

Effect of cetirizine on histamine-induced bronchoconstriction and bronchoalveolar cells

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The effect of the histamine H1 receptor antagonist (cetirizine) on histamine-induced bronchoconstriction and inflammatory cells in bronchoalveolar lavage fluid was studied in 12 healthy volunteers. In previous studies we have observed an increased number of inflammatory cells, albumin and hyaluronan in the bronchoalveolar lavage (BAL) fluid 24 h after an inhalation challenge test with histamine-chloride. In the present study cetirizine blocked the histamine-induced bronchoconstriction but did not influence the cell counts in the bronchoalveolar lavage fluid. Our result suggests that the histamine-induced bronchoconstriction but not the recruitment of inflammatory cells in the BAL fluid is mediated by histamine H1 receptors.

Key words: bronchoalveolar lavage cells; bronchoconstriction; cetirizine; histamine-chloride challenge test.

Accepted for publication 16 October 1990

Histamine is involved in the mechanisms of bronchial asthma (4,5). It can induce bronchial smooth muscle contraction, stimulate irritant receptors, increase mucus secretion and increase vascular permeability. An eosinophil chemotactic activity of histamine has also been described (6), but this effect has been subject to some controversy (15). Since inhaled histamine is able to induce bronchoconstriction it is commonly used to quantitate nonspecific bronchial responsiveness. In a previous study we noted an increased number of inflammatory cells, mainly lymphocytes and mast cells, in bronchoalveolar lavage (BAL) fluid, 24 h after inhalation challenge test with histamine-chloride in healthy subjects (13). We have also noted increased levels of albumin and hyaluronan in BAL fluid in response to inhaled histamine-chloride (12). The mechanisms involved in the recruitment of inflammatory cells to the BAL fluid are not known. Increased levels of albumin indicate that inhaled histamine increases vascular permeability in the peripheral airways. The aim of the

present study was to determine, by using a specific histamine H1 receptor antagonist, if the increase in the number of inflammatory cells in BAL fluid after inhaled histamine was mediated by H1 receptors in the airways. Cetirizine, a specific H1 receptor antagonist, was chosen because of its ability to block the bronchoconstrictor response to inhaled histamine in mild asthmatics (3).

SUBJECTS AND METHODS

Twelve healthy non-smokers (2 men, 10 women, age 22-33 years, median age 25 years) volunteered for the study. They all had a vital capacity (VC) and a forced expiratory volume in 1 s (FEV₁) of at least 80% of predicted normal values (1). None of them had a history of asthma or other lung disease. In six of the subjects, skin prick testing showed positive reactions to at least one common allergen, and three subjects had a history of mild rhinoconjunctivitis during the pollen season and/or when

Table 1.

Histamine bronchial provocation. Drop of FEV₁ (%) occurring after successive inhalation of 4, 8, 16 and 32 mg/ml of histamine chloride aerosol (*n* = 12)

	% drop FEV ₁ ± SD	(range)
Cetirizine	0.58 ± 0.67	(0-2)
Placebo	17.36 ± 13.46	(4-40)
Wilcoxon	<i>P</i> = 0.005	

in contact with animals, but none of them had any symptoms during the study that was performed in the late autumn and winter. They had not had any infection for at least 5th weeks prior to the first examination and none of them was infected during the study. The subjects gave their consent to participate in the study and the study was approved by the Ethics Committee of the University of Umeå.

Each individual was examined twice in a similar way at the same time of day. They were treated with either cetirizine 15 mg twice daily for 6th days or with a placebo in a randomized double blind cross over design. On the 5th day they took one tablet 2 h before a histamine bronchial challenge test and one tablet in the evening. On the 6th day they took one tablet 2 h before a bronchoalveolar lavage (BAL). The two periods were separated by a wash-out period of 4 weeks.

Twenty-four hours before the bronchoalveolar lavage, all subjects except one were exposed to 4, 8, 16 and 32 mg/ml of phosphate buffered histamine-dihydrochloride (MW 184). One woman once decreased her FEV₁ by 40% after inhaling 16 mg/ml and was therefore not further exposed on that occasion. The pH of the solutions was 7.05, 6.80, 6.45 and 6.07, respectively. The osmolality of the histamine-chloride solutions was 432-794 milliosmoles. The histamine aerosol was generated by a Wright nebulizer (Aerosol Product Ltd. London UK) with an output of approx. 0.13 ml/min. The aerosol was inhaled by tidal breathing for 2 min through a mask held loosely over the mouth. FEV₁ was measured after each inhalation. VC and FEV₁ were measured on a Vitalograph (Vitalograph Ltd, Buckingham, UK). The

provocation concentration causing a fall in FEV₁ of 20% is expressed as PC₂₀ value, and a PC₂₀ value of more than 8 mg histamine-chloride/ml is considered normal (9).

Bronchoalveolar lavage was performed after premedication with atropine under topical anesthesia with 250 mg of lignocaine. A flexible fiberoptic bronchoscope (BF 1T, BF 1T10 or BF 1T20, Olympus, Tokyo, Japan) was wedged in the middle lobe. Phosphate buffered saline (pH 7.4, 37°C) was infused in boluses of 60 ml and gently aspirated immediately after each bolus. Nine subjects were lavaged with 180 ml, and three individuals were lavaged with 240 ml. Identical volumes of lavage fluid were used in each individual at the two different examinations. The first 30 ml of aspirated fluid was collected as a separate portion (fraction I) and the remaining aspirated fluid was collected as a second portion (fraction II). A mixture of the first and the second portion (fraction III) was also examined.

The BAL fluid was kept on ice for a few minutes until filtered through a nylonfilter with a pore-diameter of 100 µm (Syntab Product, 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 was counted in a Bürker chamber. Slides were then prepared in a cyto-centrifuge (Cytospin Shandon, Southern Product Ltd., Runcorn UK) with 5 × 10⁴ non-epithelial cells per slide. The slides were stained with May Grünwald Giemsa for differential counting and at least 200 cells were counted. The results were expressed as total cell counts, and percentages of neutrophils, eosinophils, lymphocytes and macrophages. Mast cells were counted on slides stained with acid toluidine blue and counterstained with Mayer's acid hematoxylin (14). Mast cells were counted as the number present in 10 visual fields using an objective lens with a magnification of 16 and expressed as percentages of all non-epithelial cells.

The ratio of helper/suppressor T cells (Leu 3a/Leu 2a) was determined with the Simultest T-helper/suppressor Test (Becton Dickinson AB

Table 2.

Bronchoalveolar lavage 24 h after histamine bronchial provocation ($n = 12$)

Treatment	Total cell count $\times 10^4/\text{ml}$	Lymphocytes %	Macrophages %	Neutrophils %	Eosinophils %	Mast cells %
Cetirizine	13.07 ± 5.60 (5.80–23.20)	19.17 ± 11.00 (7–48)	78.58 ± 10.93 (51–90)	1.42 ± 0.79 (0–3)	0.75 ± 0.87 (0–1)	0.16 ± 0.12 (0.02–0.4)
Placebo	13.53 ± 10.30 (7.00–45.00)	16.75 ± 13.32 (5–53)	81.33 ± 13.44 (46–94)	1.33 ± 0.65 (0–2)	0.33 ± 0.49 (0–1)	0.16 ± 0.10 (0.02–0.35)
Wilcoxon	NS	NS	NS	NS	NS	NS

Stockholm, Sweden). One millilitre of cell suspension of 10^6 cells/ml was centrifuged at $250g$ for 3 min at $+4^\circ\text{C}$. The supernatant was aspirated and the cell pellet carefully mixed. Twenty microlitres of the reagent was added to the cells and incubated for 30 min at $+4^\circ\text{C}$ on ice. Cold phosphate buffered saline (PBS) (2 ml) was added and after mixing, the cells were again centrifuged as before. The supernatant was aspirated leaving $50\text{ }\mu\text{l}$ of fluid that was fixed with 1 % paraformaldehyde and stored at $+4^\circ\text{C}$ until analysed. After resuspension in 2 ml of cold PBS, centrifugation and aspiration of supernatant, the cells were analysed in a fluorescent lightmicroscope (Zeiss, Germany).

Statistics

For comparison between the groups, the Wilcoxon's non-parametric rank sum test was used. A value of $P < 0.05$ was considered significant.

Table 3.

Bronchoalveolar lavage 24 h after histamine bronchial provocation. Analysis of helper and suppressor T cell populations in the lavage fluid ($n = 12$)

Treatment	TH %	TS %	TH/TS
Cetirizine	60.75 ± 4.75 (55–72)	32.58 ± 5.15 (22–39)	1.94 ± 0.52 (1.41–3.27)
Placebo	60.92 ± 6.87 (51–70)	32.33 ± 6.57 (24–42)	2.00 ± 0.63 (1.26–2.88)
Wilcoxon	NS	NS	NS

RESULTS

None of the subjects had any adverse effect from the treatment. One control subject (placebo treatment) who had a drop in FEV_1 of 40 % after inhaling 16 mg histamine-chloride/ml did not inhale 32 mg/ml at that examination. All the other subjects inhaled 32 mg histamine-chloride/ml. In the control group (placebo), the drop in FEV_1 after inhaling histamine ranged from 4 % to 40 % (median 10 %, mean 17.4 %). All subjects when treated with cetirizine inhaled 32 mg histamine-chloride/ml and had a median drop in FEV_1 of only 0.5 % (mean 0.6 %, range 0 %–2 %). Cetirizine, thus, significantly ($P < 0.005$) decreased the drop in FEV_1 induced by histamine (Table 1.)

During bronchoscopy a slight reddening of the tracheal mucosa was noted in both control (three subjects) and cetirizine-treated subjects (three subjects).

There was no significant difference in the total cell counts and differential cell counts between fractions I, II or III. Therefore, only the results of fraction III, which is a pooling of fractions I and II, are shown in Tables 2 and 3.

Cetirizine had no significant effect on the total cell count or the percentage of macrophages, lymphocytes, neutrophils, eosinophils or mast cells in the bronchoalveolar lavage fluid when the subjects were treated with placebo or with cetirizine (Table 2). Neither did cetirizine have a significant effect on the helper/suppressor T lymphocytes (Table 3).

Bronchial reactivity to inhaled histamine did not differ between subjects with or without positive skin prick tests. Skin prick test results did not correlate with the cellular components of the BAL fluid.

DISCUSSION

Low concentrations of inhaled histamine induces bronchoconstriction in patients with bronchial hyperresponsiveness (9). Inhalation of high concentrations of histamine-chloride induced bronchoconstriction in some of our healthy subjects. In one of our control subjects, the FEV₁ decreased 40% in response to 16 mg/ml histamine-chloride, and in another four subjects, the FEV₁ dropped 19% or more after inhaling 32 mg histamine-chloride/ml when placebo was given before the histamine inhalation. When cetirizine was given before the histamine inhalation, all subjects inhaled 32 mg histamine-chloride/ml without a significant decrease in FEV₁. The histamine H1 antagonist, cetirizine, thus blocks histamine-induced bronchoconstriction in healthy subjects; this probably being by blockade of H1 receptors on airway smooth muscles. Our results are in agreement with Brick et al. (3) who observed that orally administered cetirizine provided a significant dose-dependent protection against histamine-chloride-induced bronchoconstriction in subjects with mild asthma.

The mechanism underlying the previously reported increase in number of inflammatory cells in the BAL fluid observed after inhalation of histamine is not known (13). The cell response to histamine in the present study was not as pronounced as in our previous study (13). Compared with the reference subjects used in the previous study (13), the number of lymphocytes was significantly increased in the cetirizine-pretreated group but did not reach significant level in the placebo-treated group. It is possible that even if histamine at the dose used caused bronchoconstriction in all control subjects its effect at the vascular level differed between the subjects. Braude et al. (2) noted that two of their seven subjects did not have an increased epithelial permeability after inhaled histamine.

Inhaled histamine can increase the permeability of the bronchial epithelium to 99Tc-DTPA (8). An increased amount of albumin found in the bronchoalveolar lavage fluid is considered to reflect a leakage from the vascular bed in the peripheral part of the lung (7). Increased levels of albumin in the BAL fluid after inhaled histamine was observed in our earlier study (12). This suggests that the inhaled histamine acts on the peripheral part of the lung either by a direct mode of action on the cells in the small airways and alveoli or by an indirect mechanism. If the high number of inflammatory cells in the BAL fluid noted in our previous study was due to an H1-mediated chemotactic effect or H1-mediated increased vascular permeability, one would have expected it to be blocked by cetirizine, but this was not the case. Thus the effect of histamine on the numbers of inflammatory cells is probably not mediated by the H1 receptors. Cetirizine did not influence the subpopulations of T lymphocytes, nor did the values differ from normal subjects (10).

It has been reported by Braude et al. (2) that increased epithelial lung permeability induced by inhaled histamine was not blocked by an H1 receptor antagonist (terfenadine) but was, however, significantly reduced by the H2 receptor antagonist, ranitidine, suggesting that histamine-induced permeability increase in the lung is mediated by H2 receptors (2). The possibility that the inflammatory cell response in BAL fluid after histamine inhalation is mediated by the H2 receptor still needs to be investigated.

In a study with sequential lavage, Lam et al. (11) noted that the number of lymphocytes and macrophages increases in the periphery of the airways while the number of neutrophils is higher in the central airways. In the present study there was no significant difference in cell proportions between the first part of the recovered fluid, which should reflect the more central part of the airways, and the second part of the recovered fluid, which should reflect the peripheral part of the bronchoalveolar system. This result suggests that the cells might have been recruited from both peripheral and more central parts of the lung or that the first portion

was large enough to contain cells from the more peripheral airways as well.

Conclusion

Inhaled histamine induced a bronchospasm in healthy subjects. This bronchospasm was blocked by cetirizine, and is thus mediated by the H1 receptors. Cetirizine, however, did not influence the cell composition in the bronchoalveolar lavage fluid, suggesting that the increased number of inflammatory cells in the bronchoalveolar lavage fluid observed after histamine challenge test is not mediated by H1 receptors.

ACKNOWLEDGEMENTS

We wish to thank Christine De Vos, Ph. D., Research and Development UCB Pharmaceutical Sector, Belgium, and Charlota Jonsen, UCB Sweden, for valuable discussions, UCB for provision of drugs, and UCB and the Medical Faculty, University of Umeå for financial support.

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