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Research Article

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Investigating the Effect of Bone Marrow-Derived Mesenchymal Stem Cells on Airway Hyper-Responsiveness of Asthma Mouse Model

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Abstract

Background and aim: Asthma is one of the main lung diseases that is identified by eosinophilic inflammation, mucus secretion, airway hyper-responsiveness (AHR) and airway obstruction. AHR is one of the important action of airways in asthma. Mesenchymal stem cells (MSCs) have regulatory effect on immune response and may be useful to the treatment of asthma. MSCs have low immunogenicity and may be safe in application. Therefore, study effect of mouse bone marrow-mesenchymal stem cells (BM-MSCs) controlling of AHR in asthma model was done.
Materials and Methods: BM-MSCs were isolated and used as treatment in asthmatic male BALB/c mice. To produce asthma animal model, mice were sensitized and challenged with OVA. On days 30 and 40, to measure of AHR, Methacholine (MCh) challenge test was applied to determine the Penh value. Finally, AHR were recorded and analyzed.
Results: Treatment of asthmatic mice with BM-MSCs could control AHR in MCh challenge test and it has significant difference ($p<0.05$) between days 30 and 40.
Conclusion: BM-MSCs are almost non-immunogenic and can be used to treat asthma and control of AHR. Using of MSCs as anti-asthma treatment presents new and applicable strategy to control of AHR in asthma.

Keywords: *Allergy, Cell Therapy, Asthma, Lung, Treatment*

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Introduction

Asthma is one of the main chronic diseases and is a complicated lung problem. This disease is identified by eosinophilic inflammation, mucus hyper-secretion, airway hyper-responsiveness (AHR) and reversible airway obstruction. AHR is one of the important action of airways in asthma and some produced anti-asthma medicines can control AHR (Roxbury & Lin, 2017; Ankermann & Brehler, 2019).

Allergy comprises the main causes of asthma. Genetic predisposition and environmental factors active of the immune response (i.e., Th2 dominant response), which is responsible for the asthma pathophysiology. IL-4 is one of the major Th2 cytokine and induces IgE isotype switching of B cells that can be bind to its receptor (FcεRI) on the mast cell's surface. Then mast cells can be activated upon allergen induced cross-linking of FcεRI-IgE and histamine will be released with other mediators, which lead to allergic symptoms such as broncho-spasm reaction (Isik *et al.,* 2019; 4. Xuan *et al.,* 2020; Miceli *et al.,* 2020).

The mesenchymal stem cell (MSC)s have regulatory and modulatory effect on immune response and inflammation and their immuneregulation capacity may be useful to the treatment of asthma. MSCs therapy is one of the new therapeutic approach that has an immunoregulatory potential on immune response-related diseases such as asthma. However, the MSCs are harvested from various tissues and may have effect on allergic asthma pathophysiology. MSCs have low immunogenicity and may be safe in clinical application (Huang *et al.,* 2021; Hou *et al.,* 2023). Furthermore, study immune-modulatory effect of MSCs in asthma was done and the controlling effect of MSCs on AHR of asthma model was evaluated.

Material and Methods

Bone marrow-mesenchymal stem cells (BM-MSCs) culture

MSCs were isolated from mouse's bone marrow according to previously studied (Huang *et al.,* 2021; Hou *et al.,* 2023). In briefly, bone marrow was flushed out of the cavity and then cultured for 5 days. The cells were re-suspended, passaged and confirmed.

Animal treatment

Male BALB/c mice (6-7 weeks old) were kept under standard conditions and divided into three groups (n = 6), which include: control group, which was sensitized with PBS and was healthy; the two other groups were sensitized and challenged with Ovalbumin (OVA) according to previously studies to produce asthma model (Cheng *et al.,* 2022; Jiang *et al.,* 2021). One of them received no treatment, and the other was treated with BM-MSCs via bronchial. Treatment was done on day 25.

Measurement of AHR

To measure of AHR on days 30 and 40, the Methacholine (MCh) challenge test was applied to determine the Penh value and under anesthetization. The mice were tracheostomized and intubed. Then initially exposed to PBS aerosol and different MCh concentrations (2, 4, 8, 16, 32, and 64 mg/ml). At least, AHR were recorded and analyzed.

Data analyzing

Data were repeated and analyzed by SPSS software version 20 with t-test and Mann-Whitney U test. Data were presented as Mean±SD by GraphPad prism and P less than 0.05 was considered as significant.

Results

After production of BM-MSCs and allergic asthma mouse model creation, AHR was measured. According to this study, treatment of mice with BM-MSCs could control AHR in MCh challenge test. This controlling has significant difference ($p<0.05$) between days 30 and 40, and in day 40 AHR was controlled better than day 30 in all MCh concentrations. Created graphs from mouse inhalation in ventilator, showed significant differences between treated and non-treated groups (Figure 1).

After analyzing of data from graphs of the ventilator, it was observed that there was significant difference between treated and non-treated groups and there was significant difference between days 30 and 40. For example, in dose 16 mg/ml, penh values in days 30 and 40 were 9.4±0.1 and 8.4±0.2 respectively that had significant difference (Figure 2).

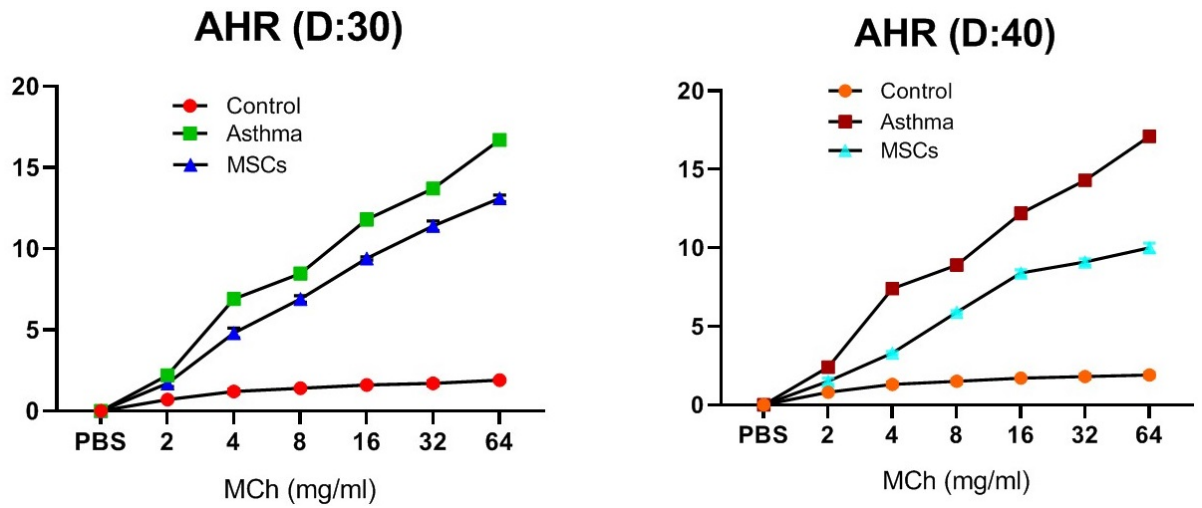

Figure 1. Penh value with increased concentration of MCh on day 30 and 40.

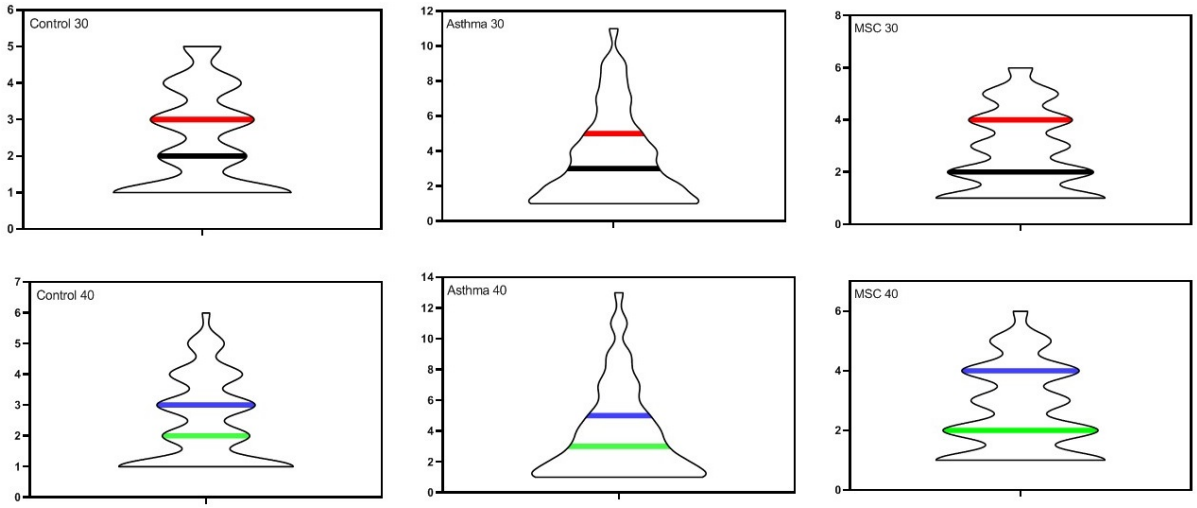

Figure 2. Penh value of MCh challenge test for each group on day 30 and 40.

Discussion

Asthma is one of the main and complicated chronic diseases of lung and leads to bronchial obstruction. The obstruction of airways is initiated with spasm of smooth muscle cells and leads to AHR. In continue, eosinophilic inflammation and mucus realizing from gablet cells lead to chronic obstruction of airways. If these factors are presented in long time, may be led to airway remodeling (Tanaka *et al.,* 2004; Booth *et al.,* 2007). Therefore, in first time and for prevent of acute airway obstruction, the AHR should be controlled, which was done in this study by mesenchymal stem cell therapy. When these cells are in airways for more time, they can be effective and have more effect on control of AHR.

Although there are notable numbers of anti-asthma therapies as short acting and long-acting drugs that can control AHR and inflammation respectively, but the most of these drugs have short time efficacy and need to be administrated (Ouyang *et al.,* 2010; Samitas *et al.,* 2015). The mesenchymal stem cell therapy can be presented as therapeutic option and can be used for long time without re-administration. Because these cells can be proliferated and have self-renovation potency, which presents new approach that not need more dose of treatment or repeating of therapeutic protocols.

In allergic diseases such as asthma, immune response modulation by cell therapy may control activation and proliferation of Th2 cells, and the therapeutic method can be used for control of

immune-related allergic response in asthma. MSCs therapy could decrease bronchoconstriction, and collagen fiber content in the airway (Yin *et al.,* 2023). MSCs as a novel therapy are developed in regenerative medicine. These cells have more plasticity and can prevent remodeling in target tissues, and also, help to repair the lung tissue and decrease lung injury. MSCs as multi-potent progenitor cells have immune-modulatory potentially (Cruz *et al.,* 2015; Lathrop *et al.,* 2014). Therefore, this approach of MSCs therapy for the control of AHR in allergic asthma can have beneficial effect.

It was showed that administration of MSCs via systemic can suppress collagen deposition and remodeling of airway, reduce goblet cells number and the response of airway to methacholine (Hu *et al.,* 2008; Urbanek *et al.,* 2016). In this study, MSCs administration could prevent AHR and can lead to acceptable airflow in obstructed airways.

Immunomodulatory effect of MSCs is regarded as a novel therapeutic approach for allergic diseases such asthma due to their immune-privileged potential. MSCs therapy for asthma via intra-trachea and/or intra-venus could decrease leukotrienes B4, and C4, which have important role in allergic asthma pathophysiology and obstruction of airway (Booth *et al.,* 2007; Lathrop *et al.,* 2014; Lin *et al.,* 2018).

In allergic reaction of airways and asthma attacks, mediators of immune cells have dominant role and can lead to spasm of the airway smooth muscle cells. One class of these mediators belong to mast cells. Type 2 cytokines such as IL-4 and its upper-hand cytokines such as IL-33 have strong effect. IL-33 belongs to IL-1 cytokine family and has effect on cytokines network signaling pathways, and controls producing of IL-4. IL-4 forces B cells to produce IgE as main allergic immunoglobulin. IgEs bind to their receptors on the mast cell and after attaching to the allergen, force mast cells to degranulation. Mediators of the mast cell granules have strong effect on spasm of smooth muscle cells and lead to airway obstruction (Zhang *et al.,* 2023). It was not evaluated in this study but, may be MSCs have effect on control of these cytokines (IL-4 and IL-33) and via this pathway, they can control degranulation of mast cells, spasm of airway smooth muscle cells and AHR.

MSC therapy is a novel immunomodulatory therapeutic approach for a variety of immune-related diseases such as allergic asthma, and immunoregulatory effect of MSC on the innate and adaptive immune response was observed. In a study, it was reported that treatment of allergic asthma with MSCs containing expression gene of IL-35 could strongly control allergo-inflammatory mechanisms, allergic immunopathology, and symptoms of asthma. It was showed that MSCs immunomodulatory effect had synergism with IL-35 immunomodulatory effect, and with together in asthma, could harness allergoinflammatory response. It was presented that expressing IL-35 vector inhibits IL-4and IgE levels. Therefore, IL-35 attenuates asthma pathogenesis. Moreover, the IL-35 attenuates proliferation of T cell and allergic immune response of Th2 cell in asthma. In predisposed patients, with high risk of specific IgE-mediated allergic response to inhaled allergens, dendritic cells with IgE, can present aeroallergens and induce an allergic inflammation. Therefore, IL-35 prevents mechanism of allergy and is a therapeutic agent for asthma and IL-35 deficiency gave rise to Th17 cells that are required to the AHR maintain after challenge of multiple allergens. It was published that MSCs can control AHR, and this will be stronger in MSCs with IL-35 gene than MSCs treatment alone without IL-35 (Bao *et al.,* 2023). MSC is almost non-immunogenic due to its embryonic state. Therefore, using BM-MSCs to treat asthma can be applicable that was done in this study and showed significant effect on control of AHR and evaluated the breathing output in asthmatic mice.

Conclusion

Therefore, using of BM-MSCs as anti-asthma treatment can present new and applicable strategy and is a useful method to control of AHR in asthma. These cells can be used as delivery system for some genes and needs more researches in this field.

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Conflict of Interest

There is no conflict of interest.

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مقاله پژوهشی

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بررسی تاثیر سلول های بنیادی مزانشیمی مشتق از مغز استخوان بر پاسخگویی بیش از حد راه هوایی مدل موشی آسم

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چکیده

زمینه و هدف: آسم یکی از بیماری های اصلی ریه است که با التهاب ائوزینوفیلیک، ترشح موکوس، واکنش بیش از حد راه هوایی (AHR) و انسداد راه هوایی مشخص می شود. AHR یکی از واکنش های مهم راه های هوایی در آسم است. سلول های بنیادی مزانشیمی (MSCs) اثر تنظیمی بر پاسخ ایمنی دارند و ممکن است برای درمان آسم مفید باشند. MSCها ایمنی زایی پایینی دارند و ممکن است در کاربرد بی خطر باشند. بنابراین، مطالعه اثر سلول‌های بنیادی مزانشیمی مغز استخوان موش (BM-MSCs) بر کنترل AHR در مدل آسم انجام شد.

مواد و روش‌ها: BM-MSCها جداسازی و به عنوان درمان در موش‌های نر BALB/c مبتلا به آسم مورد استفاده قرار گرفتند. برای تولید مدل حیوانی آسم، موش‌ها با OVA حساس شده و چلنج شدند. در روزهای ۳۰ و ۴۰ برای اندازه گیری AHR از آزمون چالشی متاکولین (MCh) برای تعیین مقدار penh استفاده شد. سرانجام، AHR ثبت و تجزیه و تحلیل شد.

یافته‌ها: درمان موش‌های مبتلا به آسم با BM-MSCs توانست AHR را در آزمون چالش MCh کنترل کند و بین روزهای ۳۰ و ۴۰ تفاوت معنی‌داری ($p<0.05$) داشت.

نتیجه‌گیری: BM-MSC تقریبا غیرایمونوژن است و می‌تواند برای درمان آسم و کنترل AHR استفاده شود. استفاده از سلول های بنیادی مزانشیمی به عنوان درمان ضد آسم، استراتژی جدید و قابل استفاده ای را برای کنترل AHR در آسم ارائه می دهد.

واژه‌های کلیدی: *آلرژی، سلول درمانی، آسم، ریه، درمان*

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