

Comparison of the efficacy of prednisolone, montelukast, and omalizumab in an experimental allergic rhinitis model*

Mete Kaan BOZKURT^{1**}, Baykal TÜLEK², Banu BOZKURT³, Nalan AKYÜREK⁴, Mehmet ÖZ⁵, Aysel KIYICI⁶

¹Department of Otolaryngology - Head and Neck Surgery, Faculty of Medicine, Selçuk University, Konya, Turkey

²Department of Pulmonary Diseases, Faculty of Medicine, Selçuk University, Konya, Turkey

³Department of Ophthalmology, Faculty of Medicine, Selçuk University, Konya, Turkey

⁴Department of Pathology, Faculty of Medicine, Gazi University, Ankara, Turkey

⁵Experimental Research Center, Selçuk University, Konya, Turkey

⁶Department of Biochemistry, Faculty of Medicine, Mevlana University, Konya, Turkey

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Aim: To compare the efficacy of prednisolone, montelukast, and omalizumab in reducing allergic symptoms and inflammation at tissue level in an experimental allergic rhinitis model.

Materials and methods: Forty Sprague Dawley rats were randomized into 5 groups as naive (NS/NC), sensitized/challenged (S/C) by subcutaneous ovalbumin antigen injection, and montelukast-, prednisolone-, and omalizumab-treated groups. A nasal allergen challenge was performed every day from day 20 to day 26. The number of sneezes and nasal/eye rubbing movements, IL-4 and CysLT levels in serum, nasal and bronchoalveolar lavage fluids determined by ELISA, and histopathological findings of nasal mucosa, sinus, and lung tissues were compared.

Results: All of the treatments significantly controlled the allergic symptoms of sneezing and nasal/eye rubbing ($P < 0.05$). IL-4 and CysLT levels on days 20 and 26 were significantly higher in the S/C group compared to the NS/NC group ($P < 0.05$). Montelukast significantly decreased serum and nasal IL-4 and CysLT levels ($P < 0.05$), prednisolone decreased nasal lavage IL-4 and CysLT levels ($P < 0.05$), and omalizumab lowered nasal lavage CysLT levels ($P < 0.05$).

Conclusion: Prednisolone, montelukast, and omalizumab were found to be effective in controlling the allergic symptoms of allergic rhinitis and upper/lower airway inflammation in an experimental allergic rhinitis model.

Key words: Experimental allergic rhinitis, airway inflammation, prednisolone, montelukast, omalizumab, immunoglobulin E, cysteinyl leukotriene, interleukin-4

1. Introduction

Allergic rhinitis (AR), the most common form of noninfectious rhinitis, is a symptomatic disorder of the nose induced after allergen exposure by an immunoglobulin E (IgE)-mediated inflammation of the membranes lining the nose (1,2). Antigen-presenting cells located in the nasal mucosa present allergens to naive T lymphocytes and initiate a complex series of events, leading to the differentiation of effector T lymphocytes and the production of T helper 2 (Th2) cytokines and antigen-specific IgE. The crosslinking of allergens to specific IgEs, bound to the surfaces of mast cells (MC) and basophils accumulated in the airway mucosa, induces

cell activation and generation of inflammatory mediators including histamine, cysteinyl leukotrienes (CysLT), prostaglandins, platelet-activating factors, and cytokines, which initiate the allergic response (1,3). The response depends on the structure of the target organ; typically, in the nose the response includes itching, sneezing, anterior or posterior rhinorrhea, and blockage; in the lungs, the response includes bronchoconstriction and wheeze due to smooth muscle contraction. The vast majority of patients with asthma have AR and 10%–40% of patients with AR have asthma, suggesting the concept of “one airway, one disease”, although there are differences between AR and asthma (4,5).

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** Correspondence: bozkurtmetekaan@hotmail.com

The increased prevalence of AR, its impact on quality of life, its economic costs, and its association with asthma underlie the need for new treatment options for this disease. In addition to well-known treatments, new pharmacological modalities such as montelukast (a CysLT receptor antagonist) and omalizumab (a recombinant humanized monoclonal anti-IgE antibody of mouse origin) have become increasingly important in the treatment of AR and asthma (6–12). Clinically, montelukast improves daytime nasal symptoms and nasal peak inspiratory flow in patients with seasonal AR (6,7). Omalizumab binds to the Cε3 domain of the free IgE molecule and downregulates the number of FcεRI on effector cells in peripheral blood and target organs. It is indicated as an add-on therapy to improve asthma control in subjects with severe and persistent allergic asthma who have a positive skin test or in vitro reactivity to a perennial aeroallergen (8,9). Omalizumab treatment decreases serum IgE levels and the number of IgE+ cells and eosinophils in the airway mucosa and the sputum (10,11), and it reduces the allergic response to adenosine 5'-monophosphate (12).

Animal models of AR and asthma are useful in evaluating the efficacy of new antiinflammatory agents in controlling the allergic response. Previously, montelukast has been shown to decrease nasal symptoms and the number of eosinophils in nasal mucosa and lung tissue, inhibit the production and release of inflammatory cytokines, and reverse structural changes in the lungs (13–17). However, there are no studies showing the efficacy of omalizumab in an experimental allergy model.

Corticosteroids are the most effective antiinflammatory agents. They decrease the production of inflammatory cells by bone marrow, the activation and migration of these cells into the tissue, arachidonic acid production by mast cells, microvascular permeability, alveolar macrophages, and the production of prostaglandin and CysLT (18). In this study, we aimed to evaluate the inflammatory changes in the nose, sinuses, and lungs in an experimental allergic rhinitis model and compared the efficacy of montelukast and omalizumab with prednisolone, a potent corticosteroid, on clinical symptoms and inflammation at tissue level.

2. Materials and methods

The study was approved by the experimental animal ethics committee of Selçuk University's Experimental Medicine Research and Application Center (date 18.02.2008; number 2008/17).

2.1. Animals

Forty Sprague Dawley rats, 6 to 8 weeks old, were randomized into 5 groups as naive [no sensitization, no allergen challenge (NS/NC)]; sensitized, challenged [no treatment (S/C)]; sensitized, challenged, montelukast

sodium-treated (MT) (Singulair, Merck, USA); sensitized, challenged, prednisolone-treated (PT) (Prednol-L Flacon, Mustafa Nevzat İlaç San., Turkey); and sensitized, challenged, omalizumab-treated (OT) (Xolair, Novartis, Switzerland) groups.

2.2 Sensitization procedures

Except for the NS/NC group, all rats were sensitized on the first day by subcutaneous (SC) injection of 1 mL of physiological saline containing 2 mg of ovalbumin antigen (OVA grade V, Sigma Labs, USA) mixed with 100 mg of AL(OH)₃ on the back. The rats in the naive group were given 1 mL of saline SC injection. The NS/NC group provided a baseline to assess the effects of challenges. Local challenges were performed every day from day 20 to day 26 by dripping OVA in physiological saline (4 mg mL⁻¹, 20 µL) into bilateral nasal cavities using a micropipette. The NS/NC rats received saline.

2.3. Treatment procedures

Montelukast sodium at 10 mg/kg was given orally from day 20 to day 26, 1 h before the local challenge. Prednisolone at 5 mg/kg was given intramuscular from day 20 to day 26, 1.5 h before the local challenge. One dose of subcutaneous omalizumab (0.5 mg) was given on day 20, 8 h before the local challenge. NS/NC and S/C rats were given saline orally.

2.4. Evaluation of nasal symptoms

The animals were placed into an observation cage (1 animal per cage) for about 10 min for acclimatization before the experiment. Ten minutes after the local challenge, the number of sneezes and nasal/eye rubbing movements were counted for 30 min according to the method described by Narita et al. (19).

2.5. Blood, nasal lavage, and bronchoalveolar lavage samples

Blood specimens were obtained from the tail vein on day 0 from the control group and on days 20 and 27 from the rats in all groups. On day 20, nasal lavage was performed by administering 0.2 mL of saline into the nasal cavities using a 22-G intracatheter. During these procedures, xylazine (10 mg/kg, intraperitoneal) and ketamine (50 mg/kg, intraperitoneal) were used for anesthesia. On day 20, 2 rats in the NS/NC and S/C groups and 1 rat in the MT group were lost. Bronchoalveolar lavage (BAL) was performed after the rats were sacrificed with cervical dislocation. The tracheas were dissected from the upper airway and 22-G catheters were placed in proximal tracheas and stabilized. BALs with 3 × 10 mL of saline containing 1 mM EDTA were performed and the samples were centrifuged at 400 × g and 4 °C for 5 min and the cells and supernatant were separated. All samples were stored at -80 °C until biochemical analysis was performed.

2.6. Biochemical examination

A rat IgE ELISA kit (Immunology Consultants Laboratory, USA), rat IL-4 ELISA kit (BenderMed, USA), and cysteinyl leukotriene EIA kit (Cayman Chemical Company, USA) were used in the study. IgE and CysLT concentrations are given as ng/mL. IL-4 concentrations are given as pg/mL.

2.7. Pathological examination

One set of nasal mucosa, sinus, and lung tissue was processed with 4% formaldehyde and embedded in paraffin for histopathological and immunohistochemical observation. The tissues were stained with hematoxylin and eosin (H&E) and the inflammation in the epithelium and subepithelium in the nasal and sinus mucosa was evaluated. The inflammation was scored as: 0, no inflammation; 1+, mild inflammation; 2+, moderate inflammation; and 3+, severe inflammation. In lung tissue, the inflammation around the bronchi, bronchioles, interstitium, and pulmonary venules was evaluated. The ratios of inflammatory cells in BAL were also calculated.

Immunohistochemical staining was performed using the streptavidin-biotin peroxidase method. CD4 (clone 1F6, 1:50 dilution, Santa Cruz Biotechnology, USA), MC tryptase (MCT, clone 10D11, 1:150 dilution, NovoCastra, UK), and MC chymase (MCC, clone CC1, 1:100 dilution, NovoCastra) antibodies were used for immunohistochemistry and the number of MCs per 100 cells was calculated.

2.8. Statistical analysis

Parameters were compared using the Kruskal–Wallis test, and in the case of any statistical differences, the values were compared between the 2 groups using the Mann–Whitney U test. The values taken on days 20 and 27 were compared using the Wilcoxon signed-rank test. The inflammation scores measured in histopathological sections and inflammatory cell numbers were compared using chi-square and Kruskal–Wallis tests and $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Clinical findings

Both sneezing and nasal/eye rubbing movements were significantly higher in the S/C group compared to the naive and treated groups ($P < 0.05$) (Table 1).

3.2. Biochemical results

In the S/C group, median IgE levels in serum and nasal lavage obtained on days 20 and 27 and in BAL samples increased significantly compared to the NS/NC group (Table 2, Mann–Whitney U test; $P < 0.05$). In treatment groups, median IgE levels in serum and nasal lavage specimens were higher than in the NS/NC group ($P < 0.05$); however, there were no significant differences in BAL IgE levels among the treatment groups and the NS/NC group ($P > 0.05$). Serum IL-4 levels on days 20 and 27 were significantly higher in the S/C group compared to the naive group (Table 2, $P < 0.05$). Montelukast treatment significantly lowered serum IL-4 levels on day 27 ($P = 0.04$), whereas the decrease was not remarkable in the PT and OT groups ($P > 0.05$). Montelukast and prednisolone treatment lowered nasal lavage IL-4 levels ($P = 0.02$ and 0.017 , respectively), whereas omalizumab treatment did not ($P > 0.05$). BAL IL-4 levels were highest in the S/C and OT groups and lowest in the MT and PT groups ($P < 0.05$). Median serum CysLT levels on days 20 and 27 were significantly higher in the S/C group than the NS/NC group (Table 2, $P < 0.05$). On day 27, montelukast, prednisolone, and omalizumab lowered serum, nasal lavage, and BAL CysLT levels compared to the S/C group ($P < 0.05$).

3.3. Pathological results

3.3.1. Nasal specimens

The nasal specimens of the S/C group showed an intense inflammation with plasma cells, lymphocytes, and eosinophils in the epithelium and subepithelium (Figure

Table 1. The number of sneezes and nasal/eye rubbing movements in the sensitized animals with and without treatment during nasal ovalbumin challenges between days 20 and 26, as compared to the NS/NC (naive) group, as monitored 30 min after the challenge.

Groups	n	Sneezes (30 min)	Nasal/eye rubbings (30 min)
NS/NC	6	1.58 ± 1.06 (1.25)	7.66 ± 2.8 (7.74)
S/C	6	17.2 ± 5.7 (15)	43.6 ± 5.16 (44.3)
Montelukast	7	8.86 ± 4.2 (7.5)	16.8 ± 6.6 (16)
Steroid	8	10.88 ± 7.2 (9.2)	18.6 ± 4.8 (16.8)
Omalizumab	8	8 ± 7.3 (5.75)	13.6 ± 7.8 (12.5)
P-value		0.002*	<0.001*

*: Kruskal–Wallis test. Significant difference among 5 groups.

Table 2. IgE, IL-4, and cysteinyl LT levels in serum, nasal lavage, and BAL.

	Serum, day 0	Serum, day 20	Serum, day 27	Nasal lavage, day 20	Nasal lavage, day 27	BAL
IgE levels ng/mL (median)						
NS/NC	113.9	116.2	119.4	114.5	124.4	60.1
S/C		218.3*	248.9*	213.7*	232.3*	106*
Montelukast		225.5*	211*	211*	207.5*	53.2
Prednisolone		224.2*	211.7*	212.6*	206.7*	53.3
Omalizumab		217.0*	209.7*	212.10*	205.3*	56.8
IL-4 levels pg/mL (median)						
NS/NC	5.48	5.66	5.92	2.44	2.47	4.25
S/C		6.13*	6.69	2.37	3.06	8.05*
Montelukast		6.34*	5.78**	2.79	2.30**	2.44
Prednisolone		6.90*	6.23	2.93	2.47**	2.72
Omalizumab		8.43*	7.04	2.82	2.86	7.52*
Cysteinyl LT (ng/mL) (median)						
NS/NC	2.13	2.5	3.15	2.13	2.43	2.28
S/C		4.36*	8.55*	2.92	3.25	3.69*
Montelukast		4.78*	1.82**	2.5	1.24**	2.99
Prednisolone		3.58*	2.09	2.39	1.85	2.78
Omalizumab		4.25*	2.32	3.24	2.11**	2.73

*: Mann–Whitney U test. Significantly higher than in the NS/NC group.

**: Wilcoxon signed-rank test. Significantly different before and after the treatment.

1b). The median inflammatory scores in the epithelium and submucosa were 3 and 2, respectively, in the S/C group (Figure 1b); 0 in the NS/NC group (Figure 1a); 0 and 0.50 in the PT group (Figure 1c); 1.5 and 2 in the OT group (Figure 1d); and 1 and 1 in the MT group (Figure 1e) ($P < 0.001$ for the epithelium and $P = 0.001$ for the submucosa, respectively).

The numbers of both MCC and MCT (number per 100 cells) in the nasal mucosa were higher in the S/C group compared to the NS/NC and treatment groups ($P < 0.001$) (Table 3). The numbers of MCT were higher in the PT group compared to the MT and OT groups, whereas the numbers of MCC did not differ among the drug groups.

3.3.2. Sinus specimens

In all groups, there was moderate-to-severe inflammation in the epithelium and submucosa of sinus specimens ($P > 0.05$). The numbers of both MCC and MCT (number per 100 cells) in the sinus mucosa were higher in the S/C group compared to the NS/NC and treatment groups ($P < 0.001$), with no differences among the 3 medications (Table 3).

3.3.3. Lung specimens

In the S/C group, a follicular bronchitis characterized by numerous lymphoid follicles in the bronchial walls and a moderate-to-intense inflammation composed of eosinophils, lymphocytes, neutrophils, and plasma cells were observed (Figure 2b). In the bronchioles and pulmonary venules, there was a moderate-to-intense infiltration of eosinophils, lymphocytes, neutrophils, and plasma cells, whereas the interstitium was normal. In the PT group, there were few eosinophils and lymphocytes in the bronchial walls (Figure 2c), whereas in the NS/NC, OT, and MT groups, a mild follicular bronchitis and eosinophil and lymphocytic infiltration were observed in the bronchial walls (Figures 2a, 2d, and 2e). Bronchioles, pulmonary venules, and the interstitium were normal.

3.3.4. Bronchoalveolar lavage specimens

The median percentage of eosinophils in BAL specimens was 35% in the S/C group, 0% in the NS/NC group, 15% in the PT group, 20% in the OT group, and 10% in the MT group, which was statistically significant ($P < 0.001$).

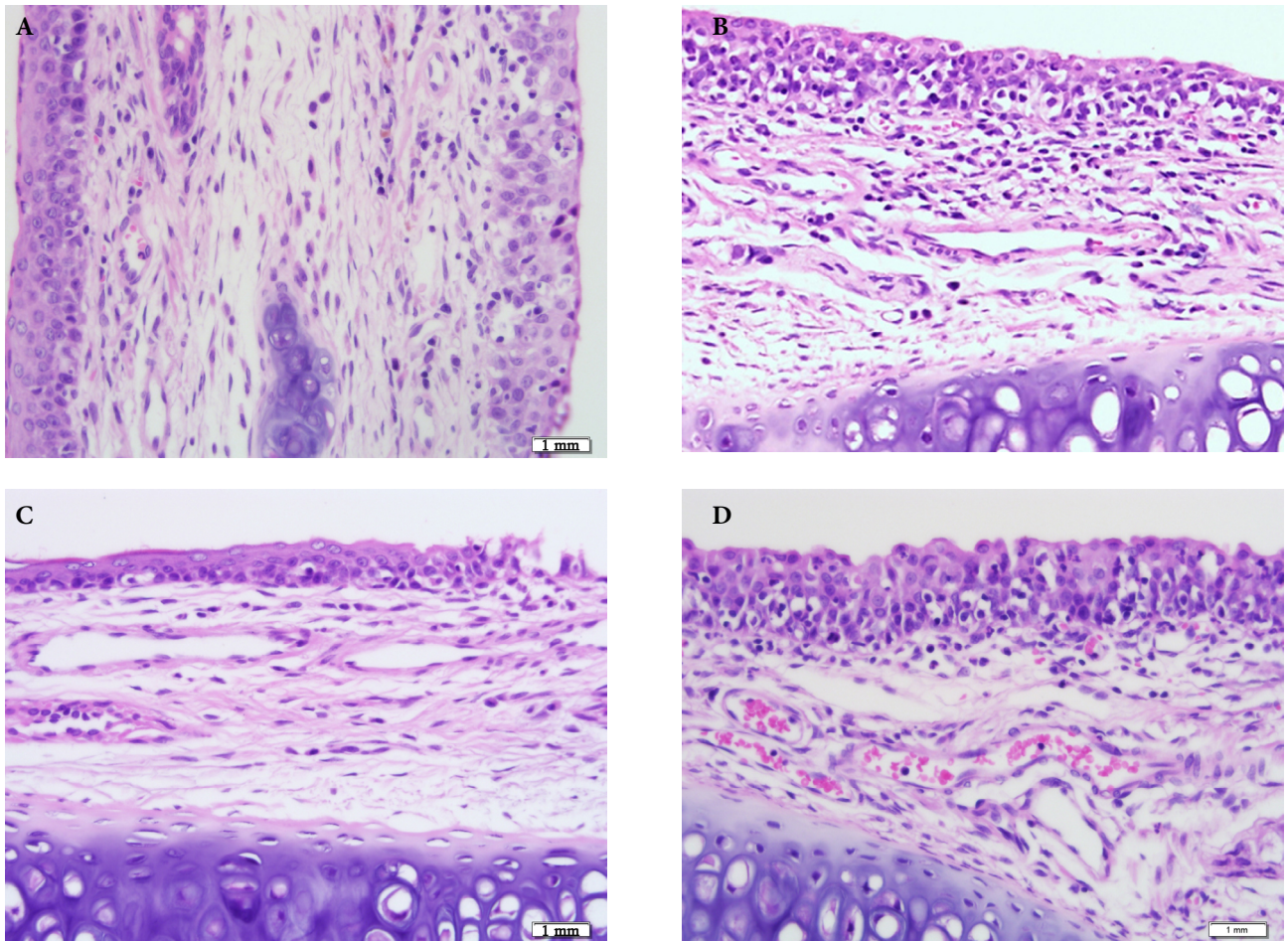


Figure 1. Nasal mucosa: A) NS/NC group. Only a few plasma cells and lymphocytes are seen (H&E, 200 $\times$). B) S/C group. Intense inflammation, including plasma cells, eosinophils, and lymphocytes in the epithelium (3+) and subepithelium (2+) (H&E, 200 $\times$). C) Prednisolone group. Mild inflammation (1+) with a few plasma cells, lymphocytes, and eosinophils in the epithelium and subepithelium (H&E, 200 $\times$). D) Omalizumab group. Mild inflammation (1+) including eosinophils, neutrophils, plasma cells, and lymphocytes in the epithelium; moderate inflammation (2+) in the plasma cells, lymphocytes, and eosinophils in the subepithelium (H&E, 200 $\times$). E) Montelukast group. Mild inflammation (1+) including a few plasma cells and lymphocytes in the epithelium and subepithelium (H&E, 200 $\times$).

4. Discussion

In the present study, 1 dose of OVA SC injection and repeated intranasal booster sensitization for 7 days resulted in a successful allergy model with clinical symptoms of sneezing and eye/nasal rubbing in Sprague Dawley rats. We also measured the levels of IgE, IL-4, and CysLT in serum, nasal lavage, and BAL after sensitization/challenge and compared the efficacy of montelukast and omalizumab with prednisolone against allergic symptoms and biochemical parameters. IL-4 is one of the most important cytokines, taking a role in allergic inflammation via B cell activation, IgE switch, specific IgE production, and the activation of other inflammatory cells. CysLTs, which are arachidonic acid-derived lipid mediators, have been linked to several processes in AR and asthma (20,21): 1) dilation of blood

vessels and increased vascular permeability, leading to nasal congestion; 2) increased mucus production and secretion, leading to rhinorrhea; 3) recruitment of inflammatory cells from the bloodstream into tissue, thus perpetuating the inflammatory response; 4) constriction of smooth muscles, leading to bronchoconstriction; and 5) collagen formation. Previously, CysLTs were recovered in the nasal washings of patients with allergic rhinitis following nasal challenges with specific allergens, and their concentrations were shown to be directly related to the dose of the allergen (22). Intranasal instillation of LTD4 was shown to induce nasal blockage (an increase of specific airway resistance) by dilatation of nasal blood vessels in S/C guinea pigs, which was largely blocked with pranlukast, a CysLT antagonist and naphazoline (23).

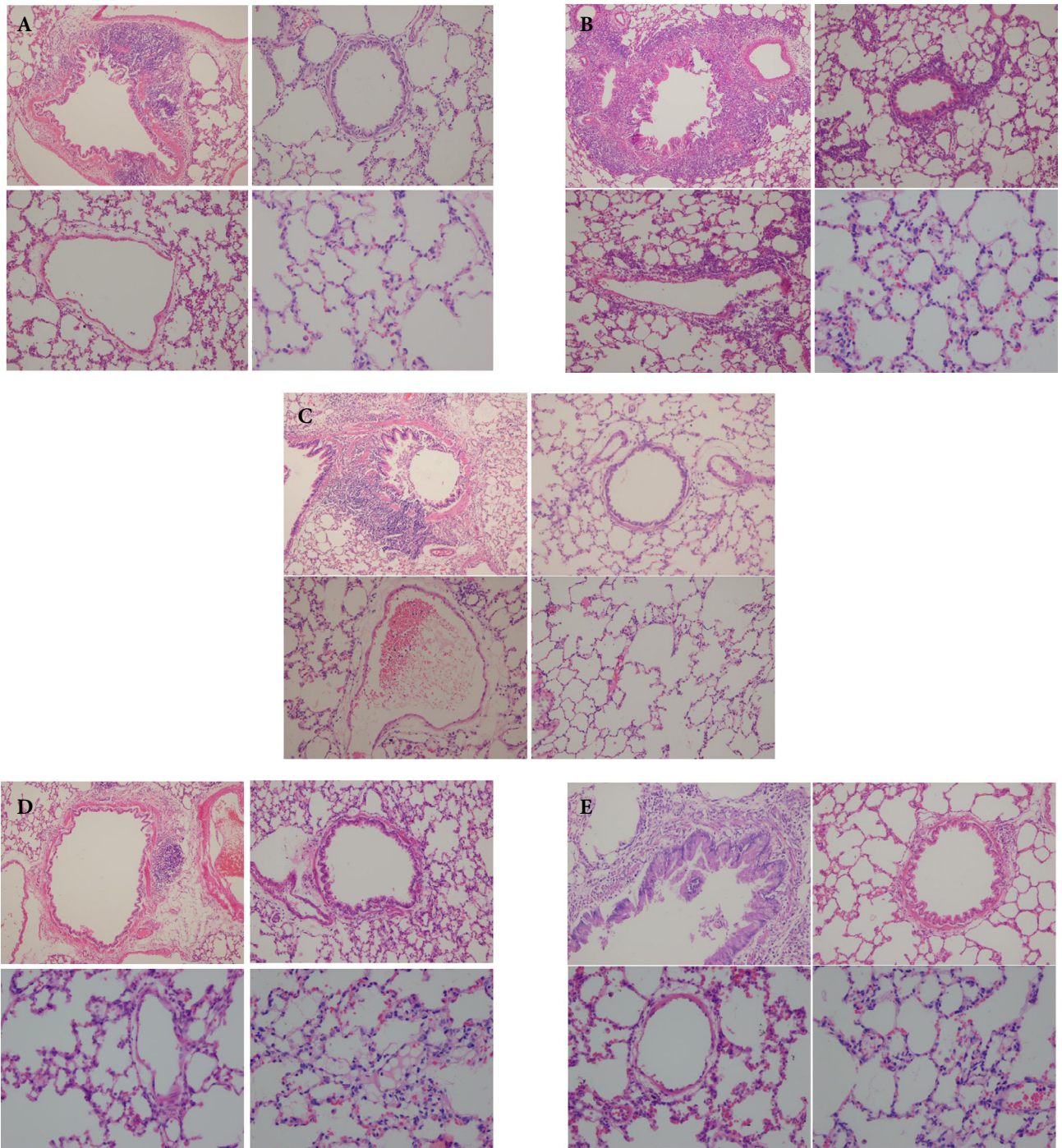


Figure 2. Lungs: bronchial wall (upper left, H&E, 100×), bronchioles (upper right, H&E, 100×), pulmonary venules (lower left, H&E, 200×), and interstitium (lower right, H&E, 200×). A) NS/NC group. Mild follicular bronchitis characterized by a few lymphoid follicles and a few eosinophils and lymphocytes in the bronchial wall. Bronchioles, pulmonary venules, and interstitium were normal. B) S/C group. Numerous lymphoid follicles with moderate infiltration of eosinophils, lymphocytes, neutrophils, and plasma cells around the bronchi, bronchioles, and pulmonary veins. The interstitium was normal. C) Prednisolone group. Mild inflammation with a few eosinophils and lymphocytes in the bronchial wall. Bronchioles, pulmonary venules, and interstitium were normal. D) Omalizumab group. Mild follicular bronchitis characterized by a few lymphoid follicles and a few eosinophils and lymphocytes in the bronchial wall. Bronchioles, pulmonary venules, and interstitium were normal. E) Montelukast group. Mild follicular bronchitis characterized by a few lymphoid follicles and a few eosinophils and lymphocytes in the bronchial wall. Bronchioles, pulmonary venules, and interstitium were normal.

Table 3. The comparison of mast cell numbers located in the mucosa of nasal and sinus specimens of S/C animals with and without treatment with the NS/NC (naive) group.

Median number of mast cells (min-max)	NS/NC	S/C	Prednisolone	Montelukast	Omalizumab
Nasal mucosa					
MCT	0 (0-1)	5 (3-5)*	2 (2)**	0 (0)	0 (0-3)
MCC	0 (0)	2 (1-5)*	0 (0)	0 (0)	0 (0-2)
Sinus mucosa					
MCT	0 (0)	3 (3-5)*	0 (0)	0 (0)	0 (0-5)
MCC	0 (0)	1 (1-2)*	0 (0-1)	0 (0)	0 (0-2)

MCT: Mast cell tryptase positive cells. MCC: Mast cell chymase positive cells.

*: Mann-Whitney U test. Significantly higher in the S/C group than the NS/NC and treatment groups.

** : Mann-Whitney U test. Significantly higher in the prednisolone group than the montelukast and omalizumab groups.

Inhaled LTD4 was also shown to elicit airway eosinophil influx in guinea pigs, which persisted as long as 4 weeks after a single exposure; pranlukast significantly inhibited both the bronchoconstriction and the eosinophilia (24). Therefore, CysLT antagonists are good options for the treatment of AR and asthma. In the study by Wu et al. (13), high doses of montelukast reduced serum IgE levels and BAL IL-4 and IL-5 levels in OVA-sensitized BALB/c mice challenged with the inhalation of OVA. In the study by Henderson et al. (21), montelukast inhibited the presence of Charcot-Leyden-like crystals in airway macrophages and the increased IL-4 and IL-13 mRNA expression in lung tissue and protein in BAL fluid seen in OVA-treated mice. In this study, IgE, IL-4, and CysLT increased significantly in sensitized groups. Montelukast and prednisolone decreased IL-4 levels remarkably, whereas omalizumab had no effect. CysLTs levels decreased after treatment with montelukast, omalizumab, and prednisolone; the most remarkable efficacy was with montelukast treatment.

Previous clinical and experimental studies evaluated the efficacy of antiinflammatory medications in controlling the nasal allergic symptoms and mucosa inflammation at the tissue level (16,23,25-27). Roa et al. (16) showed that montelukast decreased nasal symptoms in a dose-dependent manner and significantly lowered the number of eosinophils in both bone marrow and nasal tissue in mice. In the study by Shimizu et al. (25), intranasal instillation of OVA induced hypertrophic and metaplastic changes in the goblet cells of the nasal epithelium, accompanied by an increase in intraepithelial mucosubstance and eosinophilic infiltration. Dexamethasone and a CysLT antagonist decreased mucous secretion, whereas H1 antagonist, dexamethasone, and antirrat antiserum decreased eosinophil infiltration (25). Patients with perennial AR who received long-term nasal corticosteroid spray

experienced significantly less sneezing and nasal itching compared to the controls and had a significantly lower number of Langerhans cells; CD3+, CD4+, and CD8+ cells; and MC and eosinophils in the nasal mucosa (26). Jacobson et al. (27) showed that fluticasone reduced nasal symptoms significantly and inhibited seasonal infiltration of the nasal epithelium and subepithelium by MCs and eosinophils in patients with AR. In our study, prednisolone, montelukast, and omalizumab reduced allergic symptoms significantly and inhibited the infiltration of the nasal epithelium by MCs, lymphocytes, and eosinophils. The ranking of median inflammatory scores in the epithelium and submucosa in the nasal specimens from highest to lowest was the S/C group, the OT group, the MT group, the PT group, and the NS/NC group. MC infiltration of sinus mucosa epithelium also decreased after 3 treatments.

Although AR and asthma are 2 different clinical entities, these 2 conditions seem to be manifestations of 1 syndrome, the chronic allergic respiratory syndrome, in 2 parts of the respiratory tract (4,5). In most of the nonasthmatic AR patients, inflammatory changes were also detected in lower airways (28). In a previous study by McCusker et al. (29), although the allergen particles were detected in mouse nares, the eosinophilic infiltration was remarkable both in the upper and lower airways. In our study, we observed a follicular bronchitis and a moderate-to-intense inflammation composed of eosinophils, lymphocytes, neutrophils, and plasma cells in bronchi, bronchioles, and pulmonary venules, consistent with the hypothesis that the upper and lower airways are closely linked.

In asthma, chronic allergen-induced airway inflammation and IgE-mediated mast cell activation contribute to tissue eosinophilia and airway remodeling, characterized by goblet hyperplasia, mucus plugging of airways, subepithelial fibrosis, deposition of extracellular

matrix proteins, airway smooth muscle hypertrophy, and loss of pulmonary function (30). In experimental asthma models, montelukast was shown to decrease lung inflammation by inhibiting eosinophil trafficking/degranulation, Th2 cytokine release, and structural changes in the lungs (13–15,17,21). In the study by Wu et al. (13), high dose montelukast reduced IL-4, IL-5, and IL-13 mRNA expression in lungs in OVA-sensitized BALB/c mice challenged with inhalation of OVA. In the study by Henderson et al. (14), BALB/c mice, sensitized by intraperitoneal OVA, received intranasal OVA periodically from days 14 to 73 and montelukast or dexamethasone or placebo from days 73 to 163. Montelukast and dexamethasone both decreased eosinophil infiltration and goblet cell metaplasia. Only montelukast reversed the established increase in airway smooth muscle mass and subepithelial collagen deposition and reduced CysLT1 receptor expression. In the study by Muz et al. (15), montelukast treatment decreased eosinophilic infiltration, goblet cell hyperplasia, mucous occlusion, and fibrosis in lung specimens. Harrison et al. (17) showed that montelukast treatment decreased contraction in small bronchi after challenge with LTD4 and OVA. Montelukast also inhibited plasma protein extravasation and eosinophilic infiltration in the airway epithelium, airway walls, and alveolar connections. Administration of montelukast during the intranasal OVA challenge period was shown to reduce the airway eosinophil infiltration, Th2 cytokine release, mucus plugging, smooth muscle hyperplasia, and subepithelial fibrosis in mice (21). In another study, pretreatment with pranlukast significantly inhibited both the bronchoconstriction

and the eosinophilia in the bronchial epithelium and subepithelium (24). Omalizumab was also shown to inhibit the infiltration of IgE+ cells, eosinophils, and T and B lymphocytes in the airway mucosa (10,11). In our study, consistent with previous studies, prednisolone, montelukast, and omalizumab reduced inflammatory cell infiltration in the airway mucosa. On the other hand, in the allergy model of Brozmanova et al. (31), montelukast therapy did not reduce inflammation in nasal and lung specimens, even though cough was reduced. It also did not alter CysLT levels in the lung tissue.

In this study, after sensitization and challenge, eosinophil infiltration was detected in one-third of the BAL specimens and was significantly decreased by prednisolone, montelukast, and omalizumab. In the study by Careau et al. (32), the number of neutrophils, alveolar macrophages, eosinophils, and lymphocytes increased remarkably in BAL fluid after 24 h of challenge. Omalizumab (10), montelukast (13), and pranlukast (24) were shown to decrease eosinophil infiltration in sputum and BAL.

In conclusion, systemic OVA antigen sensitization and nasal challenge induced allergic symptoms and inflammation in the nasal mucosa and lung tissue, which were effectively controlled by prednisolone, montelukast, and omalizumab. CysLT, which increased significantly in serum, nasal lavage, and BAL fluid, might be an important marker in AR.

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References

1. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, Zuberbier T, Baena-Cagnani CE, Canonica GW, van Weel C et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy* 2008; 63 (Suppl. 86): 8–160.
2. Poole JA, Rosenwasser LJ. The role of immunoglobulin E and immune inflammation: implications in allergic rhinitis. *Curr Allergy Asthma Rep* 2005; 5: 252–8.
3. Stone KD, Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol* 2010; 125 (Suppl. 2): 73–80.
4. Togias A. Rhinitis and asthma: evidence for respiratory system integration. *J Allergy Clin Immunol* 2003; 111: 1171–83.
5. Bachert C, Vignola AM, Gevaert P, Leynaert B, Van Cauwenberge P, Bousquet J. Allergic rhinitis, rhinosinusitis, and asthma: one airway disease. *Immunol Allergy Clin North Am* 2004; 24: 19–43.
6. Chervinsky P, Philip G, Malice MP, Bardelas J, Nayak A, Marchal JL, Bousquet J, Tozzi CA, Reiss TF. Montelukast for treating fall allergic rhinitis: effect of pollen exposure in 3 studies. *Ann Allergy Asthma Immunol* 2004; 92: 367–73.
7. Mucha SM, deTineo M, Naclerio RM, Baroody FM. Comparison of montelukast and pseudoephedrine in the treatment of allergic rhinitis. *Arch Otolaryngol Head Neck Surg* 2006; 132: 164–72.
8. Milgrom H, Fick RB, Su JQ, Reimann JD, Bush RK, Watrous ML, Metzger WJ. Treatment of allergic asthma with monoclonal anti-IgE antibody. rhuMAB-E25 Study Group. *N Engl J Med* 1999; 341: 1966–73.
9. Bousquet J, Wenzel S, Holgate S, Lumry W, Freeman P, Fox H. Predicting response to omalizumab, an anti-IgE antibody, in patients with allergic asthma. *Chest* 2004; 125: 1378–86.
10. Djukanović R, Wilson SJ, Kraft M, Jarjour NN, Steel M, Chung KF, Bao W, Fowler-Taylor A, Matthews J, Busse WW et al. Effects of treatment with anti-immunoglobulin E antibody omalizumab on airway inflammation in allergic asthma. *Am J Respir Crit Care Med* 2004; 170: 583–93.

11. van Rensen EL, Evertse CE, van Schadewijk WA, van Wijngaarden S, Ayre G, Mauad T, Hiemstra PS, Sterk PJ, Rabe KF. Eosinophils in bronchial mucosa of asthmatics after allergen challenge: effect of anti-IgE treatment. *Allergy*. 2009; 64: 72–80.
12. Prieto L, Gutiérrez V, Colás C, Tabar A, Pérez-Francés C, Bruno L, Uixera S. Effect of omalizumab on adenosine 5'-monophosphate responsiveness in subjects with allergic asthma. *Int Arch Allergy Immunol* 2006; 139: 122–31.
13. Wu AY, Chik SC, Chan AW, Li Z, Tsang KW, Li W. Anti-inflammatory effects of high-dose montelukast in an animal model of acute asthma. *Clin Exp Allergy* 2003; 33: 359–66.
14. Henderson WR, Chiang GK, Tien Y, Chi EY. Reversal of allergen-induced airway remodeling by CysLT1 receptor blockade. *Am J Respir Crit Care Med* 2006; 173: 718–28.
15. Muz MH, Devעי F, Bulut Y, İlhan N, Yekeler H, Turgut T. The effects of low dose leukotriene receptor antagonist therapy on airway remodeling and cysteinyl leukotriene expression in a mouse asthma model. *Exp Mol Med* 2006; 38: 109–18.
16. Roa J, Morikawa H, Crawford L, Baatjes A, Duong M, Denburg JA. The effects of montelukast on tissue inflammatory and bone marrow responses in murine experimental allergic rhinitis: interaction with interleukin-5 deficiency. *Immunol* 2007; 122: 438–44.
17. Harrison S, Gatti R, Baraldo S, Oliani KL, Andre E, Trevisani M, Gazzieri D, Saetta M, Geppetti P. Montelukast inhibits inflammatory responses in small airways of the Guinea-pig. *Pulm Pharmacol Therapeut* 2008; 21: 317–23.
18. Taylor IK, Shaw RJ. The mechanism of action of corticosteroids in asthma. *Respir Med* 1993; 87: 261–7.
19. Narita S, Asakura K, Kataura A. Effects of thromboxane A2 receptor antagonist (Bay u 3405) on nasal symptoms after antigen challenge in sensitized guinea pigs. *Int Arch Allergy Immunol* 1996; 109: 161–6.
20. Busse WW. Leukotrienes and inflammation. *Am J Respir Crit Care Med* 1998; 157: S210–3.
21. Henderson WR Jr, Tang LO, Chu SJ, Tsao SM, Chiang GKS, Jones F, Jonas M, Pae C, Wang H, Chi EY. A role for cysteinyl leukotrienes in airway remodeling in a mouse asthma model. *Am J Respir Crit Care Med* 2002; 165: 108–16.
22. Shaw RJ, Fitzharris P, Cromwell O, Wardlaw AJ, Kay AB. Allergen-induced release of sulphidopeptide leukotrienes and LTB4 in allergic rhinitis. *Allergy* 1985; 40: 1–6.
23. Mizutani N, Nabe T, Imai A, Sakurai H, Takenaka H, Kohno S. Markedly increased nasal blockage by intranasal leukotriene D4 in an experimental allergic rhinitis model: contribution of dilated mucosal blood vessels. *Jpn J Pharmacol* 2001; 86: 170–82.
24. Underwood DC, Osborn RR, Newsholme SJ, Torphy TJ, Hay DW. Persistent airway eosinophilia after leukotriene (LT) D4 administration in the guinea pig: modulation by the LTD4 receptor antagonist, pranlukast, or an interleukin-5 monoclonal antibody. *Am J Respir Crit Care Med* 1996; 154: 850–7.
25. Shimizu T, Hirano H, Majima Y, Sakakura Y. A mechanism of antigen-induced mucus production in nasal epithelium of sensitized rats. A comparison with lipopolysaccharide-induced mucus production. *Am J Respir Crit Care Med* 2000; 161: 1648–54.
26. Holm AF, Godthelp T, Fokkens WJ, Severijnen EA, Mulder PG, Vroom TM, Rijntjes E. Long-term effects of corticosteroid nasal spray on nasal inflammatory cells in patients with perennial allergic rhinitis. *Clin Exp Allergy* 1999; 29: 1356–66.
27. Jacobson MR, Juliusson S, Löwhagen O, Balder B, Kay AB, Durham SR. Effect of topical corticosteroids on seasonal increases in epithelial eosinophils and mast cells in allergic rhinitis: a comparison of nasal brush and biopsy methods. *Clin Exp Allergy* 1999; 29: 1347–55.
28. Braunstahl GJ, Fokkens WJ, Overbeek SE, KleinJan A, Hoogsteden HC, Prins JB. Mucosal and systemic inflammatory changes in allergic rhinitis and asthma: a comparison between upper and lower airways. *Clin Exp Allergy* 2003; 33: 579–87.
29. McCusker C, Chicoine M, Hamid Q, Mazer B. Site-specific sensitization in a murine model of allergic rhinitis: role of the upper airway in lower airways disease. *J Allergy Clin Immunol* 2002; 110: 891–8.
30. Jeffery PK. Pathology of asthma. *Br Med Bull* 1992; 48: 23–39.
31. Brozmanova M, Plevkova J, Bartos V, Plank L, Tatar M. Antileukotriene treatment and allergic rhinitis-related cough in guinea pigs. *J Physiol Pharmacol* 2005; 56 (Suppl. 4): 21–30.
32. Careau E, Sirois J, Bissonnette EY. Characterization of lung hyperresponsiveness, inflammation, and alveolar macrophage mediator production in allergy resistant and susceptible rats. *Am J Respir Cell Mol Biol* 2002; 26: 579–86.