

Inhaled corticosteroids decrease vascularity of the bronchial mucosa in patients with asthma

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Summary

Background Increased vascularity in airway mucosa is a distinctive feature of airway remodelling in asthma. While corticosteroids have proved most effective in modifying airway inflammation, the effect of inhaled corticosteroids on increased airway mucosal vascularity in asthmatics has been little studied.

Objective We examined the effect of inhaled corticosteroid on airway vascularity in bronchial biopsy specimens taken from asthmatic patients.

Subjects and methods We studied bronchial biopsies from 28 asthmatic patients before and after treatment with inhaled beclomethasone dipropionate (BDP) 800 µg/daily, or placebo, for 6 months in a double-blind manner. Biopsy specimens were evaluated for number of vessels and percentage of area occupied by vessels, using computerized image analysis after staining for type IV collagen in vessel walls. Specimens were also examined for extent of collagen III in the subepithelial basement membrane. In addition, we compared asthmatic specimens with biopsy specimens taken from non-asthmatic control subjects.

Results There was a significant increase in number of vessels ($P < 0.01$) and percent vascularity ($P < 0.001$) in the submucosa of asthmatic patients compared with control subjects. After 6 months of treatment, we observed significant improvements in forced expiratory volume in 1 s (FEV₁), FEV₁% and airway responsiveness ($P < 0.05$, each) in the BDP treatment group compared with the placebo group. This was accompanied by significant decreases in both vessel number and percent vascularity in the airways of BDP-treated patients ($P < 0.05$, each). We also observed a significant correlation between change in percent vascularity and change in collagen III thickness in the BDP-treated patients ($r_s = 0.90$, $P < 0.001$). Furthermore, the change in percent vascularity was inversely correlated with both FEV₁ ($r_s = -0.49$, $P < 0.05$) and airway responsiveness ($r_s = -0.36$, $P < 0.05$).

Conclusion These findings suggest that inhaled corticosteroid treatment of asthma reduced airway wall vascularity during airway remodelling.

Keywords: airway inflammation, airway wall remodelling, airway responsiveness, airway vascularity, bronchial asthma, inhaled corticosteroids

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Introduction

Structural alteration of airway walls is an essential feature of asthma, and includes extracellular matrix remodelling, epithelial desquamation [1], goblet cell hyperplasia [2], collagen deposition below the basement membrane [3],

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smooth muscle hypertrophy/hyperplasia [4], and microvascular leakage and vasodilation. It has been long recognized that in fatal asthma, the airway mucosa is oedematous and contains dilated, congested blood vessels [5]. Morphometric studies have confirmed these early observations and have shown that enlarged, congested mucosal blood vessels contribute to airway wall remodeling in asthma [6]. The inflammatory process includes microvascular leakage, which may again be enhanced by structural change [7]. Recently, it was reported that both the number and percentage of vessels in biopsy specimens taken from asthmatic patients were increased compared with control subjects [8]. It is possible that increased vessel numbers and vascular enlargement, and consequent thickening of the airway wall mucosa, may lead to narrowing of the bronchial lumen.

Inhaled corticosteroids are currently the most effective anti-inflammatory medication for the treatment of asthma. Furthermore, circumstantial evidence has linked the use of inhaled corticosteroids with reversal of some of the structural changes associated with asthma [9–11]. Glucocorticoids have anti-angiogenic activity [12,13], and the mechanism of this effect appears to be related to basement membrane turnover, which is determined, in part, by the proteolytic activity associated with proliferating vessels. However, the effect of corticosteroids on increased airway mucosal vascularity observed in asthma has been little studied.

The present study was designed to examine the effects of inhaled corticosteroid treatment on airway vascularity in bronchial biopsy specimens taken from asthmatic subjects. We also examined the relationship between airway vascularity and thickness of subepithelial basement membrane, pulmonary function, and airway responsiveness.

Subjects and methods

Subjects

Twenty-eight non-smoking subjects with asthma were recruited for this study. Asthma was diagnosed according to the criteria of the American Thoracic Society [14]. Asthmatic patients had a clear history of asthma, required intermittent inhaled β_2 -agonist therapy, and had documented reversible airway obstruction (20% improvement in forced expiratory volume in 1 s (FEV₁) either spontaneously or after administration of β_2 -agonist). Atopy was defined as a positive skin prick test (weal > 3 mm in diameter in the presence of positive histamine and negative diluent controls) to one or more of the common aeroallergen extracts. Of the 28 subjects, seven were non-atopic. The duration of asthma ranged from 1 to 21 years. None of the patients received inhaled or oral corticosteroids or any

other anti-inflammatory drugs, such as sodium cromoglycate or nedocromil sodium, in the previous 3 months and inhaled β_2 -agonist was the only bronchodilator used. Nine healthy subjects were used for the control group, and were non-atopic, had never suffered from airway disease, and were all non-smokers. No subjects had any upper or lower respiratory infections for at least 2 weeks before entering the study. The Ethics Committee of Toho University School of Medicine approved this study, and written informed consent was obtained from each subject.

Study design

Seven days after the initial visit, patients returned, having omitted inhaled β_2 -agonist for at least the previous 12 h. Spirometry was performed, and airway methacholine responsiveness measured using an Astograph device (TCK-6000 CV; Chest MI. Co., Tokyo, Japan) according to the method of Takishima *et al.* [15]. Bronchial hyperresponsiveness was evaluated as the minimum cumulative dose ($D_{\min}$) of methacholine for which the reciprocal of respiratory resistance decreased linearly. $D_{\min}$ was calculated so that 1 unit $D_{\min}$ equalled 1 min of inhalation of aerosol solution at 1.0 mg/mL. A previous study reported a significant correlation between bronchial hyperresponsiveness as measured by $D_{\min}$ and values obtained by conventional methods [16]. Subjects returned 7 days later and underwent fiberoptic bronchoscopy. Patients were randomly assigned to one of two parallel treatment groups. Each patient received a 6-month course of either beclomethasone dipropionate (BDP) (Glaxo Wellcome, Tokyo, Japan) 400 μ g administered twice daily by means of a metered-dose inhaler, or a matched placebo. Six months later, patients returned to the Hospital for repeat measurements of spirometric values and airway responsiveness and examination by fiberoptic bronchoscopy.

Fiberoptic bronchoscopy

Premedication was provided by intramuscular injection of 0.5 mg atropine sulphate and 15 mg pentazocine. Throats were anaesthetized with 4% lidocaine spray, and 200 μ g salbutamol (Glaxo Wellcome) inhaled to prevent bronchoconstriction. A bronchoscope (BF type 1T-30; Olympus Co., Tokyo, Japan) was then inserted through the mouth. The pharynx, trachea and bronchi were anaesthetized with 2% lidocaine, and oxygen at 4 L/min administered through nasal cannulae throughout the procedure. Biopsy forceps (FB-15C; Olympus) were used to collect two to three biopsy specimens from the spur of a basal segment bronchus in the right lower lobe and from the subcarina of the right upper lobe. Inhalation of 200 μ g of salbutamol was given after the procedure.

Sample processing

Biopsy specimens were mounted in OCT compound (Miles Scientific, Napierville, IL, USA), frozen rapidly in dry ice and acetone, and stored at -70°C until use. Cryostat serial sections ($4\text{ }\mu\text{m}$) were cut, fixed in acetone, and stained with haematoxylin and eosin for conventional histological examination. Sections were immunostained using the alkaline phosphatase–anti-alkaline phosphatase system (Dako, Glostrup, Denmark) according to the manufacturer's instructions, and developed with the fast red substrate. Finally, sections were rinsed with distilled water, counterstained with Mayer's haematoxylin, dried by brief consecutive dips in ethyl alcohol followed by xylene, and coverslips mounted. Control slides were prepared by substitution with PBS and incubation with an irrelevant mouse mAb of the same isotype and concentration. Blood vessels were highlighted by staining the basement membrane supporting the endothelium for anti-collagen type IV (Monosan, Uden, The Netherlands; dilution 1:20). Subepithelial basement membrane was stained with mAb to detect the thickness of type III collagen deposition (Heyl Vertriebs GmbH & Co., Berlin, Germany; dilution 1:50).

Quantification

To avoid observer bias, all microscopic slides were coded before analysis by one observer and read blindly. A computerized colourimetric image analyser (Win ROOF; Mitani Corp., Fukui, Japan) was used to quantify tissue collagen type IV expression. The entire submucosal area beneath the epithelial basement membrane on the colour monitor was outlined by means of an electronic mouse cursor. Vessel numbers were counted in the submucosal area excluding mucosal glands and smooth muscle, and divided by the submucosal area examined to determine the number of vessels per mm^2 of submucosa. All structures internal to the vessel endothelial basement membrane were evaluated as vascular areas. For each subject, total vascular area was divided by the submucosal area examined to determine percent vascularity. Mean vessel size was estimated by dividing the total vascular area by the total number of vessels for each subject. Subepithelial basement membrane thickness was calculated between each point on the superficial limit and the closest point on the deeper border of specific immunoreactivity in the basement membrane. Repeatability was assessed by measuring thickness at regular intervals of $200\text{ }\mu\text{m}$ along the length of each section. The final result was the mean of the measurements performed on each biopsy specimen.

Statistical analysis

All data are expressed as means (range). The Mann–Whitney *U*-test was used for statistical comparison of

non-parametric variables for asthmatic and control groups, and Student's *t*-test used for normally distributed data. Results for clinical data were normally distributed and analysed using Student's *t*-test for within-group comparisons and the two-sample *t*-test for between asthmatic group comparisons. The number of vessels, percent vascularity, and thickness of subepithelial basement membrane exhibited positively skewed distributions, so were analysed as paired data using the Wilcoxon matched pairs signed rank test. For between-group comparisons, the Mann–Whitney *U*-test was performed on the delta values (before treatment minus after treatment) for the two treatment groups. Changes in vessel number, percent vascularity, and subepithelial basement membrane thickness were also compared by *t*-test, with Turkey-type multiple correction for multiple comparisons. Intra-observer reproducibility was assessed using the coefficient of variation, with the same observer performing at least three replicate measurements of morphometric parameters. The mean coefficient of variation for repeated measurements ranged from 4% to 12% for number of vessels, 3% to 15% for percent vascularity, and 5% to 18% for subepithelial basement membrane thickness. Correlations between individual data were tested for significance using the Spearman rank correlation test. Probability (*P*) values < 0.05 were considered significant.

Results

Fourteen subjects in the BDP group and 14 subjects in the placebo group completed the study. There were no significant differences between the two groups in sex, age, duration of asthma, FEV_1 , or airway responsiveness.

Clinical data

Asthmatic patients had significantly lower FEV_1 , $\text{FEV}_1\%$, and $D_{\min}$ compared with control subjects (Table 1). There were significant improvements in FEV_1 ($P < 0.01$), $\text{FEV}_1\%$ ($P < 0.05$), and $D_{\min}$ ($P < 0.01$) in BDP-treated, but not placebo-treated, patients. Compared with the placebo-treated group, there were significant improvements in FEV_1 , $\text{FEV}_1\%$, and $D_{\min}$ ($P < 0.05$, each) in the BDP-treated group (Table 2).

Bronchial biopsy specimens

When comparing asthmatic and control groups, significant increases were observed both in vessel number (334 mm^{-2} , range $101\text{--}814\text{ mm}^{-2}$, vs. 210 mm^{-2} , range $112\text{--}380\text{ mm}^{-2}$, respectively; $P < 0.01$) and percent vascularity (10.1% , range $2.8\text{--}18.7\%$, vs. 3.4% , range $1.4\text{--}5.7\%$, respectively; $P < 0.001$). There was no significant

Table 1. Subjects' characteristics

	Asthmatic patients (<i>n</i> = 28)	Control subjects (<i>n</i> = 9)
No. (M:F)	17:11	5:4
Age (years)	29 (14–56)	36 (23–49)
FEV ₁ (L)	2.58 (1.34–3.49)*	3.59 (2.78–4.76)
FEV ₁ (% predicted)	71.5 (54.2–85.3)**	95.6 (89.2–102.0)
D _{min} (unit)	1.25 (0.34–3.22)**	>50

Values are mean (range).

D_{min}, Airway hyperresponsiveness as the minimum cumulative unit is equal to 1 min of 1.0 mg/mL aerosol inhalation of methacholine.

P* < 0.01; *P* < 0.001 compared with control subjects.

difference in mean vessel size between asthmatics and controls (309 μm^2 , range 80–669 μm^2 , vs. 240 μm^2 , range 120–356 μm^2 , respectively). At baseline, there were no significant differences between patients who were to receive BDP or placebo treatments in either number of vessels or percent vascularity. After treatment, BDP significantly reduced the number of vessels from 314 mm^{-2} (range 114–560 mm^{-2}) to 254 mm^{-2} (range 93–672 mm^{-2}) (*P* < 0.05). However, the number of vessels remained unchanged after placebo treatment, from 353 mm^{-2} (range 101–814 mm^{-2}) to 354 mm^{-2} (range 183–641 mm^{-2}). The difference between BDP and placebo treatment in number of vessels was statistically significant (*P* < 0.05; Table 3). Within the BDP-treated group there was a significant reduction in percent vascularity from 10.4% (range 2.8–18.7%) to 4.3% (range 2.3–12.9%; *P* < 0.01) from baseline to post-treatment, respectively. Within the placebo-treated group, there was no significant change in percent vascularity from 9.8% (range 3.5–16.0%) to 9.9% (range 3.7–17.0%), from before to after treatment,

respectively. The difference in percent vascularity between the two groups was statistically significant (*P* < 0.05; Table 3).

An example of positive staining for anti-collagen IV in biopsy specimens before and after treatment with BDP, and in control subject is shown in Fig. 1. In the BDP-treated group, a significant correlation was observed between the size of blood vessels and the change in percent vascularity (r_s < 0.74, *P* < 0.01). BDP significantly reduced the thickness of subepithelial collagen III staining, from 11.3 μm (range 5.3–15.2 μm) to 8.4 μm (range 5.2–11.0 μm) (*P* < 0.01). In contrast, staining thickness remained unchanged in the placebo-treated group, from 10.1 μm (range 5.5–15.2 μm) before treatment to 10.2 μm (range 5.5–14.9 μm) after treatment. The difference between BDP and placebo treatments was statistically significant (*P* < 0.01). Moreover, a significant correlation was found between change in subepithelial basement membrane thickness and change in percent vascularity in the BDP-treated group (r_s < 0.90, *P* < 0.001, Fig. 2). No

Table 2. Clinical data before and after treatment with beclomethasone dipropionate (BDP) or placebo

	BDP		Placebo	
	Before	After	Before	After
No. (M:F)	14 (9:5)		14 (8:6)	
Age (years)	29 (17–56)		30 (14–50)	
Duration (years)	6.7 (1–16)		5.5 (1–21)	
FEV ₁ (L)	2.62 (1.34–3.49)	3.15 (1.84–4.06)**§	2.54 (1.92–3.26)	2.48 (1.80–3.29)
FEV ₁ (% predicted)	71.1 (57.9–85.3)	79.5 (69.0–95.1)*§	72.1 (54.2–85.3)	70.1 (52.4–84.0)
D _{min} (unit)	1.38 (0.34–2.29)	2.74 (0.68–5.10)**§	1.12 (0.28–3.22)	1.03 (0.20–2.35)

Values are mean (range).

D_{min}, Airway hyper-responsiveness as the minimum cumulative unit is equal to 1 min of 1.0 mg/mL aerosol inhalation of methacholine.

P* < 0.05; *P* < 0.01 for comparison between values before and after treatment (within group); §*P* < 0.05 for comparison of treatment effects on values (before minus after treatment) for BDP vs. placebo.

Table 3. Effect of inhaled corticosteroid on vascularity of bronchial mucosa in patients with asthma and control subjects

	No.	Total area (mm ²)		Vessels (mm ⁻²)		Vascularity (%)		Vessel size (µm ²)
		Before	After	Before	After	Before	After	Before
Asthma (BDP)	1	1.05	0.95	210	148	11.6	3.4	582.4
	2	0.78	0.77	420	365	7.1	2.9	133.3
	3	0.92	0.93	560	282	17.3	2.5	285.3
	4	0.93	0.76	276	266	16.4	3.6	557.5
	5	1.00	1.05	273	161	7.5	3.9	276.5
	6	0.85	0.89	179	104	7.0	5.2	331.1
	7	0.92	0.87	283	93	18.7	3.1	607.4
	8	0.86	0.80	410	355	3.8	4.1	80.2
	9	0.99	1.00	336	177	13.9	4.5	409.2
	10	0.83	0.85	245	200	8.2	2.3	285.7
	11	0.86	0.51	231	259	3.6	4.3	133.9
	12	0.91	0.81	530	672	14.9	12.9	254.7
	13	0.73	0.78	340	297	12.6	4.6	272.2
	14	0.92	0.93	114	183	2.8	3.4	223.4
	Mean	0.89	0.85	314 [†]	254* [§]	10.4 ^{††}	4.3** [§]	316.6
Asthma (placebo)	1	0.76	0.69	361	310	6.3	3.8	133.2
	2	1.02	1.02	250	267	16.0	16.6	654.5
	3	0.83	0.87	209	283	12.1	14.3	482.1
	4	0.89	1.35	530	486	6.1	3.7	103.5
	5	0.89	0.98	245	263	8.8	9.1	318.7
	6	0.87	1.00	447	336	8.1	8.0	159.7
	7	0.89	0.99	101	183	3.5	5.6	314.0
	8	0.90	0.71	468	359	8.6	7.7	164.9
	9	0.63	0.85	291	375	15.6	16.9	458.6
	10	1.01	1.01	470	599	5.6	6.4	139.5
	11	0.77	0.95	814	641	12.9	7.0	103.9
	12	0.79	0.88	181	223	15.2	17.0	669.8
	13	0.91	0.95	348	352	12.1	15.0	343.8
	14	0.78	0.78	229	279	5.8	7.5	199.1
	Mean	0.85	0.93	353 [†]	354	9.8 ^{††}	9.9	303.2
Control	1	0.94		380		3.2		203.1
	2	0.79		174		3.8		158.3
	3	0.59		146		2.7		296.7
	4	0.85		211		3.1		356.0
	5	0.73		245		5.7		164.4
	6	0.79		130		1.7		332.5
	7	0.89		112		1.4		300.0
	8	0.81		223		4.7		120.5
	9	0.65		276		4.4		231.6
	Mean	0.78		210		3.4		240.0

[†]*P* < 0.01; ^{††}*P* < 0.001: significant difference control subjects and the two other asthmatic patients.

P* < 0.05; *P* < 0.01: for comparison between values before and after treatment (within group).

[§]*P* < 0.05: for comparison of treatment effects on values (before minus after treatment) for beclomethasone dipropionate (BDP) vs. placebo.

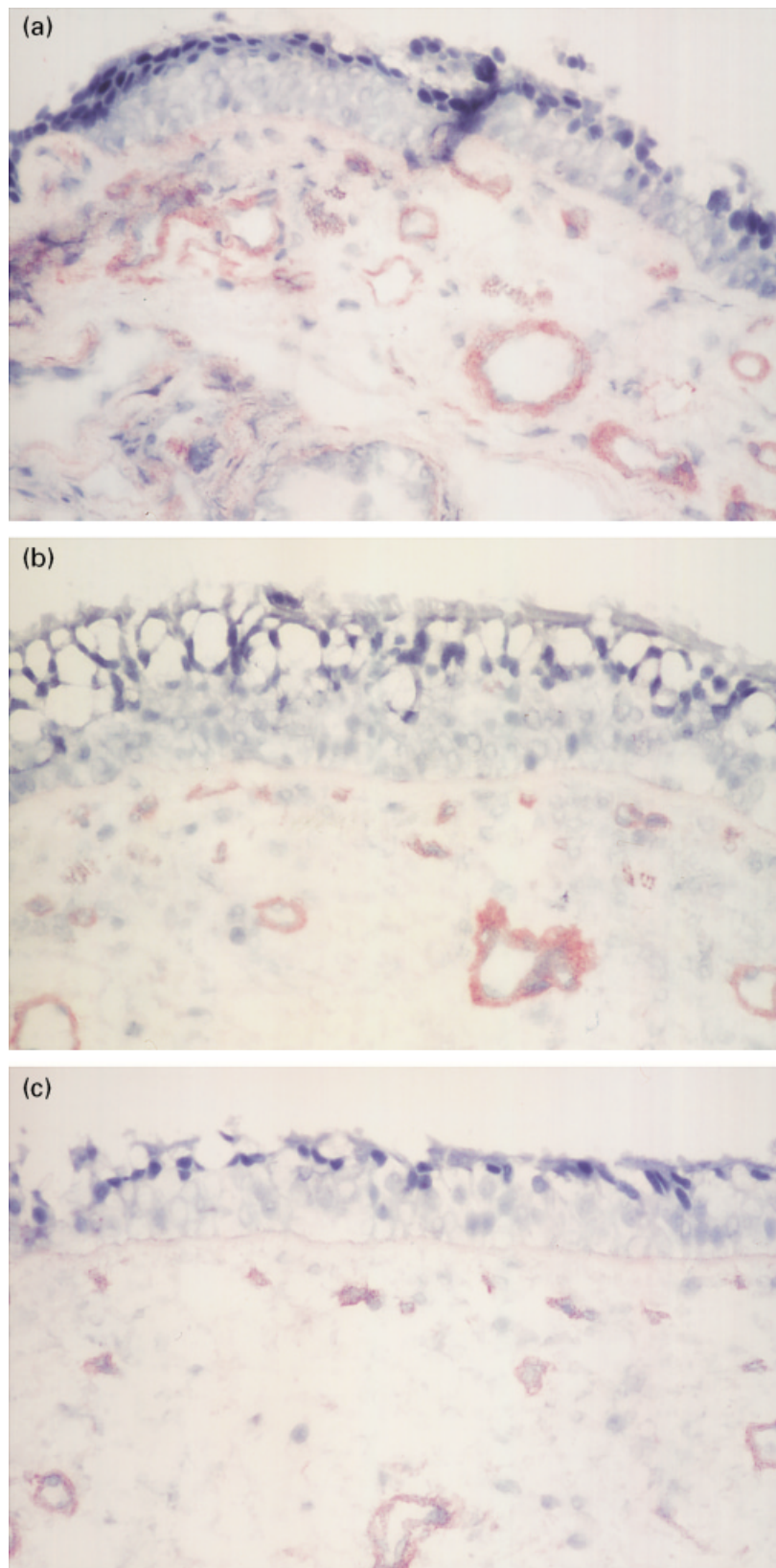


Fig. 1. Bronchial biopsy specimens stained with anti-collagen IV. (a) Vessels before and (b) after treatment with beclomethasone dipropionate (BDP) in patient with asthma (no. 9), and (c) control subject (no. 3).

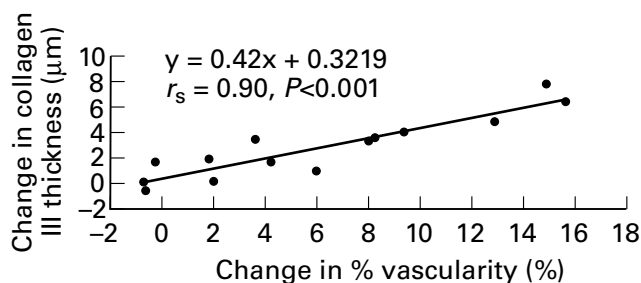


Fig. 2. Correlation between change in subepithelial basement membrane thickness and change in percent vascularity in bronchial mucosa in response to beclomethasone dipropionate (BDP) (●) treatment with asthma.

significant correlation was observed between airway responsiveness and vessel number or percent vascularity in asthmatic patients. However, a significant inverse correlation was observed between change in FEV₁ and percent vascularity ($r_s = -0.49$, $P < 0.05$) in asthmatic patients. In addition, a significant inverse correlation was found between change in $D_{\min}$ and percent vascularity ($r_s = -0.36$, $P < 0.05$, Fig. 3).

Discussion

Our results indicate that inhaled corticosteroid treatment of asthmatic patients significantly reduced bronchial mucosal vascularity. Increased vessel number and increased percent vascularity in asthmatic bronchial mucosa suggest that angiogenesis is a component of the chronic inflammatory response even in mild-to-moderate asthma. Several endothelial-specific immunological markers, such as CD31, CD34 and factor VIII, also showed positive staining for mesenchymal components and immune cells. Although type IV collagen antibody is not an endothelial-specific

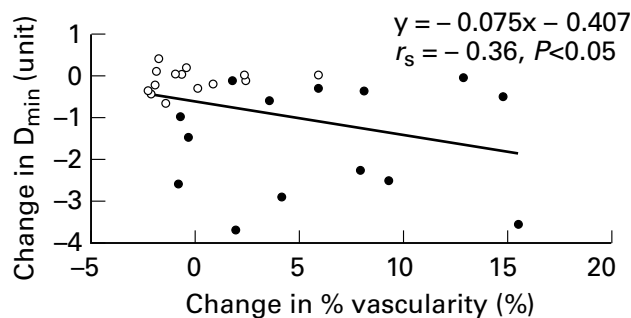


Fig. 3. Correlation between change in airway responsiveness and change in percent vascularity in bronchial mucosa in response to beclomethasone dipropionate (BDP) (●) or placebo (○) treatment with asthma. $D_{\min}$, Airway responsiveness to methacholine.

marker, it is used to define the basal lamina around vessels [17]. Blood vessel numbers recorded in our study were greater than those described by others [6,18]. The higher counts and greater sensitivity in our study may also have been due to our technique, which excluded non-vascular structures. The use of computer image analysis permitted the counting of pixels within vessels as an accurate measurement of submucosal area. The number of blood vessels and percentage of vessel area determined in our study were significantly greater than those reported by Kuwano and colleagues [6]. These differences may be due to the specimens being obtained at post mortem or by surgical resection, as used in the Kuwano study, having smaller airways compared with specimens sampled by bronchoscopy, as in the present study. The use of β_2 -agonist in asthma is associated with vascular dilatation of large-airway vessels, such as observed in canine studies [19]. The asthmatic subjects in our study were premedicated with salbutamol prior to bronchoscopy. Although unlikely to be a major factor, it is possible that premedication may have contributed to airway-vessel dilatation in asthmatic subjects. However, salbutamol would not lead to increased vessel numbers, and there were no significant correlations between vessel number or percent vascularity in bronchial mucosa and airway responsiveness in asthmatic patients. Orcida *et al.* [20] reported finding a significant inverse correlation between the number of blood vessels in bronchial mucosa and airway responsiveness in asthmatic patients, but noted that the percentage of area occupied by vessels did not correlate with airway responsiveness. The reason for this discrepancy from our findings may be that factors other than increased vascularity (e.g. degree of epithelial shedding and infiltration of inflammatory cells, basement membrane thickness and smooth muscle hypertrophy/hyperplasia) may also affect airway responsiveness.

The pathophysiological significance of increased vascularity in asthma is unclear. A model of airway wall thickening proposed by Moreno *et al.* [21] suggested that the small increase in airway wall area caused by oedema or vascular engorgement might account for the increased airway responsiveness seen upon bronchial provocation. Mitzner and colleagues [22] have used a model of blood engorgement of canine airways *in vivo* to demonstrate a correlation between wall thickening and luminal narrowing. Furthermore, in normal subjects a rapid intravenous infusion of fluid heightened responsiveness to methacholine to within the asthmatic range [23]. Thus, the indirect effect of oedema associated with vessel proliferation may contribute to both the airway narrowing and subepithelial injury typical of asthma.

Our study provides evidence that BDP reduced both vessel number and percent vascularity of asthmatic airways

compared with a placebo. While biopsy specimens taken from the same area might affect the vascularity of the second biopsy due to neovascularization, second biopsies were undertaken after a period of 6 months. In addition, neovascularization would tend to reduce the effect of BDP and increase the number of vessels in the placebo group. In the BDP-treated group, a significant correlation was observed between blood vessel size and change in percent vascularity. The decreasing effect of corticosteroid on vascularity appeared to be more dependent on the level of engorgement or dilatation of large blood vessels rather than small vessels. This effect may be based on the finding that more large vessels ($> 300 \mu\text{m}^2$) have been identified in asthmatics than in normal subjects [8]. As a significant correlation was also found between change in subepithelial basement membrane thickness and change in vascularity, we demonstrated that inhaled corticosteroid decreased not only the thickness of the subepithelium but also the vascularity of asthmatic airways. Some of the cytokines and growth factors [24] associated with airway inflammation play important roles in airway remodelling and may be responsible for the increased number and percentage of vessels in asthmatic airways. Vascular endothelial growth factor (VEGF) is known to be a potent inducer of angiogenesis and vascular hyperpermeability [25,26]. Also, tumour necrosis factor- α stimulation of angiogenesis may occur by some indirect mechanism, such as by stimulating other cells to produce angiogenic factors [27]. In the presence of corticosteroids, platelet-activating factor- and platelet-derived growth factor-induced transcription of VEGF mRNA and the secretion of VEGF protein were down-regulated [28]. This inhibitory effect of corticosteroids on VEGF expression may explain the anti-angiogenic effects of corticosteroids at a molecular level. However, further study is needed to determine which angiogenic factors are inhibited by corticosteroids.

The use of BDP resulted in marked clinical improvement as measured by FEV_1 and airway responsiveness, a finding consistent with the recognized efficacy of inhaled corticosteroids for asthma [29,30]. Our results suggest that the effect of inhaled corticosteroid on vascularity may ameliorate both the airway narrowing and increased airway responsiveness observed in asthma. Interestingly, a significant inverse correlation was found between change in percent vascularity of airways, before and after treatment with BDP, and FEV_1 and methacholine airway sensitivity. However, we observed no relationship between change in vessel number and airway sensitivity. One explanation for this finding may be that corticosteroids reduce vascular enlargement rather than vessel numbers.

In conclusion, we have demonstrated that inhaled

corticosteroid reduced both vessel number and percentage of vascular area in the bronchial mucosa of asthmatic patients. Furthermore, change in percent vascularity before and after treatment with BDP was correlated with FEV_1 and airway responsiveness. These findings suggest that the increased vascularity in asthmatic airways may play an important role in the pathophysiology of asthma, and that the ability of corticosteroids to decrease vascularity may explain, in part, their therapeutic effects in asthma.

References

- 1 Laitinen LA, Heino M, Laitinen A, Kava T, Haahtela T. Damage of the airway epithelium and bronchial reactivity in patients with asthma. *Am Rev Respir Dis* 1985; 131:599–606.
- 2 Jeffery PK, Wardlaw AJ, Nelson FC, Collins JV, Kay AB. Bronchial biopsies in asthma. An ultrastructural, quantitative study and correlation with hyperreactivity. *Am Rev Respir Dis* 1989; 140:1745–53.
- 3 Roche WR, Beasley R, Williams J, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1989; 1:520–4.
- 4 Ebina M, Takahashi T, Chiba T, Motomiya M. Cellular hypertrophy and hyperplasia of airway smooth muscles underlying bronchial asthma. *Am Rev Respir Dis* 1993; 148:720–6.
- 5 Dunnill MS. The pathology of asthma with special reference to changes in the bronchial mucosa. *J Clin Pathol* 1960; 13:27–33.
- 6 Kuwano K, Bosken CH, Pare PD, Bai TR, Wiggs BR, Hogg JC. Small airways dimensions in asthma and in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1993; 148:1220–5.
- 7 McLeish KR, Miller FN, Stelzer GT, Wellhausen SR. Mechanism by which methylprednisolone inhibits acute immune complex-induced changes in vascular permeability. *Inflammation* 1986; 10:321–32.
- 8 Li X, Wilson JW. Increased vascularity of the bronchial mucosa in mild asthma. *Am J Respir Crit Care Med* 1997; 156:229–33.
- 9 Trigg CJ, Manolitsas ND, Wang J et al. Placebo-controlled immunopathologic study of four months of inhaled corticosteroids in asthma. *Am J Respir Crit Care Med* 1994; 150:17–22.
- 10 Olivieri D, Chetta A, Del Donno M et al. Effect of short-term treatment with low-dose inhaled fluticasone propionate on airway inflammation and remodeling in mild asthma: a placebo-controlled study. *Am J Respir Crit Care Med* 1997; 155:1864–71.
- 11 Hoshino M, Nakamura Y, Sim JJ et al. Inhaled corticosteroid reduced lamina reticularis of the basement membrane by modulation of insulin-like growth factor (IGF)-I expression in bronchial asthma. *Clin Exp Allergy* 1998; 28:568–77.
- 12 Folkman J, Langer R, Linhardt RJ, Haudenschild C, Taylor S. Angiogenesis inhibition and tumor regression caused by

- heparin or a heparin fragment in the presence of cortisone. *Science* 1983; 221:719–25.
- 13 Longenecker JP, Kilty LA, Johnson LK. Glucocorticoid influence on growth of vascular wall cells in culture. *J Cell Physiol* 1982; 113:197–202.
- 14 American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987; 136:225–44.
- 15 Takishima T, Hida W, Sasaki H, Suzuki S, Sasaki T. Direct-writing recorder of the dose–response curves of the airway to methacholine. *Chest* 1981; 80:600–6.
- 16 Ishii M, Hida W, Suzuki S, Ichinose M, Sasaki H, Takishima T. Comparison of intermittent and continuous inhalation provocation tests. *Ann Allergy* 1989; 62:223–8.
- 17 Ordonez NG. Application of immunocytochemistry in the diagnosis of soft tissue sarcomas: a review and update. *Adv Anat Pathol* 1998; 2:67–85.
- 18 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; 139:806–17.
- 19 Laitinen LA, Laitinen MA, Widdicombe JG. Dose-related effects of pharmacological mediators on tracheal vascular resistance in dogs. *Br J Pharmacol* 1987; 92:703–9.
- 20 Orsida BE, Li X, Hickey B, Thien F, Wilson JW, Walters EH. Vascularity in asthmatic airways: relation to inhaled steroid dose. *Thorax* 1999; 54:289–95.
- 21 Moreno RH, Hogg JC, Pare PD. Mechanics of airway narrowing. *Am Rev Respir Dis* 1986; 133:1171–80.
- 22 Mitzner W, Wagner E, Brown RH. Is asthma a vascular disorder? *Chest* 1995; 107:97S–102S.
- 23 Rolla G, Scappaticci E, Baldi E, Bucca C. Methacholine inhalation challenge after rapid saline infusion in healthy subjects. *Respiration* 1986; 50:18–22.
- 24 D'Amore P. Mechanisms of endothelial cell growth control. *Am J Respir Cell Mol Biol* 1992; 6:1–8.
- 25 Leung DW, Cachianes G, Kuang W-J, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* 1989; 246:1306–9.
- 26 Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. *Am J Pathol* 1995; 146:1029–39.
- 27 Leibovich SJ, Polverini PJ, Shepard HM, Wiseman DM, Shirely V, Nuseir N. Macrophage-induced angiogenesis is mediated by tumor necrosis factor- α . *Nature* 1987; 329:630–2.
- 28 Nauck M, Roth M, Tamm M et al. Induction of vascular endothelial growth factor by platelet-activating factor and platelet-derived growth factor is downregulated by corticosteroids. *Am J Respir Cell Mol Biol* 1997; 16:398–406.
- 29 Juniper EF, Kline PA, Vanzieleghem MA, Ramsdale EH, O'Byrne PM, Hargreave FE. Effect of long-term treatment with an inhaled corticosteroid (budesonide) on airway hyperresponsiveness and clinical asthma in nonsteroid-dependent asthmatics. *Am Rev Respir Dis* 1990; 142:832–6.
- 30 Haahtela T, Jarvinen M, Kava T et al. Comparison of a β_2 -agonist, terbutaline, with an inhaled corticosteroid, budesonide, in newly detected asthma. *N Eng J Med* 1991; 325:388–92.