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Mechanisms of Mesenchymal Stem Cell Therapy-Decreased Endothelial Nitric Oxide Synthase Expression level in Ovalbumin-induced Allergic Rhinitis Mice Models

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KEYWORDS

Allergic rhinitis, Mesenchymal stem cells, eNOS, Ovalbumin, Nitric oxide, NF-κB signaling

ABSTRACT:

Introduction: Allergic rhinitis (AR) is a prevalent inflammatory condition characterized by nasal symptoms in response to allergen exposure. Endothelial nitric oxide synthase (eNOS) and nitric oxide (NO) signaling have been implicated in the pathogenesis of AR. Mesenchymal stem cell (MSCs) therapy has shown promise in treating AR, but the mechanisms underlying the induction of eNOS levels by MSCs in AR remain unclear.

Objectives: In this study, an ovalbumin-induced AR animal model was used to investigate the effect of MSCs on eNOS expression.

Methods: An OVA-induced allergic rhinitis rat model was used. MSCs were administered intraperitoneally, and nasal tissues were analyzed for eNOS expression. Bioinformatic analysis was conducted to identify related regulatory genes.

Results: The results demonstrated that MSCs administration significantly decreased eNOS expression in the nasal tissues of AR animals compared to the control group. Possible mechanisms for this effect include the immunomodulatory properties of MSCs, their paracrine effects on resident cells, and activation of the Nf-kB signalling pathway. Bioinformatic analysis identified 10 potential genes STAT3, RELA, IL6, TNF, IL10, NFKB1, TRL4, MAPK1, TGFB1, and CXCL8 involved in the MSC-mediated regulation of eNOS in AR.

Conclusions: Understanding these mechanisms will improve our knowledge of MSC-based therapy and facilitate the development of targeted treatments for AR and other allergic diseases. Further research is needed to elucidate the specific factors and signaling pathways involved in the induction of eNOS by MSCs in AR..

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1. Introduction

Allergic rhinitis (AR) is a prevalent inflammatory condition characterized by nasal congestion, itching, sneezing, and rhinorrhea in response to exposure to specific allergens(Varshney & Varshney, 2015). It affects a substantial portion of the global population and significantly impacts individuals' quality of life (Deka et al., 2021; Sin & Togias, 2011; Small et al., 2018). Recent study reported that the pivotal role of endothelial nitric oxide synthase (eNOS) in the development and progression of allergic diseases, including AR (Tran et al., n.d.). eNOS is an enzyme responsible for the synthesis of nitric oxide (NO), a versatile signaling molecule that plays a crucial role in various physiological processes (Sapsaprang et al., 2019). NO has been shown to exert both pro-inflammatory and anti-inflammatory effects, depending on its concentration and site of action (Janaszak-Jasiecka et al., 2023).

The expression and activity of eNOS have been found to be altered in the nasal mucosa of individuals with AR 8. Studies have demonstrated increased eNOS expression and NO production in nasal epithelial cells, as well as elevated eNOS-derived NO levels in nasal secretions of AR patients during allergen exposure (Janaszak-Jasiecka et al., 2023; Ren et al., 2019). These findings suggest a potential involvement of eNOS-mediated NO signaling in the initiation and perpetuation of nasal mucosal inflammation characteristic of AR. Histamine released by mast cells not only regulates dendritic cell response but also generate iNOS expression, nitrite oxide (NO) production, promotes loss of mitochondrial membrane potential, and produces ROS (Eifan & Durham, 2016; Korchak et al., 2022; Li et al., 2017; Novoselova et al., 2006)9. In addition, eNOS-derived NO has been implicated in modulating various immune and inflammatory processes relevant to AR (Certo et al., 2021; Ibiza & Serrador, 2008).

Mesenchymal stem cell (MSCs) therapy has emerged as a promising approach for the treatment of various inflammatory diseases, including allergic disorders (Hartanto et al., 2022; Restimulia et al., 2021, 2022; Yeole et al., 2013). MSCs have a variety of secrete paracrine factors that promote tissue repair, and suppress inflammation in AR (Hamra et al., 2021; Hartanto et al., 2022; Prajoko et al., 2022; Rantam et al., 2020; Ray, 2012; Selvi, 2017). Previous study reported that MSCs significantly decrease in the levels of eNOS and NO in the nasal mucosa, as well as a significant improvement in the symptoms of AR (Cahyono et al., 2021; Darlan et al., 2022; Drawina et al., 2022; Krainer & Glieder, 2015; Zukhiroh et al., 2022). MSCs may modulate eNOS expression and activity, thereby influencing the production and availability of NO, and subsequently, the inflammatory milieu within the nasal mucosa. However, the effect of MSCs exert their regulatory effects on eNOS levels in AR remain largely unexplored. Therefore, this study aims to evaluate the effect of MSCs on the regulation of eNOS level in the AR animal model..

2. Methods

Allergic rhinitis animal model

In this post-test only control group study design was conducted in Stem Cell and Cancer Research (SCCR) Indonesia under approved by the Ethic Committee of Universitas Sumatera Utara. (142/KEP/USU/2020). Twenty male Wistar rats aged 6 to 8 weeks were reared under controlled conditions, with a consistent 12-hour light-dark cycle and unrestricted access to OVA-free food and water. All mice used in this study were handled in accordance with an approved protocol and were randomly assigned to three groups: the control group, the sham group, the OVA+MSCs 3x106 group (T1), and the OVA+MSCs 6x106 group (T2). To induce allergic rhinitis, the mice were initially sensitized by intraperitoneal (i.p.) injection of 1 mg of OVA (Sigma-Aldrich, St. Louis, MO, USA) and 2.25 mg of aluminum hydroxide gel (alum adjuvant; Thermo Fisher Scientific, Waltham, MA, USA) in 100 µL of sterile saline on days 0, 1, and 10. Following systemic sensitization, the mice were locally challenged by intranasal (i.n.) administration of 50 µg/10 µL of OVA into their nostrils from days 15 to 21. On day 21, MSCs were intraperitoneally administered to OVA- sensitized rats, while the control group received a saline (NaCl) injection. The mice was sacrificed 3 (day 24) and 6 (day 27) days after MSCs treatment.

Immunohistochemistry

The nasal cavity paraffin-embedded sections (5 µm) were deparaffinized, extensively washed in PBS, blocked for 1 h with donkey serum, and incubated overnight at 4 °C with a rabbit anti-mouse eNOS polyclonal antibody (1:100 dilution; Thermo Fisher,

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Rockford, IL) in a humidified chamber. The slides were washed and incubated with a horseradish peroxidase-conjugated secondary antibody (1:200) from Trekkie Universal Link (Starr Trek Universal- HRP Detection Kit) for 60 min at room temperature as described previously (Tjipta et al., 2022). Immunostaining was developed with 1:400 diaminobenzidine (DAB). Mayer hematoxylin (Bio-Optica Milano S.p.A) was used as the counterstain. Representative images were obtained using an Olympus bright-field microscope, the low-magnification images were taken with a 4x objective, and the high-magnification images were taken with a 400x.

Datasets and Identification of Differentially Expressed Genes

The gene expression profiles analyzed in this study were obtained from the GEO (The Gene Expression Omnibus) database (https://www.ncbi.nlm.nih.gov/geo/). The gene involved in the eNOS regulation on AR was collected from PubMed with keywords “eNOS in Allergic Rhinitis”, and sorted by homosapien species (Hermansyah et al., 2021; Mursiti et al., 2021).

Gene Ontology and Kyoto Encyclopedia of Genes and Genomes Enrichment Analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotations were used (Amalina et al., 2021), and followed by the Database for Annotation,Visualization and Integrated Discovery (David, http://david.abcc.ncifcrf.gov/) online tool to perform enrichment analysis, to calculate the p-value, and to perform FDR correction on p-value. P-values ≤ 0.05 and gene counts >5 were considered significantly enriched (Jenie et al., 2019).

Protein–Protein Interaction Network Construction

We use the online database STRING to construct the Protein–protein interaction (PPI) network of DEGs, followed by the MCODE plug-in of Cytoscape software to perform module analysis on the constructed PPI network (node score cut-off = 0.2; max. depth = 100; k-core = 10). The CytoHubba plug-in was used for hub gene analysis was used for gene sequencing (Darlan et al., 2021; Weiss & Dahlke, 2019).

Statistical analysis

A statistical analysis was conducted with a significance level (α) of 0.05 or 5%, utilizing the Shapiro-Wilk test to assess the normality of the data. Immunofluorescent images were captured and subjected to analysis using Image J software from the National Institutes of Health, USA. The data obtained in this study demonstrated normal distribution and homogeneity. Consequently, differences between groups were evaluated through one-way ANOVA, followed by a post hoc test using the least significant difference (LSD) method.

3. Results

MSCs reduced eNOS expression on OVA-induced AR

In this study, we investigated the effects of different doses of MSCs on the expression level of eNOS in an OVA-induced allergic rhinitis mice model. The results revealed a non-significant decrease in eNOS expression level in the 3x106 MSCs treated groups compared to the NaCl group. However, the highest doses of MSCs (6x106) significantly decreased the eNOS level up to 8.01□2.05% compare to NaCl group (Figure 1). This phenomenon maybe due to the fact that on day 3, the mice were still in the inflammatory phase, and therefore the eNOS levels had not yet been influenced by MSCs. However, it is evident that there is a decreasing trend, indicating that MSCs may have the potential to lower eNOS levels, especially with longer duration of therapy.

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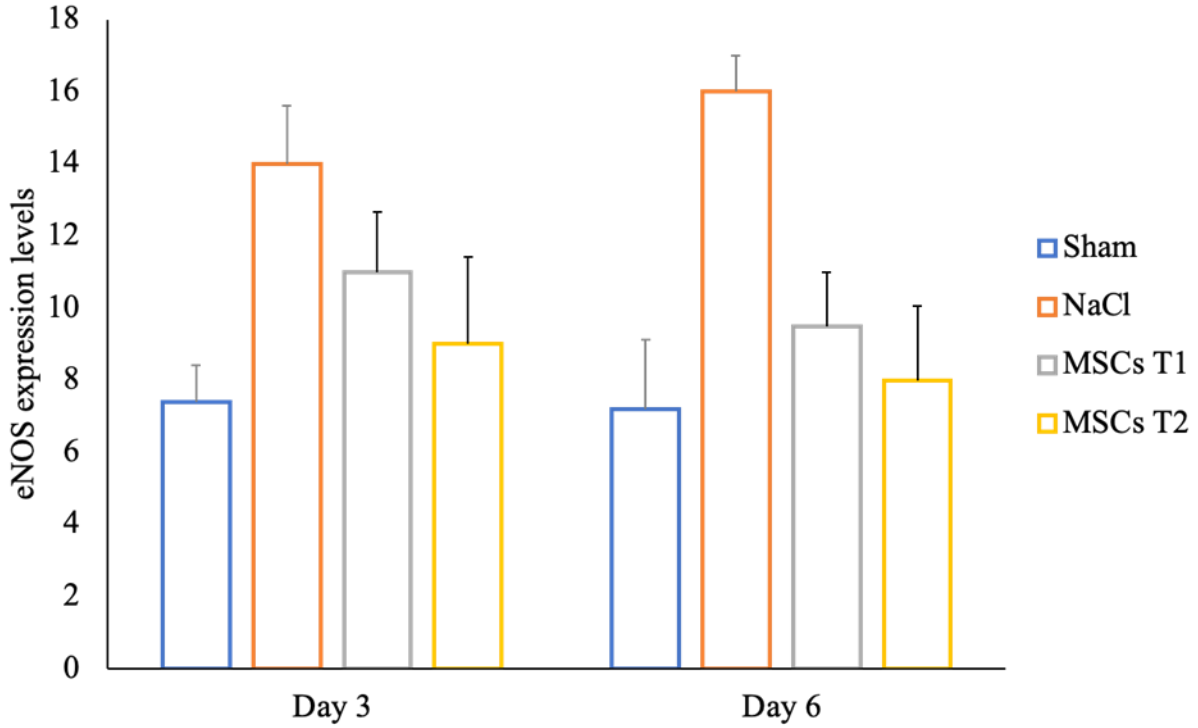

Figure 1. MSCs demonstrated a reduction in eNOS expression levels on day 3 and day 6. These data were consistent across three separate experiments.

Possible Mechanism of MSCs regulated eNOS on AR

In order to explore the possible mechanism of MSCs on the eNOS regulation on AR diseases we performed bioinformatic analysis. We obtained 57 genes that involved in the eNOS regulation in AR under PubMed Gene collection (Supplementary 1). Furthermore, in total we retrieved MSCs mediated proteins consisting 1438 genes collected from PubMed (Supplementary 2). A Venn diagram generated 45 MSCs targets in eNOS regulation in AR (TGs) (Figure 2A, Supplementary 3). To forecast the function of TGs and point out gene classes, we perform GO and KEGG pathway enrichment analysis. GO analysis was intent to checking the role of TGs in biological processes, molecular functions and cellular elements. The results of GO analysis revealed the regulation of the biological process response to lipopolysaccharide by TGs (Figure 2B). The results of the analysis of KEGG pathway enrichment revealed 7 pathways regulated by TGs, including inflammatory bowel disease, pertussis, chagas disease, amobiasis, AGE- RAGE signaling pathway in diabetic complications, toxoplasmosis, and hepatitis B (Figure 2C).

Analysis of the protein protein interaction (PPI) network (confidence level of 0.9) was conducted on TGs, which consist of 45 nodes, 67 edges, a PPI enrichment value of < 2.81e–11, and an average local clustering coefficient of 0.576 (Figure 2D). The top 10 genes with the highest degree scores were identified, including STAT3, RELA, IL6, TNF, IL10, NFKB1, TRL4, MAPK1, TGFB1, and CXCL8 (Figure 2E). These results indicated that those genes have a pivotal role in the PPI network, making them strong possible molecular mechanism.

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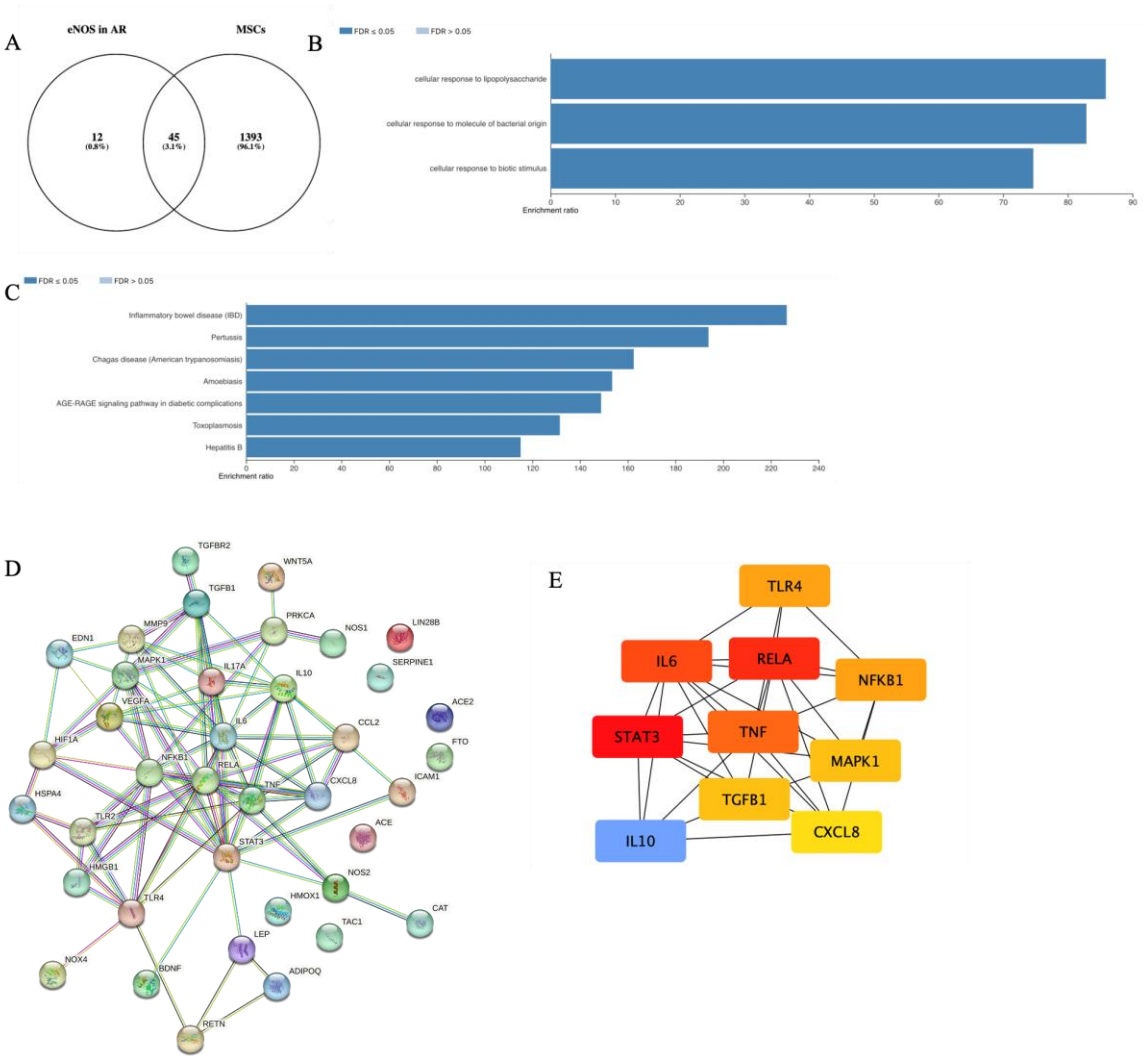

Figure 2. (A) Venn diagram of eNOS regulatory genes and MSCs-predicted targets. (B) GO enrichment analysis of potential target genes of MSCs in overcoming eNOS regulation in AR. (C) KEGG pathway of TGs under Webgestalt.

(D) PPI network of potential target genes of MSCs in eNOS regulation in AR, analysed by STRING. (E) Top 10 hub genes based on highest degree score, analysed by CytoHubba.

4. Discussion

1. Characteristics of Perinatology Room Nurses

In this study, we demonstrated that the administration of MSCs significantly decreased eNOS expression in nasal tissues of OVA-induced AR animals compared to the negative control group (NaCl treatment). This finding suggests that MSCs have the potential to decrease eNOS levels in AR, thereby influencing NO production and subsequent inflammatory processes within the nasal mucosa. Previous study also reported the same phenomenon that MSCs inhibited the eNOS level on the immunosuppressive animal models (Darlan et al., 2021). Several possible mechanisms may underlie the ability of MSCs to supress eNOS levels in allergic rhinitis including MSCs possess immunomodulatory properties, which can regulate the immune response in allergic diseases. MSCs also can modulate the activity of immune cells, such as T cells and macrophages, and suppress the production of pro-inflammatory cytokines (Putra et al., 2021). The downregulation of pro-inflammatory

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cytokines may create an anti-inflammatory environment that favors the downregulation of eNOS and NO production (Putra et al., 2020).

Furthermore, MSCs have been shown to secrete various bioactive molecules, including growth factors, chemokines, and cytokines (Azeem et al., 2018; Balaji, 2015; Dhakad et al., 2003; Diksha & Patyar, 2018; Putra et al., 2018; Sutar et al., 2022). These paracrine factors can influence the behavior of resident cells in the nasal mucosa, including endothelial cells. Interleukin-10 that contained in MSCs can inhibit the infiltration of inflammatory cells, promote tissue regeneration, and modulate the balance between Th1 and Th2 immune responses. Previous studies have suggested that MSCs can activate the PI3K/Akt pathway, which is known to play a crucial role in the regulation of eNOS expression and NO production. The activation of this pathway by MSCs may decrease eNOS expression, leading to decrease NO synthesis in the nasal mucosa of allergic rhinitis animals.

Our study also evaluated the possible mechanism of MSCs in the eNOS regulation in AR. We found that STAT3, RELA, IL6, TNF, IL10, NFKB1, TRL4, MAPK1, TGFB1, and CXCL8 were primary genes that have ability to regulated eNOS in AR. In allergic rhinitis, increased STAT3 activity may lead to the suppression of eNOS expression by interfering with its transcriptional regulation. Activation of RELA can lead to the upregulation of pro- inflammatory cytokines, such as IL6 and TNF, which can inhibit eNOS expression. Inhibition of IL6 can activate STAT3 and NF-κB signaling pathways, both of which can contribute to the downregulation of eNOS expression. TNF also can activate NF-κB signaling, which can inhibit eNOS expression and function. Furthermore, IL10 levels may be dysregulated, leading to reduced IL10-mediated suppression of pro-inflammatory cytokines like IL6 and TNF, which indirectly contribute to the downregulation of eNOS. In addition, activation of TLR4 induce downregulation of eNOS due to triggering the NF-κB signaling pathway. Overall, these genes and their associated signaling pathways modulate the inflammatory response in allergic rhinitis, leading to the downregulation of eNOS expression.

In conclusion, our study provides evidence that MSCs reduce eNOS levels in OVA-induced AR animal models. The underlying mechanisms may involve the immunomodulatory properties of MSCs, their paracrine effects, and the activation of STAT3, RELA, IL6, TNF, IL10, NFKB1, TRL4, MAPK1, TGFB1, and CXCL8 signaling pathways associated with eNOS inhibition. Further investigation is warranted to elucidate the specific factors and pathways involved in the MSC-mediated inhibition of eNOS in AR.

Limitation of Study

The tight schedules of perinatology nurses across various shifts complicated interview arrangements. To manage this, we opted for focus group discussions held at two convenient times, morning and afternoon, to ensure the interviews could be conducted smoothly.

Conclusion and Recommendation

This study demonstrates that mesenchymal stem cell (MSC) therapy significantly reduces endothelial nitric oxide synthase (eNOS) expression in the nasal tissues of ovalbumin (OVA)-induced allergic rhinitis (AR) animal models. The downregulation of eNOS by MSCs suggests a potential anti-inflammatory mechanism that may contribute to reduced nitric oxide (NO) production and inflammation in AR. These effects are likely mediated through the immunomodulatory and paracrine functions of MSCs, which influence immune cell activity and cytokine expression. Bioinformatic analysis further revealed that genes such as STAT3, RELA, IL6, TNF, IL10, NFKB1, TLR4, MAPK1, TGFB1, and CXCL8 are involved in MSC-induced regulation of eNOS, primarily through the modulation of pro- and anti-inflammatory pathways, including NF-κB and PI3K/Akt signaling. These findings offer valuable insight into the molecular mechanisms of MSC-based therapy for AR and provide a foundation for the development of targeted therapeutic strategies. Further research is needed to validate these pathways and to explore the clinical applicability of MSC therapy in allergic and inflammatory airway diseases.

Based on the findings of this study, it is recommended that further experimental and clinical research be conducted to explore the therapeutic potential of mesenchymal stem cells (MSCs) in allergic rhinitis and other related inflammatory conditions. Specifically, future studies should focus on isolating the key bioactive

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factors secreted by MSCs that are responsible for eNOS downregulation, and validating the involvement of identified genes and signaling pathways, such as STAT3, NF-κB, and PI3K/Akt, in larger and more diverse models. In addition, the development of MSC-derived products, such as secretome or exosomes, may offer a safer and more practical alternative to cell-based therapy. These efforts will help pave the way for the clinical translation of MSC therapy into personalized and targeted treatments for patients suffering from allergic airway diseases.

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