0.01). When Group 2 (only cadmium group) was compared to Group 3, 4, and 5, only in Group 4 (simultaneous melatonin administration) cadmium level in brain was significantly lower than that of Group 2 (p<0.05), whereas taurine, melatonin and NAC treatments lead to significantly less accumulation of cadmium in heart (p<0.001 for all comparisons; see Table 1). In the second part of the study (3-month cadmium treatment followed by antioxidant administration for seven days), taurine, melatonin and NAC were compared to drinking water administration. All of four interventions lead to significantly lower cadmium accumulation in brain and heart. However, the most significant preventive effect against cadmium accumulation obtained by melatonin in brain and by NAC in heart.

In the first part of the study, iron, copper and zinc levels were not significantly differed with taurine, melatonin or NAC administration when compared to cadmium alone in the brain. Selenium levels of the brain were significantly higher in taurine and NAC administered groups. In the heart, cadmium leads to selenium decrease, whereas taurine, melatonin and NAC showed preventive effects against this decrease. However, only the difference with taurine and melatonin administration reached statistical significance. Cadmium also leads to zinc increase in the heart, taurine, melatonin and NAC administration prevented this increase. In the second part of the study, 7-day water administration led to significantly lower selenium levels in the brain, 7-day taurine, melatonin or NAC administration led to similar selenium levels as in Group 2 which means they reversed selenium increase due to cadmium administration. Copper and zinc levels were similar in brain through the Groups 6, 7, 8, and 9. In the heart, selenium, iron, copper, and zinc levels were similar under conditions of 7-day taurine, melatonin, and NAC administration.

DISCUSSION

The main finding of this study is that taurine, melatonin and N-acetylcysteine (NAC) may have some protective effects in brain and heart tissues against cadmium accumulation. In addition to the levels of selenium, iron and zinc were affected by administration of taurine, mela-

tonin or NAC. In the presence of cadmium exposure, trace element levels showed some alterations which were restorated in different degrees after taurine, melatonin and NAC administration.

Previous studies investigating protective effects of taurine, melatonin and NAC against cadmium toxicity focused rather on hepatic and renal tissue oxidative damage parameters and measured SOD, GPx, CAT enzyme activities besides GSH, MDA and NO levels.[1,18,19] We, therefore, focused on cardiac and brain tissues which were not previously studied under cadmium exposure conditions. We also evaluated trace elements rather than oxidative stress enzyme activities. Measurement of cadmium levels is an important marker of cadmium exposure. Cadmium may affect intracellular trace element levels which are used as cofactors by many metalloenzymes.[20] Several studies demonstrated the protective effects of taurine, melatonin and NAC in cadmium-induced damage.[10,14,15,21-23] However, our study differs from previous studies in terms of cadmium exposure duration and administration way. To the best of our knowledge, this is the first study in which trace elements are measured under the conditions of taurine, melatonin and NAC administration and cadmium exposure.

An interesting finding of this study is that mean cadmium levels in Group 2 (only cadmium group) were almost six-fold higher in heart than in brain. This is suggestive for the hypothesis that the heart is more vulnerable for cadmium accumulation. On the other hand, when Group 1 tissue cadmium levels were compared with Group 2 tissue cadmium levels, we have seen that cadmium exposure for three months led to 18-fold accumulation in the brain whereas the same amount of exposure led only to 11-fold accumulation in the heart with respect to initial levels. These findings are in parallel with the study of Gerhardsson et al.[23] Cadmium can pass through blood-brain barrier such as lead and mercury and shows neurotoxic effects depending on the site of accumulation.[1] In humans, chronic cadmium exposure leads to headache, sleep disorders and memory loss. Evidence suggests that these neurotoxic effects are related to inhibition of superoxide dysmutase and other antioxidant enzymes and increased lipid peroxidation.

An important finding of this study is that cadmium levels were significantly lower if taurine, melatonin and NAC were administered after cadmium exposure finalized, when compared to simultaneous administration of cadmium and these substances. It is considered that simultaneous antioxidant administration failed to prevent cadmium accumulation in the heart and brain, but these substances accelerated the clearence of cadmium in these organs after exposure had been ended. From this point of view, melatonin was the most effective when compared to taurine and NAC. Melatonin was also the most effective against cadmium accumulation in the brain among these substances when administered simultaneously with cadmium (see Table 1). On the other hand, NAC was the most effective against cadmium accumulation in the heart when compared to taurine and melatonin.

Protective effects of melatonin against toxic metals such as cadmium, lead, and aluminum have been shown in many studies. Millán-Plano et al.,[24] reported that melatonin showed potentially neuroprotective effects in treatment of aluminum concentration-related disorders. Lipid-solubility of melatonin is very high which enables it to permeate through cellular barriers, thus it may affect accumulation of cadmium.[2] Melatonin restored iron concentration which was decreased by cadmium exposure. Djukić-Cosić et al.,[25] reported similar findings in rat liver. They demostrated that subacute cadmium intoxication induced a significant decrease in the levels of iron and MDA in liver. They also suggested that cadmium intoxication decreased hepatic lipid peroxidation by reducing the iron content of liver. Iron is a common redox-active transition element and is found in biological systems. It actively joins regulation of oxidant and antioxidant balance. Produced superoxide radicals may cause formation of hydroxil radicals by Fenton chemistry in presence of iron ions.[6-8,26]

It has been known from previous studies that copper and zinc ions play role in cellular defence against oxidative damage.[27] Our findings indicate that 3-month cadmium exposure did not affect copper levels in brain and heart. Thus, taurine, melatonin or NAC administration also did not affect copper levels. Zinc levels in brain was not affected from 3-month cadmium exposure and simultaneous taurine, melatonin and NAC administration. Seven-day NAC, when applied after cadmium accumulation, seemed to reduce zinc levels in the brain. Conversely in heart tissues, NAC had given orally restored zinc levels which were increased after cadmium exposure. Modi et al.[28] demonstrated that in oxidative stress induced by arsenic, zinc administration together with arsenic provided protective effects in restoring blood δ-aminolevulinic acid dehydratase (ALAD) activity. They also reported combined zinc and NAC had significantly protective effects against arsenic toxicity.

Selenium is one of the most potent scavengers of heavy metals that are toxic for brain and other organs. Selenium binds to and removes lead, mercury, arsenic and cadmium that substitute important metals such as iron, copper and zinc. Selenium can chelate these metals thus, removes these metals from brain cells.

In conclusion, we suggest that taurine, melatonin and N-acetylcysteine have some protective effects in brain and heart tissues against cadmium accumulation. Furthermore, trace element levels were restored in different degrees after taurine, melatonin and N-acetylcysteine administration.

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