

Bronchial mucosal structure after histamine inhalation

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Previous studies have shown an increased number of inflammatory cells and an increased level of hyaluronan in the bronchoalveolar lavage (BAL) fluid, 24 h after the inhalation of histamine. In the present report, the influence of histamine inhalation on the bronchial mucosa was, therefore, investigated in 20 subjects. Scanning electron microscopy (SEM) revealed that small areas of the mucosal surface were altered or lacked cilia more frequently in the bronchial biopsies taken 24 h after the inhalation of histamine than in the control biopsies. In contrast, light and transmission electron microscopy revealed no increase in epithelial damage and no changes in the subepithelial morphology. The results indicate that inhalation of histamine does not significantly alter the structure of the bronchial mucosa, which means that bronchial biopsies can be taken for routine morphological examination within 24 h after a histamine test. When using the biopsies in research, one should consider the possible influence of the histamine test.

Clinical aspects

The histamine test is widely used in clinical practice to diagnose bronchial hyperresponsiveness. The results indicate that inhalation of histamine does not significantly alter the structure of the bronchial mucosa, which means that bronchial biopsies can be taken for routine morphological examination within 24 h after a histamine test.

Key words: asthma; bronchial epithelium; bronchoalveolar lavage; bronchial mucosa; bronchial responsiveness; challenge test; histamine.

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Histamine can evoke bronchial constriction and asthma (4) and is often used to test bronchial responsiveness (4). In an earlier investigation we noted an increase in the number of mast cells and lymphocytes in the bronchoalveolar lavage (BAL) fluid from healthy subjects 24 h after the inhalation of histamine (21). Consequently, it is advisable not to perform a BAL too soon after a histamine test. However, bronchial biopsies without a BAL can also be part of an investigation that includes assessment of bronchial responsiveness. Therefore, it was of interest to determine whether a histamine test prior to the bronchoscopy influenced the morphology of the bronchial mucosa in bronchial biopsies.

Subjects and methods

Twenty healthy, non-smoking subjects, 13 men and 7 women with a median age of 33 years (quartiles 29–40 years), were investigated with a fiber-optic bronchoscopy 24 h after a histamine test. They all had a vital capacity (VC) and forced expiratory vol-

ume in 1 s (FEV₁) of at least 80% of predicted normal. None of them had a history of asthma or other lung disease. They had not had any infection for at least 5 weeks prior to the examination. The subjects were examined after their informed consent, and the study was approved by the ethics committee of the University of Umeå. The biopsies were compared with those of healthy volunteers analyzed in the same way in a previous study (23). To this control material were added six biopsies, analyzed by SEM, from another four healthy volunteers with a median age of 31 years.

Histamine exposure

The subjects were exposed to histamine according to the histamine test by Hargreave et al. and Juniper et al. (8, 11). The subjects were exposed to 8 mg/ml (1 subject), 16 mg/ml (16 subjects), or 32 mg/ml (3 subjects) of phosphate-buffered histamine-dihydrochloride or histamine-phosphate. The pH of the solutions were 6.80, 6.45, and 6.07, respectively. The

histamine aerosol was generated by a Wright nebulizer (Aerosol Products Ltd, London, UK), with an output of 0.13 ml/min. The aerosol was inhaled by tidal breathing for 2 min through a mask held loosely over the mouth. The FEV₁ was measured after each inhalation. The VC and FEV₁ were measured by a vitalograph (Vitalograph Ltd).

Flexible fiber-optic bronchoscopy

Bronchoscopy was performed after premedication with 0.5–1.0 ml of morphine-scopolamine (10 mg/ml + 0.4 mg/ml) or 0.375 mg atropine and a sedative, or only with 0.5–0.75 mg of atropine. All examinations were performed under topical anesthesia of up to 270 mg of lidocaine. One to six bronchial mucosal biopsies were taken by a flexible fiber-optic bronchoscope (Olympus BF 1T10, BF 1T20, or BFXT20) with a flexible forceps (Olympus FB20C, FB21C, or FB22C) from the middle lobe or from the right or left upper or lower lobe bronchus. The biopsies were taken from second- or third-order bronchi by three trained bronchoscopists who avoided touching the bronchial mucosa with the bronchoscope before taking the biopsies in order to avoid mechanical damage to the epithelium.

Light microscopy and transmission electron microscopy

The biopsies were collected into saline and then transferred into 2.5% glutaraldehyde within 15 min. After immersion in the fixative for at least 24 h, the specimens were rinsed in a 0.1 mol/l solution of sodium cacodylate buffer (pH 7.2) for 30 min and then postfixed overnight at 4°C in 1% osmiumtetroxide in the same buffer. After another rinse, the specimens were dehydrated in a graded series of acetone and then embedded in an epoxy resin, Polybed. Sections were cut with a thickness of 1 µm for light microscopy (LM) and 70–80 nm for transmission electron microscopy (TEM). The 1-µm sections were stained with toluidine-blue and examined by LM. For TEM, the sections were contrasted with uranyl acetate and lead citrate.

Morphometrical analyses

Of the biopsies studied in the light microscope, only those with a surface of at least 115 µm of continuous, undamaged epithelium were included in the morphometrical analysis. A light microscope with a drawing tube on a digitizing table, connected to a MOP Videoplan Image Analyzer (Kontron AG, Munich, Germany) was used for the morphometrical analysis, as described earlier (23).

The height of the epithelium and the thickness of

the lamina reticularis of the basement membrane were measured and the values for each individual were calculated. The measurements were made on sections cut perpendicular to the surface of the epithelium and at three to five different points 60–115 µm apart at an objective magnification of 40 ×.

By a point-counting method using a square lattice in the light microscope at an objective magnification of 40 ×, the volume density of polymorphonuclear granulocytes, lymphocytes, and vessels in the adjacent subepithelial tissue was measured. The measurements were made to a depth of 60–230 µm in the subjects after they had inhaled histamine.

Scanning electron microscopy

Thirty-six biopsies from 12 subjects who had, 24 h previously, inhaled histamine were analyzed by SEM. The control material analyzed by SEM consisted of 23 biopsies from 14 subjects. All the SEM biopsies were gently washed in physiological saline to remove mucus mechanically, fixed in 2.5% glutaraldehyde, critical point dried, mounted on holders and coated with gold (16). The specimens were examined in an SEM at a magnification of 600–12000 ×.

Results

The trachea and occasionally also the central bronchi were slightly reddened in half of the subjects after the histamine inhalation, and normal-looking in the remaining subjects. There was no excessive reddening in those exposed to the higher dose of histamine.

SEM studies

In the SEM, five biopsies from the subjects who had previously inhaled histamine were judged to be mechanically damaged to an extent that prevented further examination. At a low magnification, the remaining 31 biopsies were noted to have a wrinkled appearance, and in some the marks of the biopsy forceps were clearly seen. At larger magnifications, the surface of the biopsies showed areas with intact epithelium, but also areas in which the epithelium had been torn off. A damaged epithelium occurred to the same extent in the control material as in the test material. In seven of the biopsies, taken from five subjects who had inhaled 16 mg/ml of histamine, areas of epithelial cells with altered cilia or lacking cilia were seen among normally ciliated cells (Fig. 1). In the control group this was only seen in one subject. Also only one biopsy from the control group was extensively damaged mechanically.



Fig. 1. Bronchial epithelial surface from a healthy volunteer 24 h after exposure to histamine. Note the intermingled epithelial cells lacking cilia. Magnification 1200 $\times$ upper and 6000 $\times$ lower.

LM studies

In the light microscope 58% (11/19) of the biopsies were seen to have an undamaged epithelium of 115 μ m as compared to 51% of the biopsies from the controls. There was no excessive damage in the few biopsies collected from the subjects who had inhaled 32 mg/ml of histamine.

The median epithelial height was 36.3 μ m (upper

and lower quartiles 35–40 μ m), as compared to 41.5 μ m (38–45 μ m) in the control group. The median thickness of the lamina reticularis of the basement membrane after histamine inhalation was 7.9 μ m (lower and upper quartiles, 7–8 μ m), as compared to 8.5 μ m (7–9 μ m) in the controls.

The lymphocytes and polymorphonuclear leucocytes in the subepithelial tissue were few and did not differ between the groups (0–1% of the subepithelial volume). The subepithelial volume of vessels was $\leq$ 3%, except in one biopsy that had 12%.

Eight biopsies, not showing enough undamaged epithelium to be included for morphometry, exhibited either a partly denuded basement membrane with a damaged epithelium consisting mainly of basal cells, or ciliated epithelial cells in a row without subepithelial tissue. Occasional inflammatory cells and vessels were seen in the subepithelial tissue. Biopsies showing similar histology were frequently seen in the control material (23).

TEM studies

In the TEM, the cilia of the ciliated cells appeared in approximately the same number in both groups. In intact areas, the intercellular borders between adjoining epithelial cells were close under the epithelial surface. Just above the basement membrane, however, minimal intercellular space was noted, also in otherwise normal-looking epithelium.

The densely packed collagenous fibers in the lamina reticularis of the basement membrane had a similar appearance in the groups. As also noted in the light microscope, few inflammatory cells were found in the subepithelial tissue. The appearance did not differ between the histamine and the control group.

Discussion

The tracheal mucosa was slightly reddened in half of the subjects after inhaling histamine, and it is assumed that this was due to the irritant effect of histamine. A reddened trachea has also been noted in healthy subjects after exposure to sulfur dioxide, with a subsequent increase in the number of lymphocytes and mast cells in BAL fluid (20). Inflammatory cells have also been recruited in BAL fluid after histamine inhalation (21), indicating the central and peripheral deposition of histamine. Skin chamber fluids (17) and filters (2) collected from the superficial dermis of atopic patients were analyzed, after the application of histamine to the skin chambers. One study showed an elevated number of eosinophils on the filter in atopics, as compared to nonatopics (2). In another study there was no difference in the relative distribution of neutrophils, monocytes, and eosinophils of the skin chamber fluid after exposure to histamine,

as compared with exposure to compound 40/80 or with the solution vehicle (17).

That there was no increase in leukocytes in the bronchial mucosa does not imply that there was no inflammation. Histamine can induce an increase in the amount of albumin in the nasal lavage fluid (7) and of albumin and hyaluronan in BAL fluid (22). Thus, for research purposes one should be cautious when other markers of inflammation are examined and the inhalation of histamine is involved.

Epithelial damage has been associated with asthma. The epithelium of asthmatics is possibly more fragile and more prone to shed than the epithelium of healthy individuals (1, 5, 9, 12). Histamine will increase vascular permeability and evoke edema in the lower respiratory tract, and it has been speculated that this subepithelial edema could be the reason for the shedding of the bronchial epithelium described in asthmatics (1, 18). It was, therefore, of interest to examine whether histamine can give rise to epithelial damage. It is important not to confuse epithelial damage which is due to the biopsy and preparative technique with that caused by disease (15, 23). The epithelium in the present study was not more damaged than that of the healthy subjects examined without a preceding histamine test, as seen in the light microscope or the TEM (23). In the SEM, however, certain areas were seen to have altered cilia or they partly lacked cilia after the histamine test. This was found mostly in macroscopically normal-looking mucosa. Similar features have been observed in bronchial biopsies from asthmatics, although more widely spread and tending to diminish after long-term use of inhaled steroids (16). This finding may suggest that histamine has a traumatic effect on the airways if it is continuously present, as is probably the case in allergic asthmatics during the pollen season.

Histamine has been noticed to be elevated in the BAL fluid of allergic asthmatics after allergen bronchoprovocation (3) where also higher numbers of lymphocytes, eosinophils, and neutrophils have been found (6, 25). An increase in inflammatory cells has been noted in the bronchial mucosa of allergic asthmatics in a stable phase (9, 10, 13). In a study by Poulter et al. on asthmatics, frozen sections showed mononuclear cell infiltration containing activated T cells and macrophages. This was only found in one of six biopsies from their healthy controls. All the asthmatics and the control subjects had undergone a histamine test 29 h before the collection of the biopsies. The cellular infiltrate was interpreted not to be an immediate type of reaction, but to reflect an underlying chronic inflammation in the hyperresponsive asthmatic (19). Methacholine did not change the epithelium of bronchial biopsies (14).

In summary, inhalation of histamine did not cause

any major damage to the bronchial epithelium as seen in the light microscope, although in the SEM, areas of ciliary alterations or lack of cilia were noted. This has also been observed, although more widely spread, in biopsies from asthmatics (16). There was no increase in leukocytes in the subepithelial tissue. For routine morphological examination a preceding histamine test should not interfere with the result, but for strictly research purposes a histamine test should be avoided prior to a bronchoscopy (21, 22, 24).

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Material and methods

Patients

A total of 24 patients (14 men, 10 women, age range 20-45 years) with mild asthma (according to the criteria of the British Society for Allergy and Clinical Immunology) participated in the study. The patients were recruited from the outpatient clinic of the Department of Respiratory Medicine, University Hospital, Lund, Sweden. All patients gave their informed consent before entering the study. The study was approved by the Ethics Committee of the University Hospital, Lund, Sweden. The patients were divided into two groups: a placebo group and a histamine group. The placebo group received a placebo (0.9% saline) and the histamine group received a histamine solution (0.1 mg/ml). The patients were randomized into two groups by a computer program. The patients were recruited from the outpatient clinic of the Department of Respiratory Medicine, University Hospital, Lund, Sweden. All patients gave their informed consent before entering the study. The study was approved by the Ethics Committee of the University Hospital, Lund, Sweden. The patients were divided into two groups: a placebo group and a histamine group. The placebo group received a placebo (0.9% saline) and the histamine group received a histamine solution (0.1 mg/ml). The patients were randomized into two groups by a computer program.

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