

Effects of Chronic Aluminum Administration on Blood and Liver Iron-Related Parameters in Mice

Günfer Turgut¹, Bünyamin Kaptanoğlu², Sebahat Turgut¹, Yaşar Enli², and Osman Genç¹

Departments of ¹Physiology and ²Biochemistry, Pamukkale University Faculty of Medicine, Denizli, Turkey.

In this study, the effects of chronically administered aluminum on iron metabolism-related parameters of liver and blood of mice were investigated. An additional purpose to determine how chronic aluminum administration together with vitamin E as an antioxidant to mice changed the parameters related to iron metabolism. For these purposes, we used 21 adult female Balb-c mice in this study. The animals were divided into three groups: one group with aluminum administered chronically, another group with aluminum plus vitamin E administered chronically, and the control group. Serum levels of hemoglobin, ferritin, iron, transferrin, hematocrit, total iron binding capacity (TIBC), as well as percentage of transferrin saturation were determined in all groups. In addition, the liver tissue levels of ferritin and iron were analyzed. Hemoglobin and hematocrit levels of the aluminum group and aluminum plus vitamin E group were significantly decreased compared to the control. In conclusion, no changes occurred in the serum iron related parameters although Al induced anemia in mice when Al administered chronically. There was an increase in the levels of liver iron and ferritin with Al, but Vit E had no effect on the changes of all blood and liver parameters caused by Al.

Key Words: Aluminum, vitamin E, iron, blood, liver, mice

INTRODUCTION

With the industrialization and consequent pollution, aluminum (Al) is increasingly taken into our bodies through foods, air, water, and even drugs.¹ Al is present in many manufactured foods and is added to drinking water for purification purposes.^{2,3} Recent studies have showed the

ground-water pollution with Al.⁴ Although the biological effects of Al are not well known,⁵ under experimental conditions, it has been shown that Al accumulates in the kidney, stomach, brain, bone, and liver.⁶

Al might exert its toxic effects by using mechanisms which control iron homeostasis e.g. using transport proteins such as transferrin, or interfering with iron homeostasis at the level of iron regulatory proteins.⁷ Iron plays a key role in the function of many proteins, such as hemoglobin, and the cytochromes of the mitochondrial respiratory chain, and it also plays a key role in microsomal electron transfer chains and in many enzymes including several hydrolyses and catalyses.⁸

Al is not a transition metal and, hence, cannot itself initiate any oxidation/reduction reactions.⁹ However, Al has the ability to potentiate the iron-catalyzed free radical production and to initiate lipid peroxidation.¹⁰ In a study reported recently, it has been suggested that Al can induce morphological and functional alterations in erythroid cells by a direct action on circulating erythrocytes, which suggests membrane alterations due to lipid peroxidation mechanisms.¹¹ In another study, oxidative damage was contributed to Al-induced anemia by increasing erythrocyte fragility.¹²

Vitamin E (Vit E) is an essential chain breaking, peroxy radical-trapping antioxidant in membranes.¹³ Because Vit E has no effect on iron concentrations,¹³ this vitamin was used as the antioxidant in our study.

The aim of this study was to investigate the effects of chronically administered Al on iron metabolism-related parameters of the liver and blood of mice. In addition, we planned to deter-

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Reprint address: requests to Dr. Günfer Turgut, Pamukkale Üniversitesi Tıp Fak. Fiziyojisi A.D., P.K. 33 20020 Denizli, Turkey. Tel: 90-258-2134030/1367, Fax: 90-258-2132874, E-mail: gturgut@pamukkale.edu.tr

mine how chronic Al administration, together with Vit E as an antioxidant, in mice changes the parameters related to iron metabolism.

MATERIALS AND METHODS

This study was performed on 21 adult female Balb-c mice that were aged 16 to 20 weeks. The animals were divided into three groups (n=7 each). All rats were fed *ad libitum*. In the first and second groups, 877 μ mol Al₂(SO₄)₃ (aluminum sulfate)/kg body weight were daily given in the drinking water for three months.^{5,14} Vitamin E (Vit E; α -tocopherol) was also administered once a week (20 mg/kg body weight) by subcutaneous injection in to the animals of the second group during the three months.¹⁵ The animals of the control group were given only drinking water during this period. At the end of the experimental period, all the animals were anaesthetized with ether vapor, and blood was removed by cardiac puncture, after which the animals were killed by cervical dislocation. Whole blood samples and sera of the subjects were collected into tubes. Hemoglobin concentrations and hematocrit levels were determined in the whole blood, while the levels of ferritin, transferrin, iron, and total iron binding capacity (TIBC) were determined in the serum.^{5,16} The liver tissue samples were taken immediately after the sacrifices, weighed, and diluted with distillate water in the ratio of 1:1. Then, the samples were homogenated. The homogenates were centrifuged at 2000 \times g for 6 minutes, and supernatants were obtained.¹⁷ Iron levels of the supernatants and sera were analyzed with commercial kits (ILAB, Milano, Italy) by using an auto analyzer (ILAB 900, Milano, Italy). Ferritin levels of the supernatants and sera were determined by an immune-analyzer (Tosoh Corporation, Tokyo, Japan) with commercial kits (Eurogenetics, Tessenderlo, Belgium). Serum transferrin and TIBC levels were analyzed with commercial kits (ILAB, Milano, Italy) by using an auto analyzer (ILAB 900, Milano, Italy). The percentage of transferrin saturation of blood samples was calculated.

The 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 (Statistical Package for Social Sciences). The results are expressed as a mean \pm standard deviation (S.D.).

Animal care and all experimental procedures used were in accordance with those detailed in the Guide for Care and Use of Laboratory Animals, which was published by the U.S. Department of Health and Human Services.

RESULTS

The data and statistical comparisons of blood parameters for the group with chronically administered Al (Al group), the group with chronically administered Al plus Vit E (Al+Vit E group), and the control group are illustrated in Table 1. Hemoglobin and hematocrit levels of Al and Al+Vit E groups were significantly decreased compared to the control.

Table 2 shows the data and statistical comparisons of liver tissue parameters for the Al group, the Al+Vit E group, and the control group. Iron and ferritin levels of the two groups were significantly higher than those of the control.

When the Al group and the Al+Vit E group were compared, we found no significant differences in the levels of all blood and tissue parameters (Table 1, Table 2).

DISCUSSION

In this study, our goal was to investigate the changes in the levels of iron metabolism-related parameters in the liver and blood of chronically Al-administered mice, and the effects of the antioxidant Vit E on these changes. Although there have been some studies, in literature, aiming to show the effects of Al on iron metabolism, no consensus has been reached among the results.^{7,16,18-21} Some studies have indicated a reduction of iron stores in the liver of chicks and serum of rats when exposed to high levels of Al.^{16,18,19} Others have found no alterations or have discovered an increase in iron stores after exposure to Al.^{7,21} Consequently, data concerning the changes in iron concentration of tissues and body fluids after the

Table 1. Blood Parameters of Aluminum, Aluminum+Vitamin E and Control Groups (Mean \pm S.D.)

	Control (n=7)	Aluminum (n=7)	Aluminum+Vitamin E (n=7)
Hemoglobin (g/dl)	15.32 \pm 0.24 ^{a*} , ^{b*} , ^{c*}	13.18 \pm 1.60 ^{a*} , ^{c*}	13.68 \pm 1.16 ^{b*} , ^{c*}
Hematocrit (%)	35.20 \pm 1.74 ^{a*} , ^{b*} , ^{c*}	30.75 \pm 2.68 ^{a*} , ^{c*}	31.34 \pm 2.73 ^{b*} , ^{c*}
Transferrin (mg/dl)	132.85 \pm 4.59	143.14 \pm 18.39	140.85 \pm 26.92
Iron (mg/dl)	42.00 \pm 41.13	67.42 \pm 56.68	96.85 \pm 82.75
TIBC (μ g/dl)	601.00 \pm 26.94	620.71 \pm 41.12	636.00 \pm 65.17
Ferritin (ng/ml)	0.08 \pm 0.02	0.06 \pm 0.03	0.08 \pm 0.06
Transferrin saturation (%)	6.75 \pm 6.24	10.43 \pm 8.00	14.31 \pm 10.71

^a and ^b: Shows significance between two groups.

^c: Shows significance among three groups.

TIBC: Total iron binding capacity.

* $p < 0.05$.

Table 2. Liver Parameters of Aluminum, Aluminum+Vitamin E and Control Groups (Mean \pm S.D.)

	Control (n=7)	Aluminum (n=7)	Aluminum+Vitamin E (n=7)
Iron (μ g/mg protein)	2.02 \pm 0.33 ^{a*} , ^{b*}	2.64 \pm 0.47 ^{a*}	2.59 \pm 0.55 ^{b*}
Ferritin (ng/ml)	2.16 \pm 0.90 ^{a†} , ^{b†} , ^{c†}	4.69 \pm 1.69 ^{a†} , ^{c†}	4.40 \pm 1.18 ^{b†} , ^{c†}

^a and ^b: Shows significance between two groups.

^c: Shows significance among three groups.

TIBC: Total iron binding capacity.

* $p < 0.05$, [†] $p < 0.01$.

administration of Al are contradictory and seem to depend on the conditions of toxicity, which are mainly determined by different doses and different routes of administration.⁵

There have been a number of investigations that were performed with different doses and compounds of Al in literature,^{5,11,14} but we aimed to see the effects of especially high doses of Al₂(SO₄)₃, which has not been used previously in mice. Through this study, a decrease in hemoglobin and hematocrit levels of mice taking Al sulfate for three months was determined. In another study, it has also been reported that rats having aluminum chloride for three weeks showed decreases in the levels of the same parameters as our study.²² The effects of Al on erythroid progenitors and on mature erythrocytes, and the toxic effects on erythropoiesis¹¹ may be responsible for the decrease of hemoglobin and hematocrit levels.

The Al group showed no statistically significant

changes in both the transferrin level and the percentage of transferrin saturation compared to the control in our study. There are some researches supporting our results,¹¹ while some others do not.^{16,23}

Vittori et al.¹¹ did not observe alterations in plasma iron concentrations and in TIBC of rats that were chronically exposed to Al, although anemia were observed in these animals. Whereas another study illustrated that Al lowered blood iron levels.⁵ We also observed no changes in the levels of blood iron, TIBC, and ferritin of chronically Al-administered group compared to the control group. Our results like those of Vittori et al.¹¹ displayed anemia such as the decreases in hemoglobin and hematocrit levels, but there was no change in the levels of blood iron, TIBC, and ferritin.

Some studies have reported that Al increases lipid peroxidation rates.^{24,25} A long-term (8 week) dietary supplementation with Vit E might prevent

Al-stimulated oxidative injury by increasing glutathione peroxidation and decreasing lipid peroxides as thiobarbituric acid reactive substances.²⁶ For this reason, we chronically added Vit E in one of our groups. In this group, we did not find any changes in the levels of transferrin, iron, TIBC, or ferritin. Also, the percentage of transferrin saturation did not change while hemoglobin and hematocrit levels decreased significantly compared to those of the control. When compared to chronically Al-administered group, all parameters of the Al+Vit E group showed no significant changes. Among the three groups, only the hemoglobin and hematocrit levels exhibited significant changes. These findings suggest that Vit E does not influence the hematological changes exerted by Al.

It has been experimentally shown that Al accumulates in the liver.^{5,6} In our study the levels of liver iron and ferritin concentrations increased with Al exposure. Ward et al.⁷ also determined that the content of iron in liver, spleen, heart, kidney, and brain of rats increased with Al exposure. The increases in the two parameters were seen in the Al+Vit E group as well. In the comparison of iron and ferritin concentrations, there were no differences between the Al and Al+Vit E groups. This shows that Vit E exerts no influence on the changes created by Al in the liver as well as the blood. Investigations reporting that Al does not lead to lipid peroxidation in liver gave support to our results.^{5,27}

In conclusion, no changes occurred in the iron related parameters although Al did induces anemia with chronic administration of Al in mice. Another important result was the increase in iron and ferritin levels of the livers with Al. However, Vit E had no effect on the changes of all blood and liver parameters created by Al.

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