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Investigation of Behavioral Changes and Histopathological Changes in the Brain in Alzheimer's Modeled Mice with Aluminium Chloride ($AlCl_3$)

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Abstract

BACKGROUND/AIMS: Aluminium (Al) is related with many brain diseases including Alzheimer's disease, however the relation between Al and neurodegenerative diseases is still controversial. In this study behavioral and histopathological changes were investigated in the $AlCl_3$ induced Alzheimer's model in mice.

MATERIALS AND METHODS: Male and female mice divided into control (tap water) and test (50 mg/kg/day $AlCl_3$) groups. After ninety days of $AlCl_3$/water intake, rota rod, elevated plus maze, Passive Avoidance tests were performed to assess motor coordination, spatial and emotional learning and memory, respectively. After the behavioural experiments, histopathological examination was made in the brain.

RESULTS: No difference was found between the groups and the genders in the rota-rot test. Learning and memory were impaired in both gender. Long term memory impaired female mice in the test group. Neuron loss was observed in both the cerebral cortex and hippocampus CA1 regions in the test group, while an increase in pycnotic nucleated cells was observed. Neurofibrillary tangles were also observed in the hippocampus, with neurons with basophilic nuclei prominently.

CONCLUSION: As a result, long-term exposure to low doses of aluminum may cause behavioral changes and histopathological changes in the brain.

Keywords: Aluminium chloride, Alzheimer's disease, behaviour, learning, memory, mice

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative illness. The most important symptoms of progressive and irreversible AD are memory loss, decreased cognitive functions, and disorders in spatial perception, daily life activities, and speech.[1] The most important pathological findings of the disease are neurofibrillary tangles (NFT) and amyloid beta plaque formation.[2] In addition, factors such as oxidative stress, inflammation, and glutamate excitotoxicity are also thought to promote this neurodegeneration.[3-5] The pathophysiology of the disease is still not completely elucidated, and the search for new treatments continues.

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We aimed to investigate behavioral and histopathological changes by using an experimental AD model in mice in the present study.

MATERIALS AND METHODS

Animals, Chemical and Experimental Design

Study approval was obtained from the Animal Ethics Committee of Çukurova University (approval number: 2020/09, date: 10.12.2020). Swiss albino mice were obtained from Health Sciences Experimental Application and Research Center of Çukurova University. Mice were kept in standard conditions. Male and female control groups (n=20) and experimental groups (n=30) were created. Control groups were given tap water while the test ($AlCl_3$) group was given $AlCl_3$ solution (50 mg/kg/day) via a feeding bottle for ninety days.

The Rota Rod test was used to evaluate the motor coordination of the mouse on a rotating rod (10 rpm).[6] Learning and spatial memory were evaluated by using the elevated plus maze (EPM) test.[7] Long-term memory was examined using the Passive Avoidance (PA) test.[8]

Histological Analysis

The brains were fixed with 10% neutral formalin solution, and a routine paraffin embedding protocol was performed. Hematoxylin and eosin staining was performed. The differences between the groups were evaluated under a light microscope with a camera attachment.[9]

Statistical Analysis

Categorical measurements were expressed as numbers and percentages, and numerical measurements as mean and standard deviation (median and interquartile range where appropriate). Whether the numerical measurements met the assumption of normal distribution was assessed using the Shapiro-Wilk test. In the comparison of numerical measurements between the groups, Student's t-test for independent groups was used if the assumptions were met and Mann-Whitney U test was used if the assumptions were not met. The IBM SPSS Statistics version 20.0 software package was used. Statistical significance was decided when $p<0.05$

RESULTS

No significant difference was found between the $AlCl_3$ and the control groups of each sex in the Rota Rod test.

According to EPM test, there was no difference between the sexes in the control mice, while a statistically significant difference was found between male and female mice in the $AlCl_3$ group ($p<0.05$, Table 1). The latency time (LT) of male and female mice in the light arm decreased, while LT in the dark arm increased, on the second day. These time changes were statistically significant in female mice ($p<0.05$).

In the PA test setup, the time spent by mice in the light and in the dark box (LT), for 300 seconds was recorded. There was a significant difference between male and female mice with regard to LT in the light box in the $AlCl_3$ group ($p<0.05$, Table 2). No significant difference was found between male and female mice in the control group.

Histopathologic Findings

Cerebral cortex regions of all groups were examined using hematoxylin and eosin staining. The histologic structure of the cerebral cortex regions of male and female mice belonging to the control group was found to be normal. Six layers, including stratum granularis externa and interna, stratum pyramidalis externa and interna, and stratum multiformis, were preserved from the pia mater to the medulla. Especially in pyramidal neurons, the cell borders were smooth and the cell nuclei were round and centrally located (Figure 1).

Table 1. Two-day comparison of the time (sec.) spent by male and female mice in the elevated plus maze

Groups	Day 1 LT (light arm) ($\bar{X}$ ± SD, sec), p-value	Day 2 LT (light arm) ($\bar{X}$ ± SD, sec.), p-value	Day 1 LT (dark arm) (median ± IQR, sec.), p-value	Day 2 LT (dark arm) (median ± IQR, sec.), p-value
Control male (n=9); Control female (n=6)	105.1±15.5 112.3±9.1 0.325	82.1±28.7 88.7±25.2 0.657	20.0±24.0 5.0±15.0 0.388	53.0±48.0 32.0±27.0 0.689
$AlCl_3$-t male (n=12); $AlCl_3$-t female (n=11)	115.2±8.7 94.4±16.8 **0.001***	102.9±22.2 88.8±21.8 0.140	0.0±9.5 28.0±14.0 **0.001***	5.0±39.0 32.0±34.0 0.134

Data are presented as mean ± standard deviation for normally distributed groups and median ± interquartile range for non-normally distributed groups. *Shows significance in $AlCl_3$ group female mice. LT: Latency time, SD: Standard deviation, IQR: Interquartile range.

Table 2. Comparison of the time (sec.) spent by male and female mice in the Passive Avoidance test

Groups	Day 1 LT (light box) (median ± IQR, sec.)	Day 2 LT (light box) ($\bar{X}$ ± SD, sec.)	Day 2 LT (dark box) (median ± IQR, sec.)
Control male (n=9)	27.0±10.0	256.1±48.6	21.0±86.0
Control female (n=6)	30.0±34.0	287.8±24.6	2.0±7.0
p-value	0.102		0.224
$AlCl_3$-male (n=12)	27.5±16.5	298.1±5.7	0.0±0.0
$AlCl_3$-female (n=11)	26.0±47.0	274.8±48.0	0.0±43.0
p-value	**0.04***		0.566

Data are presented as mean ± standard deviation for normally distributed groups and median ± interquartile range for non-normally distributed groups. *Shows significance in $AlCl_3$ group female mice. LT: Latency time, SD: Standard deviation, IQR: Interquartile range.

It was found that the cerebral cortex of the $AlCl_3$ groups, consisted of six layers, but there was cellular damage. An increased number of cells with pyknotic nuclei and the formation of cerebral vacuoles was observed. The damage in the cerebral cortex of male mice was greater compared to that of female mice (Figure 2). Large euchromatic nuclei and smooth cell borders were seen in the CA1 region neurons in both sexes of control mice (Figure 3). In the CA1 region of the $AlCl_3$ groups, the number of normal-looking neurons decreased and was replaced by neurons with pyknotic nuclei and basophilic stained nuclei. Neurons with basophilic nuclei showed prominent NFT. Histopathologic findings in the hippocampus CA1 region of male mice were higher compared to the control group (Figure 4).

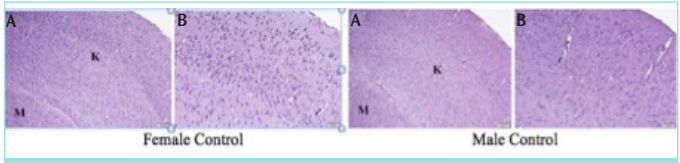

Figure 1. The histologic images of brain tissue from control female and male mice show hematoxylin and eosin staining. K: Cortex, M: Medulla. Magnifications: 100 µm (A), 50 µm (B).

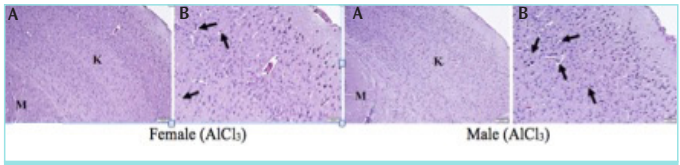

Figure 2. Histologic images of brain tissue of $AlCl_3$ group female and male mice, hematoxylin and eosin staining. K: Cortex, M: Medulla, Arrows: Cerebral vacuoles. Magnifications: 100 µm (A), 50 µm (B).

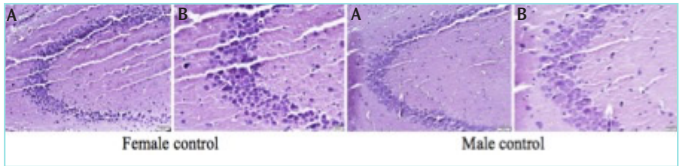

Figure 3. Histologic images of the hippocampus region of the brain of control group female and male mice, stained with hematoxylin and eosin, show characteristic features. Magnifications: 50 µm (A), 20 µm (B).

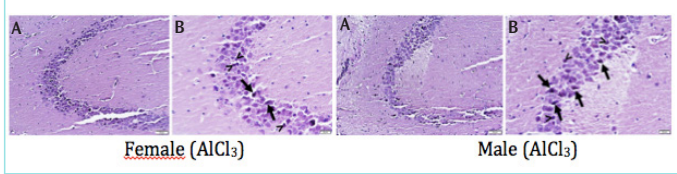

Figure 4. Histologic images of the hippocampus region of the brain of $AlCl_3$ groups female and male mice, Hematoxylin and Eosin staining. Arrows: Neurofibrillary tangles, Arrow heads: Pycnotic nucleated cells. Magnifications: 50 µm (A), 20 µm (B).

DISCUSSION

We used long-term oral administration of $AlCl_3$ to induce experimental AD, since people are exposed to aluminum in their daily lives, either in kitchen utensils or through food and medicines. Our results showed that this model is suitable for the study and in accordance with some other studies.[10] The motor coordination of both control and $AlCl_3$ groups was not impaired. This result shows that the EPM and PA tests' findings are not related to a motor coordination disorder. Exploratory, anxiety, and motor behaviors, as well as memory and spatial learning, were evaluated using the EPM test in control and $AlCl_3$ groups. In their natural habitat, mice tend to explore unfamiliar environments by sniffing and crawling, and if they feel anxious and fearful, they prefer to enter a closed, dark environment. In this study, LT in the light arm decreased and LT in the dark arm increased on the second day in the control and $AlCl_3$ groups. The decrease of LT in the light arm and the increase of LT in the dark arm, were statistically significant in the control group mice. This was an expected result, as control mice learned the safe area on the first day and remembered it on the second day, whereas memory was impaired in mice receiving $AlCl_3$. In terms of gender, no difference was found between the control group and the treatment group mice. On the other hand, a significant difference was found in female mice in the $AlCl_3$ group. In fact, AD is more common in women.[11] Emotional, and long-term memory and learning were assessed in the PA test. Female mice entered the closed box earlier, while male mice stayed in the light box longer. The findings here corroborate those of the EPM. Female mice were more stressed than male mice and had an increased desire to enter the dark zone. However, despite the electric shock on the second day, the female mice entered the closed box before the male mice. Based on these findings, it may be suggested that long-term memory is more impaired in female mice with an Alzheimer's model than in male mice.

It was observed that the histologic structure of the control group was normal, while in the $AlCl_3$ group, the cells were damaged, and the number of cells with pyknotic nuclei increased. In the $AlCl_3$ group, the normal-appearing number of neurons in the hippocampus CA1 region was decreased. NFTs were prominently seen in neurons with basophilic nuclei in the hippocampus. These findings were in accordance with some other studies.[12,13] In histopathologic examinations, no change was observed in the control group, but damage was observed in the CA1 region of the cortex and hippocampus, in male and female mice in the $AlCl_3$ group.

Study Limitations

Although we had made plans to measure, we could not measure serum Brain-Derived Neurotrophic Factor concentrations because the ELISA kits could not be obtained on time. We evaluated aluminum effects on AD, using behavioral and histopathological examinations.

CONCLUSION

In our study, aluminum exposure was found to cause changes in behavior and negatively affect learning and memory. Histopathological examinations have also shown changes in the brain.

MAIN POINTS

- Long-term oral use of low-dose $AlCl_3$ can be used in Alzheimer's disease research as an experimental model.

- Long-term exposure to aluminum can impair learning and memory.
- Female mice were more affected by aluminum than male mice.

ETHICS

Ethics Committee Approval: Study approval was obtained from the Animal Ethics Committee of Çukurova University (approval number: 2020/09, date: 10.12.2020).

Informed Consent: Patient approval has not been obtained as it is performed on animals.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: D.D.K., I.A., A.A., S.E., Concept: D.D.K., I.A., F.A., Design: D.D.K., F.A., Data Collection and/or Processing: D.D.K., I.A., A.A., Analysis and/or Interpretation: D.D.K., I.A., F.A., Literature Search: D.D.K., I.A., S.E., F.A., Writing: D.D.K., I.A., F.A.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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