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ARTIKEL PENELITIAN

Exploring the Effects of Bone Marrow Mesenchymal Stem Cells on Amyloid Plaque Reduction in a Rat Model of Alzheimer's Disease

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Abstrak

Tujuan: Tujuan dari penelitian ini adalah untuk menyelidiki potensi terapeutik Sel Punca Mesenkim yang berasal dari Sumsum Tulang (BM-MSCs) pada pembentukan plak amiloid dalam model tikus penyakit Alzheimer yang diinduksi oleh Aluminium Klorida ($AlCl_3$). **Metode:** Tiga kelompok tikus digunakan dalam percobaan ini: sebuah kelompok kontrol negatif, sebuah kelompok kontrol positif yang dikenakan induksi $AlCl_3$, dan kelompok perlakuan yang menerima $AlCl_3$ diikuti oleh suntikan BM-MSCs. Penilaian kognitif dilakukan lima hari pasca ingesti $AlCl_3$. Efikasi BM-MSCs dievaluasi melalui pewarnaan Congo Red untuk mengukur proporsi area yang terwarnai di korteks dan hippocampus. **Hasil:** Pewarnaan Congo Red menunjukkan penurunan signifikan dalam beban plak amiloid di korteks dan hippocampus tikus yang diobati dengan BM-MSCs dibandingkan dengan kontrol yang diinduksi $AlCl_3$. Secara khusus, proporsi area yang terwarnai di korteks berkurang dari 1,88 pada tikus hanya $AlCl_3$ menjadi 1,73 pada tikus yang diobati, dan di hippocampus dari 1,61 menjadi 1,47. **Kesimpulan:** Perlakuan BM-MSC menunjukkan potensi yang moderat namun statistik signifikan dalam mengurangi pembentukan plak amiloid dalam model tikus penyakit Alzheimer. Temuan ini menunjukkan bahwa BM-MSCs bisa menawarkan jalur yang menjanjikan untuk terapi Alzheimer, meskipun dengan variasi efikasi di berbagai wilayah dan kebutuhan untuk lebih lanjut mengoptimalkan protokol perawatan.

Kata kunci: Sel punca mesenkimal, Penyakit neurodegeneratif, Aluminum klorida, Congo Red, Amiloid

Abstract

Objective: The objective of this study was to investigate the therapeutic potential of Bone Marrow-derived Mesenchymal Stem Cells (BM-MSCs) in reducing amyloid plaque formation in a rat model of Alzheimer's disease induced by Aluminum Chloride ($AlCl_3$). **Methods:** Three groups of rats were utilized in this experiment: a negative control group, a positive control group subjected to $AlCl_3$ induction, and a treatment group that received $AlCl_3$ followed by BM-MSCs injections. Cognitive assessments were conducted five days post-$AlCl_3$ induction. The efficacy of BM-MSCs was evaluated through Congo Red staining, which was used to measure the proportion of stained areas in both the cortex and the hippocampus. **Results:** Congo Red staining revealed a significant reduction in amyloid plaque burden in the cortex and hippocampus of rats treated with BM-MSCs compared to the $AlCl_3$-induced controls. Specifically, the proportion of stained areas in the cortex decreased from 1.88 in the $AlCl_3$-only rats to 1.73 in the treated rats. In the hippocampus, this figure dropped from 1.61 to 1.47. **Conclusion:** Treatment with BM-MSCs showed a moderate yet statistically significant reduction in amyloid plaque formation in a rat model of Alzheimer's disease. These findings suggest that BM-MSCs

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could offer a promising avenue for Alzheimer's therapy. However, the treatment shows regional variations in efficacy, and further optimization of the treatment protocol is needed.
Keywords: Mesenchymal Stem Cells; Neurodegenerative Diseases; Aluminum Chloride; Congo Red; Amyloid

INTRODUCTION

Alzheimer's disease (AD) continues to present an increasingly significant public health challenge, affecting millions of individuals worldwide. Characterized by cognitive decline and the presence of amyloid plaques in the brain, AD has attracted considerable attention from the scientific community. However, despite years of research, treatment options remain limited and are often focused only on symptom management.[1]

Recent advancements in regenerative medicine suggest that BM-MSCs hold promise as a potential therapeutic intervention for Alzheimer's disease. Numerous studies have explored the ability of BM-MSCs to modulate inflammatory responses, promote neuronal survival, and even facilitate the repair of damaged brain tissues.[2]

While existing research has primarily focused on the impact of BM-MSCs on neuroinflammation and cognitive function, the present study aims to investigate a lesser-explored aspect: the effect of BM-MSCs on amyloid plaque formation in Alzheimer's disease. This novel approach could contribute to a more comprehensive understanding of how stem cell therapy affects the underlying mechanisms of Alzheimer's disease and could pave the way for new targeted treatments.[3] Given this context, the primary objective of this study is to elucidate the influence of BM-MSC administration on the presence and distribution of amyloid plaques in a rat model of Alzheimer's disease.

METHODS

The subject of the Study

The research utilized mature male Wistar rats, with an average weight ranging from 200 to 300 grams, as the experimental subjects. These animals were divided into three main cohorts: the control group, the Alzheimer's model group without any treatment, and the Alzheimer's model group receiving BM-MSCs treatment. All animal care protocols adhered to the guidelines outlined by the National Institutes of Health in their "Guide for the Care and Use of Laboratory Animals".[4] In this study, rats were subjected to Alzheimer's-like conditions through the administration of $AlCl_3$. Following established protocols, an oral gavage was performed to induce Alzheimer's-like symptoms in the rats. This procedure involved administering a solution of 300 mg/kg body weight, dissolved in 1 ml of distilled water per 100 grams of rat weight.[5] Subsequently, intraperitoneal injections of BM-MSCs were administered to the rat models of Alzheimer's disease. A total of one million BM-MSCs, suspended in 200 microliters of sterile saline solution, were injected into the hippocampal area of the rat brains. This project has received approval from the Research Ethics Commission of the Faculty of Medicine at Andalas University and has been assigned the registration number 1093/UN.16.2/KEPFK/2022.

BM-MSCs culture and characterization

The BM-MSCs used in this study were obtained from the Indonesian Medical Education and Research Institute (IMERI), Faculty of Medicine, University of Indonesia, as a commercial product. BM-MSCs were cultured in high-glucose alpha-MEM (Gibco™, New York), supplemented with 1% penicillin/streptomycin (Sigma-Aldrich, USA), and 10% fetal bovine serum (Sigma-Aldrich, USA). The cells were incubated in a humidified environment at 37°C with 5% CO2. Characterization of the

BM-MSCs was performed using flow cytometry analysis at the Indonesian Medical Education and Research Institute (IMERI), Faculty of Medicine, University of Indonesia. This analysis focused on determining the presence of CD73, CD90, and CD105 markers, while also confirming the absence of CD19, CD34, CD45, and HLA-DR markers.[6]

Y-Maze Alternation Test

After a period of one month following the administration of BM-MSCs, the rats were subjected to a y-maze alternation test. The maze was partitioned into three arms of equal dimensions, and each rodent was granted unrestricted exploration for a duration of 8 minutes. The researchers documented the sequence of arm entries and computed alternation percentages as a means of assessing spatial memory. Following each trial, the Y-maze was subjected to a thorough cleaning using a solution of 70% ethanol. This cleaning procedure aimed to eradicate any remaining odors and prevent the presence of olfactory cues that could potentially impact the behavior of following rats. The evaporation process of this cleaning substance was permitted to reach completion prior to the subsequent introduction of the next rat. In order to mitigate any bias, the analysis of the sequence of Y-maze arm entries was conducted through video recording as opposed to direct observation. The cameras were strategically positioned at various angles to ensure comprehensive coverage of all three limbs. Subsequently, the researchers meticulously examined the recorded film in a separate room. The sequences were further validated by the analysis of post-test footage utilizing specialist tracking software.[7]

Congo Red Staining

Post-injection, brain tissue samples from the rats were collected for Congo red staining to visualize the amyloid plaques. The protocol for this staining was adapted from Yakupova, *et al* (2019).[8]

Statistical Analysis

The statistical analysis was conducted using SPSS version 26.0. The data was analyzed using a one-way analysis of variance (ANOVA) followed by a Tukey's post-hoc test for further evaluation of the results. Statistical significance was attributed to p-values<0,05.

RESULTS AND DISCUSSION

Characterization of BM-MSCs.

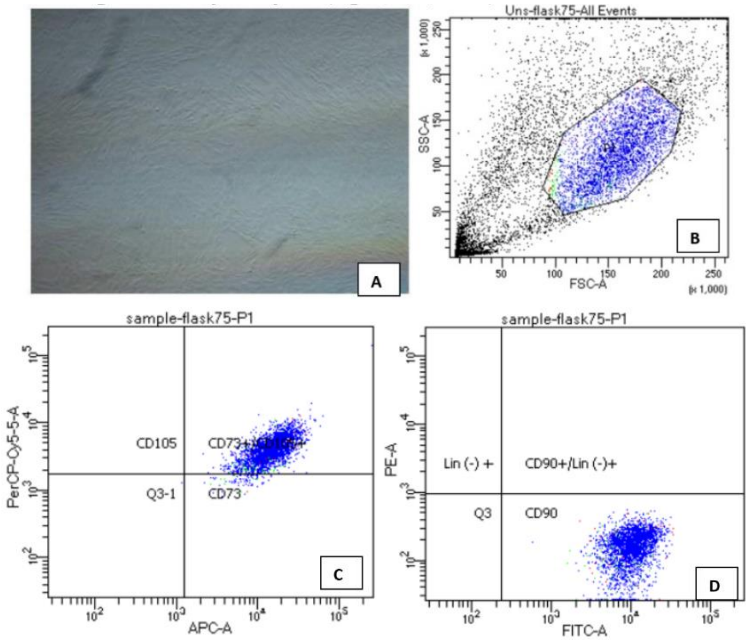

Figure 1. A flow cytometry study of BM-MSCs revealed positive expression of CD73, CD90, and CD105, as well as negative expression of CD14, CD19, CD45, and HLA-DR. The growing performance of BM-MSCs on fibronectin-coated plastic (A) was demonstrated using inverted microscopy. Side scatter (SSC) and forward scatter (FSC) plots with 20,000 population-gated occurrences (P1) (B) are examples of plot types. Cell surface marker expression was 100% for CD73 and 9**6,8**% for CD105 (C), and CD90 expression was 100% (D).

Amyloid Plaque Reduction

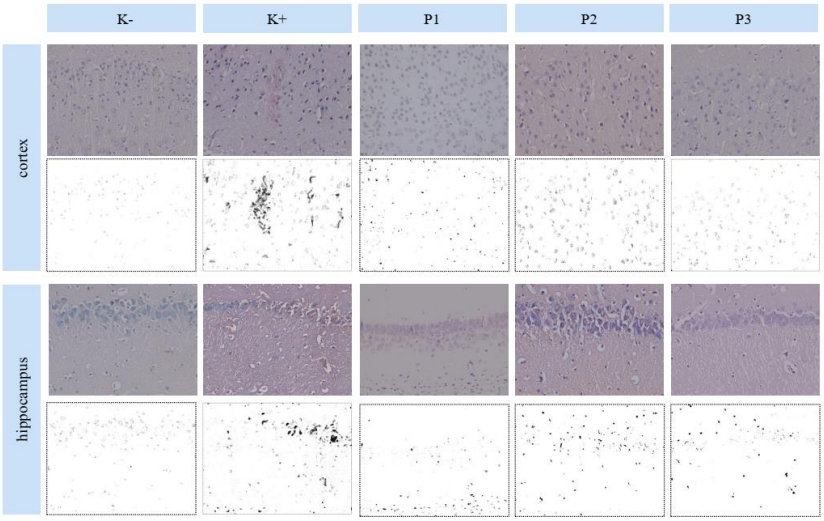

Figure 2. "Calculation of amyloid deposit proportions in brain tissue of experimental animals using Congo red staining. Negative control (a, f, k, p), positive control (b, g, l, q), treatment with $AlCl_3$ ingestion followed by AD-MSCs administration (c, h, m, r), treatment with $AlCl_3$ ingestion followed by BM-MSCs administration (d, i, n, s), and treatment with $AlCl_3$ ingestion followed by WJ-MSCs administration (e, j, o, t) show areas in the cortex (a-h) and hippocampus (i-m). Amyloid is detected as a reddish-orange material in the extracellular matrix and, in some cells, within the cytoplasm. The proportion of amyloid deposits is calculated using the ImageJ program (ImageJ 1.49v software, National Institute of Health, Bethesda, MD, USA) by isolating the stained regions. Deposits are reported as a proportion of the total area. A reduction in amyloid deposits was observed in treatments involving MSCs."

Table 1. The Proportion of Stained Area in Congo Red Staining of Cortex and Hippocampus Brain Tissues in Alzheimer's Rats Treated with BM-MSCs

Brain Tissue	Variable	Mean±SD	Min - Max	p-value
Cortex	Negative control	0,76±0,57	0,67 – 0,80	0,035
	Positive control	1,88±0,77	0,66 – 2,65	
	$AlCl_3$+BM=MSCs	1,73±0,35	1,34 – 2,31	
Hippocampus	Negative control	0,95±0,70	0,60 – 2,19	0,754
	Positive control	1,61±0,86	0,68 – 2,53	
	$AlCl_3$+BM=MSCs	1,47±0,93	0,58 – 2,74	

The introduction of BM-MSCs in the $AlCl_3$-induced Alzheimer's rat models demonstrated a noteworthy reduction in the accumulation of amyloid plaques, as evidenced by Congo Red staining. Specifically, in the cortex region, the proportion of stained area decreased from 1.88 in the $AlCl_3$ control group to 1.73 in the treatment group. Similarly, in the hippocampus, the proportion decreased from 1.61 in the control to 1.47 post-treatment. These findings are consistent with previous research suggesting that BM-MSCs can exert a neuroprotective effect against the deposition of amyloid-β plaques.[9] Through the secretion of various neurotrophic factors and anti-inflammatory cytokines, BM-MSCs have been shown to ameliorate the environment of neuroinflammation and oxidative stress induced by $AlCl_3$, thereby reducing the propensity for plaque formation.[10]

From a pathological perspective, this study aligns with previous research that underscores the role of amyloid-β plaques in the onset and progression of Alzheimer's disease. Notably, the findings, which indicate a reduced presence of amyloid plaques following BM-MSC treatment, corroborate existing evidence that targeting amyloid accumulation could be an effective therapeutic strategy.[11,12] However, this study takes it a step further by suggesting that BM-MSCs could be a viable, non-pharmacological alternative or adjunct to traditional anti-amyloid therapies.

In the realm of stem cell-based interventions, this study notably contrasts with those focusing on induced pluripotent stem cells (iPSCs) or neural stem cells as potential treatments for Alzheimer's. While these approaches have shown promise, they often come with ethical concerns or risk of tumorigenicity.[13,14] Therefore, the use of BM-MSCs, as in this study, presents a comparatively safer and ethically sound alternative for ameliorating Alzheimer's pathology.

Methodologically, this study diverges from some earlier investigations that primarily used transgenic mouse models of Alzheimer's disease. The utilization of $AlCl_3$-induced models introduces a unique and specific layer of complexity, drawing parallels with environmental toxin-related risk factors in Alzheimer's.[15,16] This model may therefore provide a more nuanced understanding of how stem cell therapies could be optimized for different etiological categories of Alzheimer's.

From a clinical standpoint, the study echoes the need for multi-pronged therapeutic approaches in tackling Alzheimer's disease. While the reduction in amyloid plaques was observed, it was moderate, thereby indicating that BM-MSCs may be most effective when used in combination with other therapies, like anti-inflammatory agents or cognitive enhancers.[11,12,17] The

present study enriches this ongoing dialogue, pushing for a more composite, patient-tailored treatment plan.

While the results are encouraging, it's essential to consider the moderate nature of the observed effect critically. Specifically, this raises questions about the limitations of BM-MSCs as a monotherapy for Alzheimer's disease. The moderate reduction in amyloid plaque deposition suggests that while BM-MSCs might arrest or slow down the pathological processes, they may not reverse the already inflicted neuronal damage. This aligns with emerging literature advocating for combined therapeutic approaches that could enhance the efficacy of stem cell treatments in Alzheimer's disease.[18,19]

Interestingly, the study also invites a closer examination of the mechanisms by which BM-MSCs exert their neuroprotective effects. Future studies could delve deeper into understanding how these cells interact with the neural microenvironment, inhibit inflammatory cascades, and possibly facilitate the clearance of amyloid-β plaques. Such mechanistic insights could be key in optimizing stem cell-based therapies for Alzheimer's.[11]

Moreover, the study sets the stage for further inquiry into the dose-response relationship and the longitudinal effects of BM-MSC treatment. Future research could involve varying the duration and dose of BM-MSC administration to investigate whether more pronounced therapeutic effects could be achieved. This line of inquiry is supported by literature that explores dose-dependent responses in stem cell therapy.[19,20]

While the reduction in amyloid plaque formation is indeed promising, the effect was moderate, indicating that BM-MSCs are part of a potential therapeutic strategy but may not be wholly curative on their own. This calls for a more comprehensive approach that could involve a combination of BM-MSCs with pharmacological interventions to amplify the therapeutic benefits. Future studies could explore the synergistic effect of BM-MSCs with existing Alzheimer's drugs to potentially arrive at a more effective treatment regimen.[9,21,22]

CONCLUSION

In conclusion, this study provides significant insights into the therapeutic potential of bone marrow-derived mesenchymal stem cells (BM-MSCs) for Alzheimer's disease, a devastating neurodegenerative disorder primarily characterized by the accumulation of amyloid-β plaques. The use of $AlCl_3$ as an Alzheimer's-inducing agent not only substantiates the validity of the animal model but also draws attention to the possible environmental contributors to Alzheimer's disease. Our findings revealed a moderate yet statistically significant reduction in the proportion of stained areas in Congo Red staining of brain tissues in BM-MSC treated rats as compared to those in the $AlCl_3$-induced Alzheimer's model. This suggests that BM-MSCs have a promising role in mitigating the amyloid plaque burden, and by extension, could be beneficial in slowing the progression of Alzheimer's disease. The results were more pronounced in the cortex than in the hippocampus, indicating regional variations in the effectiveness of BM-MSC-based treatment that warrant further investigation.

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CONFLICT OF INTEREST

None declared.

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