Simvastatin promotes alveolar epithelial cell proliferation and attenuates cigarette smoke-induced emphysema in rats

SHUYUE XIA¹,², JIAN KANG¹, YANDUO JIANG³, DESHENG HUANG⁴, SHI WANG² and BAOSEN PANG⁵

¹Institute of Respiratory Disease, The First Affiliated Hospital of China Medical University, Shenyang, Liaoning 110001; ²Department of Pulmonary Medicine, Central Hospital affiliated to Shenyang Medical College, Shenyang, Liaoning 110024; ³Department of Pathology, 202nd Hospital of PLA, Shenyang, Liaoning 110812; ⁴Department of Mathematics, College of Basic Medical Sciences, China Medical University, Shenyang, Liaoning 110013; ⁵Beijing Institute of Respiratory Medicine, Beijing Chaoyang Hospital affiliated to Capital Medical University, Beijing 100069, P.R. China

Received August 26, 2014; Accepted March 23, 2015

DOI: 10.3892/mmr.2015.4172

Abstract. Current treatments for chronic obstructive pulmonary disease (COPD) cannot reverse the pathological process of the disease, therefore, the development of novel agents and strategies for COPD treatment is required. The aim of the present study was to investigate the potential therapeutic value of simvastatin (SmSt) in cigarette smoke-induced emphysema in rats. A total of 24 male and female Wistar rats were randomly divided into four groups. The levels of vascular endothelial growth factor (VEGF) in the lung tissues and bronchoalveolar lavage (BAL) fluid of each group were measured using an enzyme-linked immunosassay. The mRNA expression of VEGF was assessed using reverse transcription-quantitative polymerase chain reaction. The protein expression levels of VEGF and proliferating cell nuclear antigen (PCNA) were determined using immunohistochemical assays. Histological investigation revealed that simvastatin reduced the total inflammatory scores significantly more in the simvastatin-treated smoke-exposed group, compared with the smoke exposed (Sm) group. Significant differences in the average inter-alveolar septal wall distance and mean alveolar numbers were also observed between the SmSt and Sm groups. The levels of VEGF in the BAL fluid and lung tissue homogenates of the SmSt group were similar to those in the simvastatin-only (St) and control (Ctl) groups, and significantly higher compared with those in the Sm group. The expression of VEGF in the alveolar and bronchial epithelial cells of the SmSt group was similar to that in the Ctl group, and significantly higher compared with that of the Sm group. The percentage of PCNA-positive alveolar epithelial cells was significantly higher in the SmSt group compared with the Sm and Ctl groups. Simvastatin exerted a significant impact on the expression of VEGF and attenuated cigarette smoke-induced emphysema in rats. Therefore simvastatin may have beneficial effects in patients with COPD.

Introduction

Chronic obstructive pulmonary disease (COPD) is a term used for diseases, which cause lung impairment and breathlessness. The World Health Organization indicated that COPD was the fifth leading cause of mortality in 2002 and was predicted to become the third leading cause of mortality in 2030 (1). A previous large population, spirometry-based epidemiological investigation reported that, in China, the prevalence of COPD in adults >40 years was 8.2% (12.4% in men; 5.1% in women) (2). Therefore, COPD is likely to become an important health care problem worldwide. COPD is associated with characteristic pathological changes in the small airways, including obstructive bronchiolitis, and the destruction of lung parenchyma, including emphysema. Current therapies for COPD, including inhaled corticosteroids and long-acting agonists, improve the pulmonary function and quality of life in patients with COPD, however, they cannot reverse the pathological process of COPD. Therefore, the identification of novel therapies for COPD is an area of intense investigation (3-5).

Liebow (6) demonstrated that the alveolar septa in patients with COPD were thin and almost avascular, suggesting that a reduction of the small capillary blood vessels may lead to subsequent alveolar septal loss. Vascular endothelial growth factor (VEGF) signaling is important for maintaining lung structure. Kasahara et al (7) demonstrated that the expression of VEGF and its receptor in the lung tissues of patients with emphysema were significantly lower than normal. Therefore, a decrease in VEGF or disruption to the VEGF signaling pathway may affect the pathogenesis of emphysema. However, the effect of levels of VEGF on airway epithelial cells, which are in direct contact with the environment, remains to be fully

Correspondence to: Professor Jian Kang, Institute of Respiratory Disease, The First Affiliated Hospital of China Medical University, 155 Nan-Jing-Bei Street, Heping, Shenyang, Liaoning 110001, P.R. China
E-mail: jiangkangcn@163.com

Key words: emphysema, lung epithelium, vascular endothelial growth factor, statins
elucidated (8). It has been suggested that statins may modulate VEGF synthesis, and the pleiotropic effects of statins with regard to their use in COPD treatment has received more attention (9,10).

Statins are potent inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase, and exhibit pleiotropic pharmacological effects (11). In addition to a cholesterol-lowering effect, they exhibit anti-inflammatory, antioxidant and immunomodulatory properties, and improve endothelial function in chronic inflammatory lung disease (10). A number of these statin pleiotropic effects are mediated by the inhibition of isoprenylation of small guanosine-5'-triphosphate-binding signaling molecules, including the Rho, Ras and Rac proteins (12). The anti-inflammatory effects include the suppression of the release of proinflammatory cytokines, chemokines, adhesion molecules and matrix metalloproteinases by inflammatory cells. Statins increase the secretion of VEGF and the expression and activity of endothelial nitric oxide synthase, which improves endothelial cell function and promotes angiogenesis (13). There is evidence that statins exert cell type-dependent effects on endothelial cell angiogenic activity and on VEGF synthesis (14). It has been reported that simvastatin may induce apoptosis in hyper-proliferative pulmonary vascular lesions (15) and may inhibit the development of colon cancer via the induction of apoptosis and suppression of angiogenesis (16).

Cigarette smoking is the most important and common risk factor for COPD, however, the underlying pathological mechanisms remain to be elucidated (17,18). Therefore, the present study performed assays in order to investigate the effects of statins on the expression of VEGF, based on the morphometric parameters in cigarette smoke-induced lung emphysema, using a rat model. A number of cell functions, including differentiation, proliferation, and apoptosis can be affected by simvastatin (19). The effect of statins on the expression of proliferating cell nuclear antigen (PCNA) was also investigated.

Materials and methods

Reagents and materials. Male (n=12) and female (n=12) Wistar rats (12-week-old; 190-220 g; Chinese Academy of Medical Sciences, Beijing, China) were included in the present study, which was approved by the Division of Laboratory Animal Medicine at the China Medical University (Shenyang, China; certificate no. SVXX, Liao, 2003-0009). The animals were housed in Plexiglas cages (Guxiu, Suzhou, China), with males and females housed separately, under a 12:12 h light-darkness cycle in temperature and humidity controlled rooms. Standard laboratory food and water were provided ad libitum. The present study was performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (20). The animal use protocol was reviewed and approved by the Institutional Animal Care and Use Committee of China Medical University.

Treatment groups. The rats were numbered in ascending order of weight and divided into four groups. The four groups of rats (n=6 in each group) were randomly assigned to the following groups: Control (CtL); smoke-exposed only (Sm); simvastatin-only (St); and smoke + simvastatin (SmSt). The Sm and SmSt groups were exposed to the smoke from 16 commercial Da-Sheng-Chan cigarettes (Hongta Liaoning Tobacco Co., Ltd., Shenyang Cigarette Factory, Liaoning, China) for 1 h each day for 16 weeks. Simvastatin (Merck Sharp & Dohme, Ltd., Hoddeston, UK) was administered orally to the SmSt and St groups at a dose of 5 mg/kg (21,22), once daily for the 16 week period.

Morphometric evaluation of the lungs. Following 16 weeks of treatment, the rats were anesthetized using pentobarbital sodium (50 mg/kg, intraperitoneally; Sigma-Aldrich, St. Louis, MO, USA) and sacrificed by cervical vertebra dislocation. The bilateral lung were excised from the chest. The right lung tissues were fixed in 10% neutral buffered formalin (Sigma-Aldrich), embedded in paraffin blocks (Leica Microsystems GmbH, Wetzlar, Germany), cut into 4-µm serial sections. The right lung was used for histological analyses. The inflammation score of the small airways (300-1,100-µm) consisted of the following: Epithelial loss, erosion and ulcer formation; goblet cell hyperplasia and hypertrophy; ciliated epithelium lodging; inflammatory cell infiltration; lymph follicle formation; bronchial stenosis; airway smooth-muscle cell proliferation disorder; connective tissue proliferation; squamous metaplasia; wall congestion, edema; and wall pigmentation. Hematoxylin-and-eosin (HE; Leica Microsystems GmbH) was used to stain hilar areas of the lung tissue, in order to assess the small-airway pathology score, using two independent investigators, in a blinded-manner [Three small-airway sections, composite score 100/3, total score 100/3 (3 x 11)] (23). The tissues were viewed under a light microscope (BX51, Olympus Corporation, Tokyo, Japan).

Standard morphometric measurements were used to determine emphysematous changes. A total of 10 randomly selected HE-stained hilar areas from each sample (magnification, low power field) were used to analyze changes in air space size, which was determined by the average interalveolar septal wall distance (mean linear intercept; MLI). The alveolar density was determined by the mean alveolar number (MAN) (24).

Measurements of VEGF and PCNA. Measurements of VEGF in the bronchoalveolar lavage fluid (BALF) were performed in order to investigate the effect of simvastatin. For preparation of BALF and measurement of VEGF in the BALF, each rats was anesthetized using 10% neutral buffered formalin (Sigma-Aldrich), the trachea were exposed and intubated and the left lung was washed three times using 2 ml sterile saline, at 0°C. BALF was collected using a 5 ml syringe and placed on ice. Following centrifugation at 400 x g for 5 min at 4°C, the VEGF concentration was measured in the supernatants using an enzyme-linked immunosorbent assay (ELISA; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), according to the manufacturer's instructions.

To determine the levels of VEGF in the lungs in each group, the lung tissues were homogenized in phosphate-buffered saline (PBS; Wake Pure Chemical Industries, Osaka, Japan), and the supernatant was obtained by ultracentrifugation at 11,100 x g for 10 min at 4°C. The level of VEGF in the supernatant was determined using a commercially available ELISA kit (Quantikine Mouse VEGF kit; R&D Systems, Inc.,
Minneapolis, MN, USA), according to the manufacturer’s instructions.

**Immunohistochemical staining and quantification.** In order to perform immunohistochemical scoring analysis for VEGF and PCNA, hilar sections (5-µm) were deparaffinized in xylene (Shenyang Chemical Reagent Plant, Shenyang, China) and rehydrated. Antigen retrieval was performed by heating the lung tissue in a microwave (MYE-1870MEG; Haier, Qingdao, China) in 10 mmol/l citric acid monohydrate for 5 min at 900 W and three times for 5 min at 600 W. Endogenous peroxidase activity was inhibited by incubating the samples with 0.5% H2O2 for 10 min. The slides were incubated overnight at 4˚C in the appropriate dilutions of the primary antibodies (mouse anti-PCNA monoclonal antibody (1:100; cat. no. sc-25280; Santa Cruz Biotechnology, Inc.) and VEGF monoclonal antibody (1:100; cat. no. sc-7269; Santa Cruz Biotechnology, Inc.). The slides were incubated for 60 min at room temperature (20-22˚C), rinsed for 2 min with PBS three times, and incubated in 3, 3′-diaminobenzidine. The slides were then evaluated using light microscopy (BX51; Olympus Corporation).

The percentages of VEGF- and PCNA-positive small-airway and alveolar epithelial cells (AECs) were calculated. The staining was scored as follows: Expression of VEGF was localized in the cytoplasm, and the expression of PCNA was localized in the cell nucleus. The 500 cells were randomly selected in the bronchial epithelium, alveolar epithelium and vascular endothelial cells in each slide at 400x magnification. The number of cells expressing VEGF or PCNA were counted, respectively.

The entire tissue samples were scored using the same magnification factor. The staining intensities of VEGF and PCNA in the SAECs, AECs and vascular endothelial cells (VECs) were scored by independent investigators in a blinded-manner, in order to avoid observer bias.

**Analysis of the expression of VEGF.** Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was used to analyze the expression levels of VEGF by amplifying the coding parts of the VEGF gene. Total lung RNA was isolated using TRIzol reagent (Invitrogen Life Technologies), according to the manufacturer’s instructions. RNA concentration was measured by absorption at 260 nm and the purity of the RNA was guaranteed based on a absorption ratio of 260 nm/280 nm. Following quantification of the RNA samples, cDNA was synthesized from 50-100 mg of total lung RNA using a Takara RNA PCR kit (AMV) Ver.3.0 (Takara, Bio, Inc.) and subjected to a semi-quantitative PCR using rTaq (Takara Bio, Inc.), according to the manufacturer’s instructions. Briefly, the first-strand cDNA was synthesized in a 10 µl reaction mixture containing total RNA, AMV Reverse Transcriptase, RNase Inhibitor, dNTP Mixture, MgCl2, 10X RT buffer, and Random 9-mer as the reverse primer. The reverse-transcription reactions were carried out at 37˚C for 10 min, 50˚C for 30 min, and 95˚C for 2 min, prior to being chilled to 5˚C for 5 min. The PCR cycle conditions were as follows: 94˚C for 5 min, followed by 28 cycles of 94˚C for 30 sec, 56˚C for 30 sec and 72˚C for 60 sec, with a final extension step of 7 min at 72˚C in a thermal cycler (Applied Biosystems Life Technologies, Foster City, CA, USA). The PCR products were electrophoresed on agarose gel (Takara, Bio, Inc.), stained with ethidium bromide (Sigma-Aldrich) and then visualized in a UV transilluminator (GAS7001B; UVItec Limited, Cambridge, UK), with images captured. GAPDH was used as an internal control. The primer sequences used in the present study were as follows: Forward: 5'-ATCTTCAAGCCGTCCTGTGT-3' and reverse: 5'-TGTTCTATCTTTCTTTGGTCTGC-3', for VEGF. Primers were obtained from Takara Bio, Inc., Dalian, China). The total lung RNA (50-100 mg) was reverse-transcribed and amplified using RT-qPCR as described.

**Statistical analysis.** The data are presented as the mean ± standard deviation (SD). Following a test of homogeneity of variance, a one-way analysis of variance was used for multiple group comparisons. Pairwise multiple comparisons were performed using Dunnett’s or the least significant difference test. A Kruskal-Wallis rank test was used for multiple comparisons of groups with unequal variances, and Dunn's method was used for pairwise multiple comparisons. All analyses were performed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). P<0.05 was considered to indicate a statistically significant difference.

**Results**

**Condition of the rats.** All 24 rats in the four groups survived the 16 week treatment period. The mean ± SD body weights in the CtL, Sm, St and SmSt groups following 16 weeks of treatment were 390.81±76.75, 371.50±62.89, 366.67±75.69 and 79.31±25.31 respectively.

<table>
<thead>
<tr>
<th>Group</th>
<th>TIS</th>
<th>MLI (µm)</th>
<th>MAN (n/nm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CtL</td>
<td>43.43±24.97</td>
<td>58.72±5.98</td>
<td>138.07±6.74</td>
</tr>
<tr>
<td>Sm</td>
<td>84.85±48.96</td>
<td>79.31±25.31</td>
<td>84.56±31.83</td>
</tr>
<tr>
<td>St</td>
<td>47.78±31.24</td>
<td>50.64±6.02</td>
<td>128.42±33.55</td>
</tr>
<tr>
<td>SmSt</td>
<td>45.96±23.27</td>
<td>60.46±4.28</td>
<td>132.11±11.22</td>
</tr>
<tr>
<td>F-statistic</td>
<td>3.321</td>
<td>4.789</td>
<td>5.700</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are presented as the mean ± standard deviation (n=6 in each group). *P<0.05, compared with the CtL group. †P<0.05 and ‡P<0.01, compared with the Sm group. TIS, total inflammation score; MLI, mean linear intercept; MAN, mean alveolar number. CtL, control; Sm, smoke exposed; St, simvastatin treated; SmSt, smoke exposed ± simvastatin treated.
XIA et al: SIMVASTATIN PROMOTES ALVEOLAR EPITHELIAL CELL PROLIFERATION

The mean concentrations of VEGF in the BALF and in the lung tissue samples were significantly lower in the Sm group, compared with those in the CtL group (P<0.01). In the SmSt group, simvastatin treatment led to significantly higher concentrations of VEGF in the BALF samples (P<0.01) and homogenate samples (P<0.01), compared with those in the Sm group (Table II).

Simvastatin increases the smoke-induced reduction in VEGF. The mRNA expression of VEGF was lower in the lung tissue samples from the Sm group than in the CtL group. However, no significant differences were observed between the SmSt and CtL groups (Fig. 3). The expression of VEGF occurred predominantly in the SAECs (51.3±2.9%) and AECs (68.3±3.3%) of the CtL group, but was also present to a lesser extent, in the VECs (8.5±0.8%) of the CtL group. Following 16 weeks of smoke exposure, the expression of VEGF was significantly lower in the Sm group compared with the CtL group in the SAECs (16.3±2.7 vs. 51.3±2.9%, respectively; P<0.01) and in the AECs (27.0±5.9 vs. 68.3±3.2%, respectively; P<0.01), however, simvastatin alone did not affect the expression of VEGF. The expression of VEGF was significantly higher in the SmSt group compared with the Sm group in the SAECs (49.0±2.9 vs. 16.3±2.7%, respectively; P<0.01) and the AECs (67.7±4.9 vs. 27.0±5.9%, respectively; P<0.01). However, no significant differences were observed in the expression of VEGF in the VECs between the SmSt and Sm groups (6.3 ± .6 vs. 4.5±1.5%, respectively; P>0.05). These findings are shown in Fig. 4A-C.

Increase of PCNA-positive AECs by simvastatin. The percentage of PCNA-positive AECs was significantly higher in the SmSt group compared with the Sm group (10.3±1.9 vs. 4.8±0.8%, respectively; P<0.01) and CtL group (7.0±1.7%, P<0.01). However, the expression of PCNA was lower in the SAECs and VECs (Fig. 5A-C; Table III).

Discussion

The present study examined whether treatment with simvastatin attenuates cigarette smoke-induced emphysema in rats...
by analyzing the expression of VEGF and lung morphology. Observations and epidemiological studies have demonstrated that statins may be beneficial for the treatment of COPD (3,5,25). Different pathological abnormalities, including emphysema and chronic bronchitis, coexist in a number of patients with COPD, and emphysema, loss of elastic recoil and intrinsic airway abnormalities synergistically contribute to disease severity. There is evidence that significant small-airway pathology not only exists in patients with a chronically and radiographically documented emphysematous phenotype of COPD, but it also affects the outcome (26). Cigarette smoke causes primary lymphocyte infiltration into the small-airways, whereas simvastatin inhibits inflammatory infiltration around the bronchial branches. Animal model systems represent useful methods to assess the impact of cigarette smoke in patients with COPD (27). Emphysema is an anatomical lesion, which has been the focus of the majority of animal models (27,28). Therefore, the present study selected a rat model to investigate the therapeutic effects of simvastatin on lung emphysema. The production of alveolar ducts was observed in lung samples from the model of smoke-induced emphysema; this change is anatomically similar to a mild form of centrilobular emphysema, commonly observed in cigarette smokers. The parenchyma between the dilated alveolar ducts and alveoli were ruptured. The results of the present study demonstrated that, following 16 weeks of cigarette smoke exposure, airway pathological scores were higher in

![Figure 2. Hematoxylin and eosin staining. (A) In the smoke exposure-only group, alveolar ducts and alveoli were dilated and a number of alveolar septa were ruptured. (B) In the smoke exposure ± simvastatin group, a number of alveoli were dilated. Magnification, x400.](image)

<table>
<thead>
<tr>
<th>Table II. Levels of VEGF in lung homogenates and BALF.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>CtL</td>
</tr>
<tr>
<td>Sm</td>
</tr>
<tr>
<td>St</td>
</tr>
<tr>
<td>SmSt</td>
</tr>
<tr>
<td>F-statistic</td>
</tr>
<tr>
<td>P-value</td>
</tr>
</tbody>
</table>

Values are presented as mean ± standard deviation (n=6 in each group). aP<0.01, compared with the control group. bP<0.01, compared with the Sm group. VEGF, vascular endothelial growth factor; BALF, bronchoalveolar lavage fluid; CtL, control; Sm, smoke exposed; st, simvastatin treated; SmSt, smoke exposed ± simvastatin treated.

<table>
<thead>
<tr>
<th>Table III. Positive expression of PCNA in rat lung tissue cells.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>CtL</td>
</tr>
<tr>
<td>Sm</td>
</tr>
<tr>
<td>St</td>
</tr>
<tr>
<td>SmSt</td>
</tr>
<tr>
<td>F-statistic</td>
</tr>
<tr>
<td>P-value</td>
</tr>
</tbody>
</table>

Values are presented as the mean ± standard deviation (n=6 in each group). aP<0.01, compared with the control group. bP<0.01, compared with the Sm group. AECs, alveolar epithelial cells; SAEC, small-airway epithelial cells; VECs, vascular endothelial cells; PCNA, proliferating cell nuclear antigen; CtL, control; Sm, smoke exposed; St, simvastatin treated; SmSt, smoke exposed ± simvastatin treated.

Figure 3. mRNA expression of VEGF in lung tissue samples from the four groups of rats. mRNA expression of VEGF198 was lower in the Sm group than in the CtL group. The mRNA expression of VEGF198 in the SmSt group was equivalent to that in the CtL group. M, molecular marker; VEGF, vascular endothelial growth factor; Sm, smoke exposed; St, simvastatin treated; SmSt, smoke exposed ± simvastatin treated. M, control.
the Sm group compared with those in the CtL group. These results suggested that the COPD rat model was a successful, appropriate experimental approach to for subsequent investigation. The results of the present study also demonstrated that simvastatin attenuated cigarette smoke-induced inflammatory infiltration, goblet-cell metaplasia and
smooth-muscle proliferation disorders in the airway walls. The results of the present study are in accordance with those of other studies. Murphy et al (29) reported the effects of simvastatin on primary bronchial epithelial cells (PBECs) derived from stable lung allografts, which demonstrated the ability of simvastatin to attenuate airway neutrophilia, remodel mediators and inhibit their upregulation in response to transforming growth factor and interleukin-17. This finding and those of other studies have demonstrated the potential of simvastatin in alleviating neutrophilic airway inflammation and causing lung remodeling (30-32).

Previous studies have identified the presence of VEGF and its receptors in several cell types of a number of organs. It has been reported that the expression levels of VEGF levels in the lungs is the highest among normal tissues (33). The observations that increased expression of VEGF or VEGF signaling causes experimental emphysema and that the lungs of patients with COPD have decreased expression levels of VEGF and VEGF receptor-2 (VEGFR) have led to the suggestion that alveolar maintenance is required for structural preservation of the lungs (34). In the present study, the expression levels of VEGF in lung tissues and BALF samples from the Sm group were lower than in the W group. Simvastatin treatment of rats exposed to cigarette smoke significantly increased the levels of VEGF, almost to the same level as the CtL group (control group). The mRNA expression levels of VEGF were similar in the lung tissues and BALF samples. In the SmSt group, simvastatin increased the expression of VEGF in the AECs compared with the Sm group. These results suggested that simvastatin treatment may prevent the cigarette smoke-induced decrease of VEGF in lung tissue and may upregulate the expression of VEGF in the AECs.

Statins are involved in improving endothelial cell function and promoting angiogenesis. They increase the production of vasodilators, including nitric oxide and VEGF, and decrease the production of vasoconstrictors, including endothelin-1, triggering oxidative stress (35). Takahashi et al (25) demonstrated that the concentration gradient of VEGF between the lungs and the circulatory system increases in response to simvastatin treatment in an emphysema model. BALF consists of an airway epithelial-cell lining fluid (ELF), which was diluted with saline in each rat. The concentration of VEGF in the ELF is suggested to be higher than in the plasma, since the measured BALF concentrations were similar to those in the plasma.

Asahara et al (36) and Brown et al (37) demonstrated that VEGF induces the mobilization of endothelial progenitor cells, and stimulation of resident AECs. In the present study, treatment with 5 mg/kg day⁻¹ simvastatin induced epithelial cell proliferation. Therefore, VEGF may directly and indirectly promote tissue-specific proliferation of AECs. The present study had several limitations. Firstly, only the direct morphological impact of simvastatin on rats was measured, and was not a mechanistic investigation. Another limitation was the use of a dose of 5 mg/kg simvastatin and 16 week time-period only. Therefore, it was not possible to investigate whether there were dose- or time-dependent effects of simvastatin. In addition, no physiological data regarding lung function were assessed and no in vitro data were available. Statins may exert pleiotropic effects in COPD via multiple pathways, and the pathogenesis of COPD is complicated. It would, therefore, be beneficial to investigate the mechanisms underlying statin involvement with COPD, in order to understand their clinical relevance and applicability.

In conclusion, the results of the present study demonstrated that simvastatin exhibits a significant impact on the expression of VEGF and attenuates cigarette smoke-induced emphysema in rats. It was hypothesized that simvastatin, at least in part, may exert beneficial effects in patients with COPD. Further investigation into the mechanisms of statins is required in order to improve the pathophysiology and to alleviate the symptoms of COPD.

Acknowledgements

This study was supported by the National Scientific Foundation of China (grant no. 2007BAI24804).

References


