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(RESEARCH ARTICLE)

Morphological brain changes in Alzheimer's-like disease in rats

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Abstract

Heavy metals are reported as neurodegenerative disorders progenitor. They play a role in the abnormal amyloid beta 40 (Aβ40) protein concentrations, the main hallmarks of Alzheimer's disease (AD). The aim of the present work is to study the morphological ultrastructural changes by transmission electron microscopy and measurements of Aluminium and Aβ40 protein concentration in rats' hippocampus due to Alzheimer's-like disease in rats. Fifty male Albino Wistar rats were divided into five groups; the first group acted as the control group, while the other four groups each received 100 mg/kg of aluminium chloride ($AlCl_3$) over the course of 2, 4, 6, and 8 weeks. The results indicated significant increase ($p<0.05$) of alumininm and significant decrease ($p<0.05$) of Aβ40 in the hippocampus of rats due to $AlCl_3$ administration starting from 2 and 6 weeks, respectivily. Histological ultrastructural hippocampus changes were observed starting from 2 weeks of $AlCl_3$ administration to reach the most damaged structure after 8 weeks, where its nucleus appeared shrunken with a condensed chromatin beside marked loss of the cytoplasmic organelles including mitochondria, rER, and Golgi apparatus. Also, vacuolation was clearly observed at the remnants of cytoplasm. According to the results of the current investigation, there is a significant link between multiple exposure to environmental heavy metals and the pathophysiology of AD by altering Aβ 40, which causes neuronal death, as well as histological and ultrastructural changes in the rat hippocampus.

Keywords: Alzheimer; Brain; Hippocampus; Rats; Amyloid beta; Electron microscopy

1. Introduction

The most common type of dementia, Alzheimer's disease (AD), is a progressive, complex neurological condition. By 2050, there will be 152.8 million individuals worldwide living with Alzheimer's disease and other dementias, up from the current estimate of 55 million [1].

Neurofibrillary tangles (NFT) inside cells and amyloid beta (Aβ) plaques outside cells are two hallmarks of Alzheimer's disease (AD). Microglia is triggered by Aβ plaques, increasing oxidative stress and the local inflammatory response, all of which are involved in neurotoxicity.

A deficiency of acetylcholine (Ach), a neurotransmitter implicated in memory, resulted from neuronal cell death. Direct neuronal injury is brought on by active oxygen, which raises intracellular Ca^{2+} and inflammation. Brain-derived neurotrophic factor (BDNF) is inhibited by the cytokines produced as a result of inflammatory responses [2].

One of the brain regions most vulnerable to the onset of early Alzheimer's disease (AD) and other neurodegenerative illnesses is the hippocampus, which is crucial for some elements of learning and memory [3].

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In large quantities, aluminium metal is found in the crust of the earth. Through the digestive and respiratory tracts, it enters the human body from the environment. Cooking utensils and medications like antacids, deodorants, and food additives all contain aluminium, which has made it easier for aluminium to enter the body [4]. Aluminium has been linked to neurological illnesses and aging-related alterations [5,6]. Long-term exposure to aluminium has been demonstrated in animal models to lead to biochemical changes similar to AD as well as neurological symptoms that resemble advanced neurodegeneration, including changes in the cerebral cortex, hippocampus, brain stem, and spinal cord [7]. In addition to causing oxidative stress in the brain, Aluminium may considerably raise the levels of malondialdehydes (MDA) and the activity of acetylcholinesterase (AChE) in the hippocampus [8]. Aluminium interacts with calcium binding sites, which are critical in neurodegeneration, to disturb calcium homeostasis as well [9].

According to Kaur et al., 2003[10], aluminium salts induce oxidative stress-related alterations such as elevated lipid peroxidation, elevated 4-HNE forms, and elevated lipofuscin accumulation. Accordingly, the most widely used animal model that imitates human AD is aluminium chloride ($AlCl_3$)-induced Alzheimer's disease in rats [11, 12]. $AlCl_3$ was utilised in several investigations at a dose of 100 mg/kg b.w. for 6 weeks, which was thought to be sufficient to hasten neurodegeneration in animal models [13-15].

The purpose of the current work is to investigate the morphological ultrastructural alterations caused by $AlCl_3$ administration for 2, 4, 6, and 8 weeks using transmission electron microscopy in concordance with measurements of Aluminium and Aβ40 protein concentration in rats' hippocampus.

2. Material and methods

2.1. Chemicals

Our tests used only chemicals purchased from Sigma- Aldrich Company (St. Louis, MO, USA). The rat Aβ 40 Elisa kits was purchased from Novus Biologicals.

2.2. Animals

For the experiment, 50 male albino Wistar rats weighing of 200±50 grames were used. The animals came from the Research Institute of Ophthalmology (RIO), Giza, Egypt's animal house facility. The Association for Research in Vision and Ophthalmology (ARVO) guidelines, local research committee recommendations, and ARRIVE standards were fully adhered to. Animals were kept with free access to water and chewing food at all times, and regulated standard ambient conditions of humidity (60±10 %), room temperature (25±2 °C), and natural day/night cycle (12:12 h).

2.3. Experimental design

Following the acclimatisation period, the animals were divided into 5 groups at random, with 10 rats placed in each group. Rats in the first group, which served as the animal's control, were given saline via gavage tube. While rats in the other 4 groups got $AlCl_3$ (100 mg/kg body weight) orally using a gavage tube over the course of 2, 4, 6, and 8 consecutive weeks, respectively, [16].

2.4. Estimation of Al Concentration

To estimate the aluminum level in the hippocampus region of the brain, a modified wet acid digestion method [17]. A mixture of concentrated nitric acid (69 %) and hydrogen peroxide (30 %) was added to the weighted samples of hippocampal tissues and let it sit overnight. The following day, we applied gentle heat to the mixture using a hot-plate until the solution color become transparent. Finally, the digests solutions were filtered. the Al levels were determined using Microwave Plasma Atomic Emission Spectroscopy (Agilent 4100 MP-AES) at wavelength 396.152 nm. The total Al concentration in hippocampal tissues was assessed in µg/gm of tissue.

2.5. Quantification of $A\beta_{40}$ protein

To evaluate the Aβ40 levels in brain tissues, the hippocampus from each experimental group were homogenized in RIPA lysis puffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.5% sodium deoxycholate, 0.1% SDS, 0.5% Triton X-100 and 0.5% NP 40) with Cocktail protease inhibitor. Then all homogenized samples were centrifuged at 14,000×g for 30 min at 4 °C. The resultant supernatant was separated and subjected to assay for the protein concentration using bicinchoninic acid (QuantiProTM BCA, Cat No: QPBCA, Sigma-Aldrich, USA) assay kit.

The status of AD biomarker Aβ40 and hippocampus of normal rats and rats orally administrated with AlCl3 for 2, 4, 6 and 8 weeks, were investigated using the research rat-specific ELISA kits (Cat No: NBP2-69916, Novusbio, USA) uses

the Sandwich-ELISA principle. The ordered instructions demonstrated by the manufacturer's manual were followed for the micro-plate preparation before reading. The absorbance of the optical density was determined with Elisa reader set to 450 nm. The concentrations of Aβ40 in hippocampus were expressed in pg/mg protein.

2.6. Hippocampus ultrastructure (Transmission electron microscopy)

TEM was employed to confirm the structural changes in hippocampus neurons caused by neurodegeneration induced by $AlCl_3$. The hippocampal tissues were quickly removed from the brains of decapitated rats on a cold plate. Segments of the hippocampus with size 1 mm^3 were immediately fixed in an EM grade primary fixative, glutaraldehyde (2.5 %) and phosphate buffer (pH 7.4) at 4 °C overnight. After that, the samples underwent three rounds of rinsing with phosphate buffer for 10 minutes each time. Subsequently, they were post-fixed at a temperature of 4°C for 2 hours in a solution consisting of 1 % osmium tetroxide. Next, all the fixed samples went through dehydration by means of an ethanol series with progressively increasing concentrations (30-100 %). Afterward, propylene oxide was used to clear the specimens before embedding them into an epoxy resin. Subsequent to embedding, ultra-thin sections (70 nm) were obtained and affixed onto copper grids. To enhance visibility under an electron microscope (JEOL JEM 1200EX II – Japan), these grids received additional contrast staining utilizing uranyl acetate and lead citrate.

2.7. Statistical analysis

All data was expressed as means ± Standard diviation. The statistical differences between groups were evaluated by one-way or two-way analysis of variance (ANOVA) using Graph Pad Prism 8.4 software (GraphPad, La Jolla, CA, United States). Student´s t-test was applied. Data were considered significant when $p < 0.05$.

3. Results

Based on the analysis conducted, it was found that there was a notable increase in aluminum concentration in the hippocampus of rats subjected to $AlCl_3$ administration compared to normal levels. The results depicted in Figure (1) clearly indicate that the Al content in the hippocampus of rats exposed to Al for 2, 4, 6 and 8 weeks exhibited a significant elevation when compared with healthy rats (0.346 ± 0.014 µg/gm of tissue), with incremental changes observed at approximately 18%,101%,179% and 237% respectively. Furthermore, our findings revealed a progressive increase in hippocampal aluminum accumulation as exposure duration extended. This suggests that prolonged exposure to aluminum may lead to an amplified uptake and retention within this particular brain region.

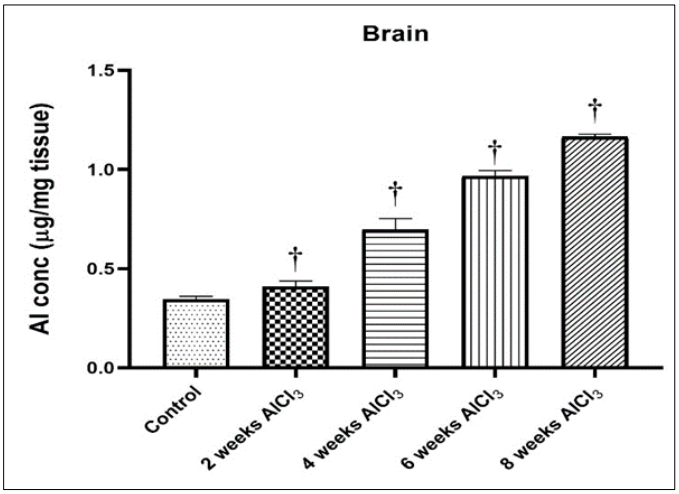

Figure 1 Concentration of Al in hippocampus. Values are expressed as mean ± S.D. (†) Level of significance ($p<0.05$) in comparison to control group

As depicted in Figure (2), extended exposure to $AlCl_3$ reduces the levels of $A\beta_{40}$ in the rat brain. The results showed that the brain concentration of $A\beta_{40}$ in the healthy control animals was 40.610 ± 3.653 pg/mg protein. The average brain content of $A\beta_{40}$ showed a significant reduction ($p<0.05$) in the brain tissues of rats subjected to Al for 6 and 8 weeks to reach 28% and 70% lower than normal levels respectively. However, no discrepancies were detected between the Aβ 40 brain content of groups exposed to Al for either 2 or 4 weeks and that of the control group.

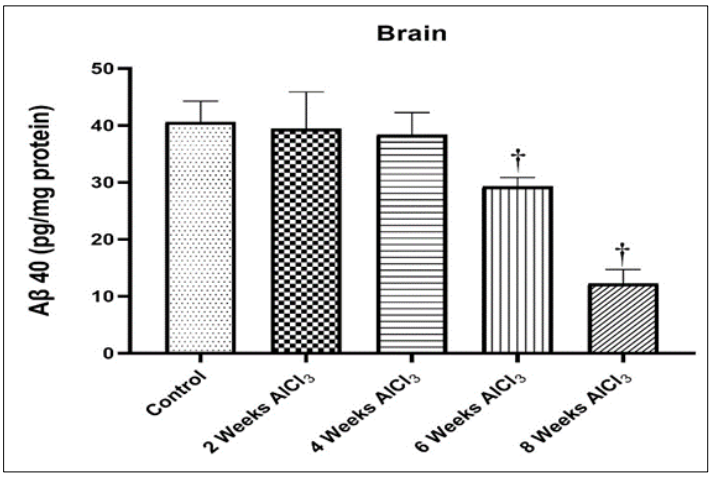

Figure 2 Concentration of $A\beta_{40}$ in hippocampus. Values are expressed as mean ± S.D. (†) Level of significance ($p<0.05$)

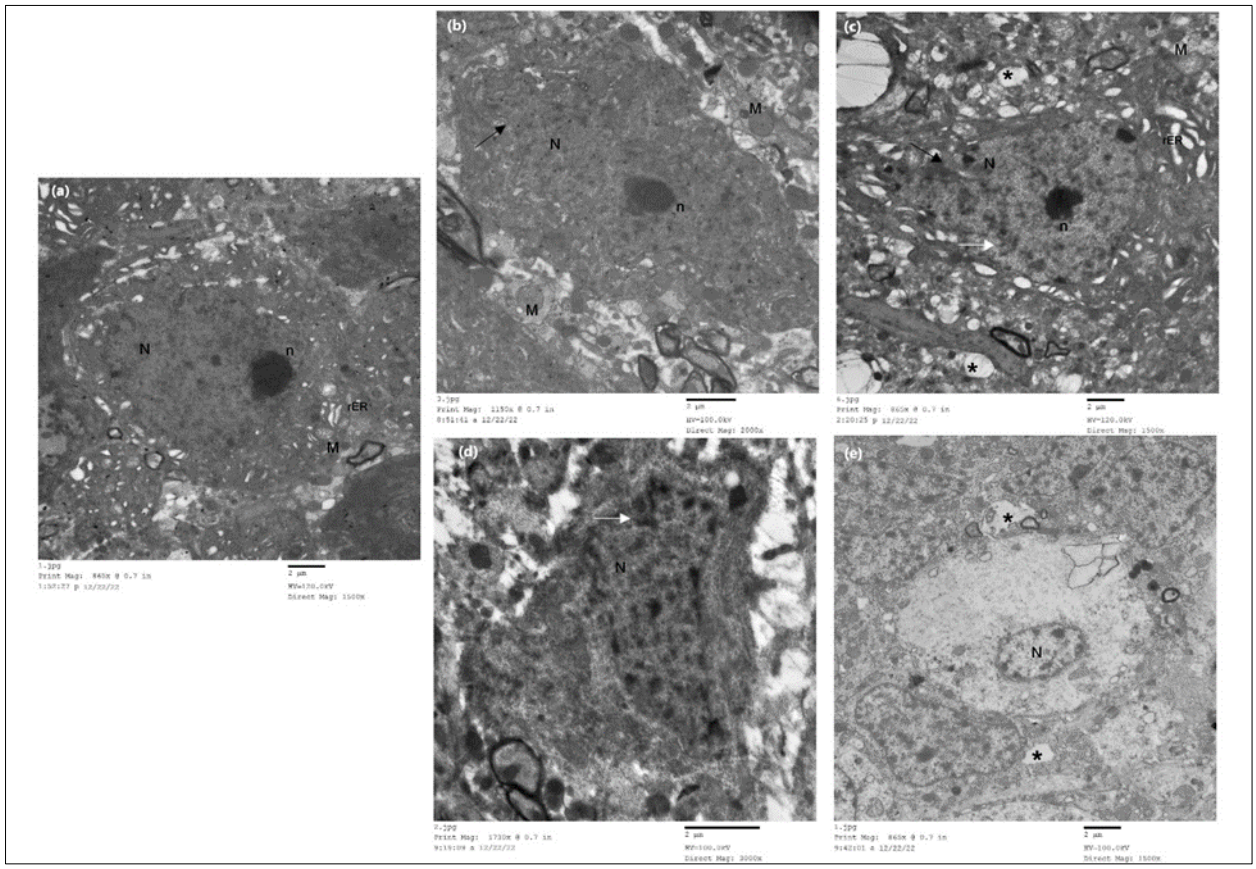

Figure 3 Transmission electron micrographs of the hippocampal sections. (a) Control group; showing a normal neuron possessing large oval nuclei (N) with presence of evenly distributed chromatin, nucleoli (n) and bounded by intact nuclear membrane. The surrounded cytoplasm contains numerus normal mitochondria (M) and rough endoplasmic reticulum (rER). (b) 2 weeks Al group; showing a near normal neuron with mild changes demonstrated with irregular shape of nuclear membrane (black arrow) and swollen mitochondria (M). (c) 4 weeks Al group; showing a nucleus with wrinkled nuclear membrane (black arrow), condensed chromatin (white arrow), electron dense nucleoli (n). While, the cytoplasm exhibited vacuolation, swollen mitochondria (M) and rER dilatation. (d) 6 weeks Al group; showing pyknotic neuron with deformed nucleus, condensed chromatin (white arrow), nucleolus disappearance and loss of cytoplasmic organelles. (e) 8 weeks Al group; showing degenerated neuron with shrunken nucleus (N) and totally loss of the cytoplasmic organelles as well as increase the cytoplasmic vacuolation (*)

Figure (3) revealed the control group (panel a) , the ultrastructure examination of hippocampal neuron revealed the ordinary fine structure of neuron with oval large nucleus contained evenly distributed chromatin, prominent nucleolus and smooth karyotheca, and its cytoplasm contains abundant organelles such as; mitochondria, endoplasmic reticulum and Golgi apparatus. After 2 weeks of Al administration, the ultrastructure examination of hippocampal neuron for group 2, shown in fig (3) panel b, demonstrated slight morphological changes recognized by the irregular shape of cell membrane, deformed nuclear structure and altered cytoplasmic organelles characterized by swollen mitochondria and cytoplasmic vacuolation. The ultrastructure examination of hippocampal neuron after 4 weeks of Al administration (fig. 3 panel c) showed marked changes in the nucleus including; wrinkled nuclear membrane, condensed chromatin and electron dense nucleoli. On the other hand, the number of vacuoles has been increased in the cytoplasm with the dilatation of rER as well. The ultrastructure examination displayed the prominent deterioration of hippocampal neuron after 6 weeks of Al administration (fig. 3 panel d). The neuron exhibited pyknosis with shrinkage nucleus, condensed chromatin and nucleolus disappearance. Noteworthy, the cytoplasm is characterized by the presence of granules, loss of organelles and mitochondria degeneration. Significant degenerative effect of Al administration was confirmed at group 5 that administrated AlCl3 for 8 weeks (fig. 3 panel e), the ultrastructure examination of hippocampal neuron revealed the damaged structure, where its nucleus appeared shrunken with a condensed chromatin beside marked loss of the cytoplasmic organelles including mitochondria, rER, and Golgi apparatus. Also, vacuolation was clearly observed at the remnants of cytoplasm.

4. Discussion

The brain is recognised to be the organ most vulnerable to Al's harmful effects, and it is particularly prone to oxidative stress caused by increased amounts of free radicals and decreased antioxidant levels [18]. The present study focused on the hippocampus for a variety of reasons. First, compared to any other region of the central nervous system, the hippocampus and neocortex are most significantly impacted by aluminium. Second, this area of the brain, which is crucial for learning and memory processes, is known to be particularly vulnerable to Alzheimer's disease. Because its pyramidal neurons may be more susceptible to aluminum-induced neurotoxicity, the hippocampus was chosen for microscopic research [19]. The present study aimed to evaluate the morphological ultrastructural changes by transmission electron microscopy in consistence with measurements of Aluminium and Aβ40 protein concentration in rats' hippocampus due to administration of AlCl3 for 2,4,6 and 8 weeks.

The results indicated significant increase ($p<0.05$) of Al concentration due to Administration of $AlCl_3$ in hippocampus of rats and this finding illustrates how Al builds up in the hippocampus during the Administration of $AlCl_3$. It stands to reason that excessive Al buildup in the hippocampus could cause memory loss, which is a hallmark of neurodegenerative disorders for which Al has been linked. In previous studies, the hippocampus showed the greatest accumulation in Wistar male rats exposed to aluminium chronically [20] and subacutely [21]. In comparison to control rats, there was a nearly 24-fold rise in aluminium accumulation in the hippocampus after chronic aluminium exposure and an 80-fold rise after subacute exposure.

Aluminium administration is regarded as a brain ROS-induced neurotoxin inducer. Aluminium buildup causes iron-induced oxidative stress in the central nervous system, which in turn stimulates amyloidogenic fragments, NFTs, and neuroinflammatory cytokines [22]. Nitric oxide and superoxide anion spontaneously combine to form peroxynitrite in activated microglia, which raises the quantity of free radicals.

Additionally, according to Wang et al., 2016 [23], oxidative stress-induced astrocyte DNA damage may be a precursor to AD pathogenesis. Additionally, researches suggested that aluminium may contribute to the deposition of Ab peptides, which severely harms brain tissues [24, 25]. The levels of Aβ, which represent a balance between their generation and removal from the brain, are composed of soluble monomeric, oligomeric, protofibrillar, and fibrillar forms. At the beginning of the fourth week of $AlCl_3$ administration, the level of Aβ 40 in the brain decreased statistically significantly ($p< 0.05$). This discovery raises the possibility that $AlCl_3$ treatment may contribute to the critical step in the progression of AD due to Aβ 40 can also change Aβ 42's solubility, stability, and shape. According to Kim et al., 2007 [26], increased Aβ40 levels had a protective impact against amyloid pathology in the brains of Tg2576 mice.

Consistently, with the biochemical results, the hippocampus histological screening through $AlCl_3$ administration indicated severe hippocampal damage.

According to results that showing pyknotic neurons with malformed nuclei, Degenerative changes that are clearly visible also include the existence of electron-dense nucleoli, which are indicative of a particular type of apoptosis and are distinguished by impressively intense cytoplasm and nucleoplasm. This outcome was consistent with previous findings [27]. On the other hand, Carageorgiou et al., 2004 [28] found that the disruption of numerous proteins,

enzymes, nucleic acids, and neurotransmitter production was a common cause of these degeneration neurons. The overt neuronal cell toxicity that resulted in biochemical anomalies was thought to be the primary cause of mitochondrial and nuclear problems [29]. Lipid peroxidation may cause the rER to enlarge [30].

In addition to harming cell membranes and some organelle membranes, lipid peroxidation may also contribute to the cytoplasmic vacuolation seen in $AlCl_3$-treated rats. The pump's ability to extrude sodium is increased due to this damage and an increase in sodium permeability. The expansion of the cytoplasm is brought on by the accumulation of sodium in the cell, which raises the water content of the cytoplasm [31].

For an effective cellular energy production and cell survival, mitochondrial integrity was crucial. In the current work, $AlCl_3$-induced rats' vacuolation and enlarged mitochondria were seen on TEM pictures. Yan et al.,2015 [32] suggested that these observed modifications may put the stability of electron transport chain complexes in risk and have an impact on mitochondrial function. Mice exposed to blasts and displaying cognitive deficits also showed similar mitochondrial changes, according to Song et al., 2018 [33].

5. Conclusion

According to the results of the current investigation, there is a significant link between multiple exposures to environmental heavy metals and the pathophysiology of AD by altering Aβ 40, which causes neuronal death, as well as histological and ultrastructural changes in the rat hippocampus.

Compliance with ethical standards

Disclosure of conflict of interest

There is no conflict of interest

Statement of ethical approval

The rules of the Association for Research in Vision and Ophthalmology (ARVO), the recommendations of the local research committee of RIO, and ARRIVE guidelines were followed. The approval number from the local ethical committee is FWA 00031860.

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