Adv Ther. 2008;25(4):342–354.
DOI:10.1007/s12325-008-0048-4

ORIGINAL RESEARCH

Budesonide/Formoterol Decreases Expression of Vascular Endothelial Growth Factor (VEGF) and VEGF Receptor 1 Within Airway Remodelling in Asthma

Ke Wang
Chun-Tao Liu
Yong-Hong Wu
Yu-Ling Feng
Department of Respiratory Medicine, West China Hospital, Sichuan University, Chengdu, China

Hong-Li Bai
Department of Radiology Medicine, West China Hospital, Sichuan University, Chengdu, China

ABSTRACT

Introduction: Angiogenesis and microvascular remodelling may play a vital role in the chronic inflammatory process within asthma. One of the most important factors involved in angiogenesis is vascular endothelial growth factor (VEGF). In this study we hypothesised that an increased expression of VEGF may be involved in airway remodelling in asthma patients. To this end, we compared the histology and expression levels of VEGF and one of its receptors ($VEGFR_1$) in bronchial tissues of patients with asthma compared with control patients. We also investigated the effect of treatment with budesonide/formoterol (Symbicort®; AstraZeneca, Lund, Sweden) in the relationship between VEGF and airway remodelling.
Methods: Bronchial tissues were obtained from patients attending the West China Hospital from April to November 2006. Thirteen patients were diagnosed with moderate asthma and 10 others were treated as control. Histological and immunohistochemical comparisons between asthmatic and control patients were made at baseline, and (for asthmatic subjects) following 6 months of treatment with budesonide/formoterol.

Address correspondence to: Chun-Tao Liu, Department of Respiratory Medicine, West China Hospital, West China Medical School, Sichuan University, Chengdu 610041, China. Email: wang2ke@yahoo.com.cn

Results: Compared with control patients, asthmatic patients had significantly decreased respiratory parameters, including forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1) (% predictive). Furthermore, patients with asthma had submucosal gland hyperplasia, increased smooth muscle mass, increased subepithelial fibrosis and neovascularisation. Asthmatic patients also exhibited increased expression of VEGF and $VEGFR_1$ within epithelial cells. The increased expression of VEGF and its receptor correlated well with airway remodelling, airflow obstruction and airway hyper-responsiveness. After treatment with budesonide/formoterol for 6 months, the expression of VEGF and $VEGFR_1$ was decreased, with correlatory decreased airway remodelling in patients with asthma.
Conclusion: The increased expression of VEGF and $VEGFR_1$ in asthmatic patients is accompanied by an increased number and size of blood vessels in asthmatic airways, as well as airway remodelling. Budesonide/formoterol therapy for 6 months can decrease the expression of VEGF and $VEGFR_1$ alongside airway remodelling in asthma. The inhibition of VEGF and its receptor may be a good therapeutic strategy for asthma.

Keywords: airway remodelling; asthma; vascular endothelial growth factor; vascular endothelial growth factor receptor 1

INTRODUCTION

Asthma is a chronic disease characterised by airway inflammation, airway hyper-responsiveness (AHR), reversible airway obstruction and airway remodelling. Airway remodelling refers to airway structural alterations including epithelial shedding, goblet cell and submucosal gland hyperplasia, increased smooth muscle mass, subepithelial fibrosis and neovascularisation.[1] Remodelling of the microvasculature is probably an important contributor to increased inner airway wall thickness, luminal narrowing, and especially AHR in asthma.[2,3] The inflammation and remodelling process are linked to aberrant activation of epithelial-mesenchymal-trophic units.[4] Many cytokines and inflammatory mediators can be involved in different ways[5] such as histamine,[6] bradykinin,[7] leucotrienes,[8] platelet-activating factors,[9] and substances released by autonomic nerves,[10] which can induce vasodilation, as well as some cytokines present in the airways that could determine the formation of new blood vessels.

Angiogenesis and microvascular remodelling are recognised features of asthma and therefore may play a vital role in chronic inflammatory processes.[11,12] Formation of new vessels and remodelling of existing ones are likely to be induced by multiple growth factors. Vascular endothelial growth factor (VEGF) is a potent angiogenic factor whose activities include endothelial cell survival, proliferation and migration.[13] VEGF acts as a pro-inflammatory cytokine by increasing endothelial

permeability and inducing expression of endothelial adhesion molecules that bind leucocytes.[14,15]

Corticosteroids are effective in achieving control of asthma. Corticosteroids reduce airway eosinophilic inflammation and expression of granulocyte macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-4, IL-5, IL-11 and IL-17. It has been reported that corticosteroids could decrease mucus production[16] and smooth muscle cell proliferation[17] in asthmatic patients. Also, beta-2 agonists reduce airway muscular tone and improve expiratory flow. Long-acting inhaled beta-2 agonists (LABA) alone, modestly decrease airway eosinophilia.[18] To be more effective, inhaled corticosteroids (ICS) have been recommended to be combined with LABA. It results in a similar control of the asthma compared with a high-dose ICS.[19] Budesonide/formoterol (Symbicort®; AstraZeneca, Lund, Sweden), a single Turbuhaler® equipped with combined ICS and LABA, is delivered and deposited preferentially in the distal airways. However, it is not clear whether budesonide/formoterol deposition in peripheral airways can influence airway remodelling.

In this study we hypothesised that an increased expression of VEGF may exist and complicate airway remodelling in asthma patients. To this end, we examined the expression levels of VEGF and $VEGFR_1$ in bronchial tissues of patients with asthma to investigate the relationship between VEGF and airway remodelling and the efficiency of budesonide/formoterol on airway remodelling.

MATERIALS AND METHODS

Study Design

A single-centre, open-label study was conducted in adult patients with moderate asthma to evaluate the effect of inhaled budesonide/formoterol on VEGF, $VEGFR_1$ and peripheral lung remodelling. The ethics committee of West China Hospital, Sichuan University approved the study, and all subjects gave written informed consent. Patients and the control subjects who met the inclusion criteria had a baseline bronchoscopy, during which transbronchial and endobronchial lung biopsy specimens were obtained. Patients were then treated with budesonide/formoterol (160/4.5 μg twice daily) for 6 months. After 6 months of treatment, a second airway biopsy specimen was taken. The effect of budesonide/formoterol on airway remodelling was measured by immunocytochemistry and Masson's trichrome stain.

Subjects

Bronchial tissues obtained from fibreoptic bronchoscopy and bronchial biopsy in West China Hospital between April and November 2006 were prepared as previously described by Hoshino and colleagues.[20] Thirteen patients were diagnosed with moderate asthma according to the Global Initiative for Asthma (GINA) 2006 (www.ginasthma.org). All asthmatic subjects had not used any steroids within the preceding 180 days. Asthmatic subjects' inclusion criteria were typical clinical symptoms, a baseline forced expiratory volume in 1 second (FEV_1) between 60%

and 85% of the predicted value (%pred) and a ≥12% increase in FEV_1 after usage of albuterol or a PC_{20} (provocative concentration of methacholine producing a 20% fall in FEV_1) of <8 mg/ml. The lung function tests were administrated according to GINA 2006. Exclusion criteria were chronic bronchitis, cystic fibrosis, bronchiectasis and diffuse panbronchiolitis.

Ten healthy control subjects were recruited from hospital personnel who answered negative to a screening questionnaire for respiratory symptoms, and who met inclusion criteria of FEV_1 values of >80%pred, PC_{20} of >10 mg/ml, and normal findings on simple chest radiograms.

All subjects were non-smokers and had no respiratory tract infections within 2 weeks preceding the operation. The site of biopsy was randomised to either the right or the left lower lobe, and five to six endobronchial biopsy specimens were taken from the tertiary carinae of the right or left lower lobes under direct visualisation.

Tissue Processing and Histological Analysis

To analyse the expression levels of VEGF and $VEGFR_1$, bronchial sections were histologically analysed. The sections were fixed with 4% neutral buffered formaldehyde. Antihuman VEGF and VEGFR1 monoclonal antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and diluted 1:100. Diaminobenzidine (DAB) was used to stain sections.

The samples were de-waxed in xylene and dehydrated in a graded series of ethanol baths. Endogenous peroxidase activity was inhibited by incubating the slides with 3% H_2O_2 for 30 minutes, followed by washes in phosphate buffered saline (PBS) and finally heated for 15 minutes in a microwave (700W) in 10 mM citric acid (pH 6.0). The sections were inactivated by normal goat serum for 20 minutes at 37°C. After repeated washes by PBS, the sections were incubated with MUC5AC antibody and put in a humid chamber at 4°C overnight. Sections were washed in PBS and incubated at 37°C for 30 minutes with biotinylated rabbit anti-goat immunoglobulin, followed by repeated washes in PBS. The tissues were incubated with streptavidin peroxidase reagents for 30 minutes at 37°C, following which fresh DAB was used to stain the sections. After repeated washes in PBS and rinsing in tap water, the slides were counterstained with haematoxylin.

Mucus-secreting submucosal glands were visualised using Alcian blue–periodic acid Schiff (AB-PAS) stain and Masson's trichrome stain was used for assessment of subepithelial fibrosis and hyperplasia of airway smooth muscle. AB-PAS[21] and Masson's trichrome[22] staining were performed following standard protocols.

Image Analysis

All images were generated by Spot Advanced software and analysed by IMAGE-Pro Plus 4.5. Two pathologists blinded to the patients' clinical data reviewed and analysed all the slides. High-power fields of airway epithelium were randomly sampled and calculations were performed on six images per section examined at ×200 to ×600 magnification. The mean of six sections from each tissue was used for sta-

tistical analysis. For immunohistochemistry, the ratios of the optical density of positively stained cell to background were recorded. For AB-PAS staining, the areas of positively stained submucosal glands were also recorded. For Masson's trichrome staining, the areas of smooth muscle mass, thickness of smooth muscle mass, subepithelial fibrosis, quantity and areas of vessels, distance between the epithelial layer and the luminal aspect of the smooth muscle layer and thickness of the reticular basement membrane were recorded.

Statistical Analysis

All data were expressed as the mean±standard error of the mean. Statistical analyses were performed using SPSS 12.0 software. Statistical significance was analysed with one-way analysis of variance (ANOVA), followed by the Student–Newman–Keuls test to isolate significant difference. Chi-square test and Pearson correlation analysis were also used. A P value of less than 0.05 (two-tailed test) was considered statistically significant.

RESULTS

Subjects

The twenty-three patients were in two groups: the study asthma group (n=13) and a control group (n=10). The patients' demographic characteristics are listed in Table 1.

The differences of age and gender between the asthma and control groups were not significant. Forced vital capacity (FVC) %pred, FEV_1 %pred, maximal expiratory flow in 75% vital capacity (V_{max75}), in 50% vital capacity (V_{max50}), in 25% vital capacity (V_{max25}), and PC_{20} were significantly decreased in the asthma group, compared with the control group.

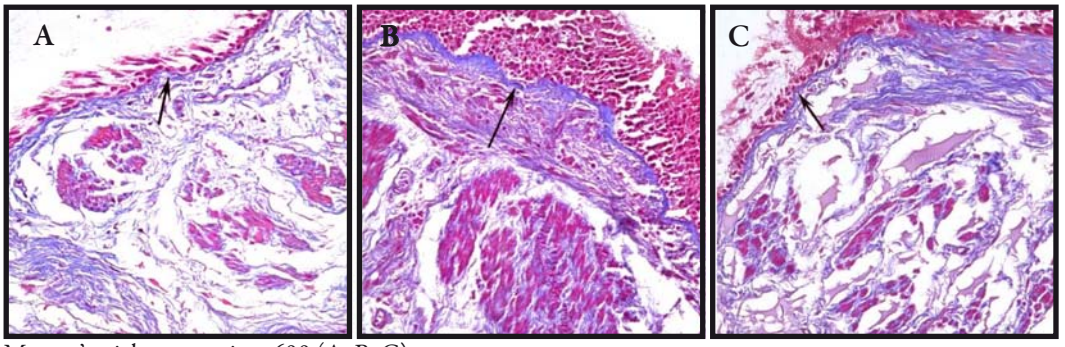
Table 1. Demographic characteristics of study subjects.

	Asthma group pretreatment (n=13)	Asthma group post-treatment (n=13)	Control group (n=10)
Mean age, years (range)	40 (35–43)	40 (35–43)	38 (30–42)
Gender, male/female	8/5	8/5	6/4
FVC, %pred	75.7±3.28†	83.5±4.71†§	95.6±4.21
FEV_1, %pred	68.3±2.86*	78.9±3.34*‡	103.2±6.95
PC_{20}, mg/ml	1.23±0.45*	4.78±0.76*‡	>32
V_{max75}	66.4±12.7*	75.3±9.8*§	102.6±8.62
V_{max50}	53.9±17.1*	72.4±8.2*‡	94.3±10.8
V_{max25}	59.1±18.5*	63.6±11.5*§	96.0±24.5

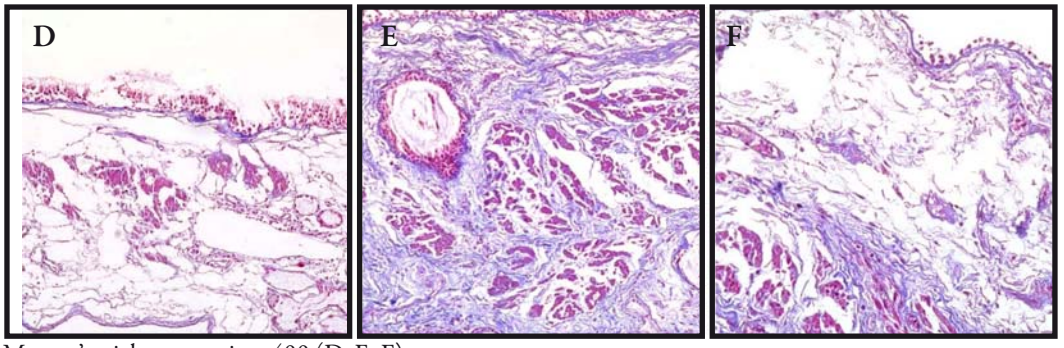
*P<0.01 compared with normal control subjects; †P<0.05 compared with normal control subjects; ‡P<0.01 compared with asthma group subjects pretreatment; §P<0.05 compared with asthma group subjects pretreatment.

FEV_1=forced expiratory volume in 1 second; FVC=forced vital capacity; PC_{20}=provocative concentration of methacholine causing a 20% fall in FEV_1; %pred=percentage of predicted value; $V_{max25–75}$=maximal expiratory flow in 25%–75% vital capacity.

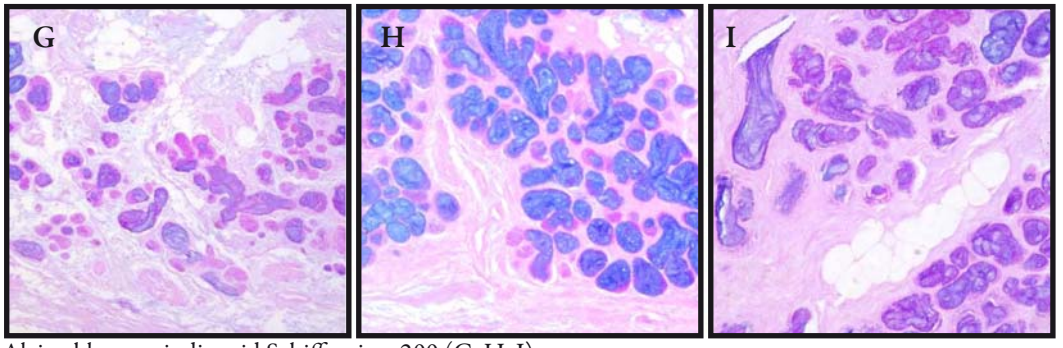
Figure 1. Assessment of airway remodelling. A, D and G were the control group; B, E and H were the asthma group pretreatment; C, F and I were the asthma group after treatment with budesonide/formoterol. With Masson's trichrome stain, the smooth muscle was stained in red colour, and the reticular basement membrane and subepithelial fibrosis were stained in blue colour. A, B and C revealed increased smooth muscle mass and thickness of reticular basement membrane in the asthma group; the arrow indicates the reticular basement membrane. D, E and F revealed increased subepithelial fibrosis. In AB-PAS stain, the sialic acid in the submucosal gland acinar cells stained blue, while polysaccharide and neutral mucin stained purple. H and I revealed submucosal gland hyperplasia in the asthma group.



Masson's trichrome stain ×600 (A, B, C)



Masson's trichrome stain ×400 (D, E, F)



Alcian blue–periodic acid Schiff stain ×200 (G, H, I)

Table 2. Assessment of airway remodelling.

	TTA, mm^2	MSA, mm^2	TASM, mm	Distance, mm	TRBM, μm	SGA, mm^2	Fibrosis, %	VQ	VA, mm^2
Asthma pretreatment	0.81±0.09	0.23±0.04†	0.34±0.02*	0.23±0.02*	22.7±2.1†	0.34±0.05*	19.3±3.3*	6.10±2.23*	0.28±0.03†
Asthma post-treatment	0.86±0.07	0.19±0.03†§	0.29±0.01*§	0.17±0.03*‡	14.5±3.7†‡	0.28±0.04*§	15.8±3.8*‡	5.44±2.38*§	0.23±0.03†‡
Control group	0.84±0.12	0.12±0.02	0.22±0.02	0.12±0.03	10.1±1.9	0.21±0.04	10.1±2.7	2.75±0.62	0.18±0.04

Data are presented as mean±standard error.
*P<0.01 compared with normal control subjects; †P<0.05 compared with normal control subjects; ‡P<0.01 compared with asthma group subjects pretreatment; §P<0.05 compared with asthma group subjects pretreatment.
Distance=distance between the luminal aspect of the smooth muscle layer and the epithelium; fibrosis=circumscribed and measured with image analysis programme and reported as percentage of total thickness of the tissue biopsy; MSA=mean smooth muscle area; SGA=mean submucosal glands area; TASM=thicknesses of smooth muscle mass; TRBM=thickness of reticular basement membrane; TTA=mean total tissue area of biopsy specimens; VA=area of vessels; VQ=quantity of vessels.

Table 3. Correlation analysis.

	MSA, mm^2	Distance, mm	TRBM, μm	SGA, mm^2	Fibrosis, %	VQ	VA, mm^2	FVC, %	FEV_1, %	$V_{max_{75}}$	$V_{max_{50}}$	$V_{max_{25}}$	PC_{20}
VEGFR	0.754*	0.682*	0.812*	0.602*	0.622*	0.437†	0.424†	−0.593*	−0.672*	−0.386†	−0.711*	−0.727*	0.845*
$VEGFR_1$	0.578*	0.694*	0.496†	0.574*	0.519*	0.491†	0.583†	−0.554*	−0.599*	−0.452*	−0.703*	−0.653*	0.615*

*P<0.01; †P<0.05.
Distance=distance between luminal aspect of the smooth muscle layer and epithelium; fibrosis=circumscribed and measured with image analysis programme and reported as percentage of total thickness of the tissue biopsy; FEV_1=forced expiratory volume in 1 second; FVC=forced vital capacity; MSA=mean smooth muscle area; PC_{20}=provocative concentration of methacholine causing a 20% fall in FEV_1; SGA=mean submucosal glands area; TRBM=thickness of reticular basement membrane; VA=area of vessels; $V_{max25-75}$=maximal expiratory flow in 25%–75% vital capacity; VQ=quantity of vessels.

After treatment with budesonide/formoterol, FVC %pred, FEV_1 %pred, V_{max75}, V_{max50} and V_{max25} increased while PC_{20} increased significantly, compared with the pretreatment asthma group.

Assessment of Airway Remodelling

Airway remodelling was assessed by determining the area and integrity of the epithelium, the smooth muscle mass, thickness of the reticular basement membrane, the degree of subepithelium fibrosis, area of the submucosal glands, quantity and areas of vessels, and the distance between the epithelium and airway smooth muscle on bronchial biopsy tissues (Figure 1).

The mean total tissue area of the biopsy specimens obtained in the asthma group did not differ significantly from that of the control group. The patients with asthma had submucosal gland hyperplasia, increased smooth muscle mass, increased thickness of reticular basement membrane, subepithelial fibrosis and neovascularisation, when compared to subjects in the control group. After treatment with budesonide/formoterol, we observed decreased submucosal gland hyperplasia, smooth muscle mass, thickness of reticular basement membrane, neovascularisation and subepithelial fibrosis (Table 2).

Correlation Analysis

Expression levels of VEGF and $VEGFR_1$ correlated well with the degree of the airway remodelling and AHR, and were negatively correlated with the lung function data (Table 3).

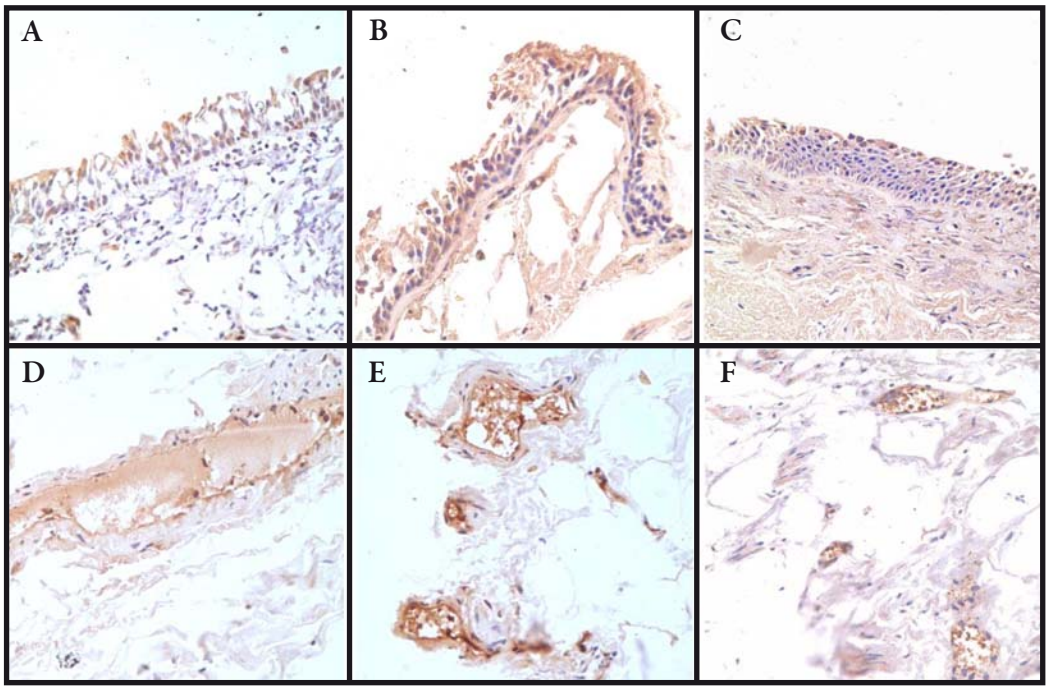
VEGF and $VEGFR_1$ Expression in Airway Epithelial Cells

To examine the correlation between angiogenesis, microvascular remodelling and airway remodelling, immunohistochemical staining for VEGF and $VEGFR_1$ were performed. VEGF was expressed mainly in the airway epithelial cells and secondarily in the smooth muscle cells from patients in all groups. $VEGFR_1$ was expressed mainly in the blood vessel endothelium and secondarily in the airway epithelial cells and smooth muscle cells. The positive signals (brown colour) for both VEGF and $VEGFR_1$ were mainly in the cytoplasm and secondarily on the cell membrane.

The more positively stained airway epithelial cells in the asthma groups showed increased expressions of VEGF and $VEGFR_1$ than the control group (Figure 2). The mean optical densities of VEGF were 1.06±0.02 in the control group, 1.18±0.03 in the asthma group pretreatment and 1.15±0.03 post-treatment. There was a significant increase in the expression of VEGF in the asthma group compared with the control group ($P<0.05$). The mean optical densities of $VEGFR_1$ were 1.49±0.06 in the control group, 2.35±0.05 in the asthma group pretreatment, and 1.83±0.03 in the asthma group post-treatment. There was a significant increase in the expression of $VEGFR_1$ in the asthma group compared with the control group ($P<0.05$).

Budesonide/formoterol treatment decreased the expression of VEGF and $VEGFR_1$ in patients with asthma ($P<0.05$ and $P<0.05$, respectively).

Figure 2. Expression of vascular endothelial growth factor (VEGF) and vascular endothelial growth factor receptor 1 ($VEGFR_1$) in airway epithelial cells (ICH ×400). A, B and C were incubated with VEGF antibody and stained with DAB (brown), while D, E and F were incubated with $VEGFR_1$ antibody and stained with DAB (brown). A and D were the control group; B and E were the asthma group pretreatment; C and F were the asthma group after treatment with budesonide/formoterol. VEGF was expressed mainly in the airway epithelial cells and secondarily in the smooth muscle cells from patients in all groups. $VEGFR_1$ was expressed mainly in the blood vessel endothelium and secondarily in the airway epithelial cells and smooth muscle cells. The asthma groups showed more positively stained airway epithelial cells than the control group. Budesonide/formoterol treatment decreased the expression of VEGF and $VEGFR_1$ in patients with asthma.



DISCUSSION

In this study, we examined the expression levels of VEGF and its receptor, $VEGFR_1$, in bronchial tissues of patients with asthma to investigate the relationship between VEGF and airway remodelling. Increased expressions of VEGF and $VEGFR_1$ were demonstrated in patients with asthma, which can result in mucosal engorgement and oedema, thus contributing to airway inner-wall thickening and subsequent airflow limitation; perhaps especially relevant in acute exacerbation of asthma. Accordingly, submucosal gland hyperplasia, smooth muscle mass, subepithelial fibrosis and neovascularisation increased in those patients. The up-regulated expressions of VEGF and its receptor correlated well with airway remodelling, airflow obstruction and AHR. It indicates VEGF and its receptor may be involved in the procedure of airway remodelling in asthma.

Treatment with ICS/LABA is the recommended maintenance treatment option for adults with persistent asthma. The formoterol component is associated with a rapid onset of bronchodilatory ef-

fect. Budesonide, on the other hand, has a prolonged dwell time in the airway tissues, resulting in a long duration of anti-inflammatory effect and modulating collagen deposition, and the expression of alpha-smooth muscle actin in peripheral airways.[23] Budesonide/formoterol decreased the expression of VEGF and $VEGFR_1$ and therefore decreased airway remodelling in patients with asthma in our study. VEGF is a specific and powerful angiogenic factor that can induce capillary hyper-permeability in vivo. It has been shown to increase fluid permeability from blood vessels when injected intradermally and promote the growth of new blood vessels when administered into healing rabbit bone grafts or rat corneas.[24] Increased vessel numbers, vascular surface area, and exaggerated expression of VEGF were demonstrated in the asthmatic airway in our findings. Increase in the number and size of vessels can contribute to thickening of airway wall, which leads to critical narrowing of bronchial lumen when bronchial smooth muscle contraction occurs.[25] VEGF also increases vascular permeability so that plasma protein, including inflammatory mediators and cells, can leak into the extravascular space to allow the migration of inflammatory cells into the airways.[26,27] This supports the concept that VEGF may indeed be an active participant in the microvascular remodelling process.

$VEGFR_1$ and $VEGFR_2$ are the two main receptors for VEGF signalling in human airways.[21] Both receptors are located on the vascular endothelium, but have divergent functions in vivo. $VEGFR_2$ has been demonstrated to be the active receptor involved in the mediation of major growth and permeability actions of VEGF, whereas $VEGFR_1$ has been postulated to act as a modulating decoy to $VEGFR_2$, thereby inhibiting $VEGFR_2$–VEGF binding.[28] Interestingly, it is the decoy receptor $VEGFR_1$ that has the higher affinity for the VEGF ligand,[29] emphasising its potential strategic importance. In our study, increased expressions of VEGF and $VEGFR_1$ correlated with the increased number and size of vessels in asthmatic airway. The expression of $VEGFR_1$ was seen to be secondarily in the airway epithelial cells and smooth muscle cells and this may indicate the influence of VEGF–$VEGFR_1$ on these cells.

Matrix metalloproteinase-9 (MMP-9) is one of the major proteinases involved in airway inflammation and bronchial remodelling in asthma.[30,31] In addition, MMP-9 induces migration of eosinophils, lymphocytes and neutrophils across basement membranes.[32,33] There is also a close relationship between VEGF and MMP-9 expression in that inhibition of $VEGFR_1$ down-regulates the expression of MMP-9, which suggests that VEGF signalling regulates MMP-9 expression and plays a critical role in initiation and maintenance of asthma.[34] This indicates that VEGF and its receptor are mediators of vascular and extravascular remodelling and inflammation that enhances antigen sensitisation and is crucial in adaptive Th2-cell-mediated inflammation. Therefore inhibition of the VEGF receptors may be a good therapeutic strategy.[35,36]

Some earlier studies have also suggested that airway structural changes are associated with airflow limitation or AHR.[37–39] Together, these findings suggest that airway structural changes can impair respiratory function and aggravate asthmatic symptoms even in patients with

mild asthma. Although inhalation of steroids can inhibit airway inflammation in asthma, whether it can reverse airway structural changes is controversial.[40]

The combination inhaler of budesonide/formoterol has been shown to reduce the expression of VEGF and $VEGFR_1$, submucosal gland hyperplasia, smooth muscle mass, thickness of reticular basement membrane, subepithelial fibrosis and neovascularisation after treating patients with asthma for 6 months. The results from our study suggest that treatment with budesonide/formoterol should be started as soon as diagnosis occurs, which could prevent airway remodelling being aggravated in the early stages of the disease.

A limitation of our study is that we do not have any data about the effects of VEGF receptor inhibitors on airway modelling in patients with asthma. This requires further investigation.

In conclusion, increased expression of VEGF and $VEGFR_1$ are accompanied by increased number and size of vessels in asthmatic airways, as well as airway remodelling. Relationships observed between VEGF and its receptors and vascularity suggest a complex, coordinated control feedback system, even within the remodelling process. Budesonide/formoterol can decrease the expressions of VEGF and $VEGFR_1$ with airway remodelling in asthma. The inhibition of VEGF and its receptor may be a good therapeutic strategy for asthma.

ACKNOWLEDGEMENTS

This project was supported by the National Science Foundation for Postdoctoral Scientists of China (Grant No. 20070411154).

REFERENCES

1. Busse WW, Banks-Schlegel S, Wenzel SE. Pathophysiology of severe asthma. *J Allergy Clin Immunol.* 2000;106:1033–1042.

2. Li X, Wilson JW. Increased vascularity of the bronchial mucosa in mild asthma. *Am J Respir Crit Care Med.* 1997;156:229–233.

3. Orsida BE, Ward C, Li X, et al. Effect of a long-acting β2-agonist over three months on airway wall vascular remodeling in asthma. *Am J Respir Crit Care Med.* 2001;164:117–121.

4. Richter A, Puddicombe SM, Lordan JL, et al. The contribution of interleukin (IL)-4 and IL-13 to the epithelial mesenchymal trophic unit in asthma. *Am J Respir Cell Mol Biol.* 2001;25:385–391.

5. Davies DE, Wicks J, Powell RM, Puddicombe SM, Holgate ST. Airway remodeling in asthma: new insights. *J Allergy Clin Immunol.* 2003;111:215–225.

6. Chediak AD, Elsasser S, Ceste ME, Gazeroglu H, Wanner A. Effect of histamine on tracheal mucosal perfusion, water content and airway smooth muscle in sheep. *Respir Physiol.* 1991;84:231–243.

7. Svensjo E, Persson CG, Rutili G. Inhibition of bradykinin induced macromolecular leakage from post-capillary venules by a β2-adrenoreceptor, terbutaline. *Acta Physiol Scand.* 1977;101:504–506.

8. Laitinen LA, Laitinen A, Widdicombe J. Effects of inflammatory and other mediators on airway vascular beds. *Am Rev Respir Dis.* 1987;135:S67–S70.

9. Bjork J, Smedegard G. Acute microvascular effects of PAF-acether, as studied by intravital microscopy. *Eur J Pharmacol.* 1983;96:87–94.

10. McDonald DM. Neurogenic inflammation in the respiratory tract: actions of sensory nerve mediators on blood vessels and epithelium of airway mucosa. *Am Rev Respir Dis.* 1987;136:S65–S72.

11. Hoshino M, Takahashi M, Aoike N. Expression of vascular endothelial growth factor, basic fibroblast growth factor, and angiogenin immunoreactivity in asthmatic airways and its relationship to angiogenesis. *J Allergy Clin Immunol.* 2001;107:295–301.

12. Thurston G, Murphy TJ, Baluk P, Lindsey JR, McDonald DM. Angiogenesis in mice with chronic airway inflammation: strain-dependent differences. *Am J Pathol.* 1998;152:1099–1112.

13. Ferrara N. Molecular and biological properties of vascular endothelial growth factor. *J Mol Med.* 1999;77:527–543.

14. Detmar M, Brown LF, Schon MP, et al. Increased microvascular density and enhanced leukocyte rolling and adhension in the skin of VEGF transgenic mice. *J Invest Dermatol.* 1998;111:1–6.

15. Melder RJ, Koenig GC, Witwer BP, Safabakhsh N, Munn LL, Jain RK. During angiogenesis, vascular endothelial growth factor and basic fibroblast growth factor regulate natural killer cell adhesion to tumor endothelium. *Nat Med.* 1996;2:992–997.

16. Laitinen LA, Laitinen A. Inhaled corticosteroid treatment for asthma. *Allergy Proc.* 1995;16:63–66.

17. Young PG, Skinner SJ, Black PN. Effects of glucocorticoids and ß-adrenoceptor agonists on the proliferation of airway smooth muscle. *Eur J Pharmacol.* 1995;273:137–143.

18. Li X, Ward C, Thien F, et al. An antiinflammatory effect of salmeterol, a long-acting ß(2) agonist, assessed in airway biopsies and bronchoalveolar lavage in asthma. *Am J Respir Crit Care Med.* 1999;160:1493–1499.

19. Kips JC, O'Connor BJ, Inman MD, et al. A long-term study of the antiinflammatory effect of low-dose budesonide plus formoterol versus high-dose budesonide in asthma. *Am J Respir Crit Care Med.* 2000;161:996–1001.

20. Hoshino M, Nakamura Y, Sim JJ, Shimojo J, Isogai S. Bronchial subepithelial fibrosis and expression of matrix metalloproteinase-9 in asthmatic airway inflammation. *J Allergy Clin Immunol.* 1998;102:783–788.

21. Burgel PR, Montani D, Danel C, et al. A morphometric study of mucins and small airway plugging in cystic fibrosis. *Thorax.* 2007;62:153–161.

22. van den Brűle S, Heymans J, Havaux X, et al. Profibrotic effect of IL-9 overexpression in a model of airway remodeling. *Am J Respir Cell Mol Biol.* 2007;37:202–209.

23. Morice AH, Peterson S, Beckman O, et al. Therapeutic comparison of a new budesonide/formoterol pMDI with budesonide pMDI and budesonide/formoterol DPI in asthma. *Int J Clin Pract.* 2007;61:1874–1883.

24. Connolly DT, Heuvelman DM, Nelson R, et al. Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. *J Clin Invest.* 1989;84:1470–1478.

25. Chetta A, Zanini A, Foresi A, et al. Vascular component of airway remodeling in asthma is reduced by dose of fluticasone. *Am J Respir Crit Care Med.* 2003;167:751–757.

26. Lee YC, Kwak YG, Song CH. Contribution of vascular endothelial growth factor to airway hyperresponsiveness and inflammation in a marine model of toluene diidocyanate-induced asthma. *J Immunol.* 2002;168:3595–3600.

27. Lee CG, Link H, Baluk P, et al. Vascular endothelial growth factor (VEGF) induces remodeling and enhances Th2-mediated sensitization and inflammation in the lung. *Nat Med.* 2004;10:1095–1103.

28. Yancopoulos GD, Davis S, Gale NW, Rudges JS, Wiegand SJ, Holash J. Vascular-specific growth factors and blood vessel formation. *Nature.* 2000;407:242–248.

29. Beck L Jr, D'Amore PA. Vascular development: cellular and molecular regulation. *FASEB J.* 1997;11:365–373.

30. Lee KS, Min KH, Kim SR, et al. Vascular endothelial growth factor modulates matrix metalloproteinase-9 expression in asthma. *Am J Respir Crit Care Med.* 2006;174:161–170.

31. Vignola AM, Riccobono L, Mirabella A, et al. Sputum metalloproteinase-9 tissue inhibitor of metalloproteinase-1 ratio correlates with airflow obstruction in asthma and chronic bronchitis. *Am J Respir Crit Care Med.* 1998;158:1925–1950.

32. Hoshino M, Takahashi M, Takai Y, Sim J. Inhaled corticosteroids decrease subepithelial collagen deposition by modulation of the balance between matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 expression in asthma. *J Allergy Clin Immunol.* 1999;104:356–363.

33. Okada S, Kita H, George TJ, Gleich GJ, Leiferman KM. Migration of eosinophils through basement membrane components in vitro: role of matrix metalloproteinase-9. *Am J Respir Cell Mol Biol.* 1997;17:519–528.

34. Leppert D, Waubant E, Galardy R, Bunnett NW, Hauser SL. T cell gelatinases mediate basement membrane transmigaration in vitro. *J Immunol.* 1995;154:4379–4389.

35. Lee KS, Kim SR, Park HS, Jin GY, Lee YC. Cysteinyl leukotriene receptor antagonist regulates vascular permeability by reducing vascular endothelial growth factor expression. *J Allergy Clin Immunol.* 2004;114:1093–1099.

36. Lee YC, Lee HK. Vascular endothelial growth factor in patients with acute asthma. *J Allergy Clin Immunol.* 2001;107:1106.

37. Boulet LP, Laviolette M, Turcotte H, et al. Bronchial subepithelial fibrosis correlates with airway responsiveness to methacholine. *Chest.* 1997;112:45–52.

38. Beasley R, Roche WR, Roberts JA, Holgate ST. Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis.* 1989;139:806–817.

39. Chetta A, Foresi A, Del Donno M, Bertorelli G, Pesci A, Olivieri D. Airways remodeling is a distinctive feature of asthma and is related to severity of disease. *Chest.* 1997;111:852–857.

40. Leigh R, Pizzichini MM, Morris MM, et al. Stable COPD: predicting benefit from high-dose inhaled corticosteroid treatment. *Eur Respir J.* 2006;27:964–971.