Environmental Toxicology and Pharmacology 62 (2018) 120–131

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

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

Reversal of behavioral decline and neuropathology by a complex vitamin supplement involves modulation of key neurochemical stressors

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ARTICLE INFO

Keywords:
Alzheimer's disease
Ascorbic acid
B-complex vitamins
Glucose bioenergetics
Metal ion homeostasis
Oxidative stress

ABSTRACT

Metal ions are crucial for normal neurochemical signaling and perturbations in their homeostasis have been associated with neurodegenerative processes. Hypothesizing that *in vivo* modulation of key neurochemical processes including metal ion regulation (by transferrin receptor-1: TfR-1) in cells can improve disease outcome, we investigated the efficacy of a complex vitamin supplement (CVS) containing B-vitamins and ascorbic acid in preventing/reversing behavioral decline and neuropathology in rats. Wistar rats (eight weeks-old) were assigned into five groups (n = 8), including controls and those administered CVS (400 mg/kg/day) for two weeks before or after $AlCl_3$ (100 mg/kg)-induced neurotoxicity. Following behavioral assessments, prefrontal cortex (PFC) and hippocampus were prepared for biochemical analyses, histology and histochemistry. CVS significantly reversed reduction of exploratory/working memory, frontal-dependent motor deficits, cognitive decline, memory dysfunction and anxiety. These correlated with CVS-dependent modulation of TfP-1 expression that were accompanied by significant reversal of neural oxidative stress in expressed superoxide dismutase, nitric oxide, catalase, glutathione peroxidase and malondialdehyde. Furthermore, CVS inhibited neural bioenergetics dysfunction, with increased labelling of glucokinase within PFC and hippocampus correlating with increased glucose-6-phosphate dehydrogenase and decreased lactate dehydrogenase expressions. These relates to inhibition of over-expressed acetylcholinesterase and increased total protein synthesis. Histological and Nissl staining of thin sections corroborated roles of CVS in reversing $AlCl_3$-induced neuropathology. Summarily, we showed the role of CVS in normalizing important neurochemical molecules linking concurrent progression of oxidative stress, bioenergetics deficits, synaptic dysfunction and cellular hypertrophy during neurodegeneration.

1. Introduction

The polygenic nature of neurodegenerative diseases has hindered comprehensive view of molecular players involved in its progression, and the development of effective therapeutic strategies for treating or managing the devastating disorders. Neural levels of metal ions needs to be tightly regulated for proper mediation of key brain functions like neurotransmission, energy metabolism, mitochondria dynamics, calcium homeostasis, vesicular trafficking, endogenous antioxidant defense as well as myelination (Lu et al., 2015; O'Rourke et al., 2014; Ximenes-da-Silva, 2016). Aberrant metal homeostasis in key brain areas and plasma have been observed in patients with neurodegenerative diseases, and is thought to enhance production of reactive oxygen species (ROS) and amyloid beta (Aβ) oligomer that results into increased extracellular plaques accumulation and intracellular NFT in Alzheimer's disease (AD) (Maynard et al., 2005), as well as selective loss of synapses and neurons in hippocampal and cerebral regions (Kanaan et al., 2013). Aβ aggregation is mediated by interaction with metals including aluminum (Al), zinc (Zn), copper (Cu) and iron (Fe), while Aβ catalysed reduction of these ions produce toxic ROS that attack cells in the absence of sufficient antioxidant mechanisms (Barnham and Bush, 2014). Also, impaired brain metal homeostasis may be initiated by abnormal metabolism of amyloid precursor protein (APP) and Aβ, as part of AD pathogenic process (Greenough et al., 2013).

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https://doi.org/10.1016/j.etap.2018.07.005
Received 29 March 2018; Received in revised form 3 July 2018; Accepted 5 July 2018
Available online 07 July 2018

Al has similar characteristics to Fe and binds to Fe-binding proteins such as ferritin (Fr) and transferrin (Tf), and can stimulate Fe-induced membrane lipid peroxidation that has been extensively shown to cause oxidative damage in the progression of neurodegeneration. Tf is the major Al binding fraction of plasma and the primary Fe transport protein in humans, and the protein has almost equal binding affinity for both metal ions (El Hage Chahine et al., 2012). The brain possesses high densities of transferrin receptors (TfR) (Moos and Morgan, 2000), localized to neurons of key brain areas such as hippocampus and entorhinal cortex (Moos et al., 2007), and to the vascular endothelium (Jefferies et al., 1984). Interestingly, altered hippocampal distribution of TfR has been documented in human post-mortem AD brain (Lehmann et al., 2012; Morris et al., 1994), suggesting a role for the receptor in the progression of the disease. Brain ion homeostasis is increasingly recognized as a potential target for the development of drug therapies for aging-related disorders (Chiang and Koo, 2014; Duce and Bush, 2010). Neuronal uptake of both Fe and Al is mainly controlled by transferrin receptor-1 (TfR-1) or by divalent metal transporter-1. Surprisingly, there has been no report on TfR-1 modulation as a potential therapy for halting or reversing neurodegenerative processes.

Several classes of vitamins are documented to exert pleiotropic effects on neural activities and are implicated in the progression of different neurodegenerative disorders. For instance, deficiency of some B-vitamins including thiamine (B1), riboflavin (B2), niacin (B3) pyridoxine (B6), folate (B9), and cobalamin (B12) are widely believed to play some roles in age-related cognitive decline. There are existing evidences that the memory decline, cognitive/psychiatric disturbances, spinal cord degeneration and peripheral neuropathy associated with B-vitamins deficiency can be resolved through dietary supplementation of the deficient B-vitamins (Clarke, 2008; Kifle et al., 2009; Morris et al., 2006; Smith, 2006). Also, we recently showed the efficacy of vitamin C (ascorbic acid; AA)-a natural organic compound with strong antioxidant properties in reversing cognitive decline and neurochemical alterations in rats (Olajide et al., 2017).

While there are increasing evidences on the beneficial potentials of vitamins supplementation in halting or slowing down propagation of molecular neurodegeneration, the exact molecular interactions involved in the process is still unclear, which is necessary for evolvement of target-based therapeutic applications. Furthermore, most of the available reports are merely observational, with conclusions drawn from very limited experimental evidences. Relationships between ion-induced neurodegeneration and neuroprotective mechanisms of vitamins have been observed, especially in the context of Fe dyshomeostasis (Arber et al., 2016; Chen et al., 2003; White et al., 2015). However, little is known about the specific mechanisms through which vitamins counteract the harmful effects of ion-induced neurodegeneration. Here we explored the novelty of a formulated vitamin complex consisting of B-vitamins plus AA in preventing/reversing behavioral, neurochemical and neuropathological alterations, while examining the role of TfR-1 modulation as show by the conceptual framework in Fig. 1.

2. Experimental procedure

2.1. Animal care and handling

Wistar rats (eight-weeks old) were held under standard laboratory conditions at the College of Health Sciences, University of Ilorin, Nigeria, where they had liberal access to rat chow and water. Ethical clearance was sought and obtained from the College of Health Sciences Ethical Committee, University of Ilorin. Animal handling protocol was carried out in strict compliance to the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

2.2. Preparation of drugs and treatment solutions

Complex vitamin supplement (CVS) was formulated by Puritan's Pride, Inc. (New York; USA) and primarily consisted of B-complex vitamins and AA. Each gram of CVS consisted of: 5 mg thiamine (thiamine mononitrate, Vitamin B-1); 5 mg riboflavin (Vitamin B-2); 25 mg niacin (niacinamide); 5 mg pyridoxine hydrochloride (Vitamin B-6); 400 mcg folic acid; 250 mcg cyanocobalamin (Vitamin B-12); 300 mcg biotin; 10 mg panthothenic acid (d-calcium panthothenate) and 120 mg ascorbic acid (vitamin c). Powdered CVS was dissolved in distilled water (40 mg/ml) and adjusted to 7.4 pH. Crystalline salt of aluminum chloride $AlCl_3$ (Sigma-Aldrich, USA) was dissolved in distilled water (20 mg/ml) and adjusted to pH 7.4. Both solutions were freshly prepared each morning of administration and stored at 4 °C before each use.

2.3. Animal grouping and treatments

A total of 40 male rats (weight = 185 ± 3 g) were randomly assigned into 5 groups (A – E: n = 8) and treated orally as follows: A – PBS (1 ml daily for 15 days); B – CVS (400 mg/kg daily for 15 days); C – $AlCl_3$ (100 mg/kg daily for 15 days); D – $AlCl_3$ then CVS (100 mg/kg $AlCl_3$ daily for 15 days followed by 400 mg/kg CVS daily for subsequent 15 days); E – CVS then $AlCl_3$ (400 mg/kg CVS daily for 15 days followed by 100 mg/kg $AlCl_3$ daily for subsequent 15 days).

2.4. Behavioral studies

2.4.1. Assessment of rats in the Y-maze

The Y-maze test was used to examine working and cognitive memory in rats following treatments. The procedure was performed in a fine-wooden apparatus with 3 arms, each measuring 75 cm in length, 15 cm in breadth and 10 cm in height. The angle between each arms measured 120°. After the last treatment (24 h), rats were placed at the center of the maze and allowed to explore freely for 5 min each. The sequence of arms movements was recorded using a video camera. Subsequently, the course of multiple arm entries and the tendency of rats to enter a less recently visited arm were analyzed. The number of arm entries and the number of triad movements were then evaluated in order to calculate the percentage of alternation. An entry occurs when all four limbs are within the arm. Spontaneous alternation behavior was calculated based on the following equation: 100 × (number of alternations/total arm entries – 2).

2.4.2. Morris water maze (MWM) test for learning and memory

This test was carried out to assess spatial learning and memory of rats in the different treatment groups. The procedure was adopted in accordance with the comprehensive description by (Vorhees and Williams, 2006). Briefly: the water maze was a pool of water measuring 100 cm in diameter and 30 cm in depth and kept at room temperature. An escape platform (made from plexiglass) which is about an inch deep from the surface of the water was placed in one of the quadrants. Rats were trained prior to the actual test with the aid of visual cues placed close to the escape platform. During the training, each rat was placed in each of the other three quadrants for a maximum of 60 s to find the escape platform at an interval of 15 min between quadrants. In the early stages, mice having difficulty in locating escape platform after 60 s were aided to the platform until escape latency period reduced to less than 15 s. During the actual test, the pool was colored and rats were placed in each of the three quadrants different from the escape platform quadrant at an interval of 15 min between quadrants. The time taken to find the escape platform was recorded as the escape latency period.

2.4.3. Testing of anxiety in the elevated plus maze (EPM)

Anxiety levels of rats were evaluated across treatment groups using this method. The rats were introduced into an elevated plus apparatus