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Effects of melatonin on aluminium-induced neurobehavioral and neurochemical changes in aging rats

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ABSTRACT

This study aimed to investigate the potential protective effects of melatonin (Mel) against aluminium-induced neurodegenerative changes in aging Wistar rats (24–28 months old). Herein, aluminium chloride (AlCl3) (50 mg/kg BW/day) was administered by gavage, and melatonin (Mel) was co-administered to a group of Al-treated rats by an intra-peritoneal injection at a daily dose of 10 mg/kg BW for four months. The findings revealed that aluminium administration induced a significant decrease in body weight associated with marked mortality for the old group of rats, which was more pronounced in old Al-treated rats. Behavioural alterations were assessed by 'open fields', 'elevated plus maze' and 'Radial 8-arms maze' tests. The results demonstrated that Mel co-administration alleviated neurobehavioral changes in both old and old Al-treated rats. Melatonin was noted to play a good neuroprotective role, reducing lipid peroxidation (TBARs), and enhancing enzymatic (SOD, CAT and GPx) activities in the brain organs of old control and old Al-treated rats. Mel treatment also reversed the decrease of AChE activity in the brain tissues, which was confirmed by histological sections. Overall, the results showed that Mel administration can induce beneficial effects for the treatment of Al-induced neurobehavioral and neurochemical changes in the central nervous system (CNS).

1. Introduction

There is increasing evidence in the literature that, during environmental stress, reactive oxygen species (ROS) can cumulatively damage biological molecules, leading to the structural and functional deterioration of cells during the aging process (Harman, 1956; Yu, 1996; Sohal et al., 2000). Oxidant radicals, such as superoxide anion ($O_2^{\cdot-}$), singlet oxygen ($^1O_2$), hydroxyl radical ($HO^{\cdot}$), peroxyl radical ($LOO^{\cdot}$), peroxynitrite anion ($ONOO^{\cdot-}$), and nitric oxide (NO) have also been reported to contribute to increased cell death (Hawkins and Davies, 2001; Arbos et al., 2008) and to have significant incidence with advanced age (Vishwas et al., 2012). Aging has also been associated with the declined ability to respond to stress (McEwen et al., 2007), imbalanced physiological functions, and increased risk of aging-related neurodegenerative diseases and conditions, including the Alzheimer's disease (AD) (Rosales Corral et al., 2012), the Parkinson's disease, and the Huntington's disease (Sood et al., 2001). In this context, several studies have demonstrated that age-related changes are associated with high imbalances between pro-oxidant and antioxidant systems in favor of ROS generations (Sahal et al., 2002; Serrano and Klann, 2004; Senthil kumaran et al., 2008). Age-related oxidative damage is, therefore, provoked by a high continuous burst of free radical generation and could induce several disorders, which in turn lead to the acceleration of aging conditions (Yu, 1996; Sahal et al., 2002; Asha Devi et al., 2012). Aging has also been associated with increased susceptibility to the failure of the neuronal system and the disruption of cerebral functions, with neurotransmitters being the biochemical interface between the neurons of the central nervous system. Cholinergic system alterations, including the acetylcholinesterase (AChE) activity and synaptic Acetylcholine (Ach) cycle, have also been reported to be implicated in central nervous system (CNS) disorders, including the Alzheimer's disease (Betancourt and Carr, 2004; Turchi et al., 2005; Herholz et al., 2005).

Several studies have recently examined the effects of environmental exposure to certain neurotoxic compounds, such as aluminum, on the risk of age-related diseases and conditions. In fact, aluminum (Al) is in present in several consumer products, including cooking utensils, drugs, cosmetics, and food additives (Yokel, 2000; Chen et al., 2010). It can also be found in various food products, especially corn, yellow cheese, salt, herbs, spices, and tea. The human body is further exposed to Al and its salts that are widely

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used as flocculants in the treatment of drinking water for purification purposes, which induces epidemiological and clinical neurotoxic manifestations that can contribute to several age-related neuropathological diseases (Colomina et al., 2002; Mui et al., 2003; Kaur and Gill, 2009; Kawahara and Kato-Negishi, 2011). Al salts have been reported to cross and alter the blood–brain barrier (Zatta et al., 2002) and to become deposited in several brain regions, such as the isocortex (Platt et al., 2001; Miu and Benga, 2006), hippocampus (Miu and Benga, 2006; Struys-ponsar et al., 1997), and cerebellum organ (Linardaki et al., 2013). Several studies (Xu et al., 1992; Bharathi Shamasundar et al., 2006; Rondeau et al., 2009) supported the hypothesis that elevated Al concentrations were present in the pathogenesis of age-related illnesses, such as the Alzheimer's disease (AD) (Romero et al., 2014), and suggested that this excess could accelerate the aging process by inflicting oxidative damage (deloncle et al., 2001; Jyoti et al., 2007; Kumar and Gill, 2009; García et al., 2009).

Increasing concerns over the relationship between enhanced ROS production and the onset of several chronic and neurodegenerative disorders and diseases have been expressed in the literature (Sood et al., 2011; Sharma et al., 2012). This has triggered a persistent search for novel therapeutic and antioxidant agents from natural origins to help prevent or alleviate the progression of oxidative stress-related diseases. Of particular relevance to this search, melatonin (Mel) has often been described as a powerful naturally-occurring antioxidant that can easily cross cell membranes and the blood–brain barrier. Melatonin (N-acetyl-5-methoxy tryptamine) is basically a pineal hormone, but can also be produced by several others tissues, including the pancreas, thyroid gland, thymus, urogenital tract, retina, and gastrointestinal tract (Kvetnoy, 1999; Bubenik and Konturek, 2011). This extensive synthesis highlights the importance of its physiological activities which include the regulation of circadian and seasonal rhythms and the immune system (Brzezinski, 1997; Carrillo-Vico et al., 2005). It has often been reported to have several beneficial effects for the treatment of certain physiological disorders, including cardiovascular diseases, circadian rhythm sleep disorders, sexual dysfunctions and neurodegenerative disorders. Several studies have investigated the strong antioxidant potential of Mel, ability to trap cellular free radicals (Ceyran et al., 2008; Reiter et al., 2010), and scavenging activity towards reactive oxygen and reactive nitrogen species (Galano et al., 2013; Tan et al., 2013; García et al., 2014). Last but not least, melatonin production has been described to decline with age and to provide an 'age clock' (Reiter, 1993; Bubenik and Konturek, 2011).

Considering the increasing concerns over the alarming rates at which aging is growing worldwide and the promising opportunities that melatonin can open for the prevention or alleviation of this and other age-related diseases, this study was undertaken to investigate the potential protective effects of melatonin (Mel) treatment against aluminium-induced neurodegenerative changes in aging Wistar rats (24–28 months old). The changes in body weight, behavioural troubles, markers of oxidative stress (lipids peroxidation level), activities of antioxidant enzymes (catalase, superoxide dismutase and glutathione peroxidase), and acetylcholinesterase (AChE) activity were monitored and brain sections were submitted to histological analyses.

2. Materials and methods

2.1. Chemicals

Aluminium chloride (AlCl3) and melatonin (Mel) compounds were purchased from Bio basic (INC., Canada). The doses used for the gavage administration of AlCl3 (50 mg/kg b.w.) (Sethi et al., 2008; Tripathi et al., 2009; Sharma et al., 2012) and intra-peritoneal injection of melatonin (10 mg/kg body weight (BW)/day) (Reiters et al., 2003; Sudnikovich et al., 2007) were selected based on the literature.

2.2. Experimental design

Male wistar rats of two age groups, young (2 months) and old (24 months), were used in this study. The animals were housed 10 per clear large plastic cage (58 cm × 38 cm × 20 cm) and maintained on a 14–10 h light–dark cycle in constant room temperature (23 ± 2 °C), with free access to standard laboratory food (SICO – Sfax) and water ad-libitum. The rats were divided into five groups: Group **1**: young control rats (2–6-month), Group **2**: old control rats (24–28-month), Group **3**: old Al-treated rats (Old Al) (AlCl3 at 50 mg/kg B.W. per gavage in NaCl physiological water at 9:00 am, for 4 months), Group **4**: old Al-treated rats co-treated with melatonin (Old Al-Mel) (at 10 mg/kg BW/day, intraperitoneally injected (IP) at 6:00 pm), and Group **5**: old control rats supplemented with melatonin only (old-Mel).

Body weight and mortality percentages were monitored throughout the treatment period. This study was approved by the local Ethical Committee for Animal Experimental Use at the Faculty of Science of Sfax, Tunisia, and the animals were treated in accordance with its guidelines.

2.3. Preparation of brain extract

About 0.5 g of brain was homogenized in 1 mL NP40-PBS buffer, pH 7.4, containing 0.5% Nonidet P-40, and protease inhibitors (10 mM EDTA, 2 mM PMSF, 5 mM NEM, 4 μg/ml of aprotinin,), and then centrifuged for 30 min at 10,000g at 4 °C. The supernatants, referred to as "NP40 extracts", were stored at −80 °C. The pellets were resuspended in PBS buffer containing 4 M urea and centrifuged (30 min, 10,000g, 4 °C). The total protein level was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard at 660 nm.

2.4. Biochemical analysis

2.4.1. Lipid peroxidation level

Lipid peroxidation level was measured by the quantification of thiobarbituric acid-reactive substances (TBARS) according to the method of Buege and Aust (1984). Briefly, 375 μl of supernatant were homogenized by sonication with 150 μl of PBS, 375 μl of TCA-BHT (trichloroacetic acid-butylhydroxytoluene) to precipitate proteins and then centrifuged (1000g, 10 min, 4 °C). 400 μl of the obtained supernatant were mixed with 80 μl of HCl (0.6 M) and 320 μl of TBA dissolved in tris, and the mixture was incubated at 80 °C for 10 min. The absorbance of the resultant supernatant was measured at 530 nm.

2.4.2. Total (Cu–Zn and Mn) superoxide-dismutase (SOD) activity

Total (Cu–Zn and Mn) superoxide-dismutase (SOD) activity was determined at 25 °C by measuring its ability to inhibit the photoreduction of nitroblue tetrazolium (NBT) into blue formazan (Sun et al., 1988). The assay was performed in 50 mM PBS (Phosphate-buffered saline) (pH 7.4) containing 13 mM methionine, 0.1 mM EDTA, 2 μM riboflavin, and 75 μM NBT. Activity was expressed as U/mg protein, one unit being the amount inhibiting the photoreduction of NBT by 50%.

2.4.3. Glutathione-peroxidase (GPX) activity

Glutathione-peroxidase (GPX) activity was assayed at 25 °C according to the method of Flohe and Gunzler (1984). Briefly, activity was measured in 250 μL of tissue extract mixed with GSH (final concentration: 0.35 mmol/L). Reaction was started with the addition of H_2O_2 (0.2 mmol/L). After the reaction was stopped, 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) was added, and absorbance was recorded at 412 nm. Activity was expressed as μmoles of GSH oxidized/min/g protein.

2.4.4. Catalase (CAT) activity

Catalase (CAT) activity was determined at 25 °C following the method of Aebi (1984) by measuring the decrease of H_2O_2 absorbance at 240 nm for 60 s. Activity was calculated using an extinction coefficient of 0.043 mM^{-1} cm^{-1} and expressed as μmoles of H_2O_2 destroyed/min/mg of protein.

2.4.5. Acetylcholinesterase (AChE) activity

Acetylcholinesterase (AChE) activity was assayed using the standard method of Ellman et al. (1961); the changes in absorbance were recorded at 412 nm using a UV spectrophotometer and expressed as μmoles of acetylcholine iodide hydrolyzed/min/mg protein.

2.5. Behavioural tests

Different groups of rats (10 rats per group) were submitted to behavioural tests designed to assess locomotor activity, anxiety level, and spatial learning ability. Accordingly, the rats were pre-trained for 7 days on all the behavioral tests employed, namely open field, elevated plus maze, and radial 8-arms maze tests. Behavioral tasks were started on the day following pre-training and continued for 13 days. Training was performed during the last treatment month, and scoring procedures are briefly described below.

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