*Research Journal of Pharmaceutical Sciences*_____ ISSN 2319 – 555X
Vol. **2(3),** 6-11, March **(2013)** *Res. J. Pharmaceutical Sci.*

Effect of Aluminum on different parts of Brainstem of Old Rats: Haematological, Biochemical and Morphological Study

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Available online at: www.isca.in
Received 8th February 2013, revised 19th February 2013, accepted 2nd March 2013

Abstract

Aluminum (Al) has identified one of the environmental factors that may cause several neurodegenerative disorders. Generally, uptake of aluminum is greater the older a person becomes. We have orally administered 100 mg $AlCl_3$ (by gavage) to +24 m male rats (n=10) for 90 days. The same number of age and sex matched rats were concurrently administered equal volume of physiological saline. Hematological and serum lipid profile were investigated on 45 and 90 days of Al exposure. Medulla, pons and mid brain were selected for biochemical investigations ie., lipid peroxide levels (LPx), superoxide dismutase (SOD), catalase and glutathione (GSH). Results showed that $AlCl_3$ increases LPO level while the activity of antioxidant enzymes and glutathione was decreased significantly in all the part of brain stem along with decreased activity of antioxidant enzyme. On the basis of results it may conclude that hematology and lipid profiles may be the markers of Al neurotoxicity and mid brain and medulla is more affected area of the brainstem.

Keywords: Aluminum, aging, brain, rats.

Introduction

Metals are of special concern because their high environmental concentrations, which may be harmful for the general population. Among toxic environmental metals[1,2], aluminum (Al) has a remarkable toxic potential for humans. Al is ubiquitous; the third most common element of the earth's crust. It is naturally released to the environment from the weathering of rocks and volcanic activity. In general, background levels of Al in the atmosphere ranging from about 0.005 to 0.18 μg/m^3. Its concentration in soils varies widely, ranging from about 7 to over 100 g/kg weight[3].

Al salts are used as coagulants to purify municipal water that is drawn from lakes or reservoirs. Aluminum compounds are also found in some antacids, food additives, and antiperspirants. It is reported that a high concentration of aluminum in drinking water may be a risk factor for Alzheimer's disease.

It enters into the biological cycle of the human body at all stages of its life. Although the elderly is the group of people that has the higher prevalence of illness, in recent decades relatively little effort has been made to investigate the alterations in toxicological responses of this wide group of population, including those concerning environmental toxins[4].

Al nduces neurofibrillary degeneration in animal brains after intracerebral injections and systemic exposure[5]. Although direct experimental evidence of Al neurotoxicity in humans is not conclusive, there is considerable clinical evidence that Al can be a neurotoxicant. Al produces neurotoxicity through many mechanisms and it promotes the formation and accumulation of insoluble amyloid beta protein and the aggregation of hyperphosphorylated tau protein, it is associated with cortical cholinergic neurotransmission deficits[6], and it increases iron-induced oxidative injury[7].

The brainstem is the region of the brain that connects the cerebrum with the spinal cord. It consists of the three main parts ie., midbrain, medulla oblongata, and the pons. Motor and sensory neurons travel through the brainstem allowing for the relay of signals between the brain and the spinal cord. The main function of the brainstem is to coordinates motor control signals sent from the brain to the body. The brainstem also controls life supporting autonomic functions of the peripheral nervous system. Experimental study[8] suggests that the brainstem may be a sensitive target exposure to methylmercury and other neurotoxicants, such as Pb, Cr, Ni and Zn[9].

The aim of this study is to investigate neurotoxic effects of Al in brainstem regions of the brain. Brain stem is the important part of the controlling cardiac rhythms and controls of involuntary movements.

Material and Methods

Nitroblue tetrazolium Cat N-5514 (NBT), thiobarbituric acid Cat T-5500 (TBA), phenazinemetho sulphate Cat N-9625 (PMS), nicotinamide adenine dinucleotide Cat N-6754 (NADH), 5.5'-dithio *bis* 2- nitrobenzoic acid Cat D-5420 (DTNB), 4,5 methyl thiazol-2-yl 2,5 diphenyl tetra zolium bromide Cat M-2128 (MTT), nicotinamide adenine dinucleotide phosphate Cat N- 7785 (NADPH) tricholoroacetic acid Cat T-

8657 (TCA) and reduced glutathione Cat G-4251 (GSH) were purchased from Sigma Chemical Co., St. Louis, MO, USA. All other reagents used were of high quality and analytical grade.

Animals: Twenty male Wistar strain rats (24m, weight 435 ±3.6 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 ^{0}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: The animals (n=10) were treated with oral dose of $AlCl_3$ (100 mg/kg body weight) once a day for 90 days. In terms of actual amount of Al, the dose works out to be 20 mg/ kg body weight. It is generally known that the rodent dose is about 10-20 times higher compared with the humans. Hence by extrapolation, for humans the dose would be ≈ 1-2 mg / kg body weight. While a group of ten rats were taken as a positive control and given equivalent volume of physiological saline.

Biochemical estimations: Hematology: The hematological tests were carried out using commercially available Qualigens kit. The hematological parameters namely hemoglobin (Hb; mg/dl), red blood corpuscles (RBC; X 10^6 cells/ mm^3 of blood), white blood corpuscles (WBC; X 10^3 cells/ mm^3 of blood), packed cell volume (PVC; percent), prothrombin time (PTT; sec) and erythrocyte sedimentation rate (ESR; mm/h) were carried out in blood samples of Al treated young and old rats and their controls.

Serum Lipid profiles: After 45 and 90 days of treatment the blood was collected from the tail vein of the rats in plain vial for serum separation. Serum total lipid, cholesterol, triglycerides, high-density lipoprotein, triacylglycerol were determined using Randox Laboratory kit reagents and VLDL was calculated using the formula TG/2.2 mmol/l. Low density lipoprotein (LDL) cholesterol was determined by differential substraction of the sum of the cholesterol fractions from the total cholesterol.

Biochemical study in Brainstem: At the end of experiment, rats were sacrificed by cervical dislocation and brain was removed immediately in an ice box. Pons, medulla oblongata and mid brain were dissected out for biochemical study. Ten percent (w/v) homogenate of different organs was prepared with the aid of York's homogenizer fitted with Teflon plunger in KCl (0.15M) or 0.1 M phosphate buffer (pH 7.1), as per requirement. The whole homogenate was first centrifuged at 2500 x g for 10 minutes in a refrigerated centrifuge. The pellet consisting of nuclear fraction and cell debris was discarded. The supernatant was further centrifuged at 11,000 x g for 15 minutes and mitochondrial fraction was separated. The clear supernatant was further centrifuged at 105,000 x g for 90 minutes and the resultant supernatant was used for enzyme activities.

The aliquots of cytosolic fraction of different region of brainstem (Pona, Medula Oblogata and midbrain) of the brain of control and experimental groups were taken for the biochemical estimation. The protein content was measured by the method of Lowery et al[10] using bovine serum albumin (BSA) as standard. Lipid peroxide level (LPO) was measured spectrophotometrically at 532 nm according to the earlier described method[11] and expressed as n mole MDA/g tissue. The Superoxide dismutase (SOD: EC 1.15.1.1.) was determined by the method of NADPH- phenanzomethosulphate, nitrobluetertrazolin formazon inhibition reaction by spectrophotometrically at 560 nm by the method of Mc Cord and Fridovich[12]. The activity of catalase (CAT: EC 1.111.6) was measured by the method of Aebi[13] using hydrogen peroxide as substrate, the decomposition of H_2O_2 was followed at 240nm. Reduced glutathione (GSH) were measured[14] in deproteinezed supernatant of homogenate with employing 5-5' dithiobes (2-nitrobenzioc acid) and recorded absorption at 412 nm. The GSH content was expressed as µ mole /g tissue.

The activity of acetylcholinesterase enzyme (AChE; EC 3.1.1.7) was determined by the method of Ellman et al[15] using acetylthiocholine iodide as substrate. The enzyme activity is measured by the increase in absorbance at 412 nm. The results were expressed as nmoles of acetylcholine hydrolyzed/min /mg protein. Nitric oxide synthase (NOS) was determined in different regions of the brainstem by the commercially available kit (Bioxytech Nitric oxide; U.S.A).

Light microscopy: For the evaluation of histopathological changes in midbrain, small section of the tissue was immediately fixed with formalin. Thereafter, the specimens were embedded in paraffin, sectioned at 5 µm and stained with hematoxyline and eosin.

Results and Discussion

The exposure over a period of time to toxic heavy metals can result in physical, muscular and neurological damages. The effect on the body when exposed long-term can resemble Parkinson's disease, multiple sclerosis, muscular dystrophy and even allergic reactions. According to the International Occupational Safety and Health Information Center, long-term exposure to heavy metals like Al, arsenic and lead can even cause neurodegenerative disorders.

Brain is a heterogeneous conglomeration of many discrete "little organs", rather than one large organ. Cerebral cortex itself exhibits around 52 distinct Brodmann's areas. Hence, instead of evaluating neurochemical parameter in the whole brain or its 3 major components (cerebrum, cerebellum and brain stem), as commonly observed[16] in the literature, we have dissected out pons, medulla oblongata and mid brain for the investigations of Al induced changes. There are no study is no study was conducted on these precious regions of the brain.

Table 1 shows the observation of haematological modifications induced by Al treated aged rats. Our results indicated that Al

treatment resulted in a significant (P<0.05) decline in the concentration of haemoglobin (Hb), total erythrocyte count (TEC) and packed cell volume (PCV), while WBCs was (TLC) increased. It is suggestive that altered peripheral blood composition is a reflection of disrupted haematopoietic process. Blood, which rapidly and constantly flows through the brain and other body organs and play an important role in the transportation of nutrients, antioxidants, hormones and some other chemicals[17]. After long term exposure of Al may cause deleterious effect on the morphology and physiology of blood cells. These findings are concomitant with the earlier published observation[18]. It is suggestive that Al may be interfering with different stages of red-cell synthesis and mature red blood cells. Mahieu et al[18] was also proposed that Al is responsible for degradation of RBCs and mycrolytic anemia. In spite of this, a persistent intoxication would cause compensating mechanisms to be triggered, leading to restoring of hematocrit and haemoglobin concentration values with a concomitant persistence of microcytosis and a decrease in MCH[18].

Table-2 shows lipid profiles following Al treatment and control rats. The concentration of lipid was found to be significantly reduced in Al treated rats on both 45 and 90 days of treatment. The reduction of lipid may be correlate with the Al induced increased lipid peroxidation. It has been reported that Al promotes the production of free radicals resulting in Al-Fe interdependence reaction[7]. Total cholesterol and triglycerides were found to be elevated in Al treated rats when compared with the controls on the day 45 and 90. The increase in serum TG may be due to hypoactivity of lipoprotein lipase in blood vessels which breaks up TG. High serum cholesterol level may results from the hepatic dysfunction. Previously it has been reported that Al exposure accelerates neurodegeneration through lipid peroxidation via free radical production. Similarly, we also observed significant increase in LPx after Al administration in aged rats. Al produces a subtle rearrangement in the membrane structure that facilitated the oxidative action of iron. Several studies are concomitant with the our finding which shows increased LPx in liver, kidney, testis and brain of different animals tissues[19], while some study shows inverse relation of Al and LPx[20].

Table-3 shows oxidative stress parameters of different brain regions of control and Al treated rats. LPx were estimated in medulla oblongata, pons and midbrain. We found that increased rate of LPx following Al exposed rats. The maximum LPx were found in medulla oblongata and least in pons. The medulla contains the cardiac, respiratory, vomiting and vasomotor centers and deals with autonomic, involuntary functions, such as breathing, heart rate and blood pressure. High MDA levels which suggest that aluminum induces superoxide anion radicals in the rat brain. The lipid peroxidation of biological membrane results in the loss of membrane fluidity, changes in membrane permeability and alteration in receptor functions. Shafiq-Ur-Rehman[21] and Sushma et al[22], also reported that brainstem is more susceptible part of the brain for metal toxicity. Brain contains various antioxidants like superoxide peroxidase (SOD), Catalase, glutathione peroxidase GSHPx and glutathione reductase GR etc. SOD is thought to be one of the major enzymes which protects against tissue damage by ROS. It is therefore possible that the decrease in SOD activities with age may be closely related to the ageing of the organism. It is reported that decrease in SOD activities with age may further accelerate the process of ageing. The effect of aging on the activities of SOD and catalase has been studied in a variety of organs and animals and found a direct correlation between the decrease in total activity of SOD and catalase, and an increase in the level of lipid peroxidation in different regions of the aging rat brain. In our previous study, we reports Al is responsible for the exceleration of aging process[7]. In the present study reduced activity of SOD and Catalase indicates cell damage via free radical production in Al exposed animals. Glutathione plays an important role in the detoxification of ROS in body. In our study the GSH levels in different parts of the brainstem were decreased. These results are concomitant with finding of Liu et al[23]. The most decreased GSH level found in mid brain and least in pons.

Table-1
Hematological profiles of control and Al treated aged rats

Parameters	45 Days		90 days	
	Control	Al treated	Control	Al treated
Hb	10.9 ± 0.11	9.83 ± 0.12*	10.2 ± 0.20	8.3 ± 0.12*
RBC	4.21 ± 0.09	3.11 ± 0.05*	3.99 ± 0.12	2.87 ± 0.08*
WBC	7.13 ±0.08	8.62 ± 0.12*	7.72 ±0.11	8.96 ±0.12*
PCV	61.2 ±3.2	58.2 ± 2.2^{ns}	59.6 ± 2.2	52.3 ±2.6*
PTT	13.3 ±0.25	16.2 ± 0.27^{ns}	13.5 ±0.19	22.6 ±2.1*
ESR	4.62 ±0.1	6.1 ± 0.2*	7.72 ±0.2	11.32 ±0.2*

Values are expressed as mean ± SEM for ten animals (N=10) in each group. Haemoglobin (Hb; mg/dl), red blood corpuscles (RBC; X 10^6 cells/ mm^3 of blood), white blood corpuscles (WBC; X 10^3 cells/ mm^3 of blood), packed cell volume (PVC; percent), prothrombin time (PTT; sec) and erythrocyte sedimentation rate (ESR; mm/h) concentration on 30, 60 and 90 day of treatment of aluminum. Statistical significance was determined by Mann-Whitney p-test. Probability, p-value less than 0.05 were considered statistically significant control and treated groups on different intervals.

Table-2
Lipid profiles and lipid peroxide levels in serum of control and Al treated aged rats

	45 Days		90 days	
	Control	**AL**	**Control**	**AL**
TL (mg/ml)	399.5± 65.8	326.7 ± 39.3*	425±27.5	313.5 ± 44.5*
TC (mg/ml)	102.4 ± 22.2	129.2 ± 19.2*	114.6 ± 23.3	155.7 ± 42.7*
TG (mg/ml)	66.7 ± 3.4	71.7 ± 2.6ns	69.3 ±5.2	82.3 ± 8.4*
LDL (mg/dl)	55.4 ± 6.9	42.7 ± 4.3*	53.6 ± 4.9	31.9 ± 3.6*
VLDL (mg/dl)	22.3 ± 1.4	19.2 ± 1.2ns	22.8 ± 1.7	14.2 ±1.2*
HDL (mg/dl)	31.2 ± 1.3	26.7 ± 1.1*	33.1 ± 1.5	21.1 ± 1.2*

Values are expressed as mean ± SEM for ten animals (N=10) in each group. The values of total lipid (TL), total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), Very low density lipoprotein (VLDL), high density lipoprotein (LDL) and lipid peroxide levels (LPx) in control and experimental groups on 45 and 90 days. Statistical significance was determined by Mann-Whitney *p*-test. Probability, *p*-value less than 0.05 were considered statistically significant control and treated groups on different intervals.

Table-3
Biochemical profiles of different parts of the brainstem of control and Al treated rats

	Medulla Oblongata		Pons		Mid Brain	
	Control	Exp	Control	Exp	Control	Exp
LPO	402.7 ± 23.8	554.8 ± 29.3*	388.3± 27.3	472.5 ± 41.2*	485.9 ± 38.2	591.1 ± 42.2*
SOD	4.0 ± 0.2	2.0 ± 0.1*	4.5 ± 0.2	3.5±0.2*	3.8 ± 0.3	2.8 ± 0.2*
CAT	1.4 ± 0.07	0.9 ± 0.09*	2.0 ± 0.1	1.2±0.8*	1.2 ± 0.9	0.6 ± 0.07*
GSH	2.6 ± 0.4	2.2 ± 0.2ns	3.6 ± 0.3	2.6 ± 0.3*	3.2 ± 0.2	2.1 ± 0.2*

Values are expressed as mean ± SEM for ten animals (N= 6) in each group. The mean values of lipid peroxide levels (LPO), activity of superoxide dismutase (SOD), Catalase and reduced glutathione content (GSH) in medulla oblongata, pons and midbrain of control and experimental aged rats. Statistical significance was determined by Mann-Whitney *p*-test. Probability, *p*-value less than 0.05 were considered statistically significant control and Al treated rats.

Al, oxidative stress and impaired cholinergic function have been all related to cognitive dysfunction. Aluminium crosses the blood brain barrier via the specific high affinity receptors for transferring (TfR)[24]. Upon entering the brain it affects the slow and fast axonal transports, induces inflammatory responses[25] inhibits long-term potentiation, and causes synaptic structural abnormalities, thereby resulting in profound memory loss. Figure-1 shows the impaired activity of AChE in Al treated and control rats. The brainstem regulates blood pressure, heart rate, and sympathetic nerve activity by the nucleus tractus solitarius (NTS)[26]. The NTS receives inputs from afferent fibers arising from arterial baroreceptors, chemoreceptors, cardiopulmonary receptors, and other visceral receptors and thus has an important role in autonomic control of the cardiovascular system[27]. In the present study we observed reduced activity of NOs in the brainstem of Al exposed rats presented in figure-2. It suggestive that reduced activity of NOs may alter the cardiac and sympathetic nerve activity in elderly. Our results similar with the finding of Hirooka et al[28], he suggest that reduced nNOS expression in the NTS, contribute to the enhanced sympathetic drive in cardiac disturb. While, Hegde et al[29], showed inverse correlation between brain NOs activity and blood pressure level. Histopathological observation revealed that increased neuronal loss and vacuolization in figure-3. These finding supports to Al overload in brain and increased oxidative damage associated with aging.

Conclusion

The results of the present study demonstrate that Al deteriorate hematological, lipid profiles and biochemical modifications. These findings suggest a possible molecular mechanism for Al-induced oxidative damage in medulla, mid brain and pons. These regions are highly regulating cardiac and parasympathetic nerve. It is suggestive that elderly people may be more prone to environmental Al toxicity and associated complications. Therefore some antioxidant intervention may be recommended. However, there is a need for further in depth studies to confirm these findings.

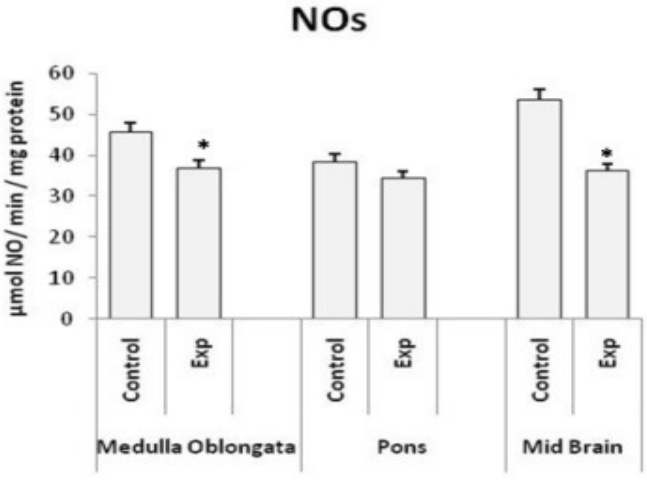

Figure-1
Activity of nitric oxide synthase (NOs) in the medulla oblongata, pons and midbrain of control and aluminum treated group. The results are expressed as Mean ± SEM in six rat of each group. Superscripts relate significant (p< 0.05) comparison with Control and Al treated rats

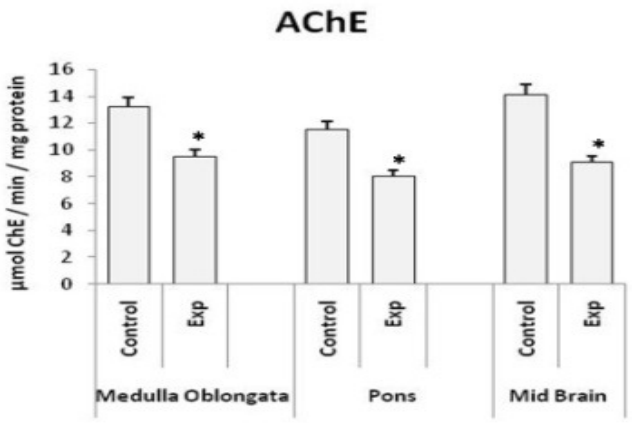

Figure-2

Activity of acetylcholinesterase (AChE) in the medulla oblongata, pons and midbrain of control and aluminum treated group. The results are expressed as Mean ± SEM in six rat of each group. Superscripts relate significant (p< 0.05) comparison with Control and Al treated rats

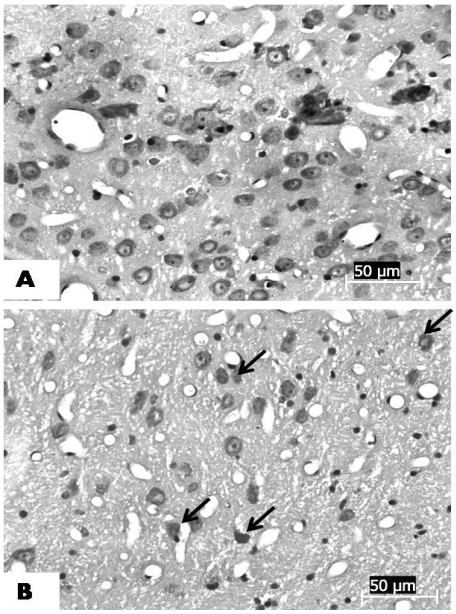

Figure-3

Light photomicrograph of H&E stained section of rat midbrain. (A) Normal histology of the midbrain tissue in the control rats showed well preserved architecture and a number of nucleated neurons. (B) The histology of 90 days Al exposed rat shows necrotic neurons and damage in both cortical and subcortical regions. The number of neurons comparatively low of the controls

Conflict of Interest: None

Acknowledgement

Author wish to thank University Grant Commission New Delhi for financial support to DKJ.

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