



Protective Effect of Melatonin on Aluminum Accumulation in Some Organs of Rats

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Abstract

This study emphasizes the protective effect of melatonin against the aluminum accumulation in some organs of rats. Twenty eight male Wistar rats were divided into four groups (n=7 animals for each), one control and three experimental, and aluminum sulphate and melatonin were applied in drinking water for three months as follows: group I: aluminum sulphate (1000 ppb in water), group II: aluminum sulphate (1000 ppb in water) and melatonin (10 mg in 100 mL water) and group III: melatonin (10 mg in 100 mL water). Control group: water. Aluminum was accumulated in liver, kidney, heart, spleen and brain of exposed rats in significantly higher amounts compared to the control group. The findings showed that melatonin administration reduced the aluminum level in studied organs and melatonin had a protective effect against aluminum accumulation.

Özet

Sıçanlarda Melatoninin Bazı Organlarda Alüminyum Birikmesi Üzerine Koruyucu Etkisi

Bu çalışma, sıçanların bazı organlarında alüminyum birikmesine karşı melatoninin koruyucu etkisini vurgulamaktadır. Yirmi sekiz erkek Wistar faresi biri kontrol, üçü deneysel olmak üzere dört gruba ayrılmıştır (her grupta n=7 hayvan); üç ay süreyle içme suyu içinde alüminyum sülfat ve melatonin belirtilen şekilde uygulanmıştır; grup I: alüminyum sülfat (suda 1000 ppb), grup II: alüminyum sülfat (suda 1000 ppb) ve melatonin (100 mL suda 10 mg), grup III: melatonin (100 mL suda 10 mg). Kontrol grup: su. Alüminyum uygulaması yapılan sıçanların karaciğer, böbrek, kalp, dalak ve beyinlerinde kontrol grubuna oranla anlamlı olarak daha yüksek miktarlarda alüminyum birikmiştir. Bulgular uygulanan melatoninin araştırılan organlarda alüminyum düzeylerini azalttığını ve melatoninin alüminyum birikmesine karşı koruyucu etkisinin olduğunu göstermiştir.

Introduction

Aluminum is the third most abundant element and it is considered to have negative effects that exceed the beneficial effects, being a trace element with toxic risk potential (Kiss and Szolnoki-Csikkel, 1993; Poston, 1991). Aluminum is released to the environment by natural processes and from various anthropogenic sources. Aluminum is not found as free metal occurring

only in oxidized state in silicates as hydrous aluminum oxides and hydroxides (Howe, 1998).

Aluminum compounds are widely used in medicine as antacids, phosphate binders, buffered aspirins, vaccines and antiperspirants (Exley, 1998; Kaehny et al., 1997).

The toxic effects of aluminum include the impairment of reproductive function (Rawy et al., 2013);

Yousef and Salama, 2009), neurological disorders (Lemier and Appanna, 2011) and oxidative stress (El-Gendy, 2011; Muselin et al., 2014; Yousef and Salama, 2009).

Some experimental investigations regarding the status of aluminum storage in different organs revealed the protective role of melatonin against the accumulation of this trivalent metallic element (Fyiad, 2007; Kametani and Nagata, 2007; Priyanka et al., 2012).

The issues regarding bio-concentration of the aluminum are important for evaluation of the effects induced by this element, aluminum being present not only in the environment (polluting the food, water, etc.) but also in some pharmaceuticals.

Melatonin or N-acetyl-5-methoxytryptamine is a ubiquitous molecule and widely distributed in nature, with functional activity occurring in unicellular organisms, plants, fungi and animals (Pandi-Perumal et al., 2006). In most vertebrates, including humans, melatonin is synthesized primarily in the pineal gland and is regulated by the environmental light / dark cycle via the suprachiasmatic nucleus (Fyiad, 2007; Pandi-Perumal et al., 2006; Reiter, 1991). Melatonin has been shown to have protective effect against the oxidative damages caused by a variety of toxins (Reiter et al., 2000).

In the present study, we tried to emphasize the protective effect of melatonin on aluminum accumulation in some organs following subchronic exposure in rats.

Materials and Methods

The study was carried out on 28 white adult male Wistar rats purchased from the animal facility of Victor Babes University of Medicine and Pharmacy, Timisoara, Romania. The rats were kept in plastic cages (3-4 individuals/cage), fed with standard diet, 25°C controlled ambient temperature, with 12 h dark/light cycle. The feed and water were given *ad libitum*. The experiment was approved by the Ethical Committee of Banat's University of Agricultural Science and Veterinary Medicine, Timisoara (no. 3558/05.06.2012).

The rats were divided into four groups (n=7 for each group): control group (C) received regular water; group I (Al) - received 1000 ppb aluminum sulphate; group II (Al+Me) - received a combination of 1000 ppb aluminum sulphate and melatonin 10 mg/100 mL water and group III (Me) - received melatonin 10 mg/100mL

water. Aluminium sulphate and melatonin were applied in drinking water during three months.

At the end of the experiment the rats were euthanatized by exsanguination, after intraperitoneal ketamine 10%, 50 mg/kg b.w. anesthesia. The organs (liver, kidney, heart, spleen and brain) were collected and kept at -18°C until the preparation. The organ samples were prepared by microwave digestion as follows: 1 g sample, 10 mL HNO₃ and 2 mL H₂O₂ in digestion plastic flask for 10 min, 120°C, 800 W using a CEM Mars X5 digestion accelerator. Aluminum was determined by atomic absorption spectroscopy at 309.3 nm wavelength using a Shimadzu AA6650 spectrometer (Shimadzu, Kyoto, Japan) with graphite furnace (pyrolytic graphite tube). Aluminum standard solution (containing 1000 mg/L) - Al(NO₃)₃ in HNO₃ 0.5 mol/L, was purchased from Merck KGaA, Darmstadt, Germany.

For the evaluation of statistical differences between studied groups, one-way ANOVA test with Bonferroni's correction was used, considering statistical difference when p<0.05 or lower. The values were expressed as mean ± SEM. The GraphPad Prism 5.0 for Windows (GraphPad Software, San Diego, USA) software used was used for statistical analysis.

Results

Aluminum level in liver (Figure 1) increased significantly (P<0.01) in rats exposed to aluminum compared to control (+418.10%). Administration of melatonin together with aluminum reduced the accumulation of aluminum in liver (-40.06%, p<0.01), however the level still remained significantly higher than in control (+210.5%, P<0.01).

In the rats kidney samples, aluminum concentration was significantly higher in exposed groups than in control (+231.88%, p<0.01). Administration of melatonin together with aluminum reduced significantly the accumulation of aluminum (-37.69%, P<0.01) but still remained significantly higher than in control – Figure 2.

Significant aluminum accumulation was recorded in the heart (figure 3) of the exposed rats compared to control (+173.67%, p<0.01). Aluminum level decreased significantly when melatonin was administered (-40.66%, p<0.01) but still remained significantly higher than in control.

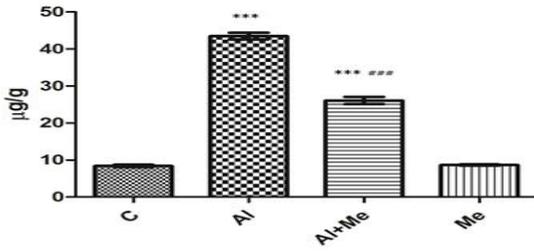


Figure 1. Aluminum level in liver of rats exposed to aluminum and melatonin.

*** Statistically different from the control group, $P < 0.01$

Statistically different from the aluminum group, $P < 0.01$

Şekil 1. Alüminyum ve melatonine maruz kalan sıçanların karaciğerlerindeki alüminyum düzeyleri.

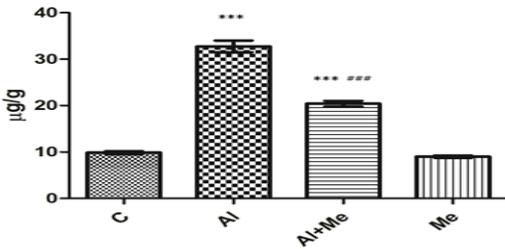


Figure 2. Aluminum level in kidney of rats exposed to aluminum and melatonin.

*** Statistically different from the control group, $P < 0.01$

Statistically different from the aluminum group, $P < 0.01$

Şekil 2. Alüminyum ve melatonine maruz kalan sıçanların böbreklerindeki alüminyum düzeyleri.

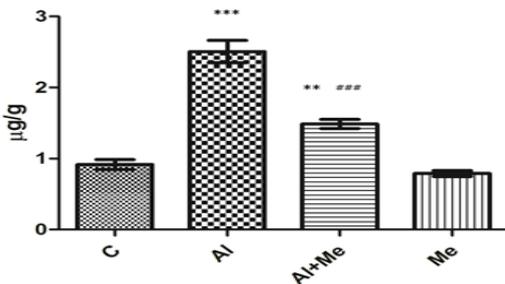


Figure 3. Aluminum level in heart of rats exposed to aluminum and melatonin.

, Statistically different from the control group
** - $P < 0.05$, *** - $P < 0.01$

Statistically different from the aluminum group, $p < 0.01$

Şekil 3. Alüminyum ve melatonine maruz kalan sıçanların kalplerindeki alüminyum düzeyleri.

Compared to control, it was observed a significant accumulation (+61.18%, $P < 0.01$) of aluminum in spleen of exposed rats (Figure 4). Aluminum level decreased when melatonin was administered (-10.45%) compared to aluminum exposed group but the differences were not significant ($P > 0.05$) and still remained higher than in control (+44.34, $P < 0.05$).

In the brain, one of the most important tissue due to aluminum implication in Alzheimer's disease, aluminum was accumulated in high amount compared to control (+1058.59%, $P < 0.01$) (Figure 5). A strong and significant ($P < 0.01$) decrease of aluminum level in brain was noted when melatonin was administered together with aluminum (-2.64%) but the level still remained higher than in control (+332.84%, $P < 0.01$).

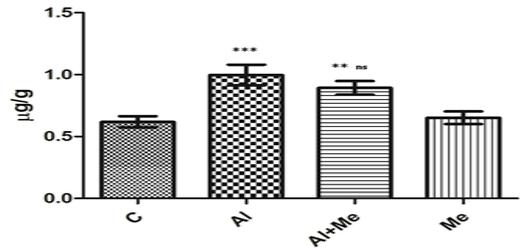


Figure 4. Aluminum level in spleen of rats exposed to aluminum and melatonin.

, Statistically different from the control group
** - $P < 0.05$, *** - $P < 0.01$

^{ns} Statistically not different from the aluminum group, $P > 0.05$

Şekil 4. Alüminyum ve melatonine maruz kalan sıçanların dalaklarındaki alüminyum düzeyleri.

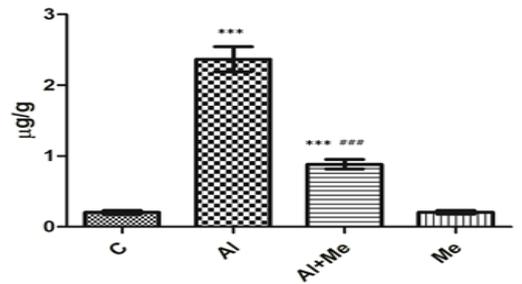


Figure 5. Aluminum level in brain of rats exposed to aluminum and melatonin.

, Statistically different from the control group
** - $P < 0.05$, *** - $P < 0.01$

Statistically different from the aluminum group, $P < 0.01$

Şekil 5. Alüminyum ve melatonine maruz kalan sıçanların beyinlerindeki alüminyum düzeyleri.

Discussion

In the present study we observed that aluminum was accumulating in studied organs and the accumulation was limited by use of melatonin.

Accumulation of aluminum in internal organs was pointed out by many authors. Spencer et al. (1995) observed that aluminum presents higher levels in rat's liver and kidney in one hour after 800 µg iv. administration compared to control. Szentmihályi et al. (2004) noted that aluminum was accumulated in liver of hyperlipidemic rats compared to control. Crafford and Avenant-Oldewage (2010) showed that aluminum was accumulated in the organs of sharptooth catfish (*Clarias gariepinus*) in high amounts especially in the liver.

The mechanism by which melatonin decreases heavy metal concentration in the tissues is not known. However, some reports suggest a possible mechanism of melatonin influence, namely through decreasing heavy metal concentration in the soft tissues and bones of animals (Chwelatyiuk et al. 2006; Limson et al., 1998). Chwelatyiuk et al. (2006) observed that melatonin co-treatment caused a dose dependent decrease in renal, hepatic and intestinal Cd concentration in mice which received drinking water containing 50 µg Cd/mL, or 50 µg Cd/mL with additional 2, 4 or 6 µg/mL melatonin for 8 weeks. Also, Limson et al. (1998) studying the metal binding affinities of melatonin noted that aluminum formed complexes with melatonin and its precursors, tryptophan and serotonin. One other possibility is that melatonin, which is capable of forming stable complexes with metals e.g. Cd (Chwelatyiuk et al. 2006; Limson et al., 1998), inhibits intestinal absorption of metal, especially its uptake from the intestinal lumen into mucosa. Another possible explanation is that melatonin, which is highly lipid-soluble, can move freely across all cellular barriers, facilitating the removal of metal from soft tissues (Reiter et al., 2002).

This study showed that aluminum sulphate exposure to rats to caused an important accumulation of this trace element in liver, kidney, heart, spleen and brain in amounts significantly higher amounts than in the control group. Melatonin significantly reduced the accumulation of aluminum in studied organs. Although the aluminum levels were still higher than the control group, this founding can be accepted as a protective effect of melatonin against the aluminum accumulation to important organs. Further studies should be

performed with different aluminum and melatonin doses for a detailed evaluation.

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