

Inhaled methacholine does not influence the cellular composition of bronchoalveolar lavage fluid

Söderberg M, Lundgren R, Bjermer L, Ångström T. Inhaled methacholine does not influence the cellular composition of bronchoalveolar lavage fluid.

Allergy 1993; 48: 173–176. © Munksgaard 1993.

A methacholine or a histamine test is often used to quantitate bronchial responsiveness, in studies investigating the influence of various inhaled stimuli on the responsiveness and composition of bronchoalveolar lavage (BAL) fluid. Histamine has been shown to evoke an inflammatory cell response in BAL fluid. In order to test the influence of the methacholine test on the cellular composition of BAL fluid, we investigated 14 healthy, nonsmoking volunteers with a methacholine test 24 h before performing a BAL. The results showed no significant changes in the cellular composition of BAL fluid, as compared with control BAL in the same persons.

Clinical aspects

A methacholine test can be performed 24 h before performing a BAL, without affecting the cellular content of BAL fluid.

Bronchoalveolar lavage (BAL) is often used to quantitate the cellular reaction in the airways to a variety of inhaled stimuli (1, 5, 6, 20, 24, 25). An inhaled stimulus can also increase bronchial responsiveness (6), the degree of which is often measured with a methacholine or a histamine test (4, 10). In earlier studies, we found that the histamine test can evoke an inflammatory cell response (27), increasing the level of hyaluronan (hyaluronic acid) (28) in the BAL fluid, and that the cellular reaction is not histamine H_1 -receptor mediated (29). The changes might be attributed to an irritative effect of histamine that is lacking in methacholine; methacholine exerts its effect more selectively, via a neuronal pathway (13). Moreover, in studies of the bronchial mucosa, in human asthmatics (12, 17, 18) and in experimental animals (11, 21), bronchial responsiveness has been examined with methacholine or acetylcholine before the collection of bronchial biopsies. To our knowledge, there are no studies of the effect of methacholine on the cellular composition of BAL.

The aim of the present study was to determine whether the inhalation of methacholine, 24 h before performance of BAL can evoke a cellular response parallel to that which has been reported for histamine (27).

M. Söderberg¹, R. Lundgren¹,
L. Bjermer¹, T. Ångström²

Departments of ¹ Pulmonary Medicine and
² Cytology, University Hospital, Umeå, Sweden

Key words: bronchoalveolar lavage; histamine test; methacholine test; lymphocytes; mast cells.

Dr Margareta Söderberg
Department of Pulmonary Medicine
University Hospital
S-901 85 Umeå
Sweden

Accepted for publication 28 August 1992

Material and methods

Fourteen healthy, nonsmoking volunteers, three men and 11 women, with a median age of 27 years (upper and lower quartiles 23–30 years), participated in the study. Six had a positive skin prick test to at least one common allergen, and two had experienced symptoms of rhinoconjunctivitis during the pollen season or in contact with animals, but none had asthma. All had a vital capacity (VC) and a forced expiratory volume (FEV₁) of 80% or more of predicted normal values (2). None had experienced symptoms of respiratory infection for at least 5 weeks before or during the examination. The study was performed out of the pollen season, with the informed consent of the volunteers and the approval of the Ethical Committee of the University of Umeå.

BAL was performed 24 h after a methacholine test according to the method described by Hargreave et al. (10). The methacholine test and the BAL were performed by the same investigator; the cytologic slides were investigated in the cytologic department, but not blinded. All subjects were exposed to 4, 8, 16, and 32 mg/ml of methacholine chloride. The pH values of the methacholine solutions were 4.2, 3.9, 3.8, and 3.5, respectively. The osmotic pressure was 471–735 mmol, measured by an osmometer (Dallchi

Kogatu Co., Japan). A control lavage was performed after at least 5 weeks.

BAL was performed as described before, after premedication with up to 1 mg of atropine, and local anesthesia with up to 250 mg of lignocaine (29). The bronchoscope was wedged in the middle lobe, and three boluses of 60 ml of saline (pH 7.4, 37°C) were infused and aspirated after each bolus. The first 15 ml was analyzed separately as representing the more central part of the bronchi (fraction I), and the rest was regarded as a sample from the more peripheral airways (fraction II). The lavage fluid was analyzed as previously described (29).

The BAL fluid was kept on ice and filtered through a nylon filter with a pore diameter of 100 µm (Syntab Products, Malmö, Sweden). To concentrate the cells, the fluid was centrifuged at 400 g for 15 min and then resuspended in a balanced salt solution at a concentration of 10^6 nonepithelial cells/ml. The total number of cells was counted in a Burkert chamber, Cytologic slides were prepared in a cytocentrifuge (Cytospin Shandon, Southern Products Ltd, Runcorn, UK) with 5×10^4 nonepithelial cells per slide. The cytocentrifuge slides were stained according to May Grunwald Giemsa before differential counting of at least 200 cells by scanning several fields. Mast cells were stained with acid toluidine blue and counterstained with Mayer's acid hematoxylin (30). Numbers of lymphocytes, neutrophils, eosinophils, and macrophages are expressed as percentages of all nonepithelial cells. Mast cells were counted as the number present in 10 visual fields with $\times 160$ magnification and were also expressed as percentages of all nonepithelial cells.

The ratio of T-helper/T-suppressor cells (Leu 3a/Leu 2a) was examined with the Simultest (Becton Dickinson AB, Stockholm, Sweden). One milliliter of cell suspension of 10^6 cells/ml was centrifuged at 250 g for 3 min at 4°C. The supernatant was aspirated and the cell pellet was carefully mixed. Twenty microliters of the reagent was added to the cells, and they were incubated for 30 min at 4°C on ice. Two milliliters of cold physiological buffered saline (PBS) was added, and, after mixing, the cells were again

centrifuged in the same way as before. The supernatant was aspirated, leaving 50 µl fluid, which was fixed with 1% paraformaldehyde and stored at 4°C until analyzed. After resuspension in 2 ml of cold BPS, centrifugation, and aspiration of supernatant, the cells were analyzed with a fluorescence microscope (Zeiss, Germany).

The albumin content of the BAL fluid was measured by nephelometry (Beckman Array Protein System, Beckman, Inc., Brea, CA, USA). The inter- and intra-assay variability were both less than 3%.

Statistics

Wilcoxon's rank sum test for paired observations was used for comparison between the values from the cell countings, and a difference with $P < 0.05$ was considered to be statistically significant.

Results

Three persons had a PC_{20} lower than 32 mg; 11.6, 19.3, and 19.7 mg/ml, respectively. The corresponding fall in FEV_1 for these persons was 37, 28, and 41%, respectively. The person with a PC_{20} of 11.6 had a positive skin prick test but no symptoms of rhinoconjunctivitis or asthma; the other two had negative skin prick tests and no symptoms. For all the other subjects, the PC_{20} was higher than 32 mg/ml, and the percent fall in FEV_1 after inhaling 32 mg/ml of methacholine was 2–18%.

The tracheal mucosa appeared to be slightly reddened in five of the subjects after they had inhaled methacholine, but it was normal-looking in the others. In the control bronchoscopy, only one subject had a slightly reddened mucosa; all the others were normal-looking.

There was no significant difference in the cellular composition between the BAL fluid collected after the methacholine test and the control BAL. Nor was there any significant difference between the first and the second portion of the BAL fluid. The ratio of T-helper to T-suppressor cells did not differ between the examination after the methacholine inhalation

Table 1. Bronchoalveolar lavage 24 h after methacholine test and in a control lavage in the same persons*

Fraction	Total cell count $\times 10^4$ /ml		Eosinophils %		Neutrophils %		Lymphocytes %		Macrophages %		Mast cells %		T-helper/T- suppressor cells
	I	II	I	II	I	II	I	II	I	II	I	II	
Control	18 (15–21)	10 (9–19)	0 (0–0)	0 (0–0)	2 (0–4)	1 (0–2)	7 (4–14)	9 (5–10)	88 (84–95)	90 (88–94)	0.03 (0.01–0.05)	0.02 (0.01–0.04)	61/37=1.65 (56–63/35–42)
Methacholine	15 (12–20)	9 (8–15)	0 (0–1)	0 (0–0)	2 (1–3)	1 (1–1)	10 (5–17)	11 (5–19)	88 (82–93)	88 (80–94)	0.03 (0.01–0.04)	0.02 (0.01–0.03)	58/36=1.61 (56–60/33–39)

* $n=15$; the same persons. Values are given as medians and, within parentheses, quartile ranges. (For T-helper/T-suppressor cells, $n=9$, and the analysis was made of the pooled fractions I and II.) No significant difference was found; $P>0.05$.

and the control BAL (measured in nine of the subjects) (Table 1).

There was no rise in the amount of albumin in the lavage fluid after methacholine inhalation, as compared with the control lavage (measured in nine of the subjects). The median values were 36 ng/l in fraction I and 35 in fraction II, and the interquartile ranges were 32–69 and 31–46, respectively. The corresponding median values in the control lavage were 34 and 46 (quartiles 16–78 and 28–84).

Discussion

The mechanisms underlying bronchoconstriction partly differ between methacholine and histamine, although they show a correlation in PC₂₀ (10). Histamine is thought to act partly through reflex, bronchoconstricting, cholinergic pathways and partly by direct effect on the histamine receptors (3, 13, 29). Methacholine is thought to have no effect on irritant receptors but to act through cholinergic receptors in smooth muscle (13). Histamine may also act directly on bronchial muscle (3, 4).

Since many investigators perform methacholine tests and BAL in the same studies, it was of interest to discover whether the methacholine test itself had any influence on BAL cells. The interval of 24 h was chosen because of a previously noted inflammatory effect of histamine on BAL cells. In the present study, methacholine had no influence. Of course this does not exclude an influence on BAL performed directly after a methacholine test, although such influence does not seem to be likely. Fabbri et al. found normal cell values in the BAL of healthy volunteers immediately after performing a methacholine test. This was done 23–24 h after exposure to toluene-di-isocyanat (6).

Seltzer et al. (26) found that ozone exposure increased responsiveness in a methacholine test and the number of polymorphonuclear cells in BAL; the methacholine test was performed the day before and 1 h after ozone exposure, and BAL fluid was collected 1–2 h later. In studies of asthmatics, the intervals between the methacholine test and BAL varied 1–14 d (7, 14, 15, 31). The relation in time between the methacholine test and BAL after endobronchial provocation was 2 weeks in a study by Wentzel (32). Pliss et al. (22) and Flint et al. (8) performed the methacholine test after BAL. Other investigators did not mention the relation in time between the methacholine test and BAL (16, 19, 20). Beasley et al. (1) found a wide range of cellular values in BAL fluid 18 h after methacholine inhalation in four asthmatics, and allergen inhalation in five asthmatics, precluding any clear distinction between the groups. Lozewicz et al. (18) found no difference in epithelial structure between biopsies from asth-

matics and control subjects collected before or the day after a methacholine test.

In a study on nasal secretion, the albumin content was increased 10 min after stimulation with methacholine (23). In the present study, however, no changes in the BAL fluid albumin concentration could be seen. Since the interval between the stimulation and the collection of fluid differed greatly between their study and ours, the studies are not really comparable.

The pH of the methacholine solution was surprisingly low. A pH of 6.5 has been observed in normal human bronchi (9). A pH of lower than 5 of the histamine solution has been found to influence slightly the PC₂₀ to histamine in a study comparing buffered and unbuffered histamine solutions (4). The present study indicates that a pH as low as 3.5 does not influence the cellular composition of BAL fluid, although it may be the cause of the redness that has been observed in the trachea of five of the subjects. This has also been noted after exposure to SO₂ and NO₂ (24, 25).

In summary, we were unable to detect any influence on the cellular composition or the albumin content of the BAL fluid 24 h after the inhalation of methacholine chloride. This was in line with our expectations, and it raises no suspicion of influence of the test in most of the other studies that have been performed involving the methacholine test before BAL. It still remains to determine whether the test has any influence on the inflammatory mediators that are currently being examined in a great number of studies.

Acknowledgments

We wish to thank Birgit Lindberg, Birgit Moberg, Marianne Brygman and Jamshid Pourazar for excellent laboratory assistance, and Harmeet Sjögren for helpful linguistic revision. The study was supported by grants from the Medical Faculty of the University of Umeå.

References

1. BEASELEY R, ROCHE R, ROBERTS IA, HOLGATE ST. Cellular events in bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis* 1989; 139: 806–17.
2. BERGLUND E, BIRATH G, BJURE J, GRIMBY I, SANDQUIST L, SÖDERHOLM B. Spirometric studies in normal subjects. I. Forced expirograms in subjects between 7 and 70 years of age. *Acta Med Scand* 1963; 173: 185–91.
3. COCKCROFT DW, KILLIAN DN, MELLON JJA, HARGREAVE FE. Bronchial reactivity to inhaled histamine; a method and a clinical survey. *Clin Allergy* 1977; 7: 235–43.
4. COCKCROFT DW, BERSCHIED BA. Effect of pH on bronchial response to inhaled histamine. *Thorax* 1982; 37: 133–6.
5. DE MONCHY JGR, KAUFFMAN HF, VENGE P, et al. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. *Am Rev Respir Dis* 1985; 131: 373–6.
6. FABBRI LM, BOSCHETTO P, ZOCCA E, et al. Bronchoalveolar neutrophilia during late asthmatic reactions induced

- by toluene diisocyanate. *Am Rev Respir Dis* 1987; 136: 36–42.
7. FERGUSON A, WONG F. Bronchial responsiveness in asthmatic children. Correlation with macrophages and eosinophils in bronchoalveolar fluid. *Chest* 1989; 96: 988–91.
8. FLINT K, LEUNG K, HUDSPITH B, BROSTOFF J, PEARCE F, JOHNSON N. Bronchoalveolar mast cells in extrinsic asthma: a mechanism for the initiation of antigen specific bronchoconstriction. *BMJ* 1985; 291: 923–6.
9. GUERRIN F, VOISIN C, MACQUET V, ROBIN H, LEQUIEN P. Apport de la pH métrique bronchique *in situ*. *Prog Respir Res* 1971; 6: 372–83.
10. HARGREAVE FE, RYAN G, THOMSON NC, et al. Bronchial responsiveness to histamine or methacholine in asthma: measurement and clinical significance. *J Allergy Clin Immunol* 1981; 68: 347–55.
11. HOLTZMAN MJ, FABBRI LM, O'BYRNE PM, et al. Importance of airway inflammation for hyperresponsiveness induced by ozone. *Am Rev Respir Dis* 1983; 127: 686–90.
12. JEFFERY PK, WARDLAW AJ, NELSON FC, COLLINS JV, KAY AB. Bronchial biopsies in asthma. An ultrastructural, quantitative study and correlation with hyperactivity. *Am Rev Respir Dis* 1989; 140: 1745–53.
13. JUNIPER EF, FRITH P, DUNNETT C, COCKCROFT DW, HARGREAVE FE. Reproducibility and comparison of responses to inhaled histamine and methacholine. *Thorax* 1978; 33: 795–810.
14. KELLY C, WARD C, STENTON C, BIRD G, HENDRICK D, WAALTERS E. Number and activity of inflammatory cells in bronchoalveolar lavage fluid in asthma and their relation to airway responsiveness. *Thorax* 1988; 43: 684–92.
15. KIRBY J, HARGREAVE F, GLEICH G, O'BYRNE P. Bronchoalveolar cell profiles of asthmatic and nonasthmatic subjects. *Am Rev Respir Dis* 1987; 136: 379–83.
16. LIU M, BLEECKER E, LICHTENSTEIN L, et al. Evidence for elevated levels of histamine, prostaglandin D₂, and other bronchoconstricting prostaglandins in the airways of subjects with mild asthma. *Am Rev Respir Dis* 1990; 142: 126–32.
17. LOZEWICZ S, GOMEZ E, FERGUSON H, DAVIES RJ. Inflammatory cells in the airways in mild asthma. *BMJ* 1988; 297: 1515–16.
18. LOZEWICZ S, WELLS C, GOMEZ E, et al. Morphological integrity of the bronchial epithelium in mild asthma. *Thorax* 1990; 45: 12–15.
19. METZGER W, RICHESON H, WORDEN K, MONICK M, HUNNINGHAKE G. Bronchoalveolar lavage of allergic asthmatic patients following allergen bronchoprovocation. *Chest* 1986; 4: 477–83.
20. METZGER WJ, ZAVALA D, RICHESON HB, et al. Local allergen challenge and bronchoalveolar lavage of allergic asthmatic lungs. Description of the model and local airway inflammation. *Am Rev Respir Dis* 1987; 135: 433–40.
21. O'BYRNE PM, WALTERS EH, GOLD BD, et al. Neutrophil depletion inhibits airway hyperresponsiveness induced by ozone exposure. *Am Rev Respir Dis* 1984; 130: 214–19.
22. PLISS L, INGENITO E, INGRAM R, PICHURKO B. Assessment of bronchoalveolar cell and mediator response to isocapnic hyperpnea in asthma. *Am Rev Respir Dis* 1990; 142: 72–6.
23. RAPHAEL G, DRUCE H, BARANIUK J, KALINER M. Pathophysiology of rhinitis. *Am Rev Respir Dis* 1988; 138: 413–20.
24. SANDSTRÖM T, STJERNBERG N, ANDERSSON MC, et al. Cell response in bronchoalveolar lavage fluid after exposure to sulfur dioxide: a time-response study. *Am Rev Respir Dis* 1989; 140: 1828–31.
25. SANDSTRÖM T, ANDERSSON MC, KOLMODIN-HEDMAN B, STJERNBERG N, ÅNGSTRÖM T. Bronchoalveolar mastocytosis and lymphocytosis after nitrogen dioxide exposure in man: a time kinetic study. *Eur Respir J* 1990; 3: 138–43.
26. SELTZER J, BIGBY B, STULBARG M, et al. O₃-induced change in bronchial reactivity to methacholine and airway inflammation in humans. *J Appl Physiol* 1986; 60: 1321–6.
27. SÖDERBERG M, LUNDGREN R, BJERMER L, STJERNBERG N, ROSENHALL L. Inflammatory response in bronchoalveolar lavage fluid after inhaling histamine. *Allergy* 1988; 43: 98–102.
28. SÖDERBERG M, BJERMER L, HÄLLGREN R, LUNDGREN R. Increased hyaluronan (hyaluronic acid) levels in bronchoalveolar lavage fluid after histamine inhalation. *Int Arch Allergy Appl Immunol* 1989; 88: 373–6.
29. SÖDERBERG M, LUNDGREN R, ÅNGSTRÖM T. Effect of Cetirizine on histamine induced bronchoconstriction and bronchoalveolar cell composition. *Allergy* 1991; 46: 217–21.
30. STROBEL S, MILLER H, FERGUSON A. Human interstitial mucosal mast cells: evaluation of fixation and staining techniques. *J Clin Pathol* 1981; 34: 851–8.
31. WARDLAW AJ, DUNETTE S, GLEICH GJ, COLLINS JW, KAY AB. Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma. Relationship to bronchial hyperactivity. *Am Rev Respir Dis* 1988; 137: 62–9.
32. WENTZEL S, LARSEN G, JOHNSTON K, VOELKER N, WESTCOTT J. Elevated levels of LTC₄ in bronchoalveolar lavage fluid from atopic asthmatics after endobronchial allergen challenge. *Am Rev Respir Dis* 1990; 142: 112–19.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.