COUGH REFLEX SENSITIVITY TESTING IN ASTHMATIC PATIENTS AND HEALTHY VOLUNTEERS

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Abstract

Asthma and allergic diseases are complex conditions caused by a combination of genetic and environmental factors. Cough is a major symptom in some people with asthma. Cough reflex sensitivity (CRS) test with capsaicin is one of the most important method for cough studying. Cough reflex sensitivity was defined as the lowest concentration of capsaicin that elicited 2 or more (C2) and 5 or more coughs (C5). The aim of this study was to find out a cough reflex sensitivity in patients with asthma bronchiale and in healthy volunteers. In the present study we conclude that cough reflex sensitivity was significantly increased in asthmatic adult patients compared with healthy adult volunteers.

Key words: cough, cough sensitivity, asthma bronchiale, adult healthy volunteers

INTRODUCTION

Asthma is a chronic inflammatory condition of the airways. Chronically inflamed airways are hyperresponsive. They become obstructed and airflow is limited (by bronchoconstriction, mucus plugs, and increased inflammation) when airways are exposed to various risk factors (GINA update 2012), in the forms of infections, allergens, and environmental irritants (1). During an asthma attack, airways will produce excess mucus and swell, and muscles around the airways will tighten leading to airway obstruction, tightness in the chest, coughing, and wheezing (1).

Bronchial responsiveness is characterized by a change in airway caliber in response to bronchoconstrictor and/or bronchodilator stimuli. Bronchial hyperresponsiveness is defined as increased responsiveness in comparison with an expected response. This increased response of the airways can be measured by inhalation of specific or non-specific stimuli, and it should not be observed in normal subjects. Bronchial hyperresponsiveness is one of the main pathophysiological characteristics of asthma and is present in nearly all asthmatic patients, especially during symptomatic episodes. It can also be found in other diseases, such as allergic rhinitis, chronic obstructive pulmonary disease, cystic fibrosis and heart failure, or after a respiratory infection (2).

The cough reflex protects the airway and lungs from aspiration, inhaled irritants, particulates and pathogens and clears the air spaces of accumulated secretions. Studies in animals provide conclusive evidence that cough is initiated by activation of vagal afferent nerves (3). Airway nerves have the capacity to control airway functions via neuronal reflexes and through neuromediators and neuropeptides. Neuronal mechanisms are known to play a key role in the initiation and modulation of airway hyperresponsiveness and inflammation. In addition to the traditional transmitters such as norepinephrine in postganglionic sympathetic nerve fibres and acetylcholine in parasympathetic nerve fibres, a large number of neuropeptides have been identified to have different pharmacological effects on the

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muscle tone of the vessels and bronchi, mucus secretion and immune cells. Meanwhile, a broad range of stimuli including capsaicin, bradykinin, hyperosmolar saline, tobacco smoke, allergens, ozone, inflammatory mediators and even cold, dry air have been shown to activate sensory nerve fibres to release neuropeptides such as the tachykinins substance P and neurokinin A to mediate neurogenic inflammation (4). Other stimuli are known to initiate coughing in animals and in human subjects include articulate (dust, mechanical) vibratory stimulation of the airway mucosa, larynx or chest wall, chemical irritants such as resiniferatoxin, cinnamaldehyde and allyl isothiocyanate, anandamide and prostaglandin E2. Although seemingly varied in origin and chemical composition, many of these stimuli share common modes of action. For example, acids protons, capsaicin, resiniferatoxin and anandamide all act in part or entirely by activation of the ion channel receptor TRPV1 (3, 5). Cinnamaldehyde, allyl isothiocyanate and several other known respiratory irritants (e. g. cigarette smoke) activate the ion channel TRPA1 (3, 6, 7). The cough reflex is initiated by activation of bronchopulmonary C-fibres and the mechanically-sensitive cough receptors. Stimuli initiating cough through activation of one or both of these vagal afferent nerves include capsaicin, acid, or bradykinin. Multiple ion channels and cell surface receptors regulating the response to these tussive stimuli have been identified. The cough receptors and C-fibres may interact centrally to produce a heightened sensitivity to challenge. Interactions of afferent pathways innervating the esophagus and upper airways may contribute to the heightened cough sensitivity in chronic diseases such as gastroesophageal reflux disease, asthma and upper airways disorders (upper airway cough syndrome) – three of the most common causes of chronic cough (3).

Cough can be partitioned into four phases (3). The initial phase comprises the encoding of action potentials by the afferent nerves directly responding to the tussive stimulus, and the subsequent reconfiguration of the respiratory motor drive within the brainstem. This initial phase immediately precedes any change in respiratory muscle activity.

The second phase is the enhanced inspiratory effort.

The expiratory phase of cough has 2 components:

1. An initial compressive phase when expiration is initiated against a restricted or occluded upper airways,
2. The expulsive phase, when the upper airways are dilated, allowing forceful expiration and the high airflow velocities that facilitate airways clearance.

Depending on the stimulus and situation the number and forcefulness of the coughs can vary substantially. Given this complexity and the multiple elements involved lower and upper airways, respiratory muscles, brainstem, it is apparent that multiple afferent nerve subtypes act in concert to regulate the sensitivity, forcefulness and repetitions of coughs in response to all tussive stimuli (3).

The aim of our study was to evaluate difference of sensitivity of the cough reflex in adult asthmatic patients and healthy subjects, using the current European Respiratory Society guidelines (8) on the assessment of cough.

MATERIAL AND METHODS

The study was approved by Ethics Committee of Jessenius School of Medicine in Martin, Slovakia and Ethics Committee of Žilina Self-Governing Region, Slovakia. Informed consent was obtained from all asthmatic patients and healthy volunteers after the purpose of the test had been explained.

Subjects

The study population consists of 38 healthy volunteers (19 females, 19 males; mean age 32 years) and 19 adult patients with asthma bronchiale (14 females, 5 males; mean age 33 years). They were dispensed at allergology outpatient departments. Healthy volunteers

had no clinical respiratory symptoms, normal lung function test and no history of acute respiratory infection (last 4 weeks) and were recruited consecutively. They had no history of allergic, respiratory, cardiovascular, gastrointestinal and endocrine diseases, they were not treated with ACE-inhibitors. Required information was obtained from the questionnaire. Tiffeneau index (FEV1/FVC) in healthy volunteers was $> 85\%$. Smokers were excluded from the study.

The asthmatic patients had a characteristic history of asthma bronchiale. Asthmatic patients in our study had intermittent or mild persistent asthma bronchiale (GINA, 2012). All asthmatic patients were non-smokers. They had no history of acute respiratory infection (last 4 weeks) before or during the study. They were treated with inhaled glucocorticoids (beclomethasone dipropionate 100-250 μg , budesonide 200-400 μg , ciclesonide 80 μg , fluticasone furoate 100-250 μg). They were not treated with antihistamines and inhaled glucocorticoids at the time of examination. The last dose of the inhaled glucocorticoids were taken 24 hours before the cough reflex sensitivity test. Short-acting β -agonists were withheld for at least 12 hours before the CRS test. Tiffeneau index (FEV1/FVC) in asthmatic patients was $> 80\%$.

Study design

Based on a structured, interview-led questionnaire each subject was asked about history of asthma bronchiale, allergic, cardiovascular, gastrointestinal, endocrine, respiratory diseases, ACE inhibitor treatment. Pulmonary function testing was performed before and after the cough reflex sensitivity test. Subjects reported their subjective feeling during the cough reflex sensitivity test. All subjects gave written informed consent. Healthy volunteers were examined in the laboratory, bronchodilator therapy were available. Asthmatic patients were examined at outpatient department, where all of the medicaments of first aid were available.

Assessment of cough reflex sensitivity

European Respiratory Society published guidelines on the assessment of cough to the standardisation of cough challenge methodology (8). According to the guidelines, the single-breath dose-response method was preferred. The cough reflex sensitivity to capsaicin (SIGMA, St. Louis, MO) was determined using a compressed air-driven nebuliser (KoKo DigiDoser; nSpire health Inc, Louisville, CO, USA), that is modified by the addition of an inspiratory flow rate to $0.5 \text{ L}\cdot\text{s}^{-1}$ regardless of excessive inspiratory effort with each breath. Each subject inhaled up to 12 capsaicin aerosol concentrations ($0.49\text{--}1000 \mu\text{mol/l}$) during 1200 ms at 1 min intervals. Each subject inhaled randomly between incremental concentration of capsaicin the aerosol of a control solution (physiological saline) in order to increase cough challenge blindness. This strategy reduced the effect of voluntary suppression or conditioned responses in subjects, who would otherwise be anticipating progressively higher concentrations of capsaicin. When employing the single-breath method of capsaicin administration, the tussive response to each dose of aerosol is immediate and brief (8). Therefore, only coughs occurring within 15 s of capsaicin delivery were counted. For each test, the lowest concentration of capsaicin inducing two or more (C2) and five or more (C5) cough efforts were reported. Each concentration of tussigenic agent was inhaled once. Subjects were unaware that the induction of a specific number of coughs was the end-point of the study.

Data analysis

The values of the capsaicin cough reflex sensitivity were expressed as geometric mean value with 95 % confidence interval (CI) of the capsaicin concentration causing two or more (C2) and five or more (C5) cough efforts. C2 and C5 was calculated for each group. Differences between groups were analysed by non-parametric analysis of the Wilcoxon test, a value of $P < 0.05$ was considered to be significant.

RESULTS

The C2 capsaicin concentration was $2.03 \mu\text{mol/l}$ ($1.16\text{--}3.55$) in asthmatic patients. That was significantly less than $8.71 \mu\text{mol/l}$ ($5.62\text{--}13.50$) in healthy volunteers ($P < 0.001$). Cough reflex sensitivity expressed as C2 concentration of capsaicin was significantly increased in asthmatic adult patients (*Fig. 1*).

Fig. 1 Cough reflex sensitivity expressed as geometric mean and 95 % confidence interval of capsaicin concentration inducing 2 or more cough in healthy adult subjects (control C2) and in adult patients with asthma bronchiale (asthma C2)

The C5 capsaicin concentration was $3.02 \mu\text{mol/l}$ ($1.77\text{--}5.17$) in asthmatic patients. That was significantly less than $31.81 \mu\text{mol/l}$ ($20.52\text{--}49.33$) in healthy volunteers ($P < 0.001$). Cough reflex sensitivity expressed as the C5 capsaicin concentration was also significantly increased in the adult patients with asthma bronchiale (*Fig. 2*).

Spirometric indices (forced expiratory volume in 1 second – FEV1; forced vital capacity – FVC; Tiffeneau index – FEV1/FVC) did not show any significant changes before and after the CRS test in asthmatic adult patients and healthy control. During the testing of CRS, we did not observe any serious adverse effects. 96.5 % of subjects complained about transient throat itching accompanying cough at higher concentrations of inhaled capsaicin.

Fig. 2 Cough reflex sensitivity expressed as geometric mean and 95 % confidence interval of capsaicin concentration inducing 5 or more cough in healthy adult subjects (control C5) and in adult patients with asthma bronchiale (asthma C5)

DISCUSSION

Reflex coughing is induced by multiple mechanical, chemical, and irritant stimuli directed toward laryngeal and/or tracheobronchial mucosa (9). Cough is a common symptom of many respiratory and non-respiratory disorders (8). Chronic cough is one of the most common symptoms in patients with persistent asthma and may be the sole presenting feature of this disease (10). Asthma is characterized by bronchospasms accompanied with frequent coughing, but the pathogenesis is still not clear (11).

In the present study we assessed changes in cough reflex sensitivity by capsaicin in adult asthmatic patients and healthy adult volunteers. There is significant difference of sensitivity of the cough reflex among the patients with asthma bronchiale and the control group. We found that asthmatic patients have a lower cough thresholds than control group of healthy volunteers. Doherty et al. (10) showed that a reduced cough threshold is a frequent finding in airways disease associated with asthma. Use of the capsaicin challenge gives objective information about cough susceptibility which may prove more discriminatory than just questioning about the presence or absence of cough or sputum production. Patients with asthma have an increased sensitivity to capsaicin which is most marked in those who complain of cough (10). Nakajima et al. (12) showed that in asthmatics in whom cough is the sole or predominant symptom, CRS is significantly enhanced.

In our study cough reflex was assessed according to the guidelines of European Respiratory Society on assessment of cough. Each administration of capsaicin aerosol was performed at 1 minute interval with the inhalation time set at 1200 ms (8). In the study of Doherty et al. (10) subjects inhaled from the dosimeter beginning with saline control and then, with a minimum of 30 second intervals, increasing strengths of capsaicin until a given

inhalation caused five coughs (C5). This dose was repeated to ensure that a reproducible C5 response had been attained and, if so, that the value was recorded as the patient's value (C5 capsaicin). In the study of Nakajima et al. (12) was used continuous capsaicin inhalation (1 minute) at each concentration of capsaicin and they compared the results with those obtained using intermittent inhalation. Data showed that the threshold was significantly lower in asthmatic patients than in healthy individuals.

Asthmatic patients involved in the present study were not treated with inhaled glucocorticoids and antihistamines at the time of examination. In the study of Doherty et al. (10) the response to capsaicin was not related to treatment with inhaled corticosteroids but was increased in those using anticholinergic agents. In the study of Ekstrand et al. (13) the asthmatic patients displayed higher capsaicin cough sensitivity than the controls before the treatment period, but not afterward. Before treatment, capsaicin cough reaction correlated significantly with levels of exhaled nitric oxide, but not with methacholine sensitivity. After treatment with inhaled corticosteroids, the capsaicin cough sensitivity and the inflammatory parameters were normalized. In comparison to the control group, the patients reported more affective reactions to and behavioral disruptions induced by environmental irritants. In steroid-naïve patients with a positive methacholine test, there is a link between that part of the airway inflammation that is reflected by exhaled nitric oxide and that followed by an augmented reactivity of capsaicin-sensitive sensory nerves. This association disappears after steroid treatment (13).

Our data suggest that the capsaicin cough reflex sensitivity testing can reflect functional changes in the airway afferent nerve-endings mediating cough during asthma bronchiale. We conclude that airway afferent nerve endings reactivity in asthmatic subjects was significantly increased compared with healthy volunteers. Our data about increased CRS in asthmatics are consistent with data of other studies (10, 12).

A recent review of the 20-yr clinical experience with capsaicin failed to find a single serious adverse event associated with capsaicin cough challenge testing in humans (in the 122 published studies that described 4833 subjects). In the small fraction of studies that mentioned any adverse reactions to capsaicin inhalation, these were most commonly transient throat irritation, some degree of retrosternal discomfort accompanying cough at higher concentrations of inhaled capsaicin (14). Capsaicin does not induce clinically significant bronchoconstriction in healthy volunteers or asthmatics (15, 16).

The relation between CRS and respiratory function has been studied, but the result is contentious. In the present study we did not observe a significant difference between spirometric parameters (FEV1, FVC, FEV1/FVC) before and after capsaicin challenge in healthy volunteers and asthmatic patients. In the study of Fujimura et al. (17) inhaled capsaicin significantly decreases FEV1 at the dose, that elicited 5 or more coughs (C5) in patients with asthma bronchiale. In the study of Doherty et al. (10) CRS was independent of the degree of airway obstruction. Airway hyperresponsiveness and cough sensitivity arise through different mechanisms (12).

The capsaicin cough challenge test is a simple and reproducible laboratory method for the assessment of cough susceptibility in a wide range of diseases. In this study we show that a reduced cough threshold is present in asthmatic patients. The cough reflex sensitivity measurement can add valuable information besides commonly used spirometry and inflammatory methods in clinical practice in management of asthmatic patients.

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