

Fiberoptic bronchoscopy and bronchial mucosal biopsies in asthmatics undergoing long-term high-dose budesonide aerosol treatment

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Mucosal biopsies from the pharynx, right main stem bronchus and right lower lobe were obtained during flexible fiberoptic bronchoscopy and were examined with light microscopy (LM) and electron microscopy (EM) in 10 asthmatics after 11 months' (range 7-15 months) treatment with high doses of inhaled budesonide via the Nebuhaler®, i.e. 1600 µg daily. Results were compared with biopsies from 10 controls suspected of having focal, malignant lung diseases. Visual inspection of the tracheobronchial tree showed no signs of atrophy, ulcerations or thrush patches, and LM and EM showed no specific signs of mucosa and connective tissue atrophy; however, epithelial desquamation was seen in the asthmatics. No complications were observed.

Key words: asthma; bronchial mucosal biopsies; high-dose budesonide.

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Glucocorticoids cause atrophy when repeatedly applied to the skin (4) and this has caused considerable concern when corticosteroids were introduced for long-term inhalation treatment of severe asthmatics. However, earlier studies have shown no evidence of epithelial damage to the airways even after 3 years of treatment with conventional doses of beclomethasone dipropionate (9, 10, 12).

Inhaled budesonide in high doses (1600 µg/day) applied via the Nebuhaler® is an effective antiasthmatic treatment (6), and this study was performed in order to investigate whether long-term treatment with this new steroid caused bronchial epithelial atrophy.

Bronchial washings and culturing of swabs taken from the back of the pharynx were performed to evaluate whether high-dose inhaled budesonide treatment produced increased frequency of bacterial or fungal infection in the airways. Earlier studies with beclomethasone dipropionate in conventional doses have failed

to show increased incidence of infection. However, oropharyngeal candidiasis is seen in about 5% of patients treated with inhaled steroids (8).

PATIENT AND METHODS

Ten steroid-dependent asthmatics (5 females and 5 males) were selected consecutively from a group of 45 out-patients undergoing long-term treatment with high daily doses of inhaled budesonide (800 µg b.i.d.) via the 750 ml Nebuhaler. Patient data are given in Table 1. The control group consisted of 10 patients (3 females and 7 males) suspected of malignant lung diseases, where fiberoptic bronchoscopy was indicated for diagnostic purposes (Table 1).

We were not able to match groups according to age, partly for ethical reasons. Controls were not suffering from disseminated lung diseases or bronchial obstruction, nor were they taking oral steroids (Table 1). None of the

subjects in the two groups suffered from respiratory tract infections within the 4 weeks prior to bronchoscopy. The patients gave their informed consent to participate, and the study was approved by the local Ethics Review Committee of Copenhagen.

Flexible fiberoptic bronchoscopy was performed by an experienced bronchoscopist. The asthmatic patients were all sedated with 10 mg benzodiazepine (Stesolid®) 1 h prior to bronchoscopy, and oropharynx was anaesthetized with lidocain aerosol and 4% lidocain swabs. A flexible fiberoptic bronchoscope (Olympus BF-B3R) with an outer diameter of 5.3–5.9 mm was guided transnasally, and lidocain 0.5 ml of a 4% solution was applied through the bronchoscope at the vocal cords, larynx, intertracheal and carina level. Specially designed biopsy forceps were used to obtain the best preservation of specimens (2) resulting in sections with an area of 1.7 mm² for histological examination.

Throughout the entire procedure patients were kept fully informed of how the examination was proceeding so as to avoid anxiety. Immediately before biopsy bronchial washings from the right mainstem using 5 ml isotonic sodium chloride solution were performed. The bronchial washings were later cultured for bacteria and fungi. Two biopsies were obtained from the bronchus at the lower lobe, from the right stem bronchus and the pharynx. Biopsies for LM were kept in a formaldehyde 4% solu-

Table 2

Cultures for fungi and bacterias from swabs taken from pharynx and bronchial washings in asthmatic and control patients

Asthmatics: Patient No.	Pharynx	Bronchial washings
1	–	–
2	<i>C. albicans</i>	<i>Staph. aureus</i> <i>Pneumococ</i> <i>C. albicans</i>
3	–	–
4	–	–
5	–	<i>Alpha streptococ</i> <i>Staph. albus</i>
6	<i>C. albicans</i>	<i>Staph. aureus</i>
7	<i>E. coli</i>	<i>E. coli</i>
8	<i>C. albicans</i>	<i>C. albicans</i>
9	–	–
10	<i>C. albicans</i>	<i>Staph. aureus</i> <i>C. albicans</i>
Controls:		
1	–	–
2	–	–
3	–	–
4	<i>E. coli</i>	–
5	–	–
6	<i>Klebs. pneumoniae</i> <i>Staph. aureus</i> <i>C. albicans</i>	<i>Staph. albus</i>
7	–	–
8	–	<i>Klebs. pneumoniae</i>
9	–	–
10	<i>Klebs. pneumoniae</i>	<i>H. influenzae</i> <i>C. albicans</i> <i>Neisseria art.</i>

Table 1

Patient data; mean (range)

	Asthmatics (n = 10)	Control (n = 10)
Male	5	7
Female	5	3
Age (years)	36 (17–45)	60 (17–76)
Asthma duration (years)	18 (3–40)	–
Oral prednisone (years)	8 (3–18)	–
Budesonide treatment (months)	11 (7–15)	–
Smokers	3	7
Ex-smokers	3	0
Non-smokerrrs	4	3
Prednisone intake mg/day	5.5 (0–10)	0

Table 3

Light microscopy of biopsies from the right stem bronchus

Histopathological changes	Number of patients	
	Controls	Asthmatics
Metaplasia	1	2
Ulceration	0	0
Dysplasia	0	0
Eosinophils	1	0
Mast cells	0	0
Chronic inflammation	1	3
Fibrosis	1	3
Atrophy	0	0

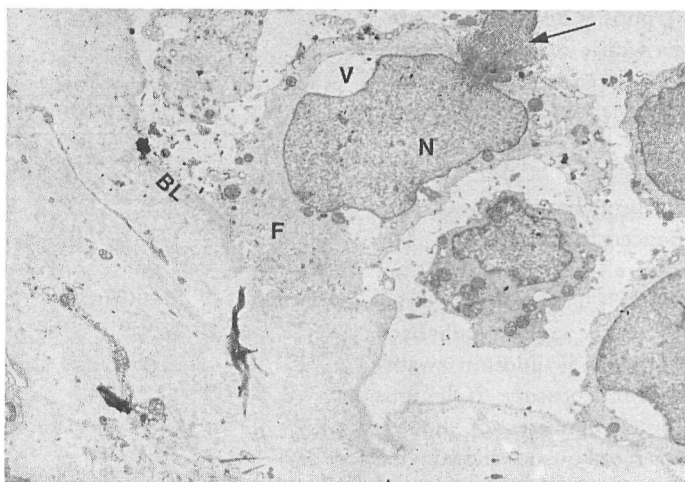


Fig. 1. Epithelial cells of small bronchus. Epithelial cells show filamentous cytoplasm (F) with perinuclear vacuoles (V). Nuclei (N) show coarse granules of chromatin without perinuclear condensation. An epithelial cell shows cytolysis with partly extruded nucleus (arrow). The epithelial cells are released from basal lamina (BL). $\times 3,700$.

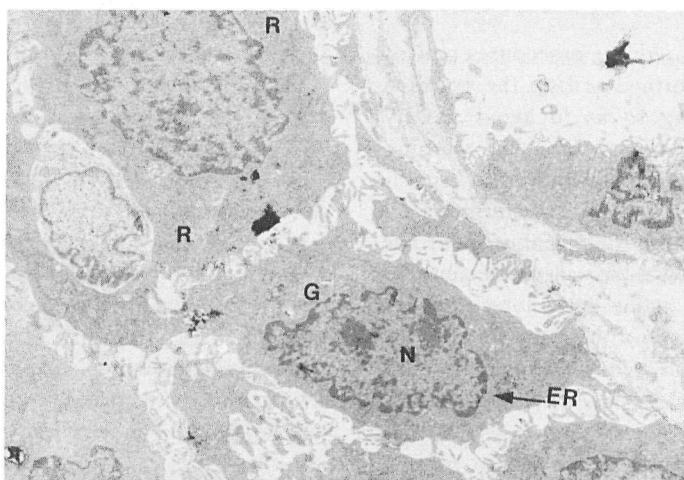


Fig. 2. Epithelial cells of small bronchus. The epithelial cells show cellular structures indicating active cell growth, i.e. large Golgi apparatus (G), numerous ribosomes (R) and granular endoplasmic reticula (ER), together with large chromatin-rich nuclei (N). $\times 3,700$.

tion and stained with haematoxylin and eosin. Biopsies for EM were kept in glutaraldehyde 2% before incubation in epon. Sections were routinely prepared. Biopsies from asthmatics and controls were randomized before LM and EM examination and the code was broken after evaluation by the pathologist.

RESULTS

Bronchoscopy. Direct visual inspection of the tracheobronchial tree with the flexible fiberoptic bronchoscope revealed no specific signs of ulceration, bleeding, atrophy or thrush patches in either the asthmatic group or the controls.

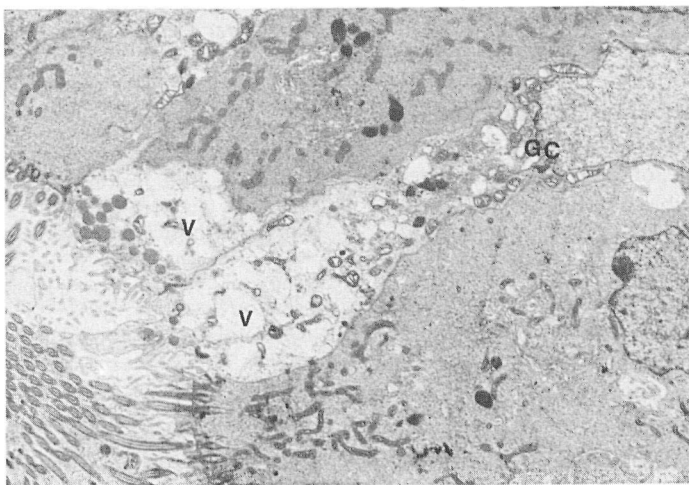


Fig. 3. Ciliated epithelium shows well-preserved ciliar epithelial cells and goblet cells (GC). The granules of the goblet cells are seen as empty cavities (V). $\times 4,700$.

No complications, such as vagally mediated laryngospasms or bronchospasms, were seen. No patient experienced haemorrhage.

Bronchial washings. Five asthmatics and three controls harboured bacteria and three asthmatics and one control harboured *Candida albicans* in the oropharynx (Table 2).

Light microscopy. Evaluation of sections from biopsies taken from the pharynx, the right main stem bronchus, and the right lower lobe showed no specific differences between the two groups, and it is noteworthy that although there was identity between histopathological findings in the two groups, the number and quality of these findings could not be classified as abnormal (Table 3). Among other things the number of goblet cells was equal in biopsies from the two groups of patients.

Electron microscopy. In the budesonide-treated asthma patients the epithelial cells were either undergoing active proliferation or desquamation indicating an increased epithelial cell turnover (Figs. 1, 2). Furthermore, goblet cells contained secretory granules with sparse contents (Fig. 3). Connective tissue appeared oedematous with a few infiltrating cells, i.e.

lymphocytes, macrophages, and, occasionally, neutrophilic granulocytes, some showing cytolysis. Vessels were small and dilated, and vascular endothelial cells showed vacuolated cisterna of granular endoplasmic reticula with a few ribosomes. Collagen fibrils appeared normally distributed, except in one asthma patient with fibrosis where numerous thin collagen fibrils were seen.

DISCUSSION

We found it of importance to evaluate bronchial mucosal biopsies in patients undergoing long-term treatment with inhaled budesonide in high doses, because this treatment has shown good anti-asthmatic effects (6), and documentation of possible airway epithelium damage produced by the high-dose treatment is lacking. We refrained from a study design where mucosal biopsies were obtained before and after long-term treatment with budesonide using the patients as their own controls for ethical reasons – bronchoscopy is a potentially hazardous procedure especially in asthmatics (3). For the same reasons it was impossible to match the two groups according to age or smoking habits or to obtain biopsies from a group of healthy volunteers. Many studies evaluating the effects on

airway epithelium produced by inhaled steroids have failed to show airway epithelial damage, but in all studies the dose of inhaled steroid – mainly beclomethasone dipropionate – has been much lower than in our study.

Visual inspection of the respiratory tract mucosa using a flexible fiberoptic bronchoscope did not reveal any abnormalities, e.g. atrophy, and no significant differences in LM histopathology between the two groups of patients, while the EM findings indicated some non-specified differences between asthmatic patients and controls. Further studies using the EM technique are needed to determine the exact origin of these cellular changes, which from a theoretical point of view could be caused either by asthma, long-term oral steroid therapy or by topical budesonide therapy. Laitinen et al. (5) have described similar airway epithelial changes in asthmatics, which strengthens the view that various degrees of epithelial damage of the airways are present in asthma. The changes in the goblet cells may imply steroid influences on the production of secretory granules (7).

Bronchial washings showed that a variation of bacteria and *Candida albicans* may be present in the major airways without causing clinical signs of infection. The oropharyngeal bacterial and fungal flora is often similar to the flora found in the bronchial tree, indicating contamination of the bronchial washings (1). More asthmatic patients harboured *Candida albicans* in the pharynx compared with the controls; however, this is a well-known phenomenon in patients treated with inhaled steroids (8).

Our results support those from others (9, 10, 12): that there is no evidence of bronchial mucosal atrophy caused by inhaled steroids even when used in high doses. The reason for this could be that the doses of inhaled steroid are deposited over a large area in contrast to local application of steroids to the skin, and that lung fibroblasts might be less sensitive to steroids than skin fibroblasts (11).

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