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ORIGINAL ARTICLE

The effect of atorvastatin on lung histopathology in a murine model of chronic asthma

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Asthma;
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Abstract
Introduction: Atorvastatin is a statin group medicine that reduces the level of serum cholesterol; thus it is used to treat hypercholesterolaemia. Independent of the cholesterol-lowering property of statins they also have anti-inflammatory and immunomodulating effects. This study aimed to investigate the effect of atorvastatin on histological changes in the lungs in a murine model of chronic asthma.
Materials and methods: Twenty-eight BALB/c mice in Group I, II, III and IV were divided into four groups. All the mice except the control group (Group I) were sensitised with ovalbumin. Intraperitoneal injection with saline, atorvastatin (10 mg/kg), dexametazon (1 mg/kg) was administered to Group II, Group III, and Group IV respectively for five consecutive days. Mice were sacrificed 24 h after the last drug administration. All the histological properties of lung tissue samples from all groups were evaluated with light and electron microscopy. In addition, IL-4 and IL-5 levels of the lung tissue were measured.
Results: When Group II and Group III (atorvastatin) were compared, thicknesses of basement membrane and subepithelial smooth muscle layer, height of epithelium, number of mast and goblet cells were significantly lower in Group III. In comparing Group III (atorvastatin) and Group IV (dexamethasone), all the improvements in histological parameters were similar. In addition, the IL-4 and IL-5 levels of the lung tissue were significantly lower in atorvastatin group (Group III) compared to placebo-treated group.
Conclusion: Atorvastatin had a beneficial effect on histological changes in a chronic murine model of asthma.

Introduction

Asthma is formed by airway hyperresponsiveness to airflow obstruction and airway remodelling. This chronic

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inflammatory is most common in childhood. In the last decade the morbidity and mortality of asthma has been increasing.[1]

The current approach for asthma treatment aims to suppress airway inflammation. The most common drugs in asthma therapy are inhaled glucocorticoids in spite of several side effects when they are used in high doses or for a prolonged time.[2] Airway remodelling consists of progressive structural changes in the composition, content, and organisation of the cellular and molecular constituents of the airway wall.[3] Although current asthma therapies are effective in reducing inflammation, airway remodelling is poorly responsive to current therapies.[4,5] New therapy strategies which have a potent effect on airway remodelling are required.

Statins reduce the biosynthesis of cholesterol by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A reductase. They were mostly used in patients with high cholesterol level to reduce the morbidity and mortality of coronary artery disease.[6] Statins have been recently recognised as anti-inflammatory agents and shown to possess immunomodulating effects.[7,8] Statins also have a potential therapeutic role in respiratory disease. Bronchial smooth muscle hyperresponsiveness induced by ovalbumin stimulation has been found to be significantly attenuated by an intraperitoneal injection or inhalation of statins.[9,10]

However, the accurate benefits of atorvastatin in asthma treatment have not been investigated yet. Therefore, in this study our aim is to investigate the efficacy of atorvastatin on lung histopathology in a murine model of chronic asthma.

Materials and methods

Experimental animals

Twenty-eight conventionally raised, 6- to 8-week-old male BALB/c mice weighing 18–20 g were used for the study. They were maintained in the experimental animal laboratory of Dokuz Eylul University and kept in hygienic macrolene cages and fed a standard laboratory diet ad libitum in air-conditioned rooms on a 12 h light/12 h dark cycle. All experiments were carried out according to the protocols approved by the local animal use and care committee (Dokuz Eylul University). The protocols complied with the standards in the Guide for the Care and Use of Laboratory Animals, prepared by the Institute of Laboratory Animal Resources and published by the National Academy Press, National Research Council, Commission of Life Sciences, Institute of Laboratory Animal Resources.

Sensitisation and inhalational exposure

Mice were divided into four groups. Mice in the study groups except for the control group were sensitised on days 0 and 14 by intraperitoneal (i.p.) injections of 10 mg chicken egg albumin (ovalbumin, grade V, 98% pure; Sigma, St. Louis, MO, USA) with alum as an adjuvant. Mice in study Groups II, III, and IV were then challenged with an aerosol of 2.5% ovalbumin in saline for 30 min/day for three days of the week for eight weeks beginning from the 21st day of the study. Mice in control group (Group I) received normal saline with alum i.p. on days 0 and 14 of the experiment and aerosolised saline without alum for 30 min/day for three days of the week for eight weeks beginning from the 21st day of the study[11] (Fig. 1). Exposures were carried out in a whole body inhalation exposure system. Temperature and relative humidity were maintained at 20–25 °C and 40–60%, respectively. A solution of 2.5% ovalbumin in normal saline was aerosolised by delivery of compressed air to a sidestream jet nebuliser and injected into a chamber. The aerosol generated by this nebuliser comprised >80% particles with a diameter of <4 mm. Particle concentration was maintained in the range of 10–20 mg/mm^3.[12]

Study drugs

Mice in Group I received saline; Group II received atorvastatin at dose of 10 mg/kg/day, and Group III dexamethasone at dose of 1 mg/kg/day intraperitoneally once a day for the last five days of the challenge period. Intraperitoneal doses of atorvastatin (Ator, Sanovel, Turkey) and dexamethasone 1 mg/kg were chosen from other studies also conducted with BALB/c mice.[13]

Histopathological analysis

Animals were sacrificed by an overdose of ketamine 24 h after the last drug administration. Two investigators who were blinded to the treatment groups interpreted the histopathology. Tissue specimens were obtained from the mid zone of the left lung of mice. Samples were fixed in 10% formalin for light microscopic evaluation. Some tissue samples of 1–2 mm^3 obtained from adjacent regions were stocked in 2.5% gluteraldehyde for electron microscopic evaluation. After fixation, samples were embedded in paraffin for light microscopic evaluation and serial sections of 5-μm thickness were prepared. After choosing the first section randomly, 10 sections in each mouse were selected by skipping over 10 sections and proceeded to the staining process. For light microscopic evaluation, three different staining processes were used. The first 10 samples were stained with haematoxylin and eosin (H&E). In these samples, general tissue features were examined and thicknesses of epithelium and subepithelial smooth muscle layers of the medium and small airways were measured. In order to evaluate the thicknesses of epithelium and subepithelial smooth muscle layers, measurements were performed from four points of each airway at levels of 3, 6, 9, and 12 o'clock. Considering that each section contained approximately two to three airways, around 20 or more airways were evaluated for each mouse.

Photomicrographs were taken by JVC TK-890-E camera (Japan), which was adapted on an Olympus BH-2 RFCA model microscope (Olympus Optical, Tokyo, Japan). The histological analysis was carried out with UTHSCSA Image Tool for Windows Version 3.00 software.

The consecutive 10 sections were stained with toluidine blue and the other 10 sections with periodic acid-Schiff (PAS). Photomicrographs were taken randomly from five fields of each section which were stained with toluidine blue. For mast cell enumeration, a standard transparent counting frame representing an area of 20,000 μm^2 was used