

Short Communication

Docosahexaenoic Acid Prevents Aluminum Induced Neuronal Cell Death

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Abstract: In the present study, protective efficacy of docosahexaenoic acid (DHA) on Aluminum (Al) induced neuronal death were tested. This mode of administration induces caspase -3 and Bax activation along with increased rate of ROS production and release of LDH. Morphologically increased astrocytosis were observed. Coadministration of DHA inhibited caspase-3 and bax expression and abolish ROS. Furthermore, treatment with DHA dramatically inhibits apoptosis, as assessed by the transmission electron microscope as reduced rate of astrocytosis. Treatment with DHA may represent a therapeutic strategy to reverse the neuronal death associated with aluminum toxicity and may exert its effect on apoptosis-regulatory proteins.

Keywords: Aluminum; apoptosis; docosahexaenoic acid; astrocytosis

INTRODUCTION

Aluminum (Al) is the third most abundant element in the earth crust (8.3%). It can enter into the body via diet, drinking water, medicine, inhaled fumes and particles from occupational exposures. Also, it is well known to be involved in the etiology of several human pathologies such as Alzheimer type (AD) [1]. Al plays an important role in the degeneration of nerve cells. Cortical neurons in susceptible of the AD brain through ROS induced DNA damage, nuclear apoptotic bodies and chromatin condensation. Al leads to the alteration of the Bcl-2, Bax ratio in the brain (Ghribi et al., 2001). This alteration is an important indication in the development of extensive apoptosis. Apoptosis under mitochondrial control has been implicated in the neuronal death process [2] and involves the release of lactate dehydrogenase, cytochrome c and activation of caspase-9 and of caspase-3 (Ghribi et al., 2001) into the cytoplasm and initiation of the apoptosis cascade.

The docosahexaenoic acid (DHA, 22:6n-3) is an omega (n)-3 polyunsaturated fatty acid (PUFA) and it is a major component of membrane phospholipids in brain [3]. It has been taken on a central role as a target for therapeutic intervention in AD [4]. In view of the aforementioned considerations, the present study was designed to assess Al induced neuronal death and protective efficacy of docosahexaenoic acid was tested.

METHODOLOGY

Animals

Thirty male albino rats (weight 220 ±10 grams) were taken from NIMS University animal house. The animals were separately housed in polypropylene cages in a room, which was maintained

at a temperature of 22±2 °C, relative humidity of 50±10 % and 12h light dark cycles. They were fed a commercial pellet diet and allowed access to water ad libitum. The Institutional Animal Ethics Committee approved the study prior to the initiation of the experiment and also approved all experimental protocols.

Treatment

Animals were randomly divided into five groups (n = 6) viz. Group 1 served as control treated with normal saline, Group 2 treated with 100mg / kg body weight of aluminum chloride, group thired treated with only 100mg of DHA, Group four treated with 100 mg DHA along with Aluminum chloride and fifth group treated with 100 mg of vit E with aluminum chloride for 90 days. Dose was directly introduced into the rat pharynx via a feeding cannula.

Estimations of total ROS and LDH levels

The basal level of ROS was determined by the procedure of Montoliu et al., [5] using fluorescent dye dichlorofluorescein and measurements with a Hitachi 850 spectrofluorometer at 488 nm for excitation and 525 nm for emission wavelengths. Lactate dehydrogenase (LDH, EC 1.1.1.27) was assayed by the method of Cabaud and Wroblewski [6]. LDH catalyses the reduction of pyruvate to lactate and NAD⁺ by NADH and it is determined from the rate of reduction in NADH, measured spectrophotometrically at 340nm.

Protein extraction and Western blotting

Cerebral cortex suspended in 10 volumes of lysis buffer (pH 7.5), 50 mM sodium chloride and 1% Triton-X-100 containing phenylmethylsulfonyl fluoride

(1 mM) and protease inhibitor cocktail (a mixture of 4-(2-aminoethyl) benzenesulfonyl fluoride, pepstatinA, E-64, bestatin, leupeptin, and aprotinin (Sigma, St. Louis, MO) and kept on ice for 10 min. Tissues were then homogenized using a Teflon homogenizer and centrifuged at 12,000Xg for 15 min at 4°C, and the supernatant was collected. Protein concentration was determined in supernatant using Lowery method (1951). Thereafter, were subjected to SDS-PAGE and electrotransferred onto nitrocellulose membrane and incubated with either anti-caspase3 or anti-β actin (Cell signalling technology, MA USA) antibody followed by incubation with horseradish peroxidase conjugated secondary antibodies (Santa Cruz biotechnology, CA USA). The signals were detected using an enhanced chemiluminescence detection system. Relative expression of each protein was determined.

Electron Microscopic studies:

Ultrastructural changes in cerebral cortex were assessed as prescribed method [8] using Karnovsky’s fixative (0.1 M paraformaldehyde and glutaraldehyde solution in cacodylate buffer, pH 7.3). The samples were post fixed for three hours at 4°C in 1% osmium tetroxide prepared in 0.1 M cacodylate buffer and stained with 1% aqueous uranyl acetate overnight. Following dehydration, the specimens were embedded in Epon 812 at room temperature. Sections were cut on an LKB-Ultramicrotome with a glass knife. Thereafter, sections were mounted on 300 mesh copper grids, stained with 1% uranyl acetate and lead citrate and examined with a Phillips (FEI Tecnai 12 twin) Transmission Electron Microscope.

RESULTS

Biochemical studies:

The level of ROS was found to be significantly increased ($p < 0.001$) in AL treated groups as compared with the controls. The co administration of DHA and Vit E were significantly ($p < 0.01$) reduces ROS levels when compared with the Al treated rats (fig-1). The concentration LDH was markedly increased in AL treated rats when compared with the control while co administered with DHA and vitamin were reduces these changes as compared with the AL treated rats (fig-2).

Molecular studies:

The western blot analysis showed (fig-2A) increased capase-3 activation in Al treated rats and its relative density was found to be significantly ($p < 0.001$) elevated when compared the controls (fig-2B). While treatment with DHA and Vit E in Al treated rats exhibited reduction ($p < 0.01$) in density. The Bax were also showed similar pattern (fig-2C) to the caspase-3. It

is found to be activated significantly ($P < 0.001$) as compared with the controls and the co administration of DHA and Vit E reduces the activation of bax near to controls. On the other hand bcl-2 showed significant ($p < 0.05$) activation in DHA + Al treated rats when compared with the Al treated rats.

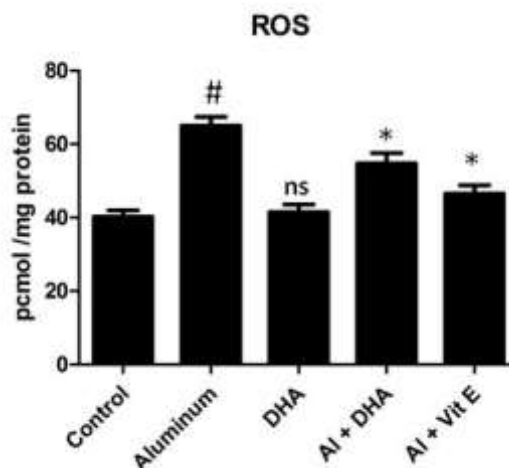


Figure- 1: Levels of reacting oxygen species (ROS) in cerebral cortex control and experimental group. The results are expressed as Mean ± SD in six rat of each group. Superscripts relate significant ($p < 0.05$) comparison with control vs aluminum (#) and aluminum vs DHA + Al and Vit E + Al.

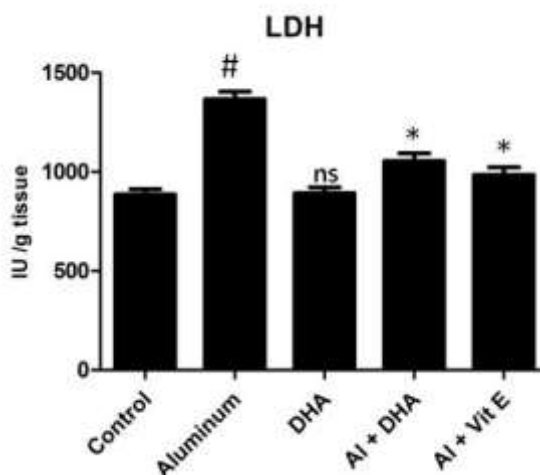


Figure- 2: Levels of lactate dehydrogenase in cerebral cortex control and experimental group. The results are expressed as Mean ± SD in six rat of each group. Superscripts relate significant ($p < 0.05$) comparison with control vs aluminum (#) and aluminum vs DHA + Al and Vit E + Al.

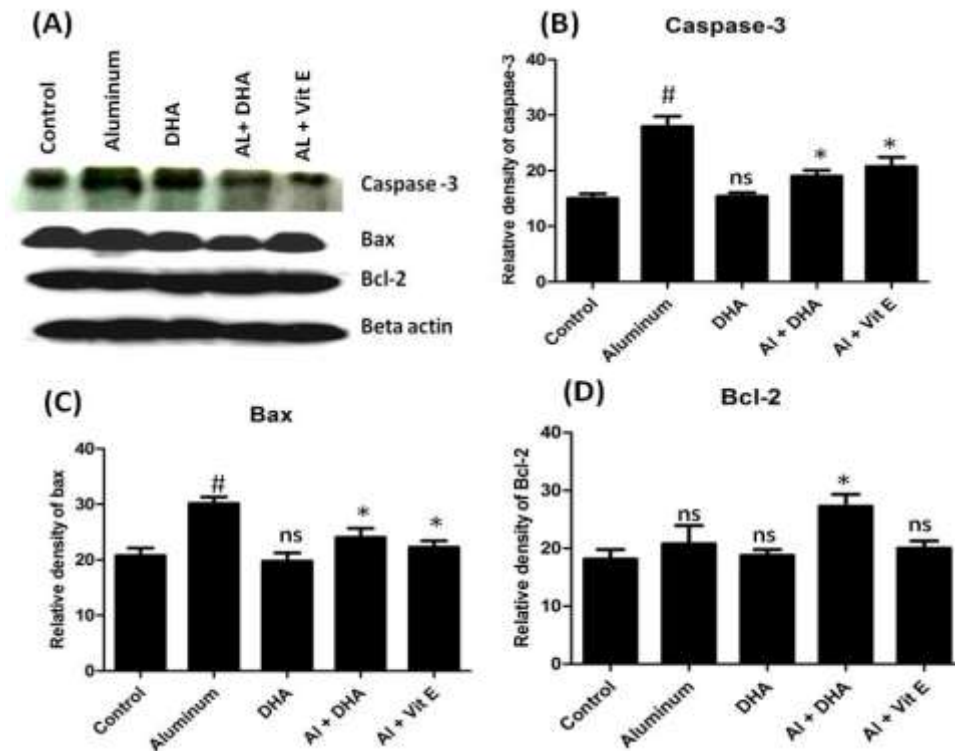


Figure- 3: western blot analysis of cell lysates of control and experimental group (A). Densitometry analysis of caspase-3 (B), Bax (C) and Bcl(D).The results are expressed as Mean \pm SD in six rat of each group. Superscripts relate significant ($p < 0.05$) comparison with control vs aluminum (#) and aluminum vs DHA + Al and Vit E + Al.

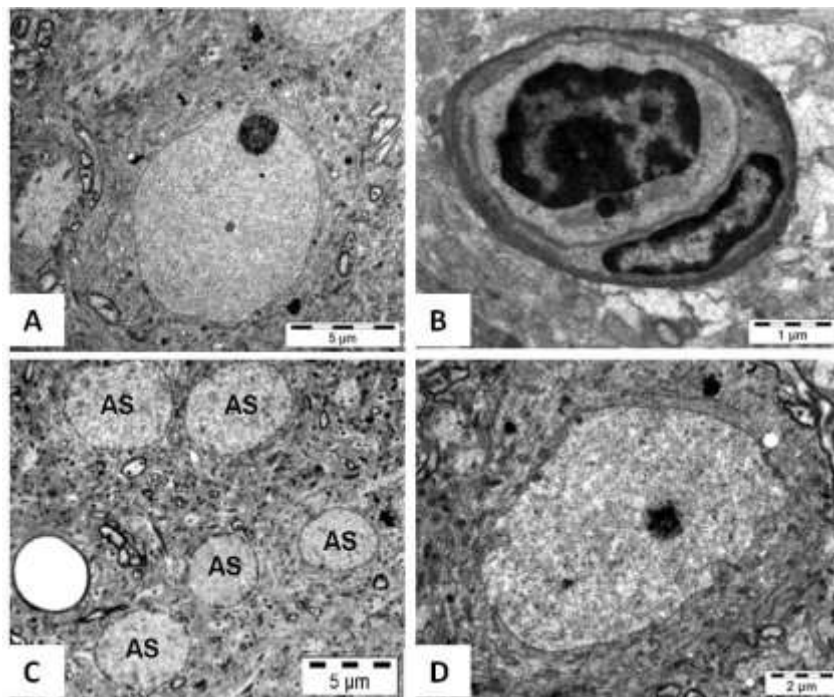


Fig-4. Electron micrograph is showing a peripheral segment of perikaryon of a control neuron (A). The clustered of dead neurons (B) and increased astrocytosis (C) in Al treated rats. Well architected neuron with nucleolus were seen in DHA co-administrated rats.

DISCUSSION

The Al radially access to the brain by the blood-brain barrier [9]. Al has been shown to induce the production of reactive oxygen species [10] and

oxidative stress. Aluminum (Al) neurotoxicity is well established but its mechanism of action is poorly understood. We reported here Al increases oxidative burden in terms of increased production of ROS. This

may be due to the altered axonal mitochondrial turnover, disruption of the cellular organelles i.e. golgi, or reduction of synaptic vesicles induced by Al treatment. This may be due to the result in the release of oxidative products like malondialdehyde and protein carbonyl content and due to decreased activity of antioxidant enzymes within the neurons [11]. We also observed that alteration in LDH. It is suggested that, elevated level of LDH the potent marker of cell death. It may be due to increased production of ROS. Apoptosis is one of the mechanisms contributing to neuronal loss. It is evidenced by DNA damage, nuclear apoptotic bodies and chromatin condensation. Bax is a pro-apoptotic protein present in the cytosol. The getting apoptotic stimuli translocated to the mitochondrial membrane and release of many apoptotic proteins bound to membrane [12]. In the present study we observed increased expression of Bax levels in Al treated rats as compared to control ones (Figure 2A). This result indicates the increased translocation of Bax to mitochondria and mitochondrial mediated cell death. While, Bcl-2 is an anti-apoptotic protein bound to the mitochondria and inhibit the release of apoptotic protein bound to mitochondrial membrane [13]. Mitochondrial mediated apoptosis depends on the Bax/Bcl-2 ratio. Increase in the Bax/Bcl-2 ratio diverts the cell towards apoptosis. In our study there significant changes were noted in antiapoptotic bcl-2 activation (Figure 2D).

The electron microscope stands out as an invaluable tool for the resolution of the ultrastructural details of neuronal cell death. The pathogenesis of cell death induced by Al indicated that a number of alterations occur in neurons that share certain common characteristics. In the present study there is two distinct characteristics were noted one is the condensation of dead neurons (fig 4B) and increased astrocytosis (4C). Cell death leading to cell loss in the aluminum treated old rats was also apparent by the proliferation of astrocytes seen in these cases as many as five astrocytes were found in one field. It is suggested that, neuronal loss substantiated by the astrocytosis. These results indicate that proliferations indicate that the spaces created by the loss of neurons are occupied by the proliferating astrocytes in the CNS.

Effect of DHA: In the present study, we observed significant changes in DHA and vit E co-administration. DHA reduces ROS levels and LDH. It may be due to the chemical property of DHA that ameliorating free radicals and regeneration of membrane. DHA also regulates the expression of apoptotic protein. It is suggested that DHA supplementation enhances the lipid modulation and prevent cell death.

CONCLUSION

On the basis of results it may conclude that the treatment with DHA may represent a therapeutic strategy to reverse the neuronal death associated with

aluminum toxicity and may exert its effect on apoptosis-regulatory proteins.

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