Inhibition of antigen-induced bronchial smooth muscle hyperresponsiveness by lovastatin in mice

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Abstract

Statins have been proposed as a novel treatment of respiratory diseases. To determine the beneficial effects of statins on the airway hyperresponsiveness, a characteristic feature of allergic bronchial asthma, the effect of systemic treatment with lovastatin on antigen-induced bronchial smooth muscle hyperresponsiveness was investigated in mice. Male BALB/c mice were sensitized and repeatedly challenged with ovalbumin antigen. Animals were also treated with lovastatin (4 mg/kg/day, *i.p.*) once a day prior to and during the antigen inhalation period. The bronchial smooth muscle responsiveness to acetylcholine, but not to high K⁺-depolarization, was markedly and significantly augmented in the repeatedly antigen challenged mice. The bronchial smooth muscle hyperresponsiveness to acetylcholine induced by antigen exposure was significantly attenuated by the systemic treatment with lovastatin. Thus, lovastatin might have therapeutic potential to ameliorate airway hyperresponsiveness in allergic bronchial asthma.

Key words: asthma, airway hyperresponsiveness, bronchial smooth muscle, lovastatin, mouse

Introduction

Increased airway narrowing in response to nonspecific stimuli, called airway hyperresponsiveness (AHR), is a characteristic feature of allergic bronchial asthma. One of the factors that contribute to the exaggerated airway narrowing in asthmatics is an abnormality of the nature of airway smooth muscle (Martin *et al.*, 2000; Seow *et al.*, 1998). It is thus important for development of asthma therapy to understand changes in the contractile signaling of airway smooth muscle cells associated with the disease. To date, there is increasing evidence that a monomeric GTP-binding protein RhoA and its downstream target Rho-kinase are involved in the agonist-induced Ca²⁺ sensitization of airway smooth muscle contraction (Chiba *et al.*, 1999; 2005, Ito *et al.*, 2001; Yoshii *et al.*, 1999). Recent studies revealed an augmentation of the RhoA/Rho-kinase-mediated Ca²⁺ sensitization in animal models of allergic bronchial asthma (Chiba *et al.*,

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1999; 2005), and the RhoA/Rho-kinase pathway has now been proposed as a new target for the treatment of AHR in asthma (Gosens *et al.*, 2006).

Statins are a class of cholesterol lowering drugs that decrease mortality from cardiovascular disease (Shepherd *et al.*, 1995) and stroke (Briel *et al.*, 2004). The beneficial effects of statins have been attributed to reduce cholesterol biosynthesis through competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which converts HMG-CoA to L-mevalonate. Statins have pleiotropic effects beyond lowering cholesterol levels, such as anti-inflammatory actions and anti-oxidant activity (Kumar and Reynolds, 2005; McKay *et al.*, 2004), and have also been proposed as a novel treatment of respiratory diseases (Hothersall *et al.*, 2006). One of the mechanisms of the pleiotropic effects may result from the depletion of the downstream metabolites farnesylpyrophosphate and geranylgeranylpyrophosphate. These isoprenoid pyrophosphates are precursors required for the posttranscriptional prenylation and functional activation of certain proteins, including RhoA. It is thus likely that statins have an ability to reduce the Ca^{2+} sensitization of smooth muscle contraction via an inhibition of RhoA function indirectly. Indeed, lovastatin can inhibit translocation of RhoA to plasma membrane, which is modulated by its geranylgeranylation, resulting in an inhibition of RhoA-mediated function in cultured tumor cell lines (Lee *et al.*, 2006; Zhong *et al.*, 2003).

In the present study, the effects of lovastatin on the augmented contraction of bronchial smooth muscle in mice with experimental asthma were investigated to determine the beneficial effects of this agent on the AHR in allergic bronchial asthma.

Methods

Male BALB/c mice were purchased from the Charles River Japan, Inc. (Kanagawa, Japan) and housed in a pathogen-free facility. All animal experiments were approved by the Animal Care Committee of Hoshi University (Tokyo, Japan).

Preparation of a murine model of allergic bronchial asthma was performed as described previously (Chiba *et al.*, 2004; 2005; 2006). In brief, mice (8 weeks of age) were actively sensitized by intraperitoneal injections of 8 μg ovalbumin (OA; Seikagaku Co., Tokyo, Japan) with 2 mg Imject Alum (Pierce Biotechnology, Inc., Rockford, IL, USA) on days 0 and 5. The sensitized mice were challenged with aerosolized OA-saline solution (5 mg/ml) for 30 min on days 12, 16 and 20. A control group of mice received the same immunization procedure but inhaled saline aerosol instead of OA challenge. Animals also received intraperitoneal injection with lovastatin (Toronto Research Chemicals, Inc., Ontario, Canada; 4 mg/kg/day; dissolved in 50% DMSO in PBS) (Chiba *et al.*, 2008) or its vehicle once a day for 10 days from Day 11 to Day 20. The treatment was carried out 1 h before the antigen inhalation in case of the days of the antigen challenge (Days 12, 16 and 20). Twenty-four h after the last OA challenge, mice were sacrificed by exsanguination from abdominal aorta under urethane (1.6 g/kg, *i.p.*; Sigma, St. Louis, MO) anesthesia. The smooth muscle responsiveness of the left main bronchial tissues was measured as previous papers (Chiba *et al.*, 2004; 2005). Epithelium was removed by gently rubbing with sharp-edged tweezers.

All the data are expressed as the mean $\pm$ s.e.mean. Statistical significance of difference was determined by unpaired Student's *t*-test or two-way analysis of variance (ANOVA).

Fig. 1. Effects of systemic treatments with lovastatin on the bronchial smooth muscle hyperresponsiveness induced by repeated antigen exposure. The acetylcholine (ACh; A and B)- and high K⁺ depolarization (C and D)-induced contractions of bronchial smooth muscles isolated from non-sensitized control (Control; A and C) and repeatedly antigen-challenged (Challenged; B and D) mice were measured isometrically. Lovastatin (4 mg/kg/day) or its vehicle (50% DMSO in PBS) was also pretreated intraperitoneally for 10 days as described in the METHODS section. Each point represents the mean $\pm$ SEM from 5–6 different animals. Note that the bronchial smooth muscle responsiveness to ACh of the challenged group (Vehicle-Challenged in B) was significantly augmented as compared with that of the control one (Vehicle-Control in A; $P < 0.05$ by two-way ANOVA), whereas the responsiveness to high K⁺ depolarization was not changed (C and D). The augmented ACh responsiveness induced by antigen challenge was significantly inhibited by pretreatment with lovastatin (Vehicle-Challenged vs. Lovastatin-Challenged; $P < 0.05$ by two-way ANOVA). *, $P < 0.05$ vs. Lovastatin-Challenged by unpaired Student's *t*-test.

Results and discussion

Using our well-established mouse model of allergic bronchial asthma (Chiba *et al.*, 2004; 2005; 2006), the effect of systemic treatment with lovastatin on bronchial smooth muscle hyperresponsiveness induced by antigen exposure was determined.

As shown in Figs. 1A and 1B, the contractile response to acetylcholine (ACh) of bronchial smooth muscle from the repeatedly antigen-challenged mice was markedly augmented, *i.e.*, bronchial smooth muscle hyperresponsiveness, as compared to that from the control animals: ACh concentration-response curve was significantly shifted upward by the antigen exposure (Vehicle-Control in Fig. 1A vs. Vehicle-Challenged in Fig. 1B; $P < 0.05$). The value of maximal

contraction induced by ACh (Emax; g) of the Vehicle-Challenged group (0.49 ± 0.03) was significantly greater than that of the Vehicle-Control one (0.34 ± 0.04 , $P < 0.05$), whereas no significant change in the 50% effective concentration (EC50; -logM) was observed (5.07 ± 0.15 , and 5.14 ± 0.25 , respectively). On the other hand, no significant change in the contractile response induced by high K⁺-depolarization (in the presence of 10^{-6} M atropine and 10^{-6} M indomethacin) was observed between the groups (Figs. 1C and 1D).

To determine the effect of lovastatin on the bronchial smooth muscle hyperresponsiveness observed in the repeatedly antigen-challenged mice, animals also received intraperitoneal injection with lovastatin (4 mg/kg/day) or its vehicle (50% DMSO in PBS) prior to and during the period of repeated antigen exposure. In the non-sensitized normal control animals, the lovastatin treatments had no effect on the contractility of bronchial smooth muscle to ACh (Fig. 1A) or high K⁺-depolarization (Fig. 1C). However, the bronchial smooth muscle hyperresponsiveness to ACh observed in the repeatedly antigen-challenged mice was significantly inhibited by the systemic treatments with lovastatin (Fig. 1B; $P < 0.05$). The bronchial smooth muscle responsiveness to ACh of the antigen-challenged animals completely returned to the control level: no significant difference was observed between the Lovastatin-Challenged (Fig. 1B) and the Vehicle-Control (Fig. 1A) groups. Similar results have also been obtained in a rat model of allergic bronchial asthma (Chiba *et al.*, 2008), indicating that lovastatin could inhibit bronchial smooth muscle hyperresponsiveness induced by allergen in various species of mammals.

Statins are known to have pleiotropic effects beyond lowering cholesterol levels. It has been reported that simvastatin (McKay *et al.*, 2004) and pravastatin (Yeh and Huang, 2004) showed an anti-inflammatory property in experimental allergic asthma. It is thus possible that the attenuation of bronchial smooth muscle hyperresponsiveness by lovastatin currently observed may result from an inhibition of the antigen-induced airway inflammation. Indeed, airway inflammation determined by total cell counts in bronchoalveolar lavage fluid was also inhibited by lovastatin (4.31 ± 1.08 for Vehicle-Challenged and 1.95 ± 0.38 ($\times 10^6$ cells/ml) for Lovastatin-Challenged groups, respectively; $P < 0.01$). Alternatively, direct effect of lovastatin on the bronchial smooth muscle might also be considerable. Statins inhibit HMG-CoA reductase and thus can reduce the downstream metabolite geranylgeranylpyrophosphate, a precursor required for the posttranslational geranylgeranylation and functional activation of RhoA. RhoA is a critical protein that is involved in the agonist-induced Ca²⁺ sensitization of smooth muscle contraction. Our previous study revealed an augmentation of acetylcholine-induced translocation of RhoA to the plasma membrane, *i.e.*, an augmented activation of RhoA, in association with the bronchial smooth muscle hyperresponsiveness in a rat model of AHR (Chiba *et al.*, 2001). The augmented RhoA translocation was inhibited by the systemic treatment with lovastatin (Chiba *et al.*, 2008).

In conclusion, the present study demonstrates that lovastatin inhibits the antigen-induced bronchial smooth muscle hyperresponsiveness in mice. Statins, such as lovastatin, may have therapeutic potential to ameliorate AHR in allergic bronchial asthma.

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