Capsaicin-sensitive cough receptors in lower airway are responsible for cough hypersensitivity in patients with upper airway cough syndrome

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Background:
Cough hypersensitivity may be related to the pathogenesis of upper airway cough syndrome (UACS). The purpose of the study was to investigate the role of capsaicin-sensitive cough receptors on the laryngopharynx and lower airway in the cough hypersensitivity of patients with UACS.

Material/Methods:
59 patients with UACS, 33 patients with rhinitis/sinusitis without cough, and 39 healthy volunteers were recruited for the study. Cough threshold C5, defined as the lowest concentration of capsaicin required for the induction of ≥5 coughs upon exposure to capsaicin, were determined at baseline and after laryngopharyngeal anesthesia with lidocaine in all the subjects. After induced sputum cytology, the concentrations of histamine, prostaglandin E2 (PGE2), and calcitonin-gene-related peptide (CGRP) in the induced sputum were measured by ELISA. In 15 patients with UACS, sputum cytology and measurement of the above mediators were repeated after successful therapy.

Results:
C5 response to capsaicin was significantly lower in the UACS group than in the rhinitis/sinusitis group and healthy control groups [3.9 (0.98, 7.8) µmol/L vs. 7.8 (3.9, 93.75) µmol/L vs. 31.2 (15.6, 62.5) µmol/L, H=40.12, P=0.000]. Laryngopharyngeal anesthesia with lidocaine dramatically increased C5 to capsaicin in the subjects of all 3 groups by a similar degree, but the increase in the UACS group was still the lowest, with an increased level of histamine, PGE2, and CGRP in the induced sputum. When cough resolved with the treatment of cetirizine alone or in combination with erythromycin, the levels of CGRP and histamine in the induced sputum decreased significantly in 15 patients with UACS, with no obvious change in cell differential or concentration of PGE2 in the induced sputum.

Conclusions:
Laryngeal TRPV1 plays an important role in cough sensitivity, but sensitization of capsaicin-sensitive cough receptors in the lower airway may be more responsible for the cough hypersensitivity in patients with UACS.

Key words: upper airway cough syndrome • rhinitis • sinusitis • cough sensitivity • laryngopharynx

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Background

Upper airway cough syndrome (UACS), previously referred to as postnasal drip syndrome (PNDS) [1], is the leading cause of chronic cough in Western countries, with a prevalence of 24–52% [2–4], and is the second leading cause of chronic cough in China, with a prevalence of 25–26% [5,6]. The pathogenesis of UACS is still poorly understood.

It has been proposed that either mechano- or chemo-stimulation of the afferent nerves innervating the pharynx and larynx or the lower airway by secretions emanating from the nose and/or sinuses dripping down into these areas is related to the elicitation of cough in UACS. However, postnasal drip is a common physiological event [7], and about 20–40 milliliters of mucus are secreted from the nose every day in healthy people, which is moved backward by the waves of epithelium cilia into the nasopharynx, where it is swallowed or expected by throat-cleaning, but does not induce cough [8]. In an elaborate study, Bardin et al. found that the secretions in the maxillary sinus were not seeded in the lung of the patients with only sinusitis, as well as patients with asthma and sinusitis [9], suggesting the aspiration is not a significant mechanism underlying UACS.

Currently, cough hypersensitivity is considered as a universal feature of chronic cough and may explain the pathogenesis of UACS [10]. With the afferent limb sensitization of the cough reflex, people with UACS probably have an exaggerated response to cough stimuli at threshold or sub-threshold levels. Due to the nature of upper airway disorders as a cause of UACS, the involvement of local sensory nerves innervating the hypopharynx or larynx in the cough hypersensitivity may be a logical speculation. However, the available data on the issue are conflicting [11,12].

Therefore, a prospective cross-sectional study was conducted to examine the effect of capsaicin-sensitive sensory nerves located in the laryngeal and lower airway on cough hypersensitivity in patients with UACS by comparing these patients to those with rhinitis/sinusitis without cough, and to healthy volunteers.

Material and Methods

Subjects

Fifty-nine patients who had UACS as the only cause of their chronic cough and who came to our respiratory clinic between January 2009 and June 2011 were recruited into the study and designated as the UACS group. The Control group included 33 patients with rhinitis/sinusitis who did not cough (Rhinitis/sinusitis group) and 39 healthy volunteers (Healthy control group).

UACS was diagnosed according to the guideline for the diagnosis and management of chronic cough [13,14]. Diagnostic criteria included: 1) cough lasted for at least 8 weeks; 2) a history of chronic rhinitis or sinusitis; 3) 1 or more of the following symptoms: the sensation of secretions dripping down into the throat, the need to clear the throat often (throat-clearing sign), nasal discharge, nasal obstruction, or sneezing; 4) 1 or more of the following signs: the erythema and “cobblestone” appearance of the posterior pharyngeal mucosa or mucoid/purulent secretions dripping into the pharynx; 5) the presence of mucosal blur or thickening of more than 6 mm, or air-fluid level on sinus roentgenogram; and 6) symptoms including cough responding favorably to antihistamine (oral cetirizine 10 mg once daily) or antihistamine plus macrolide (erythromycin, 0.25 g, twice daily) in the case of chronic sinusitis. When the patients met the criteria of 1, 3, 4, and 6 and either one or both of criteria 2 and 5, UACS was definitely confirmed [15]. Rhinitis/sinusitis without cough was diagnosed by ENT doctors in our hospital the same as described above. Healthy volunteers came from the population who visited the hospital for regular physical examination.

All the subjects were life-time non-smokers or had stopped smoking more than 2 years ago, and had had no upper respiratory tract infections within the 2 months preceding the study. Their characteristics are shown in Table 1. Except for cough, there were no significant differences in age, sex distribution, and lung function parameters among the 3 groups. The study protocol was approved by the Ethics Committee of Tongji Hospital and written informed consent was obtained from all the participants.

Detection of cough sensitivity to capsaicin

Cough sensitivity to capsaicin was detected according to the method described by Fujimura et al. [16,17] with modifications adapted to ERS guidelines [18]. Briefly, subjects wearing nose clips inhaled an aerosolized control solution of physiological saline followed by progressively increasing double concentrations (0.49–1000 μmol/L) of the capsaicin solution (Wako Pure Chemical Ind., Japan), delivered through a PARI BOY N085 air-compressed nebulizer (PARI GmbH, German) at an output rate of 0.5 ml/min with a 3.7-μm mass median diameter of the particles. Each concentration of solutions was inhaled by tidal mouth-breathing for 15 s, and the number of cough was counted during the inhalation. The cough threshold was defined as the lowest concentration of capsaicin required for the induction of ≥5 coughs (C5) and was used as an indicator of cough sensitivity.
Sputum induction and cytology

Sputum was induced and processed as described previously [19]. Briefly, the subjects continuously inhaled the 4% hypertonic saline solution through an ultrasonic nebulizer (YS9801, Yisheng Corp., Shanghai, China) and were asked to expectorate sputum into a sterile pot every 5 min, after blowing their noses and rinsing their mouths. Sputum with minimal saliva contamination was immediately collected, mixed with 4 volumes of 0.1% dithiothreitol by gentle aspiration and then was mixed on a bench rocker for 20 min. The filtrate through a 48-mm gauze was centrifuged at 3000 rpm for 10 min, and the cell-free supernatant was removed and stored at –80°C until assay. The cell pellet was re-suspended in 1 mL of PBS and smeared on glass slides, followed by total cell counting using a standard hemocytometer. The air-dried preparations were stained with HE stain, and then cell differential was performed on 400 nucleated cells according to standard morphological criteria.

Measurement of mediators in supernatants of induced sputum

The levels of mediators in the induced sputum were determined in duplicate by ELISA, according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN, USA). The lower limits of measurement for histamine, prostaglandin E2 (PGE2), and calcitonin gene-related peptide (CGRP) were 0.01 ng/mL, 0.01 pg/mL, and 7.8 pg/mL, respectively. The intra-assay and inter-assay variability of the measurements were 5% and 10%, respectively, across the range of concentrations measured.

Procedures

All the subjects in the healthy control group underwent spirometry and the induction of the sputum in the first day with the preceding collection of general information. Twenty-four hours later, after the baseline cough threshold C5 to inhaled capsaicin was detected, 5 ml of physiological saline were placed in the laryngopharynx and was held for 5 min in a supine position. C5 was re-measured immediately (0 min) and at 5, 10, 15, and 20 min. When it was completed, the subjects were asked to rest for 30 min. Then, the above procedure was repeated, except physiological saline was replaced with 2% lidocaine for local anesthesia at the laryngopharynx.

The patients in the UACS group and rhinitis/sinusitis group were treated in the same way as those in the healthy control group. However, their cough threshold C5 to inhaled capsaicin was only measured at baseline and 0 min after local anesthesia at the laryngopharynx with 2% lidocaine based on the preliminary results in the healthy control group.

In addition, the induced sputum examination was performed once more in 15 patients with UACS when they were treated with oral cetirizine for 10–14 day (10 mg once daily) and cough resolved.

Statistical analysis

Data with normal distribution are expressed as mean ± SD while those with skewed distribution are shown as median (25%, 75% interquartile). The log-transformed cough threshold C5 (Log C5) is expressed as geometric means ± standard error (SEM). Comparisons among the 3 groups and between the 2 groups were performed using the Kruskal-Wallis test followed by the Mann-Whitney U test for data with skewed distribution, or one-way analysis of variance (ANOVA) followed by Newman-Keuls test for data with normal distribution. Data with non-normal distribution were compared using the Wilcoxon test between 2 groups. Difference in gender distribution among 3 groups was analyzed using the chi-square test. Statistical analysis

Table 1. General information of subjects in three groups.

|                      | UACS          | Rhinitis/sinusitis | Healthy control |
|----------------------|---------------|--------------------|-----------------|
| Subjects (male)      | 59 (23)       | 33 (14)            | 39 (13)         |
| Age (yr)             | 46.8±15.1     | 42.5±15.9          | 40.2±15.8       |
| Duration (months)    | 6 (4–92)      | 0                  | 0               |
| FEV1% predicted      | 97.02±14.68   | 99.29±13.86        | 94.82±11.28     |
| FVC% predicted       | 102.38±18.08  | 101.82±10.90       | 95.84±11.25     |
| FEV1/FVC%            | 80.53±8.67    | 80.99±8.89         | 84.05±6.05      |
| C5 (μmol/L)          | 3.9 (0.98, 7.8)* | 7.8 (3.9, 93.75)* | 31.2 (15.6, 62.5) |

Data are presented as mean ± SD except for duration and C5 which are presented as median (25%, 75% interquartile). * P<0.05 vs. rhinitis/sinusitis group; ** P<0.05 vs. healthy control group.
was done with SPSS version 17.0 (Chicago, IL, USA). A value of \(P<0.05\) was considered statistically significant.

**Results**

**Cough threshold C5 to inhaled capsaicin and the change with laryngopharngeal anesthesia**

The baseline C5 was lower in UACS patients (3.9 [0.98, 7.8] \(\mu\)mol/L) than in the rhinitis/sinusitis group (7.8 [3.9, 93.75] \(\mu\)mol/L, \(Z=-3.44, P=0.001\)) and healthy control group (31.2 [15.6, 62.5] \(\mu\)mol/L, \(Z=-6.27, P=0.000\)). Moreover, C5 was also significantly lower in the rhinitis/sinusitis group than in the control group (\(Z=-2.10, P=0.04\)).

Laryngopharngeal anesthesia was successful in 36 of 39 healthy volunteers, and failed in 3 subjects because of intolerance to the adverse effects of lidocaine. Compared to the baseline, lidocaine significantly increased C5 at 0 min (\(Z=-3.03, P=0.002\)), 5 min (\(Z=-3.03, P=0.002\)), 10 min (\(Z=-3.03, P=0.002\)), and 15 min (\(Z=-2.75, P=0.006\)), but not at 20 min (\(Z=-1.91, P=0.057\)). The effects of lidocaine did not change over time between 0–15 min (Figure 1). Physiological saline had no influence on C5.

Two patients in the UACS group and 2 patients in the rhinitis/sinusitis group withdrew because of severe discomfort caused by lidocaine. With laryngopharngeal anesthesia, C5 was dramatically increased from 2.93 (1.71,7.8) \(\mu\)mol/L to 7.8 (3.9, 15.6) \(\mu\)mol/L in UACS group (\(Z=-4.54, P=0.000\)), and from 15.6 (1.47,62.5) \(\mu\)mol/L to 31.2 (3.9,125) \(\mu\)mol/L in rhinitis/sinusitis group (\(Z=-2.94, P=0.003\)) (Figure 2). C5 was not different between males and females (Table 2), and the increase in C5 (\(\Delta\log C5 = \log C5' - \log C5\)) was comparable among the 3 groups (\(H=1.87, P=0.39\)). After laryngopharngeal anesthesia, C5 in the UACS group was still the lowest (\(H=6.80, P=0.03\)), followed by the rhinitis/sinusitis group (\(Z=-2.24, P=0.03\)) and the control group (\(Z=-2.98, P=0.003\)).

**Cell counting and detection of mediators in induced sputum**

The total number of cells in the induced sputum was significantly higher in the UACS group and rhinitis/sinusitis group than in the healthy control group, but the difference between the UACS group and the rhinitis/sinusitis group was not obvious.

### Table 2. Difference in C5 between males and females.

|                      | Female (N) | Male (N) | \(X^2\) | \(P\)  |
|----------------------|------------|----------|----------|--------|
| UACS                 | Increased  | 26       | 18       | 1.018  | 0.503  |
|                      | Decreased or unchanged | 9 | 3 |  |
| Rhinitis/sinusitis   | Increased  | 15       | 6        | 2.820  | 0.127  |
|                      | Decreased or unchanged | 4 | 6 |  |
| Healthy Control      | Increased  | 17       | 8        | 0.007  | 1.000  |
|                      | Decreased or unchanged | 8 | 4 |  |
| Total                | Increased  | 58       | 32       | 0.077  | 0.836  |
|                      | Decreased or unchanged | 21 | 13 |  |
Table 3. Total cells and differential cells in the induced sputum of different groups.

| UACS                  | Rhinitis/sinusitis | Healthy control | H     | P   |
|-----------------------|-------------------|-----------------|-------|-----|
| Total cells (×10⁶/ml) | 3.1 (2, 5)*        | 5 (2, 12.5)*    | 2     | 11.69 | 0.003 |
| Neutrophils,%         | 6.75 (1, 19)      | 20.75 (0, 48.38) | 5     | 1 (1, 10) | 4.20 | 0.122 |
| Macrophages,%         | 63.75 (38.88, 78) | 56.25 (34.50, 79.88) | 77.25* | (67, 82) | 10.85 | 0.004 |
| Lymphocytes,%         | 18.88 (13, 26.13) | 14.25 (8.69, 24.55) | 17    | (11, 27) | 2.22 | 0.330 |
| Eosinophils,%         | 0 (0, 2)**        | 0 (0, 0)        | 0     | 13.427 | 0.001 |

* P<0.05 vs. healthy control group; * P<0.05 vs. rhinitis/sinusitis group. Data are presented as median (25%, 75% interquartile).

Table 4. Comparison of mediator levels in induced sputum supernatants between subjects in each group.

| UACS                  | Rhinitis/sinusitis | Healthy control | H     | P   |
|-----------------------|-------------------|-----------------|-------|-----|
| Histamine (ng/ml)     | 918.5 (363.4, 1766.1)** | 439.2 (344.2, 552.9)* | 273.0 | (175.3, 475.3) | 12.51 | 0.002 |
| Prostaglandin E2 (pg/ml)| 82.84 (62.24, 134.4)** | 65.11 (20.84, 83.55)* | 38.53 | (11.39, 46.09) | 7.23 | 0.03 |
| CGRP (pg/ml)          | 35.07 (29.0, 112.3)** | 28.2 (20.9, 34.4) | 25.6 | (20.2, 29.7) | 8.08 | 0.02 |

* P<0.05 vs. rhinitis/sinusitis group; * P<0.05 vs. healthy control group. Data are presented as median (25%, 75% interquartile). CGRP – calcitonin gene-related peptide.

Similarly, the UACS group had more eosinophils as compared to the rhinitis/sinusitis group and healthy control group (Table 3). Furthermore, the concentrations of histamine, prostaglandin E2, and CGRP in the induced sputum from the UACS group and the rhinitis/sinusitis group were significantly higher than those in the healthy control group (Table 4). The treatment with cetirizine alone or in combination with erythromycin in 15 UACS patients resulted in cough resolution and decrease in histamine from 963.7 (195.7, 10217) ng/ml to 458.8 (153.3, 851.7) ng/ml (Z=–1.96, P=0.05) in the induced sputum, but did not change the number of differential cells and concentration of PGE2 in the induced sputum.

**Discussion**

The current study showed that cough sensitivity in UACS was significantly higher than in rhinitis/sinusitis patients without cough and healthy subjects, as indicated by lower cough threshold C5. Laryngopharyngeal anesthesia partially reduced the cough sensitivity in the 3 groups, but reinforced the fact that cough hypersensitivity was a feature in the patients with UACS, since local treatment with lidocaine decreased C5 among all the 3 groups by a similar degree and C5 in the UACS group was still lowest.

Cough sensitivity to capsaicin is related to the excitability of the capsaicin-sensitive C afferent nerve fibers in the airway, which contain capsaicin receptor-transient receptor potential vanilloid 1 (TRPV1) and widely innervates the interepithelial space or subepithelia on the mucosa in the upper airway (nose and larynx) and lower airway (trachea, bronchus, and lung), but is absent in the pharynx [20,21]. In the patients with chronic cough, the overexpression of TRPV1 in sensory nerve endings in the airway has been confirmed and is considered to contribute to the increased cough sensitivity [22,23]. In UACS, it was speculated the cough hypersensitivity may be caused by the sensitization of C afferents located in the upper airway, due to postnasal drip and local inflammation [24], but our results show that this may not be true. In our study, capsaicin was inhaled orally and patients wore nose clips, thus C-fiber on nasal mucosa had no chance to be stimulated by capsaicin. Although laryngopharyngeal anesthesia with lidocaine blocked the impetus transmission in the local afferents and increased cough threshold C5 by a similar degree in all 3 groups, it did not abolish the increased cough sensitivity in the patients with UACS. Therefore, the sensory afferents in the upper airway (including nose and larynx) may not be important for cough hypersensitivity detected in the patients with UACS, even though they are involved in some way, implying that cough receptors in the lower airway may be a major contributing factor.

Several lines of evidence have demonstrated that airway inflammation in rhinitis/sinusitis is not limited to the upper airway. Eosinophilia in the lower airway is common in patients with allergic rhinitis who only presented with nasal symptoms, and the capsaicin-sensitive C afferent nerve fibers in the airway.
as evaluated by bronchoalveolar lavage fluids, bronchial mucosa biopsies, and induced sputum [25,26]. Furthermore, sinusitis is usually characterized by chronic upper and lower airway inflammation [27]. In patients with diverse causes of chronic cough, including UACS, lower airway inflammation has been universally observed [28,29], but how the lower airway inflammation occurs remains unclear. Our procedure for sputum induction minimized the possible contamination of nasolaryngeal secretions by blowing noses and rinsing mouths before the collection of induced sputum. Airway injury caused by coughing itself was impossible to attribute to the lower airway inflammation [30,31] because rhinitis/sinusitis without cough presented with the similar cell profile in the induced sputum. The absence of airway inflammation in the healthy subjects did not support the potential proinflammatory effect of hypertonic saline inhalation during the sputum induction [32]. As demonstrated by Bardin et al. [9], aspiration of nasolaryngeal secretions into the lung could not explain the occurrence of cough in the patients with UACS, even though the possibility could not completely excluded in our study. We believe the lower airway inflammation in the patients with UACS may represent the airway manifestation of systematic allergic reaction in the case of allergic rhinitis [26,33] or the extension of upper airway inflammation by unknown mechanisms as observed in sinusitis [34].

Lower airway inflammation can trigger local tissue injury and epithelial shedding, and expose the subepithelial sensory afferents to tussive stimuli [35]. The inflammatory mediators such as histamine, PGE2, and CGRP released during inflammation [36] can cause cough directly. In addition, these mediators may also sensitize the sensory nerve by upregulating the expression of TRPV1 on C-fibers innervating the airway, altering the synaptic function through modification of the release and responsiveness of neurotransmitters, and reducing the transmission threshold of primary afferents, and ultimately lead to cough hypersensitivity [37]. Our study revealed an increase of histamine, PGE2, and CGRP released in the induced sputum from the patients with UACS and a recovery of histamine and CGRP but not PGE2 when cough resolved with the treatment of cetirizine alone or in combination with erythromycin, suggesting histamine and CGRP are mainly responsible for the occurrence of cough and cough hypersensitivity in the patients with UACS.

Laryngeal hyperresponsiveness, defined as the concentration of inhaled histamine required for 25% decrease in the maximal inspiratory flow, was found in 76% of patients with UACS and is considered as a mechanism underlying UACS [38]. However, laryngeal hyperresponsiveness seems to be non-specific for UACS, since the patients with the other causes of chronic cough presented with a similar frequency of laryngeal hyperresponsiveness and the causal relation between laryngeal hyperresponsiveness and cough has not been confirmed [38]. Moreover, whether laryngeal hyperresponsiveness is prevalent in patients with rhinitis/sinusitis without cough and healthy subjects or not remains to be established. Laryngeal hyperresponsiveness represents the exaggerated reactivity of the larynx to extrinsic stimuli and is associated with local muscular contraction, airway constriction, and the activation of related nerve fibers such as C-fibers and Aδ-fibers [39]. Nevertheless, cough hypersensitivity and laryngeal hyperresponsiveness are different concepts. The reactivity of the extrathoracic airway does not necessarily reflect the sensitization of C fiber related to cough elicitation. Therefore, our finding that the sensory afferents in the laryngopharynx are not involved in cough hypersensitivity are not conflicting with the possible coexisting laryngeal hyperresponsiveness in the patients with UACS.

Conclusions

Laryngeal TRPV1 plays an role in cough sensitivity, while in the patients with UACS, the sensitization of capsaicin-sensitive cough receptors in the lower airway may be more responsible for the cough hypersensitivity. Further studies are needed to elucidate how the lower airway inflammation occurs and how it is related to cough hypersensitivity in patients with chronic cough caused by upper airway disorders.

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