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Effect of Intrahippocampal Injection of Aluminum on Active Avoidance Learning in Adult Male Rats

[1]A. Sarkaki, [2]S. Zahedi Asl and [3]R. Assaei
[1]Physiology Research Center, Department of Physiology, Faculty of Medicine,
Ahwaz Jondishapour University of Medical Sciences, 61357-15794, P.O. Box 45, Ahwaz, Iran
[2]Institute of Endocrinology and Metabolism,
Shaheed Beheshti University of Medical Sciences, Tehran, Islamic Republic of Iran
[3]Department of Physiology, Faculty of Medicine,
Ahwaz Jondishapour and Lorestan Universities of Medical Sciences, Ahwaz, Iran

Abstract: Aim of this research was to study the effect of intrahippocampal injection of different doses of $AlCl_3$ in adult male rats on active avoidance learning. Thirty five adult male Wistar rats (250-300 g) were used into five groups: (1) Control, (2) Test-I received daily 1 μL $AlCl_3$ 1%, pH = 7.2, 3); Test-II received daily 1 μL $AlCl_3$ 0.5%, pH = 3.4, 4); Sham-I received daily 1 μL aCSF, pH = 7.2, 5); Sham-II received daily 1 μL aCSF, pH = 3.4. All rats in test and sham groups treated 10 min before training. Animals were anaesthetized with ketamine HCl/xylazine (90/10 mg kg^{-1} b.wt.$^{-1}$, i.p.) and underwent a stereotaxic surgery for implant of two stainless steel guide cannula into the hippocampus bilaterally. Every day 10 min after above treatments all rats were used to assess the spatial learning performing using Y-maze. Criterion Correct Response (CCR) was 90% in last session of training. There were no significant differences between training sessions to receiving CCR in control, Sham-I and Sham-II groups. Cognition in animals received $AlCl_3$ 1%, pH = 7.2 was impaired significantly with compare to other groups (*$p<0.0001$). Present results show that intrahippocampal injection of $AlCl_3$ 1%, causes active avoidance learning impairment significantly. The exact mechanism of Al_3 effect on brain and cognition is remains unknown.

Key words: Al_3, hippocampus, active avoidance learning, Y-maze, rat

INTRODUCTION

Aluminum (Al_3), a known neurotoxin, causes extensive damage to the nervous system, including the impairment of learning and memory. Chronic Al_3-exposure in rats is associated with neuronal apoptosis in brain and impaired learning and memory (Niu *et al*., 2007). Aluminum salts are added to a range of commercially-prepared foods and beverages: to clarify drinking water, make salt free-pouring, color snack/dessert foods and make baked goods rise (Walton, 2007). $AlCl_3$ in drinking water for 8 months causes deficits in rat spatial learning and memory (Luo *et al*., 2007). Investigators have suggested that learning and memory deficit of rats could be induced by $AlCl_3$ solution and acetylcholinesterase (AChE) expressions in hippocampus were increased (Gong *et al*., 2006). Studies have shown that Wistar rats were given daily aluminum chloride 500 mg kg^{-1}, i.g, for one month, followed by continuous exposure via the drinking water containing 1600 ppm aluminum chloride for up to 5 months, significantly increased escape latency and searching distance when tested by Morris water maze, indicative of brain dysfunction (Gong *et al*., 2005). In the other hand, aluminum-induced learning and memory impairment model was established by gavage of aluminum chloride (600 mg kg^{-1}) for 3 months (Shi-Lei *et al*., 2005). In compare with controls, the synapses in aluminum-induced rats exhibited significant changes such as decreased thickness of postsynaptic density (PSD), increased width of synaptic cleft, increased numbers of flat synapse, decreased numbers of positive curvature synapse and perforated synapse and significantly increased aluminum deposits in hippocampus and frontal cortex. These findings indicate that aluminum can decrease the ability of rats to learning and memory and induce their synaptic configuration changes, that may be related to synaptic efficacy and may be one of the mechanisms for Al_3 to induce Alzheimer's Disease (AD) (Jing *et al*., 2004). Excess aluminum exposure impairs neurocognitive in humans and animals.

Corresponding Author: Alireza Sarkaki, Physiology Research Center, Department of Physiology, Faculty of Medicine, Ahwaz Jondishpour University of Medical Sciences, 61357-15794, P.O. Box 45, Golestan Blv., Ahwaz, Islamic Republic of Iran

Epidemiological studies have shown a potential link between chronic Al_3 exposure and Alzheimer's disease. So, aluminum has been etiologically and epidemiologically related to several neurologic conditions, including AD (Zhang *et al*., 2003). Al_3 overload caused significantly increased level of Al_3 in serum. Brains of experimental animals, studied by optical microscopy, displayed a massive cellular depletion in the hippocampal formation, particularly, the CA_1 field and also in the temporal and parietal cortex. These behavioral and neuropathological modifications associated with long-term exposure to Al_3 are reminiscent of those observed in AD (Miu *et al*., 2003). After exposure young and old male rats to 100 mg/kg/day of Al_3 as Al $_3$nitrate nona hydrate in drinking water concurrently with citric acid (356 mg/kg/day) for a period of 100 days, there were no significant effects of Al_3 exposure between groups could be detected on behavior, while the total number of synapses decreased with age and Al_3 exposure (Colomina *et al*., 2002).

Although the neurotoxic actions of aluminium have been well documented, its contribution to cognitive impairment such as avoidance learning and memory remains controversial. In this study the effect of intrahippocampal bilateral administration of different doses of $AlCl_3$ in adult male Wistar rats on active avoidance learning in equal 3-arms Y-maze was studied.

MATERIALS AND METHODS

Animals: Thirty five adult male Wistar rats (250-300 g) were used as subjects in the present experiment (from Lab Animal Care and Breeding Center of Ahwaz Jondishapour University of Medical Sciences AJUMS, Iran). This study was conducted from 25th December of 2006 to 20th December of 2007. All animals were housed individually per cage under a 12 h light/dark cycle, 20±2°C temperature and 60-65% humidity controlled room with food and water *ad libitum*. All procedures were approved by the Institute Research Ethics, Animal Care and Use Committee of AJUMS. Rats were divided randomly into five groups 7 in each: (I) Control without any surgery or Al_3 administration, (II) Test-I received 1 μL $AlCl_3$ 1% and pH 7.2, (III) Test-II received 1 μL $AlCl_3$ 0.5% and pH 3.4, (IV) Sham-I received 1 μL aCSF and pH 7.2, in order to evaluate the effect of injected volume on learning, (V) Sham-II received 1 μL aCSF and pH 3.4, in order to evaluate the effect of acidic pH on learning. All animals received drugs into hippocampi bilaterally daily 10 min before training.

All rats in test and sham groups were anaesthetised with ketamine hydrochloride/xylazine (90/10 mg kg^{-1}, i.p.) and underwent a stereotaxic surgery. In groups undergoing microinjection, two stainless steel guide cannula (0.6 mm, O.D.) with an inner needle guide (0.3 mm, O.D.) were inserted into the hippocampus bilaterally at stereotaxic coordinates: P, 2.2 from bregma, L, ±2; H, 3 mm from skull surface (Paxinos and Watson, 1986). All implants were fixed to the skull with acrylic dental cement and two anchor screws. All injections were done 7-10 days post surgery recovery. Drugs were injected at the rate of 1 μL min^{-1}. The needle remained in place for an additional 5 min following the infusion and then it was slowly withdrawn. The animals in the sham groups were injected with an equivalent dose of aCSF with the same method.

Training procedure: Every day 10 min before the above treatments, the five groups of rats were used to assess the spatial learning performing using an equal 3-arms Y-maze with using an A/D converter, a special software on a PC as active avoidance learning as reported previously (Sarkaki and Karami, 2004). The device is composed of three arms (of equal length, separated from each other at an angle of 120 degrees), with an array of stainless steel rods on the floor of the arms through which an electric current can be applied. Each arm has a lamp on the end top and the electric power and the lamp can be turned on individually when needed. Training was done as one session, 30 trials daily. Animals were conditioned using a 15 watts light as Conditioned Stimulus (CS) and 20-25 V electrical foot shock (AC, 150-200 mA, 50 Hz, 200 μsec pulse wide) as unconditioned stimulus (UCS). Inter-Trials Interval (ITI) and Inter-Stimuli Interval (ISI) were 60 and 5 sec, respectively. Trained animals left the dark arms and enter in light arm during 5 sec delay time (ISI). This effort was counted as conditioned response. Criterion Correct Responses (CCR) were 90% in last session of training. Training session number was same for control, test and sham groups.

Statistical analysis: The data of percents of correct responses in each group presented as Mean±SEM were analyzed for significant differences by one-way ANOVA followed by Tukey's post hoc test. A p-value less than 0.05 were assumed to denote a significant difference and level of significance is indicated by asterisk: *$p<0.0001$.

RESULTS

Data showed that training sessions in control group to receiving 90% correct responses (Criterion Correct Responses or CCR) of 30 trials per session daily was 5.33±0.56. Training sessions to CCR in Sham-I group that

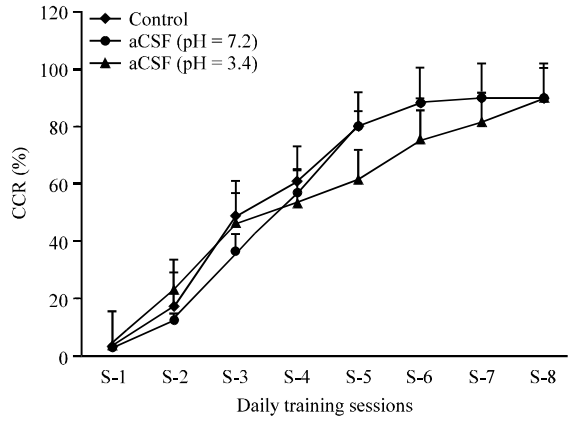

Fig. 1: Percents of criterion correct responses of control, aCSF with pH = 7.2 and aCSF with pH = 3.4 groups during training sessions in Y-maze

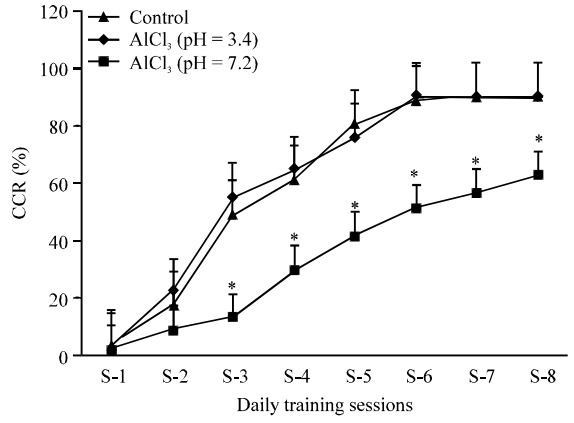

Fig. 2: Percents of criterion correct responses of control, $AlCl_3$ with pH = 7.2 and $AlCl_3$ with pH = 3.4 groups during training sessions in Y-maze. CCR% was reduced significantly in group receiving $AlCl_3$ pH =7.2 (*p<0.0001)

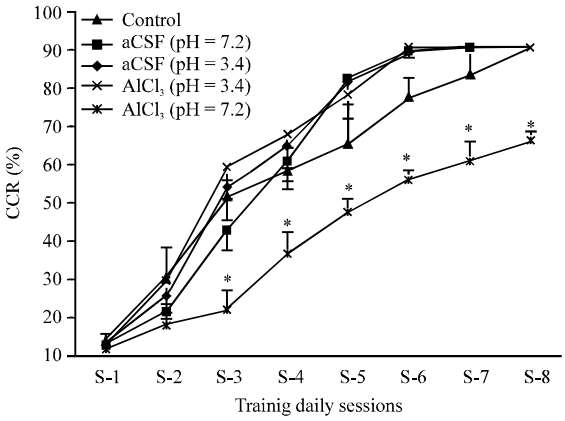

Fig. 3: Comparison of criterion correct responses percents in control, aCSF with pH = 7.2, aCSF with pH = 3.4, $AlCl_3$ with pH =7.2 and $AlCl_3$ with pH = 3.4 groups during training sessions in Y-maze. CCR% was reduced significantly only in group receiving $AlCl_3$ pH = 7.2 (*p<0.0001)

received intrahippocampal 1 μL aCSF, pH = 7.2 bilaterally was 5.5±0.43. In other hand mean training sessions to CCR in Sham-II group that received intrahippocampal 1 μL aCSF, pH = 3.4 bilaterally was 6±0.45. There were no significant differences between mean training sessions to receiving CCR level in control with both Sham-I and Sham-II groups (Fig. 1).

Mean training sessions to receiving CCR in test-I group that treated with intrahippocampal 1 μL $AlCl_3$, pH = 3.4 bilaterally was 5±0.52 and did not different with control group significantly. So, acidic pH solutions (pH = 3.4) such as aCSF or $AlCl_3$ could not affect mean training sessions to receiving CCR. Mean training sessions to receiving CCR in test-II that treated with intrahippocampal 1 μL $AlCl_3$, 1%, pH = 7.2 bilaterally was different significantly from third training session ($p<0.0001$) in compare to control and all other groups. While animals in test-II group trained extra three sessions could not receive to CCR level (Fig. 2), other groups usually trained 6 to 7 sessions to receiving CCR.

As it appears animals in two groups including control and sham-1(aCSF with pH = 7.2) received to CCR after seven training sessions, while animals in group test-1 (0.5% $AlCl_3$, pH = 3.4) received to CCR one session later (Fig. 2).

But cognition in animals that received $AlCl_3$ 1%, pH = 7.2 was impaired significantly with compare to other groups ($p<0.0001$). So, central administration of $AlCl_3$ could impair cognitive function as dose-dependent (Fig. 3).

DISCUSSION

Results of this research show that intrahippocampal injection of 1% $AlCl_3$ impairs active avoidance learning significantly.

Intraperitoneal injection of $AlCl_3$ for 60 days in Sprague-Dawley rats could decrease active avoidance response and spontaneous motor activity in the shuttle-box test and the open field test significantly. Granulovacuolar degeneration (GVD) of nerve cells in hippocampus was observed and the number of GVD cells increased significantly. The incidence of GVD per 300 nerve cells was significantly related to the dosage of aluminum (Sun *et al*., 1999). Intracerebroventricular (ICV)

injection of aluminum tartrate produces transient regional cerebral glucose uptake (rCGlu) depression in caudate-putamen, geniculate bodies and periaquaeductal gray (Provan and Yokel, 1992).

Al_3, after 4 weeks administration, had a deleterious effect on the activities of biosynthetic (choline acetyltransferase) and hydrolytic (acetylcholinesterase) enzymes of the neurotransmitter acetylcholine. The levels of acetylcholine were also significantly lowered in different brain regions at the end of the dose regimen. There was a significant decrease in high-affinity choline uptake following Al_3 exposure and number of binding sites of muscarinic acetylcholine receptor decreased with the maximum effects being manifested in the hippocampus. The impaired cholinergic functioning had severe effects on cognitive functions. These results suggest that Al_3 exerts its toxic effects by altering cholinergic transmission, which is ultimately reflected in neurobehavioral deficits (Julka *et al*., 1995).

Although Al_3 contributes to a variety of cognitive dysfunctions and mental diseases, the underlying mechanisms of Al_3 interactions with the nervous system are still unknown. The negative action of Al_3 on synaptic transmission and Long-Term Potentiation (LTP) by performing electrophysiological recordings both *in vivo*, using freely moving animals and *in vitro*, using hippocampal slices was confirmed (Platt *et al*., 1995). Studies on the effect of aluminum on the brain of rats exposed to this metal (500 mg Al_3 L^{-1} in drinking water) daily for 180 days showed significant reduction in the spontaneous locomotor activity was noticed after 90 and 180 days of Al_3 exposure to the rats, the magnitude of the change being almost identical at both the time intervals. Aluminum exposure also produced significant deficits in acquisition and retention of learned response in rats, these changes being time dependent. They indicated significant increase in the lipid peroxidation and decrease in the activity of Mg^{2+}-ATPase and Na^+, K^+-ATPase in the brain of rats. Al_3 may is responsible to initiate neurotoxic effects by producing changes in the structure and function of the plasma membrane needs further investigations (Lal *et al*., 1993).

Investigators tested the hypothesis that Al_3-induced inhibition of learning may be due to its effect on glutamate release secondary to changes in calcium channel function and/or intracellular events triggering glutamate release. It is suggesting an Al_3-induced alteration of Ca^{2+}channel function. These effects were prevented by the Gi protein inhibitor N-ethylmaleimide, suggesting an effect of Al_3 on the Gi protein to inhibit glutamate release. Suggesting an Al_3 modulation of protein kinase C (PKC)-evoked glutamate release. These results demonstrate an Al_3 inhibition of glutamate release that may be mediated by multiple, but interconnected mechanisms (e.g., via interactions with Ca^{2+} systems), providing multiple targets for an Al_3-induced alteration of neuronal function (Provan and Yokel, 1992).

It was found that after long-term exposure to Al_3, it was concentrated in white matter of the medial striatum, corpus callosum and cingulate bundle and the spontaneous motor ability in the open field and the latency of passive avoidance in aluminum treated rats were decreased as compared with the controls. In hippocampus, the contents of aspartate (Asp) and glutamine (Gln) were significantly decreased while taurine (Tau) was significantly increased at higher doses of Al_3 as compared with controls. The altered content of amino acid neurotransmitters in hippocampus might be one of the important mechanisms of aluminum neurotoxicity (Jia *et al*., 2001). Damage of the cingulate bundle in Al_3-treated animals led to a severe anterograde degeneration of cholinergic terminals in cortex and hippocampus, as indicated by acetylcholinesterase labelling. It was suggested that the enhancement of inflammation and the interference with cholinergic projections may be the modes of action through which Al_3 may cause learning and memory deficits and contribute to pathological processes in AD (Platt *et al*., 2001).

Intracerebroventricular (ICV) microinjection of aluminum (5.0 μg in 2.0 μL), once a day for 5 days could cerebral damage. Using meloxicam, a selective inhibitor of cyclooxygenase-2 (COX-2), has putative protective effects on the oxidative damage induced by aluminum overload in mouse brain. These evidences approved impairment of learning and memory function was caused by aluminum overload (Jun-Qing *et al*., 2006).

According to the World Health Organization, oral ingestion of aluminum additives is the main form of aluminum exposure for the general public. Wistar rats that chronically consumed aluminum in an amount (1.5 mg kg^{-1} b.wt.) equivalent to the high end of the total aluminum range ingested daily by humans living in contemporary urban society, their hippocampal neurons stained for aluminum, showing some but not all features of aluminum accumulation that occur in human hippocampal neurons. In view of evidenced linkages of aluminum with beta-amyloid plaque and neurofibrillary tangle formation in humans with Alzheimer's disease, the findings suggest this protocol is worsting in larger groups of animals (Walton, 2007).

Compared with the rats in the control group, the learning and memory abilities of the Al_3-exposed rats were significantly decreased. The content of MDA was increased while the activity of SOD was decreased. The

membrane structure of neurons in cerebrum cortex of the Al_3-exposed rats were broken, dissolved and gone. Aluminum can accelerate lipid peroxidation in rat's brain, which may be one of the important intoxication mechanisms of aluminum (Zhang and Yu, 2002; Yang *et al*., 2006; Li *et al*., 2006; Kaneko *et al*., 2006).

An investigation on possible effects of chronic aluminium exposure on neurofilament phosphorylation and its subsequent disruption in various regions of the rat brain showed that an intra-gastric dose of aluminium (10 mg kg^{-1} b.wt. for 12 weeks) resulted in a marked enhancement of Ca^{2+}/CaM dependent protein kinase activity as compared to cAMP dependent protein kinase. The levels of phosphoprotein phosphatase were found to be significantly depleted only in the cerebral cortex. The cytoskeletal proteins were found to be aggregated and disrupted neuronal regions following 12 weeks of aluminium treatment. This study lends further support to the possible role of aluminium as a potent neurotoxic agent and in the etiopathogenisis of various neurodegenerative diseases (Kaur *et al*., 2006).

Cognition in animals that received $AlCl_3$ 1%, pH = 7.2 was impaired significantly with compare to other groups ($p<0.0001$) due to Al_3 accumulation in brain tissue. So, everybody needs to know that any contact with aluminum (beverages, foods, industrial and etc.) may act as toxic and impairs cognitive function as dose-dependent.

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