RESULTS OF ALZHEIMER'S DISEASE ANIMAL MODEL INDUCTION BASED ON THE INTRAHIPPOCAMPAL INJECTION OF AMYLOID B-PEPTIDE (1-42) AT VIETNAM MILITARY MEDICAL UNIVERSITY

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Abstract

Objectives: To evaluate some behaviors and histological results of the hippocampus in a rat model of Alzheimer's disease (AD). ***Methods:*** A longitudinal, descriptive study was conducted on 21 Wistar rats. Intrahippocampal amyloid beta (Aβ) injection was performed, followed by behavioral testing and analysis and hippocampal histological examination. ***Results:*** Rats in the AD model showed learning impairment with a reduced average latency time (52.09 ± 10.67s) and reduced latency time on the 5th day (27.08 ± 6.88s) during the learning phase of the Morris water maze (MWM) test. Memory impairment was indicated by a decreased percentage of spontaneous alternations (34.45 ± 7.03%) in the Y-maze test, reduced time spent (14.84 ± 4.61s), and shorter distance traveled (2.26 ± 0.82m) in the target quadrant during the probe trial of the MWM test. Neurodegeneration in the hippocampus was observed, with an increased degeneration score (1.83 ± 0.31). ***Conclusion:*** Intrahippocampal Aβ injection is an effective method for inducing the AD model in rats, characterized by learning and memory impairments as well as neurodegeneration in experimental animals.

Keywords: Alzheimer's disease model; Hippocampus; Stereotaxic surgery.

INTRODUCTION

Alzheimer's disease is a progressive, age-related degenerative brain disorder with a multifactorial and heterogeneous etiology. Among many hypotheses proposed for the pathogenesis of AD, the amyloid hypothesis is the most widely accepted pathological mechanism. Extracellular amyloid plaques, intracellular neurofibrillary tangles, neuronal degeneration, and consequent memory impairment are hallmark features of AD [1].

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AD models serve as valuable tools for replicating pathological changes, deciphering the disease's pathogenesis, and testing novel therapeutic approaches [1]. Many AD models have been developed on a global scale, with the rat intrahippocampal Aβ injection model being demonstrated as one of the most representative and reliable AD animal models [2]. However, this specific AD model has not yet been reported in Vietnam. Therefore, this study aims to: *Evaluate behaviors and histological outcomes of AD rat model induction via intrahippocampal Aβ injection.*

MATERIALS AND METHODS

1. Subjects

Including 21 adult healthy Wistar rats experimented in strict compliance with The Animal Center Guidelines for Care and Use of Laboratory Animals at Vietnam Military Medical University.

* *Location and time:* The study was conducted at the Practical and Experimental Surgery Department from September 2022 to July 2024.

2. Methods

* *Study design:* A longitudinal, descriptive study.

* *Sample size:* The sample size was determined using the formula:

$$n = DF/k + 1$$

n: Sample size of each group; DF: Degree of freedom with a value of 10 or 20; k: Number of comparison groups. With k = 2, the sample size of each group is: $6 \leq n \leq 11$ [3]

* *Research procedure:*

21 rats were divided into 3 groups: The control group (n = 6), the sham operation (SO) group (n = 8), and the Aβ injection (AB) group (n = 7).

Drug: $A\beta_{1-42}$ (ab120959) was purchased from Abcam and was dissolved in PBS (2 μg/μL).

Surgery: Rats were anesthetized with intraperitoneal ketamine and xylazine, and their heads were shaved and fixed onto the stereotaxic apparatus (Japan). Injection points (coordinates: AP = -4mm, ML = ± 2.2mm) were marked in stereotaxic frame. The hole was drilled into the skull using an electric driller (Komax drill, Germany). The Hamilton needle (Hamilton 10μL pump, USA) was lowered into the hippocampus at a depth of DV = -3.2mm. For the AB group, 2.5μL of Aβ (2 μg/μL) was injected bilaterally. In the SO group, the needle was inserted without injecting any substance. After the injection, the needle was carefully withdrawn, the skull opening sealed with composite solder, and the skin sutured [2].

Behavioral test:

+ Y-maze test: The Y-maze test was performed according to the procedures

described in a previous study (Prieur E et al., 2019). The Y-maze is a three-arm maze with equal angles between all arms of 75cm in length and 15cm in width, with 15cm-high walls. The maze floor and the walls were constructed using black-painted wood. Rats were initially placed within one arm, and the sequence and number of arm entries were recorded over an 8-minute period for each rat and monitored using a video tracking system (ANY-maze, Stoelting, USA) [9].

+ MWM test: The MWM test was performed according to the procedures described in a previous study (Vorhees CV et al., 2006) [4]. The MWM is a black circular pool (150cm in diameter and 60cm in height). The circular pool was filled with water at a temperature of 21°C ± 1°C. The pool was divided into four equal quadrants. A transparent platform (10cm in diameter and 28cm in height) was centered in one of the four quadrants of the pool and submerged 2cm below the water's surface so that it was invisible at the surface. The water maze experiment was performed on 6 days. On the 5th of learning, the rat received swimming training for 120 seconds in the presence of the platform. The rat underwent a daily session of four training trials each, with an inter-trial interval of 2 minutes. In each training trial, rats were placed in water facing the wall of the pool in a randomly selected pool quadrant. Once the rat located the platform, it was allowed to remain on it for 10 seconds. If the mouse failed to locate the platform within 120 seconds, it was placed on it for 10 seconds and then removed. On the 6th day, in the probe trial, the platform was removed, and rats were tested for memory retrieval by swimming for 60 seconds. The trajectory of each rat while swimming was monitored using a video tracking system (ANY-maze, Stoelting, USA).

Histological analysis: At the end of the experimental phase, the hippocampus of 6 rats of each group (AB and SO) was extracted and fixed in 10% formalin, then paraffin block was cast and cut into 3 - 4µm slices and stained with Hematoxylin-Eosin (HE). The specimens were observed under an optical microscope.

** Research variables and indicators:*

Behavioral assessment indicators: Determined by Anymaze software:

+ Number of alternating movements is the number of times the rat successfully enters three consecutive wings (ABC, ACB, BCA, etc.) and percent alternation [9]. % Alternation = (Number of alternating movements)/(Total number of times entering the wings -2) x 100%.

+ The average escape latency (s) on each day of learning. The swimming time (s) and distance (m) at the target quadrant in the probe trial [4, 5].

Pathological indicators:

Neurodegeneration is determined by cell damage in the hippocampus on HE staining, which includes cytoplasm eosinophilic, vacuole, dispersed chromatin, and loss of nuclear membrane integrity. Each damage is given 1 score; the neurodegenerative score is the sum of the scores of the lesions [6].

* *Data collection and processing methods:* Behavioral data were extracted from the Excel files of Anymaze software. Statistical analysis was performed using SPSS software. Quantitative variables were expressed as Mean ± SEM. Alternation (%) in the Y-maze test, the time and distance of swimming in the target quadrant in the MWM probe trial were analyzed using one-way ANOVA, the Tukey's post-hoc test in case of multiple comparisons. Escape latencies and swimming distance in the training trials in the MWM test were analyzed using repeated-measures two-way ANOVA and Bonferroni adjustment for multiple comparisons. Neurodegenerative scores were analyzed using the Independent-Sample T-test. The differences were considered statistically significant with $p < 0.05$.

3. Ethics

The research was conducted according to Decision No. 3424/QĐ-HVQY dated September 19, 2022. The Department of Practical and Experimental Surgery granted permission for the use and publication of the research data. The authors declare to have no conflicts of interest in the research.

RESULTS

1. Learning performance of AD model in rats

Repeated measure two-way ANOVA test showed that there was a difference in latency time between groups [($F(2, 10) = 14.07$; $p = 0.001$]. Bonferroni adjustment for multiple comparisons showed that the latency time of the AB group (52.09 ± 10.67s) was higher than that of the SO group (22.84 ± 2.62s) and the control group (20.94 ± 1.93s). On the 5th day of the learning phase, the latency time of the AB group (27.08 ± 6.88s) was higher than that of the SO group (7.18 ± 0.88s) and the control group (6.73 ± 0.83s); the difference was statistically significant with $p < 0.05$. There was no difference in latency time between the SO group and the control group during learning days with $p > 0.05$.

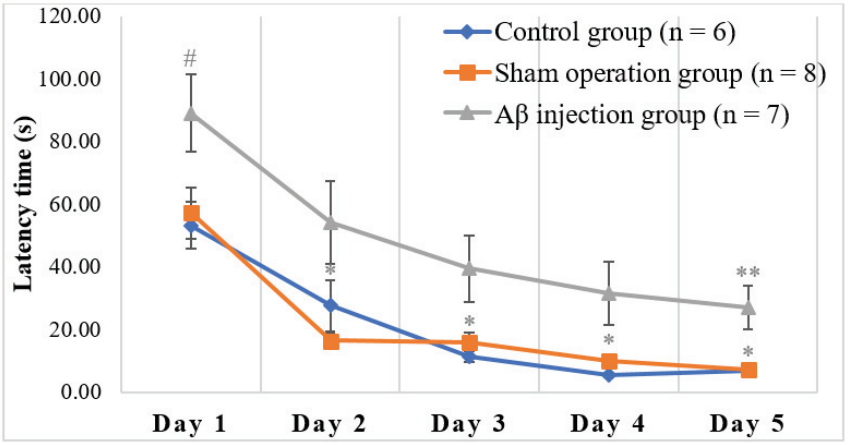

Figure 1. The swimming time to the platform of rats in the MWM.

*(**: Compare between the AB group and SO group, control group (** p < 0.05); *: Compare between the SO and control group (* p > 0.05). #: Compare between three group (# p > 0.05))*

2. Memory impairment of AD model in rats

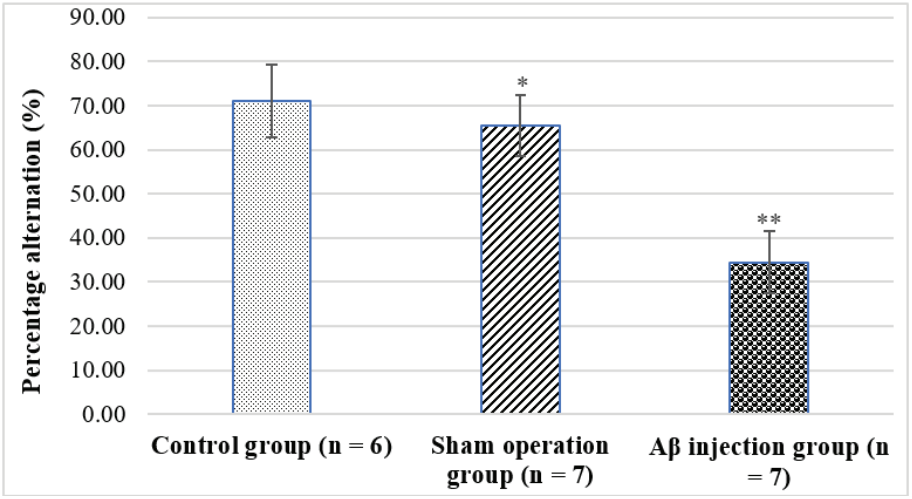

Figure 2. Percentage alternation of rats in Y-maze.

*(**: Compare between the AB group and SO group, control group (** p < 0.05); *: Compares between the SO and control group (* p > 0.05))*

One-way ANOVA test showed that there were significant differences in % alternation between the three groups [F(2, 19) = 7.36; p = 0.005], Tukey's post-hoc test showed that % alternation in the AB group (34.45 ± 7.03%) was lower than that in the SO group (65.44 ± 6.84%) and control group (71.09 ± 8.19%); the

difference was statistically significant with $p < 0.05$. There was no difference in % alternation between the SO group and the control group with $p > 0.05$

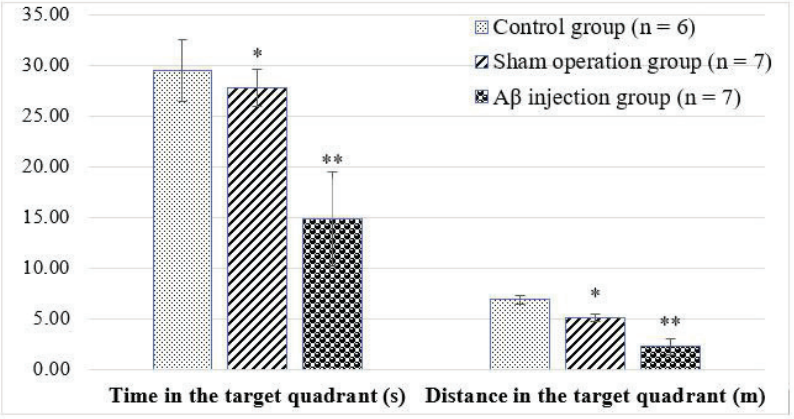

Figure 3. Time and distance of the rats at the target quadrant.

*(**: Compare between the AB group and SO group, control group (** $p < 0.05$); *: Compare between the SO and control group (* $p > 0.05$))*

One-way ANOVA test showed that there were significant differences in time [$F(2, 20) = 5.89$; $p = 0.01$], distance [$F(2, 20) = 15.32$; $p < 0.001$] of swimming in the target quadrant between the three groups. Tukey's post-hoc test showed that the swimming time of the AB group (14.84 ± 4.61s) was shorter than that of the SO group (27.79 ± 1.78s) and control group (29.52 ± 3.06s); the swimming distance of the AB group (2.26 ± 0.82m) was shorter than that of SO group (5.11 ± 0.34m) and control group (6.87 ± 0.48m).

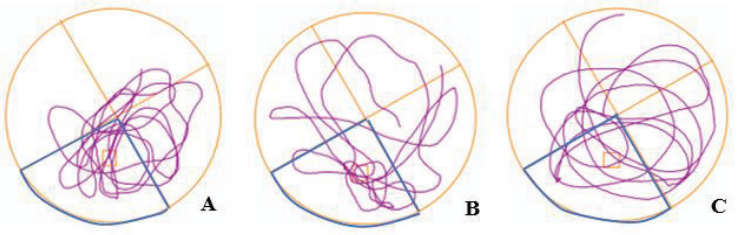

Figure 4. Illustration of the swimming path of rat in the MWM.

(A: The swimming path of rats in the control group; B: The swimming path of rats in the SO group; C: The swimming path of rats in the AB group)

Rats in the AB group spent less time swimming in the target quadrant (blue border) than those in the control group and SO group.

Figure 5. Neurodegenerative scores in the hippocampus of model rats.

*(**: Compare between the AB group and SO group (**p < 0,05))*

Independent Sample T-test showed that the neurodegenerative score in the hippocampus of the AB group (1.83 ± 0.31) was higher than that of the SO group (0.67 ± 0.33); the difference was statistically significant with $p < 0.05$.

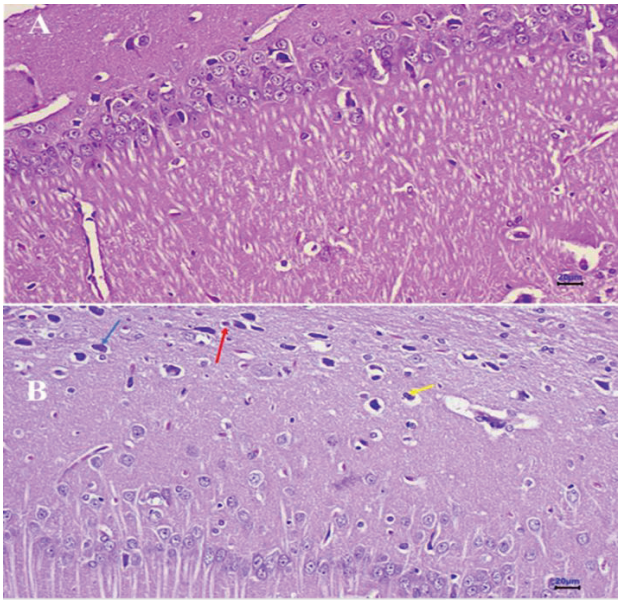

Figure 6. Microscopic image of rats' hippocampus.

(Normal hippocampus in SO rats (A), neurodegeneration in AB rats (B) with dispersed chromatin (green arrow), vacuoles (yellow arrow), eosinophilic bodies (red arrow))

DISCUSSION

1. Intrahippocampal Aβ injection impairs learning and memory performance in AD model rats

Memory is the brain's ability to categorize, encode, store, and retrieve acquired information. Declarative memory is stored in the middle of the temporal lobe and the hippocampus. The hippocampus plays a significant role in spatial learning and memory. The hippocampal subregions support the creation of episodic memory; the CA3 (cornus ammonis: CA) region generates sharp wavelets to consolidate memories. CA1 and CA3 have neurons that represent points in the space of an environment, which Nadel L et al. (1971) called place cells; this finding is called the place cell theory [4]. Neuronal connections in the CA of the hippocampus form a trisynaptic loop that plays a role in converting short-term memory into long-term memory and maintaining by unidirectional progression of synaptic connections through the loop.

Memory impairment is a hallmark symptom of AD, characterized by impaired acquisition of new information and progressive loss of information retrieval over time. Prieur E et al. (2019) showed that rats with a high % alternation had good working memory because they recalled the wings they had visited and tended to visit the recently visited wings less. This requires interaction across several brain regions, such as the hippocampus and prefrontal cortex [9].

In the SO group, 1 rat had a stimulating behavior on the behavioral test day on Y-maze; to avoid data interference, we did not include it in the analysis. The study showed that AB rats had impaired memory compared to SO rats and control rats. The % alternation of the AB group (34.45%) was lower than that of the SO group (65.44%) and control group (71.09%). This result is consistent with the study of Jeong H et al. (2021) on ICR mice (n = 10/group), showing that AB mice had memory impairment with % alternation (50%) lower than the control group (70%) [10].

The MWM test to assess learning ability and spatial memory is based on the survival instinct of animals; when put in water, they have to swim to survive. Learning ability was assessed in the training phase. Othman M et al. (2022) showed that the latency time gradually decreased, reflecting increased learning ability [4]. The results of the study showed that AB rats had reduced learning ability compared to SO and control rats. The average latency time of 5 days of learning in the AB group was higher than that of the SO group and control group; the difference was

on the 5th day of the learning phase. This result is similar to previous studies, such as Rahman S et al. (2020) on the AD model of intrahippocampal Aβ injection in Wistar rats (n = 8/group), which showed that latency time in AB rats was higher than that in the control group [6].

Spatial memory was assessed in the probe test phase (on the 6th day of the MWM test). Time and distance swimming within the target quadrant were the most frequently used parameters. Previous studies have shown that longer time spent in the target quadrant reflects better memory retrieval because they remember the area as safe [4, 5]. The results showed that AB rats had impaired memory compared to SO and control rats. The swimming time in the target quadrant of the AB group (14.84s) was lower than that of the SO group (27.79s) and control group (29.52s). The swimming distance in the target quadrant of the AB group (2.26m) was lower than that of the SO group (5.11m) and control group (6.87m). This result is similar to the study of Rahman S et al. (2020), which shows that the time of the AB group (13s) was smaller than that of the control group (22s). Jeong H et al. (2021) showed that this time in the AB mice (15s) was lower than that of the control group (25s) [10].

2. Aβ injection causes hippocampal neurodegeneration

The pathological features of AD include extracellular amyloid plaques, intracellular neurofibrillary tangles, and degeneration and loss of neurons and synapses [1]. Aβ causes synaptic dysfunction, changes in cell membrane permeability, calcium homeostasis, oxidative stress, inflammation, and neurodegeneration [8]. Faucher P et al. (2016) showed in animal models that Aβ causes molecular and cellular changes in the hippocampus, resulting in cognitive impairment even in the absence of amyloid deposition [7]. The microscopic pathological finding of the hippocampus by HE staining showed that the AB rats group had a higher neurodegenerative score (1.83) than the SO rats group (0.67). The results of the study are consistent with previous studies that intrahippocampal Aβ injection causes neurodegeneration, such as Rahman S et al. (2020) showed that the number of degenerated neurons in the AB rats (70) was higher than the control group (5) [6].

CONCLUSION

This study successfully established an AD model using intrahippocampal Aβ injection in rats characterized by learning and memory impairments as well as neurodegeneration in the hippocampus.

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