Capsaicin-evoked cough responses in asthmatic patients: Evidence for airway neuronal dysfunction

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Background: Cough in asthmatic patients is a common and troublesome symptom. It is generally assumed coughing occurs as a consequence of bronchial hyperresponsiveness and inflammation, but the possibility that airway nerves are dysfunctional has not been fully explored.

Objectives: We sought to investigate capsaicin-evoked cough responses in a group of patients with well-characterized mild-to-moderate asthma compared with healthy volunteers and assess the influences of sex, atopy, lung physiology, inflammation, and asthma control on these responses.

Methods: Capsaicin inhalational challenge was performed, and cough responses were analyzed by using nonlinear mixed-effects modeling to estimate the maximum cough response evoked by any concentration of capsaicin (E_{max}) and the capsaicin dose inducing half-maximal response (ED_{50}).

Results: Ninety-seven patients with stable asthma (median age, 23 years [interquartile range, 21-27 years]; 60% female) and 47 healthy volunteers (median age, 38 years [interquartile range, 29-47 years]; 64% female) were recruited. Asthmatic patients had higher E_{max} and lower ED_{50} values than healthy volunteers. E_{max} values were 27% higher in female subjects ($P = .006$) and 46% higher in patients with nonatopic asthma ($P = .003$) compared with healthy volunteers. Also, patients with atopic asthma had a 21% lower E_{max} value than nonatopic asthmatic patients ($P = .04$). The ED_{50} value was 65% lower in female patients ($P = .0001$) and 71% lower in all asthmatic patients ($P = .0008$). ED_{50} values were also influenced by asthma control and serum IgE levels, whereas E_{max} values were related to 24-hour cough frequency. Age, body mass index, FEV_1, PC_{20}, fraction of exhaled nitric oxide, blood eosinophil counts, and inhaled steroid treatment did not influence cough parameters.

Conclusion: Patients with stable asthma exhibited exaggerated capsaicin-evoked cough responses consistent with neuronal dysfunction. Nonatopic asthmatic patients had the highest cough responses, suggesting this mechanism might be most important in type 2–low asthma phenotypes. (J Allergy Clin Immunol 2017;139:771-9.)

Key words: *Atopy, transient receptor potential vanilloid type 1, vagus, pharmacodynamic modeling*

Asthma affects an estimated 300 million persons worldwide and is characterized by symptoms of cough, wheeze, chest tightness, and shortness of breath. Current dogma suggests asthma symptoms arise as a consequence of airway narrowing, bronchial hyperresponsiveness, and airway inflammation. Yet despite effective treatments targeting each of these components of asthma, many patients have substantial residual symptoms. Even in a clinical trial setting with optimal inhaled treatment, up to 50% of asthmatic patients have symptoms that are not well controlled.[1] Although for some patients adherence might be an issue,[2] it is also likely that undiscovered mechanisms explain the heterogeneity in asthma clinical phenotypes and treatment responses.

Symptoms are often challenging to study because they can only be reported subjectively; however, cough is readily amenable to objective quantification.[3] Cough in asthmatic patients is not only a common[4] and troublesome[5] symptom but also predicts disease severity[6] and poor prognosis,[7] suggesting it reflects important

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Abbreviations used
ACQ: Asthma Control Questionnaire
ATS: American Thoracic Society
C2: Concentration of capsaicin inducing at least 2 coughs
C5: Concentration of capsaicin inducing at least 5 coughs
ED_{50}: Capsaicin dose inducing half-maximal response
E_{max}: Maximum cough response evoked by any concentration of capsaicin
FENO: Fraction of exhaled nitric oxide
LCQ: Leicester Cough Questionnaire
TRPV1: Transient receptor potential vanilloid type 1

pathophysiologic processes, yet remarkably little is understood about the underlying mechanisms. The general assumption is that airway afferent nerves activating the cough reflex are stimulated by inflammatory mediators, mucus, and bronchospasm, and the possibility that these neuronal pathways are dysfunctional is rarely considered.

Vagal afferent fibers innervate the airways and are responsible for mediating symptoms and airway reflexes.[8,9] Coughing is readily evoked by activation of C fibers; these networks of unmyelinated and chemically sensitive afferents are characteristically sensitive to capsaicin (chili pepper extract) through activation of the transient receptor potential vanilloid type 1 (TRPV1) channel. Aδ fibers are sparsely distributed, thinly myelinated fibers in the proximal airways that also evoke cough. They protect the airways by responding to mechanical stimuli (eg, foreign objects) and changes in osmolarity and acidity. Importantly, they are typically insensitive to capsaicin and inflammatory mediators and do not usually express TRPV1.

Experimentally evoked cough responses to inhaled irritants are an established tool for studying the cough reflex and thus airway nerve function. Capsaicin is the most widely used agent, and the concentration of capsaicin causing 5 or more coughs (C5) is considered a measure of cough reflex sensitivity.[3] However, previous studies in asthmatic patients have produced conflicting results, with some studies suggesting sensitization of the cough reflex (reduced C5) and others finding no difference from healthy control subjects.[10-13]

We have recently investigated capsaicin-evoked cough responses using repeat inhalations of capsaicin and concentrations beyond C5. Nonlinear mixed-effects modeling of these data found maximum cough response evoked by any concentration of capsaicin (E_{max}) best discriminated patients with chronic cough from healthy control subjects/patients with mild asthma; the difference between healthy and asthmatic subjects did not quite reach *a priori* statistical significance.[14] Therefore we have studied capsaicin-evoked cough responses in a larger group of patients with well-characterized mild-to-moderate asthma and healthy volunteers. We also investigated the influence of sex, atopic status, lung physiology, inflammation, and asthma control on capsaicin-evoked cough responses. Some of the results of these studies have been previously reported in the form an abstract.[15,16]

METHODS

Subjects

Subjects with a physician's diagnosis of asthma were recruited but not selected for symptoms of cough. Treatment with salbutamol as required and/or inhaled corticosteroids at 500 μg or less of fluticasone propionate equivalent daily with or without a long-acting bronchodilator was permitted. Subjects with uncontrolled symptoms according to Global Initiative for Asthma classification or not receiving stable medication over the previous 4 weeks were excluded. Healthy control subjects approximately matched for age were also recruited. We excluded current smokers, those with a recent chest infection or exacerbation, and those using any medication that might alter the cough responses (eg, opiates, gabapentin, anticholinergics, and theophylline). The study protocols for healthy control subjects and asthmatic patients were approved by the local research ethics committee (13/COA/002 and 13/CLU/001), and all subjects provided written informed consent.

TABLE I. Comparison of asthmatic patients and healthy volunteers

	Asthmatic patients	Healthy volunteers	*P* value
Participants (no.)	97	47	
Age (y)	23.0 (21.0-27.0)	38.0 (29.0-47.0)	<.001
Sex (male/female)	39/58	17/30	.64
BMI (kg/m^2)	24.1 (21.8-27.0)	25.0 (22.2-28.6)	.25
Smoking history (pack years)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	.34
FEV_1 (% predicted)	95 (87.0-103.0)	103.0 (97.0-115.0)	<.001
FVC (% predicted)	102 (95-110)	106.0 (99.0-118.0)	.02
Cough frequency (coughs/h)			
24 h	1.1 (0.5-2.4)	0.2 (0.0-0.9)	<.001
Day	1.6 (0.7-3.8)	0.2 (0.0-1.3)	<.001
Night	0.0 (0.0-0.4)	0.0 (0.0-0.1)	.25

Data are presented as medians and interquartile ranges and compared by using the Mann-Whitney *U* test.
BMI, Body mass index; *FVC*, forced vital capacity.

Study protocol and procedures

For full details, see the Methods section in this article's Online Repository at www.jacionline.org. Asthmatic patients attended on 3 occasions. On visit 1, subjects underwent history and examination and completed the Asthma Control Questionnaire (ACQ), Leicester Cough Questionnaire (LCQ), fraction of exhaled nitric oxide measurement (FENO; NIOX, Aerocrine, Solna, Sweden), spirometry, and bronchodilator reversibility measurement, and an ambulatory cough monitor (VitaloJAK; Vitalograph, Buckinghamshire, United Kingdom) was fitted for the next 24 hours. At visit 2, at least 48 hours later, subjects underwent full blood count, serum IgE measurement, skin prick testing, and PC_{20} measurement. Subjects completed a peak flow diary twice a day for 7 days after visit 2.

Visit 3 took place at least 1 week later, and a capsaicin cough challenge was performed, as previously described,[14] by using a dosimeter (KoKo Dosimeter; Ferraris, Hertford, United Kingdom) and a nebulizer pot (Model 646; Devilbiss Healthcare, Somerset, Pa) with an inspiratory flow limiter. Briefly, 4 inhalations were administered, 30 seconds apart, of doubling doses of capsaicin (0.48-1000 μmol/L). After each inhalation, the number of coughs in the first 15 seconds was counted and later verified by using a cough monitor (VitaloJAK). The challenge was completed when the patient reached the final dose or the maximal tolerated dose. Spirometry was performed before and after each challenge.

Healthy volunteers attended on 2 occasions. On visit 1, consent was obtained, screening and spirometry were performed, and the ambulatory cough monitor was attached. On visit 2, the capsaicin challenge was performed.

Statistical analysis

Cough responses to capsaicin were analyzed by using nonlinear mixed-effects modeling software (NONMEM 7.3; ICON Development Solutions, Ellicott City, Md) and the Laplace estimation method.[17,18] Additional investigations of the NONMEM output and statistical and graphic analyses were performed in Matlab R2014a (MathWorks, Natick, Mass). We applied