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hWJ-MSC Reduces IL-1β Gene Expression in Alzheimer's Rat Models

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ABSTRACT

Objective: This study aims to study effect of Wharton's Jelly MSCs regulate IL-1β gene expression and to evaluate the potential of these MSCs as an innovative therapy for Alzheimer's disease;
Method: This study was post test only control group design. Male Wistar rats were divided into three groups: Control, $AlCl_3$, and $AlCl_3$ + hWJ-MSC. AlCl3 (300 mg/kg/day) was administered orally for 5 days. hWJ-MSCs ($1x10^6$ cells) were injected intraperitoneally on day 11. Brain tissue RNA was isolated using TRIzol®, and cDNA synthesis was conducted. Gene expression was analyzed by real-time PCR;
Result: The results of this study showed that the average of IL-1β levels in Negative Control, Positive Control and Treatment were 0.536 ± 0.342, 0.804 ± 0.801, 0.694± 0.303;
Conclusion: Our study indicates that human Wharton's Jelly Mesenchymal Stem Cells (hWJ-MSCs) can reduce IL-1β levels in an Alzheimer's disease model, suggesting their potential to alleviate inflammation. Although the decrease was not statistically significant, these findings open new avenues for exploring hWJ-MSCs as a promising therapeutic option for Alzheimer's disease.

Keywords: *Alzheimer, hWJ-MSC, IL-1β, $AlCl_3$*

INTRODUCTION

Alzheimer's disease is one of the largest degenerative diseases among the elderly and continues to increase as the elderly population grows worldwide. [1-4]
Alzheimer's disease is the leading cause of disability in the elderly and ranks fifth in causing death among the elderly. Severe dementia, which is common in people with Alzheimer's disease, can lead to complications such as immobility, swallowing disorders, and malnutrition, increasing the risk of acute life-threatening conditions. People with Alzheimer's disease are reported to survive four to eight years after diagnosis, but some can live up to 20 years post-diagnosis. This illustrates the slow and uncertain progression of Alzheimer's disease, which results in patients spending the rest of their lives dependent on family or caregivers. [1,5]
Neural tissue damage in Alzheimer's occurs due to the accumulation of amyloid plaques (Aβ) and neurofibrillary tangles (NFT) in neural tissue. The excessive accumulation of Aβ in neural tissue due to gene mutations or imperfect phagocytosis leads to plaque formation, which gradually spreads and covers the neural tissue. This condition stimulates the activation of microglia to

eliminate Aβ and secrete pro-inflammatory cytokines.[6-9]

Exposure to aluminium (Al) in mouse models has frequently been used as an experimental model of neurodegenerative diseases. In animal models, AlCl3 has been shown to promote inflammation, disrupt synaptic function, and cause neurodegeneration in various regions of the brain and spinal cord. Mechanistic studies indicate that inflammation activates the IκB kinase protein, leading to the phosphorylation of inhibitor kappa B (NF-κB). This allows NF-κB dimers to move into the nucleus and bind to promoter sites of specific genes. This pathway is initiated by pro-inflammatory mediators such as interleukin-1 β (IL-1β).[10] Inflammatory cytokines such as IL-1β are released by microglia and contribute to tau hyperphosphorylation. Pro-inflammatory cytokines can act in an autocrine fashion and promote Aβ production and reduce Aβ clearance (Xiu 2015). Research shows that high levels of IL-1β in the brains of Alzheimer's patients are associated with disease severity and faster cognitive decline.[11]

In recent years, research on mesenchymal stem cell (MSC) therapy has attracted great attention in the effort to develop effective treatments for various neurodegenerative diseases, including Alzheimer's. MSCs derived from Wharton's Jelly (WJ) in human umbilical cords show great potential in this field due to their unique regenerative and immunomodulatory properties. The umbilical cord, which is usually cut and discarded, contains stem cells with various advantages. MSCs from Wharton's Jelly can migrate to injured areas, interact with the local microenvironment, and modulate immune responses and inflammatory processes.[12-14] Infused WJ-MSCs are able to recruit microglial cells during the initial acute stage after transplantation in the AD mouse model. After the initial stage, WJ-MSCs maintain a lower number of resident activated microglial cells, despite the pro-inflammatory environment. Wharton's Jelly mesenchymal stem cell (WJ-MSC) treatment significantly reduced pro-inflammatory microglial activation and the expression of pro-inflammatory cytokine IL-1β.[15]

This study aims to further explore the effects of Wharton's Jelly MSCs on IL-1β gene expression in an Alzheimer's mouse model. The Alzheimer's mouse model is used to replicate the main characteristics of this disease in humans, including amyloid plaque accumulation, neurofibrillary changes, and neuroinflammation. The use of this animal model allows for a deeper understanding of the molecular and cellular mechanisms involved in MSC therapy responses.

MATERIALS & METHODS

Experimental Design

This experimental study used a posttest only control group design. The research was conducted from January 2023 to January 2024 at the Animal House, Biomedical and Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Andalas. The protocol of this study was approved by the Ethics Committee of Faculty of Medicine, Universitas Andalas (No. 456/UN.16.2/KEP-FK/2023).

Animal Subjects

Male Wistar rats (Rattus norvegicus) aged 2 months and weighing 200-300 grams were used. The subjects were divided into three groups:

Group I (negative control): standard diet.

Group II (positive control): standard diet with $AlCl_3$.

Group III (treatment): standard diet with $AlCl_3$ and hWJ-MSC.

The sample size was determined using the WHO formula, with 5 rats per group. A total of 18 rats were used, including 1 additional rat per group to account for potential dropouts.

Acclimatization and Treatment

The rats were acclimatized for 7 days in cages with a constant temperature of approximately 26°C and provided with a standard pellet diet daily. On day 7, the rats

were weighed and randomly assigned to the three groups.

Aluminum Chloride Administration

AlCl3 was purchased from Milipore Merck, USA (cat no 1.01084.1000) and administered orally (300 mg/kg body weight/day) for 5 days. On days 6 and 7, the rats were given only the standard diet without $AlCl_3$.

Stem Cell Injection

hWJ-MSC were obtained from the IMERI FKUI laboratory, identified using CD73, CD105, and CD90 markers. Thawing and subculturing to passage 3 were performed at the Biomedical Laboratory, FK Unand. Cells were harvested at 95% confluence and counted using a hemocytometer. On day 11, hWJ-MSC (human Wharton's Jelly Mesenchymal Stem Cells) were injected intraperitoneally at a concentration of 1x10^6 cells in 500 µl PBS.

Y Maze Test

The Y maze test assesses working and short-term spatial memory in animals. The Y-shaped maze has arms 1200 units long. Animals explore the maze, and those with good memory tend to visit new arms. Entry into an arm is counted when all limbs are inside. Alternation is recorded if the animal visits all three arms without repeating the same arm consecutively.

RNA Isolation

Total RNA was isolated from brain tissue using TRIzol® reagent (Thermo Fisher Scientific, CA, USA). Tissues were homogenized and chloroform was added followed by centrifugation. The aqueous phase was transferred to a new tube, and RNA was precipitated with isopropanol. The RNA pellet was washed, resuspended in RNase-free water, and quantified.

cDNA Synthesis and PCR

cDNA was synthesized using the Thermo Fisher Scientific kit (Vilnius, Lithuania). The reaction included 5 µg RNA, 1x RT buffer, 20 pmol oligo(dT), 4 mM dNTPs, 40 U SuperScript II RTase, and Nuclease-Free Water to a final volume of 20 µl. The reaction was incubated at 52°C according to the iScript cDNA synthesis protocol (Bio-Rad). PCR amplification was performed for 40 cycles: initial denaturation at 95°C for 3 minutes, denaturation at 94°C for 5 minutes, followed by 94°C for 45 seconds, 55°C for 30 seconds, 72°C for 45 seconds, and a final extension at 72°C for 7 minutes. [16]

Gene Expression Analysis

Relative quantification of gene expression was calculated using the 2^(-ΔΔCT) method:
- ΔCT (experimental) = CT(target, experimental) – CT(housekeeping, experimental)
- ΔCT (control) = CT(target, control) – CT(housekeeping, control)
- ΔΔCT = ΔCT (experimental) – ΔCT (control)
- Relative gene expression = 2^(-ΔΔCT)

Analysis Statistic

Data normality was tested using the Shapiro-Wilk test (for samples <30). If data were normally distributed, parametric tests were conducted using One-way ANOVA, followed by Post Hoc tests with Least Significant Differences (LSD).

RESULT

After being exposed to AlCl3 for 5 days, the percentage of Y maze in the positive control and treatment groups was lower than the negative control After the treatment group was injected with hWJ-MSC intraperitonealyl and observed 1 month later, the Y maze of the treatment group was almost the same as the negative control group (figure 1).

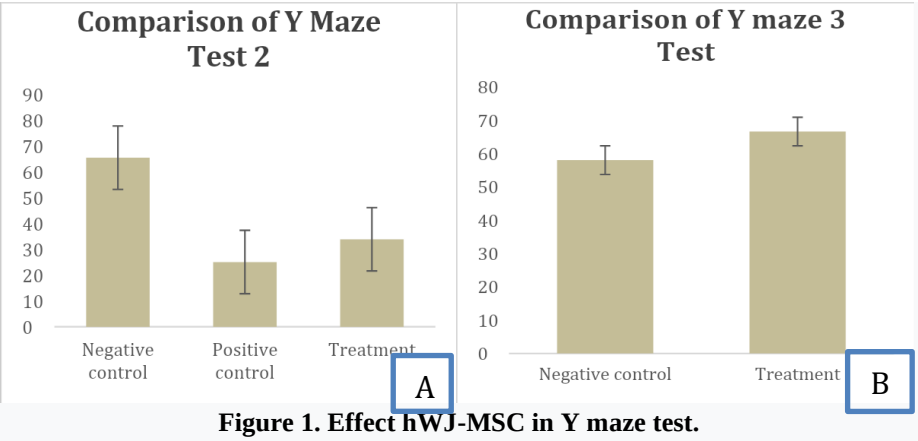

Figure 1. Effect hWJ-MSC in Y maze test.

The results of this study indicate that IL-1β levels increased in the group treated with AlCl3 (positive control) and decreased after treatment with hWJ-MSCs (treatment group) (Figure 2). This outcome suggests a reduction in inflammation levels by hWJ-MSCs, although it was not statistically significant (p value > 0.05).

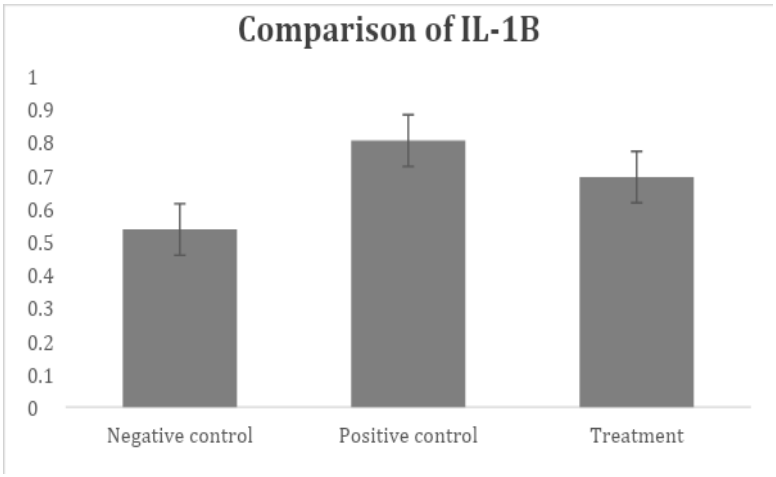

Figure 1. Effect of hWJ-MSC on IL-1β level on alzheimer rat model induced $AlCl_3$.

DISCUSSION

Administration of $AlCl_3$ can induce Alzheimer's-like conditions in experimental animals due to its high toxicity to brain neurons and surrounding cells. The exact mechanism triggering this effect is still unknown, but $AlCl_3$ can easily reach the central nervous system through the blood-brain barrier (BBB), forming stable complexes with L-glutamic acid and accumulating in various regions, particularly the striatum, hippocampus, and cortex, causing damage to neurons and glial cells. Al exposure can alter serotonin, norepinephrine, GABA, dopamine, and glutamate levels, drastically reducing norepinephrine and dopamine levels in the striatum, cerebral cortex, and hippocampus while increasing glutamate and GABA levels in various brain regions. These mechanisms contribute to cognitive impairment and loss of motor function. Al exposure can activate microglial cells, leading to neurotoxicity and neural inflammation through the production of pro-inflammatory cytokines via various pathways, including increased expression of signal transducer and activator of transcription 3 (STAT3), resulting in elevated IL-1β levels.[17-18]

The Y maze test is used to assess the spatial memory function of test animals. Research results show that in Y maze 1, there was no significant difference since no intervention had been applied. After the administration of $AlCl_3$, changes in the behavior and cognition of the test animals were observed, though not statistically significant. This could be due to some animals already having low baseline scores prior to any intervention, affecting the statistical results. In Y maze 3, comparing two groups of animals also yielded statistically insignificant results. This indicates that after treatment with hWJ-MSC, there was no significant difference between the treatment group and the negative control. Focusing on the treatment group, the mean score in maze 1 decreased compared to maze 2, and the mean score in maze 3 increased compared to maze 2. This suggests that $AlCl_3$ induces cognitive dysfunction, which can be ameliorated by hWJ-MSC, although not statistically significant (Figure 1).

The Y maze spatial memory test has several bias factors. Previous research indicates that intrinsic rotation in rats is an element unrelated to memory, having only a marginal effect on the rate of spontaneous alternation in each maze. Quantitative analysis shows that locomotor behavior, such as the number and ratio of arm visits and distance travelled, does not correlate with the alternation rate for each arm. Another confounding factor influencing previous research results is stress in test animals, which can bias the outcomes. The stress level in test animals can affect their brain capacity percentage, explaining why some animals have low Y maze test scores even without any intervention. Therefore, confirming Alzheimer's markers cannot be reliably achieved using only the Y maze test.[19-20]

The results of this study indicate that IL-1β levels increased in the positive control group and decreased after treatment with hWJ-MSCs (treatment group) (Figure 2). This outcome suggests a reduction in inflammation levels by hWJ-MSCs, although it was not statistically significant (p value > 0.05). In this inflammatory state, treatment with hWJ-MSCs was administered intraperitoneally, showing a decrease in IL-1β levels, although not statistically significant. This finding suggests that the potential of hWJ-MSCs as inflammation regulators remains a challenge and requires further evidence, given the numerous inflammatory cytokines associated with alzheimer's disease. The observed reduction in IL-1β following hWJ-MSC administration may be due to the potential of hWJ-MSCs to suppress STAT3 expression, thereby reducing IL-1β levels.[21]

These results are consistent with previous studies, such as Gao H et al., which investigated mesenchymal stem cells using neural stem cell-derived extracellular vesicles, where IL-1β levels decreased after treatment. Similarly, Liuka et al. found that bone marrow-derived mesenchymal stem cells also reduced IL-1β levels in their research.[22-23]

CONCLUSION

Interleukin-1β levels increased in rats with AlCl3-induced neurotoxicity, mimicking Alzheimer's disease, and decreased following treatment with hWJ-MSCs, although the reduction was not statistically significant. This suggests that while hWJ-MSCs have potential anti-inflammatory effects, their efficacy in reducing IL-1β levels in this model requires further investigation.

Declaration by Authors

Ethical Approval: Approved

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