Tissue and Cell 46 (2014) 409–414

Contents lists available at ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

Aluminum chloride impacts dentate gyrus structure in male adult albino Wistar rats

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ARTICLE INFO

Article history:
Received 11 December 2013
Received in revised form 12 May 2014
Accepted 14 May 2014
Available online 3 July 2014

Keywords:
Aluminum chloride
Necrosis
Neuroblasts
Dentate gyrus
Histopathology
Toxicity

ABSTRACT

To get better insights into the aluminum neurotoxicity, rats were treated with $AlCl_3$ for increasing doses and periods. Body and brain weights, plasma and brain $AlCl_3$ levels were assayed. Light microscopy observation of brain was performed. $AlCl_3$ exposure showed a significant decrease ($p < 0.05$) on body and brain weight with the highest dose at 18 months. Statistical analysis confirms no significant interaction during 6 months ($\rho = 0.357$; $p > 0.05$) while, significant correlation was observed during 12 ($\rho = 0.836$; $p < 0.001$) and 18 months ($\rho = 0.769$; $p < 0.001$) between body and brain weight. Plasma and brain $AlCl_3$ concentration increased significantly ($p < 0.05$) with dose and period dependent manner. Statistical analysis confirms significant interaction between brain concentrations of $AlCl_3$ and administrated doses during 6 ($\rho = 0.969$; $p < 0.001$), 12 ($\rho = 0.971$; $p < 0.001$) and 18 months ($\rho = 0.965$; $p < 0.001$). Similar relation was established between plasma $AlCl_3$ concentration and administrated doses during 6 ($\rho = 0.970$; $p < 0.001$), 12 ($\rho = 0.971$; $p < 0.001$) and 18 months ($\rho = 0.964$; $p < 0.001$). Significant relation was confirmed between plasma and brain $AlCl_3$ concentration during 6 ($\rho = 0.926$; $p < 0.001$), 12 ($\rho = 0.983$; $p < 0.001$) and 18 months ($\rho = 0.906$; $p < 0.001$). Morphological alterations mainly targeted the subgranular layer with modulation of the dentate gyrus appearance. This study highlights the toxic effect of $AlCl_3$ on the brain which may affects learning and memory and seems to be different according to dose and duration of exposure.

1. Introduction

Aluminum is widely distributed in the ambient environment and used in various fields. Oral ingestion including by food and water currently comprises the main form of aluminum exposure for humans (Walton, 2007). However, the Al accumulation is of no biological or physiological function in mammalian species (Walton, 2012) and can even induce neurotoxicity (Wu et al., 2012). Its effects on human health should not be ignored. So far, Al has been reported as an important etiological factor of some neurodegenerative disorders such as Alzheimer's diseases (Percy et al., 2011), Parkinson's diseases (Oyanagi, 2005) and other chronic neurodegenerative diseases (Bondy, 2010). With the increasing concern on human health it is necessary to develop further research and find more data about the detailed mechanism of the adverse effects induced by aluminum.

Animal studies and epidemiological investigations showed that Al is a neurotoxicant which could cause learning and memory impairment (Liang et al., 2012), cognitive dysfunction and result in neurodegenerative diseases (Riihimaki et al., 2000). Exposure to Al at long-term low level could affect dopaminergic metabolism and neurocognitive function in workers, Al could cause behavioral deterioration and dendritic atrophy (Petit et al., 1985) and may be associated to the neurofibrillary tangles and amyloid plaques of patients with Alzheimer's disease (Khachaturian, 2006).

It is well known that aluminum affects the hippocampus (Deloncle and Guillard, 1990) and cortex regions (Urano et al., 1997) more severely than any other area of the central nervous system. These brain regions are known to be particularly susceptible in Alzheimer's disease and have an important role in learning and memory functions. For these reasons dentate gyrus, part of the hippocampal formation was chosen.

In the present study, we were looking to determinate $AlCl_3$ impacts dentate gyrus structure among male adult albino Wistar rats.

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http://dx.doi.org/10.1016/j.tice.2014.05.006
0040-8166/

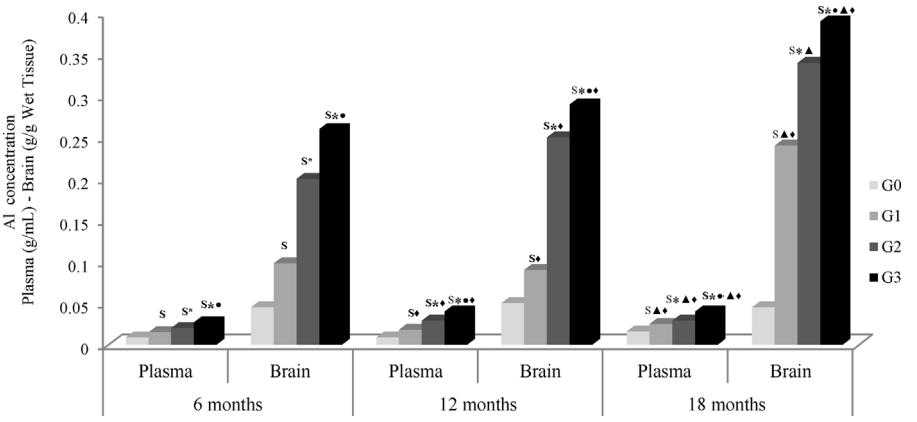

Fig. 1. Plasma and brain aluminum concentration at different doses and periods of treatment with $AlCl_3$. (Results were expressed as median; $n = 5$ rats.) ^{S} Significant compared to control (G_0). * Significant compared to (G_1) at same period. ● Significant compared to (G_2) at same period. ▲ Significant compared to the same dose (12 months). ♦ Significant compared to the same dose (6 months).

2. Materials and methods

2.1. Animals and treatment

Sixty adult male Wistar rats (Pasteur Institute of Tunis, Tunisia) weighing 200–250 g were used in this study. The animals were acclimatized to housing conditions (22 °C room temperature and a 12-h light/dark period) with proper aeration for 2 weeks in the Experimental Medicine Unit of the Faculty of Medicine of Tunis and were given food and water *ad libitum*. Standard rat pellet diet (Industrial Society of Food, Sfax, Tunisia) contained 1.75 mg Al and 0.0625 mg Fe. After two weeks of acclimation, animals were divided into four equal groups ($n = 15$; 5 rats/cage). Control rats (G0) received distilled water as drinking water. The other groups received aluminum chloride ($AlCl_3 \cdot 6H_2O$ Sigma Company (St. Louis, MO, USA)) in drinking distilled water (similar exposure route to human). $AlCl_3$ was dissolved in distilled water without neutralization or filtration. The limpid $AlCl_3$ solution was used as drinking water. Respectively, G1 received 0.18 g $AlCl_3$/L; G2 received 0.72 g $AlCl_3$/L and G3 received 3.6 g $AlCl_3$/L distilled water all during 6, 12 and 18 months. Rats were weighed regularly. Sacrifices were performed at 6, 12 and 18 months. Blood sample was taken from each rat and put in a sterile heparinized tube before centrifugation at 3000 × *g* for 15 min. The plasma was collected and stored at −20 °C until analysis by atomic absorption spectrophotometry. Brains were entirely dissected, weighed and conserved for mineralization and histology.

Ethical approval for the study was duly obtained from the responsible Ethical committee.

2.2. Aluminum determination

Aluminum concentrations were determined using atomic absorption spectrophotometry with a graphic oven (Zeenit "analytik Jena": model 700). In order to avoid contamination with aluminum all the glassware and bottles were cleaned using a 0.01 N HNO_3 solution for 48 h, then rinsed with ultrapure water. 0.5 g of tissue or 1 mL of plasma was placed in a polypropylene tube; 2 ml of pure nitric acid were added and mineralization was performed in an oven at 50 °C for 72 h.

After mineralization, 20 μl of the mineralized liquid sample were introduced in the furnace, heating that leads to the atomization was carried out in an inert argon atmosphere following the different steps of drying, decomposition, atomizing cleaning and cooling.

2.3. Histopathology

Histopathological evaluations of brain were performed on all animals, of the control and treated groups. Organs were formaldehyde fixed and embedded in paraffin by a routine procedure. They were then sectioned, stained with Hematoxylin–Eosin and examined under an Olympus optical light microscope.

2.4. Statistical analysis

Statistical analyses were performed using SPSS 19.0 for Windows (SPSS, Chicago, IL, USA). To determine whether there were differences between all groups, the Kruskal–Wallis and *U* Mann–Whitney tests were performed ($p < 0.05$). A value of $p < 0.05$ was considered to be significant. All data are presented as medians. Spearman correlation was performed to ascertain whether correlation was significant or not.

3. Results

During this study, all the rats survived to the different treatments.

3.1. Plasma and brain $AlCl_3$ concentration

Data in Fig. 1 showed that plasma and brain $AlCl_3$ concentration increased significantly ($p < 0.05$) with a dose and period dependent manner.

Statistical analysis confirmed a significant correlation between brain $AlCl_3$ concentrations and administrated doses during 6 ($\rho = 0.969$; $p < 0.001$), 12 ($\rho = 0.971$; $p < 0.001$) and 18 months ($\rho = 0.965$; $p < 0.001$).

A similar relation was established between plasma $AlCl_3$ concentration and administrated doses during 6 ($\rho = 0.970$; $p < 0.001$), 12 ($\rho = 0.971$; $p < 0.001$) and 18 months ($\rho = 0.964$; $p < 0.001$).

A significant relation was confirmed between plasma and brain concentration of $AlCl_3$ during 6 ($\rho = 0.926$; $p < 0.001$), 12 ($\rho = 0.983$; $p < 0.001$) and 18 months ($\rho = 0.906$; $p < 0.001$).

3.2. Effect of $AlCl_3$ on body and brain weight

Following Figs. 2 and 3, chronic Aluminum Chloride exposure showed no significant decrease ($p > 0.05$) of body and brain weight except with the highest dose at 18 months where body and brain weight decreased significatively ($p < 0.05$) by 24.4% and 23.9%