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SULFORAPHANE RESCUES MEMORY DYSFUNCTION AND SYNAPTIC AND MITOCHONDRIAL ALTERATIONS INDUCED BY BRAIN IRON ACCUMULATION

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Abstract—Iron overload contributes to the development of neurodegeneration and the exacerbation of normal apoptosis rates, largely due to its participation in the Fenton reaction and production of reactive oxygen species (ROS). Mitochondria constitute the major intracellular source of ROS and the main target of attack by free radicals. They are dynamic organelles that bind (fusion) and divide (fission) in response to environmental stimuli, developmental status, and energy needs of the cells. Sulforaphane (SFN) is a natural compound that displays antioxidant and anti-inflammatory activities. This study aims to investigate the effects of SFN on memory deficits and changes in markers of mitochondrial function, DNM1L and OPA1, and the synaptic marker, synaptophysin, induced by neonatal iron treatment. Male rats received vehicle or carbonyl iron (30 mg/kg) from the 12th to the 14th postnatal day. In adulthood, they were treated with saline or SFN (0.5 or 5 mg/kg) for 14 days every other day. Memory deficits were assessed using the object recognition task. DNM1L, OPA1, and synaptophysin levels in the hippocampus were quantified by Western blotting. Results showed that SFN was able to reverse iron-induced decreases in mitochondrial fission protein, DNM1L, as well as synaptophysin levels in the hippocampus, leading to a recovery of recognition memory impairment induced by iron. These findings suggest that SFN may be further investigated as potential agent for the treatment of cognitive deficits associated with neurodegenerative disorders. © 2015 Published by Elsevier Ltd. on behalf of IBRO.

Key words: sulforaphane, iron, mitochondria, recognition memory, synapse, neurodegenerative disorders.

INTRODUCTION

Iron is the most abundant transition metal in the brain and is involved in metabolic processes such as oxidative phosphorylation and synthesis of DNA, RNA and proteins; acting as a cofactor for many enzymes (Youdim et al., 1991; Crichton et al., 2008). In neurons, iron plays a role in the production of various neurotransmitters including dopamine, norepinephrine, serotonin and GABA (Todorich and Connor, 2004; Lee et al., 2006). Iron entry in the neonatal brain is essential for normal neurodevelopment and for the establishment of the final iron concentration in the adult brain, as brain iron absorption is maximal during the neonatal period (Connor et al., 1995; Moos, 2002).

Evidence suggest that iron overload contributes to the development of neurodegeneration, through the exacerbation of apoptosis rates, mainly due to its participation in the Fenton reaction and production of reactive oxygen species (ROS) that result in cell damage (oxidative stress) (Lee et al., 2006). In neurological diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), dementia with Lewy bodies, and Huntington's disease (HD), iron accumulation occurs in regions most susceptible to neuronal degeneration (cortex, hippocampus, and substantia nigra). The mechanisms underlying iron accumulation in the brain are still a matter of controversy. It has been hypothesized that both genetic and non-genetic factors may be involved. Although it is known that the absorption of iron in the brain is higher during development of the nervous system, there is a continuous absorption of iron resulting in the accumulation of iron during the aging process. Thus it is possible that dietary iron can represent a modifiable risk factor for neurodegenerative disorders associated with aging (Quintana et al., 2006; Bartzokis et al., 2007). In previous studies we have demonstrated that adult rats treated with iron in the neonatal period (12th to 14th day

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Abbreviations: AD, Alzheimer's disease; ANOVA, analysis of variance; EDTA, ethylenediaminetetraacetic acid; HD, Huntington's disease; HDAC, histone deacetylase; OPA1, optic atrophy type 1; PD, Parkinson's disease; ROS, reactive oxygen species.

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of life) have greater oxidative damage in the hippocampus and cortical areas where there is increased apoptosis, resulting in loss of memory (Dal-Pizzol et al., 2001; de Lima et al., 2005; Miwa et al., 2011; da Silva et al., 2014). Over the years, we have been using this model of cognitive impairment to investigate the pharmacological properties of compounds in the search of novel potential treatments for memory dysfunction associated with neurodegenerative disorders (de Lima et al., 2008; Fagherazzi et al., 2012; Silva et al., 2012; Garcia et al., 2013). Mitochondria are the main intracellular source of superoxide anion (O^{2-}) or ROS as well as the main target of attack by free radicals (Harman, 1972; Miquel et al., 1980). Mitochondria are dynamic organelles that actively divide (fission) and join each other (fusion) to combine metabolites and copies of mtDNA to suit the ever-changing energy demands of the cell (Labrousse et al., 1999; Smirnova et al., 2001). Mitochondrial fission is a regular event during cell division, allowing the cells to divide as a means of maintaining adequate energy supply (McBride et al., 2006). The balance between mitochondrial fission and fusion is controlled by large dynamin-related GTPases, which have antagonistic effects (Liu et al., 2012). Fission is regulated and maintained by at least two proteins: the cytosolic dynamin-related protein 1 (Drp1 or DNM1L) and the transmembrane fission 1 (Fis1) protein. Mitochondrial fusion provides a mechanism by which the population of the organelle is kept evenly and facilitates inter-complementation of mtDNA (Chen and Chan, 2005), and the protein optic atrophy type 1 (OPA1), is required for this event, causing mitochondrial inner membrane fusion (Meeusen et al., 2006). In neurons, mitochondria are distributed not only in the cell body, but also migrate to the long processes, including synaptic terminals, which require large amounts of energy (Chen and Chan, 2006). Mitochondrial fission and fusion are critical for proper synaptic functioning, as the defects in the regulation of the dynamic properties of the mitochondria may be involved in energy failure, which may, ultimately, lead to neurodegeneration (Van Laar and Berman, 2013).

The synapses are formed by the functional link between the axons with post-synaptic terminals of their target neurons. Cognitive deficits have been correlated with changes in synaptic morphology, including loss of structural pre or post-synaptic proteins (such as synaptophysin) and the progressive loss of synaptic density especially in the hippocampus (Rapp and Gallagher, 1996; Rosenzweig and Barnes, 2003; Burke and Barnes, 2006; Driscoll et al., 2006). Synaptophysin is located exclusively in synaptic vesicles, where it is involved in several steps of synaptic function including exocytosis, synapse formation, biogenesis and endocytosis (Daly et al., 2000; Arthur and Stowell, 2007).

Sulforaphane [SFN, 1-isothiocyanate-(4R)-(methylsulfinyl)butane] is an isothiocyanate formed in mammals by gut bacteria-derived myrosinase acting on a precursor compound glucoraphanin, which is found in cruciferous vegetables of the genus *Brassica* such as cauliflower, broccoli, cabbage, Brussels sprouts, mustard, and cress (Van Poppel et al., 1999; Fahey et al., 2001; Atwell et al., 2015). SFN displays antioxidant, anti-inflammatory, and anticarcinogenic properties (Ping et al., 2010; Guerrero-Beltrán et al., 2012). Studies have shown that SFN protects against renal, hepatic, and cardiac damage (for a review see Guerrero-Beltrán et al., 2012). Recently, it has been demonstrated that SFN may be a promising neuroprotective compound. In a model of neonatal ischemia–hypoxia in rats, SFN was able to reduce the infarct volume and to decrease the number of apoptotic cells, as well as to reduce caspase-3 activity and to suppress oxidative stress (Ping et al., 2010). In the 6-hydroxydopamine (6-OHDA) experimental model of PD in rats, SFN was shown to protect against nigral damage, alleviating behavioral changes such as motor coordination and rotational behavior, increasing antioxidant defenses, and protecting against oxidative damage and apoptosis (Morroni et al., 2013). Dash and coworkers (2009) have demonstrated that SFN was able to ameliorate cognitive deficits induced by traumatic brain injury in rats. SFN also reduced infarct volume in brains of adult rats submitted to cerebral ischemia (Zhao et al., 2006).

Although evidence suggests that SFN exhibits neuroprotective properties having mitochondria as its main target, its functional properties and mechanisms of action are not completely understood. Here, we used the iron-induced model of memory impairment, which is associated with oxidative stress and increases in apoptotic markers, to investigate the effects of SFN on memory deficits as well as mitochondrial and synaptic alterations, by measuring hippocampal levels of mitochondrial fission and fusion proteins, DNM1L and OPA1, and the synaptic marker, synaptophysin. We also analyzed the expression of three genes encoding antioxidant enzymes.

EXPERIMENTAL PROCEDURES

Animals

Pregnant Wistar rats were obtained from the Centro de Modelos Biológicos Experimentais (CeMBE), Pontifical Catholic University, Porto Alegre, RS, Brazil. After birth each litter was adjusted within 48 h to eight rat pups, and to contain offspring of both genders in about equal proportions. Each pup was kept together with its mother in a plastic cage with sawdust bedding in a room temperature of 21 ± 1 °C and a 12/12-h light/dark cycle. At the age of 3 weeks, pups were weaned and the males were selected and maintained in groups of three to five in individually ventilated cages with sawdust bedding. For postnatal treatments, animals were given standardized pellet food and tap water *ad libitum*.

All behavioral experiments were performed at light phase between 09:00 a.m and 4:30 p.m. All experimental procedures were performed in accordance to the Brazilian Guidelines for the Care and Use of Animals in Research and Teaching (DBCA, published by CONCEA, MCTI) and approved by the Institutional Ethics Committee of the Pontifical Catholic University (CEUA 13/00366). All efforts were made to minimize the number of animals and their suffering.

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