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ANTILEUKOTRIENE TREATMENT AND ALLERGIC RHINITIS-RELATED COUGH IN GUINEA PIGS

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Experimental allergic rhinitis produces enhanced cough response in awake guinea pigs. Leukotriene receptor antagonists, as anti-inflammatory agents, have been effective in treatment of asthma and allergic rhinitis to inhibit the early and late allergic response. In the present study, we evaluated the effect of montelukast (Singulair, Merck, USA) on the cough reflex in an experimental model of allergen-induced rhinitis in guinea pigs. Guinea pigs (n=16) were sensitized with intraperitoneal ovalbumin (OVA). The animals were then used to develop a model of allergic rhinitis by repeated intranasal instillation of 0.5% OVA at weekly intervals for 8 weeks. Allergic rhinitis was evaluated from the occurrence of typical clinical symptoms including sneezing, conjunctival and nasal secretion, or nasal acoustic phenomenon. Between the 6th and 8th nasal challenge (NCh) the animals (n=8) were treated daily for 14 days with oral montelukast (10mg/kg). Cough was induced by citric acid aerosol inhalation in gradually increasing concentration (0.05-1.6 M) and was evaluated before sensitization and then after the 1st, 6th, and 8th nasal challenge when rhinitic symptoms were most conspicuous. The intensity of cough was significantly increased after the first and repeated nasal OVA challenges, and reduced after the 8th NCh that was preceded with montelukast treatment [9(6-14) vs. 16.5(14-22) vs. 25.5(23-42) vs. 8.5(8-13); P=0.0003]. We conclude that antileukotriene therapy suppresses the stimulating effect of experimental allergic rhinitis on the chemically-induced cough in awake guinea pigs.

Key words: *allergic rhinitis, antileukotrienes, citric acid-induced cough, guinea pig, montelukast*

INTRODUCTION

Many theories have been postulated concerning the link between allergic rhinitis and asthma. Given that the upper and lower airways do have the same epithelial lining, it is perhaps hardly surprising that each shows similar reaction to inhalant allergens and irritants (1). Increased levels of inflammatory factors in the blood, and propagation of inflammation through the airway or systemic pathway, can be a possible mechanism for lower airway dysfunction among patients with upper airway disease such as rhinitis (2). Nevertheless, the underlying pathology of both asthma and rhinitis is inflammation in which eosinophilia is a characteristic feature. Recent studies supporting this idea have shown the migration of eosinophils into the upper and lower respiratory mucosa after segmental allergen challenge in patients with allergic rhinitis (2).

The most common cause of chronic cough is a group of related conditions of chronic rhinitis, sinusitis, and postnasal drip (3). In these cases, the cough reflex may be sensitized through an action of inflammatory mediators from the nasal mucosa, a reflex sensitization of airway sensory nerves, or the facilitation of the central cough generator from nasal reflex input (3-5).

It is now recognized that chronic inflammation plays a key role in asthma and allergic rhinitis, and the release of numerous mediators contribute to the inflammatory reaction. It has been postulated that the cysteinyl-leukotrienes, including LTC₄, LTD₄, and LTE₄, may be important mediators in airway inflammation. The main source of these leukotrienes is an increased number of airway inflammatory cells, including eosinophils, basophils, and mast cells after allergen inhalation by sensitized subjects. Hence, if their production/synthesis or action could be inhibited then the underlying inflammatory process should be controlled (6, 7).

On the basis of experimental and clinical practice we supposed that antileukotrienes, as anti-inflammatory agents, may prevent the action of various proinflammatory mediators including leukotrienes, histamine, prostaglandins, kinins, and therefore reduce enhanced cough sensitivity. In this study we followed the effects of montelukast, a potent and selective CysLT₁ receptor antagonist that is effective in inhibiting the early and late allergic response (6).

MATERIAL AND METHODS

Animals

The study was approved by the Ethics Committee of the Jessenius Faculty of Medicine in Martin. Twenty seven adult male Trik strain guinea pigs, weighing 250-350 g were used for the study. The animals were acclimatized in cages at a mean temperature of 24°C for 1 week after delivery to the animal house and allowed free access to water and standard rodent diet. All guinea pigs received humane care in compliance with the national guidelines.

Ovalbumin sensitization

After acclimatization to laboratory conditions, the animals were sensitized. They were passively sensitized with intraperitoneal ovalbumin (10 µg, Sigma-Aldrich, St. Louis, MO) administered together with aluminum hydroxide (100 mg) in 1 ml saline using a method described by Underwood et al (8). Twenty-one days later, successful sensitization was confirmed by the intradermal injection of OVA (25 µl of 200 µg ml⁻¹) into the dorsal back surface. Sensitized animals were used for the experiments 7 days later.

Model of allergic rhinitis

Experimental guinea pigs (n=16) were used to develop a model of allergic rhinitis by repeated intranasal instillation of 0.015 ml of 0.5% OVA separately for each nostril using a thin catheter. The animals were challenged at 7-day intervals, 8 times in total. Control animals (n=11) were repeatedly challenged intranasally with saline in the same doses as the experimental ones.

Evaluation of clinical symptoms

After nasal provocation, the allergic rhinitis was evaluated from the occurrence of typical clinical symptoms including sneezing, conjunctival and nasal secretion, and nasal acoustic phenomenon reflecting the degree of nasal obstruction. The symptoms were monitored during a period of 1 hr after nasal challenge in each animal.

We evaluated the number of sneezes per 1 h starting after intranasal OVA challenge and other nasal symptoms, such as nasal acoustic phenomenon and lacrimation, using a scoring system. Symptom scores were graded on a 4-point scale. Each grade was assigned a numerical score (0-3), and data were analyzed as both separate symptoms and a total symptom score. Nasal acoustic symptom scores were graded in points as follow: 0 - none; 1 - impaired inspiration, alar breathing; 2 - nasal crackles; 3 - intensive nasal crackles and severe breathing impairment. Lacrimation scoring was done as follows: 0 - none; 1 - hazy eyes; 2 - intensive lacrimation; 3 - manifested conjunctivitis. Both phenomenon scores were summed up to obtain one value. The maximum total score might be 6.

Antileukotriene treatment

Between the 6th and 8th nasal challenge (for 14 days) the experimental guinea pigs (n=8) received daily oral montelukast (10 mg/kg, Singulair, Merck, USA). The other OVA animals were given saline in the same manner.

Experimental groups

Three groups of guinea pigs were studied: (a) OVA-sensitized guinea pigs (OVA, n=8); (b) OVA-sensitized animals treated with montelukast (OVA-MK, n=8); (c) guinea pigs with instilled saline (control, n=11).

Chemically-induced cough

Awake guinea pigs were individually placed in a body plethysmograph box (type 855, Hugo Sachs Electronic, Germany) and were exposed to citric acid aerosol (Lachema) in double gradually increasing concentrations (from 0.05 to 1.6 M). Physiological saline was used as the first challenge. The citric acid aerosol was generated by a jet nebulizer (Pariprovocation test I, Pari Starneberg, Germany; output 5 l min⁻¹, particles mass median diameter 1.2 µm) and delivered to the head chamber of the plethysmograph box. Respiratory changes in the airflow were measured with a

pneumotachograph (Godart, Germany) and a Fleish head connected to the head chamber and were recorded with a moving pen recorder (Multiscriptor Hellige 21, Germany). The appearance of cough was detected with a microphone placed in the roof of the head chamber and connected to a tape recorder. The airflow signal and cough sound were simultaneously recorded in PC for an off-line analysis.

Cough was recorded during 30 s inhalation of each concentration of the tussigen. The interval between some exposures was 1 min. To quantify the intensity of cough reaction, the cough response was expressed as a total number of coughs during all citric acid challenges. The cough response was evaluated on the basis of sudden enhancement of expiratory airflow accompanied by a typical cough sound. The cough sound was analyzed from power spectra using fast Fourier transformation (9). Cough was elicited before sensitization and then 30 min after the 1st, 6th, and 8th NCh, corresponding to successive weeks, when rhinitic symptoms were most conspicuous.

Histological assessment

At the end of the experiment, samples of nose, larynx, trachea, bronchi, and lungs were removed to describe histomorphological findings. All tissues were fixed in 10% buffered neutral formalin, dehydrated in graded alcohol concentrations, and embedded in paraffin. Material in paraffin blocks was cut and stained routinely with hematoxylin and eosin. All slides were evaluated by two observers-pathologists for both qualitative and quantitative analysis, using a semiquantitative approach based on the agreement of the observers. The histopathological assessment was performed by light microscopy.

Statistical analysis

Values are expressed as median and interquartile range. Statistical analysis was performed using one-way analysis of variance. If a significant difference was detected, the individual group differences were determined by the Duncan multiple range test. A probability value $P < 0.05$ was considered as significant.

RESULTS

Effects of repeated nasal challenges on clinical symptoms of allergic rhinitis

Repetitive nasal OVA challenge in the sensitized animals led to a significant increase in the frequency of sneezing in the experimental group compared with controls, starting from the 1st challenge (*Table 1*) and continuing to the end of challenges. The results showed a gradual increase in sneeze response that reached a significant difference at the 5th challenge, as comparing with initial value [21(13-30) vs. 8(5-11); $P < 0.05$] (*Table 1*). Other symptoms, such as nasal acoustic phenomenon and lacrimation, increased in the experimental group, starting from the 3rd and continuing to the last challenge (*Table 2*). Significant differences in the symptom score between the experimental and control groups occurred from the 1st week of nasal challenges and persisted till the 6th nasal provocation. Sneezes were rare and there were no other symptoms in the control animals. Our data suggest individual variability in sneezing and other clinical symptoms. Clinical symptoms of allergic rhinitis arose in 5-10 min and variably

Table 1. The number of sneezes in the sensitized animals over the course of weekly nasal ovalbumin challenges compared with those in the control group challenged with saline, as monitored during 1 h after the challenge.

	Nasal antigen challenges (weeks)					
	1	2	3	4	5	6
Experiment (n=16)	8(5)*	10(8)*	14(16)*	15(9)*	21(17)* #	15(14)*
Control (n=11)	1(2)	2(2)	1(2)	3(5)	0(1)	2(5)

Data are median and interquartile range. *Difference between experimental and control animals at the corresponding challenges; #different from the initial value; $P < 0.05$ for all.

Table 2. Symptom scores, summed for nasal acoustic phenomenon and lacrimation, in the sensitized animals over the course nasal ovalbumin challenge compared with those in the control animals challenged with saline, as monitored during 1 h after nasal challenges.

	Nasal antigen challenges (weeks)					
	1	2	3	4	5	6
Experiment (n=16)	2(1)*	3(1)*	3(1)*#	3(1)*#	4(1)*#	4(1)*#
Control (n=11)	0	0	0	0	0	0

Data are median and interquartile range. *Difference between the experimental and control animals at the corresponding challenges, #different from the initial value; $P < 0.05$ for all.

persisted from 20 to 40 min. In addition, no guinea pig died of anaphylactic shock during sensitization or challenge procedures. *Table 1* and *Table 2* show symptoms of experimental allergic rhinitis only over the time course of 6 nasal challenges, because after the 6th NCh the experimental group (n=16) was divided into two subgroups: OVA animals with montelukast treatment (n=8) and OVA animals without the treatment (n=8). There were no significant differences in symptoms between the two groups of animals observed after the 7th and 8th NCh, although a mild tendency to a decrease in symptoms occurred in the treated animals.

The effects of repeated nasal OVA challenges and antileukotriene therapy on citric acid-induced cough

The citric acid cough response, induced 30 min after the 1st nasal OVA challenge, significantly increased in the sensitized animals compared with the control nonsensitized animals challenged with saline [15.5(10-18) vs. 7(4-9); $P < 0.05$] (*Fig. 1*). The increase in the citric acid-induced cough persisted in the same animals after the 6th nasal OVA challenge compared with controls [20.5(13-25.5) vs. 6(4-8); $P < 0.01$] (*Fig. 1*). There were no significant differences between the experimental animals before sensitization and the control ones (*Fig. 1*).

When the citric acid-induced cough is analyzed in the group of the sensitized animals alone, its intensity significantly increased after nasal OVA challenge at 1 week (OVA 1) and 6 weeks (OVA 6) compared with the initial value, and then was reduced after the 8th challenge that was preceded by montelukast treatment

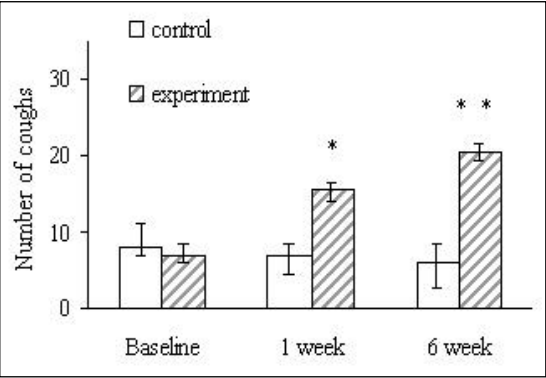


Fig. 1. Citric acid-induced cough before sensitization (baseline) and at 1 and 6 weeks' nasal challenges in experimental (ovalbumin; hatched bars) and control (saline; empty bars) animals. The number of coughs is expressed as median and interquartile range. * $P<0.05$ and ** $P<0.01$ compared with the corresponding control.

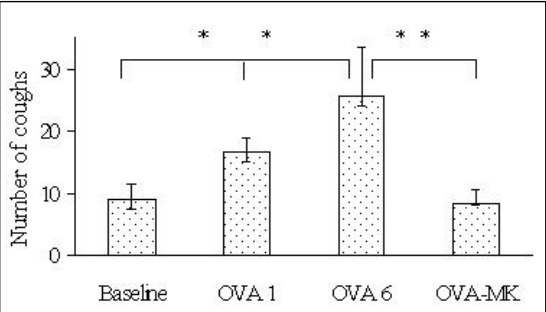


Fig. 2. Citric acid-induced cough in experimental animals before sensitization (baseline) and at the 1st (OVA 1), 6th (OVA 6), and 8th nasal ovalbumin challenge (OVA-MK), the latter preceded by montelukast treatment. The number of coughs is expressed as median and interquartile range. ** $P<0.01$.

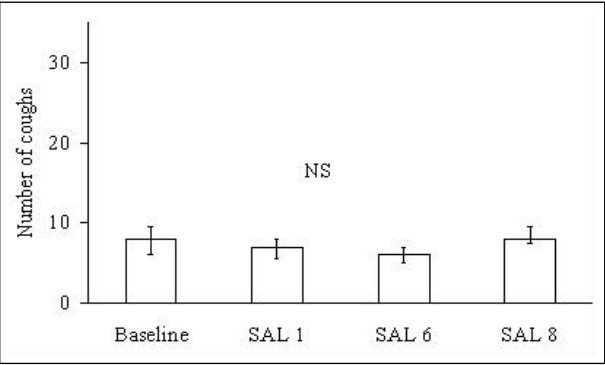


Fig. 3. Citric acid-induced cough in control animals before sensitization (baseline) and at the 1st (SAL 1), 6th (SAL 6), and 8th nasal saline challenge (SAL 8). The number of coughs is expressed as median and interquartile range. There were no significant differences (NS).

[9(6-14) vs. 16.5(14-22) vs. 25.5(22.5-42) vs. 8.5(8-13); $P=0.0003$] (Fig. 2). In contrast, there was no significant difference in the citric acid cough response in the control group of animals challenged with saline at the same time intervals as those for OVA sensitized animals [8(4-11) vs. 7(4-9) vs. 6(4-8) vs. 8(7-11); $P=0.427$] (Fig. 3).

Finally, comparison of the citric acid cough response at the 8th NCh among the three groups of animals showed a stimulating effect of repeated nasal ovalbumin challenge (OVA, $n=8$) and retraction of the stimulation after montelukast (OVA-

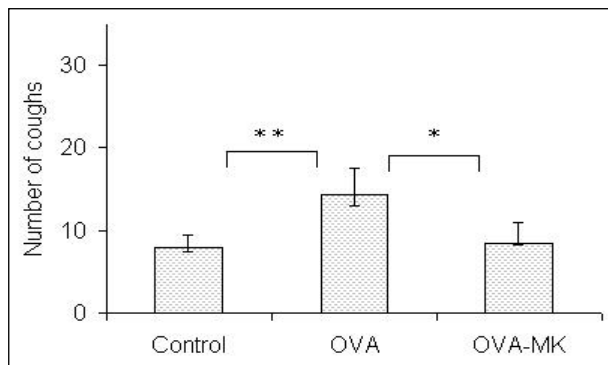


Fig. 4. Stimulation of citric acid-induced cough by nasal ovalbumin challenges (OVA) in sensitized animals and its reversal by montelukast (OVA-MK), compared with nonsensitized animals (Control). The number of coughs is expressed as median and interquartile range. Difference from control at ** $P < 0.01$ and * $P < 0.05$.

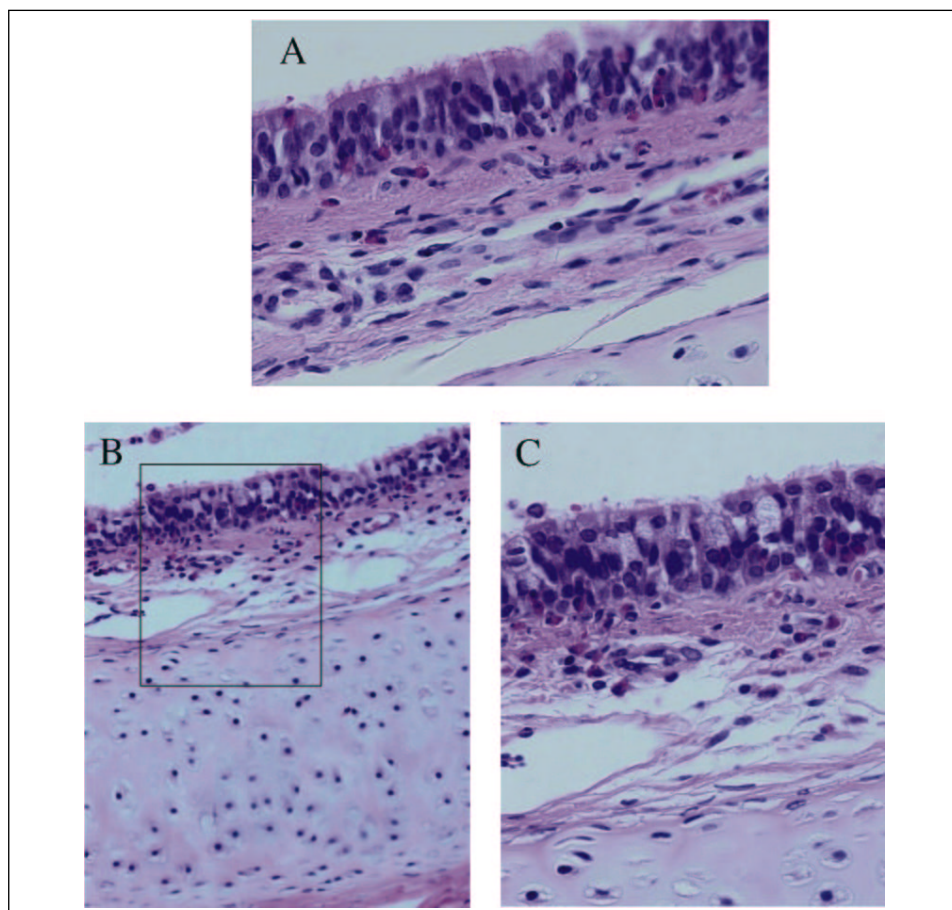


Fig. 5. Morphology of the laryngeal mucosa in control nonsensitized (A) and sensitized allergic rhinitis (B) guinea pigs. The area confined with the black square in B is blown up in C to underscore the excessive vascular dilatation, congestion, edema, and eosinophilic infiltration in the mucosa. Magnification - 4x in A and B, and 40x in C.

MK, n=8) in the sensitized animals toward the level seen in the nonsensitized animals (Control, n=11); [8.0 (7-11) vs. 14.5(13-20.5) vs. 8.5(8-13) for the nonsensitized, sensitized, sensitized + montelukast, respectively]. The differences in cough in these groups were significant (see *Fig. 4*).

Histological examination of the nasal, laryngeal and tracheal mucosae revealed excessive vascular dilatation, congestion, and edema in the OVA challenged animals. There were diffuse mucosal infiltrations with eosinophils, occasionally also with lymphocytes. In addition, diffuse mild hyperplasia of serous glands and epithelium was observed. In the lungs, there were some eosinophils around small bronchi. In the group of OVA-challenged animals with montelukast therapy (OVA-MK), the morphological picture, surprisingly, was similar, even though the cough was reduced. In contrast, the nasal, laryngeal, and tracheal mucosa of control animals was covered by columnar epithelium without pathological changes (*Fig. 5*).

DISCUSSION

A number of studies have focused on the early and late allergic response, especially in relation to pulmonary hyperresponsiveness and eosinophilic accumulation. It is evident that cysteinyl leukotrienes, released in concert with mucosal inflammation, contribute to rhinitis (7). They increase nasal secretion, nasal airway resistance, and nasal eosinophilic infiltration. Release of cysteinyl leukotrienes occurs in both naturally occurring and experimentally induced rhinitis (7, 10). It is known that the intensity of airway inflammation and cough is closely related to the number of mediators. Our previous studies have shown increased cough sensitivity in the early, but not late, allergic response in conscious guinea pigs (11, 12). The count of coughs was highest 24 h after nasal antigen challenge in sensitized conscious guinea pigs (13). The hyperreactive cough reflex, which demonstrates the correlation between chronic airway inflammation and cough response, has also been observed in immunochallenged guinea pigs, alongside increases in eosinophils in the airway epithelium, submucosa, and bronchoalveolar lavage compared with normal or passively sensitized animals (14). Exacerbated rhinitic clinical symptoms and enhanced cough sensitivity during the early allergic phase were confirmed in the present study in which multiple nasal OVA challenges were employed. The underlying mechanisms of these findings may have to do with increased sensitivity of nerve endings in the respiratory mucosa.

In the present study we also examined the efficacy of leukotriene D4 (LTD4) receptor antagonism in chemically-induced cough with respect to relief of symptoms of allergic rhinitis. We used montelukast, a selective LTD4 antagonist that may reduce cellular and non-cellular components of airway inflammation induced by antigen challenge. Clinical trials with montelukast have shown an

improvement in pulmonary function, symptoms, and a reduction in sputum and airway eosinophilia in asthma and allergic rhinitis (6, 15). In asthma, montelukast attenuates LTD4-induced bronchoconstriction and early and late airway responses to allergen (16). In this study we showed a significant decrease in the citric acid-induced cough after the commencement of montelukast administration in multiple-challenged guinea pigs. These findings indicate that oral antileukotriene therapy could inhibit cough of allergic rhinitis in concert with a reduction of symptoms and airway eosinophilia.

There is limited direct evidence, both clinical and experimental, to support the use of leukotriene antagonists as a first-line anti-eosinophilic inflammatory treatment. Likewise, data are conflicting in regard to improvement of symptoms, airway function, and reduction of airway eosinophilia by anti-leukotriens (7, 15). Our morphological findings showed some reduction in airway eosinophilia after montelukast treatment, although the effect was rather meager, which is in line with the findings of others (6, 7, 15). But montelukast was evidently effective in reducing the number of coughs evoked by inhalation of citric acid. The mixed data raise the possibility of intertwined and not yet full clear disease pathogenesis. It appears that the development of new anti-asthmatic treatments, including antileukotriene agents requires further investigations into rhinitis and asthma.

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