

STAMINA A NOVEL PROSPECT IN ASTHMA MANAGEMENT

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ABSTRACT

Background: Current therapy for asthma includes bronchodilators and anti-inflammatory drugs. Inhibition of RhoA/ROCK pathway to control hyperresponsiveness presents a novel area in asthma therapy.

Study Design: Experimental randomized control trial. "To examine the effect of statins on the airway hyperresponsiveness." Statins are lipid-lowering agents exhibiting pleiotropic effects in decreasing oxidative stress and inflammation. Several published studies have reported using statins in treating asthma patients, but their results could be more consistent. This study aims to determine whether statins are beneficial for asthma administration and explore the covariables that may affect their clinical effectiveness.

Materials and Maths: Five groups of six similar guinea pigs animals under similar laboratory conditions were selected. The animal model of asthma was reproduced via intraperitoneal ova albumin in all except negative control group 1. These groups were Group I negative control, II positive-control, III atorvastatin treated, IV Simvastatin treated, and V lovastatin treated group. After 28 days, animals were sacrificed. Bronchoalveolar fluid (B.A.L.) analysis was taken, and Smooth muscle contractions in tracheal strips to increasing concentrations of acetylcholine were recorded by isometric transducers using a computerized data recording system. The effects of statins were assessed by comparing concentration-response curves of experimental groups with those of controls.

A systematic literature search was performed in PubMed, Embase, and Cochrane Center Register of Controlled Trials from inception to September 2012. Randomized controlled trials (R.C.T.), retrospective studies, and controlled clinical trials that reported the use of statins in the treatment of asthma patients were eligible. Quality evaluation was conducted for R.C.T. using Jadad criteria.

Results: Comparison of B.A.L. of positive control with that of negative confirmed the induction of allergic inflammatory model. The concentration-response curve was significantly shifted upward in positive control compared to negative control ($P < 0.05$). In the case of statin-treated groups, there was a significant downward shift in concentration-response curves compared to positive control, showing inhibition of airways hyperresponsiveness by statins. Significant differences were observed between them and positive control ($P < 0.05$). A total of 18 articles were included. Our study found no conclusive evidence to demonstrate that statins could enhance lung function in asthmatics, although they may reduce airway inflammation. The results were inconsistent across studies concerning symptoms, quality of life, maintenance medication, and asthma hospitalization/emergency department (E.D.) visits. The present study demonstrates that statins inhibit hyperresponsiveness in the allergic airway inflammatory model of the guinea pig. Statins may have a therapeutic potential to ameliorate airway hyperresponsiveness in allergic bronchial asthma. Human trials are needed to include these drugs in asthma treatment guidelines.

Keywords: Asthma Guinea pig Model Statin B.A.L.

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INTRODUCTION

Bronchial asthma, an epidemic of dysregulated immunity¹⁹, is now defined in pathological terms as a chronic inflammatory disorder much more than the clinical defining criteria of reversible bronchospasm to put physicians on a proper therapeutic path. The smooth muscles of the tracheobronchial tree tend

to contract more intensely in response to a given stimulus than they do in normal individuals¹. Airway hyperresponsiveness occurs due to enhancement in Ca²⁺ sensitivity², which proposes that an increase in the intracellular RhoA/ROCK pathway is the cause of the increased Ca²⁺ sensitivity. The primary anti-inflammatory drugs that maintain asthma under clinical control include glucocorticoids, leukotriene modifiers, and anti-IgE antibodies, mostly because of their anti-inflammatory properties. However, some groups of people react badly to the above, such as smokers, obese asthmatics⁵, and non-Th2-high asthmatics. Even with large doses of bronchodilators and corticosteroids, some people's asthma is not well managed. Glucocorticosteroid resistance instances and sensitivity to beta-agonist¹⁰ pharmaceutical side effects are among them. They reduce cholesterol and have pleiotropic anti-inflammatory properties³. In inflammatory and immune-related conditions such as rheumatoid arthritis, asthma, stroke, and organ transplant rejection, these effects may be advantageous⁴. As hydroxymethylglutaryl coenzyme A (HMG-A) reductase inhibitors, statins can block the mevalonate pathway and the production of downstream intermediates that post-translationally modify small guanosine triphosphatases (GTPases), such as farnesylpyrophosphate (F.P.P) and geranylgeranyl pyrophosphate (GGPP)⁹. Due to their potential to promote smooth muscle contraction and proliferation of the airway and airway hyperresponsiveness, GTPases may be involved in the pathogenesis of asthma. Research has shown that statins limit the growth and contraction of airway smooth muscle in vitro and decrease the overall inflammatory cell infiltration and eosinophilia in bronchoalveolar lavage fluid in an animal model of asthma. A retrospective analysis conducted in 2009 by Stanek et al. revealed that statin medication was independently linked to a substantial 33% relative risk reduction for hospitalizations or emergency department (E.D.) episodes due to recurrent asthma. Recently, the therapeutic efficacy of statins in treating asthma has been studied in several asthmatics.

Short-term statin therapy may improve lung function, enhance the anti-inflammatory effect of inhaled corticosteroids (I.C.S.), and score better on the Asthma Control Questionnaire (A.C.Q.) and Asthma Quality of Life Questionnaire (AQLQ), according to certain studies²⁰, Simvastatin^{13,16,17}, Lovastatin¹⁴, and Rosuvastatin¹⁵. There is evidence that every statin

has a unique set of therapeutic actions. For instance, in vitro, atorvastatin is less effective than Simvastatin and lovastatin against human smooth muscle cells.

Moreover, cerivastatin is the most effective in decreasing inflammation mediated by NF- κ B. Hydrophilic pravastatin has far less of an impact on inflammatory responses in human monocytes in vitro and mouse leukocytes in vivo than lipophilic statins like atorvastatin and Simvastatin. Therefore, because statins may have varied effects, future clinical studies should assess the effectiveness of various statins. The efficacious dosage of statins in animal asthma models has been validated by research on animals; Simvastatin's high dose is 40 mg/kg, while lovastatin's is 4 mg/kg. For males, an atorvastatin dose of up to 80 mg per day is advised. However, in every study that qualified, the daily dose of statins was less than 40 mg.

Meanwhile, other research could not confirm these findings^{21,22}. The first step in creating the animal model was intraperitoneal sensitization to the ovalbumin antigen. Intranasal administration used Ovalbumin for a repeat antigen challenge two weeks after sensitization. Inflammation and increased airway responsiveness are caused by this kind of therapy^{11,12}. Additionally, it increases smooth muscle cell RhoA translocation¹

MATERIALS AND METHODS

Experimental randomized control study. The sample size was 6 animals per group at a 95% confidence interval, 90% research power, and 1:2 case-control ratio using StatCalc (EpiInfo). Male guinea pigs were randomly assigned to groups I (negative control), II (positive control), III (atorvastatin), IV (Simvastatin), and V using the lottery method. The animals lived at Malakand University Animal House. Each group was assigned a 5 x 3-foot cage with natural light and dark cycles, a 22-24°C temperature range, and 45-65% humidity. Regular rat feed and limitless water were provided. All groups except group 1 (negative control) employed sensitization and airway challenge to construct an airway inflammatory model (Chiba et al., 2005). On days 0 and 14, sensitization was performed by intraperitoneal injection of 100 μ g ovalbumin (Sigma-Aldrich et al.) and 200mg Al (OH) 3 (Biosector Denmark) in PBS solution. On days 25, 26, and 27, 1% ovalbumin in P.B.S. solution was inhaled nasally for 20 minutes as an airway challenge. Group III, IV, and V animals were gavaged 1.5 mg/kg of atorvas-

atin, simvastatin, and lovastatin 30 minutes before each challenge. Drugs were in distilled water. 2004 (Mackay et al.). Cervical dislocation killed animals on day 28. The respiratory system was dissected in one block. B.A.L. was added to the syringe to count total and differential cells. Ahzad et al. (2009). Krebs solution was added to tracheal tissue. At least three cartilages were in each tracheal tube ring. A longitudinal incision on the ventral side of each ring opened it up and generated a tracheal strip with cartilaginous edges and smooth muscle in the core (Gillani et al., 2005). Stitching connected the cartilaginous ends. The tissue was then mounted in a 15 ml tissue bath. The tissue bath included Krebs solution aerated with oxygen at 37°C (Gillani et al., 2008). In the organ bath, one thread was hooked. One had an isometric transducer (0-50g sensitivity). An isometric transducer was connected to a Powerlab AD data recorder from Australia. After a five-minute baseline, tissue was administered acetylcholine at increasing doses until peak reactivity. Responses were recorded in grams. The effects of each concentration were documented for four minutes. We utilized 0.003, 0.01, 0.03, 0.1, 0.3, 0.1, 0.3, 10, 30, and 100 (µM) concentrations.

Two concentrations were analyzed statistically. Reaction-1 was generated by concentration-1 at 0.3µM, which produced 45% of the maximal reaction in the negative control. Concentration-2 (10µM) provided 75% of the highest reaction in the negative control, resulting in reaction-2. Other organizations used the same method. Each group's answers were in grams for the two concentrations mentioned.

RESULTS

We compared the statin, positive control, and negative control groups. According to data analysis, response-1 for the negative control was much lower than for the positive control. A significant difference was found using Tukey's Multiple Comparison Test ($p < 0.001$). Significant differences ($p < 0.001$) were seen when Response-1 from Statin-treated groups III, IV, and V were compared to positive control. A comparison of the Response-2 data between the positive and negative control groups showed significant differences ($p < 0.001$). Between the statin-treated and positive control groups, there was a significant difference in Response -2 ($p < 0.001$). Responses -1 and -2 showed no discernible changes between the statin-treated groups III, IV, and V and the negative control group.

Groups treated with statins also showed no significant differences. Testing bronchoalveolar lavage (B.A.L.) in positive and negative groups demonstrated the materials and techniques' ability to induce allergic airway inflammation consistently.

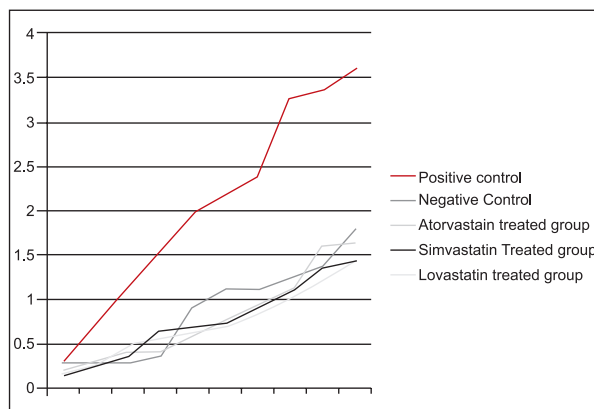


Fig 1: Percentages of MCQs according to C1, C2 and C3.

Table 1: Turkey's Multiple Comparison Test

Groups	Mean Diff.	q	Significant? p < 0.05?	Summary
I versus II	-2.302	15.31	Yes	***
I versus III	-0.1383	0.9203	No	ns
I versus IV	0.08500	0.5655	No	ns
I versus V	-0.3333	2.218	No	ns
II versus III	2.163	14.39	Yes	***
II versus IV	2.387	15.88	Yes	***
II versus V	1.968	13.10	Yes	***
III versus IV	-0.2233	1.486	No	ns
III versus V	-0.1950	1.297	No	ns
IV versus V	-0.4183	2.783	No	ns

DISCUSSION

The current research shows that the concentration-response curve in the statin-treated groups was substantially altered lower than the positive control group (increasing concentration of acetylcholine vs amplitude of airway muscle contraction). There was a significant difference ($p < 0.001$) between the groups treated with statins and the positive control at both response -1 and response -2.

Our current research is compared to several other investigations. These investigations used inflammatory

cell infiltrates, inflammatory mediators, and inflammatory markers as measures, decreasing when statins were taken. In an animal model, Mackey et al. (2004) found that Simvastatin decreased cytokines in bronchoalveolar lavage (B.A.L.) fluids and blocked the infiltration of inflammatory cells. Yeh et al. (2004) showed the same anti-inflammatory effects of pravastatin in a comparable animal paradigm of allergic airway inflammation. In addition, Chiba et al. (2008) found that lovastatin decreased the amount of inflammatory cells that invaded airways while simultaneously attenuating the hyperresponsiveness of the bronchial smooth muscle caused by antigens. Simvastatin reduced allergic airway inflammation, enhanced lung physiology, and reduced hyperreactivity in a mouse model of asthma, as shown by Zeki et al. (2009). The authors reported that the mevalonate route seems to regulate inflammation in the allergic airways. However, Simvastatin's beneficial effects on lung compliance and airway hyperreactivity could be unrelated to the mevalonate system.

Several studies have shown that various statins have distinct anti-inflammatory effects, in contrast to the current research, which found no significant difference between the three statin-treated groups. According to Kiener et al., hydrophilic pravastatin has less impact on inflammatory responses in human and animal models than lipophilic statins like atorvastatin and Simvastatin. According to Knapp et al., atorvastatin had little impact, and pravastatin exhibited no action on human smooth muscle cells exposed to apoptotic agents. However, lovastatin and Simvastatin had a strong sensitizing effect. Various statins showed distinct dose-response effects, as revealed by Hilgendorff et al. They demonstrated that cerivastatin is much more effective than fluvastatin when it comes to inhibiting NF- κ B activation in human blood monocytes. Takahashi et al. demonstrated that various statins had distinct effects on protein expression. For instance, pravastatin and fluvastatin may boost the production of TNF- α and IL-18 in monocytes driven by lipopolysaccharide (L.P.S.), whereas atorvastatin and Simvastatin may suppress the production of TNF- α .

A clinical experiment was conducted by Fehr et al. on 27 healthy participants. The immune responses to atorvastatin and simvastatin therapy were observed to vary significantly. Simvastatin significantly increased the expression of the CD38 activation marker and the human leukocyte antigen (H.L.A.)-D.R. on peripheral T lymphocytes. In contrast, atorvastatin significantly

decreased the expression of both markers. On the other hand, Simvastatin reduced, and atorvastatin increased T cell activation induced by superantigen. The different species employed as animal models in the experiments might be one reason for this discrepancy.

Very few clinical studies have been carried out on this topic. Medco Health Solution Inc. conducted massive research with 6,574 patients. They concluded that corticosteroid users who simultaneously use statin drugs had a one-third lower risk of requiring E.R. care or hospitalization. This provides substantial support for our research.

CONCLUSIONS

This research shows that statins prevent hyperresponsiveness in the guinea pig allergic airway inflammation model. In cases of allergic bronchial asthma, statins may be able to treat airway hyperresponsiveness. Further human studies must include these medications in well-known asthma therapy recommendations such as the Gena guidelines. Statins are generally safe medications, particularly when used to treat hypercholesterolemia in heart patients. As a result, step 3 asthma therapy may try their inclusion as an adjunct therapy. The people who work on these standards with expertise will make this choice.

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