

Inflammatory response in bronchoalveolar lavage fluid after inhaling histamine

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To examine the influence of the histamine chloride challenge test on the bronchoalveolar lavage cell population, lavage fluid from 15 subjects was collected 24 h after the histamine test, and was compared with the lavage fluid from a reference group of 25 subjects. Inhaled histamine is commonly used to quantitate non-specific bronchial responsiveness. Increase in airway responsiveness after exposure to ozone or allergen is associated with airway inflammation. Bronchoalveolar lavage, has therefore become a valuable tool in the study of bronchoalveolar cells and mediators in subjects with asthma and bronchial hyperresponsiveness. The total cell number and differential cell counts in bronchoalveolar lavage fluid 24 h after inhalation challenge test with histamine-chloride were studied. There was a significant increase in lymphocytes, mast cells and neutrophils after histamine test. The conclusion was that inhaled histamine-chloride can induce an inflammatory cell response in the lung. Thus the histamine-chloride test should not be performed before bronchoalveolar lavage.

Key words: bronchoalveolar lavage; challenge test; histamine-chloride; lymphocytes; mast cells; neutrophils

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CLINICAL ASPECTS

An increase in inflammatory cells in the bronchoalveolar lavage (BAL) fluid was noted 24 h after histamine-chloride challenge test. From a practical point of view, histamine-chloride challenge test should not be performed before BAL, because the BAL cell population can be influenced by the histamine-chloride test.

Bronchoalveolar lavage (BAL) has frequently been used to study the alveolar cell population in such interstitial lung diseases as sarcoidosis and extrinsic allergic alveolitis. Lymphocytosis as well as an increased number of neutrophils, mast cells and eosinophils have been reported in different interstitial lung disorders. So far, examination of cellular distribution in BAL fluid has, however, been of minor clinical value (16).

In recent years, BAL has also been performed in patients with bronchial asthma to study the mechanisms behind asthma and bronchial hyperresponsiveness (1, 2, 4–8, 12, 14, 15). According to BAL studies, eosinophils and their mediators might be involved in the

development of late asthmatic reactions (4). BAL following local allergen bronchoprovocation has showed an increase in neutrophils, lymphocytes and eosinophils, suggesting that airway inflammation is a consequence of bronchoprovocation (15). Histamine is one of the mediators considered to play an important role in bronchial asthma. Histamine can induce smooth muscle spasm, stimulate irritant receptors, increase mucous secretion and also increase vascular permeability. An eosinophilotactic effect of histamine has also been reported (3).

Inhaled histamine is commonly used to quantitate non-specific bronchial responsiveness (9). In the present study the influence of histamine-

chloride (HC) challenge test on the bronchoalveolar cell population has been examined.

SUBJECTS AND METHODS

Subjects

Thirty-three healthy non-smoking volunteers (9 women, 24 men, age 21–57 years, median age 34 years) were included in the study. The subjects had no history of asthma or other lung disease and they had no history of respiratory tract infection within 6 weeks prior to the examination. All subjects participated as volunteers after their informed consent. The study was approved by the Ethics Committee of the University of Umeå.

Histamine chloride test group. Bronchoalveolar lavage was performed in 15 subjects (4 women, 11 men, age 25–50 years, median age 36 years) 24 h after HC-test. All subjects inhaled 16 mg/ml of histamine chloride and none of them were hyperreactive.

Reference group. Twenty-five of the subjects (5 women, 20 men, age 21–57 years, median age 32 years) underwent BAL as a reference sample without previous HC-test. Seven of the subjects served as their own controls. These subjects were examined both before and after HC-test with an interval of more than 6 weeks between the examinations.

Histamine test

Phosphate-buffered histamine-dihydrochloride (MW184) in concentrations from 0.03 mg/ml to 16 mg/ml was used for the histamine tests. The 0.125 mg/ml solution had a pH of 7.46 while the 16 mg/ml solution had a pH of 6.48. The test aerosol was generated by a Wright nebulizer (Roxon Medi-Tech Ltd., Quebec, Canada) with an output of about 0.13 ml/min. The aerosol was inhaled by tidal breathing for 2 min through a mask held loosely over the mouth. Histamine chloride, in concentrations from 0.03 mg/ml and in doubling concentrations to a

maximum of 16 mg/ml, was inhaled at 5-min intervals (9). The provocation concentration causing a fall in forced expiratory volume in 1 s (FEV₁) of 20 %, was expressed as PC₂₀-value. A PC₂₀-value of more than 8 mg/ml was considered to be normal. Vital capacity (VC) and FEV₁ were measured by a vitalograph (Vitalograph Ltd., Buckingham, England).

Bronchoalveolar lavage

Flexible fiberoptic bronchoscopy with BAL was performed under topical anesthesia with approximately 250 mg lidocaine (Xylocain®, Astra, Sweden). The subjects were premedicated with 0.5 mg Atrophine sulphate (ACO, Sweden) or 0.5–0.75 ml of Morphine-Skopopolamine® (10 mg/ml + 0.4 mg/ml, ACO, Sweden) subcutaneously. All examinations were performed with the subject in the supine position.

A flexible fiberoptic bronchoscope (Olympus BF-1T, BF-10T or BF-4B2, Olympus, Tokyo, Japan) was introduced through the mouth without intubation. After wedging the bronchoscope, the lingula segment of the left lung was lavaged with 4 × 60 ml of sterile Kreb's Ringer phosphate buffer (pH 7.3, 37°C). The fluid was gently aspirated immediately after each instillation.

The aspirated BAL fluid was kept on ice and filtered through a nylon filter with a pore diameter of 100 µm (Syntab Product AB, Malmö, Sweden). To concentrate the cells, the fluid was centrifuged at 400 g for 15 min and then resuspended in a balanced salt solution to a concentration of 10⁶ non-epithelial cells/ml. The total number of cells in the lavage fluid were counted in a Brüker chamber. Cytological slides were prepared in a cytocentrifuge (Cytospin Shandon, Southern Product Ltd., Runcorn, England) with 5 × 10⁴ non-epithelial cells per slide. The cytocentrifuge slides were stained with May-Grünwald-Giemsa before differential counting. Mast cells were stained with acid toluidine blue and counter stained with Mayer's acid hematoxylin (17).

Numbers of lymphocytes, neutrophils, eosinophils and macrophages were expressed

both as percentages of 200 cells (except epithelial cells) and as actual lavage fluid concentrations. Mast cells were counted as the number present in 10 visual fields with $\times 16$ magnification and expressed as percentages of all non-epithelial cells and as actual lavage fluid concentrations.

Statistical analysis

Wilcoxon's non-parametric rank sum test was used for statistical comparison between groups.

RESULTS

In seven of the 15 examinations performed after HC-test, a slight reddening of the bronchial mucosa in the central airways was noted macroscopically during bronchoscopy.

The total number of cells/l in the BAL fluid was the same in the subjects examined after HC-test as in the reference subjects. There was, however, an increase in the number of lymphocytes, neutrophils and mast cells, but not in the number of eosinophils or macrophages after HC-test. The total cell number and relative count of cells are given in Table. 1.

Nine of the 15 subjects examined after HC-test had more than 20 % of lymphocytes in the lavage fluid, compared with three of the 25 subjects in the reference sample.

Among the seven subjects examined as their own controls, six had more than 20 % lymphocytes after HC-test while only one had 20 % lymphocytes when BAL was performed as control without previous HC-test. This control group did not differ from the other control subjects.

DISCUSSION

The observation of an increased number of inflammatory cells in bronchoalveolar lavage fluid 24 h after histamine-chloride challenge test was unsuspected, since there have been no previous reports on such effects of histamine. An increase in lymphocytes, neutrophils and mast cells was noted in this investigation. A chemotactic effect of histamine on these cells has not,

Table 1

	Total cell count $\times 10^7/l$	Lymphocytes		Monocytes/Macrophages		Neutrophils		Eosinophils		Mast cells	
		%	$\times 10^7/l$	%	$\times 10^7/l$	%	$\times 10^7/l$	%	$\times 10^7/l$	%	$\times 10^4/l$
After histamine chloride test ($n = 15$)	8.2 4.8-9.4	24.0 8-30	1.1 0.8-1.9	73.0 61-91	6.5 2.3-7.5	3.0 1-4	0.2 0.1-0.3	0.0 0-1	0.0 0-0.1	0.2 0.1-0.4	10.8 3.4-38.0
Reference subjects ($n = 25$)	6.0 5.0-8.0	7.0 5-15	0.4 0.3-0.7	90.0 82-95	5.3 4.3-7.0	1.0 0-2.0	0.1 0-0.1	0 0-0	0 0-0	0 0-1	0 0-6
Comparison between groups	NS	$P < 0.005$	$P < 0.001$	$P < 0.001$	NS	$P < 0.01$	$P < 0.005$	NS	NS	$P < 0.005$	$P < 0.001$

NS: no significant differences.

as far as we know, been reported. An eosinophilotactic effect of histamine has been reported (3), but has been questioned (19). In the present study there was no significant increase in eosinophils after inhalation of histamine.

The possibility of some laboratory fault, e.g. loss of macrophages during the preparation procedure of the lavage fluid, has been considered as an explanation of the present findings. The fact that the total number of inflammatory cells was increased in the HC-group makes such an explanation less probable. Seven subjects were examined both with and without previous HC-test. These had an increase in inflammatory cells in the BAL fluid after HC-test, while the cellular distribution without previous HC-test did not differ from the reference group.

Bronchoalveolar lavage was repeated after HC-test in three of the subjects with high levels of lymphocytes; each still had more than 20 % lymphocytes in the lavage fluid. These three subjects also underwent BAL without previous HC-test, and this time each had a lower number of lymphocytes.

The mechanisms behind the increase in inflammatory cells in the BAL fluid after inhalation of histamine chloride are not clear. One possible explanation could be that inhalation of acidic substances, such as histamine-chloride, can induce an inflammatory response in the airways. Inhaled SO_2 , which can also induce bronchoconstriction, has recently been shown to induce bronchoalveolar lymphocytes together with an increase in the total number of macrophages and lysozyme-positive macrophages 24 h after exposure (Sandström et al., to be published). Buffered histamine chloride with a pH of 6.5 in the 16 mg/ml concentration was used. It therefore seems unlikely that the acidity of the inhaled substance is responsible for the increase in inflammatory cells in the BAL fluid (10). It seems more likely that the increase is an effect of the histamine-chloride itself rather than an effect of the acidity of the inhaled aerosol. Besides inducing smooth muscle spasm, histamine can stimulate irritant receptors and increase vascular permeability. Our results suggest that histamine may also play a part in the cellular inflammatory response. However, the

mechanism by which histamine-chloride attracts inflammatory cells to the lung is not yet identified.

One possible mechanism could be that histamine penetrates the epithelium in the central airways and causes a reflex-induced increase in inflammatory cells. Some of the subjects examined after HC-test showed a slight reddening of the bronchial mucosa, suggesting that the inhaled aerosol might have had an irritative effect.

Another possibility could be that the inhaled histamine might have a direct chemotactic effect on the cells of the peripheral airways.

Inhaled allergen, as well as local application of allergen on the airway epithelium of asthmatic patients and animals, have been reported to increase inflammatory cells in BAL fluid (6, 13, 15). The factors attracting these cells to the lung after allergen exposure have not been identified. Bronchial hyperresponsiveness is associated with an increase in mast cells (7, 11). Flint et al. (7) found increased numbers of mast cells within the bronchoalveolar cell population in asthmatics with a corresponding increase in total cellular histamine. Atopic asthmatics have elevated levels of histamine in the BAL fluid (2). From our findings it is suggested that histamine may play a role in the inflammatory response of the lung to inhaled substances such as allergen.

Not all of the subjects showed an increase in inflammatory cells in BAL fluid after HC-test. Besides individual differences in response to histamine, differences could be related to the dose deposition of histamine-chloride in the airways (18).

From a practical point of view, histamine-chloride challenge test should not be performed before BAL, because the BAL cell population can be influenced by HC-challenge test. The duration of the effects of the HC-test has to be examined further.

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