The Effects of Sulfur-Containing Compounds on Total Antioxidant Capacity Levels of Liver, Kidney and Brain in Lead-Exposed Rats

SÜLFÜR-İÇEREN BİLEŞİKLERİN KURŞUNA MARUZ KALMIŞ RATLARDA KARACİĞER, BÖBREK VE BEYİN TOTAL ANTİOKSİDAN KAPASİTESİ DÜZEYLERİNE ETKİLERİ

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Abstract

Objective: Lead is a non-essential toxic heavy metal widely present in the environment and chronic exposure to has been a matter of public health concern in many countries. Considering the sulfur-containing compounds with antioxidant activity to prevent or treat experimental lead toxicity in animals, this study was carried out to investigate the antioxidative effects of some sulfur-containing compounds in rats where oxidative stress was induced by lead acetate.

Material and Methods: Wistar albino rats were exposed to 2000 ppm of lead, as lead acetate, alone or with daily to sulfur-containing compounds [L-methionine, lipoic acid (LA), N-acetylcysteine (NAC), L-homocysteine (Hcy)] for five weeks. The parameters of oxidative stress, and the levels of total antioxidant capacity (TAC) were determined [malondialdehyde (MDA) in sera by Satoh-Yagi's method; TAC in liver, kidney and brain by $ABTS^{\cdot+}$ method] in rats following the experiment (Analysis of the data was performed by one-way analysis of variance (ANOVA) and subsequent analysis was performed using the Tukey test. In lead group MDA levels in sera were determined to be significantly high compared to controls ($p< 0.01$)].

Results: In lead group MDA levels in sera were determined to be significantly high compared to controls ($p< 0.01$). MDA levels in sera were reduced in all groups compared to lead group ($p< 0.01$). In all lead-antioxidants groups liver TAC levels were determined to be slightly lower compared to lead group while brain's levels were higher. Kidney TAC levels in lead-methionine, and lead-LA groups were significantly high ($p< 0.01$).

Conclusion: These results suggested the oxidative stress induced by lead was reduced, and the antioxidant capacities in lead-exposed rats were altered by sulfur-containing compounds.

Key Words: Lead, methionine, thioctic acid, acetylcysteine, homocysteine

Özet

Amaç: Kurşun çevrede yaygın olarak bulunan esansiyel olmayan toksik bir ağır metaldir ve kronik maruz kalma birçok ülkeyi etkileyen bir halk sağlığı sorunudur. Antioksidan aktiviteli sülfür içeren bileşiklerin hayvanlarda deneysel olarak kurşun zehirlenmesini önlediği veya tedavi ettiği göz önüne alınarak, bu çalışma kurşun asetat ile oksidatif stres oluşturulan ratlarda sülfür içeren bazı bileşiklerin antioksidan etkilerinin araştırılması amaçlandı. Bu çalışma kurşun asetat ile oksidatif stres uyarılmış ratlarda bazı sülfür içeren bileşiklerin antioksidan etkilerinin incelenmesi için düzenlendi.

Gereç ve Yöntemler: Wistar albino ratlara 2000 ppm kurşun, kurşun asetat formunda, tek başına ya da sülfür-içeren bileşikler [L-metiyonin, lipoik asit (LA), N-asetilsistein (NAC), L-homosistein (Hcy)] ile birlikte günlük olarak verildi. Ratlarda oksidatif stres parametreleri ve total antioksidan kapasitesi seviyeleri (TAK) [serumda malondialdehit (MDA) Satoh-Yagi metoduyla; karaciğer, böbrek ve beyinde (TAK) ise $ABTS^{\cdot+}$ metoduyla] deney bitimini takiben ölçüldü (Verilerin analizi one-way ANOVA) variyans analizi kullanılarak yapıldı ve müteakip analizlerse Tukey testi kullanılarak yapıldı).

Bulgular: Kontrol grubuna göre kurşun grubunda serum MDA seviyeleri anlamlı yüksek ($p< 0.01$) bulundu. Kurşun grubuna göre tüm gruplardaki serum MDA seviyeleri anlamlı düşüktü ($p< 0.01$). Kurşun grubuna göre tüm kurşun-antioksidan gruplarındaki karaciğer TAK seviyeleri hafif düşükken; beyin seviyeleri yüksek bulundu. Kurşun-metiyonin ve kurşun-LA gruplarındaki böbrek TAK seviyeleri ise önemli yüksekti.

Sonuç: Bulgular sülfür içeren bileşiklerin kurşunla uyarılan oksidatif stresi azalttığını ve kurşuna maruz kalan ratlardaki antioksidan kapasitelerini değiştirdiğini gösterdi.

Anahtar Kelimeler: Kurşun; metiyonin; tioktik asid; asetilsistein; homosistein

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Lead, common environmental occupational toxic heavy metal, is known to have indirect oxidative effects on biological systems or cells.[1] Emerging evidences suggest that lead induces the production of reactive oxygen species (ROS) that result in lipid peroxidation, DNA damage, and depletion of cell antioxidant defense systems.[2]

Change in lipid peroxidation production reactions and antioxidant defense systems were associated with changes in a variety of biochemical pathways.[3]

Most of the literature regarding that the sulfur-containing compounds have pro-oxidant or antioxidant actions depending on the physiological circumstances, but are generally considered antioxidants.[4] The primary sulfur-containing compounds of interest in medical science are methionine, cysteine, Hcy taurine, LA, coenzyme A, glutathione (GSH), and etc. With the exception of the two sulfur-containing vitamins, thiamin and biotin, all of these sulfur compounds are synthesized from just one parent compound, methionine.[5] But, NAC is a derivative of the sulfur-containing amino acid cysteine and an intermediary in the conversion of cysteine to GSH.

In the present study, we targeted to investigate the beneficial effects of some sulfur-containing compounds [L-metiyonin, LA, NAC, L-Hcy] on altered oxidative stress parameters and antioxidant status of tissues with lead treatment. We determined MDA as lipid peroxidation indicator and oxidative stress status have been described with determination TAC levels of liver, kidney and brain. Another aim of our study to investigate whether Hcy, that is known to induces atherogenesis and cardio-vascular diseases, is ability an antioxidant effect to lead exposure.[6]

Material and Methods

The lead acetate, NAC and, 2.2-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS), hydrogen peroxide, were purchased from Merck (Darmstadt, Germany). The water-soluble analogue of vitamin E (Trolox; 6-hydroxy-2,5,7,8-tetra-methylchroman-2-carboxylic acid) and all other chemicals were purchased from Sigma (St. Louis, MO, USA). All chemicals were ultra pure grade, and type I reagent-grade deionized water was used.

This study protocol was approved by our institutional ethical board. All animal experimentations were performed in accordance with guidelines of Fırat University medical board. The animals were randomized into 6 groups of 10 animals each. All groups were given only standard rat feed and water during the 1st week. After this habition period to environment, group I (n=10) served as the control and was given only standard rat chow and water for 5 weeks. Group II (n=10) received 2000 ppm lead acetate in its drinking water for 5 weeks. Group III (n=10) received 2000 ppm lead acetate in its drinking water for 5 weeks and, 100 mg/kg/day methionine dissolved in water. Group IV (n=10) was treated like group III, except that it received 25 mg/kg/day intraperitoneolly. LA for 5 weeks. At the end of the study 2 rats were died from peritonitis. Group V (n=10) received water containing 2000 ppm lead acetate and, 800 mg/kg/day NAC. Group VI (n=10) received 2000 ppm lead acetate and, 50 mg/kg/day Hcy in their water.

At the end of the 6. week, the animals were sacrificed by cervical decapitation and the liver, kidney and brain were excised immediately. The tissues were maintained at -20°C until assayed. They were weighed and homogenized (1 mg tissue: 4 mL 140 mM phosphate buffer saline pH 7.4) in ice-cold buffer using 10 strokes in a Teflon/glass homogenizer. The homogenate was centrifuged for 15 minutes at 1000g at 4°C and the supernatant centrifuged again at 18.000 g for 15 min at 4°C. The blood samples were collected to uncontaining lead tubes using heparin and, EDTA as an anticoagulant or tubes without anticoagulants. Sera were removed by centrifugation for 10 min at 3000 rpm. The red blood cells were washed three times with an equal volume of cold saline. The samples were maintained at -20°C for assays (not longer than 7 days).

MDA Determination

MDA concentrations were measured as Thiobarbituric Acid Reactive Substances (TBARS) according to a modified version of Satoh's and Yagi's methods.[7,8] In brief, 0.3 mL serum was mixed with 2.4 mL 1/12 N H_2SO_4 in a centrifuge

tube and shaken gently. After 0.3 mL 10% (v/v%) phosphotungstic acid was added to the tube and it was left to stand at room temperature for 5 minutes, the mixture was centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded and the sediment was mixed with 1.5 mL water. The centrifugation was repeated and the supernatant was discarded again. The sediment was resuspended in 4.0 ml water and in fresh 1 mL thiobarbituric acid (TBA) reagent [1:1 (v/v) 0.67% TBA and glacial acetic acid], mixed thoroughly and heated in a bath of boiling water for one hour. After cooling in cold water, the resulting chromogen was extracted with 3.0 mL n-butyl alcohol by vigorous shaking. The organic phase was separated by centrifugation at 3000 rpm for 10 minutes, and its absorbency was recorded at a wavelength of 530 nm. The level of absorbency was converted into nmol/mL MDA from a standard curve generated with 1,1,3,3- tetraethoxypropane (SIGMA).

TAC Determination

The final supernatant was used to evaluation of TAC levels. TAC values of tissues was determined using a novel automated colorimetric measurement method for TAC developed by Erel.[9] In this method the hydrogen peroxide radical reacts with the colourless substrate ABTS to produce the $ABTS^{\cdot+}$ radical, which is blue-green in colour. Upon the addition of a tissue sample, the oxidative reactions initiated by the hydrogen peroxide present in the reaction mix are suppressed by the antioxidant components of the tissue, preventing the colour change and thereby providing an effective measure ment of the TAC of the tissue. Briefly, 200 μL of TAC reagent I (acetate buffer 0.4 mol/L pH 5.8), 20 μL of TAC reagent II (the $ABTS^{\cdot+}$ in acetate buffer 30 mmol/L pH 3.6) was vortex mixed with 5 μL test sample and standards. The test was performed at 37°C. Absorbance at 660 nm was read against a reagent blank at a predetermined time (0-5 min) after sample-reagent mixing in an Aeroset analyzer (Abbott) at 37°C. Thus the results were expressed as μmol Trolox equiv. /L.

Protein Measurement

The protein concentration assayed in 1 mL aliquot of homogenate used for TAC was estimated by method of Lowry et al. using bovine serum as a standard.[10] Results of TAC were corrected by protein content in each sample as μmol. trolox. Eq/mg.protein.

Statistical Analysis

The results are expressed as mean ± SD. Analysis of the data was performed by one-way ANOVA and subsequent analysis was performed using the Tukey test. The p values smaller than 0.05 were selected to indicate statistical significance between groups.

Results

MDA concentrations

Table 1 shows the results of serum MDA concentrations in all groups. The MDA level of lead-group ($p< 0.01$) and lead-LA group ($p< 0.05$) were significantly higher than the control values. Furthermore, methionine and Hcy administrated lead-exposed rats showed lower serum MDA concentrations. Statistically significant decreases were observed serum MDA concentrations in all lead-antioxidants groups by supplement sulfur containing antioxidants when compared to lead-group ($p< 0.01$).

TAC of Tissues

The tissue TAC levels are given in Table 2. In liver TAC values, no significant increasing was observed in sulfur containing antioxidant administrated

Table 1. Serum MDA concentrations (nmol/mL).

Groups	n	Serum
Control	10	0.35 ± 0.06
Lead	10	0.66 ± 0.06 [a]
Lead-methionine	10	0.29 ± 0.03 [c]
Lead-lipoic acid	8	0.45 ± 0.10 [b, c]
Lead-acetylcysteine	10	0.40 ± 0.09 [c]
Lead-homocysteine	10	0.28 ± 0.07 [c]

All the values are expressed mean ± SD, a: $p< 0.01$ vs control; b: $p< 0.05$ vs control; c: $p< 0.01$ vs lead group.

rats when compared to lead-group or controls. A significant increase in TAC of kidney were recorded in lead-methionine and lead-LA groups as compared with controls and lead-group ($p< 0.01$) although a slight increase could be noted in TAC levels of kidney from NAC and Hcy supplement groups.

Increasing in brain TAC levels in lead-LA or decreasing those in lead-methionine and lead-NAC and lead-Hcy were not significant compared with lead-group. Therefore, decrease of brain TAC levels was remained slightly in rats given lead or lead with methionine, NAC or Hcy than in controls while no significant increase was recorded in LA supplemented group.

Discussion

The present study was designed to investigate oxidative stress parameters (MDA in sera) and TACs of tissues administrating of rats to lead and sulfur containing antioxidants. We found that serum MDA levels in lead group were significantly higher compared to controls ($p< 0.01$), whereas serum MDA levels in all groups were lower than that of lead group ($p< 0.01$). The results of our study demonstrated that administration of lead acetate for 5 weeks induced a toxic effect on erythrocytes by increasing MDA concentrations, These results are agreement with those Kasperczyk et al. and Jiun et al. who found increased MDA levels in lead-exposed workers.[11,12] Here we asssumed that lead might have a direct peroxidative activity or act indirectly, facilitating creation or propitious conditions for lipid peroxidation. Direct peroxidative activity of lead may be bound up with ROS generation such as H_2O_2, atomic oxygen and hydroxyl radicals.[13-15] rate may have two activities. First, peroxidative activity of lead may be bound up with ROS generation which can be induced delta-aminolevulinic acid (ALA) due to inhibition of delta-amino-levulinic acid dehydratase (ALAD) activity on heme synthesis by Pb.[16,17] Indirect activity of lead may be connected to cell membrane composition and increase its sensitivity to ROS or modify cell antioxidant defense.[18]

In this research, we also reported that the TAC levels of liver and kidney of rats exposed to lead were slightly higher that those of controls although brain's TAC levels were lower. Experimental researches on lead-exposed animals have much divergence in the influences of lead on TAC levels in different tissues. Mousa et al. observed a rising at 7th day and a reducing 14th day in TAS values in plasma of goats exposed to 5.46 mg/kg lead acetate oral for 2 weeks.[19] Damage in renal tissues, chronic exposure to lead affects glomerular filtration, renal clearance and tubular reabsorption. Moreover, damage to the renal cells by lead and ALA resulted in an increasing accumulation them in kidney.[20] Like the Demasi's research, increases in TAC values of liver and kidney may be caused by response to lead-induced oxidative stress.[21]

Methionine is a sulfur-containing essential amino acid and it's a low molecular mass antioxidant.[4] Here we reported that administering methionine to prevent oxidative damage of lead elevated TAC values in kidney, whilst reduced serum MDA levels significantly. In the tissues, although the methionine decreased the lipid peroxi-

Table 2. Total antioxidant capacities of tissues (µmol.trolox.Eq/mg.protein).

Groups	n	Liver	Kidney	Brain
Control	10	16.60 ± 1.27	16.85 ± 2.33	17.94 ± 1.03
Lead	10	17.52 ± 1.70	17.87 ± 1.54	16.85 ± 1.47
Lead-methionine	10	17.49 ± 1.73	20.91 ± 1.98[a, b]	17.61 ± 1.72
Lead-lipoic acid	8	15.76 ± 2.48	21.72 ± 1.84[a, b]	18.70 ± 1.97
Lead-acetylcysteine	10	16.98 ± 1.99	18.75 ± 1.50	17.33 ± 1.12
Lead-homocysteine	10	17.17 ± 2.18	18.51 ± 1.35	17.18 ± 1.05

All the values are expressed mean ± SD, a: $p< 0.01$ vs control; b: $p< 0.01$ vs lead group.

dation, the effect did not reach statistical significance. However, slightly rising TAC levels in liver and decreases TAC values in brain were found in rats exposed to lead and supplemented with methionine.

LA, is co-enzyme which involved in carbohydrate utilization necessary for the production of ATP in mitochondria and it is shown to have antioxidant potential to inhibit some of the toxic effects of lead.[22] In the present study comparing to lead group, supplementing LA to prevent lead's oxidative effects decreases in serum MDA levels, and TAC values in kidney, whereas no significant changes in total antioxidant statusof tissues were detected.

NAC is a thiol containing antioxidant which has been used to reduce effects of oxidative stress.[23] In the present study, in group which administered NAC to prevent to lead's effects since it was found that serum MDA level decreased in the presence of NAC when compared to lead exposed group. Supporting these findings, many investigators have shown that NAC was found i) to stimulate GSH synthesis maintaining intracellular GSH levels and scavenging ROS[23,24] ii) to have some chelating actions on lead.[25,26]

Hcy is a thiol formed by the demethylation of methionine that was shown to be synthesized by animals and humans. Hcy can converse to methionine or cysteine when its concentration is low.[4,6] In this study, we also found that Hcy prevent oxidative stress. Since it reported that serum MDA level decreased by giving Hcy when compared with lead exposed group. Here we assumed that the thiol group in the structure of Hcy raised the possibility that Hcy might chelate heavy metals thereby increasing its excretion when a group that received it.

Conclusion

Evaluated results showed that sulfur-containing compounds support antioxidant capacity in rats, suggesting that supplementation of sulfur- bearing compounds might be helpful in treatment lead poisoning in human and animals.

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