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EFFECTS OF INHALED CORTICOSTEROIDS ON COUGH IN AWAKE GUINEA PIGS WITH EXPERIMENTAL ALLERGIC RHINITIS - THE FIRST EXPERIENCE

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Allergic rhinitis is a common cause of chronic cough. Topical corticosteroids are regarded as the most effective first-time treatment in allergic rhinitis. In this study we evaluated the cough sensitivity during the early and late allergic responses in guinea pigs with experimental allergic rhinitis. Another aim of the study was to follow up the effect of inhaled beclomethasone dipropionate on the cough in guinea pigs with allergic rhinitis. 31 guinea pigs were sensitized with ovalbumin (OA). Animals were intranasally challenged with OA (experiment) or saline (control) in 7-day intervals for 9 weeks. Cough was induced by inhalation of citric acid aerosols in gradually increasing concentrations for 30 s and was evaluated 1 h after the 8th nasal challenge (NCH) and 17 h after the 9th NCH. Cough was significantly increased only during an early allergic response, 1 h after repeated NCH [18 (14-23) vs. 8 (3-10); $P<0.001$]. Five experimental animals were inhaling aerosol of beclomethasone dipropionate seven days between the 8th and the 9th NCH and cough was evaluated 1 h after the 9th NCH. Inhaled corticosteroids significantly inhibited the enhanced allergic rhinitis related cough [4 (1-9) vs.19 (9-37) vs. 6 (3-9); $P<0.05$].

Key words: *experimental allergic rhinitis, citric acid-induced cough, inhaled corticosteroids, guinea pig*

INTRODUCTION

It has been recognized that there is an association between allergic inflammation in the upper airway (allergic rhinitis) and in the lower airway (asthma) in many patients (1). The chronology of rhinitis and asthma is still under

discussion. From many studies, it appears that rhinitis often occurs before the onset of asthma and may therefore be a predictor of asthma. Several pathomechanisms could explain the association between rhinitis and asthma: nasal obstruction leading to mouth breathing, an increased deposition of inhaled allergen in lower airways, a nasal-bronchial reflex, microaspiration of nasal secretion, postnasal drip syndrome and an increased bronchial hyperresponsiveness in subjects with allergic rhinitis (2).

Asthma and allergic rhinitis are the most frequent causes of chronic cough (3). Both conditions have similar immunological mechanisms and underlying pathogenesis (4). The pathophysiological events following allergen exposure are described as biphasic, composed of an early (mediated by mast cells-derived mediators) and a late phase response (occurs from 4 h to 12 h after exposure and persists to 24 h) and involves increased recruitment and activation of inflammatory cells, such as T cells, neutrophiles, macrophages and eosinophiles. In the case of allergic rhinitis the cough reflex may be sensitized through an action of inflammatory mediators from the nasal mucosa or through sensitization of airway sensory nerves or facilitation of the central cough generator by nasal reflex inputs (5, 6).

Previous experimental evidence has demonstrated significantly enhanced cough reflex sensitivity during exudative allergic rhinitis in awake sensitized guinea pigs immediately after a nasal challenge (7). We supposed that airway inflammation during the late phase response after repeated nasal antigen challenges might affect afferent sensory nerve endings and modulate the cough response. Therefore, the goal of our study was to evaluate the cough reflex sensitivity in the early and late allergic response in sensitized awake guinea pigs after repeated nasal antigen challenges.

Topical corticosteroids are regarded as the most effective first-time treatment in allergic rhinitis (8). They are effective in relieving all of the symptoms of rhinitis and can be administered for long periods of time without the risk of significant side effects. They inhibit the secretion of cytokines and infiltration of inflammatory cells such as neutrophiles, eosinophiles, T-lymphocytes, and mast cells leading to improvement of nasal itching, watery rhinorrhea, sneezing, and nasal obstruction in rhinitic patients. On the base of experimental and clinical practice, we supposed that inhaled corticosteroids, as anti-inflammatory agents, may prevent the action of various inflammatory mediators and therefore may reduce enhanced cough reflex sensitivity.

MATERIAL AND METHODS

Animals

Thirty one adult male Trik strain guinea pigs weighing 250-350 g were used in the study. This experiment was approved by the Ethics Committee of the Jessenius Faculty of Medicine.

Ovalbumin sensitization

All animals were passively sensitized with ovalbumin (10 μg, Sigma) administered intraperitoneally together with aluminium hydroxide (100 mg) in 1 ml saline, by using a modified method of Underwood and co-workers (9). Twenty-one days later, successful sensitization was confirmed by an intradermal injection of ovalbumin (25 μl of 200 μg ml^{-1}) into the dorsal back surface. Sensitized animals were used for experiments 7 days later.

Model of allergic rhinitis

Sensitized experimental animals were used to develop model of allergic rhinitis by repeated intranasal instillation of 0.015 ml of 0.5% OA into both anterior nares using a thin catheter. Animals of the experimental group (n=20) were repeatedly intranasally challenged at 7-day intervals for nine weeks. Control animals (n=11) were challenged in a similar manner by using saline.

Evaluation of clinical symptoms

Immediately 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. Having a standard method, we individually monitored these symptoms during a period of 1 h after nasal challenge in each animal. We evaluated the frequency of sneezes and other nasal symptoms such as nasal acoustic phenomenon and lacrimation using a scoring system. Symptom scores were graded on a four-point scale. Each grade was assigned a numerical score (0-3), and data were analyzed both as separate symptoms and as a total symptom score. Nasal acoustic symptoms scores were graded in points as follow: 0 - none; 1 - impaired inspiration, alar breathing; 2 - nasal cracles; 3 - intensive nasal cracles and severe breathing impairment. Lacrimation scoring was done as follows: 0 - none; 1 - hazy eyes; 2 - intensive lacrimation; 3 - conjunctivitis. Both phenomenon scores were summed up to one value. The maximum total score might be 6.

Inhaled corticosteroids therapy

Between the 8^{th} and 9^{th} nasal antigen challenges five experimental animals were daily inhaling the aerosol of beclomethasone dipropionate (Aldecin, Schering-Plough Central East AG, Switzerland) in a body plethysmograph box (type 855, Hugo Sachs Electronic, Germany). The total dose of 350 μg of aerosol was sprayed into the plethysmograph chamber and animals were inhaling the aerosol for 3 minutes.

Chemically-induced cough

Awake guinea pigs were individually placed in a body plethysmograph box and were exposed to citric acid aerosol (Lachema) in double gradually increasing concentrations (from 0.05 to 1.6 M); each for 30 s. 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 body plethysmograph. Respiratory changes in the airflow were measured by using a pneumotachograph (Godart, Germany) with a Fleish head connected to the head chamber and were recorded with a moving pen recorder (Multiscriptor Hellige 21, Germany). Respiratory sounds, including cough and sneezing, were recorded with a microphone placed in the roof of the head chamber and connected to a tape recorder. Pneumotachograph and tape recorder outputs were simultaneously recorded on a PC for the off-line analysis.

Cough was recorded during 30 s inhalation of each concentration of the tussigen and during the subsequent 60 s observation time. Therefore, the interval between exposures was 1.5 min. The number of coughs was evaluated from the airflow trace on the basis of sudden enhancement of expiratory flow accompanied by a typical cough sound. Cough sound was analyzed from power spectra using fast Fourier transformation. This method is able to differentiate cough from sneezing (10). Cough response was expressed as the total number of coughs during all citric acid challenges to quantify the intensity of cough reaction.

The cough reflex was elicited 1 h after the 8th NCH and 17 h after the 9th NCH in both experimental (n=15) and control (n=11) animals. Five other experimental animals underwent inhaled corticosteroids between the 8th and 9th NCH and cough was provoked before the 1st NCH and 1 h after the 8th and 9th NCH.

Statistical analysis

All data (coughing, sneezing, clinical symptoms) 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 multiple range tests. A probability value of $P<0.05$ was considered as significant.

RESULTS

The effect of repeated nasal challenges on clinical symptoms of allergic rhinitis

Repetitive nasal ovalbumin challenge in sensitized animals led to a significant increase in the sneeze response in the experimental groups, starting from the 6th challenge (*Table 1*) and continuing to the end of challenges. In the control animals, sneezes rarely appeared. Significant differences in sneeze frequency were present between the experimental and control animals from the 4th to the end of challenges. The intensity of nasal acoustic phenomenon and lacrimation, expressed as the symptom score, was significantly enhanced in the experimental group, starting from the 6th and continuing to the last challenge (*Table 2*). Significant differences in the symptom score between the experimental and control groups occurred from the 3rd week of nasal challenges and persisted till

Table 1. Sneeze response in experimental allergic rhinitis.

	Weeks of nasal antigen challenge								
	1	2	3	4	5	6	7	8	9
Experiment (n=20)	2 (5)	1 (4)	5 (5)	6 (7)*	7 (12)*	13 (12)*#	15 (15)*#	15 (18)*#	13 (11)*#
Control (n=11)	1 (3)	1 (2)	3 (5)	1 (1)	1 (2)	2 (3)	2 (3)	3 (3)	2 (4)

The number of sneezes from the 1st to 9th weekly nasal ovalbumin challenges in the sensitized experimental animals compared with those in the control group challenged with saline, monitored during 1 h after the challenges. Data are expressed as median and intequartile range. *P <0.05 - significant difference between the experimental and control animals at the same week of challenge; #P<0.05 - significant difference compared with the initial value in the experimental group.

Table 2. Symptom score in experimental allergic rhinitis.

	Weeks of nasal antigen challenge								
	1	2	3	4	5	6	7	8	9
Experiment (n=20)	0 (1)	0 (2)	1 (3)*	1 (3)*	1 (5)*	2 (2)*#	3(2)*#	2(4)*#	2(3)*#
Control (n=11)	0	0	0	0	0	0	0	0	0

Symptom score (nasal acoustic phenomenon and lacrimation) in the sensitized experimental animals from the 1st to 9th weekly nasal ovalbumin challenges compared with those in the control group challenged with saline, monitored during 1 h after the challenges. Data are expressed as median and intequartile range. Both phenomenon scores were summed up to one value (the total score 0-6). *P<0.05 - significant difference between the experimental and control animals at the same week of challenge; #P<0.05 - significant difference compared with the initial value.

the 9th nasal provocation. In addition, there were no symptoms in the control animals. Taken together, these results clearly indicate that the 6th nasal challenge is the key time point of enhanced nasal responsiveness.

Our data suggest quite a large individual variability in sneezing and other clinical symptoms. Clinical symptoms of allergic rhinitis, manifesting as sneezing, rhinorrhea, and nasal crackles, arose in 5-10 min and individually persisted. In many animals, ovalbumin-induced allergic rhinitis with clinical symptoms lasted from 20 to 40 min, in others for about 1 h. A few animals showed an extremely long period of exudative allergic rhinitis-induced clinical symptoms, up to 3 h. However, no guinea pig died of anaphylactic shock during sensitization or challenging procedures.

The effect of repeated nasal challenges on citric acid-induced cough

Our findings have shown that the cough response to citric acid was significantly increased 1 h after the 8th nasal ovalbumin challenge compared with the control animals [18 (14-23) vs. 8 (3-10); P<0.001] (*Fig. 1*). The intensity of the cough reflex evoked 17 h after repeated NCH was not significantly different between the experimental and control groups [14 (10-18) vs. 9 (6-15); P=0.124] (*Fig. 1*), although there was a mild tendency to increase.

In the second experimental group (n=5), the intensity of citric acid-induced cough was significantly increased 1 h after the 8th NCH compared with the control value and significantly decreased after the 9th NCH that was preceded by inhaled beclomethasone dipropionate therapy (*Fig. 2*). Therefore, inhaled corticosteroids significantly inhibited the enhanced allergic rhinitis-related cough [4 (1-9) vs. 19 (9-37) vs. 6 (3-9); P<0.05].

DISCUSSION

Recently many researchers have extensively focused on the early and late allergic response especially in relation to pulmonary hyperresponsiveness and

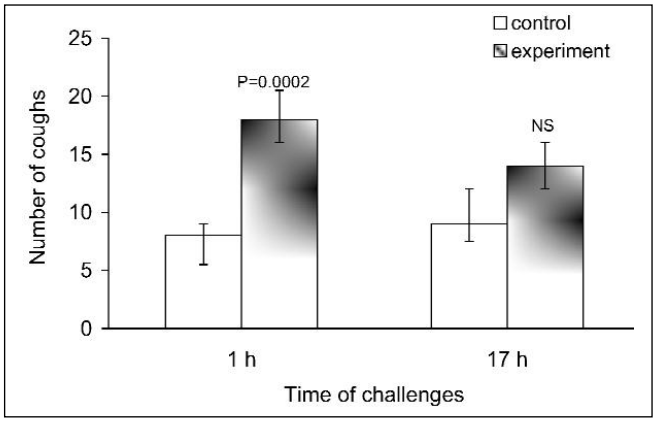

Fig. 1. Changes in citric acid-induced cough in guinea pigs 1 h after the 8th nasal challenge and 17 h after the 9th nasal challenge between the experimental animals sensitized with ovalbumin and the control animals challenged with saline. Data are expressed as median and interquartile range. NS - nonsignificant.

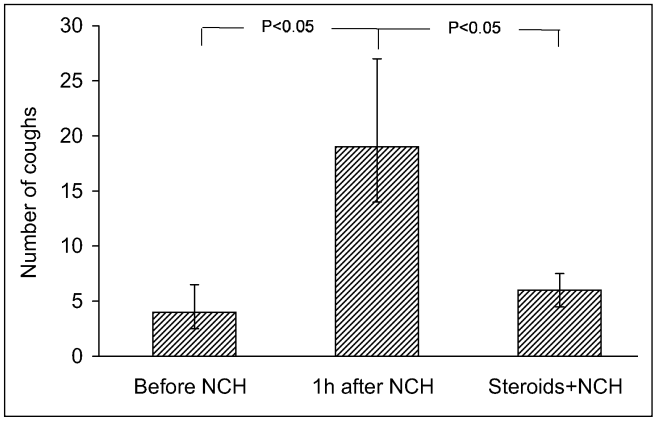

Fig. 2. Comparison of citric acid-induced cough changes in the sensitized experimental group of animals before nasal ovalbumin challenge, 1 h after the 8th nasal challenge, and 1 h after the 9th nasal challenge preceded by inhaled cortico-steroids therapy. Data are expressed as median and interquartile range.

eosinophil accumulation. The guinea pig has long been preferentially used as a model of allergic diseases, because the animal shows a very similar pulmonary response and histological findings following the antigen challenge to those seen during asthmatic attacks in patients (11). Despite some studies to the contrary, Nabe et al. (11) have convincingly indicated that the first, second, or third antigen challenge hardly produce the late allergic response. These authors further reported that repeated inhalations of antigen for long intervals of time lead to highly reproducible pulmonary dysfunction with the early and late airway responses to every repeated antigen challenge in the sensitized guinea pigs.

Our studies were focused not only on the cough response in the early phase after nasal antigen challenge but were expanded to include the late phase response following nine nasal antigen challenges. The present findings showed significantly enhanced cough sensitivity to citric acid in the guinea pigs, which corresponded only to the early allergic response after repeated nasal challenges. As above-mentioned, sensitivity of the cough reflex was increased during

ovalbumin-induced exudative rhinitis in guinea pigs (7). Riccio et al. (12) have shown that an acute allergen challenge lowers the mechanical threshold for activation of rapidly adapting receptors in guinea pig trachea in vivo. The hyperreactive cough reflex, which demonstrates the correlation between cough response and chronic airway inflammation, has been observed in the guinea pigs immunochallenged multiple times and was accompanied by a significant increase in eosinophiles in the airway epithelium, submucosa, and bronchoalveolar lavage, compared with normal or passively sensitized animals (13). Similar results have been obtained when cough reflex sensitivity to capsaicin challenge was studied in pollen-sensitive patients with seasonal allergic rhinitis. Their cough sensitivity to capsaicin was significantly increased not only in the pollen season but also out of season, compared with healthy volunteers (14).

On the other hand, our data revealed that the cough response provoked 17 h after repeated NCH was not significantly different, although there was a mild tendency for its increase. Our findings support the clinical observation of Minoguchi et al. (15) who reported no correlation between cough reflex sensitivity and airway inflammation 24 h after allergen challenge in asthmatic patients, although the airway responsiveness to histamine significantly increased.

It is generally known that beclomethasone dipropionate is a topically active corticosteroid used in the treatment of asthma and allergic rhinitis with marked anti-inflammatory, antiallergic and antiproliferative effects (16). This study was undertaken to examine the efficacy of inhaled beclomethasone dipropionate in chemically-induced cough with respect to relief of symptoms of allergic rhinitis. Our findings showed a significant decrease in citric acid-induced cough after the 9th nasal antigen challenge that was preceded by inhaled corticosteroids treatment. Therefore, our results indicated that the inhaled corticosteroid therapy completely blocked the stimulating effect of experimental allergic rhinitis on the chemically-induced cough in awake guinea pigs. These results conform to a recent study that showed that treatment with inhaled steroids increases the cough threshold to inhaled citric acid in patients with asthma (17). Similarly, treatment with inhaled steroids decreases the number of eosinophiles in sputum and also sensitivity to capsaicin in patients with eosinophilic bronchitis, another cause of chronic cough (18).

At the present stage, there are still many uncertain points in the understanding of the mechanisms of allergic rhinitis and asthma-related cough.

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