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RESEARCH ARTICLE

Aluminum chloride impaired spatial memory, but not senile plaques formation in the rat model of Alzheimer's disease

Muhammad Ihwan Narwanto[1,*], Masruroh Rahayu[2], Setyawati Soeharto[3], Nurdiana[3]

[1] Department of Anatomy, Faculty of Medicine, University of Jember, Jember, Indonesia.
[2] Department of Neurology, Faculty of Medicine, University of Brawijaya, Malang, Indonesia.
[3] Department of Pharmacology, Faculty of Medicine, University of Brawijaya, Malang, Indonesia.
* Corresponding author, email: muhammadnarwanto@unej.ac.id

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ABSTRACT

Aluminum compounds can be easily found in the environment. Aluminum contamination is the environmental factor as one of the risk factors for Alzheimer's disease (AD). In the animal model, aluminum chloride ($AlCl_3$) induces inflammation and oxidative stress. Inflammation and oxidative stress are important pathogenesis pathways in the AD. This study was conducted to determine whether $AlCl_3$ can impair spatial memory and induce senile plaques formation. A total of 24 young adult Wistar rats were used in this study. The rats were divided into four groups; one control group and three $AlCl_3$ treated groups with doses of 150 mg/kg, 300 mg/kg, and 600 mg/kg, respectively for 8 weeks. The spatial memory test was measured using Morris water maze and the histopathology was done by identification of senile plaques formation in the hippocampal tissue. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. The level of statistical significance was set at a p value < 0.05. This study showed that there are significant differences ($p<0,05$) between the control group and all the $AlCl_3$ treatment groups in the memory test, however, there is no change in the senile plaque's expression in all groups. Administration of $AlCl_3$ for 8 weeks can cause the impaired of spatial memory without senile plaques formation.

1. Introduction

Aluminum compounds are abundant in the environment. Aluminum is widely used for mixture material in the metal, paper industry, food packages, cooking utensils, water purifiers, and pharmaceuticals. Exposure of the aluminum in the human body, mainly from the food, beverages, pharmaceuticals, and other oral intakes (Aguilar *et al.*, 2008). Environmental contamination by aluminum is one of the risk factors for Alzheimer's disease (AD) (Rondeau *et al.*, 2009; Killin *et al.*, 2016).

The sporadic form of Alzheimer's disease was the most prevalent. Sporadic Alzheimer's was a form of AD in which age and environmental factors were more likely than gene mutations to cause the disease (Lee and Han, 2013). Memory impairment is the major clinical symptoms of AD (Jayakar and Huang, 2010). Memory impairment in an Alzheimer's animal model induced intra-hippocampus amyloid beta40, was accompanied by the formation of senile plaques and induced neurodegeneration in CA1 hippocampus (Nobakht *et al.*, 2011). Inflammation and oxidative stress were significant AD pathogenesis pathways (Agostinho *et al.*, 2010). Senile plaques are extracellular deposits of amyloid-beta protein in the brain tissue and histophatology hallmark of Alzheimer's disease (Deture and Dickson, 2019).

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Administration of $AlCl_3$ in animal models induces inflammation and oxidative stress. $AlCl_3$ administered orally to rats for three months causes the production of proinflammatory cytokines in the brain (Cao *et al.*, 2016). Reatment of neonatal rats with aluminum increases lipid peroxidation products and decreases superoxide dismutase activity in brain tissue (Yuan *et al.*, 2012). In this study, aluminum chloride was used as an inducer to simulate AD in an animal model. The purpose of this study is to quantify the deficit in spatial memory and formation of senile plaques caused by chronic $AlCl_3$ administration in rats as a model for sporadic Alzheimer's disease.

2. Materials and Methods

2.1. Animal and treatment

Twenty-four young adult male Wistar (3 months olds) with approximately 200 g of body-weight used in this experiment. The rats were kept in an individual cage, under a 12-h light-dark cycle, and ad libitum access to food and water. After three days of acclimatization, the rats were randomly divided into four groups, one control group and three groups of $AlCl_3$ treatment. The rats in the control group were administered distillate water orally every day. $AlCl_3$ (TCI, Portland, USA) was administered orally daily to the treatment groups at doses of 150 mg/kg, 300 mg/kg, and 600 mg/kg for eight weeks. This study evaluated the dose and duration of $AlCl_3$ administration based on a previous study that administered 100 mg/kg for six weeks (Lin *et al.*, 2015). The previous study showed that there was no impairment of spatial memory and the formation of senile plaques (unpublished data). Five grams of $AlCl_3$ dissolved in 100 ml distillate water (Baydar *et al.*, 2003). Each week, the body weight of the rats was measured to adjust the $AlCl_3$ dosage. After eight weeks of $AlCl_3$ treatment, the Morris water maze was used to evaluate the spatial memory of rats. All treatments are performed during the day. Ether was used to sacrifice rats, followed by cervical dislocation. The brain was extracted for staining with Thioflavin S (Santa Cruz Biotechnology, Dallas, Texas). The research ethics approval was obtained from the Research Ethics Committee of Faculty of Medicine University of Jember according to letter number 1151/H25111/KE/2017.

2.2. Morris water maze test

After 8 weeks of administration of $AlCl_3$, rat spatial memory was measured using the Morris water maze (Bromley-Brits *et al.*, 2011). The apparatus consists of a black circular swimming pool diameter of 150 cm, depth of 50 cm constructed using waterproof material. It was divided into four quadrants, marked spatial cues at the inner wall of the pool above the water surface and on a room wall. A clear 10 cm in diameter of the plexiglass platform was placed at the middle of one quadrant. The pool filled water at 22 °C to 30 cm height. To record the rats' time taken to reach the platform, the camera positioned above the pool. The measurement was carried out for 5 successive days, with two trials each day.

Five consecutive days of measurements were conducted, with two trials per day. On day one, the platform was positioned 1 cm above the surface of the water. The rat was placed gently in the pool water, facing the pool wall. Then, release it and exit the testing area immediately. Time the rat's arrival on the platform. If the rat reached it within 120 seconds, the trial was concluded, and the rat was allowed to remain on the board for 5 seconds before being returned to its cage. If the rat could not locate the platform within 120 seconds, it was guided to it and allowed to remain for 20 seconds before being returned to its cage. When releasing the rat, repeat the test in each of the four quadrants. On days 2-5, after water was added to a height of 1 cm above the surface platform, the same experiment was conducted as on day one.

2.3. Senile plaque

The brain was fixated in 10% neutral buffer formalin for two weeks. The hippocampal slide for histopathology observation is cut at a coronal section -3.8 mm from the bregma and stained with Thioflavin S. Briefly, each section of hippocampal tissue was deparaffinized in Xylene, hydrated graded alcohol, and distilled water, in that order. The tissue was then incubated for 8 minutes at room temperature in filtered 1 percent aqueous Thioflavin-S; washed for 3 minutes in 80 percent ethanol twice; for 3 minutes in 95% ethanol; and for 3 minutes in distilled water. Finally, the slides were coverslipped in glycerol and dried in the dark overnight for histology observation (Rajamohamedsait and Sigurdsson, 2012). Analyze slides were done within the next few days-weeks using the fluorescent microscope with 490-550 nm filter.

2.4. Statistical analysis

All data are expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. The level of statistical significance was set at a $p\text{-value} < 0.05$.

3. Results

Five days of observation revealed that all groups learned to locate the platform. When the rats were released, their spatial memory was recorded until they reached the platform. The difference in the amount of

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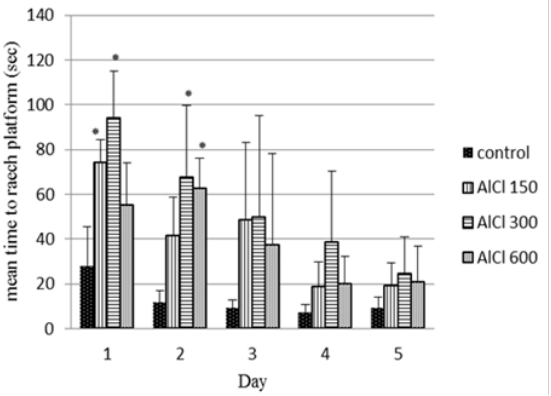

Figure 1. Mean time to find the platform. Data expressed as mean ± SD of each group. *p<0.05 compared to control group.

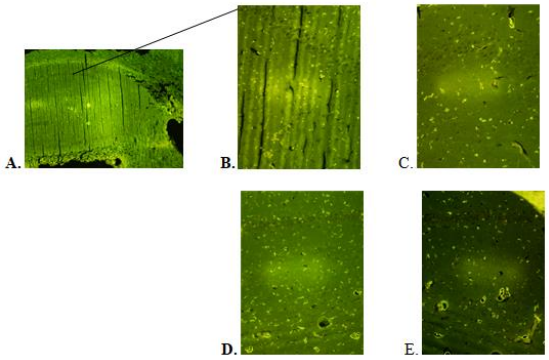

Figure 2. Hippocampus. Staining with Thioflavin S, senile plaques cannot identify in all the group. A. 100× magification of hippocampus, B. Control group, C. $AlCl_3$ 150 mg/kg group, D. $AlCl_3$ 300 mg/kg group, E. AlCl3 600 mg/kg group. B-E 400× magnification.

time it took the treated animals to reach the platform compared to the time it took the untreated animals to reach the platform indicated a deficit in spatial memory. We demonstrated that there are significant differences between treated and control animals on days 1 and 2, but their spatial memory returns to normal on day 3. Specifically, administration of 150 mg/kg $AlCl_3$ decreased memory performance only on the first day ($p<0.05$), after which it returned to normal. On day 1 and day 2, the $AlCl_3$ 300 mg/kg treatment group takes longer to reach the platform ($p<0.05$). Only on day 2 following the water maze test did $AlCl_3$ 600 mg/kg-treated animals demonstrate memory impairment ($p<0.05$) (Figure 1).

Our histopathology analysis using Thioflavin-S staining did not reveal the formation of senile plaques in response to $AlCl_3$ treatment, and there is no difference between treated animals and the control group (Figure 2).

4. Disscussion

Our results indicated that $AlCl_3$ significantly impaired the spatial memory of rats. This result is consistent with previous research demonstrating that administration of 100 mg/kg to rats for six weeks impairs spatial memory (Lin *et al.*, 2015). We discovered that the middle dose has a greater number of day-significant tests than the highest dose. Theoretically, the larger dose should impair memory faster and for a longer duration. The possible explanation is that $AlCl_3$ concentrations in the brain are not proportional to oral dose administration. The $AlCl_3$ levels in the brain should serve as the measurement standard for the drug. This research is incomplete, so we are aware of its limitations. We hypothesized that administration of $AlCl_3$ would induce inflammation and oxidative stress in the rat's brain and impair spatial memory. Interleukin 1 beta (IL1ß) and tumor necrosis factor alpha (TNFa) mRNA expression in the brain of rats has been shown to increase after 3 months of $AlCl_3$ administration, according to a prior study (Cao *et al.*, 2016). The other studies demonstrated that intra basal brain injection with TNFa can reduce the number of cholinergic neurons, while in vitro study also proved that administration of TNFa impaired long term potentiation (LTP) (Wenk *et al.*, 2003; Wall *et al.*, 2016). In brain tissue, aluminum increased the amount of free radicals. Increased levels of free radicals can impair spatial memory.(Kawahara and Kato-Negishi, 2011; Massaad and Klann, 2011).

Aluminum's role in the pathogenesis of Alzheimer's disease remains unclear. Some evidence supported the role of aluminum in the pathogenesis of Alzheimer's disease, but few data demonstrated its involvement. In a recent study, administration of $AlCl_3$ did not result in the formation of senile plaques as determined by Thioflavin-S histopathological staining. We suspect that 8 weeks of administration of $AlCl_3$ is still short, thus it did not enough to induce senile plaques formation. A study done by Chen *et al.*, 2013 demonstrated that $AlCl_3$ administration required at least 5 months to induce the formation of diffuse senile plaques. The other possibility is that the 3-month administration of $AlCl_3$ in our study may produce amyloid-beta as the primary component of senile plaques, but it has not yet aggregated to form senile plaques. In fact, a previous study demonstrated that amyloid-beta levels increased in the cortex and hippocampus after 6 weeks of $AlCl_3$ administration (Lin *et al.*, 2015). According to the other study, oral

administration of $AlCl_3$ to rats for eight weeks increased amyloid-beta levels in brain tissue and impaired their memory. (Promyo *et al.*, 2020). This study's limitation is that aluminum concentrations in rat brain tissue were not measured.

5. Conclusions

Oral administration of 150 mg/kg, 300 mg/kg, and 600 mg/kg $AlCl_3$ for 8 weeks can impair spatial memory, but did not induce senile plaques formation. A longer duration of administration of $AlCl_3$ is required to reveal the senile plaques.

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