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Chronic exposure to aluminum and melatonin through the diet: Neurobehavioral effects in a transgenic mouse model of Alzheimer disease

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ABSTRACT

Aluminum (Al) is a known neurotoxic element involved in the etiology of some serious neurodegenerative disorders such as Alzheimer disease (AD). Antioxidants like melatonin might protect neurons against the damage produced in AD. The APPSWE (Tg2576) transgenic mouse is one of the most used animal models developed to mimic AD damage. In the present study, wild type and Tg2576 mice were orally exposed during 14 months to Al, melatonin, and citric acid, as well as to all possible combinations between them. At 17 months of age, mice were evaluated for behavior using the open-field test and the Morris water maze. Transgenic animals exposed to melatonin only and to Al plus citric acid plus melatonin showed a good acquisition. No effects on acquisition in the Morris water maze were observed in wild type mice. With respect to the retention of the task, only melatonin wild type animals, and Al plus citric acid plus melatonin transgenic mice showed retention during the acquisition. Control wild type animals and Al plus citric acid plus melatonin transgenic mice showed good long term retention. Melatonin improved learning and spatial memory in Al-exposed transgenic mice.

1. Introduction

Aluminum (Al) is a highly neurotoxic element without any known biological function. Human exposure to Al occurs primarily through contaminated food and water or airborne dust, as well as through antiperspirants, immunizations, allergy injections and antacids among others (Walton, 2012a, 2013; Yokel, 2000; Yumoto et al., 2009). Another possibility is occupational exposure. Thus, workers in the Al industry and miners are much vulnerable to Al exposure as a result of breathing dust containing Al (Percy et al., 2011). However, the most usual Al exposure for the general population is through the diet, mainly from dietary additives (Fekete et al., 2013; Walton, 2012a, 2013; WHO, 1989). The primary human dietary sources of Al in the US are foods and beverages. Coffee, wine, black and green tea, and drinking water contain Al (Yokel and Florence, 2008). In a total diet study in France, Arnich et al. (2012) reported that the main contributors to Al adult exposure were hot beverages other than coffee (13%) and vegetables excluding potatoes (11%). In children, the main contributors were vegetables, excluding potatoes (8%), pasta (7%), pastries and cakes (6%), and dairy-based desserts (6%). On the other hand, most unprocessed foods contain less than 5 mg Al/kg, while processed foods such as cake mix or frozen pizza cheese contain around 400 mg Al/kg (Yokel, 2013). This high amount of Al in processed foods is due to the presence of Al additives used as rising agents, dyes, anti-caking agents and pH adjusting. One of the most used Al-containing additives is sodium aluminum phosphate (SALP) (Walton, 2012a, 2013). Another potential Al source is the transfer from cookware and utensils into the food (Karbouj et al., 2009.)

Although oral Al is poorly absorbed, it has been shown that some Al compounds such as maltolate, ascorbate, succinate, lactate or citrate are much more easily absorbed. Specifically, citric acid increases Al absorption from 5 to 10 times in both humans and animals (Domingo et al., 1993; García et al., 2009; Walton, 2012b; Zhou et al., 2008) and it can easily access to the bloodstream crossing the intestinal mucosa (Domingo et al., 1993; Whitehead et al., 1997; Yokel, 2013). In addition, citric acid is a very common component of human diets and is widely used as food additive (Golub and Keen, 1999; Magaia et al., 2013; Yilmaz et al., 2012).

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Since human Al toxicity is clearly demonstrated, the use of Al compounds has been regulated. In 2007, the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (FAO/WHO) reduced the tolerable weekly intake (PTWI) for humans from 7 mg Al/kg bw to 1 mg Al/kg bw (Walton, 2012a; WHO, 2007). It is estimated that about one-half of the American population is consuming up to 25 mg Al per day, while a 5% may ingest more than 95 mg Al/day (Greger, 1993; Walton, 2012a). On the other hand, normal human body burden of Al is ~60 mg, but only a 0.25% reaches the brain. In spite of that relatively low quantity, Al affects the entorhinal cortex, hippocampus, subiculum, septum, olfactory lobe, piriform cortex, temporal cortex, parietal cortex, frontal cortex, cingulate cortex, amygdala, substantia nigra, basal nucleus of Meynert, dorsal raphe nucleus and locus coeruleus (Walton; 2009, 2012a). These brain regions are particularly vulnerable to deterioration in AD (Braak and Braak, 1991; Kováks et al., 2001; Walton, 2009, 2012a).

Early onset AD usually has a familial link due to gene mutations, which result in increased secretion of neurotoxic Aβ protein. No specific gene mutations have been associated with sporadic forms of AD, which account 85–95% of AD cases. The lack of identified hereditary links for most AD cases suggests that environmental factors probably interact with other factors to cause the disease. In recent decades, Al has been suggested as a potential environmental contributor to AD (Walton, 2012a, 2013; Yokel 2013). *In vitro* and *in vivo* studies relate Al with the propensity of Aβ-42 to form β sheets by two ways: the up-regulation of gene expression of APP, and the divergence of APP metabolism from its non-amyloidogenic pathway to its amyloidogenic pathway. Both result in the formation of Aβ oligomers, fibrils and plaques (Kawahara et al., 2001; Rodella et al., 2008; Walton, 2012a).

Melatonin (N-acetyl-5-methoxytryptamine) (Mel) is a by-product of serotonin produced mainly by the pineal gland and is one of the main output signals of the central nervous system (Bubenik and Konturek, 2011; Corrales et al., 2013; Mauriz et al., 2013). During the day, the intense light blocks the production of Mel in the pineal gland, while at dusk the blood concentration of pineal-produced Mel begins to increase (Bubenik and Konturek, 2011; Reiter, 1993). This increase of Mel levels provides a signal to all body cells about the onset of night, which is called the daily clock. Similarly, the yearly clock inform from the season using the changes on day length. It is thought that changes on pineal-produced melatonin with aging might also provide the "age clock". From puberty, Mel levels start a steady decline until the old age. This age-dependent decline is controversial since it is not resolved yet whether the Mel changes are the cause, or a consequence of the aging process (Bubenik and Konturek, 2011; Reiter, 1995). In AD patients, profound reduction in Mel secretion and expression of melatonin MT1/MT2 receptor occur. These changes in Mel signaling may contribute to the circadian rhythm alterations and cognitive impairments associated with AD (Baño Otalora et al., 2012).Mel has a strong antioxidant capacity, being able to protect DNA and other macromolecules against free radicals damage (Bubenik and Konturek, 2011; Reiter, 1995; Reiter et al., 2010). Mel also up-regulates the activity of other antioxidants (Corrales et al., 2013; Esparza et al., 2005; García et al., 2009; Gómez et al., 2005; Olcese et al., 2009). Moreover, Mel has antitumoral, immunomodulatory, anti-inflammatory and neuroprotective properties (Baño Otalora et al., 2012; Mauriz et al., 2013) preventing lipid peroxidation and other destructive processes related to oxidative stress (Rodella et al., 2013). A number of investigations have demonstrated the role of Mel in prevention of degenerative disorders in which free radicals generation is involved (i.e., Parkinson's disease and AD) (Antunes Wilhelm et al., 2013; Baño Otalora et al., 2012; Bubenik and Konturek, 2011).

Factors such as the different chemical forms of the Al-administered compound, the doses of both Al and Mel, the low gastrointestinal Al absorption, differences in Al absorption depending on the age, the animal model and the behavioral assessment procedures selected contribute to found a great variability of results (Domingo et al., 1993; García et al., 2009, 2010; Golub, 2001; Walton, 2009). One of the most popular animal models of AD is the APPSWE (Tg2576) transgenic mouse. The main characteristics of this animal model are the overexpression of the Aβ polypeptide gene and the favoring of the cleavage of amyloid polypeptide by the enzyme β-secretase against α-secretase. This leads to an excess of β products, which finally deposit and form senile plaques. These deposits begin approximately at 9–12 months of age (Ribes et al., 2008). The behavioral phenotype is characterized by spatial memory deficits after 11–15 months of life, which was shown by altered performance in Y and Morris water mazes. These behavioral alterations correlate well with the development of amyloid plaques in the frontal, temporal, and entorhinal cortex, as well as in the cerebellum (García et al., 2009; King and Arendash, 2002; Ribes et al., 2008; Zhang et al., 2012).

Based on the above, the main goal of the present study was to evaluate the effects of chronic exposure to Al plus citric acid on spatial learning and memory in wild type and APPSWE transgenic mice, as well as to assess the protective role of Mel administration.

2. Material and methods

2.1. Chemicals

Aluminum and citric acid were administered through the diet. Regular chow was supplemented with Al lactate (1 mg of Al per g of chow) (Harlan Ibérica, Barcelona, Spain) (Gómez et al., 2008) and with 3.2% of citric acid (Sigma Chemical, St. Louis, MO, USA) (Cit) (Golub and Keen, 1999). Mel (Sigma Chemical, St. Louis, MO, USA) was dissolved in absolute ethanol and added to the drinking water to a final ethanol concentration of 0.066%, it was administered in feeding bottles protected from the light. Control drinking water contained also a 0.066% of ethanol. A fresh Mel solution was prepared twice a week.

2.2. Animals and treatment

Adult transgenic (Tg2576) and wild-type mice were used. Parental APPSWE hemizygous male mice were obtained from Taconic Europe (Lille Skensved, Denmark). After a quarantine week, males were backcrossed with female mice (C57BL6/SJL) obtained from Charles River (Barcelona). Because of the aggressive behavior among males, which results in frequent injuries, only females were used in the current study. At 2 months of age, female mice were selected, genotyped using mouse tail, and separated according to the genotype. Mice were housed in an animal room maintained at 22 ± 2 °C, a relative humidity of 50 ± 10%, and a 12-h light/dark automatic light cycle (light: 08.00–20.00 h). All animals were allowed free access to food and water. The experimental procedures used in the current study were approved by the Ethics Committee of Animal Research, "Universitat Rovira i Virgili" (Tarragona, Catalonia, Spain). They were carried out according to the Spanish Government Guide and the European Community Guide for animal care.

From 3 months of age, mice were fed with one of the three different diets during 14 consecutive months: regular chow, regular chow supplemented with citric acid (3.2%), and regular chow supplemented with both citric acid (3.2%) and Al lactate (1 mg Al/g chow). Melatonin-treated animals received also 10 mg/kg/day of Mel through drinking water. Animals were divided into the ten following experimental groups: control wild (C-W), control Tg2576 (C-Tg), melatonin wild (Mel-W), melatonin Tg2576 (Mel-Tg), citric acid wild (Cit-W), citric acid Tg2576 (Cit-Tg), aluminum lactate and citric acid wild (AlCit-W), aluminum lactate and citric acid Tg2576 (AlCit-Tg), aluminum lactate and citric acid plus melatonin wild (AlCit-Mel-W), and aluminum lactate and citric acid plus melatonin Tg2576 (AlCitMel-Tg). 12–14 animals per group were selected. After 14 months of treatment (17 months of age), behavioral tests were initiated.

2.3. Behavioral tests

All mice were evaluated for behavior. General activity was assessed in an open-field, while spatial learning and memory were evaluated in a Morris water maze spatial reference task.