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MESENCHYMAL STEM CELL THERAPY FOR ASTHMA: CLINICAL AND HISTOPATHOLOGICAL EVALUATION IN A MOUSE MODEL

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ABSTRACT

Asthma is a chronic respiratory condition characterized by inflammation, airway obstruction, edema, and mucus production in the lungs. Mesenchymal stem cells (MSCs) have gained attention for their potential therapeutic benefits due to their ability to self-renew and promote tissue repair. This study explores the effects of MSCs in treating asthma in a mouse model.

In this experiment, MSCs were administered to asthmatic mice. We then measured key markers of asthma, including the percentage of eosinophils in the blood and bronchoalveolar lavage fluid (BALF), as well as levels of interleukin-4 (IL-4) and Immunoglobulin E (IgE). Additionally, we performed histopathological analysis of lung tissue to assess the degree of inflammation and structural changes.

The results showed that MSC treatment significantly reduced eosinophil counts in both the blood and BALF, and lowered levels of IgE and IL-4, which are key markers of allergic inflammation. Furthermore, MSCs helped reduce eosinophilic inflammation, mucus production, and goblet cell hyperplasia in the lungs.

Overall, our findings suggest that MSC therapy may offer a promising approach for controlling asthma symptoms and improving lung function, making it a potential treatment option for asthma management.

KEYWORDS: *Cell therapy, Regenerative medicine, pulmonary disease. Certainly! Here's a more concise and human-readable version of the introduction, focusing on the key points:*

1. INTRODUCTION

Asthma is a widespread chronic disease characterized by inflammation, airway obstruction, edema, and excessive mucus secretion. These factors lead to airway narrowing and hyperreactivity. The condition involves varying degrees of mononuclear cell infiltration, eosinophilia, mucus production, epithelial damage, smooth muscle thickening, and airway remodelling.

The overreaction of the airways to both internal and external stimuli is a hallmark of asthma. This overreaction is triggered by the direct stimulation of smooth muscle cells around the airways, as well as the release of pharmacologically active substances from mast cells and sensory neurons. Airway hyperresponsiveness (AHR) correlates with the severity of asthma, and lung function can be measured using spirometry and challenges to test responsiveness to bronchodilators or exercise. T lymphocytes are crucial in regulating airway inflammation, but current anti-inflammatory treatments for asthma are often ineffective at preventing disease progression, which can lead to structural changes such as airway remodelling and fibrosis.

Stem cells, particularly mesenchymal stem cells (MSCs), have shown potential in regenerative medicine due to their ability to self-renew, differentiate into various cell types, and aid in tissue repair. MSCs can also modulate the immune system, promoting a more balanced immune response. These stem cells are capable of homing to sites of inflammation, differentiating into needed cell types, and secreting growth factors and cytokines to support tissue repair and lung function. Given the limitations of current asthma treatments, MSC-based therapies represent a promising alternative for managing asthma and reducing its symptoms. This study investigates the effect of MSCs on the clinical and pathological improvement of asthma in a mouse model.
Sure, here's the rewritten section in a more human-readable format:

2. MATERIALS AND METHODS

Isolation and Cultivation of Stem Cells
Mesenchymal stem cells (MSCs) were isolated from the bone marrow of mice using a flushing method, where the bone marrow was flushed out from the femur. After collection, the cells were washed and verified using specific markers to confirm they were

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indeed MSCs. The cells were then cultured in a growth medium, allowing them to multiply. Before being administered to the asthmatic mice, the MSCs were counted and their viability was checked to ensure they were healthy and suitable for treatment.
Here's the section rewritten in a more human-friendly and clearer way:

3. ANIMAL MODEL OF ASTHMA

In this study, we used 48 male Balb/c mice, aged 8 weeks, to create an allergic asthma model. The mice were divided into four groups and then sensitized and challenged with ovalbumin (OVA) to trigger asthma symptoms.

The process began by injecting OVA into the peritoneal cavity of the mice on days 1 and 14. Starting on day 24, the mice inhaled OVA via the trachea on days 24, 26, 28, and 30. For the group receiving MSC treatment, the mice were administered MSCs on days 25, 27, and 29.
The groups were as follows:
- Mice sensitized with OVA but not treated with MSCs.
- Mice sensitized with OVA and treated with MSCs.
- Mice sensitized with OVA and treated with budesonide (a common asthma medication).
- Healthy control mice that were not sensitized to OVA and received only normal saline.

On day 31, samples were collected to assess and compare the effects of the treatments on asthma symptoms, inflammation, and lung function in the treated and control groups.

3.1. Counting Eosinophils in Mouse Blood

Blood samples were collected, and slides were prepared to count the number of eosinophils. The percentage of eosinophils compared to the total number of cells was then calculated.

3.2. Counting Eosinophils in Bronchoalveolar Lavage Fluid (BALF)

BALF was collected from the mice's lungs. After anesthetizing the mice, a small incision was made in the trachea to insert a tube for flushing the lungs with a saline solution (PBS). The fluid was then collected. To examine the cells, the fluid was processed using a cytospin technique, and the slides were stained with Giemsa dye. The number of eosinophils and their percentage in the total cell population were counted.

3.3. Measuring Interleukin 4 (IL-4)

The amount of the cytokine IL-4 in the BALF was measured using an enzyme-linked immunosorbent assay (ELISA) kit designed specifically for IL-4 detection.

3.4. Measuring Total Immunoglobulin E (IgE)

Blood was collected from the mice, and the serum was separated. The total IgE level in the serum was measured using an ELISA kit designed to detect IgE.

3.5. Histopathological Analysis

Lung tissue samples were prepared for histopathological analysis. The tissue was stained with Hematoxylin and Eosin (H&E) to assess general inflammation, and with Periodic Acid-Schiff (PAS) stain to evaluate mucus production and goblet cell hyperplasia. These samples were then examined under a light microscope to assess inflammation around the airways and blood vessels.

3.6. Statistical Analysis

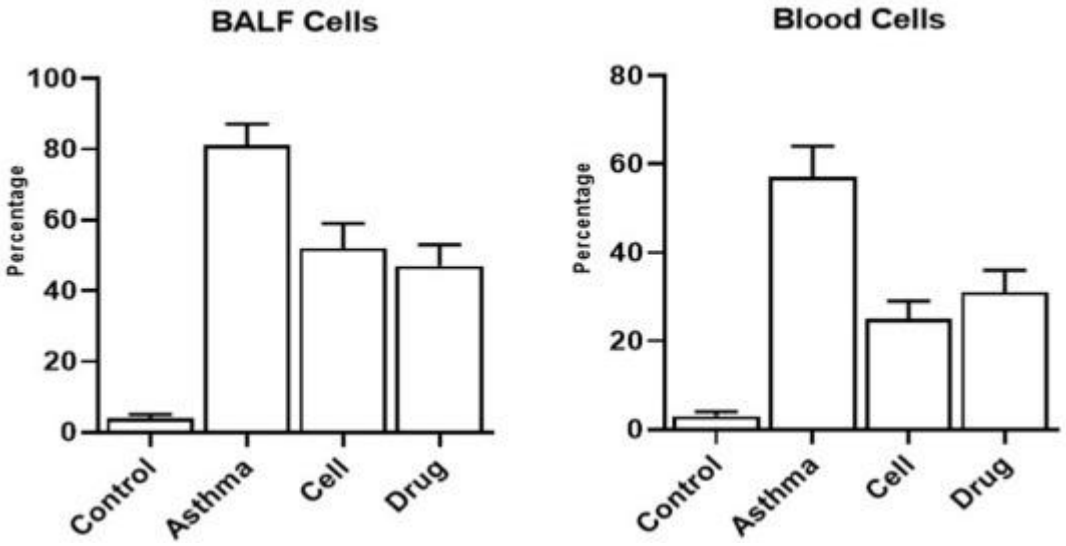
Data collected from the experiments were entered into SPSS software (version 20) for statistical analysis. The T-test was used to compare the results between groups, and a p-value of less than 0.05 was considered statistically significant.

4. RESULTS

4.1. Eosinophils in the Blood

The percentage of eosinophils in the blood was significantly higher in the asthma group (57 ± 7%) compared to the healthy control group (3 ± 1%). However, treatment with cell therapy (MSCs) reduced the eosinophil percentage to 25 ± 4% in the asthmatic mice (Fig. 1).

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4.2. Eosinophils in the Bronchoalveolar Lavage Fluid

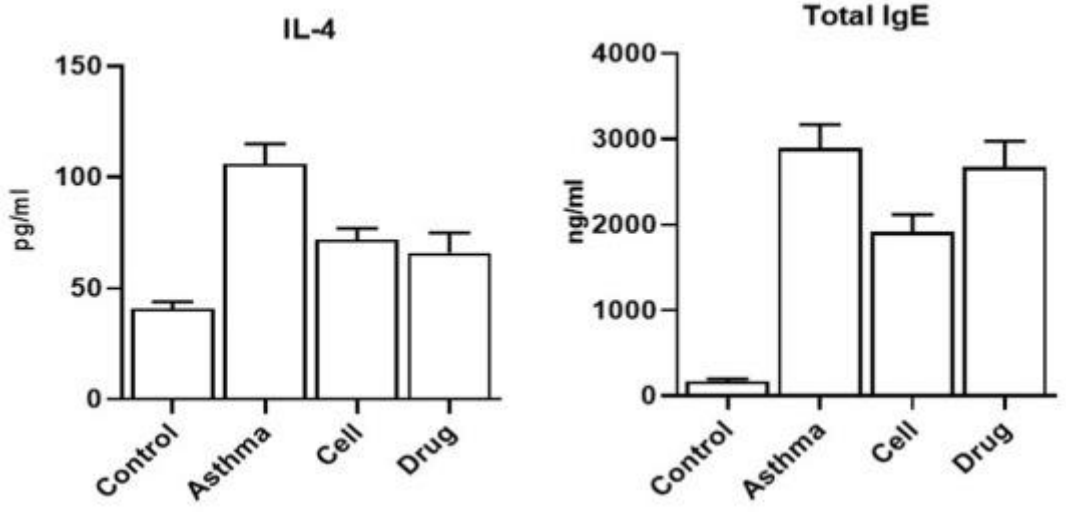
The percentage of eosinophils in the bronchoalveolar lavage fluid was significantly higher in the asthma group (81 ± 6%) compared to the healthy control group (4 ± 1%). However, treatment with cell therapy (MSCs) reduced the eosinophil percentage to 52 ± 7% in the asthmatic mice.

4.3. Total IgE

The level of IgE was significantly higher in the serum of asthmatic mice (2894 ± 274 ng/ml) compared to the control mice (169 ± 28 ng/ml). However, cell therapy (MSCs) reduced the IgE levels to 1914 ± 204 ng/ml in the asthmatic mice.

4.4. IL-4

The level of IL-4 in the bronchoalveolar lavage fluid (BALF) was significantly higher in asthmatic mice (106 ± 9 pg/ml) compared to healthy control mice (41 ± 3 pg/ml). However, cell therapy (MSCs) reduced IL-4 levels to 72 ± 5 pg/ml in the asthmatic mice (Fig. 2).



4.5. Histopathological Analysis

Eosinophilic inflammation around the bronchi and blood vessels was significantly higher in the asthma group (3.8 ± 1 and 3.7 ± 3, respectively) compared to the healthy control group (0.5 ± 0.2 and 0.5 ± 0.2, respectively). However, cell therapy (MSCs) reduced inflammation around the bronchi (1.9 ± 0.3) and vessels (1.8 ± 0.3) in the asthmatic mice.

Additionally, mucin production and goblet cell hyperplasia were increased in the asthma group (3.9 ± 1 and 3.8 ± 1, respectively) compared to the healthy controls (0.5 ± 0.1 and 0.5 ± 0.2, respectively). Cell therapy significantly reduced mucin production (2.2 ± 0.3) and goblet cell hyperplasia (1.7 ± 0.2) in the treated asthmatic mice.

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5. DISCUSSION

Asthma is a chronic inflammatory disease that causes changes in the structure and function of the airways, leading to symptoms like wheezing, coughing, and shortness of breath. Inflammation in the airways is a key factor in the development of asthma, and it results from the activation of immune cells and the release of inflammatory mediators. Over time, chronic inflammation can lead to structural changes in the airways, such as airway remodeling, smooth muscle thickening, and subepithelial fibrosis, which can make asthma less responsive to treatment and harder to control.

In asthma, the immune system becomes imbalanced, particularly in the T-helper (Th) lymphocytes, with an overactivation of Th2 cells. These cells release pro-inflammatory cytokines like IL-4, IL-5, and IL-13, which contribute to airway inflammation, mucus production, and the characteristic symptoms of asthma. Chronic inflammation and mucus buildup in the airways can also contribute to airway remodelling, further impairing lung function.

One promising approach to treating asthma is the use of mesenchymal stem cells (MSCs). These cells are pluripotent, meaning they can develop into a variety of cell types. MSCs were first identified in bone marrow but have since been found in other tissues like fat, dental pulp, and umbilical cord. MSCs have unique properties, such as the ability to escape immune system attacks, making them a potential therapy for inflammatory diseases.

MSCs have been studied for their ability to reduce inflammation and promote tissue repair in diseases like asthma. They secrete growth factors, including hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF), which help repair tissue, reduce inflammation, and promote cell survival. MSCs can also secrete exosomes that carry signaling molecules to help repair lung tissue, reduce fibrosis, and modulate the immune response.

In our study, we found that MSC therapy reduced key markers of asthma in mice. Specifically, levels of IL-4 in bronchoalveolar lavage fluid (BALF) and total IgE in serum were significantly lower in MSC-treated mice compared to untreated asthmatic mice. Additionally, we observed a reduction in eosinophils in both the blood and BALF of MSC-treated mice, suggesting that MSCs were able to control eosinophilic inflammation.

Our findings are consistent with previous studies on MSCs in asthma. For example, a study by Castro et al. (2019) found that multiple injections of MSCs in mice with asthma caused by occupational allergens led to reduced airway inflammation, lower levels of IL-4 and IL-13, and decreased numbers of eosinophils. The study also showed that MSCs reduced collagen production and improved lung tissue structure. MSCs are thought to work by activating T regulatory cells (Tregs) that produce anti-inflammatory cytokines like IL-10 and TGF-β, which help suppress the immune response and reduce inflammation. MSCs can also change the behavior of macrophages, shift them from a pro-inflammatory M1 type to a more healing M2 type, and reduce the proliferation of airway smooth muscle cells.

In another study by Neza Adamik et al. (2022), MSCs derived from adipose tissue were found to reduce inflammatory cytokines and improve severe asthma in horses, providing further evidence of the therapeutic potential of MSCs in asthma treatment. Similarly, Shin et al. (2021) found that MSCs derived from human umbilical cord blood helped suppress the cytokines that drive asthma, improving lung function in mice with severe asthma.

In our study, MSCs not only reduced inflammation but also controlled mucin production and goblet cell hyperplasia, which are key features of asthma pathology. The administration of MSCs was able to reduce the eosinophilic inflammation around the bronchi and blood vessels, and the overall pathophysiology of asthma in the treated mice was improved.

These findings suggest that MSC therapy could be a promising treatment for asthma, particularly for patients who do not respond well to conventional therapies. Given their ability to modulate the immune response, reduce inflammation, and promote tissue repair, MSCs offer a potential new approach to managing and treating asthma.

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