Effectiveness of atorvastatin in suppressing *MUC5AC* gene expression in human airway epithelial cells

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Background: We recently reported that chronic cholesterol depletion in NCI-H292 cells by lovastatin suppresses interleukin (IL)-1β–induced *MUC5AC* gene expression. However, as there are numerous statins affect *MUC5AC* expression, we sought to determine which statin is most effective in reducing *MUC5AC* expression, and whether this activity of statins is related to IL-1 receptor (IL-1RI) and mitogen-activated protein kinase (MAPK) activity.

Methods: Four statins, namely atorvastatin, fluvastatin, lovastatin, and simvastatin, were tested. Cholesterol depletion was measured via modified microenzymatic fluorescence assay and filipin staining. NCI-H292 cells were pretreated with 10 μM of each statin for 1 hour, 10 ng/mL of IL-1β was added, and cells were then co-incubated with statin and IL-1β for 24 hours. *MUC5AC* mRNA expression was measured via real-time polymerase chain reaction (PCR). The phosphorylation levels were assayed by Western blot.

Results: Cholesterol in the plasma membrane was markedly decreased by all 4 statins, of which atorvastatin was the most potent. IL-1β–induced *MUC5AC* messenger RNA (mRNA) expression was most significantly decreased by 10 μM atorvastatin, to 1.4 ± 0.2-fold of the level of the untreated control group, as opposed to an increase to 4.7 ± 0.5-fold for IL-1β alone, and this suppression of *MUC5AC* expression was dose-dependent. This decrease in *MUC5AC* expression by atorvastatin was mediated via the IL-1 receptor and the MAPK pathway, including both phospho-p38 MAPK and phospho–extracellular signal-regulated kinase (phospho-ERK).

Conclusion: These results suggest that atorvastatin is the most potent of the assayed statins with respect to suppression of IL-1β–induced *MUC5AC* mRNA expression, and may be considered as an anti-hypersecretory agent.

Key Words:
cholesterol; atorvastatin; *MUC5AC*; MAP kinases; IL-1β; mucin; airway epithelial cell

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Statins are a class of drugs that lower cholesterol levels by inhibiting the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. Statins, one of the most widely prescribed agents in medicine, have protective effects against cardiovascular diseases, as well as anti-inflammatory, antioxidant, and pleiotropic effects.[1–3] Statins also have neuroprotective activities, with studies finding that statin use may be associated with a reduction in the incidence of dementia, Alzheimer's disease, and Parkinson's disease.[4–7] Thus, various potential uses of statins have been actively studied.

Hypersecretion of airway mucus is a characteristic feature of chronic airway diseases such as cystic fibrosis and chronic rhinosinusitis. In the conducting airways of the lung and nasal cavity, mucins are a major contributor to the viscoelastic property of mucus secretion. Among human mucin genes, *MUC5AC*, which encodes the mucin 5A protein, is recognized as a major airway mucin gene in the airway epithelium. Thus, regulation of *MUC5AC* may serve as a new strategy for treating respiratory diseases.[8–11] Chronic cholesterol depletion in the cell membranes of NCI-H292 cells by the statin, lovastatin, causes suppression of interleukin (IL)-1β–induced expression of the *MUC5AC* gene via IL-1β receptor-specific and mitogen-activated protein kinase (MAPK)-dependent pathways.[12] Thus, the role

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of statins in mucin production is considered to be clinically important. As numerous statins exist, we sought to investigate which statin is the most effective in reducing *MUC5AC* expression and whether this activity of statins is related to IL-1 receptor and MAPK.

Materials and methods

Cell culture

Human pulmonary mucoepidermoid carcinoma cell lines (NCI-H292 cells) from the American Type Culture Collection (Rockville, MD) were cultured in RPMI 1640 (Gibco BRL, Grand Island, NY) and Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL) supplemented with 10% fetal bovine serum (FBS) (Cellgro, Hemdon, VA) in the presence of 2 mM L-glutamine, penicillin (100 μg/mL), and streptomycin (100 μg/mL) at 37°C in a humidified chamber with 95% air and 5% CO_2. When cultures reached 60% to 80% confluence, the cells were incubated in each medium containing 0.5% FBS for 24 hours, after which they were rinsed with phosphate-buffered saline (PBS) and exposed to 10 ng/mL human recombinant IL-1β (R&D Systems, Minneapolis, MN). Certain cultures were pretreated with individual statins. Four statins, namely atorvastatin, fluvastatin, lovastatin, and simvastatin (Sigma Chemical Co., St. Louis, MO) were administered for 1 hour before exposure to IL-1β, which was dissolved in PBS containing 0.1% bovine serum albumin.

Materials

IL-1β was purchased from R&D Systems; anti-phospho-Type I IL-1 receptor (phospho-Y496) antibody was purchased from Abcam (Cambridge, MA); and anti-phospho-p44/42 MAPK (Thr^{202}/Tyr^{204}) antibodies and anti-phospho-p38 MAPK (Thr^{180}/Tyr^{182}) antibodies were purchased from Cell Signaling Technology (Beverly, MA). Four statins, namely atorvastatin, fluvastatin, lovastatin, and simvastatin (Sigma Chemical Co.), were used. Dimethyl sulfate (DMSO) was used as solvent, as the selected statins were lipophilic.

Determination of cell viability

Cell viability was determined via MTS (3-[4,5-dimethylthiazol-2-yl]-5-[3-carboxymethoxyphenyl]-2-[4-sulfophenyl]-2H-tetrazolium, inner salt) and electron coupling reagent (phenazine ethosulfate) assays, using a previously described method.[12]

Measurement of intramembranous cholesterol

Cells were rinsed twice with 1 mL cold PBS, and lipids were extracted with chloroform-methanol 2:1 (vol/vol). Homogenized cell lysates were centrifuged for 10 minutes at 14,000 rpm (160,250 rcf), and the organic phase was transferred to a clean tube, dried under vacuum, and redissolved in 20 μL 2-propanol and 10% Triton X-100. One microliter (1 μL) per assay was used, adjusting to 50 μL with cholesterol reaction buffer in the wells of a 96-well plate. Cholesterol levels were measured via a modified microenzymatic fluorescence assay (Cayman Chemical Company, Ann Arbor, MI) according to the manufacturer's protocol, using a previously described method.[12] The results were described as the ratio of cholesterol to cell protein (μg/mg).

Filipin staining

Cells cultured on polylysine-coated cover slips were divided into 2 groups: a control group and an experimental group, which was subdivided according to each statin. The cells were subsequently rinsed with cold PBS and fixed on ice using 4% paraformaldehyde. The cells were again rinsed with cold PBS for 10 minutes and stained at room temperature for 2 hours with 100 μg/mL of filipin (Sigma Chemical Co.), which is highly fluorescent and binds specifically to cholesterol, hence its widespread use as a histochemical stain for cholesterol. After being rinsed once with PBS, the cells were observed through a fluorescence microscope with a ultraviolet (UV) filter set (340-380 nm excitation, 40 diachronic, 430 nm long-pass filter).

Real-time polymerase chain reaction of *MUC5AC* mRNA

Primers and probes were designed using Applied Biosystems Primer Express software and purchased from Applied Biosystems (Carlsbad, CA). Commercial reagents (TaqMan Universal PCR Master Mix; Applied Biosystems) were used in accordance with the manufacturer's protocols. One microgram (1 μg) of complementary DNA (cDNA) (reverse transcription mixture) and oligonucleotides at final concentrations of 800 nM for primers and 200 nM for TaqMan hybridization probes were analyzed in a 25-μL volume, using a previously described method.[12] Relative quantities of *MUC5AC* mRNA were calculated using a comparative cycle threshold method and normalized to tubulin, which was used as an endogenous control. Expression was reported as the ratio of *MUC5AC* to tubulin intensity.

Western blot analysis

Cell lysates were prepared in radioimmunoprecipitation assay buffer (Sigma Chemical Co.). Protein quantities were measured via bicinchoninic acid protein assay using bovine serum albumin as a standard. Next, 30 μg per lane of each protein lysate was resolved by electrophoresis. We used 4% to 12% gels (Invitrogen, Grand Island, NY) for IL-1RI analysis and 8% sodium dodecyl sulfate (SDS)-polyacrylamide gels for *MUC5AC* protein and MAPK analysis. Resolved proteins were transferred to nitrocellulose membranes, and Western blot analysis was performed using a previously described method.[12] p-IL-1RI (1:500), phospho–extracellular signal-regulated kinase (p-ERK; 1:1000), and p-p38 MAPK (1:1000) antibodies were used, and in addition, expression of *MUC5AC* protein was assessed with anti-*MUC5AC* antibody (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA).

TABLE 1. Cell viability by statin*

	1 μM	10 μM	100 μM
Atorvastatin (%)	99 ± 1	97 ± 2	65 ± 4
Fluvastatin (%)	99 ± 2	91 ± 3	58 ± 3
Lovastatin (%)	96 ± 2	91 ± 2	55 ± 4
Simvastatin (%)	100 ± 1	98 ± 2	25 ± 3

*Values are the percentage of living cells compared to the control group, which was not treated with any statins. The control group was set to 100% of living cells.

Statistics

Each experiment was performed at least 3 times, and the mean value and the standard deviation were calculated. A repeated measures analysis of variance (ANOVA) test was used to analyze differences, and multiple comparisons were added. Values of p less than 0.05 ($p < 0.05$) were defined as significant.

Results

Cytotoxicity of statins

To evaluate the cytotoxicity of the 4 statins, we treated cells with statins at various concentrations (0, 1, 10, 100 μM) for 24 hours and examined cell viability. Cells exposed to 10 μM of each statin had normal viability; however, those exposed to 100 μM of each statin showed some loss of viability (Table 1). Therefore, we used statins at 10 μM in subsequent experiments.

Depletion of cholesterol by statins

To confirm the depletion of cholesterol by statins, an intramembranous cholesterol level analysis and filipin staining were performed for each statin. After treatment with 10 μM of each statin for 24 hours, the intramembranous cholesterol/protein ratio was calculated. Compared with the control, the relative ratios of intramembranous cholesterol to protein after treatment with atorvastatin, fluvastatin, lovastatin, and simvastatin were 0.59 ± 0.14, 0.63 ± 0.03, 0.70 ± 0.09, and 0.68 ± 0.05, respectively (Fig. 1A). On filipin staining, filipin was observed to adhere specifically to cholesterol along the cell membrane in the control group. In the statin treatment groups, the intensity of fluorescence markedly decreased (Fig. 1B). These results indicate that all of the statins decreased cholesterol levels in the cell membrane and that atorvastatin was the most potent cholesterol-lowering drug.

Changes in IL-1β-induced *MUC5AC* expression by statins

To study the effect of statins on *MUC5AC* expression in NCI-H292 cells, each statin was treated for 25 hours without IL-1β and *MUC5AC* expression was examined using real-time polymerase chain reaction (PCR). Under this statin-only condition, simvastatin significantly increased *MUC5AC* expression compared to the control (control : simvastatin = 1 : 4.6 ± 0.3; $p < 0.05$). However, other statins did not cause significant increases in *MUC5AC* expression (control : atorvastatin : fluvastatin : lovastatin = 1 : 1.2 ± 0.2 : 1.5 ± 0.3 : 1.3 ± 0.1; respectively, Fig. 2). To reveal the effect of statins on *MUC5AC* expression in IL-1β–treated NCI-H292 cells, cultured cells were pretreated with 10 μM of each statin for 1 hour, 10 ng/mL of IL-1β was added and co-treated with each statin for 24 hours, and *MUC5AC* expression was then examined using real-time PCR. Under this condition of IL-1β

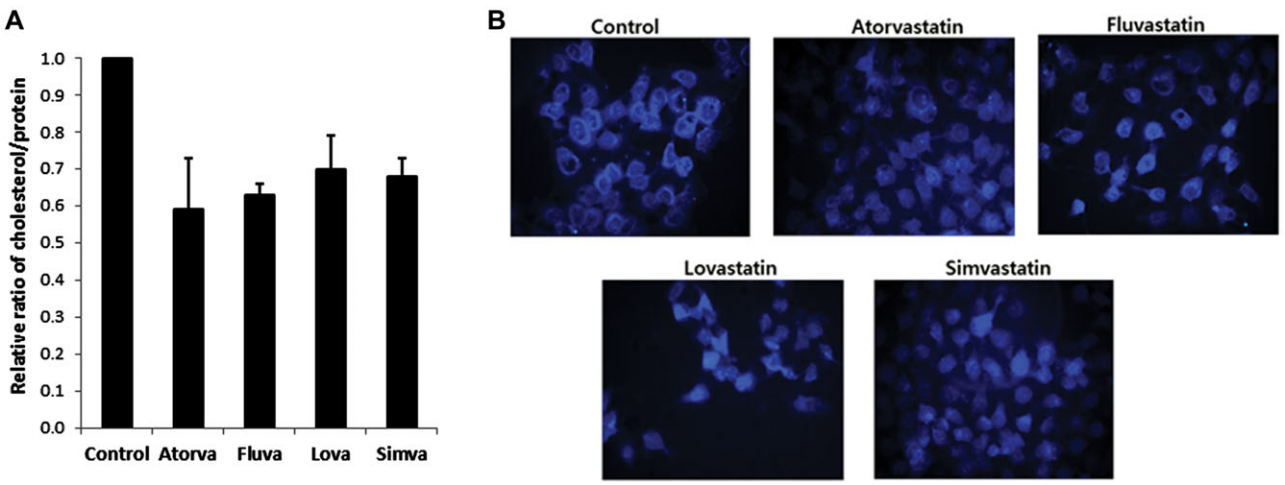

FIGURE 1. Determination of intramembranous cholesterol level via modified microenzymatic fluorescence assay (A) and filipin staining (B). (A) The intramembranous cholesterol level was determined using a modified microenzymatic fluorescence assay. The amount was expressed as a cholesterol/protein ratio, and the data of the experimental group were compared with those of the control group (no treatment with statins). The cholesterol/protein ratio in NCI-H292 cells was decreased by treatment with 10 μM statins. Graphs depict mean ± standard deviation of 4 independent experiments. (B) Filipin staining in NCI-H292 cells after treatment with statins. Filipin staining was performed after treatment with 10 μM statins for 24 hours and was observed with a fluorescence microscope. Compared to the control group, the staining intensity along the cell membrane was significantly decreased. Atorva = atorvastatin; Fluva = fluvastatin; Lova = lovastatin; Simva = simvastatin.

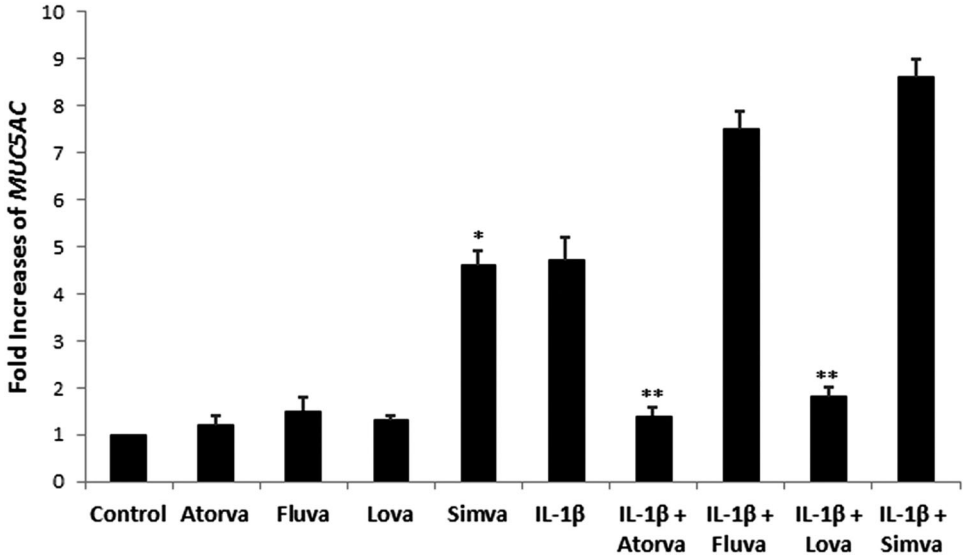

FIGURE 2. Changes in *MUC5AC* expression in response to statin treatment. Cultured cells were pretreated with 10 μM statins for 1 hour, and were then treated with 10 ng/mL of IL-1β for 24 hours. Graphs depict mean $\pm$ standard deviation of three independent experiments. In these conditions, simvastatin increased *MUC5AC* expression ($p < 0.05$). Significant decreases in IL-1β–induced *MUC5AC* expression were observed following treatment with atorvastatin and lovastatin ($p < 0.05$). *$p < 0.05$ compared to controls that were treated with neither IL-1β nor atorvastatin. ** $p < 0.05$ compared to IL-1β-treated cells. Atorva = atorvastatin; Fluva = fluvastatin; IL = interleukin; Lova = lovastatin; Simva = simvastatin.

stimulation and co-treatment with statins, a significant decrease in IL-1β–induced *MUC5AC* expression was observed following treatment with atorvastatin and lovastatin compared to IL-1β–only treated cells (control : IL-1β : IL-1β + atorvastatin : IL-1β + lovastatin = 1 : 4.7 $\pm$ 0.5 : 1.4 $\pm$ 0.2 : 1.8 $\pm$ 0.2; $p < 0.05$). In contrast, fluvastatin and simvastatin treatment caused an increase in IL-1β–induced *MUC5AC* expression (control : IL-1β : IL-1β + fluvastatin : IL-1β + simvastatin = 1 : 4.7 $\pm$ 0.5 : 7.5 $\pm$ 0.4 : 8.6 $\pm$ 0.4; $p > 0.05$; Fig. 2). As for *MUC5AC* protein expression, in comparison to the control that was treated with neither IL-1β nor atorvastatin, expression of *MUC5AC* protein induced by IL-1β was significantly decreased by atorvastatin (control : atorvastatin : IL-1β : IL-1β + atorvastatin = 1 : 1.2 $\pm$ 0.2 : 3.6 $\pm$ 0.5 : 1.4 $\pm$ 0.3; $p < 0.05$; Fig. 3). These results indicate that atorvastatin and lovastatin decrease IL-1β–induced *MUC5AC* expression in cell membranes and that atorvastatin is the most potent in suppressing *MUC5AC* expression. Thus, atorvastatin was used in subsequent experiments.

Dose-dependent suppression of *MUC5AC* expression by atorvastatin

NCI-H292 cells were pretreated with 0.1, 1, or 10 μM of atorvastatin for 1 hour and then treated with 10 ng/mL of IL-1β for 24 hours. The *MUC5AC* expression in cells treated with IL-1β alone increased by 4.5 $\pm$ 0.4-fold relative to the control group, which was treated with neither IL-1β nor atorvastatin. Additionally, in cells pretreated with 0.1, 1, or 10 μM of atorvastatin, the *MUC5AC* expression significantly decreased with 1 and 10 μM atorvastatin (control : 0.1 μM atorvastatin : 1 μM atorvastatin : 10 μM atorvastatin : IL-1β : IL-1β + 0.1 μM atorvastatin : IL-1β + 1 μM atorvastatin : IL-1β + 10 μM atorvastatin = 1 : 1.1 $\pm$ 0.1 : 1.2 $\pm$ 0.2 : 0.9 $\pm$ 0.1 : 4.5 $\pm$ 0.4 : 3.2 $\pm$ 0.4 : 1.9 $\pm$ 0.2 : 1.2 $\pm$ 0.2; $p < 0.05$; Fig. 4).

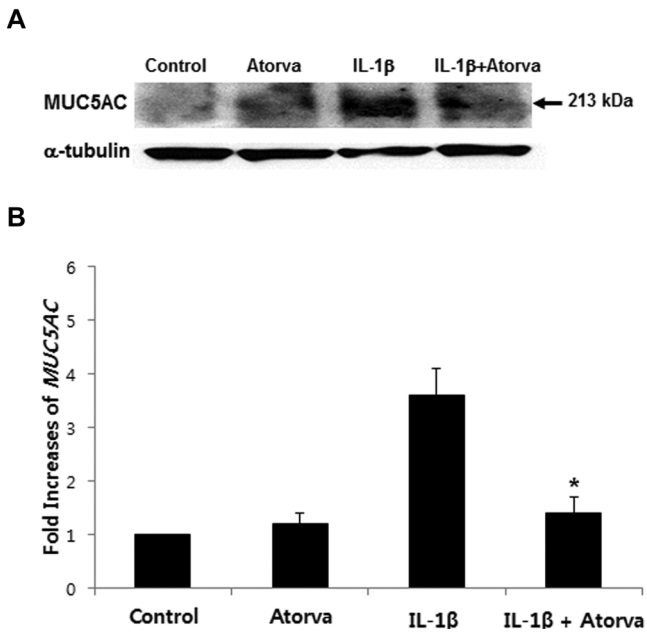

FIGURE 3. Suppression of IL-1β–induced *MUC5AC* protein expression by atorvastatin. NCI-H292 cells were pretreated with 10 μM atorvastatin for 1 hour and then treated with 10 ng/mL of IL-1β for 24 hours. Graphs depict mean $\pm$ standard deviation of 3 independent experiments. Western blot analysis for *MUC5AC* protein was respectively performed. Expression of *MUC5AC* protein (213 kDa) induced by IL-1β was suppressed by pretreatment with atorvastatin. Housekeeping band used was α-tubulin. *$p < 0.05$ compared to IL-1β-treated cells. Atorva = atorvastatin; IL = interleukin.

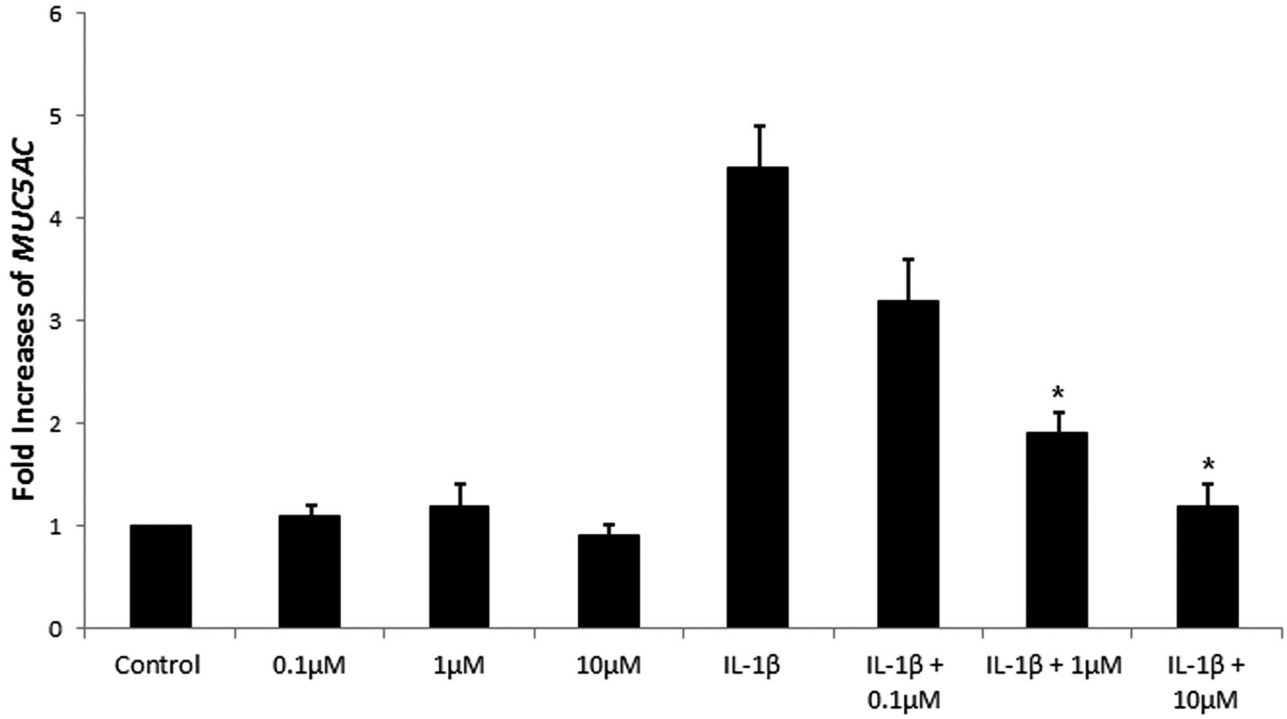

FIGURE 4. Dose-dependent suppression of *MUC5AC* expression by atorvastatin. NCI-H292 cells were pretreated with 0.1, 1, or 10 μM atorvastatin for 1 hour and then treated with 10 ng/mL of IL-1β for 24 hours. Graphs depict mean $\pm$ standard deviation of 3 independent experiments. In comparison with cells treated with IL-1β alone, cells pretreated with 1 or 10 μM atorvastatin exhibited significantly decreased *MUC5AC* expression. *$p < 0.05$ compared to IL-1β–treated cells.IL = interleukin.

Changes in MAPK and IL-1 receptor phosphorylation induced by atorvastatin

To determine whether the decrease in *MUC5AC* expression induced by atorvastatin was dependent on MAPK activity, we pretreated cells with 10 μM of atorvastatin for 24 hours and then incubated them with IL-1β for 20 minutes. A group exposed to neither atorvastatin nor IL-1β was used as a negative control group, and a group treated only with IL-1β was used as a positive control group. Significant decreases in both phospho-p38 (Fig. 5A, B) and phospho-ERK (Fig. 5A, C) levels were noted in IL-1β–treated and atorvastatin-treated cells, compared with cells treated with IL-1β alone (for phospho-p38 expression, control : atorvastatin : IL-1β : IL-1β + atorvastatin = 1 : 1.2 $\pm$ 0.2 : 3.9 $\pm$ 0.4 : 1.5 $\pm$ 0.3; for phospho-ERK expression, control : atorvastatin : IL-1β : IL-1β + atorvastatin = 1 : 1.4 $\pm$ 0.3 : 4.6 $\pm$ 0.5 : 2.0 $\pm$ 0.2; $p < 0.05$). Expression of phospho-IL-1RI was also significantly decreased in cells treated with IL-1β plus atorvastatin compared with those treated with IL-1β alone (control : atorvastatin : IL-1β : IL-1β + atorvastatin = 1 : 1.2 $\pm$ 0.2 : 4.2 $\pm$ 0.4 : 1.6 $\pm$ 0.3; $p < 0.05$; Fig. 5A, D).

Discussion

This study initially sought to determine which statin is the most effective drug at suppressing *MUC5AC* expression in human airway epithelial cells. This question arose from 2 observations: first, that chronic cholesterol depletion in the plasma membrane suppresses *MUC5AC* expression, and second, that plasma cholesterol-lowering efficacies differ among statins.[12,13] Our reason for selecting the 4 lipophilic statins in this study is as follows. The difference between lipophilic and hydrophilic statins may lay on their differing chemical structures, pharmacokinetic profiles, and lipid-modifying efficacy. Unlike the lipophilic statins that penetrate the cell membrane, hydrophilic statins such as pravastatin cannot penetrate the cell membrane. Thus, the physiochemical property of lipophilic statins is an important factor in modulating the fluidity of the cell membrane and the metabolism of the cell. Schaefer et al. compared the effects of various statins and found that atorvastatin was the most effective statin tested in lowering LDL cholesterol, whereas fluvastatin, lovastatin, and simvastatin had about 33%, 60%, and 85% of the efficacy of atorvastatin, respectively.[14] Thus, we hypothesized that these differences in the cholesterol-lowering ability of the statins may affect their mucin-suppressing ability. Our results demonstrated that atorvastatin decreased IL-1β-induced *MUC5AC* expression more potently than other tested statins. Therefore, atorvastatin is the most effective statin in suppressing *MUC5AC* expression in human airway epithelial cells, such as NCI-H292 human pulmonary mucoepidermoid cancer cells.

We also attempted to determine whether the cholesterol-lowering effect of statin is related to the suppression of *MUC5AC* expression. We hypothesized that the cholesterol-lowering effect of statin is related to the suppression

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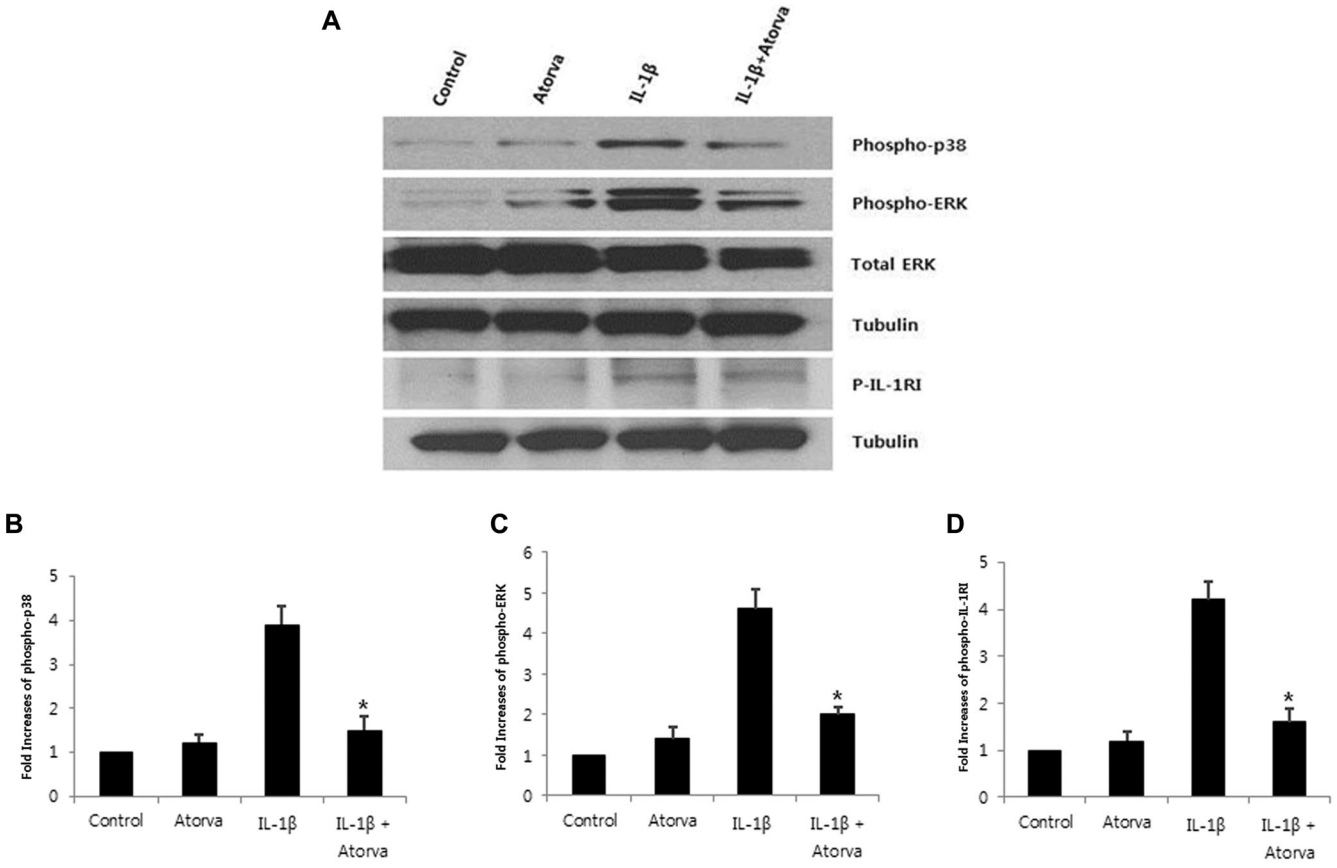

FIGURE 5. Changes in MAPK and IL-1 receptor phosphorylation in response to atorvastatin. NCI-H292 cells were pretreated for 24 hours with 10 μM atorvastatin and then incubated with IL-1β for 20 minutes. A group without exposure to atorvastatin or IL-1β was used as a negative control and a group treated with only IL-1β was used as a positive control. Graphs depict mean ± standard deviation of three independent experiments. A significant decrease in phospho-p38 (A, B) and phospho-ERK (A, C) was observed in cells treated with IL-1β- plus atorvastatin relative to levels in IL-1β-treated cells ($p < 0.05$). Expression of phospho-IL-1RI was also significantly decreased in cells treated with IL-1β- plus atorvastatin relative to levels in IL-1β-treated cells ($p < 0.05$) (A, D). Housekeeping band used was α-tubulin. *$p < 0.05$ compared to IL-1β–treated cells. Atorva = atorvastatin; ERK = extracellular signal-regulated kinase; IL = interleukin; MAPK = mitogen-activated protein kinase.

of *MUC5AC* expression based on the following reasons. First, the mean intramembranous cholesterol/protein ratio resulting from atorvastatin treatment was 0.59, indicating that it was the most potent among the tested statins in this regard. Second, atorvastatin showed dose-dependent suppression of IL-1β–induced *MUC5AC* expression, and its cholesterol-lowering ability in humans is the most potent among statins. These results indicate that cholesterol depletion in the plasma membrane by atorvastatin affects the lowering of the cholesterol level in the plasma membrane and may cause alterations in kinase with anti-inflammatory activity. Moreover, this *MUC5AC*-suppressing activity of statins varies among statins. Although fluvastatin and simvastatin caused cholesterol depletion in the plasma membrane, as determined by intramembranous cholesterol analysis and filipin staining, these statins did not suppress *MUC5AC* expression. Meanwhile, it has been shown that high plasma cholesterol levels enhance the expression of proinflammatory genes and cytokines, thus promoting a low-grade inflammation. Instead of a cholesterol-lowering effect, statins have been shown to exhibit independent anti-inflammatory properties by reducing proinflammatory cytokines.[15–17] Thus, further studies on the anti-inflammatory effects of statins such as decrease in mucus secretion should be followed.

In contrast to atorvastatin and lovastatin, simvastatin and fluvastatin tended to increase IL-1β–induced *MUC5AC* expression. According to recent studies, proinflammatory cytokines, chemokines, and mucin secretion are decreased by simvastatin and fluvastatin. Chen et al.[18] demonstrated that simvastatin attenuates acrolein-induced mucin production via the Ras/ERK pathway using a rat model. Liu et al.[19] revealed that simvastatin attenuates airway responsiveness and allergic inflammation. Additionally, simvastatin is considered to be a novel medicine for expectorant therapy.[20] Although the effect of fluvastatin on mucin has not been studied, Jouneau et al.[21] proved the existence of an anti-inflammatory effect of fluvastatin on cystic fibrosis patients stimulated by *Pseudomonas aeruginosa* and *Aspergillus fumigatus* antigens. These studies imply that statins are effective in controlling inflammation, although the mucin-suppressing and anti-inflammatory activities of statins may differ according to cell type or genotype. In a study using primary mouse tracheal

epithelial cells, simvastatin inhibited the expression of IL-13–inducible proinflammatory genes pertinent to asthma; however, it also induced the expression of several genes potentially relevant to inflammation.[22] In an animal study, the downregulation of immunoglobulin E (IgE)-mediated cytokine production by fluvastatin was dependent on genetic background, as C57BL/6J mast cells were sensitive to fluvastatin, whereas 129/SvImJ mast cells were resistant.[23] Thus, additional studies using normal nasal or bronchial cells and animals are needed to solve the discrepancy regarding the actions of simvastatin and fluvastatin.

Based on our results, the atorvastatin-mediated suppression of IL-1β–induced *MUC5AC* expression is mediated via the IL-1 receptor and the MAPK pathway, including both phospho-p38 MAPK and phospho-ERK. As atorvastatin lowers cholesterol in the plasma membrane, it can also lower the activity of the IL-1 receptor, as known to occur with lovastatin.[12] We also proved that lovastatin-mediated suppression of IL-1β–induced *MUC5AC* expression operates only via the p38 MAPK-dependent pathway.[12] In this study, atorvastatin-mediated suppression of IL-1β–induced *MUC5AC* expression occurred specifically at the IL-1 receptor and operated via both the ERK-dependent and the p38 MAPK-dependent pathways. Specifically, this discrepancy could be explained by the type of cells or cholesterol-lowering agents used in the experiment. In human airway epithelial cells such as NCI-H292 cells, acute depletion of cholesterol by methyl-β-cyclodextrin involves p-p38 MAPK and p-ERK, and chronic depletion of cholesterol by atorvastatin is mediated via the same pathways; however, depletion by lovastatin involves only the p38 MAPK pathway.[12,24] Meanwhile, numerous studies have reported that atorvastatin reduces the activation of nuclear factor κB (NF-κB) and that this signal transduction differs according to cell type; the signal transduction is mediated only through an ERK-dependent pathway in meningioma cells and human aortic endothelial cells, a p38-dependent pathway in Mardin-Darby canine kidney epithelial cells, and a c-Jun N-terminal kinase (JNK)-dependent pathway in coronary artery smooth muscle cells.[25–28] Thus, the signal transduction of atorvastatin-mediated suppression of *MUC5AC* expression could be explained specifically in terms of either cell types or cholesterol-lowering agents used in the experiment.

In chronic rhinosinusitis, thick discharge or rhinorrhea due to an increase of mucin secretion is a troublesome symptom.[29,30] Given our findings in a previous study that statins suppress *MUC5AC* expression, we thought that the use of statins might decrease the prevalence of chronic rhinosinusitis.[12] However, based on our epidemiologic study, hyperlipidemia itself is a risk factor for chronic rhinosinusitis regardless of the use of statins.[31] This discrepancy between the use of statins and the prevalence of chronic rhinosinusitis can be explained as follows. First, we were unable to determine what kind of statin the patients took. As simvastatin somewhat increased basal *MUC5AC* expression according to our results, the kind of statin must be considered in analyses. Second, statins may have anti-inflammatory effects in addition to lipid-lowering effects. Thus, this anti-inflammatory effect may negatively influence the prevalence rate. The study on the anti-inflammatory effects of statins in chronic rhinosinusitis is beyond the scope of this study, and further study on anti-inflammatory effects of statins is required.

The main drawback of this study was the cells that were used. In this study, we used human airway epithelial cells; however, if normal nasal or bronchial cells had been used, the data might have been different. Future studies using these normal cells are needed. Additionally, animal studies using a chronic rhinosinusitis model will be required to verify the in vivo effect of statins on chronic rhinosinusitis.

Conclusion

Among the statins evaluated, atorvastatin appeared to be the most effective statin in suppressing IL-1β–induced *MUC5AC* mRNA expression in human airway epithelial cells, such as NCI-H292 human pulmonary mucoepidermoid cancer cells, and may be considered to be an anti-hypersecretory agent.

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