



Study of Atorvastatin in experimental allergic airway inflammation in mice[☆]

Mohamed Nabih Abdel Rahman, Ahmed A.M. Abdelmotelb^{*}

Department of Pharmacology, Tanta Faculty of Medicine, Tanta, Egypt

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ABSTRACT

Background: Inflammation and oxidative stress are associated with airway diseases. There is growing evidence that Atorvastatin could be used as a therapy for these conditions.

Objective: On these bases, we evaluated Atorvastatin as a protective and reversal treatment for the allergic airway diseases in mice model. We also looked at the possible interaction with the currently used effective medication.

Methods: Mice were sensitized and challenged with ovalbumin (OVA) to develop features of allergic airway diseases mainly of bronchial inflammation. Atorvastatin was injected during or after the sensitization and challenge process to evaluate its protective or reversal effects, respectively. Total and differential cells in the BAL fluids together with IL-4, IL-5 and IL-10 cytokine levels were evaluated. Total IgE and cholesterol levels in serum were studied.

Results: In the protective phase, Atorvastatin inhibited the OVA-induced cellular infiltration of lung bronchi, decreased IL-4 and IL-5 and prevented the increase in IL-10 cytokine levels. Also, it reduced the OVA-induced high serum total IgE level. Injection of Atorvastatin after challenge was not effective in reversing the inflammatory process, with no major contribution towards augmenting the actions of Dexamethasone. The cholesterol lowering effect was marked in the protective phase while less effective for the reversal phase.

Conclusion: Our results indicate that Atorvastatin reduced the allergic inflammatory features in mice and it could be useful towards developing a better therapeutic regimen for the treatment of allergic diseases.

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1. Introduction

The prevalence of allergic diseases, such as allergic rhinitis, conjunctivitis, asthma, food allergies and anaphylaxis has constantly increased in the developed world during the last decades [1]. The current therapy is inadequate and also the majority of the prescribed drugs have side effects [2]. Consequently, a major thrust has been given to identify new molecules for mitigating these disorders.

These diseases are caused by a dysfunction of the immune system in response to normally harmless environmental agents and are characterized by an excessive activation of mast cells, basophile and T helper cells of the Th2 lineage cells [3]. It has also a substantial economic impact on public health spending [4].

Mice models of allergy replicate many features of the human asthma, including airway hyper-reactivity and airway inflammation [5]. Therefore, examination of disease models in mice has been used to elucidate asthma pathology and to identify and evaluate new therapeutic agents.

Important roles for a number of cytokines, including IL-4, IL-5 and IL-13 have been shown in the development of allergic asthma in humans and increased airway responsiveness in experimental models [6,7]. Similarly, a group of negative regulators of allergic inflammation

also have been implicated, among them IL-10, with a critical role for the development of AHR after allergic sensitization [8].

Atorvastatin is a member of the drug class known as statins, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors, used for lowering blood cholesterol. It also stabilizes plaque and prevents strokes through anti-inflammatory and other mechanisms. Statins are dramatically effective, treating hyperlipidemia and preventing cardiovascular disease, particularly in high risk populations [9]. Recently it has been shown to be useful in treating aortic aneurysms [10], inflammatory colitis [11], chronic renal disease [12], nasal poly disease [13] and chronic lymphocytic leukemia [14].

We already have extremely effective medications for the treatment of allergic asthma in the form of corticosteroid, with the dilemma of either it is not working or it has not been taken as prescribed [15] with the beneficial effectiveness of combined to statins still remain unclear.

In this study, we investigated the possible use of those potentials for Atorvastatin in an allergy model and studied the interaction with corticosteroid as an effective anti-allergic drug.

2. Material and methods

2.1. Animals

Laboratory-bred male Swiss albino mice (20–25 g in weight) were used in this study. Mice were housed in accordance with the institutional and national guidelines. All mice received standard

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^{*} Corresponding author. Tel.: +20 101414090.

E-mail address: ahmedasem72@gmail.com (A.A.M. Abdelmotelb).

mouse chow throughout the experiment. All investigations were in agreement with the ethical principles in animal experimentation, adopted by the ethics committee in animal experimentation of our Institution.

2.2. Sensitization and challenge protocol

Mice were sensitized by intraperitoneal injection (i.p.) of ovalbumin (OVA), 10 µg Grade V chicken egg (Sigma Chemical Co., Egypt) and 1 mg aluminum potassium sulfate (alum; Sigma Chemical) in 0.5 ml saline, on days 0, 7 and 14 [16]. Mice were then challenged for 30 min by nebulization of 1% (wt/vol) OVA in saline solution on days 19–23. Aerosol challenge was performed on groups of up to 12 mice in a whole-body inhalation exposure chamber attached to an ultrasonic nebulizer (NE-U07; Omron Corporation, Tokyo, Japan) with an output of 1 ml/min and 1 to 5 µm particle size. Control mice were sensitized with 1 mg of alum in 0.5 ml saline and challenged with nebulized saline solution.

2.3. Preparation of treatments

Atorvastatin (Ator) (Lipitor, Pfizer, Egypt) was dissolved in saline–10% ethanol solution at 2 mg/ml (pH 7.6) and administrated by i.p. (20 mg/kg) according to our protocol. Dexamethasone (Dex) (Sigma-Aldrich, Egypt) was injected i.p. as 1 mg/kg dissolved in saline and used as anti-allergic model.

2.4. Mice groups and experimental design

Mice were divided into seven groups ($n = 10$ –12/group). All mice were sensitized by i.p. injection of OVA on days 0, 7 and 14 of the experiment. Treatment or vehicle was given early in the protective phase of the study from day 0 till day 23 and on the reversal phase from day 24 till day 34 (Fig. 1). Group 1; mice were saline challenged. Group 2; mice were OVA challenged and ethanol (vehicle) treated. Group 3; OVA challenged and Ator treated.

After OVA challenge, mice in the reversal phase of the study (day 24 till day 34), were treated as follow: group 4; mice were ethanol (vehicle) treated, group 5; mice treated with Ator, group 6; mice treated with Dex and group 7; mice co-treated with Ator and Dex.

2.5. Analysis of serum

The mice were sacrificed 12 h after the last OVA challenge for the protective phase and on day 35 for the reversal phase. Blood samples were collected by heart puncture and centrifuged (10 min, 4 °C, 1500×g), and plasma was kept at –70 °C until use. The total serum IgE was determined by ELISA (Abnova, Taiwan) and the serum total cholesterol level was determined according to the manufacturer's protocol (Abcam, Egypt).

2.6. Bronchial lavage fluid

Tracheal cannulation with total 2.0 ml saline wash was performed and around 1.5 ml of bronchoalveolar lavage (BAL) fluid was recovered after 4 lavages. After centrifugation (400g for 6 min at 4 °C), cells were resuspended in 2.5 ml MEM (minimal essential medium) media, stained with 0.4% trypan blue, and enumerated using haemocytometer and the supernatant kept at –70 °C till used. Cells were subjected to cyto-centrifugation (Cytospin; Shandon Southern, Runcorn, UK) with 10^4 cells in 100 µl/slide and slides left to dry for 12 h. One slide from each sample was stained with rapid Romanowsky stain (according to the manufacturer's instructions). Differential cell counting was performed using standard morphological criteria, counting a minimum of 500 cells using Nikon plan microscope (100×/1.3 Oil) Nikon (Kingston, Surrey, UK). Data from animals which received the same treatment on different occasions were pooled and analyzed together.

Bronchial lavage supernatants were used to measure the levels of IL-4 (Lingmei Biotech Company, Shanghai, China), IL-5 (R&D Systems, Minneapolis, USA) and IL-10 (eBioscience, Hatfield, United Kingdom) and were determined by ELISA according to the manufacturer's protocol.

2.7. Statistical analysis

The values are presented as median (interquartile range). Multiple group comparison were made with Kruskal–Wallis 1-way ANOVA and two-group statistical comparisons were made using Mann–Whitney U test. GraphPad Prism version 5 for Windows, GraphPad Software, San Diego California USA, <http://www.graphpad.com> was used. P values of less than 0.05 were taken as statistically significant.

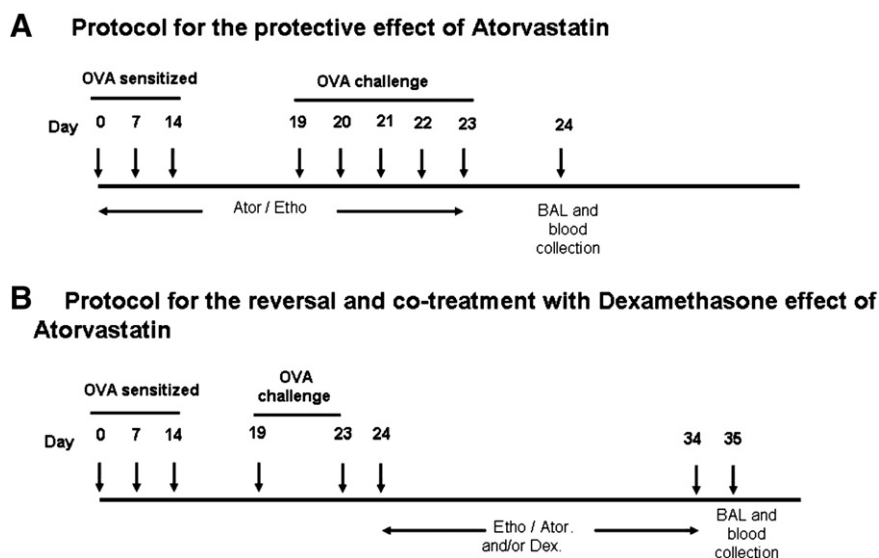


Fig. 1. Experimental protocols. Mice were sensitized and challenged as described in Section 2. A. To evaluate the protective effect of Atorvastatin (Ator), mice were sensitized with OVA and challenged with saline or OVA, and were given vehicle or Ator. by i.p. injections daily (20) till the end of this phase on day 23. B. To evaluate the reversal effect, mice were sensitized with OVA and challenged with OVA to develop allergic airway disease and then treated with Veh or Ator or Dexamethasone (Dex) or combined treatment of Ator + Dex as described in Section 2. Mice were treated from day 24 till the end of this phase (day 34) before sacrificing animals on day 35. No booster allergen dose was given in the reversal phase.

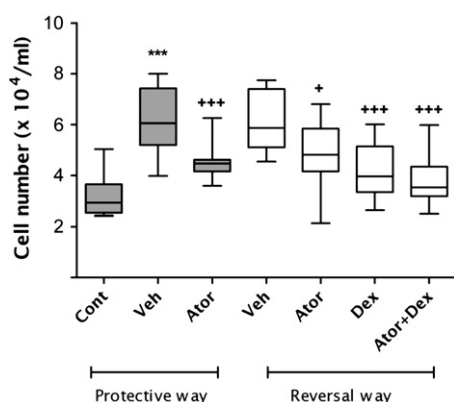


Fig. 2. Atorvastatin treatment reduces leukocyte recruitment in an OVA-induced model of allergic airway inflammation. Mice were sensitized and challenged as described in the Section 2. Groups were compared with respect to the total cell counts of the control group for the protective phase (gray) and for the Veh counts in the reversal phase (clear) of the BAL fluid. Data are displayed as box blots with the median and interquartile range and the largest and smallest values that are not outliers (whiskers) for nine to twelve mice in each group. The (*) for the significant increase and (+) for the decrease compared with response for the control (Cont) and Vehicle (Veh) treated in the protective and reversal phases (Mann–Whitney U test). (+) $P < 0.05$. (++) $P < 0.01$. (+++) $P < 0.001$.

3. Results

The study design is indicated diagrammatically in Fig. 1. Essentially mice received treatment on day 0 of the protective phase till 12 h after the last day of challenge (day 23), while in the second phase (reversal) treatment started on day 24 and lasted for 10 days when mice were sacrificed 12h after the last dose. OVA exposed mice showed significant increase in the inflammatory markers, denoting the establishment of inflammatory lung disease. The injection of ethanol alone (Vehicle) did not alter OVA-induced airway inflammation.

3.1. Leukocyte accumulation

In the protective phase, injections of Atorvastatin significantly reduce ($P < 0.001$) BAL fluid total leukocyte cell influx by about 30% over those in the vehicle injected mice (Fig. 2). Injections of Atorvastatin after OVA-challenge (reversal phase) reduced ($P < 0.05$) the number recovered by only 20% compared to the vehicle only treated animals. Dexamethasone injection was able to reduce the influx of cells to a greater level (30%) with an augmented effect for the co-treated group (37%), both compared to the vehicle only treated animals.

Table 1

Effect of Atorvastatin on the recruitment of inflammatory cells into BAL fluid in OVA-challenged mouse allergic airway inflammation. Data are displayed as median and interquartile range values for nine to twelve mice in each group. The (*) for the significant increase and (+) for the decrease compared with response for the control and Veh treated in the protective and reversal phases (Mann–Whitney U test). (+) $P < 0.05$. (++) $P < 0.01$. (+++) $P < 0.001$.

A. protective phase				
	Cont	Veh	Ator	
Macrophage (10^3 /ml)	17.8 (15.3–24.3)	39.4*** (35.5–48.8)	28.5 ⁺ (22.1–29.3)	
Lymphocyte (10^3 /ml)	5.4 (3.5–6.5)	9.3*** (8.5–11.4)	6.8 ⁺⁺ (6.6–10.5)	
Neutrophil (10^3 /ml)	1.6 (1.5–1.8)	4.1*** (3.6–4.9)	2.8 ⁺⁺⁺ (2.2–3.1)	
Eosinophil (10^3 /ml)	2.5 (2.0–3.2)	4.7*** (4.2–5.9)	3.6 ⁺⁺⁺ (3.5–5.5)	
Mast cells (10^3 /ml)	1.2 (1.1–1.4)	2.9*** (2.1–3.7)	2.1 ⁺⁺⁺ (1.8–2.3)	
B. Reversal phase				
	Veh	Ator	Dex	Ator + Dex
Macrophage (10^3 /ml)	38.2 (33.7–49.3)	30.4 ⁺ (27.6–37.7)	26.5 ⁺ (19.1–36.7)	23.9 ⁺⁺ (19.9–30.7)
Lymphocyte (10^3 /ml)	8.8 (7.8–11.4)	7.2 (6.6–10.2)	5.3 ⁺⁺ (3.6–7.9)	4.4 ⁺⁺ (3.6–8.1)
Neutrophil (10^3 /ml)	3.9 (3.4–4.6)	3.2 (2.6–4.3)	2.2 ⁺⁺⁺ (1.7–3.0)	2.1 ⁺⁺⁺ (1.7–2.9)
Eosinophil (10^3 /ml)	4.7 (4.1–6.1)	3.9 (3.4–5.1)	3.5 ⁺ (2.8–4.5)	3.3 ⁺ (2.3–4.5)
Mast cells (10^3 /ml)	2.8 (2.1–3.3)	2.4 (1.9–2.6)	2.0 ⁺ (1.5–2.2)	1.8 ⁺⁺ (1.2–2.2)

3.2. Differential cell response

Identification of the inflammatory cells recruited following Atorvastatin injections reveals distinct patterns of cell accumulation (Table 1). Injection of Atorvastatin from day 0 was associated with great reduction ($P < 0.001$) in neutrophil, macrophage and mast cell numbers but to a lesser extent in other cell types under the conditions examined (Table 1-A) while injection of the drug after day 23 failed to alter the increased cell number except for a slight reduction ($P < 0.05$) in the macrophage counts (Table 1-B).

Injection of Dexamethasone was able to decrease ($P < 0.001$) the neutrophil counts, while its reducing effects ($P < 0.05$) on the other cell types were less marked. The animals received combined Atorvastatin and Dexamethasone treatment, and their cell counts tended to be lower with some 6–17% more reduction in their cell counts.

3.3. Cytokine profile

Atorvastatin injections alter some of Th2 cytokines known to be important in allergic airway diseases. In the protective phase of the study, Atorvastatin treatment was able to reduce the increased IL-4 and IL-5 cytokine levels in BAL fluid by 25% and 37%, respectively, both compared to the vehicle treated mice (Fig. 3 A and B). Treatment started after the challenge failed, for the conditions applied, to reduce the high IL-4 and IL-5 observed in this model. Dexamethasone treatment effectively reduced both cytokine levels with marked effect toward IL-5. Combined injection of Atorvastatin and Dexamethasone induced more reduction of IL-4 level with no effect on IL-5 level. The lower limit of detection was 1 pg/ml for IL-4 and IL-5.

In the protective phase, injection of Atorvastatin induced high level of IL-10 compared to control or vehicle treated animals. In the reversal phase, Atorvastatin tended to increase IL-10 level to a lesser level with no effect for the injected Dexamethasone either alone or in combination with Atorvastatin.

3.4. Total serum IgE level

To test the effect of Atorvastatin on IgE levels, sera samples were collected from mice and total IgE levels were measured as described in Section 2 (Fig. 4). The level of total IgE was eight fold higher in OVA-challenged compared to the saline challenged animals. Atorvastatin injections were able to reduce the level by 40%. Introducing Atorvastatin after OVA challenge failed to alter the high IgE level induced. Dexamethasone injections induced a reduction of about 70% while combined treatment did not show any difference to the effect of the Dexamethasone alone.

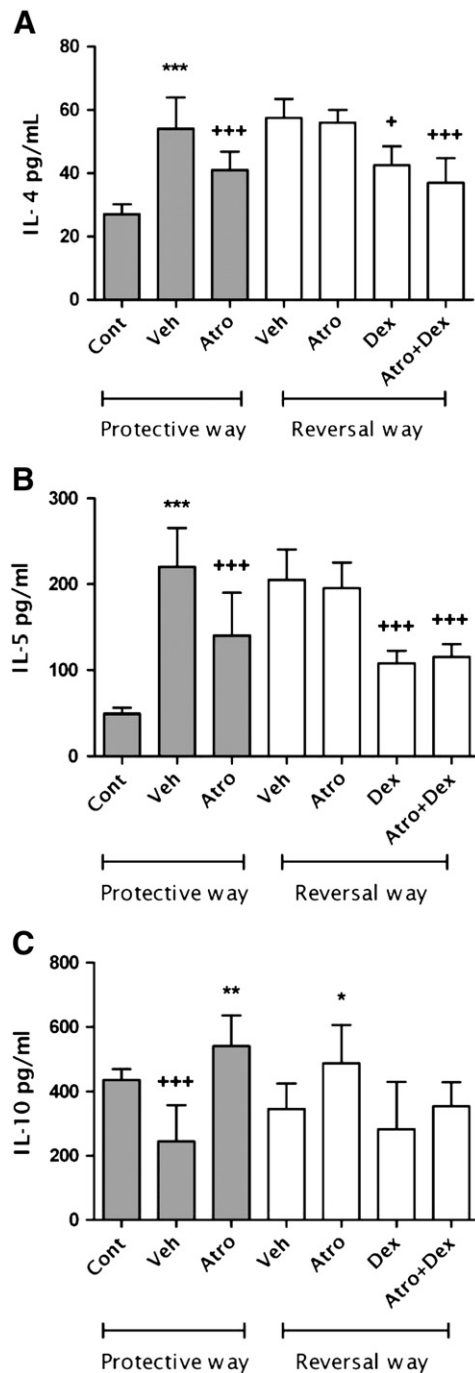


Fig. 3. Effects of Atorvastatin on the studied cytokine levels in the BAL fluids of OVA-challenged mice for the protective and reversal phase of the study. Th2 cytokines, IL-4 (A), IL-5 (B) and IL-10 (C) were determined by ELISA. Gray panels represent the protective experiment and the clear panels represent the reversal study. Data are represented as outlined in the legend of Fig. 2. (*) $P < 0.05$. (**) $P < 0.01$.

3.5. Total cholesterol level

To confirm the cholesterol lowering effect for the Atorvastatin dose used and to find possible interaction with Dexamethasone, total cholesterol levels were determined. In this model, the total cholesterol level in OVA-challenged mice was leveled to the control group. The injection of Atorvastatin induced a lower (18%) cholesterol level in the protective phase of this study while injecting the drug in the reversal phase, has only 7% less, both compared to the relevant controls. The use of Dexamethasone alone has no effect, but there was a trend

($P = 0.06$) toward an additive lowering effect when combined with Atorvastatin in the reversal phase.

4. Discussion

In this report, Atorvastatin significantly reduced the OVA-induced inflammatory cellular infiltrates of the lung bronchial system. In addition, it reduced total IgE levels in serum and IL-4, IL-5 levels in BAL fluid. On the other hand, it prevented the decrease in IL-10 in the BAL fluid. Injection of Atorvastatin after the induced allergic airways had limited effects. Minor interactions were detected for Atorvastatin when injected in Dexamethasone treated animals in the applied conditions.

The murine models of allergic airways as well as human atopic asthma showed inflammatory lung infiltrates together with other phenotypic changes [17] that would facilitate the use of these models to study allergic airway diseases. The dose of antigen used in animal models is a major determinant of the polarized development of Th2 cells in vivo, therefore in the current study the 10 μ g sensitization dose was used to ensure detectable levels for cytokines [18].

The response to allergen challenge in human is associated with infiltration of lung with inflammatory cells especially eosinophils [19,20]. In this study, the OVA-challenged animals demonstrated high cellular infiltrate in their bronchial trees, an effect that was significantly reduced by early Atorvastatin treatment. This effect may be related to the anti-inflammatory or the anti-oxidant properties of Atorvastatin previously shown [21,22].

The eosinophilia induced by the allergen challenge was partly reduced by Atorvastatin early treatment but marked for mast cell counts. These effects may be explained by the fact that Atorvastatin could inhibit the growth and the mediators release from mast cells [23], but a direct effect on other inflammatory cells cannot be excluded.

To find out the mode of action of Atorvastatin in allergy, we studied the BAL fluid cytokines profile. The Th2 cytokines, IL-4 and IL-5, have been shown to be produced during allergic response and also to

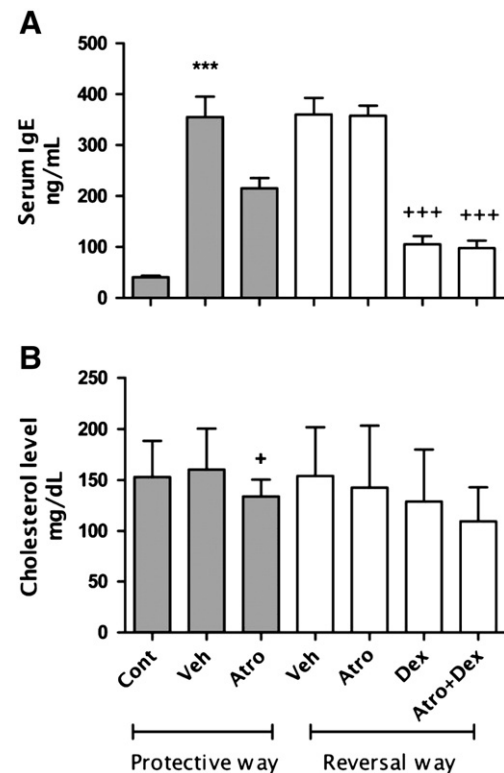


Fig. 4. Effects of Atorvastatin on serum IgE level and total cholesterol level for the protective and reversal phases. Data shown as listed in Figs. 2 and 3.

promote allergic inflammation [24]. Interestingly, our results showed that early treatment with Atorvastatin reduced the IL-4 and IL-5 levels (Fig. 3). These results are similar to the observations shown by Simvastatin treatment in a similar model [25]. Also, the increase in IL-10 cytokine level by Atorvastatin is a novel finding in this model. IL-10 has been demonstrated to be essential for the presence of airway hyperresponsiveness but not essential for the pulmonary inflammation [8]. The effect of Atorvastatin on these cytokines would be explained in the view of studying its effects on Th1 cytokine, for better understanding of its possible immune modulatory effects in allergy as shown in previous studies [26,27].

The early airway response in asthma is IgE dependant and mostly caused by activation and degranulation of pulmonary mast cells with several mediator release causing the immediate airway constriction [28]. The higher production of IgE is associated with more degranulation, more release of mediators and more airway inflammation [29]. In the present work, OVA-challenged animals showed high serum total IgE levels, which were significantly ($p=0.002$) reduced with early Atorvastatin treatment. This may be explained in the view of the inhibitory effect of Atorvastatin on IL-4 production and consequently the reduction in the IgE production [30] but other mechanisms could exist.

The cholesterol lowering effect was studied to evaluate the effectiveness of the Atorvastatin dose applied. Cholesterol level was not affected by the allergen treatments but early treatment with Atorvastatin was showing significant hypocholesterolemic effect which shows working dose of the drug.

In the second part of the study, we looked at the possible reversal role for Atorvastatin on the established allergic conditions compared with Dexamethasone as anti-allergic drug. Compared to the Vehicle treated animals, Atorvastatin reduced the total cell recruited in bronchi but less markedly than the early treatment. Injection of Atorvastatin with Dexamethasone was associated with more reduction compared to the injection of Dexamethasone alone. This augmented effect may be coming from affecting the same target (s), as shown for IL-4, or other different targets [31], but a clear conclusion would need further study.

The treatment with Dexamethasone in the reversal phase was also associated with more reduction in serum total IgE levels compared to the vehicle only treated animals but with no added effect when injected with Atorvastatin. Dexamethasone injections in a similar model showed no effect on serum IgE level which may be related to the use of higher antigen dose that would decrease the IgE production [18]. There was a trend toward more hypocholesterolemic, anti-inflammatory effects for the combined treatment but a conclusion would need further studies.

In conclusion, our findings indicated that Atorvastatin can prevent but not reverse the inflammatory hallmarks of allergy, with no adding effects to corticosteroids in the applied conditions. It would provide a possible new therapeutic regimen in treating allergic airway diseases.

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References

- [1] Devereux G. The increase in the prevalence of asthma and allergy: food for thought. *Nat Rev Immunol* 2006;6:869–74.
- [2] Barnes PJ. Therapeutic strategies for allergic diseases. *Nature* 1999;402:B31–8.
- [3] Galli SJ, Tsai M, Piliponsky AM. The development of allergic inflammation. *Nature* 2008;454:445–54.
- [4] Fireman P. Symposium: understanding asthma pathophysiology. *Allergy Asthma Proc* 2003;24:79–83.
- [5] Shin YS, Takeda K, Gelfand EW. Understanding asthma using animal models. *Allergy Asthma Immunol Res* 2009;1:10–8.
- [6] Wills-Karp M. Immunologic basis of antigen-induced airway hyperresponsiveness. *Annu Rev Immunol* 1999;17:255–81.
- [7] Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998;282:2261–3.
- [8] Makela MJ, Kanehiro A, Borish L, Dakhama A, Loader J, Joetham A, et al. IL-10 is necessary for the expression of airway hyperresponsiveness but not pulmonary inflammation after allergic sensitization. *Proc Natl Acad Sci USA* 2000;97:6007–12.
- [9] Paraskevas KI, Tzovaras AA, Briana DD, Mikhailidis DP. Emerging indications for statins: a pluripotent family of agents with several potential applications. *Curr Pharm Des* 2007;13:3622–36.
- [10] McNally MM, Agle SC, Parker FM, Bogey WM, Powell CS, Stoner MC. Preoperative statin therapy is associated with improved outcomes and resource utilization in patients undergoing aortic aneurysm repair. *J Vasc Surg* 2010;51:1390–6.
- [11] Lee JY, Kim JS, Kim JM, Kim N, Jung HC, Song IS. Simvastatin inhibits NF-kappaB signaling in intestinal epithelial cells and ameliorates acute murine colitis. *Int Immunopharmacol* 2007;7:241–8.
- [12] Paraskevas KI, Tzovaras AA, Stathopoulos V, Mikhailidis DP. Statin use in patients with chronic kidney disease stages 2–4: targeting beyond improved mortality rates. *Int Urol Nephrol* 2010;42:711–13.
- [13] Folli C, Descalzi D, Bertolini S, Riccio AM, Scordamaglia F, Gamalero C, et al. Effect of statins on fibroblasts from human nasal polyps and turbinates. *Eur Ann Allergy Clin Immunol* 2008;40:84–9.
- [14] Friedman DR, Magura LA, Warren HA, Harrison JD, Diehl LF, Weinberg JB. Statin use and need for therapy in chronic lymphocytic leukemia. *Leuk Lymphoma* 10–7–2010.
- [15] Rubin BK. What does it mean when a patient says, “my asthma medication is not working?”. *Chest* 2004;126:972–81.
- [16] Ram A, Das M, Gangal SV, Ghosh B. Para-bromophenacyl bromide alleviates airway hyperresponsiveness and modulates cytokines, IgE and eosinophil levels in ovalbumin-sensitized and -challenged mice. *Int Immunopharmacol* 2004;4:1697–707.
- [17] Blyth DI, Pedrick MS, Savage TJ, Hessel EM, Fattah D. Lung inflammation and epithelial changes in a murine model of atopic asthma. *Am J Respir Cell Mol Biol* 1996;14:425–38.
- [18] Sakai K, Yokoyama A, Kohno N, Hiwada K. Effect of different sensitizing doses of antigen in a murine model of atopic asthma. *Clin Exp Immunol* 1999;118:9–15.
- [19] Beasley R, Roche WR, Roberts JA, Holgate ST. Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis* 1989;139:806–17.
- [20] Bradding P. Asthma: eosinophil disease, mast cell disease, or both? *Allergy Asthma Clin Immunol* 2008;4:84–90.
- [21] Kumar VL, Guruprasad B, Wahane VD. Atorvastatin exhibits anti-inflammatory and anti-oxidant properties in adjuvant-induced monoarthritis. *Inflammopharmacol* 2010;18:303–8.
- [22] Furuya DT, Poletto AC, Favaro RR, Martins JO, Zorn TM, Machado UF. Anti-inflammatory effect of atorvastatin ameliorates insulin resistance in monosodium glutamate-treated obese mice. *Metabolism* 2010;59:395–9.
- [23] Krauth MT, Majlesi Y, Sonneck K, Samorapompichit P, Ghannadan M, Hauswirth AW, et al. Effects of various statins on cytokine-dependent growth and IgE-dependent release of histamine in human mast cells. *Allergy* 2006;61:281–8.
- [24] Perkins C, Wills-Karp M, Finkelman FD. IL-4 induces IL-13-independent allergic airway inflammation. *J Allergy Clin Immunol* 2006;118:410–9.
- [25] Kim DY, Ryu SY, Lim JE, Lee YS, Ro JY. Anti-inflammatory mechanism of simvastatin in mouse allergic asthma model. *Eur J Pharmacol* 2007;557:76–86.
- [26] Zhang A, Zhang H, Wu S. Immunomodulation by atorvastatin upregulates expression of gap junction proteins in coxsackievirus B3 (CVB3)-induced myocarditis. *Inflamm Res* 2010;59:255–62.
- [27] Cheng X, Ding Y, Xia C, Tang T, Yu X, Xie J, et al. Atorvastatin modulates Th1/Th2 response in patients with chronic heart failure. *J Card Fail* 2009;15:158–62.
- [28] Nadler MJ, Matthews SA, Turner H, Kinet JP. Signal transduction by the high-affinity immunoglobulin E receptor Fc epsilon RI: coupling form to function. *Adv Immunol* 2000;76:325–55.
- [29] Prussin C, Metcalfe DD. 4. IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol* 2003;111:S486–94.
- [30] Fish SC, Donaldson DD, Goldman SJ, Williams CMM, Kasaian MT. IgE generation and mast cell effector function in mice deficient in IL-4 and IL-13. *J Immunol* 2005;174:7716–24.
- [31] Mondo CK, Yang WS, Zhang N, Huang TG. Anti-oxidant effects of atorvastatin in dexamethasone-induced hypertension in the rat. *Clin Exp Pharmacol Physiol* 2006;33:1029–34.