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Simvastatin and bone marrow-derived mesenchymal stem cells (BMSCs) affects serum IgE and lung cytokines levels in sensitized mice

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ABSTRACT

Introduction: The effects of bone marrow-derived mesenchymal stem cells (BMSCs) and simvastatin combination therapy on serum total and specific IgE levels and lung IL-13 and TGF-β levels in sensitized mouse were examined.

Material and methods: Control (n = 5), Sensitized (S), (n = 5), S + BMSC (n = 6), S + simvastatin (n = 6) and S + BMSC + simvastatin (n = 4) groups of BALB/c mice were studied. Mice were sensitized by ovalbumin. Sensitized mice were treated with a single intravenous injection of BMSCs (1×10^6) or daily intraperitoneal injection of simvastatin (40 mg/kg) or both BMSCs and simvastatin on the last week of challenge. Serum total and ovalbumin specific IgE levels as well as IL-13 and TGF-β levels in broncho-alveolar lavage (BAL) fluid were evaluated.

Results: Serum total and specific IgE levels as well as lung IL-13 and TGF-β levels were significantly increased in S group compared to control group ($P < 0.001$ for all cases). Treatment with BMSCs, simvastatin and their combination significantly decreased serum total and specific IgE levels ($P < 0.05$ to $P < 0.01$). However, IL-13 and TGF-β levels were significantly decreased by BMSCs and BMSC + simvastatin combination therapy ($P < 0.05$ for all cases). The effect of simvastatin and BMSCs combination therapy on serum specific IgE levels as well as lung IL-13 and TGF-β levels were significantly higher than the effect of BMSCs and simvastatin alone ($P < 0.001$ for IL-13 and $P < 0.01$ for other cases).

Conclusions: Simvastatin and BMSCs combination therapy affects serum IgE as well as lung IL-13 and TGFβ levels more than BMSC therapy and simvastatin therapy alone which may be due to increased BMSCs migration into the lung tissue.

1. Introduction

Bronchial asthma is the chronic respiratory disease characterized by airway inflammation [1]. Airway inflammation causes airway structural changes known as airway remodeling [2].

Increased number of Th_2 cells in the airways of asthmatic patients was reported. These cells secrete IL-4, IL-5, IL-9, and IL-13 which play a key role in the chronic lung inflammation and remodeling since they stimulate B cells to synthesize immunoglobulin E (IgE), eosinophil and neutrophil recruitment to tissues, airways inflammation, excess mucus production and fibrosis [3–5].

Mesenchymal stem cells are the multipotent cells [6] which can play the role in control of inflammatory and immunologic responses [7–9]. Moreover, the therapeutic effects of these cells on remodeling have been shown in various diseases [10–12].

In the previous study, the inhibitory effect of BMSCs on airway inflammation, goblet cell hyperplasia and inflammatory cell infiltration to the BAL fluid but not subepithelial fibrosis in the mouse model of asthma was showed [13]. It was postulated that the inability of BMSCs to ameliorate fibrosis may be due to the low migration capacity of these

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cells, and a strategy which enhances BMSCs migration into the lung tissue may augments their therapeutic effects [13]. In fact, in another study increased BMSCs migration into the lung tissue in the mouse model of asthma due to simvastatin and BMSCs combination therapy was observed [14]. It was also found that combination therapy is more effective in reducing airway inflammation, goblet cell hyperplasia and inflammatory cell infiltration to the BAL fluid than BMSCs alone, and, unlike the BMSCs therapy alone, combination therapy can reduce subepithelial fibrosis [14].

However, the mechanism by which combination therapy exerts these effects remained unclear. Therefore, in the present study, the mechanism underlying the effects of simvastatin and BMSCs combination therapy on airway remodeling in an ovalbumin-induced asthma model in mouse was examined.

2. Material and methods

2.1. Animal sensitization, experimental groups and treatment protocol

Male BALB/c mice (6–8 weeks old) were obtained from Pasteur Institute, Iran, and bred in the animal laboratory of Tehran University of Medical Science. Mice were maintained in regular cages under the controlled environmental conditions (20 ± 2 °C and 12 h light–dark cycle) and allowed free access to standard lab chow and water. Animal care and the general protocols for animal use were approved by the Animal Ethics Community of Tehran University of Medical Sciences.

Animals were divided into five groups including; control group (animals were not sensitized, n = 5), sensitized group (S, animals were sensitized by OVA, n = 5), S + BMSC group (sensitized animals were treated with BMSCs, n = 6), S + simvastatin group (sensitized animals were treated with simvastatin, n = 6), and S + BMSC + simvastatin (sensitized animals were treated with simvastatin and BMSCs, n = 4).

Sensitized mice, S + BMSC, S + simvastatin and S + BMSC + simvastatin groups were sensitized by intra-peritoneal injection of OVA (10 μg) (OVA, Sigma grade 5) and aluminum hydroxide (2 mg) on day 0 and 14. From forth week, animals were exposed to aerosolized OVA (3%) in a closed chamber (dimensions 40 × 40 × 70 cm) using a compressor nebulizer (Omron CX3, Japan, particle size 3–5 μm and output of 5 l/min) for 30 min/day, three days/week for eight weeks [15]. Animals in S + BMSC group were treated with single intravenous injection of BMSCs (1×10^6) on day 67, animals in S + simvastatin group were treated with daily intraperitoneal injection of simvastatin (40 mg/kg) from day 67 to day 74, and animals in S + BMSC + simvastatin group received both BMSCs and simvastatin drugs on the last week of challenge. Animals of control group only received normal saline instead of OVA.

The stock solution of simvastatin (Arasto Pharmaceutical Chemical Inc, Tehran-Iran, SIM-F8-24–88) was prepared as follows: simvastatin (40 mg) was dissolved in ethanol (1 ml) and 0.1 N NaOH (1.5 ml) and incubated at 50 °C for 2 h. The PH was then adjusted to 7.2. Immediately prior to use, the stock solution was diluted in sterile PBS to achieve the desired concentration [16].

2.2. BMSCs generation and characterization

BMSCs were harvested from adult male Balb/c mice bone marrow and their phenotype was verified by flow cytometric analysis of following antigens: CD31, CD45, CD90 and CD44, plus adipogenic and osteogenic differentiation assays as described previously [11,12].

2.3. Collection of BAL fluid and serum

Mice were anesthetized one day after the last challenge. Blood samples were collected by cardiac punctures, kept at room temperature for 2 h, and centrifuged at 3000*g* for 5 min. Obtained sera were stored at -20 °C for subsequent immunoglobulin assays. Trachea was cannulated and lungs were lavaged with three 0.3 ml sterile phosphate buffered saline. BAL fluid was centrifuged at 1000*g* for 5 min and supernatant was stored at − 70 °C for cytokine assays.

2.4. Measurement of cytokines and immunoglobulin

For each animal IL-13 (IL-13; eBioscience, San Diego, CA) and TGF-β (TGF-B; eBioscience, San Diego, CA) levels in BAL fluid, and total IgE (IgE; eBioscience, San Diego, CA) and ovalbumin specific IgE (IgE; Biolegend, San Diego, CA) levels in serum were quantified by enzyme-linked immunosorbent assay described by the manufacturer. Results are expressed as picograms per milliliter for IL-13 and TGF-β, and nanograms per milliliter for IgEs.

2.5. Data analyses

Data were analyzed with the analysis of variance followed by the Tukey test and are presented as the mean ± SEM. A probability value of < 0.05 was considered statistically significant.

3. Results

3.1. BMSCs characterization

Under appropriate conditions, the BMSCs appeared as a monolayer of large, fibroblast-like flattened cells which were able to adopt an osteogenic or adipogenic phenotype (Fig. 1). BMSCs were positive for CD44 and CD90, but they did not express CD31 and CD45 on flow cytometric analysis of BMSCs within 3–5 passages (Fig. 2).

3.2. Serum total and specific IgE levels

In group S, serum total and specific IgE levels were significantly higher compared to control group ($p < 0.001$ for both cases). Treatment of sensitized mice with both BMSCs and simvastatin significantly decreased serum total and specific IgE levels compared to

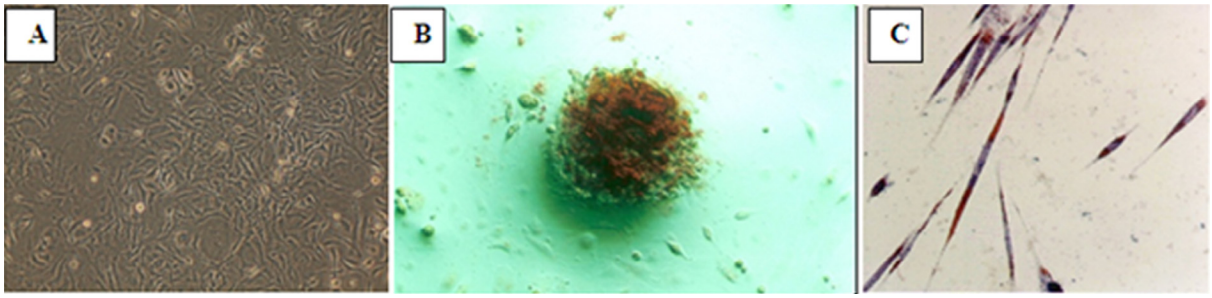

Fig. 1. Undifferentiated BMSCs indicating a spindle-shaped morphology under phase contrast microscopy (A). Alizarin red staining of mineralized bone tissue indicating BMSCs differentiation to osteoblasts (B). Oil red O positive intracellular lipid droplets indicating BMSCs differentiation to adipocytes (C).

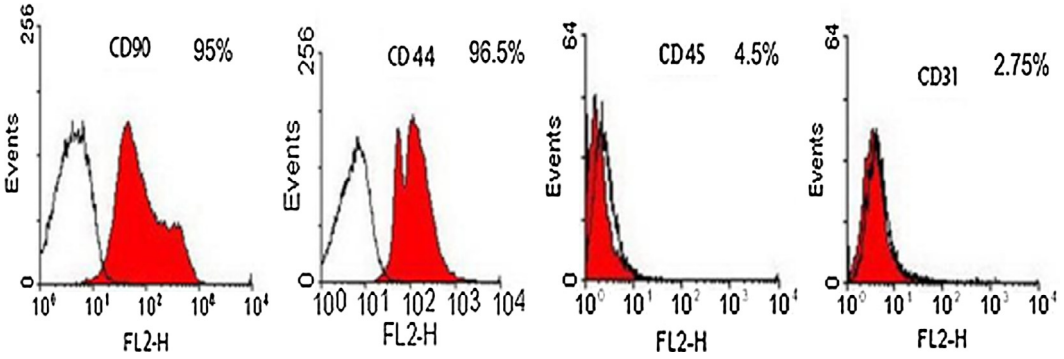

Fig. 2. Flow cytometric analysis indicating that the cells were BMSCs since CD44 and CD90 were positive and CD31 and CD45 were negative.

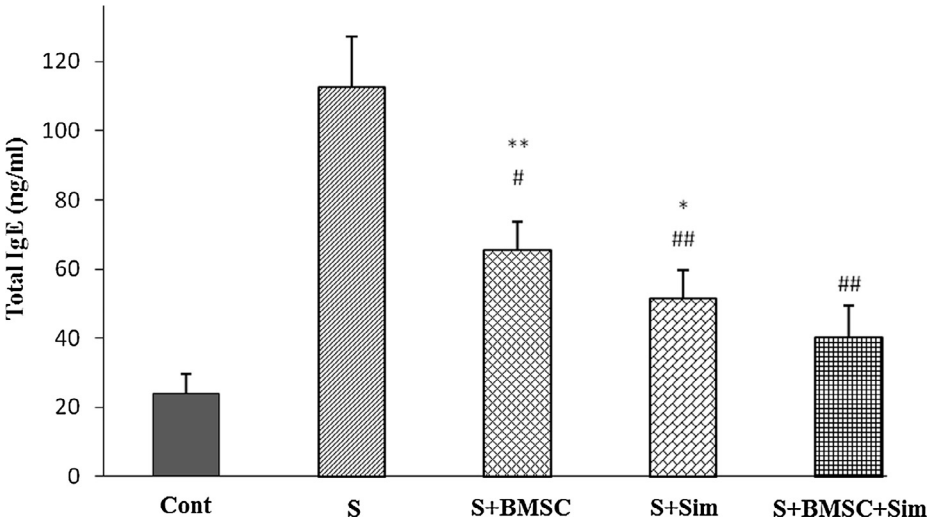

Fig. 3. Serum total IgE level in: control animals (Cont, n = 5), sensitized animals (S, n = 5), sensitized animals treated with BMSCs (S + BMSC, n = 6), simvastatin (S + Sim, n = 6) and BMSCs and simvastatin (S + BMSC + Sim, n = 4). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared to sensitized group. Statistical comparison between groups was performed using analysis of variance followed by the Tukey test.

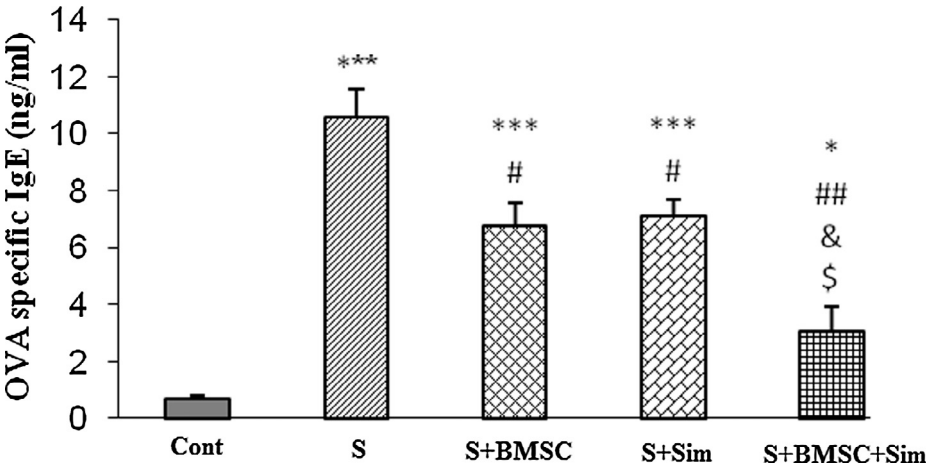

Fig. 4. Serum specific IgE level in: control animals (Cont, n = 5), sensitized animals (S, n = 5), sensitized animals treated with BMSCs (S + BMSC, n = 6), simvastatin (S + Sim, n = 6) and BMSCs and simvastatin (S + BMSC + Sim, n = 4). $^{*}P < 0.05$, $^{***}P < 0.001$ compared to control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared to sensitized group, $^{\&}P < 0.05$ compared to S + BMSC group, $^{\$}P < 0.05$ compared to S + simvastatin group. Statistical comparison between groups was performed using analysis of variance followed by the Tukey test.

non-treated S group ($P < 0.01$ for total IgE in asthma + simvastatin group and $P < 0.05$ for other cases). Serum total and specific IgE levels were also significantly decreased due to combination therapy of the two drugs ($P < 0.01$ for both cases). The effect of combination therapy of BMSC + simvastatin on serum specific IgE levels were significantly higher than BMSC and simvastatin therapy alone ($P < 0.05$ for both cases). However, serum total and specific IgE levels of S + BMSC and S + simvastatin groups were significantly higher than those of control group ($P < 0.05$ to $P < 0.001$) but there was not statistical difference in serum total IgE levels between combination therapy of BMSC + simvastatin and control group (Figs. 3 and 4).

3.3. IL-13 and TGFβ levels in BAL fluid

IL-13 and TGF-β levels were significantly increased in sensitized mice compared to control animals ($P < 0.001$ for both cases). Treatment of sensitized animals with BMSCs alone and BMSCs plus simvastatin significantly decreased IL-13 ($P < 0.01$ and $P < 0.001$, respectively) and TGF-β ($P < 0.05$ and $P < 0.01$, respectively) levels. The effect of combination therapy of BMSC + simvastatin on IL-13 and TGF-β were significantly higher than simvastatin therapy alone ($P < 0.05$ for both cases). However, IL-13 in all three treated groups and TGF-β levels in BMSCs and simvastatin treated groups alone were significantly higher than those of control animals ($p < 0.05$ to

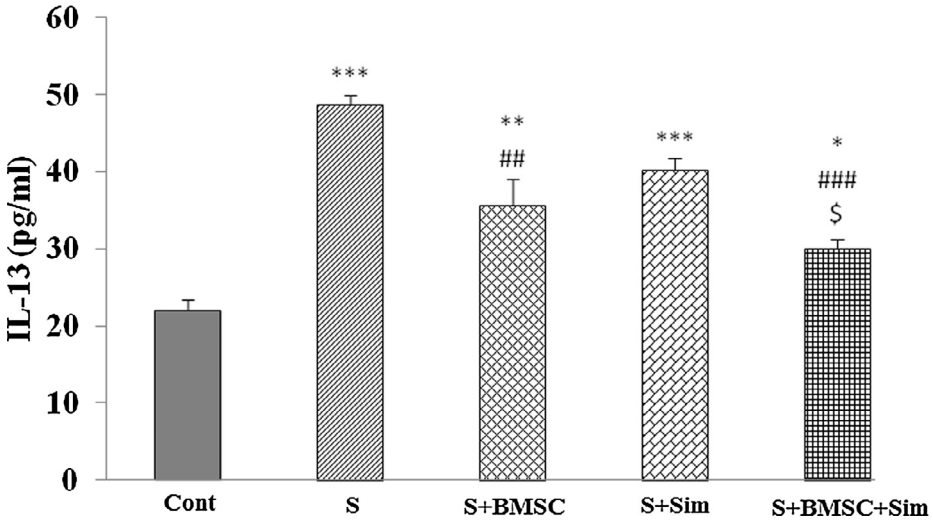

Fig. 5. IL-13 level in BAL fluid of: control animals (Cont, n = 5), sensitized animals (S, n = 5), sensitized animals treated with BMSCs (S + BMSC, n = 6), simvastatin (S + Sim, n = 6) and BMSCs and simvastatin (S + BMSC + Sim, n = 4). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to control group, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared to sensitized group, $^{\$}P < 0.05$ compared to S + simvastatin group. Statistical comparison between groups was performed using analysis of variance followed by the Tukey test.

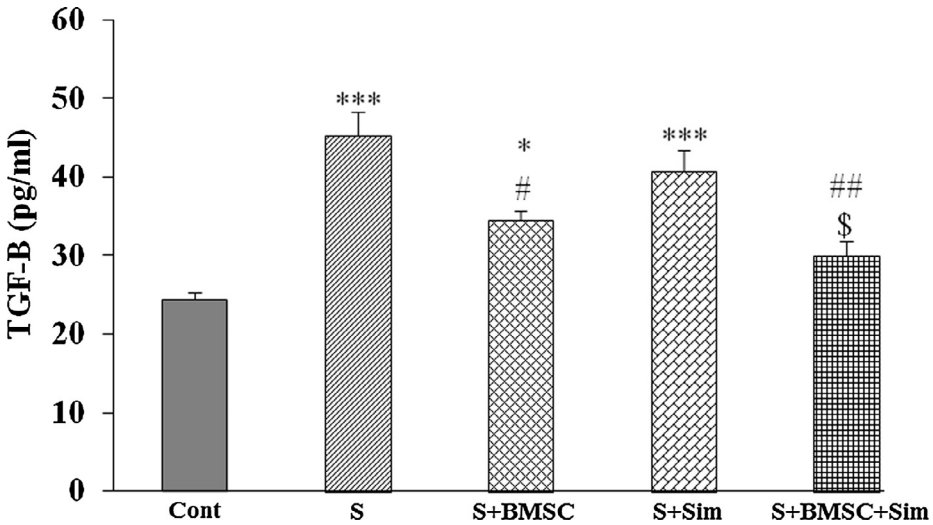

Fig. 6. TGF-β level in BAL fluid of: control animals (Cont, n = 5), sensitized animals (S, n = 5), sensitized animals treated with BMSCs (S + BMSC, n = 6), simvastatin (S + Sim, n = 6) and BMSCs and simvastatin (S + BMSC + Sim, n = 4). $^{*}P < 0.05$, $^{***}P < 0.001$ compared to control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared to sensitized group, $^{\$}P < 0.05$ compared to S + simvastatin group. Statistical comparison between groups was performed using analysis of variance followed by the Tukey test.

$p < 0.001$), (Figs. 5 and 6).

4. Discussion

In the present study there were significant increase in serum total and specific IgE as well as IL-13 and TGF-β levels in BAL fluid in sensitized animals compared to control group. Treatment with simvastatin reduced serum total and specific IgE levels but was unable to change IL-13 and TGF-β levels in BAL fluid. Treatment with BMSCs decreased serum total and specific IgE levels as well as IL-13 and TGF-β levels in BAL fluid. Simvastatin and BMSCs combination therapy not only reduced total and specific IgE levels and IL-13 and TGF-β levels in BAL fluid, but was also more effective in reducing most measured parameters than simvastatin and BMSCs alone.

The possible mechanism of the higher therapeutic effect of simvastatin and BMSCs combination therapy compared to treatment with each drug alone and especially BMSCs therapy is increased BMSCs migration to and localization in the lungs of sensitized animals. In fact in the previous study, it was shown that BMSCs migrate to and localize in the lungs of sensitized mice treated with BMSCs and effectively inhibit airway inflammation, goblet cell hyperplasia and inflammatory cell infiltration including neutrophil and eosinophil to the BAL fluid, but are unable to reduce subepithelial fibrosis [13]. However, the results of other study, showed that administration of BMSCs with simvastatin resulted in a higher migration of BMSCs into the lung tissue of sensitized mice and decreases neutrophil and eosinophil infiltration to the BAL fluid, airway inflammation and goblet cell hyperplasia. Furthermore, the combination therapy was more effective in reducing some above parameters than simvastatin and BMSCs treatment alone and also can reduce subepithelial fibrosis.

In the present study, treatment with simvastatin reduced serum total and specific IgE levels but did not change IL-13 and TGF-β levels in BAL fluid. It has been shown that statins inhibit Th2 response and thereby reduce Th2 cytokines levels, inflammatory cell infiltration to the airways, IgE production, goblet cell hyperplasia and collagen deposition [17–19]. The anti-inflammatory properties of simvastatin in COPD and particularly in asthma was shown by modulation of T-cell activity. Simvastatin is able to activate monocytes to modulate cytokine release from T-cells. It might suppressTh2/Tc2-cytokines in asthma and COPD but could increase IFNγ in COPD [20]. The results of the present study showed that simvastatin reduce serum total and specific IgE levels and thereby decrease airway eosinophilia and neutrophilia. However, simvastatin failed to completely eliminate these patho-physiological changes and reverse them to normal level. Furthermore, simvastatin treatment could not reduce IL-13 and TGF-β levels and therefore failed to decrease goblet cell hyperplasia and collagen deposition. IL-13 stimulates the goblet cell proliferation and, by increasing TGF-B production, causes collagen deposition and fibrosis [2]. Therefore, the absence effect of simvastatin treatment on reduction of IL-13 level may leads to failure to reduce the TGF-β level, collagen deposition and goblet cell

hyperplasia.

However, treatment with BMSCs reduced serum total and specific IgE levels as well as IL-13 and TGF-β levels in BAL fluid. Th2 to Th1 shift may attenuate inflammatory and allergic responses during asthma treatment and the potential therapeutic effect of increased Th1/Th2 ratio in animal model of asthma was shown [21–23]. Previous studies have shown that BMSCs therapy can reduce IL-4, IL-5, IL-13, IgE and TGF-β levels in ovalbumin sensitized animals and shift the immune response from Th2 to Th1 [24–26]. The effect of BMSCs treatment on pulmonary inflammation may mediate by their immune system regulatory effect, because these cells secrete immunomodulatory substances disrupting chronic inflammatory processes [24,27]. Furthermore, BMSCs can inhibit Th2 production, suppress T-cell responses and disrupt T cells mitosis [26]. Reduction of airway inflammation, mucus hypersecretion and bronchoconstriction index as well as Th2 cytokines levels and inflammatory cells infiltration in animal models of asthma and chronic obstructive pulmonary disease (COPD) due to intravenously administration of MSCs were shown which support the findings of the present study [8,9,27–30]. The results of the present study as well as our previous studies showed that treatment with BMSCs caused reduction in serum total and specific IgE as well as IL-13 and TGF-β levels in BAL fluid, reduced airway inflammation, goblet cell hyperplasia and inflammatory cell infiltration to BAL fluid. It seems that the results of BMSCs therapy could be due, at least, in part, by modulating the immune system and inhibiting the Th2 immune response since BMSCs can reduce Th2 Cytokines levels. However, BMSCs therapy was unable to completely eliminate pathophysiological changes, and there was significant differences in most measured parameters, specially collagen deposition and subepithelial fibrosis between the BMSC treated group and control group which supported by previous studies [30,31].

The results of the present study showed that simvastatin and BMSCs combination therapy could reduce total and specific IgE levels as well as IL-13 and TGF-β levels in BAL fluid, furthermore, it was more potent in reducing some parameters than simvastatin therapy and BMSCs therapy. The greater effect of simvastatin and BMSCs combination therapy than the effect of each drug alone is perhaps due to increase BMSCs migration to the lung tissues as has been shown in the previous study [14]. Perhaps for this reason simvastatin and BMSCs combination therapy led to an increase in the BMSCs therapeutic effects, since combination therapy not only lead to reduction of some parameters such as total IgE and TGF-β levels, but also could reverse them to normal values. In addition, the decrease in some factors including serum specific IgE level and IL-13 and TGF-β levels in BAL fluid in the combination therapy group was significantly higher compared to simvastatin and BMSC therapy alone. In addition, combination therapy could take IL-13 levels closer to normal value than the other two treatments. Cui et al [32] and Pirzad et al [16] also showed that simvastatin can increases the therapeutic effect of BMSCs on stroke by increasing their migration into the brain tissue which support the findings of the present and our previous studies.

5. Conclusions

In conclusion, the results of the present study indicated the superiority of therapy than simvastatin therapy and BMSCs therapy alone on various pathophysiological changes involved in asthma which may be due to increase in BMSCs migration into the lung tissue by simvastatin.

Taken together the findings of the present study showed that combination therapy of simvastatin and BMSC may also be a possible therapy for asthma, but caution and solid preclinical data building are warranted.

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