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Rosuvastatin suppresses cytokine production and lung inflammation in asthmatic, hyperlipidemic and asthmatic-hyperlipidemic rat models

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ABSTRACT

Background: Given the role that T lymphocytes play on the pathogenesis of allergic asthma, drugs targeting Th2 and Th17 cells may be a hopeful therapeutic strategy. This study aimed to evaluate the effect of rosuvastatin treatment on cytokine production and lung inflammation in allergic asthma.

Methods: The animals were assigned into control (C), asthmatic (A), hyperlipidemic (H), asthmatic-hyperlipidemic (AH), rosuvastatin (40 mg/kg/day intraperitoneally, for 3 weeks)-treated asthmatic (AR), rosuvastatin-treated hyperlipidemic (HR) and rosuvastatin-treated asthmatic-hyperlipidemic (AHR) groups (n = 6 in each group). The levels of IL-4, IFN-γ and IL-17, total and differential WBC counts in bronchoalveolar lavage fluid (BALF), Th1/Th2 balance, and pathological changes were evaluated.

Results: The BALF level of IL-4 in A, H and AH groups, and IL-17A in A and AH groups were significantly higher than that in C group ($p < 0.05$ to $p < 0.001$). IFN-γ level and Th1/Th2 balance (IFN-γ/IL-4 ratio) in A and AH groups were significantly decreased ($p < 0.05$ to $p < 0.01$). Inflammatory cells infiltration, muscle hypertrophy and emphysema were also observed in A and AH groups. The BALF levels of IL-4 in AR, HR and AHR groups, IFN-γ level in HR group, and IL-17A level in AR and AHR groups showed a significant improvement compared to that of A, H and AH groups ($p < 0.05$ to $p < 0.001$). Rosuvastatin treatment increased Th1/Th2 balance in all treated groups ($p < 0.05$ to $p < 0.01$), decreased total WBC counts, neutrophilia, eosinophilia and lung inflammation in AR and AHR groups, and improved muscle hypertrophy and emphysema in AHR group.

Conclusions: Rosuvastatin treatment improved lung pathological changes by suppression of Th2 and Th17-mediated cytokines which was unrelated to its lipid-lowering activity. Therefore, rosuvastatin might be a candidate immunomodulatory drug for treatment of patients with allergic asthma.

1. Introduction

Asthma is a chronic inflammatory disorder of the airways with an alteration in the balance of T helper (Th)1/Th2, and an imbalance of regulatory T cells (Tregs)/Th17 ratio. The inflammatory responses in asthmatic airways are mainly mediated by activated Th2 cells, particularly interleukin (IL)-4, IL-5 and IL-13. Th1 cells can inhibit the development of Th2 cells, reducing the Th2-induced asthmatic response [1]. The Th17 cells are a subset of pro-inflammatory T helper cells which promote the release of other pre-inflammatory cytokines, recruit neutrophils, promote secretion of mucus by the mucous glands, and strengthen the airway hyper-responsiveness [2].

Hyperlipidemia is a potential risk factor for asthma independent of obesity [3] and associate with an elevated ratio of IL-4/IFN-γ cells, and switching of the T-cell responses from a healing Th1 response to a non-healing Th2 response in animal study [4]. Lipid rafts act as signaling

Abbreviations: Th, T helper; Tregs, regulatory T cells; IL, interleukin; HMG–CoA, 3-hydroxy-3-methylglutaryl-CoA; BALF, bronchoalveolar lavage fluid; TC, total cholesterol; TG, triglycerides; LDL-C, low density lipoprotein; HDL-C, high density lipoprotein; ANOVA, analyzed by the one-way analysis of variance; VCAM, vascular cell adhesion molecule

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platforms, connecting molecules essential for activation of immune cells [5]. Therefore, crosstalk between lipid metabolism and host immunity may affect allergic diseases. Accordingly, the existence of close interactions between pathophysiology of asthma and hyperlipidemia conditions is suggested [6]. The studies reported inconsistent findings regarding the effect of hyperlipidemia on immune system [7–10]. Up to date, only a few and inconsistent studies have explored the relationship between lipid profiles and allergic asthma.

Statins, the 3-hydroxy-3-methylglutaryl-CoA (HMG–CoA) reductase inhibitors, are one class of lipid-lowering medications that potentially could be used as an alternative treatment for patients with chronic respiratory diseases, which possibly mediated by other pleiotropic actions such as antioxidant, anti-inflammatory and immunomodulatory effects [11]. The novel immunomodulatory aspects of statins in conjunction with the wide use of these medications highlight the importance of examining the potential effects of these medications [12]. Rosuvastatin is a synthetic statin that has a high affinity for the active site of HMG–CoA reductase and exhibits greater potency in inhibiting enzyme activity and cholesterol synthesis in vitro than other statins [13]. This statin showed higher pleiotrophic effects such as anti-oxidant, anti-inflammatory and immunomodulatory properties than other statins [11].

The present study aimed to examine the possible interaction of hyperlipidemia on allergic asthma, and evaluate the effect of rosuvastatin as a potential anti-inflammatory agent on allergic asthma through measurement of IL-4, IFN-γ and IL-17A levels, total white blood cell (WBC), eosinophil and neutrophil counts in bronchoalveolar lavage fluid (BALF), Th1/Th2 balance, and pathological changes in asthmatic, hyperlipidemic and asthmatic-hyperlipidemic rat models. This study was conducted in three different conditions, which has not been done before.

2. Materials and methods

2.1. Animals

Forty-two male Wistar rats (8 weeks old and weighing 140–160 g) were obtained from Animal house, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran and were placed in plexiglas cages (n = 3 per cage) under controlled conditions of 12 h light/dark cycle, 22 ± 2 °C and humidity of 54 ± 2%. Food and water were provided *ad libitum* throughout the experimental period. This experimental study was done according to the Ethics Committee Guidelines of Mashhad University of Medical Sciences for Animal Experiments (number 940997).

Animals were randomly divided into seven groups (n = 6 in each group) as: control (C), asthmatic (A), hyperlipidemic (H), asthmatic-hyperlipidemic (AH), rosuvastatin-treated asthmatic (AR), rosuvastatin-treated hyperlipidemic (HR) and rosuvastatin-treated asthmatic-hyperlipidemic (AHR) groups. Treated groups received 40 mg/kg/day rosuvastatin (Sigma Chemical Ltd, UK) intraperitoneally for 3 weeks [14], (Fig. 1).

For hyperlipidemia induction, rats received normal diet plus 10% ethanol (Sanaeh Shemicali Daro Hamon Teb Markazi, Zarandieh, Iran) and 10% fructose (Sigma Chemical Ltd, UK) in drinking water during 9 weeks [14]. Each rat in all groups averagely drank 40 ml/day drinking water which was not significantly different among experimental groups. After 9 weeks, sensitization of animals with ovalbumin (Sigma Chemical Ltd, UK, 98% pure) in the A, AH, AR and AHR groups was performed using previously described standard protocol [14].

2.2. Determination of lipid profile

At the end of 12 weeks period, the rats were anesthetized by intrapritoneal administration of ketamine (50 mg/kg) and xylazine (5 mg/kg). Blood samples (5 ml per rat) were taken out by cardiac puncture and centrifuged at 2000 revolution per minute (rpm) for 10 min. The serum was collected using pasture pipette and stored at −20 °C and thawed just before use for the determination of blood lipids. Serum total cholesterol (TC), triglycerides (TG), Low density lipoprotein (LDL-C) and high density lipoprotein (HDL-C) were measured on the basis of colorimetric method for single point measurement using colorimetric kits (Pars Azmoon Co, Iran) following the manufacturer's instructions.

2.3. Measurement of BALF IL-4, IFN-γ and IL-17A levels

For BALF preparation, immediately after blood sample collection through cardiac puncture and opening the chest, the left lung was clamped and preserved for histological analysis. A cannula carefully introduced into the trachea and the right lung lavage was performed with one mL normal saline for five times (total 5 ml), [15,16]. After BALF centrifugation (3500 rpm at 4 °C for 10 min), supernatant was collected and stored at −70 °C until analysis. Finally, BALF levels of IL-4, IFN-γ (Bioassay Technology Laboratory, China) and IL-17A (Karmania Pars Gene, Iran) were measured by commercial ELISA kits [17].

2.4. Total WBC, eosinophil and neutrophil counts in BALF

One mL of BALF was stained with Turk solution and counted by Neubauer chamber. The smear of centrifuged BALF was examined under a light microscope and eosinophils and neutrophils were counted and classified based on their appearance. After determining total WBC, eosinophil and neutrophil counts, the absolute number of each type of WBC was calculated by multiplying the percentage of each subset in an individual sample by the total number of cells in that sample [14].

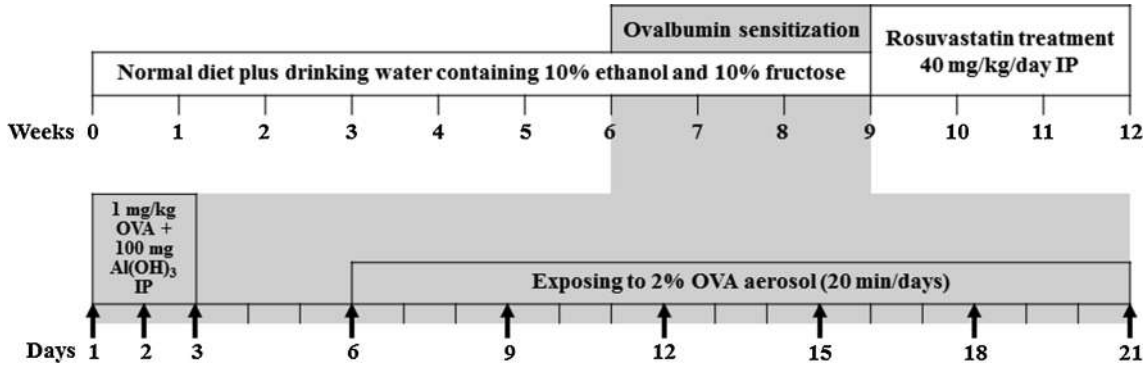

Fig. 1. Time table of the study and protocol of animal sensitization and treatment. Lower panel shows the method of animal sensitization and upper panel indicates the method for hyperlipidemia induction and treatment with rosuvastatin.

2.5. Lung histopathological evaluation

After bronchoalveolar lavage had been performed, the left lung was removed. The specimens were fixed in 10% formalin (37%, Merck, Germany), embedded in paraffin and were cut into 4 μm slices and stained with hematoxylin-eosin (H&E) solution. Subsequently, the histology slides were viewed under a light microscope. Lung pathologic changes of different groups such as inflammation, muscle hypertrophy and emphysema were evaluated. The scoring of pathological changes was: 0, no pathological changes; 1, patchy changes; 2, local changes; and 3, severe changes, [17].

2.6. Statistical analysis

Data were analyzed by the one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test and results were presented as mean ± SEM. Values of $p < 0.05$ were considered statistically significant. Ranked data were presented as box plots showing the minimum, first quartile, median, third quartile and maximum of the lung pathological changes. For comparison of the lung pathological results, the Kruskal-Wallis test and the Mann-Whitney *U* test were used.

The percentage change for each variable was calculated in non-treated groups, using the following equations:

$$\text{A/C, H/C or AH/C} = \frac{(\text{Value in A, H or AH groups} - \text{Value in C group}) \times 100}{\text{Value in C group}}$$

And in treated groups as follows:

$$\text{AR/A, HR/H or AHR/AH} = \frac{(\text{Value in AR, HR or AHR groups} - \text{Value in A, H or AH groups}) \times 100}{\text{Value in A, H or AH groups}}$$

Regarding the scoring of pathological changes and the presence of samples with zero score (no pathological changes) in the C group, the percent change of the pathological changes in A, H and AH groups compared to the C group could not be calculated.

3. Results

3.1. Serum lipid profile

The serum levels of TC, TG and LDL-C in H and AH groups were significantly higher than C group ($p < 0.05$ to $p < 0.001$, Fig. 2). There were no significant difference in the lipid profile between H and AH groups. A significant reduction in the serum level of TC was seen in HR and AHR groups compared to H and AH groups ($p < 0.01$ for both cases, Fig. 2a). Rosuvastatin treatment decreased serum level of TG in AR ($p < 0.05$), HR, and AHR groups compared to A, H and AH groups respectively ($p < 0.001$ for both cases, Fig. 2b). A significant reduction in the serum level of LDL-C was seen in AHR group compared to AH group ($p < 0.05$, Fig. 2c).

3.2. BALF levels of IL-4, IFN-γ and IL-17A

The BALF level of IL-4 in A, H and AH groups was significantly higher than that in C group ($p < 0.05$ for A group and $p < 0.001$ for H and AH groups, Fig. 3a). The percent change of IL-4 in AH/C was significantly higher than that of H/C ($p < 0.05$, Fig. 4a). The BALF level of IL-4 in AR, HR and AHR groups showed significant decrease compared to those of A, H and AH groups ($p < 0.05$ to $p < 0.001$, Fig. 3a).

Compared to C group, the BALF level of IFN-γ in A and AH groups was significantly decreased ($p < 0.01$ and $p < 0.05$, respectively), while IFN-γ level in H group was increased ($p < 0.05$, Fig. 3b). The percent change of BALF IFN-γ level in AH/C was significantly lower than those of A/C ($p < 0.05$), and higher than those of H/C ($p < 0.001$, Fig. 4b). The BALF level of IFN-γ in HR group showed a significant decrease compared to that of H group ($p < 0.05$, Fig. 3b). The percent change of IFN-γ level in AHR/AH was significantly higher than that of HR/H ($p < 0.001$, Fig. 4b).

The Th1/Th2 balance (IFN-γ/IL-4 ratio) was decreased in A and AH groups compared to that of C group ($p < 0.01$ and $p < 0.05$, respectively, Fig. 3c). The percent change of Th1/Th2 (IFN-γ/IL-4) ratio

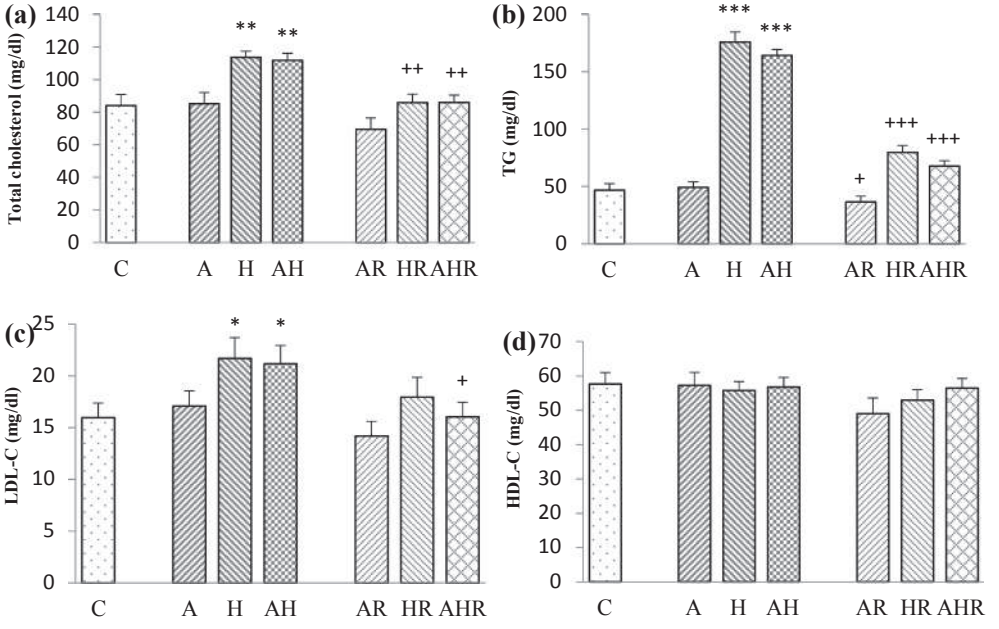

Fig. 2. The lipid profiles of total cholesterol (a), triglycerides (b), LDL-C (c), and HDL-C (d) in the control (C), asthmatic (A), hyperlipidemic (H), asthmatic-hyperlipidemic (AH), rosuvastatin-treated asthmatic (AR), rosuvastatin-treated hyperlipidemic (HR), and rosuvastatin-treated asthmatic-hyperlipidemic (AHR) groups. Data are shown as mean ± SEM (n = 6 in each group). *;P < 0.05, **;P < 0.01 and ***;P < 0.001 compared to control group. +;P < 0.05, ++;P < 0.01 and +++;P < 0.001 compared to untreated groups. Statistical analysis were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

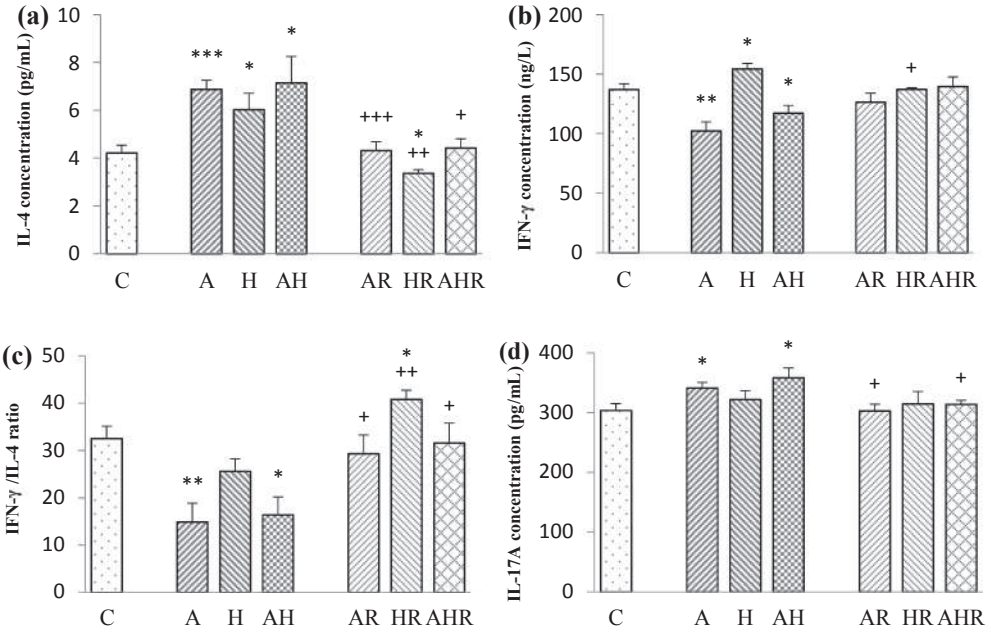

Fig. 3. The levels of IL-4 (a) and IFN-γ (b), Th1/Th2 balance (IFN-γ/IL-4 ratio), (c), and IL-17A level (d) in BALF of the control (C), asthmatic (A), hyperlipidemic (H), asthmatic-hyperlipidemic (AH), rosuvastatin-treated asthmatic (AR), rosuvastatin-treated hyperlipidemic (HR), and rosuvastatin-treated asthmatic-hyperlipidemic (AHR) groups. Data are shown as mean ± SEM (n = 6 in each group). *;P < 0.05, **;P < 0.01 and ***;P < 0.001 compared to control group. +;P < 0.05 compared to untreated groups. Statistical analysis were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

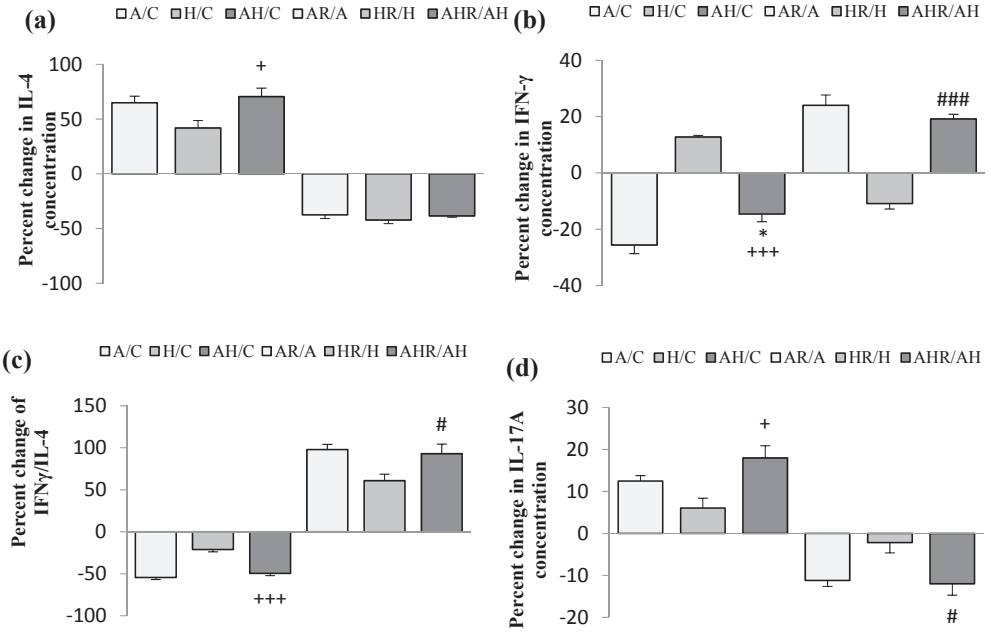

Fig. 4. Percent change of BALF levels of IL-4 (a), IFN-γ (b), Th1/Th2 balance (IFN-γ/IL-4 ratio), (c), and IL-17A (d) in the asthmatic relative to the control group (A/C), hyperlipidemic relative to the control group (H/C), asthmatic-hyperlipidemic relative to the control group (AH/C), rosuvastatin-treated asthmatic relative to the asthmatic group (AR/A), rosuvastatin-treated hyperlipidemic relative to the hyperlipidemic group (HR/H), and rosuvastatin-treated asthmatic-hyperlipidemic relative to the asthmatic-hyperlipidemic group (AHR/AH). Data are shown as mean ± SEM (n = 6 in each group). +;P < 0.05, ++;P < 0.0 and +++;P < 0.001 compared to H/C group. #;P < 0.05 compared to HR/H group. Statistical analysis were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

in AH/C was significantly higher than that of H/C ($p < 0.001$, Fig. 3c). Rosuvastatin treatment increased the Th1/Th2 balance (IFN-γ/IL-4 ratio) in AR, HR and AHR groups compared to those of A, H and AH groups ($p < 0.05$ for AR and AHR groups, and $p < 0.01$ for HR group, Fig. 3c). The percent change of Th1/Th2 (IFN-γ/IL-4) ratio in AHR/AH was significantly higher than that of HR/H ($p < 0.05$, Fig. 3c).

The BALF value of IL-17A in A and AH groups was significantly higher than that in C group ($p < 0.05$ for both groups, Fig. 3d). The percent change of IL-17A level in AH/C was significantly higher than that of H/C ($p < 0.05$, Fig. 4d). The BALF level of IL-17A in AR and AHR groups showed a significant decrease compared to those of A and AH groups ($p < 0.05$ for both groups, Fig. 3d). The percent change of IL-17A level in AHR/AH was significantly higher than that of HR/H ($p < 0.05$, Fig. 4d).

3.3. Total WBC, eosinophil and neutrophil counts in BALF

Total WBC, eosinophil and neutrophil counts of A and AH groups were significantly higher than those of controls ($p < 0.001$ for all cases, Fig. 5a–c). A higher number of neutrophils was also seen in H group compared to C group ($p < 0.05$, Fig. 5c). Significant reduction in total WBC, eosinophil and neutrophil were seen in AR and AHR groups compared to A and AH groups ($p < 0.01$ for all cases, Fig. 5a–c).

3.4. Lung histopathological changes

Inflammatory cell infiltration in peribronchial and perivascular areas, airway epithelial thickening, bronchial dilation and airspace enlargement were observed in A and AH groups (Fig. 6b and d). H

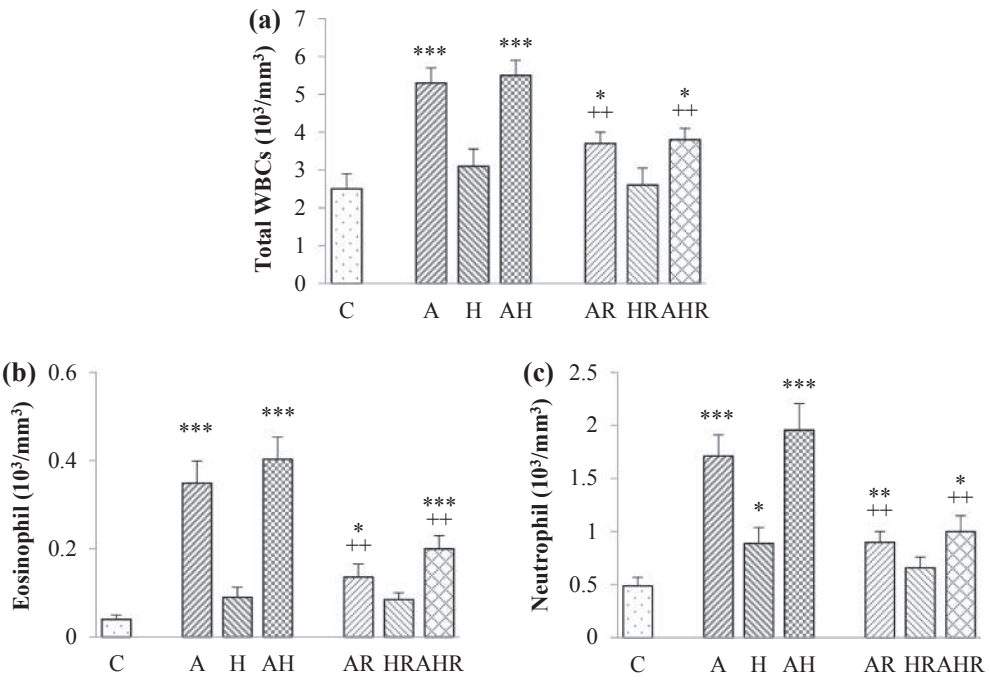

Fig. 5. Total WBC (a), eosinophil (b), and neutrophil (c) counts in BALF of the control (C), asthmatic (A), hyperlipidemic (H), asthmatic-hyperlipidemic (AH), rosuvastatin-treated asthmatic (AR), rosuvastatin-treated hyperlipidemic (HR), and rosuvastatin-treated asthmatic-hyperlipidemic (AHR) groups. Data are shown as mean ± SEM (n = 6 in each group). *;P < 0.05, **;P < 0.01 and ***;P < 0.001 compared to control group. +;P < 0.05 and ++;P < 0.01 compared to untreated groups. Statistical analysis were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

group showed only a patchy change in inflammatory cell infiltration (Fig. 6c). The pathological changes of lung including inflammation, muscle hypertrophy and emphysema in A and AH groups were significantly higher than those of C group ($p < 0.05$ to $p < 0.001$, Fig. 7).

Reduced inflammatory cell infiltration in the peribronchial and perivascular areas was observed in AR and AHR groups (Fig. 6e and g). Rosuvastatin treatment decreased lung inflammation in AR and AHR groups, and improved muscle hypertrophy and emphysema in AHR group compared to untreated groups ($p < 0.05$ to $p < 0.001$, Fig. 7). The percent change of muscle hypertrophy in AHR/AH was significantly higher than that of HR/H ($p < 0.05$, Fig. 8b).

4. Discussion

The results of the present study showed an increase in total WBC, eosinophil and neutrophil counts, and the levels of IL-4 and IL-17A, and a decrease in IFN-γ level and IFN-γ/IL-4 ratio in BALF of asthmatic group. Moreover, pathological changes including inflammation, muscle hypertrophy and emphysema confirmed allergic asthma induction in the current study similar to the results of previous studies [14,17,18]. In this study, induction of hyperlipidemia caused a significant increase in the serum levels of TC, TG and LDL-C compared to controls. The ethanol-fructose combined diet significantly worsened plasma lipid profiles in rats which was in line with the previous study [14]. Hyperlipidemia also resulted in increased levels of IL-4 and IFN-γ in BALF which has been reported previously in rats [19,20] and human [21,22] with hyperlipidemia.

Alternation in lipid metabolism and abnormalities in lipid profile have a potent influence on immunity and inflammation [19,22]. IL-4 stimulates lipolysis by enhancing the activity of hormone-sensitive lipase and it may participate in lipid metabolism [19].

In this study, induction of hyperlipidemia together with allergic asthma caused a significant increase in total WBC, eosinophil and neutrophil counts, and the levels of IL-4 and IL-17A, and a significant decrease in IFN-γ level and IFN-γ/IL-4 ratio in BALF which may be due to allergic asthma, hyperlipidemia or both conditions. The percent change of IFN-γ in asthmatic-hyperlipidemic group relative to control group (AH/C) was significantly lower than those of asthmatic group relative to control group (A/C) which could be due to hyperlipidemia condition. The percent change of IL-4, IFN-γ, IL-17A and IFN-γ/IL-4 ratio in asthmatic-hyperlipidemic group relative to control group (AH/C) was significantly higher than those of hyperlipidemic group relative to control group (H/C) which shows that this increase in AH group was due to allergic asthma condition.

Increase in total WBC counts, especially BALF eosinophilia, is a characteristic of allergic asthma [11]. In this study, rosuvastatin reduced total WBC counts, neutrophilia and eosinophilia in BALF. In the mice models of ovalbumin-induced asthma, treatment with simvastatin [23] and rosuvastatin [14,24] reduced the total WBC, lymphocyte, macrophage, neutrophil and eosinophil counts in BALF dose-dependently. Rosuvastatin also improved peripheral eosinophilia in asthmatic patients [25] which supported the results of this study.

Beyond blood lipid lowering, systemic treatment with rosuvastatin decreased IL-4 level in all treated groups, and IL-17 level in AR and AHR groups. The percent changes in IFN-γ, IL-17A and IFN-γ/IL-4 ratio in treated asthmatic-hyperlipidemic group relative to untreated asthmatic-hyperlipidemic group (AHR/AH) was significantly higher than those of treated hyperlipidemic group relative to untreated hyperlipidemic group (HR/H) indicating the response to rosuvastatin treatment in AHR group was due to the improvement of allergic asthma conditions. Moreover, pathological changes including inflammation, muscle hypertrophy and emphysema were improved in the AR and AHR groups.

In fact the effect of treatment with simvastatin, lovastatin, fluvastatin and rosuvastatin on reduction of Th2 cytokines levels in a mouse allergic asthma model were shown previously [5,23,24,26–28]. Anti-inflammatory effects of pravastatin and pitavastatin through RhoA inhibition and suppression of inflammatory cytokine production were also demonstrated. Simvastatin and lovastatin also reduced serum cholesterol level and Rho expression in the lungs of murine model of asthma [29,30]. Therefore, inhibition of HMG-CoA reductase and the RhoA/Rho-kinase pathway may be considered as a useful target for the treatment of allergies and asthma. In another study, pravastatin

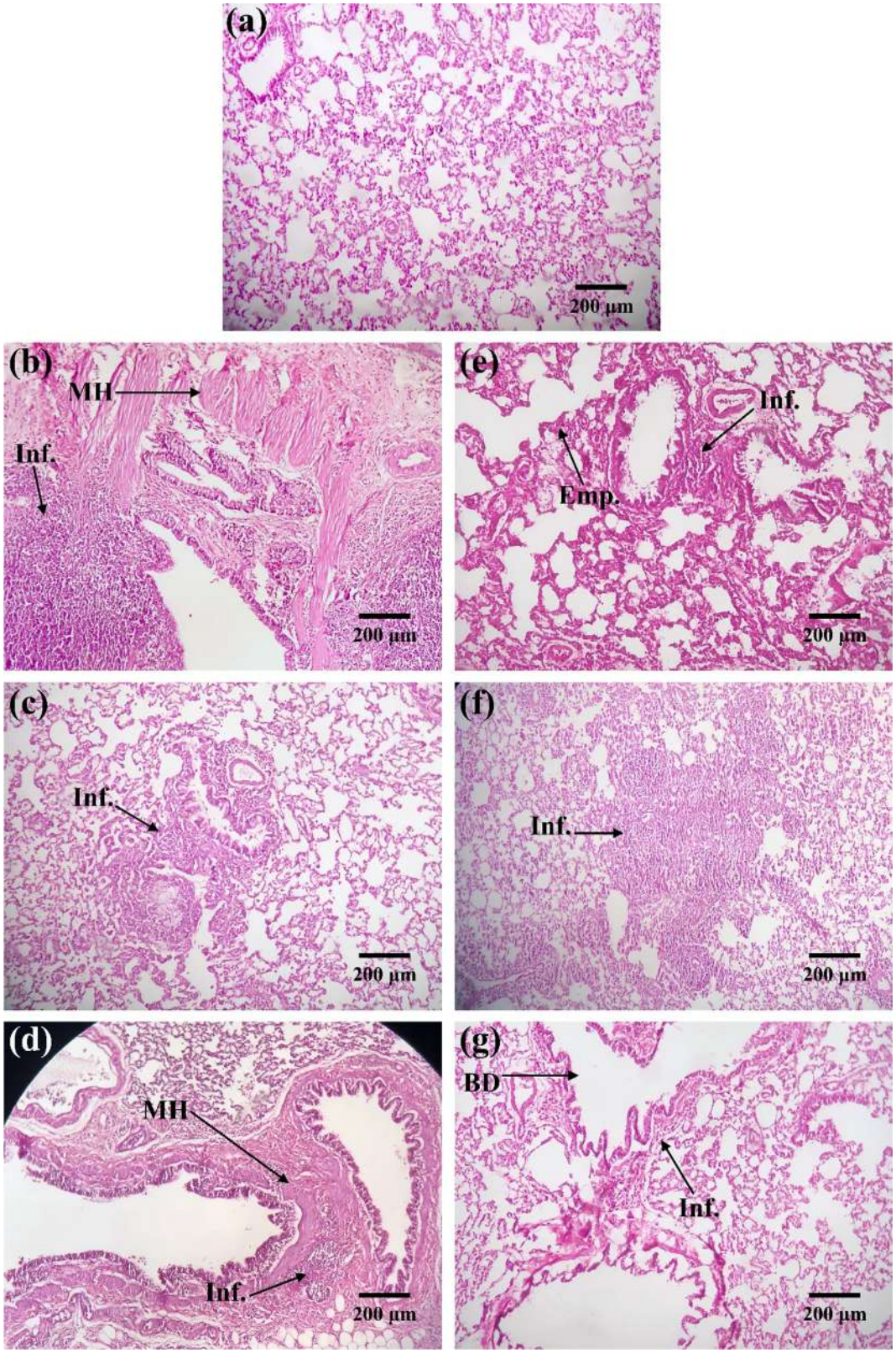

Fig. 6. Photographs of a lung specimen in the control (a), asthmatic (b), hyperlipidemic (c), asthmatic-hyperlipidemic (d), rosuvastatin-treated asthmatic (e), rosuvastatin-treated hyperlipidemic (f), and rosuvastatin-treated asthmatic-hyperlipidemic (g) groups. BD: bronchial dilation, Emp.: emphysema, Inf.: inflammatory cell infiltration, and MH: muscular hypertrophy (Magnification: 10 × 20; scale bar: 200 µm).

suppressed airway inflammation by inhibiting IL-17 production [31]. The role of IL-17 in immune responses has been recently highlighted, but whether rosuvastatin affect IL17 production has not been well studied. In other animal models, rosuvastatin ameliorated Th17/Treg functional imbalance in hypertensive patients with carotid atherosclerosis [32]. Rosuvastatin treatment also reduced Th2 and Th17 cell

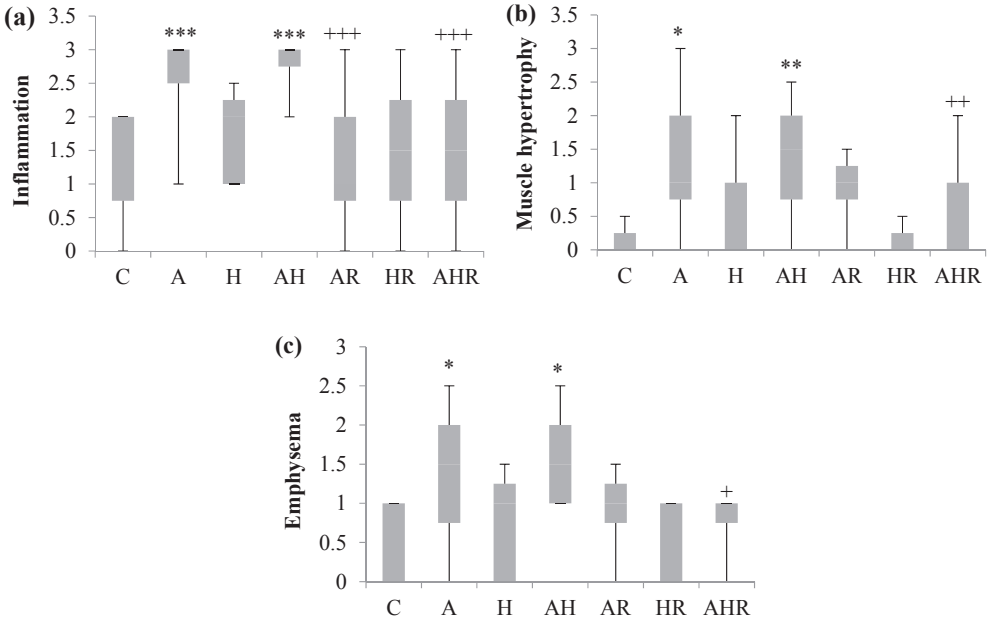

Fig. 7. Lung pathological score in the control (C), asthmatic (A), hyperlipidemic (H), asthmatic-hyperlipidemic (AH), rosuvastatin-treated asthmatic (AR), rosuvastatin-treated hyperlipidemic (HR), and rosuvastatin-treated asthmatic-hyperlipidemic (AHR) groups. Box plot graph showing the minimum, first quartile, median, third quartile and maximum of the lung pathological changes (n = 6 in each group). For comparison of the lung pathological results, the Kruskal-Wallis test and the Mann-Whitney *U* test were used. *;P < 0.05, **;P < 0.01 and ***;P < 0.001 compared to control group. +;P < 0.05, + +;P < 0.01 and + + +;P < 0.001 compared to untreated groups.

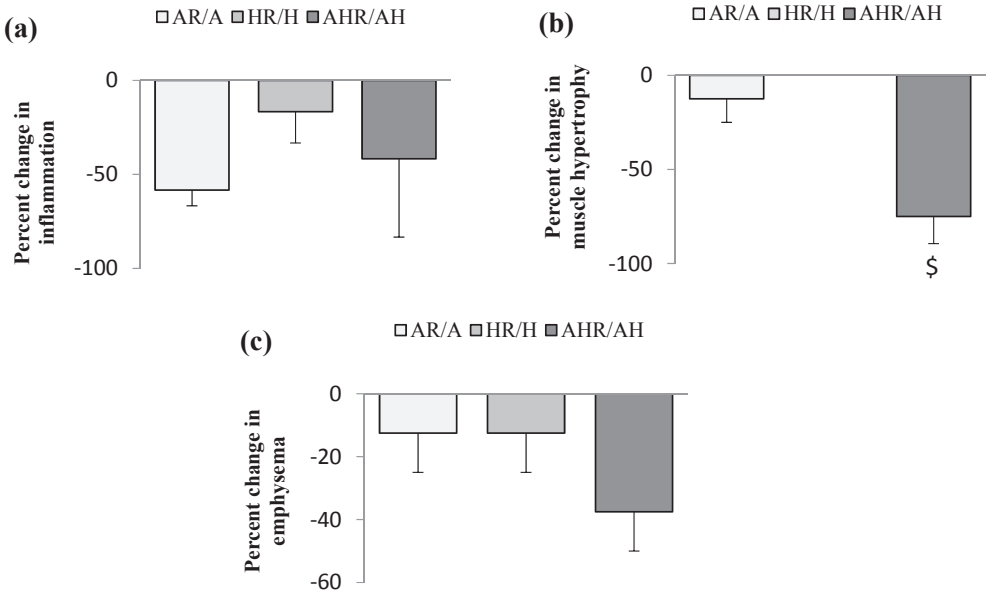

Fig. 8. Percent change of pathological changes including inflammation (a), muscle hypertrophy (b) and emphysema (c) in rosuvastatin-treated asthmatic relative to the asthmatic group (AR/A), rosuvastatin-treated hyperlipidemic relative to the hyperlipidemic group (HR/H), and rosuvastatin-treated asthmatic-hyperlipidemic relative to the asthmatic-hyperlipidemic group (AHR/AH). Data are shown as mean ± SEM (n = 6 in each group). $;P < 0.05 compared to AR/A group. Statistical analysis were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

cytokines in a dextran sulfate sodium-induced colitis model [33] which was in line with the findings of the present study.

The inflammatory cell infiltration in the airways, allergic airway inflammation and airway remodeling are related to expression of vascular cell adhesion molecule (VCAM)-1 in endothelial cells by antigen. Th2-mediated cytokines such as IL-4 increases VCAM-1 expression in the airway endothelial cells [26]. In this study, rosuvastatin treatment ameliorated lung inflammation, smooth muscle hypertrophy and emphysema by suppressing Th2 and Th17-mediated cytokines. Given the major role that T lymphocytes play on the pathogenesis of allergic asthma, drugs targeting Th2 and Th17 cells may be a candidate therapeutic strategy.

There are a few limitations to this study that need to be addressed in further studies. First, only one dose of rosuvastatin has been studied. It has already been shown that the pleiotropic effect of rosuvastatin is dose-dependent. In previous studies, the administration of rosuvastatin at dose of 40 mg/kg showed an anti-hyperlipidemic effect, whereas higher doses induced adverse effects [34]. Second, currently there is no reference drug that can be administrated simultaneously to treat hyperlipidemia and allergic asthma.

5. Conclusions

Rosuvastatin showed the anti-inflammatory and immunomodulatory effects on allergic asthma model which was unrelated to its lipid-lowering activity. The therapeutic effect of rosuvastatin on allergic asthma is not only due to its lipid lowering effect because it affects both non-hyperlipidemic and hyperlipidemic asthmatic groups. Therefore, rosuvastatin might be a candidate immunomodulatory drug for the treatment of patients with allergic asthma.

CRediT authorship contribution statement

Saeideh Saadat: Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Visualization. **Nema Mohamadian Roshan:** Methodology, Visualization. **Mohammad Reza Aslani:** Resources. **Mohammad Hossein Boskabady:** Conceptualization, Methodology, Validation, Writing - review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] C. Yang, J. Tian, W. Ko, C. Shih, Y. Chiou, Oligofucoidan improved unbalance the Th1/Th2 and Treg/Th17 ratios in asthmatic patients: An ex vivo study, Exp. Ther. Med. 17 (2019) 3–10.
[2] J. Zhu, X. Liu, W. Wang, X. Ouyang, W. Zheng, Q. Wang, Altered expression of regulatory T and Th17 cells in murine bronchial asthma, Exp. Ther. Med. 14 (2017) 714–722.
[3] B. Al-Shawwa, N. Al-Huniti, G. Titus, M. Abu-Hasan, Hypercholesterolemia is a potential risk factor for asthma, J. Asthma 43 (2006) 231–233.
[4] A.K. Robertson, X. Zhou, B. Strandvik, G. Hansson, Severe hypercholesterolaemia leads to strong Th2 responses to an exogenous antigen, Scand. J. Immunol. 59 (2004) 285–293.
[5] T. Tschernig, Controversial data on simvastatin in asthma: What about the rat model? J Asthma Allergy. 3 (2010) 57–63.
[6] Y.C. Chen, K.Y. Tung, C.H. Tsai, M.W. Su, P.C. Wang, C.H. Chen, et al., Lipid profiles in children with and without asthma: interaction of asthma and obesity on hyperlipidemia, Diabetes Metab. Syndr. 7 (2013) 20–25.
[7] Á. Baldán, A.V. Gomes, P. Ping, P.A. Edwards, Loss of ABCG1 results in chronic pulmonary inflammation, J. Immunol. 180 (2008) 3560–3568.
[8] Y.-F. Yeh, S.-L. Huang, Enhancing effect of dietary cholesterol and inhibitory effect of pravastatin on allergic pulmonary inflammation, J. Biomed. Sci. 11 (2004) 599–606.
[9] A. McKay, B.P. Leung, I.B. McInnes, N.C. Thomson, F.Y. Liew, A novel anti-inflammatory role of simvastatin in a murine model of allergic asthma, J. Immunol. 172 (2004) 2903–2908.
[10] M.B. Fessler, M.W. Massing, B. Spruell, R. Jaramillo, D.W. Draper, J.H. Madenspacher, et al., Novel relationship of serum cholesterol with asthma and wheeze in the United States, J. Allergy Clin. Immunol. 124 (967–74) (2009) e15.
[11] P. Bradbury, D. Traini, A.J. Ammit, P.M. Young, H.X. Ong, Repurposing of statins via inhalation to treat lung inflammatory conditions, Adv. Drug Deliv. Rev. 133 (2018) 93–106.
[12] M. Ostroukhova, R.W. Kouides, E. Friedman, The effect of statin therapy on allergic patients with asthma, Ann. Allergy Asthma Immunol. 103 (2009) 463–468.
[13] V. Capra, G.E. Rovati, Rosuvastatin inhibits human airway smooth muscle cells mitogenic response to eicosanoid contractile agents, Pulm. Pharmacol. Ther. 27 (2014) 10–16.
[14] S. Saadat, A. Mokhtari, M.H. Boskabady, Rosuvastatin affects tracheal responsiveness, bronchoalveolar lavage inflammatory cells, and oxidative stress markers in hyperlipidemic and asthmatic rats, Iran J Allergy Asthm. 18 (2019) 624–638.
[15] S. Saadat, M. Mohammadi, M. Fallahi, M.R. Aslani, The protective effect of α-hederin, the active constituent of Nigella sativa, on tracheal responsiveness and lung inflammation in ovalbumin-sensitized guinea pigs, J. Physiol. Sci. 65 (2015) 285–292.
[16] S. Saadat, F. Beheshti, V.R. Askari, M. Hosseini, N.M. Roshan, M.H. Boskabady, Aminoguanidine affects systemic and lung inflammation induced by lipopolysaccharide in rats, Respir. Res. 20 (2019) 96.
[17] R. Keyhanmanesh, S. Saadat, M. Mohammadi, A.A. Shahbazfar, M. Fallahi, The protective effect of α-hederin, the active constituent of nigella sativa, on lung inflammation and blood cytokines in ovalbumin sensitized guinea pigs, Phytother. Res. 29 (2015) 1761–1767.
[18] H. Ebrahimi, M. Fallahi, A.M. Khamaneh, M.A. Ebrahimi Saadatlou, S. Saadat, R. Keyhanmanesh, Effect of α-hederin on IL-2 and IL-17 mRNA and miRNA-133a levels in lungs of ovalbumin-sensitized male rats, Drug Dev. Res. 77 (2016) 87–93.
[19] J.B. Braun, J.B. Ruchel, A.G. Manzoni, F.H. Abdalla, E.A. Casalli, L.G. Castilhos, et al., Pretreatment with quercetin prevents changes in lymphocytes E-NTPDase/E-ADA activities and cytokines secretion in hyperlipidemic rats, Mol. Cell. Biochem. 444 (2018) 63–75.
[20] M.-X. Xu, M. Wang, W.-W. Yang, Gold-quercetin nanoparticles prevent metabolic endotoxemia-induced kidney injury by regulating TLR4/NF-κB signaling and Nrf2 pathway in high fat diet fed mice, Int. J. Nanomed. 12 (2017) 327.
[21] S.R. Mirhafez, M. Tajfard, A. Avan, A. Pasdar, R. Nedaeinia, M. Aghasizade, et al., Association between serum cytokine concentrations and the presence of hypertriglyceridemia, Clin. Biochem. 49 (2016) 750–755.
[22] A. Santi, I.B.M. da Cruz, V.L. Loro, M.M.M.F. Duarte, F. Barbisan, T. Duarte, et al., Overt hypothyroidism is associated with blood inflammatory biomarkers dependent of lipid profile, J. Appl. Biomed. 14 (2016) 119–124.
[23] L. Xu, X-w Dong, L-l Shen, F-f Li, J-x Jiang, R. Cao, et al., Simvastatin delivery via inhalation attenuates airway inflammation in a murine model of asthma, Int. Immunopharmacol. 12 (2012) 556–564.
[24] Z. Tao, W. Zhang, D-x Wang, N-w Huang, B. Hong, D. Wang, et al., Rosuvastatin attenuates mucus secretion in a murine model of chronic asthma by inhibiting the gamma-aminobutyric acid type A receptor, Chin. Med. J. 125 (2012) 1457–1464.
[25] S. Olgun Yildizeli, D. Kocakaya, B. Balcan, A. Ikinci, R. Ahiskali, B. Ceyhan, Influence of rosuvastatin treatment on airway inflammatory markers and health related quality of life domains in asthmatic patients, Marmara Med. J. 30 (2017) 73–81.
[26] D.Y. Kim, S.Y. Ryu, J.E. Lim, Y.S. Lee, J.Y. Ro, Anti-inflammatory mechanism of simvastatin in mouse allergic asthma model, Eur. J. Pharmacol. 557 (2007) 76–86.
[27] K.T.R. Samson, K. Minoguchi, A. Tanaka, N. Oda, T. Yokoe, Y. Yamamoto, et al., Inhibitory effects of fluvastatin on cytokine and chemokine production by peripheral blood mononuclear cells in patients with allergic asthma, Clin. Exp. Allergy 36 (2006) 475–482.
[28] C.-J. Liou, P.-Y. Cheng, W.-C. Huang, C.-C. Chan, M.-C. Chen, M.-L. Kuo, et al., Oral lovastatin attenuates airway inflammation and mucus secretion in ovalbumin-induced murine model of asthma, Allergy Asthma Immunol Res. 6 (2014) 548–557.
[29] T. Ahmad, U. Mabalirajan, A. Sharma, J. Aich, L. Makhija, B. Ghosh, et al., Simvastatin improves epithelial dysfunction and airway hyperresponsiveness: from asymmetric dimethyl-arginine to asthma, Am. J. Respir. Cell Mol. Biol. 44 (2011) 531–539.
[30] Y. Chiba, J. Arima, H. Sakai, M. Misawa, Lovastatin inhibits bronchial hyperresponsiveness by reducing RhoA signaling in rat allergic asthma, Am. J. Physiol. Lung Cell. Mol. Physiol. 294 (2008) 705–713.
[31] M. Imamura, K. Okunishi, H. Ohtsu, K. Nakagome, H. Harada, R. Tanaka, et al., Pravastatin attenuates allergic airway inflammation by suppressing antigen sensitisation, interleukin 17 production and antigen presentation in the lung, Thorax 64 (2009) 44–49.
[32] Z. Liu, Y. Zhao, F. Wei, L. Ye, F. Lu, H. Zhang, et al., Treatment with telmisartan/rosuvastatin combination has a beneficial synergistic effect on ameliorating Th17/Treg functional imbalance in hypertensive patients with carotid atherosclerosis, Atherosclerosis. 233 (2014) 291–299.
[33] S.K. Shin, J.H. Cho, E.J. Kim, E.-K. Kim, D.K. Park, K.A. Kwon, et al., Anti-inflammatory and anti-apoptotic effects of rosuvastatin by regulation of oxidative stress in a dextran sulfate sodium-induced colitis model, World J. Gastroenterol. 23 (2017) 4559.
[34] H. Dodiya, V. Kale, S. Goswami, R. Sundar, M. Jain, Evaluation of adverse effects of lisinopril and rosuvastatin on hematological and biochemical analytes in wistar rats, Toxicol Int. 20 (2013) 170.