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Original Article

The Effect of Bone Marrow Mesenchymal Stem Cells on Nestin and *Sox-2* Gene Expression and Spatial Learning (Percent Alternation Y-Maze Test) against $AlCl_3$-Induced Alzheimer's-like Pathology in a Rat Model

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Abstract

Background: Alzheimer's disease (AD) is a neurodegenerative condition characterized by gradual cognitive impairment, including loss of synapses and nerve cells involved in learning, memory, and habit formation processes. Bone Marrow Mesenchymal Stem Cells (BM-MSCs) are multipotent cells. Because of their self-renewable, differentiation, and immunomodulatory capabilities, they are commonly used to treat many disorders. Hence, the current study intends to examine the effect of BM-MSCs transplantation on Aluminum chloride ($AlCl_3$)-induced cognitive problems, an experimental model resembling AD's hallmarks in rats.

Methods: The study was conducted in 2022 at The Biomedical Laboratory Faculty of Medicine, Andalas University, Indonesia. Adult male Wistar rats (three groups: negative control; no intervention+treatment with PBS; positive control: $AlCl_3$+treatment with aqua dest; $AlCl_3$+BM-MSCs: $AlCl_3$+treatment with BM-MSCs, n=5 each) were treated daily with $AlCl_3$ orally for five days. Stem cells were intraperitoneally injected into rats at a dose of $1x10^6$ cells/rat. The same quantity of phosphate-buffered saline was given to the control group. One month after stem cell injection, the rat brain tissue was removed and placed in the film bottles that had been created. The expression of neural progenitor cell markers, including nestin and sex-determining Y-box 2 (SOX-2), was analyzed using real-time polymerase chain reaction (RT-PCR). Rats' cognitive and functional memory were examined using Y-maze. Data were analyzed using SPSS software (version 26.0) with a one-way analysis of variance (ANOVA) test.

Results: The gene expression of nestin (29.74±0.42), SOX-2 (31.44±0.67), and percent alternation of Y-maze (67.04±2.28) increased in the $AlCl_3$+BM-MSCs group compared to that in the positive control group. RT-PCR analysis indicated that nestin ($P<0.001$) and SOX-2 ($P<0.001$) were significantly enhanced in the $AlCl_3$+BM-MSCs group compared to the positive control group. This group also indicated an increased percent alternation of Y-maze ($P<0.001$) in the $AlCl_3$+BM-MSCs group compared to the positive control group.

Conclusion: Due to its potential effects on cell therapy, BM-MSCs were found effective in a rat model of AD on the impairment of the rats' behavior and increased expression of neural progenitor cell markers.

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Keywords ● Mesenchymal stem cells ● Neurodegenerative diseases ● Aluminum chloride ● Reverse transcriptase polymerase chain reaction ● Nestin

What's Known

- Stem cell therapy has been investigated as a potential method for treating Alzheimer's disease in animal models.
- Neural cells express nestin as a neural precursor marker.

What's New

- Intraperitoneal injection of bone marrow mesenchymal stem cells improves the efficacy of Alzheimer's disease therapy.
- Bone marrow mesenchymal stem cell injection could cause behavior alterations in rats and increased expression of markers indicative of neural progenitor cells.

Introduction

Alzheimer's Disease (AD) is the primary form of dementia, a neurodegenerative disorder that arises with age and is characterized by progressive memory loss and cognitive impairment. The deposit of amyloid-β (Aβ) peptides that form senile plaques, hyperphosphorylation of tau protein, which forms neurofibrillary tangles, and significant neuronal cell death are all major pathological markers of AD. The hippocampus is the most injured region in the brain during the AD process. It is one of the first areas to be impacted, and the pathological abnormalities are most visible there.[1] Nevertheless, the exact pathomechanisms of AD are not reasonably obvious.

Environmental toxic metals exert well-known effects on brain development. Numerous studies have found that toxic metals are associated with neurodegenerative disorders, including AD and Parkinson's diseases. Aluminum (Al) is one of the poisonous metals linked to neurodegenerative diseases since it disrupts various cellular metabolic pathways in the central nervous system (CNS). This material can be found in packaged foods, toothpaste, medicines, and purified drinking water. The $AlCl_3$-induced mouse model of AD has been reported as the primary animal model commonly used in research on AD in humans.[2]

Merely five medicines have been licensed by the US Food and Drug Administration for AD therapy, including galantamine, donepezil, cholinesterase inhibitors tacrine, rivastigmine, and the glutamate receptor antagonist memantine.[3] These five compounds can only aid with AD symptoms, but they do not stop or reduce the brain pathology of AD nor stop the disease development. Current drug clinical trials are based on "one drug, one mechanism". Meanwhile, the pathogenesis of AD is very complicated. Targeting merely one clinical hallmark, such as Amyloid Beta (Aβ), Tau, or neuroinflammation, is not likely to result in therapeutic results.[4]

Stem cell therapy has been studied as a potential approach for treating AD in animal models. Therapeutic stem cells are categorized into autologous and allogenic groups based on their tissue origins.[5, 6] Autologous stem cells are obtained from the bone marrow, brain, dental pulp, and fat. Allogenic stem cells, on the other hand, are derived from the umbilical cord, placenta, or embryonic tissue. Allogenic stem cells have two limitations. The first is an ethical problem, and the second is allogeneic immunogenicity. As these limitations cannot be handled in the short term, allogeneic stem cells may not be suitable for treating AD. Therefore, autologous bone marrow or fat stem cells are recommended. Remarkably, therapeutic stem cells obtained from bone marrow outperform those obtained from adipose tissue.[7, 8]

Numerous proofs show that Bone Marrow Mesenchymal Stem Cells (BM-MSCs) can help with neurodegeneration, memory loss, and behavioral issues. According to research, decreasing the number of Aβ plaques improves both young and old Tg2576-APP Swe, PS1 M146V (TASTPM) mice. Morris water maze test, Y-maze alternation test, plus-maze discriminative avoidance task, social recognition test, and open-field evaluation demonstrate decreased cognitive impairment (*i.e.*, learning and spatial memory skills).[9] Besides, single gene therapy of bone marrow-derived mononuclear cells might result in an encouraging outcome.

Stem cells have been shown to have medicinal value through paracrine effects.[4] Mesenchymal stem cells (MSCs) therapy have shown increased secretion of neurotrophic and angiogenic factors through paracrine pathways, especially vascular endothelial growth factor (VEGF), glial cell-derived neurotrophic factor (GDNF), insulin growth factor (IGF), and brain-derived neurotrophic factor (BDNF).[10-12] Another significant pathway of MSCs treatment is neuroinflammation regulation. Neuroinflammation is crucial in the etiology of AD. Numerous earlier researches showed that MSCs can transform proinflammatory M1 and A1 phenotypes of microglia and astrocytes into anti-inflammatory M2 and A2 phenotypes.[13, 14]

Given the importance of identifying viable therapeutic alternatives for AD therapy, this research aimed to study the effect of intraperitoneal injection of BM-MSCs on the expression of neurogenesis genes and spatial learning in AlCl3-induced Alzheimer's-like pathology in male rats.

Materials and Methods

Animals

Adult male Wistar rats (n=15), weighing 200-250 g, were purchased from the Animal House of the Biomedical Laboratory Faculty of Medicine, Andalas University, Indonesia. The animals were housed in carefully regulated conditions with a light/dark cycle of 12:12 hours, a temperature of 23 °C, and a humidity level of 60%. They were acclimated for a week and given unrestricted access to water and a regular rat diet before any experimental procedures. The study methodology and regulations were

approved by The Research Ethics Committee of the Medical Faculty of Andalas University, Indonesia (code: 1093/UN.16.2/KEP-FK/2022). Every attempt was made to minimize animal pain.

$AlCl_3$ Preparation

The rats were divided into three groups (n=5 for each group) using a simple randomization method, and $AlCl_3$ was used to induce AD, except for the control group. The Sumatran Biota Laboratory at Andalas University in Indonesia offered $AlCl_3$ (Sigma-Aldrich® Brand, Merck, Germany). $AlCl_3$ was administered orally to rats for 5 days, 300 mg/Kg body weight in 1 mL of distilled water/100 Kg of rats.[15]

Negative control: No intervention+treatment with PBS

Positive control: $AlCl_3$+treatment with *aqua dest*

$AlCl_3$+BM-MSCs: $AlCl_3$+treatment with BM-MSCs

Culture and Cell-Surface Marker Analysis

The BM-MSCs were purchased from the Indonesian Medical Education and Research Institute (IMERI), Faculty of Medicine, Indonesia University. BM-MSCs were verified using Flow Cytometry to assess the expression of MSC-specific surface markers (including CD73, CD90, and CD105).

Intra-Peritoneal Injection of BM-MSCs

To induce anesthesia, intraperitoneal injections of 15 mg/Kg xylazine (Sigma-Aldrich® Brand, Merck, Germany) and 50 mg/Kg ketamine (Sigma-Aldrich® Brand, Merck, Germany) were given to all experimental animal groups.[16, 17] Rats received intraperitoneal injections of stem cells at a dose of $1x10^6$ cells/mice.[1] In the control group, the same quantity of PBS (Sigma-Aldrich® Brand, Merck, Germany) was given to every rat. One month following the stem cell injection, the rat brain tissue was removed and placed in the film bottles that had been created.

Brain Tissue Specimen

Cervical dislocation was employed as a method for sacrificing animals. The brain tissue was aseptically exposed and dissected. Certain specimens were subjected to fixation in a 10% paraformaldehyde solution for histological examination, while others were promptly frozen in liquid nitrogen, and then, stored at a temperature of -80 °C for PCR analysis.[6]

RNA Isolation

The TRIzol reagent (Sigma-Aldrich® Brand, Merck, Germany) was utilized to extract total RNA from all experimental group tissues. The tissues (50-100 mg of tissue per sample) were homogenized with 1 mL of the TRIzol reagent using a homogenizer. The tube was filled with 200 µm of chloroform, then turned upside down and kept at room temperature for 5 min. After that, the samples were centrifuged with a centrifuge (Thermo Scientific, US) at 12,000 g for 15 min at 4 °C, and the top transparent layer was transferred into a fresh, sterile microtube. The mixture was incubated once again for 10 min at room temperature after adding 2x isopropanol (Sigma-Aldrich® Brand, Merck, Germany). At 12,000 g and 4 °C, the centrifugation was repeated for 10 min. After removing the supernatant, the particles were washed with 350 µL of 70% ethanol.

The tube was then progressively inverted and vortexed. It was re-centrifuged for 5 min at 7500 xg and 4 °C. The supernatant was discarded after a 10 min vacuum. The pellets were resuspended in 25–40 µL RNAse Free Water (Sigma-Aldrich® Brand, Merck, Germany) after the vacuum was complete (depending on the number of pellets). Then, RNAs at a concentration of 1000 ng were measured and equated.[13]

Synthesis of cDNA

cDNA was produced using a synthesis kit from Sigma-Aldrich® Brand, Merck, Germany.[4] The full cDNA synthesis mixture included 5 g total RNA, 1x RT buffer, 20 pmol oligodT, 4 mM dNTP, 10 mM DTT, 40 U of SuperScript TMII RTase, nuclease-free water, and a reaction volume of 20 L. Total cDNA synthesis was performed using the manual kit guide at 52 °C for 50 min. The reaction was amplified in a thermal cycler (C1000 Thermal Cycler, Biorad).[18]

PCR Gradient Amplification

All PCR processes were completed within the 40-cycle limit, consisting of a predenaturation phase lasting 3 min at 95 °C, an initial denaturation lasting 5 min at 94 °C, a core cycle comprising 94 seconds at 94 °C, 55 seconds at 55 °C, 45 seconds at 72 °C, and extension lasting 7 min at 72 °C.[19]

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) (CFX96 Real-Time System, Biorad) was used to measure the changes in the mRNA levels of several genes, including nestin and SOX-2. The *GAPDH* gene was utilized as a housekeeping gene. Brain tissue was harvested from rats and ground with a mortar and pestle. Total RNA was extracted and examined as previously reported. Table 1 illustrates the sequence of particular primers.[20]

Table 1: Primer sequences for RT-PCR

Gene name	Cell type	Primer sequence (5'-3')
Nestin	Neural stem cells	F- GAGGTGGCTACATACAGGACTC R- AAGAGAAGCCTGGGAACCTC
SOX-2	Neural stem cells	F- AACGCAAAAACCGTGATGCC R- TTGAGAACTCCCTGCGAAGC
GAPDH	Housekeeping gene	F-TCAACAGCAACTCCCACTCTTCCA R-ACCCTGTTGCTGTAGCCGTATTCA

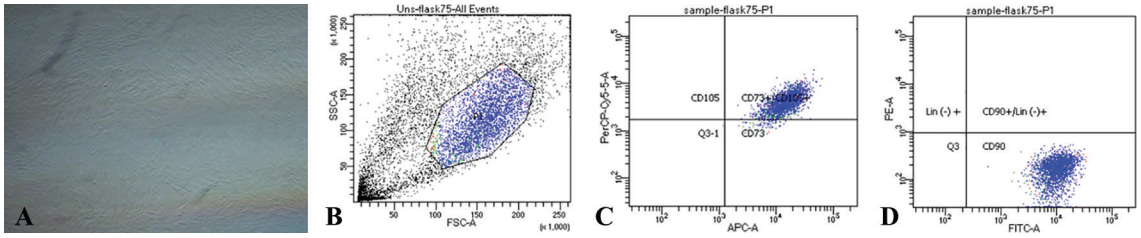

Figure 1: The flow cytometry analysis of human mesenchymal stem cells isolated from bone marrow showed the histograms of CD73, CD90, and CD105 positive expression, and CD14, CD19, CD45, and HLA-DR negative expression. The inverted microscopy showed the growth performance of bone marrow-derived MSCs on fibronectin-coated plastic (A). Plot types include sides scatter (SSC) and forward scatter (FSC) with the 20,000 population-gated occurrences (P1) (B). The cell surface marker expression showed 100% for CD73-APC and 96% for CD105-PerCP-Cy5.5 (C), while the Lin (-)-PE expression was 0%, and CD90-FITC expression was 100% for cell surface markers (D).

Y-Maze Test

Examining the study rats' cognitive and functional memory was the goal of this examination. The research used a wooden contraption with three arms of 75 cm long, 15 cm wide, and 10 cm tall.[21] Each arm was at a 120-degree angle. Following 24 hours of therapy, rats were placed in the center of the device and given 5 min to explore at their own pace. The progression of the arm movements was documented on video. The animals' inclination to enter less recently visited arms and the approaches of various rat arms were then examined. The following equation was used to determine the sudden behavioral changes: 100 (total entries/changes for arms-2).[22]

Statistical Analysis

The results were presented as mean±SEM of five repetitions of the same procedure. Statistical analysis was performed using SPSS software version 26 (IBM, Chicago) with a one-way analysis of variance (ANOVA) followed by the Tukey *post hoc* test. Statistical significance levels were $P<0.05$.

Results

Two to three passes after the primary culture was initially plated, the BM-MSCs were developed into a monolayer of broad, flat cells. The cell became reasonably homogenous in appearance and grew into some elongated or spindle-shaped cells after three passes (figures 1 A and B). The BM-MSCs featured smaller, more spindle-shaped cells mixed in with low-contrast flat cells, giving them a heterogeneous appearance. According to the flow cytometry data, the markers CD73, CD90, and CD105 were present in the cells (figures 1 A, B, C, and D).

Two neural progenitor markers, nestin, and SOX-2, were significantly less expressed in $AlCl_3$ group rats than the control group (figures 2 and 3).

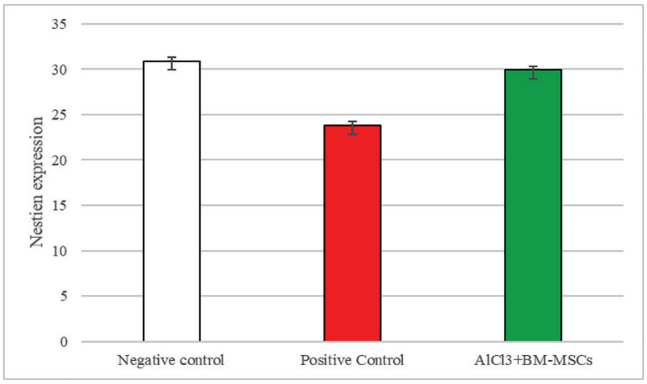

Figure 2: The measured levels of nestin in brain tissue are illustrated. One-way analysis of variance (ANOVA) followed by the Tukey *post hoc* test was used to evaluate significant differences between groups.

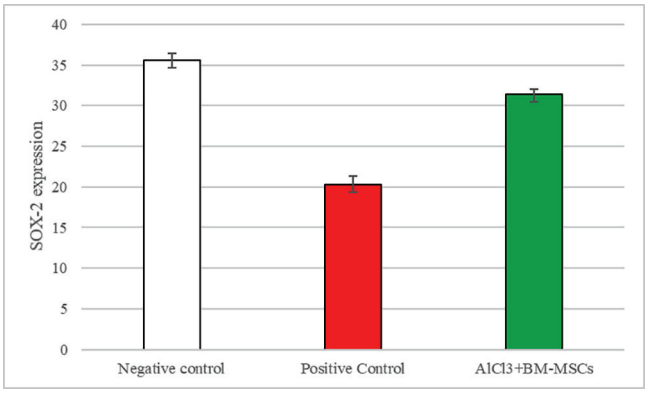

Figure 3: The measured levels of SOX-2 in brain tissue are illustrated. One-way analysis of variance (ANOVA) followed by the Tukey *post hoc* test was used to evaluate significant differences between groups.

Table 2: Gene expression in Alzheimer's rats model after BM-MSCs therapy

Group	Negative control	Positive control		$AlCl_3$+BM-MSCs	
	Mean±SEM	Mean±SEM	P	Mean±SEM	P
Nestin	30.91±0.49	23.77±0.59	<0.001	29.74±0.40	0.027
SOX-2	35.61±0.94	20.30±1.16	<0.001	31.44±0.67	<0.001

Data are expressed as mean±SEM. The scores were analyzed using Tukey *post hoc* analysis. A significant difference was found compared to the negative control group. Statistical significance levels were P<0.05. $AlCl_3$: Aluminum chloride; BM-MSCs: Bone marrow-mesenchymal stem cells

Table 3: Percent alternation of Y-maze test results in Alzheimer's rats model after BM-MSCs therapy

Group	Negative control	Positive control		$AlCl_3$+BM-MSCs	
	Mean±SEM	Mean±SEM	P	Mean±SEM	P
Percent alternation Y-maze test	72.67±2.61	24.00±2.99	<0.001	67.04±2.28	0.014

Data are expressed as mean±SEM. The scores were analyzed using Tukey *post hoc* analysis. Significant differences from the negative control group. Statistical significance levels were P<0.05. $AlCl_3$: Aluminum chloride; BM-MSCs: Bone marrow-mesenchymal stem cells

In the $AlCl_3$+BM-MSCs group, BM-MSCs treatment boosted the expression of the neural progenitor markers (nestin and SOX-2). The nestin expression in the negative control group showed significant differences from the positive control group (P<0.001) and also from the $AlCl_3$+BM-MSCs group (P=0.027). The SOX-2 expression in the negative control group had substantial differences from the positive control group (P<0.001) and also from the $AlCl_3$+BM-MSCs group (P<0.001) (table 2). The percent alternation of the Y-maze test in the negative control group showed substantial differences from the positive control group (P<0.001) and also from the $AlCl_3$+BM-MSCs group (P=0.014) (table 3). BM-MSCs may not only boost nasopharyngeal carcinoma (NPC) survival against $AlCl_3$ toxicity but also may, through modulatory action, cause NPCs to commit to a neuronal fate by inducing neuronal differentiation.

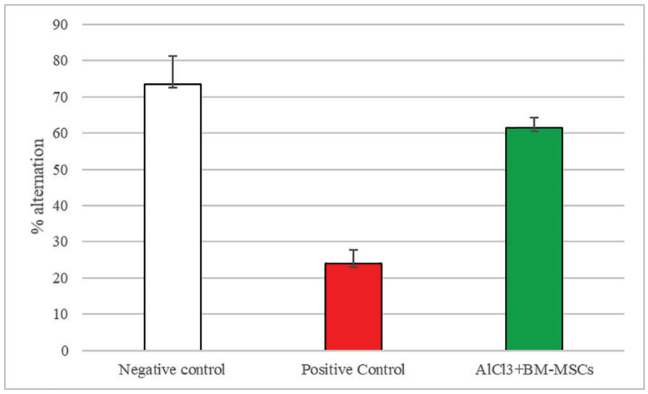

Figure 4: The percent alternation of the Y-maze test is illustrated. One-way analysis of variance (ANOVA) followed by the Tukey *post hoc* test was used to evaluate significant differences between groups.

According to the findings of the behavioral study, rats' propensity for exploring novel settings was tested using the Y-maze test. Typically, rats will opt to explore a new arm of the maze rather than go back to the one they have already studied. Comparing the $AlCl_3$ group with the control and $AlCl_3$+BM-MSCs groups showed a discernible reduction in the number of entries to the intended arm (figure 4).

Discussion

The intraperitoneal injection of BM-MSCs had beneficial effects on rats with AD. Following the intraperitoneal injection of these stem cells, a substantial rise in the nestin and SOX-2 gene expression was observed. This study delves into the therapeutic potential of BM-MSCs in addressing the Alzheimer's-like pathology induced by $AlCl_3$ in a rat model. Given the escalating prevalence of AD and its profound ramifications, there is an urgent need for promising solutions that can mitigate or halt the disease progression.

A pronounced characteristic of AD is the decline in the expression of genes associated with neural repair and proliferation, such as nestin and SOX-2. As per the findings, there was a decrease of 6.17 units in the expression of the nestin gene in the positive control, compared to the negative control. Interestingly, the introduction of BM-MSCs caused reverting of this expression almost to the levels observed in the negative control, with a mere difference of 0.20 units. This aligns with prior research suggesting that mesenchymal stem cells foster neuroprotection by secreting a variety of supportive factors.[23]

Similarly, SOX-2, another focal gene in this study, displayed a parallel trend. Its expression dipped by 15.31 units post-$AlCl_3$ treatment. Yet again, the BM-MSCs intervention resulted in a significant rebound, nearing the expression levels found in the negative control. SOX-2 is renowned for its pivotal role in the sustenance and differentiation of neural stem cells.[24] The outcomes of this study echo the sentiments of related research that vouch for the potential of BM-MSCs in amplifying SOX-2 expression.[25]

Impaired learning and memory functions are clinical telltales of AD. In this research endeavor, the alternation in the Y-maze test percentage served as a barometer for spatial memory function. The data unveiled a marked reduction in the learning and memory aptitudes in the $AlCl_3$-induced group, with an alternation percentage standing at a scanty 24.00%. However, the administration of BM-MSCs led to a recovery rate of almost 43.04% when juxtaposed with the negative control. The literature also echoes this sentiment, showcasing how BM-MSCs have the potential to rejuvenate cognitive faculties.[26]

One pronounced characteristic of AD is the decline in the expressions of genes crucial for neural repair and proliferation. The nestin gene, fundamental to neural stem cells and brain plasticity, demonstrated a significant reduction in its expression in the $AlCl_3$-induced group. This reduction aligns with the observations of Khan and colleagues who reported diminished nestin levels in AD models.[27] However, upon introducing BM-MSCs, this decline was notably countered, corroborating the discoveries of Jo and others (2014).[28] Their work highlighted the neuroprotective attributes of MSCs attributed to the upregulation of vital genes.

In a parallel vein, the SOX-2 gene, pivotal for the maintenance and differentiation of neural stem cells,[29] showcased a similar pattern. Its expression sharply decreased following $AlCl_3$ induction, a trend consistent with reduced SOX-2 levels identified in other AD models.[30] BM-MSCs administration nearly restored it to the baseline levels. This resurgence of SOX-2 expression underscores the reparative capabilities of BM-MSCs, echoing the findings of Zhang and colleagues (2018) that emphasized BM-MSCs role in promoting neural regeneration.[31]

The study's cognitive facet was assessed via the percentage alternation in the Y-Maze test, representing spatial memory. A marked decline post $AlCl_3$ induction mirrored the cognitive impairments prevalent in AD patients.[32] Yet, the introduction of BM-MSCs heralded an encouraging recovery, reflecting the insights of Cui and colleagues that championed the therapeutic potential of stem cells in reviving cognitive functions.[33]

The substantial rebound in gene expressions and cognitive functions following BM-MSCs' intervention underscores the therapeutic promise of these stem cells. This assertion finds resonance in an emerging body of evidence highlighting the MSCs' ability to release neurotrophic factors, curb inflammation, and positively influence the neural milieu for regeneration.[34]

In summation, the results from this study highlight the therapeutic prowess of BM-MSCs in combatting Alzheimer's-like pathology. By reviving the expression levels of nestin and SOX-2 genes and enhancing spatial memory function, BM-MSCs might pave the way for novel treatment modalities for AD.

Nevertheless, it is pivotal to accentuate the inherent limitations of this study. While the

$AlCl_3$-induced rat model is widely recognized, its efficacy in mirroring human AD pathology is still under debate. Hence, expansive research employing diverse models and focusing on human subjects is essential to corroborate the findings of our short-duration (experimental study with a one-month) experimental study. Using an AD model over a long period is recommended for higher data reliability.

Conclusion

Based on these findings, it can be concluded that intraperitoneally transplanted BM-MSCs improved Y-maze alternation and the expression of neural progenitor markers (nestin and SOX-2) in rats with diseases similar to AD, caused by $AlCl_3$. The potential clinical application of MSCs is supported by their solid proliferative capacity, abundance, absence of immunological rejection, and easy intraperitoneal injection. Our study might provide a preclinical basis for using BM-MSCs to treat AD. More research is required to determine the precise molecular pathways and signaling networks associated with the effects of BM-MSCs.

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Authors' Contribution

A: conception and design, performed the computations, investigated the study, and supervised the findings of this work; G.R: conception and design, data analysis; H.A: conception and design, data analysis; Al: conception and design, data analysis. All authors discussed the results and contributed to the manuscript writing. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest: None declared.

References

1 Zeng Q, Zheng M, Zhang T, He G. Hippocampal neurogenesis in the APP/PS1/nestin-GFP triple transgenic mouse model of Alzheimer's disease. Neuroscience. 2016;314:64-74. doi: 10.1016/j.neuroscience.2015.11.054. PubMed PMID: 26639620.

2 Aboelwafa HR, El-Kott AF, Abd-Ella EM, Yousef HN. The Possible Neuroprotective Effect of Silymarin against Aluminum Chloride-Prompted Alzheimer's-Like Disease in Rats. Brain Sci. 2020;10. doi: 10.3390/brainsci10090628. PubMed PMID: 32932753; PubMed Central PMCID: PMCPMC7564174.

3 Si Z, Wang X. Stem Cell Therapies in Alzheimer's Disease: Applications for Disease Modeling. J Pharmacol Exp Ther. 2021;377:207-17. doi: 10.1124/jpet.120.000324. PubMed PMID: 33558427.

4 Hu J, Wang X. Alzheimer's Disease: From Pathogenesis to Mesenchymal Stem Cell Therapy - Bridging the Missing Link. Front Cell Neurosci. 2021;15:811852. doi: 10.3389/fncel.2021.811852. PubMed PMID: 35197824; PubMed Central PMCID: PMCPMC8859419.

5 Lee M, Ban JJ, Yang S, Im W, Kim M. The exosome of adipose-derived stem cells reduces beta-amyloid pathology and apoptosis of neuronal cells derived from the transgenic mouse model of Alzheimer's disease. Brain Res. 2018;1691:87-93. doi: 10.1016/j.brainres.2018.03.034. PubMed PMID: 29625119.

6 Estakhri F, Reza Panjehshahin M, Tanideh N, Gheisari R, Azarpira N, Gholijani N. Efficacy of Combination Therapy with Apigenin and Synovial Membrane-Derived Mesenchymal Stem Cells on Knee Joint Osteoarthritis in a Rat Model. Iran J Med Sci. 2021;46:383-94. doi: 10.30476/IJMS.2020.83686.1301. PubMed PMID: 34539013; PubMed Central PMCID: PMCPMC8438345.

7 Shen Z, Li X, Bao X, Wang R. Microglia-targeted stem cell therapies for Alzheimer disease: A preclinical data review. J Neurosci Res. 2017;95:2420-9. doi: 10.1002/jnr.24066. PubMed PMID: 28643422.

8 Naaldijk Y, Jager C, Fabian C, Leovsky C, Bluher A, Rudolph L, et al. Effect of systemic transplantation of bone marrow-derived mesenchymal stem cells on neuropathology markers in APP/PS1 Alzheimer mice. Neuropathol Appl Neurobiol. 2017;43:299-314. doi: 10.1111/nan.12319. PubMed PMID:

26918424.
9 Qin C, Lu Y, Wang K, Bai L, Shi G, Huang Y, et al. Transplantation of bone marrow mesenchymal stem cells improves cognitive deficits and alleviates neuropathology in animal models of Alzheimer's disease: a meta-analytic review on potential mechanisms. Transl Neurodegener. 2020;9:20. doi: 10.1186/s40035-020-00199-x. PubMed PMID: 32460886; PubMed Central PMCID: PMCPMC7251864.
10 Duncan T, Valenzuela M. Alzheimer's disease, dementia, and stem cell therapy. Stem Cell Res Ther. 2017;8:111. doi: 10.1186/s13287-017-0567-5. PubMed PMID: 28494803; PubMed Central PMCID: PMCPMC5427593.
11 Liu H, Zhang H, Ma Y. Molecular mechanisms of altered adult hippocampal neurogenesis in Alzheimer's disease. Mech Ageing Dev. 2021;195:111452. doi: 10.1016/j.mad.2021.111452. PubMed PMID: 33556365.
12 Mashhouri S, Meysam Abtahi Froushani S, Asghar Tehrani A. Non-Adherent Bone Marrow-Derived Mesenchymal Stem Cells Ameliorate Clinical Manifestations and Inflammation in an Experimental Model of Ulcerative Colitis in Rats. Iran J Med Sci. 2020;45:341-51. doi: 10.30476/ijms.2020.72514.0. PubMed PMID: 33060877; PubMed Central PMCID: PMCPMC7519406.
13 Wei Y, Xie Z, Bi J, Zhu Z. Anti-inflammatory effects of bone marrow mesenchymal stem cells on mice with Alzheimer's disease. Exp Ther Med. 2018;16:5015-20. doi: 10.3892/etm.2018.6857. PubMed PMID: 30542456; PubMed Central PMCID: PMCPMC6257159.
14 Qin C, Li Y, Wang K. Functional Mechanism of Bone Marrow-Derived Mesenchymal Stem Cells in the Treatment of Animal Models with Alzheimer's Disease: Inhibition of Neuroinflammation. J Inflamm Res. 2021;14:4761-75. doi: 10.2147/JIR.S327538. PubMed PMID: 34566422; PubMed Central PMCID: PMCPMC8456430.
15 Sisca DY. Pengaruh pemberian mesenchymal stem cells wharton jelly terhadap ekspresi gen CALM1 dan CASP9 pada tikus alzheimer. Indonesia: Andalas University; 2021.
16 Yu S, Hei Y, Liu W. Upregulation of seladin-1 and nestin expression in bone marrow mesenchymal stem cell transplantation via the ERK1/2 and PI3K/Akt signaling pathways in an Alzheimer's disease model. Oncol Lett. 2018;15:7443-9. doi: 10.3892/ol.2017.7543. PubMed PMID: 29731895; PubMed Central PMCID: PMCPMC5920825.
17 Lee C, Willerth SM, Nygaard HB. The Use of Patient-Derived Induced Pluripotent Stem Cells for Alzheimer's Disease Modeling. Prog Neurobiol. 2020;192:101804. doi: 10.1016/j.pneurobio.2020.101804. PubMed PMID: 32464173.
18 Khan AA, Huat TJ, Al Mutery A, El-Serafi AT, Kacem HH, Abdallah SH, et al. Significant transcriptomic changes are associated with differentiation of bone marrow-derived mesenchymal stem cells into neural progenitor-like cells in the presence of bFGF and EGF. Cell Biosci. 2020;10:126. doi: 10.1186/s13578-020-00487-z. PubMed PMID: 33133516; PubMed Central PMCID: PMCPMC7594431.
19 Kumar K, Kumar A, Keegan RM, Deshmukh R. Recent advances in the neurobiology and neuropharmacology of Alzheimer's disease. Biomed Pharmacother. 2018;98:297-307. doi: 10.1016/j.biopha.2017.12.053. PubMed PMID: 29274586.
20 Ferri AL, Cavallaro M, Braida D, Di Cristofano A, Canta A, Vezzani A, et al. Sox2 deficiency causes neurodegeneration and impaired neurogenesis in the adult mouse brain. Development. 2004;131:3805-19. doi: 10.1242/dev.01204. PubMed PMID: 15240551.
21 Sadatpoor SO, Salehi Z, Rahban D, Salimi A. Manipulated Mesenchymal Stem Cells Applications in Neurodegenerative Diseases. Int J Stem Cells. 2020;13:24-45. doi: 10.15283/ijsc19031. PubMed PMID: 32114741; PubMed Central PMCID: PMCPMC7119211.
22 Zhao Y, Dang M, Zhang W, Lei Y, Ramesh T, Veeraraghavan VP, et al. Neuroprotective effects of Syringic acid against aluminium chloride induced oxidative stress mediated neuroinflammation in rat model of Alzheimer's disease. Journal of Functional Foods. 2020;71:104009.
23 Lee HJ, Lee JK, Lee H, Carter JE, Chang JW, Oh W, et al. Human umbilical cord blood-derived mesenchymal stem cells improve neuropathology and cognitive impairment in an Alzheimer's disease mouse model through modulation of neuroinflammation. Neurobiol Aging. 2012;33:588-602. doi: 10.1016/j.neurobiolaging.2010.03.024. PubMed PMID: 20471717.
24 Bylund M, Andersson E, Novitch BG, Muhr J. Vertebrate neurogenesis is counteracted by Sox1-3 activity. Nat Neurosci. 2003;6:1162-8. doi: 10.1038/nn1131. PubMed PMID: 14517545.
25 Wang X, Yang G. Bone marrow mesenchymal

stem cells-derived exosomes reduce Abeta deposition and improve cognitive function recovery in mice with Alzheimer's disease by activating sphingosine kinase/sphingosine-1-phosphate signaling pathway. Cell Biol Int. 2021;45:775-84. doi: 10.1002/cbin.11522. PubMed PMID: 33300254.

26 Guo S, Perets N, Betzer O, Ben-Shaul S, Sheinin A, Michaelevski I, et al. Intranasal Delivery of Mesenchymal Stem Cell Derived Exosomes Loaded with Phosphatase and Tensin Homolog siRNA Repairs Complete Spinal Cord Injury. ACS Nano. 2019;13:10015-28. doi: 10.1021/acsnano.9b01892. PubMed PMID: 31454225.

27 Khan MB, Hoda MN, Vaibhav K, Giri S, Wang P, Waller JL, et al. Remote ischemic postconditioning: harnessing endogenous protection in a murine model of vascular cognitive impairment. Transl Stroke Res. 2015;6:69-77. doi: 10.1007/s12975-014-0374-6. PubMed PMID: 25351177; PubMed Central PMCID: PMCPMC4297613

28 Koura SM, Salama M, El-Hussiny M, Khalil MEA, Lotfy A, Hassan SA, et al. Fluoxetine induces direct inhibitory effects on mesenchymal stem cellderived osteoprogenitor cells independent of serotonin concentration. Mol Med Rep. 2019;19:2611-9. doi: 10.3892/mmr.2019.9924. PubMed PMID: 30720108; PubMed Central PMCID: PMCPMC6423613.

29 Ellis P, Fagan BM, Magness ST, Hutton S, Taranova O, Hayashi S, et al. SOX2, a persistent marker for multipotential neural stem cells derived from embryonic stem cells, the embryo or the adult. Dev Neurosci. 2004;26:148-65. doi: 10.1159/000082134. PubMed PMID: 15711057.

30 Luo Y, Coskun V, Liang A, Yu J, Cheng L, Ge W, et al. Single-cell transcriptome analyses reveal signals to activate dormant neural stem cells. Cell. 2015;161:1175-86. doi: 10.1016/j.cell.2015.04.001. PubMed PMID: 26000486; PubMed Central PMCID: PMCPMC4851109.

31 Zhang R, Liu Y, Yan K, Chen L, Chen XR, Li P, et al. Anti-inflammatory and immunomodulatory mechanisms of mesenchymal stem cell transplantation in experimental traumatic brain injury. J Neuroinflammation. 2013;10:106. doi: 10.1186/1742-2094-10-106. PubMed PMID: 23971414; PubMed Central PMCID: PMCPMC3765323.

32 Cacabelos R. Pharmacogenomics of Cognitive Dysfunction and Neuropsychiatric Disorders in Dementia. Int J Mol Sci. 2020;21. doi: 10.3390/ijms21093059. PubMed PMID: 32357528; PubMed Central PMCID: PMCPMC7246738.

33 Vizoso FJ, Eiro N, Cid S, Schneider J, Perez-Fernandez R. Mesenchymal Stem Cell Secretome: Toward Cell-Free Therapeutic Strategies in Regenerative Medicine. Int J Mol Sci. 2017;18. doi: 10.3390/ijms18091852. PubMed PMID: 28841158; PubMed Central PMCID: PMCPMC5618501.

34 Kim DS, Lee MW, Yoo KH, Lee TH, Kim HJ, Jang IK, et al. Gene expression profiles of human adipose tissue-derived mesenchymal stem cells are modified by cell culture density. PLoS One. 2014;9:e83363. doi: 10.1371/journal.pone.0083363. PubMed PMID: 24400072; PubMed Central PMCID: PMCPMC3882209.