Capsaicin cough threshold test in diagnostics

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Summary
Background: Among patients with chronic unexplained cough, there is a recognized subgroup with respiratory symptoms induced by environmental irritants like chemicals and odours. The diagnosis of sensory hyperreactivity (SHR) has been suggested for this group of patients and can be made using a tidal breathing capsaicin inhalation test. The aim of the present study was to evaluate the ability of a single-breath, dose-response capsaicin threshold test to discriminate such patients from control subjects.

Methods: A total of 46 patients with chronic cough and SHR who had previously shown a positive reaction in accordance with limits set for a tidal breathing capsaicin test were tested once with a single-breath, dose-response capsaicin cough threshold test, assessing capsaicin concentrations to evoke 2 (C2), 5 (C5) or 10 (C10) coughs. Twenty-nine subjectively healthy control subjects were also included and tested with the threshold method.

Results: Patients had significantly lower C2, C5 and C10 in comparison to controls. From the results among patients and controls, sensitivity and specificity were calculated, and a receiver operating characteristic curve was constructed, showing excellent ability for C5 and C10 to discriminate patients from control subjects.

Conclusions: For patients with SHR and chronic cough, capsaicin cough sensitivity was once again confirmed to be increased, in this case, using the single-breath dose-response method. Limits set for cough reactions regarded as more sensitive than normal can be useful in diagnostics and further research. C5 seems to be the best measure to use in research and differential diagnostics.

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Background

Inhaled capsaicin (8-methyl-N-vanillyl-6-nonenamide) has long been used to induce cough in a safe and dose-dependent manner, and the degree of induced cough reflects the reactivity of sensory C-fibres in the respiratory mucosa [1–4]. In cough research the use of capsaicin has been important, and has good short- and long-term reproducibility [5–12]. The most commonly used method to assess capsaicin cough sensitivity is the single-breath dose-response method: Doubling doses are given at 1-min intervals to assess the cough thresholds, stating the concentration of capsaicin causing 2 (C2), 5 (C5), or 10 or more (C10) coughs during the 1-min period between each dose. This single-breath method is recommended in the European Respiratory Societies (ERS) guidelines [13], and some studies suggest that C5 is the clinically superior value [6].

It has been suggested that patients with upper and lower airway symptoms induced by odours and chemicals, such as flower scents, perfume, cleaning agents, car exhaust fumes and tobacco smoke, suffer from airway sensory hyperreactivity (SHR) [5]. Common symptoms are cough, hoarseness, difficulty in getting air, rhinorrhea, and eye irritation. Most of these patients could also be diagnosed with chronic cough, having coughing that persists for more than 8 weeks [4,11]. The symptoms may be misinterpreted as asthma and/or allergy, except that the clinical picture shows no bronchial obstruction or IgE-mediated reactions. Such patients are often diagnosed as having idiopathic or unexplained chronic cough, and might also be included in the newly established cough hypersensitivity syndrome [14–18]. In a recent report there was a high degree of agreement among opinion leaders as to the concept that cough hypersensitivity underlies the aetiology of chronic cough in the majority of patients [19].

Stimulation of the unmyelinated C-fibres of the trigeminal and vagal nerves is likely involved in chemical-induced airway symptoms [20,21]. In line with the hypothesis of SHR, these patients react to inhaled capsaicin with more coughing and other symptoms than healthy individuals and asthmatic patients, and the reactions can be blocked by preinhalation of a local anaesthetic [5,22]. The suggested diagnosis of SHR can, together with pronounced airway symptoms from scents and chemicals, be assessed using a standardized capsaicin inhalation test to identify patients and to differentiate between healthy subjects, patients with asthma and those with SHR [23,24]. In this test, incremental concentrations of capsaicin are inhaled to induce coughing using a tidal breathing method, and limits for the number of coughs in a normal reaction are set. This capsaicin inhalation test has shown good reproducibility using a simple device for tidal breathing (Pari Boy or Maxin MA3), and no influence on lung function has been found [5,23,24]. However, for research purposes, and in clinics with access to more sophisticated nebulizer systems, the single-breath method may have advantages, giving more specified data on delivered aerosol and being in accordance with ERS guidelines. In this study we aimed to evaluate the extent to which capsaicin inhalation testing in conjunction with the single-breath dose-response method can distinguish patients with chronic cough and SHR from healthy control subjects.

Methods

Patient group

The study group included 46 non-smoking patients, 41 women and 5 men, 21–74 years of age (mean 56 years). They were referred to an asthma and allergy outpatient clinic because of cough and airway symptoms suggestive of asthma or allergy. The patients were screened using a questionnaire on airway symptoms and on symptoms in response to environmental irritants, and all had a history of at least two years of coughing and pronounced upper and/or lower airway symptoms induced by irritants like chemicals and scents. They had within the previous five years had positive reactions to a capsaicin inhalation test administered with the tidal breathing method according to the method described by Johansson et al. [23], and were diagnosed as having SHR as an explanation for their airway symptoms. All patients had negative skin-prick test results when tested with a standard panel of 10 allergens in sources common to Sweden and had also undergone a methacholine test within the previous five years. The methacholine test was performed in accordance with international guidelines [25] and was negative for all patients, indicating the absence of bronchial asthma.

The patients were asked to take no medication for at least 4 h, and no long-acting β2-agonists for at least 72 h, prior to the inhalation tests.

Control group

The control group consisted of 29 non-smoking, subjectively healthy individuals, 25 women and 4 men, 27–66 years of age (mean 52 years). They were also screened using questions on airway symptoms and on symptoms in response to chemicals and scents. None had a history of asthma, allergies or airway symptoms in response to environmental irritants, and none was taking any medication for the airways.

Study design

Each participant visited the clinic once and was tested with a single-breath capsaicin provocation. The cough thresholds of the study participants were registered manually during the provocations. Cough was defined as the characteristic sound that follows a forced expiratory effort against a closed glottis and distinguished from other sounds such as clearing the throat [13,26], by a discretionary decision of the investigator upon observation of the subjects. The total time for each provocation was about 15 min.

Provocations were not carried out on subjects who had experienced respiratory infections in the past month. The participants could not be using angiotensin-converting enzyme inhibitors or any medication for gastro-oesophageal reflux. Pregnancy and breastfeeding were exclusion criteria.
Written informed consent was obtained from all patients and controls after they were provided with verbal and written information. The Regional Ethics Review Board of Gothenburg, Sweden, approved the study.

Capsaicin solutions

A stock solution of capsaicin (Sigma–Aldrich, Sweden AB, Stockholm, M2028) [1 mmol/L in ethanol (99.5%)] was prepared and then dissolved in 0.9% saline to provide a stock solution of 500 μmol/L capsaicin. Fresh serial dilutions were prepared from this stock solution, using saline diluent to produce doubling concentrations from 0.49 to 500 μmol/L.

Capsaicin provocation with the single-breath method

Doses were administered from a compressed air–driven sidestream nebulizer (MedicAid Pro, Sussex, UK) controlled by an aerosol provocation system (APS version 5.02 software, Viasys Healthcare GmbH, Hoechberg, Germany). The nebulizer’s output was 240 mg/min, and the mass median particle diameter was 3.2 μm. The duration of aerosol delivery was programmed to 0.4 s, thereby providing 0.0016 ml per breath. The subjects inhaled, without a nose clip, three single, vital capacity breaths before the capsaicin solution was ejected in the fourth inhalation. Doubling doses were given at 1-min intervals, and the concentration of capsaicin causing 2 (C2), 5 (C5) or 10 or more (C10) coughs during the 1-min period between each concentration was registered. A value of 1000 μmol/L capsaicin was assigned if C2, C5 and/or C10 values were >500 μmol/L.

In all participants the forced expiratory volume during 1 s (FEV1) was measured using a MasterScope (version 4.67 software, Viasys Healthcare GmbH, Hoechberg, Germany) before and after each completed capsaicin provocation. The participants used a nose clip, and the higher of two values was recorded.

Statistical analysis

The Wilcoxon signed-rank test was used for paired data. For comparison between groups Fisher’s exact test was used for dichotomous variables and the Mann–Whitney U-test was used for continuous variables. All tests were two-tailed and results were considered significant if \( P < 0.05 \). Data were analysed using version 16.0 of the SPSS software package (SPSS Inc., Chicago, IL, USA) and SAS® 9.2 (SAS Institute Inc., Cary, NC, USA).

The single-breath method was evaluated by constructing a receiver operating characteristic (ROC) curve, in which sensitivity versus 1-specificity for each possible cough cut-off level was plotted [27,28]. If the area under the curve is 0.5, this suggests no discrimination. An area under the curve of more than 0.9 indicates that a method has outstanding discrimination ability, for example, the ability to distinguish two groups from each other [29]. A corresponding value of more than 0.8 indicates excellent discrimination ability, and more than 0.7 is considered as acceptable discrimination ability.

Results

Patients and controls coughed dose-dependently in terms of growing cough reaction with increasing capsaicin concentrations. The cough thresholds for C2, C5 and C10 were significantly lower in patients compared to controls \( (P < 0.005 \) for C2 and \( P < 0.0001 \) for C5 and C10) (Fig. 1).

The mean value of FEV1 before the capsaicin provocation was 106% of predicted value (95% CI: 101–110) among the patients and 101% of predicted value (95% CI: 93–109) among the controls (ns). The results did not differ significantly between recordings before and those after the provocations (data not shown) in either group.

Ability to distinguish patients from controls

Based on values for sensitivity and specificity with different “cut-offs”, limits for a positive test were suggested for the capsaicin concentrations that evoked C2, C5 and C10.

C2

From the individual threshold results a limit for a positive test was set when C2 was evoked from a capsaicin concentration of <2 μmol/L, giving sensitivity for C2 of 0.65 and a specificity of 0.66.

C5

The corresponding limit for C5 was set to a capsaicin concentration of <15.6 μmol/L, giving sensitivity for C5 of 0.96 and a specificity of 0.86.

Figure 1 Mean log concentrations (μmol/L) of capsaicin eliciting 2 (C2), 5 (C5) or 10 or more (C10) coughs in 46 patients with chronic cough and SHR and in 29 control subjects. Error bars represent 95% confidence intervals (CI).
ROC curves of optimal cough cut-off levels were calculated, and a ROC curve was constructed showing the discrimination ability, and C2 also had large individual divergence. Limits for discrimination of patients from healthy controls were set and could be of use in the future.

**Discussion**

The main findings in this study were that SHR patients previously diagnosed with a tidal breathing inhalation capsaicin cough test had significantly lower C2, C5 and C10 in comparison to controls using a single-breath dose-response method. From the cough threshold outcomes among patients and controls, sensitivity and specificity were calculated, and a ROC curve was constructed showing excellent ability for both C5 and C10 to discriminate patients from control subjects, even better ability in this aspect than earlier results with the tidal breathing method [8]. The corresponding curve for C2 demonstrated less discrimination ability, and C2 also had large individual divergence. Limits for discrimination of patients from healthy controls were set and could be of use in the future.

The ROC curve is often used to evaluate whether a method can distinguish patients from healthy subjects, and in this study the single-breath method of capsaicin inhalation showed a high degree of such discrimination ability. However, attention must be paid to the previously known increased capsaicin cough reaction with the tidal breathing method among patients — a necessary qualification to the study. To evaluate a method without introducing bias, no such assumptions should be set, but the current results show that the threshold method has a corresponding good ability to distinguish patients.

Increased cough sensitivity to capsaicin has been found in patients with several reasons for chronic cough. Patients with gastro-oesophageal reflux [30,31], bronchitis, chronic obstructive pulmonary disease [32–34] and pulmonary fibrosis [35] have shown increased cough sensitivity to inhaled capsaicin, while for asthmatics, the results are more contradictory [5,24,32–34,36]. When using a capsaicin inhalation test to identify SHR patients, it is important to carefully exclude other diagnoses that may influence the cough sensitivity. On the other hand, patients with unexplained chronic cough should be questioned about chemical sensitivity as an inducing factor, in light of results showing a majority of a group of patients with chronic unexplained cough having chemicals and scents as inducing factors and being positive to a capsaicin inhalation test [37,38]. The narrow selection of chronic cough patients in the present study, all having cough induced by environmental irritants, may explain why in contradiction to other reports [31,39], the capsaicin cough threshold test showed excellent ability to discriminate patients from healthy control subjects.

The recently introduced **cough hypersensitivity syndrome** throws new light on cough evoked from environmental stimuli with origin in diverse respiratory conditions and emphasizes the value of capsaicin use in cough research [14–19]. The current results are in accordance with the ERS guidelines recommending the single-breath method as preferable [13], and now this method has proven to be useful also in diagnosing and studying chronic cough related to SHR. Further, the clinical impression from the provocations was that the method was easy handled and quick to carry out, and caused few symptoms among the participants. A disadvantage, however, is that the necessary equipment is expensive and requires some expert knowledge.

With regard to the present findings of high sensitivity and specificity for C5 and C10, the cut-off limits for these thresholds seem to be preferable to the limit for C2. We also found a variety among the control subjects for C2, indicating a difficulty in separating patients from healthy individuals that is in accordance with other researchers’ findings of less reproducibility for C2 [6]. Using C5 seems to be the simplest way to assign heightened capsaicin cough sensitivity also in regard to the short time needed to perform the provocation and the minor trouble caused to the patient, and is further in agreement with earlier suggestions [6]. With regard to many earlier reports of safety and reproducibility of capsaicin cough sensitivity [5–12,40], this method seems harmless and trustworthy. As both the tidal breathing and the threshold method have proven in several earlier studies to have good short- and long-time reproducibility [5–12,40], this aspect was not evaluated in the present study.
We conclude that in patients with SHR and chronic cough, capsaicin cough sensitivity is once again confirmed to be increased, in this study using the single-breath dose-response method, and to discriminate SHR patients from subjectively healthy individuals. Limits set for cough reactions assessed as more sensitive than normal can be useful in further research.

Conflict of interest statement

The authors declare that they have no conflict of interest, financial or otherwise, related to this study.

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