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Orjinal Article

Changes in the levels of MDA and GSH in mice serum, liver and spleen after aluminum administration

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Abstract. Aluminum (Al) is widely distributed in the environment and enters the human body by air, water, food and drugs. It is claimed that Al toxicity increases the rate of lipid peroxidation and hence the formation of free radicals in some investigations. As an index of lipid peroxidation, serum and tissue malondialdehyde (MDA) levels increase, whereas there is a decrease in the anti- oxidant glutathione (GSH). It has been observed that the increase in the levels of MDA recovers with the administration of vitamin E. twenty four adult mice were divided into three groups; Al administered, Al+vitamin E and controls. 300 mg/kg body weight of Al sulfate were given to Al and Al+vitamin E groups for three months orally. 20 mg/kg body weight of vitamin E was additionally given to Al+vitamin E group subcutaneously once a week during this period of time. Liver and spleen tissue as well as serum samples were obtained. MDA and GSH levels of the samples were analyzed. We found statistically significant increase in MDA levels of both serum and tissue samples while there was a decrease in the GSH levels. We also observed a recovery on these changes caused by chronic Al administration with vitamin E addition. Chronic high dose of Al sulfate can lead to tissue oxidative injury, and Vitamin E is capable of preventing the deleterious effects of Al^{+3} ions.

Key words: Aluminum, lipid peroxidation, serum, liver, spleen

1. Introduction

Aluminum (Al) is the third most abundant element and the most common metal in the earth's crust, existing primarily as polymorphous aluminosilicates (Al_2O_5Si) in rocks and soils (1). The ongoing acidification of our environment has increased both the solubilization and conversion of these inert forms of Al in biologically active species (2). However, biological function of Al is not well understood at present (3). This element enters the human body via food, air, water and drugs (4), and is present in many manufactured foods such as processed cheese, baking powders, cake mixes, frozen dough, pancake mixes (5, 6) and pharmaceutical products, especially antacids (1-7). It is also added to drinking water for purification purposes (5). It has been shown that Al accumulates in kidney, brain and especially in liver experimentally (3, 8, 9).

Free radicals, which have unpaired electrons on outer orbital, are generated during several metabolic reactions (10-12). These radicals are very reactive species and may cause tissue damage and even cell death (10-13). The occurrence of free radicals increases in some pathological conditions and has some deleterious effects on several critical molecular and cellular components such as proteins, DNA, and membrane lipids (13). The primary targets of reactive oxygen species are cell-membrane polyunsaturated fatty acids, which, in turn, lead to damage in the cell structure and function (14). It has been shown that lipid peroxidation have

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serious effect on some vital functions such as fluidity and selective permeability of membranes as well as signal transduction (15). Additionally, the decomposition of lipid hydroperoxides leads to a wide variety of end products, one of which is malondialdehyde (MDA), which is now accepted as a reliable marker of lipid peroxidation (16).

There are some antioxidant mechanisms against free radical damage. The antioxidant mechanisms are mainly divided into two groups; enzymatic antioxidants such as superoxide dismutase, glutathione peroxidase and catalase, and non-enzymatic antioxidants such as vitamin E (Vit E; á-tocopherol), ascorbic acid and â-carotene as well as reduced glutathione (GSH) and uric acid (15, 17, 18). GSH is the most abundant intracellular thiol-based antioxidant, prevalent in millimolar concentrations in all living aerobic cells, and plays an important role in the cellular defense cascade against oxidative injury (19-21). It also serves to detoxify some endogenic and exogenic compounds with conjugation reactions catalyzed by glutathione S-transferases (19, 20). GSH is a cofactor for glutathione peroxidase, which catalyzes the reduction of hydrogen peroxide to water and oxygen, hence limiting the formation of hydroxyl radical, the highly toxic reactive oxygen species (21).

Vit E, an important lipid-soluble antioxidant placed in a special region of membranes, is a well-characterized chain-breaking antioxidant with the particular function of preventing lipid peroxidation in membrane systems (22, 23). The loss of Vit E will be accompanied by increased rate of lipid and protein oxidation, destruction of membrane function, and inactivation of membrane enzymes and receptors (24). Therefore, Vit E has received attention as a potential therapeutic agent to prevent or reduce clinical disease states thought to be associated with excess free radical production (25).

Our aim was to determine to what extend chronic Al administration changes the serum, liver and spleen MDA and GSH levels, and how addition of Vit E influences the effects of Al on these changes in mice.

2. Material and method

This study was performed on 24 adult Balb-c mice. The animals were divided into three groups. Mice were fed ad libitum with pellet mice food in 22 ^{0}C heated rooms. In the first (n=7) and second (n=8) groups, 300 mg Al sulfate [$Al_2(SO_4)_3$]/kg body weight was daily given in the drinking water for three months. Second group was additionally administered 20 mg/kg body weight of Vit E, as used in the dose of most studies (26, 27), once a week during this period of time subcutaneously. The third group (n=9) was given nothing and accepted as controls. In the end of the three months of the study, blood samples were collected and then liver and tissue samples were obtained immediately after sacrifice. The liver and spleen tissues were placed into petri dishes after being washed with cold water and then stored at –70°C (28), until assayed by the procedure of Ohkawa (16). After thawing, each sample was weighed, homogenized in 0.15 M potassium chloride solution, and 0.4 ml of homogenate was mixed with 1.5 ml thiobarbituric acid, 1.5 ml acetic acid (pH 3.5) and 0.2 ml sodium dodecyl sulfate. A set of MDA standards was freshly prepared. After mixing, all samples and standards were heated at 100°C for one hour and cooled by using water. The absorbance was recorded at 532 nm and compared with those obtained from MDA standards. All procedures except homogenization were applied to serum samples but 0.5 ml serum was used instead of 0.4 ml tissue homogenate.

GSH estimation was achieved by the modification of the procedure described by Moron et al. (29). The modification is briefly as following: After homogenization of tissue samples with potassium chloride, 0.5 ml homogenate is mixed with 3 ml of deproteinization solution (sodium chloride, metaphosphoric acid, EDTA and distilled water) and 1.5 ml potassium chloride solution. Each sample was centrifuged at 1000 g for 5 minutes, and 0.5 ml of supernatant was added into 2 ml of Na_2HPO_4 and 0.5 ml Ellman reactive (DTNB; dithiodinitrodibenzoic acid, sodium citrate, distilled water). The absorbance of supernatants were recorded at 412 nm and compared with those obtained from GSH standards. The same procedure was followed for the serum samples except homogenization, and results are given as mg per dl of serum.

Protein levels of tissue samples were determined by biuret method, and the results of liver and spleen MDA and GSH levels were given as nmol MDA/mg protein and µg/mg protein, respectively. Animal care and all experimental procedures used were in accordance with those detailed in the Guide for Care and Use of Laboratory Animals published by the U.S. Department of Health and Human Services. Results were evaluated with Mann Whitney U and Kruskal Wallis tests for the significance between and among groups, respectively. Statistical analysis was made with SPSS 9.0 program (Statistical Package for Social Sciences),

Table 1

Results and comparisons of serum MDA and GSH levels of mice in control, aluminum administered and aluminum+vitamin E administered groups.

	Group Control (n=9)	Al Administered Group (n=7)	Al+Vit E Administered Group (n=8)	P
MDA (nmol/ml)	12.98±1.71 a***, b*	18.97±3.22 a***, c***	10.65±1.32 b*, c***	P<0.001
GSH (mg/dl)	4.65±1.78 a*	4.00±3.10 b*	6.58±2.17 a*, b*	P<0.05

*MDA: Malondialdehyde, GSH: Reduced glutathione, Values as mean ± S.D. a, b and c: Shows significance between two groups (Mann Whitney U test) P: Shows significance among three groups (Kruskal Wallis test) * p<0.05, ** p<0.01, *** p<0.001*

Table 2

Results and comparisons of liver MDA and GSH levels of mice in control, aluminum administered and aluminum + vitamin E administered groups.

	Control Group (n=9)	Al Administered Group (n=7)	Al+Vit E Administered Group (n=8)	P
MDA (nmol MDA/mg protein)	2.08±0.47 a***, b**	3.30±0.30 a***, c***	2.60±0.20 b**, c***	P<0.001
GSH (µg/mg protein)	9.82±2.15 a**	6.57±1.38 a**, b**	9.77±2.19 b**	P<0.01

*MDA: Malondialdehyde, GSH: Reduced glutathione, Values as mean ± S.D. a, b and c: Shows significance between two groups (Mann Whitney U test) P: Shows significance among three groups (Kruskal Wallis test) * p<0.05, ** p<0.01, *** p<0.001*

Table 3

Results and comparisons of spleen MDA and GSH levels of mice in control, aluminum administered and aluminum + vitamin E administered groups.

	Control Group (n=9)	Al Administered Group (n=7)	Al+Vit E Administered Group (n=8)	P
MDA (nmol MDA/mg protein)	3.22±0.65 a**	3.44±0.33 b***	2.50±0.37 a**, b***	P<0.01
GSH (µg/mg protein)	7.64±1.35 a*	6.89±1.26 b**	11.33±3.54 a*, b**	P<0.01

*MDA: Malondialdehyde, GSH: Reduced glutathione, Values as mean ± S.D. a, b and c: Shows significance between two groups (Mann Whitney U test) P: Shows significance among three groups (Kruskal Wallis test) * p<0.05, ** p<0.01, *** p<0.001*

and the results are expressed as mean ± standard deviation (S.D.).

3. Results

Results and comparisons of serum MDA and GSH levels of the three groups were given in Table 1. Serum MDA levels of mice were found to be increased in aluminum administered group compared to control group ($p<0.001$). Serum levels of MDA were recovered in aluminum+vitamin E group when compared to aluminum administered group ($p<0.001$). Serum GSH levels of aluminum administered group were lower but this was not statistically significant. However serum GSH levels of aluminum+vitamin E administered group were higher than both controls and aluminum administered group ($p<0.05$).

Table 2 illustrates the results and comparisons of liver MDA and GSH levels of the groups while Table 3 shows those of spleen MDA and GSH levels. Liver MDA levels of both aluminum and aluminum+vitamin E administered groups elevated compared to those of controls ($p<0.001$, $p<0.01$, respectively). In addition, the MDA levels of aluminum administered group were higher than aluminum+vitamin E administered group ($p<0.001$). Liver GSH levels of aluminum administered group decreased compared to controls ($p<0.01$), while GSH levels of aluminum+vitamin E administered group recovered ($p<0.01$). Spleen MDA levels did not show any difference between control and aluminum administered group.

However aluminum+vitamin E administered group had lower MDA levels than controls ($p<0.01$) and aluminum administered group ($p<0.001$). Also spleen GSH levels showed no difference between controls and aluminum administered group while aluminum+vitamin E administered group had higher levels of spleen GSH than controls ($p<0.05$) and aluminum administered group ($p<0.01$).

4. Discussion

Although biological function of Al is not understood very well (3) it has been reported that Al exposure can increase lipid peroxidation rates (30, 31). Some authors suggest that Al^{+3} interacts with cell membrane directly (32) whereas Al salts were shown to accelerate peroxidation of membrane lipids induced by Fe(II) salts (33). Al^{+3} ions produce a subtle rearrangement in the membrane structure that facilitated the oxidative action of iron (33). Effects of Al on lipid peroxidation in various tissues such as liver, kidney, testis and brain of different animals were investigated and Al increased the rate of lipid peroxidation in some studies (30, 31, 34, 35) while these changes were not observed in some studies (3, 36, 37).

These controversial results may be due to the dose and type of Al compounds, duration of Al administration and the kind of animal used. For example, it was shown that lipid peroxidation rate increased with Al phosphate, leading to the rise of MDA and the decrease of GSH (31, 38, 39). In one study lipid peroxidation rate and levels of its markers did not change with Al sulfate, which we used in our study as well (3). In our study we found that levels of serum, liver and spleen MDA as a marker of lipid peroxidation increase in mice elevated with three months of Al administration while the levels of GSH decreased.

The increase in lipid peroxidation rate in our study may be owing to the high dose of Al used. GSH provides powerful antioxidant protection to body systems heavily exposed to reactive oxygen species (40, 41). Its deficiency causes oxidant damage and greater lipid peroxidation which in turn leading to cell damage (42-46).

Investigations have shown that the decrease of glutathione peroxidase activity caused by Al was parallel to the increase of lipid peroxidation rate (35). Vit E is a major lipid soluble antioxidant present in plasma and erythrocyte membrane, and spare GSH and prevents its oxidation together with vitamin C. A positive correlation between vitamin E and lipid peroxide formation has been documented (47, 48).

It has been observed that 8 weeks of Vit E administration resulted in the recovery of MDA and GSH levels in Al administered animals (49). We also found this recovery in the serum, liver and spleen tissue MDA and GSH levels of mice in our study even if a high dose of Al sulfate was used in the study for relatively longer period of time.

The current study suggests that a high dose of Al sulfate administration for three months of period shows an increase in MDA levels of serum, liver and spleen tissue of mice as an index of lipid peroxidation while the GSH levels decreased. Administration of the antioxidant agent Vit E together with Al sulfate resulted in the recovery of MDA and GSH levels in Al administered animals in our study. That is, a chronic high dose of Al sulfate can lead to tissue oxidative injury, and Vit E is capable of preventing the deleterious effects of Al^{+3} ions.

References

1. Wang M, Ruan D, Chen J, Xu Y: Lack of effects of vitamin E on aluminium-induced deficit of synaptic plasticity in rat dentate gyrus in vivo. Food Chem Toxicol 2002;40: 471-478.
2. Lukiw WJ, Mclachlan DR: Neurotoxicology of aluminium. In Effects and Mechanisms, Handbook of Neurotoxicology (Edited by Chang L, Dyer R), Marcel Dekker, New York, 2002;70-80.
3. Farina M, Lara FS, Brandao R, Jacques R, Rocha JBT: Effects of aluminum sulfate on erythropoiesis in rats. Toxicol Lett 2002;132: 131-139.
4. Kim MS, Lenore S, Clesceri LS: Aluminum exposure: a study of an effect on cellular growth rate. Sci Total Environ 2001;278: 127-135.
5. Levesque L, Mizzen CA, McLachlan DR, Fraser PE: Ligand specific effects on aluminium incorporation and toxicity in neurons and astrocytes. Brain Res 2000;877: 191-202.
6. Nayak P: Aluminum: Impacts and disease. Environ Res 2002;89:101-115.
7. Roberts NB, Zhu H, Kim JY, Shin HR, Kim JI, Choi SY: Further studies on the interrelationship of aluminum and silicon in patients receiving aluminum hydroxide therapy for dyspepsia and factors that relate to the solubilization of aluminum. J Trace Elem Exp Med 2002;15: 9-19.
8. Dlugaszek M, Fiejka MA, Graczyk A, Aleksandrowwicz JC, Slowikowska M: Effects of various aluminium compounds given orally to mice on Al tissue distribution and tissue concentrations of essential elements. Pharmacol Toxicol 2000;86: 135-139.
9. Schetinger MR, Bonan CD, Morsch VM, Bohrer D, Valentim LM, Rodrigues SR: Effects of aluminum sulfate on delta- aminolevulinate dehydratase from kidney, brain, and liver of adult mice. Braz J Med Biol Res 1999;32: 761-766.
10. Athar M, Abdulla H, Sultana S, Favier A, Pero R: Free radicals and trace elements. J Trace Elem Exp Med 1993;6: 65-73.
11. Frei B: Molecular and biological mechanisms of antioxidant action FASEB 13: 963-964, 1999.
12. Barry H: Antioxidants and human disease. A general introduction. Nutr Rev 1997;55: 544-549.
13. Halliwell B: Free radicals, antioxidants and human disease: curiosity, cause or consequence? The Lancet 1994;344: 721-724.
14. Floyd RA: Role of oxygen free radicals in carcinogenesis and brain ischemia. FASEB 1990;4: 2587-2597.
15. Halliwell B, Gutteridge J: Lipid peroxidation, oxygen radicals, cell damage, and antioxidant therapy. Lancet 1984;23: 1396-1398.
16. Ohkawa H, Ohishi N: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95: 351- 358.
17. Singh V: A current perspective on nutrition and exercise. J Nutr 122: 760-765, 1992.
18. Witt E, Reznick A, Viguie C, Starke R, Packer L: Exercise, oxidative damage and effects of antioxidant manipulation. J Nutr 1992;122: 766-773.
19. Armstrong RN: Structure, catalytic mechanism, and evolution of the glutathione transferases. Chem Res Toxicol 1997;10: 2-18.
20. Van Bladeren PJ: Glutathione conjugation as a bioactivation reaction. Chem Biol Interact 2000;129: 61–76.
21. Hsu CH, Chi BC, Liu MY, Li JH, Chen CJ Chen RY: Phosphine-induced oxidative damage in rats: role of glutathione.Toxicology 2002;179: 1-8.
22. Burton GW, Joyce A, Ingold KU: First proof that vitamin E is major lipid-soluble, chain-breaking antioxidant in human blood plasma. Lancet 1982;2: 327-329.
23. Horvath ME, Faux SP, Smith AG, Blazovics A, Looij M, Feher J, Cheeseman KH: Vitamin E protects against iron- hexachlorobenzene induced porphyria and formation of 8-hydroxydeoxyguanosin in the liver of C57BL/10SCSn mice. Toxicol Lett 122: 97-102, 2001.
24. Parker L: Protective role of vitamin E in biological systems. Am J Clin Nutr 1991;53: 1050S-1055S.
25. Horvath ME, Blazovics A, Kemeny T, Vasarhelyi B, Weinbrenner Z, Feher J: The effect of vitamin E on experimental hyperlipidemia. Orv Hetil 1993;134: 1757-1760.
26. Omera FO, Blakley BR: Vitamin E is protective against iron toxicity and iron-induced hepatic vitamin E depletion in mice. J Nutr 1993;123: 1649-1655.
27. Latchoumycandane C, Chitra KC, Mathur PP: The effect of methoxychlor on the epididymal antioxidant system of adult rats. Reprod Toxicol 2002;16: 161–172.
28. Erol U, Gurdal M, Erol A, Aslan R, Konukoğlu D, Onmus H: Is midazolam effective as an antioxidant in preventing reperfusion injury in rat kidney? Int Urol Nephrol 2002;34: 121- 127.
29. Moron MS, Depierre JW, Mannervik B: Level of glutathione, glutathione reductase and glutathione-S-transferase activity in rat lung and liver. Biochem Biophys Acta 1979;82: 67-78.
30. Deloncle R, Huguet F, Babin P, Fernandez B, Quellard N, Guillard O: Chronic administration of aliminum L-glutamate in young mature rats-effects on iron levels and lipid- peroxidation in selected brain areas. Toxicol Lett 1999;104: 65-73,.
31. Chugh SN, Arora V, Sharma A, Chugh K: Free radical scavengers and lipid peroxidation in acute aluminium phosphide poising. Indian J Med Res 1996;104: 190-193.
32. Fraga CG, Oteiza PI, Golub MS, Gershwin ME, Keen CL: Effects of aluminum on brain lipid peroxidation. Toxicol Lett 1990;51: 213-219.
33. Zatta P, Kiss T, Suwalsky M, Berthon G: Aluminium (III) as a promoter of cellular oxidation. Coordin Chem Rev 2002;228: 271-284.
34. Guo CH, Huang CJ, Chiou YL, Hsu GSW: Alteration of trace element distribution and testis ACE activity in mice with high peritoneal aluminum. Biol Trace Elem Res 2002;86: 145-157.
35. Julka D, Gill KD: Effect of aluminium on regional brain antioxidant defense status in Wistar rats. Res Exp Med 1996;196: 187-194.
36. Swain C, Chainy GBN: Effects of aluminium sulfate and citric acid ingestion on lipid peroxidation and on activities of superoxide dismutase and catalase in cerebral hemisphere and liver of developing young chicks. Mol Cell Biochem 1998;187: 163-172.

37. Swain C, Chainy GB: Aluminum effect on lipid peroxidation and on the activities of superoxide dismutase and catalase in the cerebral hemisphere and liver of young chicks. J Trace Elem Med Biol 1997;11: 77-82.
38. Chugh SN, Kolley T, Kakkar R, Chugh K, Sharma A: A critical evaluation of antiperoxidant effect of intravenous magnesium in acute aluminium phosphide poisoning. Magnes Res 1997;10: 225-230.
39. Hsu C, Han B, Liu M, Yeh C, Casida JE: Phosphine- induced oxidative damage in rats: attenuation by melatonin. Free Radic Biol Med 2000;28: 636-642.
40. Rahman I, MacNee W: Lung glutathione and oxidative stress: implications in cigarette smoke-induced airway disease. Am J Physiol 1999;277:: L1067–L1068.
41. Kelly FJ: Glutathione: in defence of the lung. Food Chem Toxicol 1999;37: 963–966.
42. Sokol RJ, Taylor SF, Devereaux MW, Khandwala R, Sondheimer NJ, Shikes RH, Mierau G: Hepatic oxidant injury and glutathione depletion during total parenteral nutrition in weanling rats. Am J Physiol 1996;270: G691–G700.
43. Wang X, Kanel GC, DeLeve LD: Support of sinusoidal endothelial cell glutathione prevents hepatic veno-occlusive disease in the rat. Hepatology 2000;31: 428–434.
44. Bouchard G, Yousef IM, Barriault C, Tuchweber B: Role of glutathione and oxidative stress in phalloidin-induced cholestasis. J Hepatol 2000;32: 550–560.
45. Scholz RW: Mechanism of interaction of vitamin E and glutathione in the protection against lipid peroxidation. Ann N Y Acad Sci 1989;570: 514–517.
46. Scholz RW, Reddy PW, Wynn MK, Graham KS, Liken AD, Gumpricht E, Reddy CC: Glutathione-dependent factors and inhibition of rat liver microsomal lipid peroxidation. Free Radic Biol Med 1997;23: 815–828.
47. Julka D, Gill KD: Effects of aluminium on regional brain antioxidant defense status in wistar rats. Res Exp Med 1996;196: 187-194.
48. Broquist HP: Buthionine sulfoximine an experimental tool to induce glutathione deficiency: elucidation of glutathione and ascorbate in their role as antioxidants. Nutr Rev 1991;50**:** 110–111.
49. Fattah AA, Yousef HM, Bekairi AM, Sawaf HA: Vitamin E protects the brain against oxidative injury stimulated by excessive aluminium intake. Biochem Mol Biol Int 1998;46: 1175-1180.