Food and Chemical Toxicology 70 (2014) 252–259

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Melatonin does not modify the concentration of different metals in AβPP transgenic mice

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ARTICLE INFO

Article history:
Received 28 March 2014
Accepted 27 May 2014
Available online 5 June 2014

Keywords:
Aluminum
Dietary exposure
Citrate
Melatonin
Essential elements
AβPP transgenic mice

ABSTRACT

Metals such as aluminum, iron, copper, and zinc have been implicated in the etiology of certain neurodegenerative disorders. On the other hand, it is well known that citric acid enhances Al absorption through the diet, while melatonin may bind such metals and decrease ROS production. In this study, we determined the concentrations of Al, Cu, Zn, Fe, and Mn in various tissues of Tg2576 Al-treated mice. Female mice and wild type littermates were exposed to 1 mg Al/g plus 3.2% of citric acid and melatonin 10 mg/kg/day for 15 months. At 18 months of age, metal concentrations were measured in bone, liver, kidney and spleen, as well as in three brain regions. In the citric plus Al group, Al levels were higher in hippocampus than in cortex and cerebellum, while Al concentration in bone was higher than those in kidney, liver and spleen, The current results show that exposure to Al plus citric acid did not produce relevant changes in metal levels related with genotype. Moreover, co-administration of melatonin with Al did not modify significantly metal concentrations in tissues. The present results do not support that melatonin can diminish Al or Fe concentrations in various tissues.

1. Introduction

Aluminum (Al) is distributed throughout the environment. Although it is the third most abundant element on earth, there is not essential function in humans. Concern about Al toxicity in humans, including exposure through food, is present since has shown Al was a potential neurotoxic (ATSDR 2008; Yokel, 2012a,b; Walton 2012; Exley and Vickers, 2014). Herein exposure to Al is mainly from food, water, airborne dust, antiperspirants, immunizations, allergy injections and antacids. In general, trace elements, including Al, in food may come from environmental sources, as they are naturally present in earth's crust. Processing and packaging may also contribute to the presence of metals in food (Arnich et al., 2012). Foods and beverages are largest contributor of Al intake for humans, providing about 3.5–10 mg/day (Yokel, 2012a). This suggests being food the largest single source of Al for humans (Yokel and Florence 2006; Yokel, 2012a).

The release of trace elements, coming from a ceramic object, depends not only on its manufacturing but it is also greatly determined by its use and the type of food in contact with it. In a recent study, Dermont et al. (2012) analyzed the migration of number of metals. The first group (boron, cobalt, copper, potassium, lithium, manganese, sodium, nickel, antimony, strontium, titanium, vanadium, zinc and zirconium) showed higher values when the migration was induced by citric or malic acid. The opposite effect was seen in the second group in which acetic acid was clearly the stronger leaching agent. This group included aluminum, barium, chromium, iron and magnesium.

Al absorption from the gastrointestinal tract appears to be primarily in the distal intestine. Free Al^{3+} and smaller Al complexes are able to pass more easily thorough the glycocalyx to access and traverse the enterocytes, for absorption into the bloodstream. This absorbed bioavailable Al fraction is the most interesting to medical scientist rather than the much larger, non-absorbed Al fraction (Whitehead et al., 1997; Yokel, 2013).

On the other hand, citrate, in the diet and pharmaceuticals, is well-known to increase Al absorption 5 or 10. Formation of the Al citrate complex: (a) maintains Al solubility over a relatively large pH range, (b) holds Al in a low molecular weight complex, and (c) increases mucus permeability so the Al citrate complex can cross the mucosa an enterocyte lining of the intestine to access the bloodstream (Domingo et al., 1993; Whitehead et al., 1997; Walton 2012).

Aluminum was the first metal linked with neurodegenerative diseases. Various mechanisms have been proposed for Al-induced

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http://dx.doi.org/10.1016/j.fct.2014.05.025
0278-6915/

neurotoxicity, including free radical damage via enhanced lipid peroxidation, impaired glucose metabolism, effects on signal transduction and protein modification, alterations on the axonal transport, and alteration of phosphorylation levels of neurofilaments (Esparza et al., 2003, 2005; Exley, 2004; Gómez et al., 2005, 2008; Shcherbatykh and Carpenter, 2007; Kumar et al., 2009; Li et al., 2012; Walton, 2012; Yokel, 2012b; Ayton et al., 2013). However, the link of Al to the etiology of the most serious neurological disorders such as Alzheimer's disease (AD) remains still unclear (McLachlan et al., 1991; Yokel, 2000; Exley, 2004; Esparza et al., 2005; Gómez et al., 2005; Percy et al., 2011; Kawahara and Kato-Negishi, 2011; Zhang et al., 2012).

Alzheimer's disease (AD) is the most common cause of dementia in humans. Neurofibrillary tangles and neuritic senile plaques are the two pathological hallmarks that define AD. AD prevalence rates vary by sex, ethnicity, and geographic areas, suggesting an important modulating role for environmental factors (Maynard et al., 2006; Shcherbatykh and Carpenter, 2007; Kawahara and Kato-Negishi, 2011).

The environmental component of AD has been suggested to involve prions, viruses and metal neurotoxicants (Walton, 2013). For the most AD cause, the lack of identified hereditary links, suggests that environmental factors are likely to interact with other factors to cause this disease. Aluminum is one of the suggested environmental contributors (Walton, 2013; Yokel, 2013). In addition to age and female gender, a number of risk factors have been also associated with the potential development of AD. Some metals, especially Al, copper (Cu), iron (Fe) and zinc (Zn), have been included among commonly suggested risk factors (Domingo, 2006; Shcherbatykh and Carpenter, 2007; Akiyama et al., 2012; Roberts et al., 2012; Yokel, 2012b). The amount of Al that gradually accumulates in brain from frequent ingestion of dietary Al over the life span may be sufficient to produce Al neurotoxicity in neurons that might take the form of AD in especially susceptible individuals (Walton, 2012).

The results of a number of studies suggest the importance of transition metals in AD. Copper, Zn and Fe accumulated in and around amyloid plaques in the AD brain, where their levels were three-to-five higher than those of age matched controls (Maynard et al., 2002, 2005; Bush, 2003; Shcherbatykh and Carpenter, 2007; Duce and Bush, 2010; Roberts et al., 2012). While metal ions play an important role in biology, experimental evidence and clinical data suggest that brain biometal dysregulation and possible environmental metals exposure, could be related to the decline of cognitive functions and contribute to AD pathogenesis (Liu et al., 2006; Duce and Bush, 2010; Roberts et al., 2012; González-Domínguez et al., 2014).

Melatonin (Mel) is derived from the aminoacid tryptophan and is produced by the pineal gland during the dark phase of the circadian cycle. Melatonin has a number of physiological functions, including regulating circadian rhythms, clearing free radicals, improving immunity and generally inhibiting the oxidation of biomolecules. Melatonin also decreases during the aging process, having patients with AD and other neurodegenerative disorders more profound reductions of this substance (He et al., 2010; Luchetti et al., 2010; Rosales-Corral et al., 2012; Polimeni et al., 2014). In recent years, the role of Mel as a metal chelator attracted the interest of some investigators (Limson et al., 1998; Gulcin et al., 2003). It is well known that Cu (II) and Zn (II), as well other metal ions, binding to amyloid β, accumulate in AD plaques. Abnormality high levels of Fe have been also demonstrated in a number of neurodegenerative disorders including AD (Polimeni et al. 2014; Campbell et al., 2001). In turn, *in vitro* studies have shown that Mel has a inhibitory effect on free radical production. This effect is particularly evident in the presence of Fe(II) > Cu(II) > Zn(II) > Mn(II) > Al(III) when they interact with amyloid β peptides (Zatta et al., 2003).

The amyloid precursor protein (AβPP) (SW) transgenic mouse is an animal that express high levels of the mutant amyloid-peptide (Aβ). This mouse is currently considered as an experimental model of AD. Nowadays, information about concentrations of the above indicated metals in brain and other tissues in this animal model as well as the possible protective action of Mel is very scarce. In this study, we have used citric acid in order to enhance the absorption and accumulation of Al in various tissues. It was designed to test if long-term oral intake of excess Al, with citrate supplementation in the diet, would enhance Al, Cu, Zn, Fe and Mn absorption and deposition in brain and other tissues. We also investigated if oral co-administration of Mel could reduce metal deposition in Tg2576 and wild type mice.

2. Materials and methods

2.1. Chemicals, animals and treatment

Aluminum was administered through the diet as Al lactate (Sigma Chemical, St. Louis, MO, USA). Regular chow was supplemented with 1 mg of Al per g of chow plus 3.2% of citric acid (Harlan, Barcelona, Spain) to promote Al uptake (Golub and Keen, 1999). Melatonin (Sigma Chemical) was dissolved in absolute ethanol and added to the drinking water at a final ethanol concentration of 0.066% in feeding bottles protected from the light. Animals treated with Mel received 10 mg/kg/day (García et al., 2010a,b). Other reagents were of the highest quality available and obtained from commercial sources.

AβPP transgenic mice were bred from a breeding pair of Tg2576 mice (Taconic Europe, Ejby, Denmark). An authorization from Taconic Europe was obtained to do the cross breeding. The Tg2576 colony was maintained by crossing Tg2576 males with C57BL6/SJL (wild type) F1 females. At 2 months of age, the transgene status was determined by PCR of tail DNA. Female animals were then separated into transgenic and wild type. Only females were used because of the aggressive behavior among males results in frequent injuries. Animals (15–20 g) were housed in a room equipped with automatic light cycles (12 h light/dark), and maintained at 22 ± 2 °C and relative humidity of 40–60%. Food and tap water were offered *ad libitum* throughout the study. The procedures used in the study were approved by the Ethics Committee of Animal Research, "Rovira i Virgili" University (Tarragona, Spain).

At 3 months of age, treatment was initiated. Experimental animals were AβPP transgenic mice and wild type littermates. We have used 10 experimental groups, 5 wild type and 5 Tg mice. Control groups (C) were fed with regular chow, while the second groups were fed with regular chow plus 3.2% of citric acid (Cit group), and the third groups received Al lactate supplemented in the diet (1 mg of Al/g diet, plus 3.2% of citric acid) (Cit+Al group) (Golub and Keen, 1999), Finally, the fourth groups received (Mel) at doses of 10 mg/kg/day in drinking water, and the fifth groups received Al lactate supplemented in the diet (1 mg of Al/g diet, plus 3.2% of citric acid) plus Mel in drinking water (Cit+Al+Mel group).

Fresh diet and water were replaced twice per week and continued until the end of the study at 18 months of age. Food consumption was measured during the first month of treatment. No differences between control and treated animals were found (data not shown). At the end of the experimental period, animals were anesthetized by an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg) dissolved in 0.9% saline. Subsequently, animals were decapitated and brains were removed. Brains were placed on an ice-cold plate and cortex, cerebellum and hippocampus were dissected. Samples of liver, spleen, bone, and kidney were also collected.

2.2. Metal analysis

Tissue samples were weighed in a microsampling quartz insert, being 65% nitric acid (Supapur, E. Merck) added to digest the samples. The microsampling inserts were then introduced in Teflon vessels and put into a microwave oven Star D (Milestone, Sorisole, Italy) (Gómez et al., 2008). All materials were previously washed with 10% nitric acid in order to avoid any potential contamination. For quality control, tissues reference standards (prepared in-house) and NIST Standard Reference Material (Bovine liver 1577b, NIST, Gaithersburg, MD, USA) was measured in each assay. Aluminum, Fe, Mn, Cu, and Zn concentrations were determined by means of a computer-controlled sequential inductively coupled plasma spectrometer (Perkin Elmer Elan 6000) according to DIN EN ISO 17294-2. Detection limits were the following: 1.00 μg/kg for Al, 0.010 μg/g for Fe, 0.10 μg/kg for Mn and Cu, and 0.020 μg/g for Zn while Si detection limit was 0.050 μg/ml in chow and water samples.

2.3. Statistics

Statistical analysis was performed using the software Statistical Package for the Social Sciences (SPSS Statistics 19.0). To evaluate the homogeneity of variances, the Levene test was used. When the variances of different treatment groups were