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Ascorbic acid ameliorates behavioural deficits and neuropathological alterations in rat model of Alzheimer's disease

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ABSTRACT

Exploring the links between neural pathobiology and behavioural deficits in Alzheimer's disease (AD), and investigating substances with known therapeutic advantages over subcellular mechanisms underlying these dysfunctions could advance the development of potent therapeutic molecules for AD treatment. Here we investigated the efficacy of ascorbic acid (AA) in reversing aluminium chloride ($AlCl_3$)-induced behavioural deficits and neurotoxic cascades within prefrontal cortex (PFC) and hippocampus of rats. A group of rats administered oral $AlCl_3$ (100 mg/kg) daily for 15 days showed degenerative changes characterised by significant weight loss, reduced exploratory/working memory, frontal-dependent motor deficits, cognitive decline, memory dysfunction and anxiety during behavioural assessments compared to control. Subsequent analysis showed that oxidative impairment-indicated by depleted superoxide dismutase and lipid peroxidation (related to glutathione-*S*-transferase activity), cholinergic deficits seen by increased neural acetylcholinesterase (AChE) expression and elevated lactate dehydrogenase underlie behavioural alterations. Furthermore, evidences of proteolysis were seen by reduced Nissl profiles in neuronal axons and dendrites which correspond to apoptotic changes observed in H&E staining of PFC and hippocampal sections. Interestingly, AA (100 mg/kg daily for 15 days) significantly attenuated behavioural deficits in rats through inhibition of molecular and cellular stressor proteins activated by $AlCl_3$. Our results showed that the primary mechanisms underlying AA therapeutic advantages relates closely with its abilities to scavenge free radicals, prevent membrane lipid peroxidation, modulate neuronal bioenergetics, act as AChE inhibitor and through its anti-proteolytic properties. These findings suggest that supplementing endogenous AA capacity through its pharmacological intake may inhibit progression of AD-related neurodegenerative processes and behavioural alterations.

1. Introduction

The multiple etiopathologic mechanisms involved in the initiation and progression of neurodegeneration underlying neurodegenerative diseases (NDD) have hampered comprehensive understanding of subcellular manifestations of the diseases and the development of effective therapy (Bedse et al., 2015). A common form of NDD is Alzheimer's disease (AD) that affects millions of people worldwide, with about 13% of the American population currently affected or at risk (Reitz et al., 2011). While figures of sufferers keep increasing, profound efforts are geared towards actualizing therapeutic molecules that can improve behavioural decline by ameliorating/palliating the parallel neuropathological changes seen in key brain areas (Chen and Zhong, 2013; Exley et al., 2006; Kim et al., 2013). However, only few of such discoveries sparingly reduce behavioural deficits in AD patients, while the progression of the disease at the microstructural levels strongly persists. Primarily, the pathological hallmark of AD includes increased deposition of extracellular amyloid plagues and intracellular neurofibrillary tangles (NFTs) in selective brain regions, with both responsible for neuronal loss and inflammation (Nisbet et al., 2015). However, several studies have shown that aggregated Amyloid-β proteins (Aβ) and hyperphosphorylated Tau filaments seen in AD brains are preceded by other very crucial subcellular neuropathological events (Giacobini and Gold, 2013; Šimić et al., 2016; Walter and van Echten-Deckert, 2013). Therefore, different molecular players promoting the proteolysis and aggregation of these proteins may

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be associated with the important molecular mechanisms responsible for pathogenesis of AD. Oxidative stress inducing metal ions like aluminium (Al) have been shown to substantially promote Aβ and NFTs accumulation which damages neurons through several downstream pathways (Puzzo et al., 2015). Once in the neuronal environment, Al cleaves to organelles and endomolecules within axons and synaptic terminals, disrupting physiological functions of crucial cellular ions, thereby interfering with phosphorylation and dephosphorylation of polypeptides (Cavallucci et al., 2013; Murakami and Yoshino, 2004). Through these processes, Al may interfere with mitochondria functions (Selfridge et al., 2013) and cause endoplasmic reticulum stress (Bermales et al., 2012), oxidative damage (Campbell, 2002), bioenergetics deficits, synaptic dysfunction (Tu et al., 2014), disruption of cell signalling pathways and neurotransmission (Kumar and Gill, 2009), abnormal neuronal gene expression and neuronal death (Manczak et al., 2011). Such degenerative cascades associated with Al-toxicity are widely known to underlie behavioural deficits similar to those manifested by AD sufferers.

The crucial roles played by vitamins in neural health and diseases have been increasingly debated. Vitamins are viewed as natural sources for treating neurological conditions, given that their deficiencies in humans (and other higher mammals) are known to initiate neurodegenerative conditions. For example deficiencies or lack of major vitamins components in the biological system have been linked with leading forms of NDD including AD and Huntington's diseases (Keeney and Butterfield, 2015; Moore et al., 2012; Warner et al., 2015). A critically important vitamin to normal brain development and function is Ascorbic acid (AA, Vitamin C), which is a natural organic compound with strong antioxidant properties. AA is commonly considered as the most important antioxidant in the body because it scavenges several kinds of free radicals. The review by Harrison, (2012) indicated that deficit or depletion in levels of AA occurs in about 30% of Westerners, most of whom are either aged or hospitalized. Harrison also stated that AA intake is critical to slowing the onset and progression of AD. Furthermore, it was shown that vitamin C and E supplements reduced the rate of cognitive decline in people who took them alongside a non-steroidal anti-inflammatory drug in a clinical trial (Fotuhi et al., 2008). However, despite documented evidences on beneficial potentials of AA against behavioural deficits caused by NDD, most of the available reports are merely observational, with conclusions drawn from very limited facts. It remains largely unclear if AA supplementation can halt or slow down molecular and histopathological processes that propagates behavioural decline in AD. Specifically, the therapeutic potentials of AA on corticohippocampal-dependent learning, cognitive and memory impairment, motor and exploratory imbalance and anxiety levels resulting from the combination of oxidative redox impairment, altered cholinergic neurotransmission, dysfunctional neuronal energy bioenergetics, reduction of ribosomal protein synthesis and cell death is unknown. Here we examined the involvement of these degenerative pathways in $AlCl_3$-induced AD and their implications on important behavioural paradigm. We further evaluated the corresponding therapeutic potentials/mechanisms of AA within the prefrontal cortex (PFC) and hippocampus of Wistar rats.

2. Materials and methods

2.1. Chemicals

Crystalline salts of $AlCl_3$ (Cat. No.: 563919) and AA (Cat. No.: 1043003) are products of Sigma-Aldrich (USA) sourced from Lab Trade Limited®, Ilorin, Nigeria. Phosphate buffered saline (PBS; pH 7.4) was freshly prepared. Assay kits for superoxide dismutase (SOD: Cat. No.: ab65354), acetylcholinesterase (AChE: Cat. No.: ab2803), lactate dehydrogenase (LDH: Cat. No.: ab102526) and glutathione-*S*-transferase (GST: Cat. No.: ab65326) were products of Abcam acquired through Bio legend Inc., San Diego, CA, USA. Other materials and reagents used were of analytical grade and were sourced within our laboratory.

2.2. Animal care and ethical approval

Wistar rats were procured from the Faculty of Veterinary Sciences, University of Ilorin and bred at the animal holding facility of the Faculty of Basic Medical Sciences, College of Medicine, 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, Nigeria. Animal handling and protocols were 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).

2.3. Animal grouping and treatments

Adult male rats (24, with average weight of 185 ± 3 g) were randomly assigned into 4 groups (A–D), each consisting of 6 rats (n = 6). The groups were treated orally as follows: PBS group (received 1 ml daily for 15 days); AA group (received 100 mg/kg of ascorbic acid daily for 15 days); $AlCl_3$ group (received 100 mg/kg aluminium chloride only daily for 15 days); $AlCl_3$ + AA group (received 100 mg/kg aluminium chloride daily for 15 days followed by 100 mg/kg ascorbic acid daily for the subsequent 15 days). In the $AlCl_3$ + AA group, AA daily treatment commenced 24 h after the 15th day of $AlCl_3$ administration. Treatment doses adopted in this study were as reported by; $AlCl_3$ (Mathiyazahan et al., 2015) and AA (Tutkun et al., 2015). Summary of experimental procedure is illustrated in Fig. 1. Rats were weighed twice a week (72- h intervals), beginning from the first day of administration using a digital weighing balance. Percentage weight change for each rat was obtained by subtracting the initial weight (day 1 of administration) from final weight (24 h after last administration) and converted to percentage. The average weight change in each group was then determined and compared across groups.

2.4. Preparation of other treatment solutions

$AlCl_3$ solution was prepared by dissolving the crystalline salt in distilled water (20 mg/ml) and adjusted to pH 7.4 with 0.1 M PBS. Also, AA was prepared in distilled water (40 mg/ml) and adjusted to pH 7.4 with 0.1 M PBS. Both solutions were freshly prepared each morning of administration and stored at 4 °C before administration. Oral administration of treatment solutions (PBS, $AlCl_3$ and AA) to rats across all groups was done using a modified oral cannula.

3. Behavioural studies

3.1. Morris water maze (MWM)

This test was carried out to assess spatial learning and memory of rats in the different treatment groups. The procedure was done 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 which is about an inch deep from the surface of the water was placed in one of the quadrants, outside of which were visual cues. Rats were trained 24 h prior to the actual test. 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 until the escape