Inhalation of menthol reduces capsaicin cough sensitivity and influences inspiratory flows in chronic cough

Eva Millqvist a,*, Ewa Ternesten-Hasséus a, Mats Bende b

a Department of Internal Medicine/Respiratory Medicine and Allergology, The Sahlgrenska Academy at University of Gothenburg, Sweden
b Department of Oto-Rhino-Laryngology, Head and Neck Surgery, Central Hospital, Skövde, Sweden

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Capsaicin;
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Cough;
Human;
Menthol;
Sensory hyperreactivity

Summary
Introduction: Chronic cough is a common clinical problem and there is a shortage of effective treatments for it. Within the group of transient receptor potential ion channels a receptor for the cooling substance menthol has been identified. This study aimed to assess whether pre-inhalation of dissolved, nebulised menthol could increase capsaicin cough thresholds and influence spirometric values.

Methods: Fourteen patients with chronic cough and airway sensitivity to environmental irritants and 15 control subjects were tested on three occasions. Each one inhaled a 1 mL of nebulised menthol solution of 0.5% or 1% or placebo (saline with 0.05% menthol) at each visit in a randomized and double-blind order. They were then provoked by capsaicin inhalation.

Results: Patients’ cough thresholds differed significantly from the controls’ on all three provocations (P < 0.0001). After inhalation of 1% menthol, the patients’ cough thresholds were significantly higher (P < 0.02) compared to after placebo inhalation and to after 0.5% menthol inhalation (P < 0.05). The patients’ peak inspiratory flows were significantly reduced after inhalation of the placebo (saline) (P < 0.05) but not after inhalation of 0.5% or 1% menthol. Forced inspiratory flows 50% were lowered after inhalation of placebo and of 0.5% menthol (P < 0.05) but not after 1% menthol. Among the controls, forced inspiratory flows 50% were lowered after only placebo inhalation (P < 0.05).

Conclusions: In patients with chronic cough, pre-inhalation of menthol reduces cough sensitivity to inhaled capsaicin and influences inspiratory flows. The findings may provide scientific support for the common practice of using menthol as a reliever for variant airway discomfort. The use of menthol in different cigarette brands could be questioned since it could conceal the natural irritation following smoking.

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* Corresponding author. Department of Internal Medicine/Respiratory Medicine and Allergology, Sahlgrenska Academy at University of Gothenburg, Bruna Stråket 11A, 5-413 45 Gothenburgh, Sweden. Tel.: +46 708 43 38 19; fax: +46 31 82 49 04.
E-mail address: eva.millqvist@medfak.gu.se (E. Millqvist).

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Introduction

Cough is one of the most common causes to seek medical help in the Western world. When it persists for more than 2 months, it is regarded as chronic, though the definition of “chronic cough” varies in the literature. The pathophysiology behind coughing is fairly well understood in a number of conditions, although it is not always possible to attribute a persistent cough to an airway disease or any other medical disorder. In some cases, chronic cough is related to reflux and indigestion and some authors claim an upper airway cough syndrome as a common reason for enduring cough. Still, there remains a group of patients with no obvious explanation for their coughing; these patients are often described as presenting with chronic idiopathic cough. In chronic cough, both labelled as idiopathic and with an evident cause as asthma or chronic obstructive pulmonary disease (COPD), augmented cough sensitivity to inhaled capsaicin is often present and may mirror a general up-regulation of the cough reflex, described by the term cough hypersensitivity syndrome.

Menthol (C\textsubscript{10}H\textsubscript{20}O) is contained in non-prescription products for short-term relief of minor mouth, nasal and throat irritation, for example in lip balms, cough medicines and nasal sprays. In addition, it is used as an additive in certain cigarette brands, both for flavour and to reduce and “hide” the throat and sinus irritation caused by smoking. The sensation of cold evoked by menthol was explained by the recently identified transient receptor potential melastin 8 (TRPM8) receptor, which is triggered by cool temperatures and menthol. The increasing knowledge of the role of transient receptor potential (TRP) ion channels in respiratory diseases and of the TRPM8 may indicate a function for menthol in cough treatment.

Several studies have provided evidence of a condition called airway sensory hyperreactivity (SHR), which is distinct from asthma, COPD, or allergy. SHR patients with cough and pronounced airway sensitivity to environmental irritants like odorous chemicals and scents, but without asthma (bronchial obstruction) or allergy, have increased cough sensitivity to inhaled capsaicin.

The aim of this study was to evaluate any influence of inhaled, nebulised menthol on capsaicin cough sensitivity and on lung function and inspiratory flow values in patients with chronic cough and SHR in comparison with healthy control subjects.

Methods

Subjects

Fourteen non-smoking patients (12 women and 2 men) were consecutively selected from the Asthma and Allergy Outpatient Clinic at Sahlgrenska University hospital Gothenburg Sweden, during 2010. Their mean age was 51 years [95% confidence interval (CI): 45–58] and they had a history of at least one year (mean 14; 95% CI: 10–18) of cough and other airway symptoms induced by environmental irritants. They had a negative skin prick test testing ten of the most common respiratory allergens in Sweden, a negative methacholine test in accordance with international guidelines, and no signs of spirometric reversibility or variability in lung function. Further they had no diagnosis or symptoms of gastro-oesophageal reflux. They were screened with a capsaicin inhalation cough test and with questionnaires on airway symptoms in response to chemicals and scents and all had pronounced airway symptoms, including cough, induced by such items. After fulfilling the criteria for a positive capsaicin inhalation cough test and reaching the cut-off limit for the diagnosis of SHR, the patients were consecutively asked to take part in the study. Demographic data are shown in Table 1.

The control group consisted of 15 non-smoking individuals, 13 women and 2 men, and was recruited from among hospital workers and the researchers’ friends and relatives.

<table>
<thead>
<tr>
<th>Patient no</th>
<th>Sex</th>
<th>Age</th>
<th>FEV\textsubscript{1} % of predicted value</th>
<th>Duration of disease (years)</th>
<th>Inducing factors</th>
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<tbody>
<tr>
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<td>F</td>
<td>61</td>
<td>116</td>
<td>15</td>
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</tr>
<tr>
<td>2</td>
<td>F</td>
<td>52</td>
<td>119</td>
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<td>70</td>
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<td>F</td>
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<td>100</td>
<td>10</td>
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<td>F</td>
<td>54</td>
<td>126</td>
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<tr>
<td>7</td>
<td>F</td>
<td>50</td>
<td>116</td>
<td>24</td>
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<td>8</td>
<td>F</td>
<td>53</td>
<td>108</td>
<td>10</td>
<td>Chemical irritants, exercise, cold air</td>
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<tr>
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<td>28</td>
<td>91</td>
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<tr>
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<td>M</td>
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<td>103</td>
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<tr>
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<td>F</td>
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<td>Chemical irritants, cold air</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>36</td>
<td>117</td>
<td>1</td>
<td>Chemical irritants, exercise, cold air</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>64</td>
<td>100</td>
<td>25</td>
<td>Chemical irritants</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>58</td>
<td>102</td>
<td>15</td>
<td>Chemical irritants, cold air</td>
</tr>
</tbody>
</table>
Inhaled menthol reduces the capsaicin cough response

Their mean age was 52 years (95% CI: 46–58). They were to consider themselves as healthy, report no airway symptoms and use no airway-related drugs. No further physical examination was done.

The participants were not allowed to be treated with 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 Ethical Review Board of Gothenburg, Sweden approved the study.

Study design

All participants visited the clinic on three occasions with about one week’s interval apart each occasion. At each visit they inhaled a 1 mL menthol solution of one of two concentrations or a solution to represent placebo as near as possible (saline with 0.05% menthol added so the participants couldn’t guess which was a placebo by the lack of menthol). After the participant rested for five minutes, a capsaicin inhalation provocation was performed.

The cough thresholds of all study participants were registered manually during the provocations by the same investigator. 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 by a discretionary decision of the investigator upon observation of the subjects.

Menthol inhalation

As noted, before each capsaicin test, a pre-inhalation was given in a double-blind and randomized fashion, of either 1 mL 0.05% menthol (placebo), or 1 mL of 0.5% or 1% menthol.

Five gram of pure (99%) solid menthol (Sigma–Aldrich, Sweden AB, Stockholm, M2772) was dissolved in 5 mL 99.5% ethanol and 5 mL Tween-80 (Sigma–Aldrich, Sweden AB, Stockholm, P1754) providing a stock solution of 50% menthol. Menthol solutions of 0.05%, 0.5% and 1% were prepared from this stock solution with 0.9% saline (9 mg/mL).

Doses were administrated 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 nebulizers’ output was 240 mg/min and the mass median particle diameter 3.2 μm. Each dose of 1 mL was inhaled through a mouthpiece, without a nose clip, by tidal volume breathing to completion, or for a maximum of 4 min, followed by 5 min of rest before the capsaicin provocation.

Capsaicin cough provocation

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 512 μmol/L capsaicin. Fresh serial dilutions were prepared from stock solution, using saline diluent to produce doubling concentrations from 0.5 to 512 μmol/L.

Doses were administrated using the nebulizer described above (MedicAid Pro, Sussex, UK).

The capsaicin cough challenge was performed in accordance with the ERS guidelines using the single breath method. 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 breathes before the capsaicin solution was given in the fourth inhalation. Doubling doses were given every minute and the concentration of capsaicin causing five (C5) or ten or more (C10) coughs during the 1-min period between each dose was registered. A value of 1024 μmol/L capsaicin was assigned if C5 and/or C10 values were >512 μmol/L.

Results

All participants performed the three provocations according to the protocol.

Statistical analysis

The Mann–Whitney U-test was used for non-paired data and the Wilcoxon signed-rank test was used for paired data. Data are presented as means with 95% confidence intervals (CIs) or medians. Results were considered significant if \( P < 0.05 \). All data were analysed using version 16.0 of the SPSS software package (SPSS, Inc., Chicago, IL, USA).

As shown in Table 2, patients’ capsaicin cough thresholds were significantly lower than the control subjects’ on all three provocation occasions (\( P < 0.0001 \)).

After inhalation of 1% menthol, the patients’ C10 cough thresholds were significantly higher compared to thresholds after placebo inhalation (\( P < 0.02 \)) and in comparison to the thresholds after inhalation of 0.5% menthol (\( P < 0.05 \)) (Fig. 1). The C5 was not significantly changed. The cough thresholds after the inhalation of 0.5% menthol did not differ significantly from those after placebo inhalation (0.05% menthol).

The capsaicin cough threshold values of the control group showed no significant differences after each of the three inhalations (Table 2).
Spirometry

Expiratory values
The mean value of forced expiratory volume in 1 s (FEV₁) before the placebo provocation was 103% of predicted value (95% CI: 94–112) among the patients and 101% of predicted value (95% CI: 95–108) among the controls (ns). It did not differ significantly between recordings before or after any of the provocations (data not shown) in either group.

Inspiratory values
Before inhalation of menthol or placebo the inspiratory values from the three provocation occasions did not differ significantly among the patients or the controls or between the two groups.

After inhalation of placebo there was a significant fall in peak inspiratory flow (PIF) in the patient group from a mean value of 3.7 L/s (95% CI: 2.8–4.6) to 3.3 L/s (95% CI: 2.6–4.1) (P < 0.05). There was also a fall in forced inspiratory flow 50% (FIF₅₀) from 3.5 L/s (95% CI: 2.6–4.5) to 3.1 L/s (95% CI: 2.4–3.9) (P < 0.05) (Fig. 2). After inhalation of 1 mL 0.5% menthol, the patients’ mean FIF₅₀ decreased significantly from 3.7 L/s (95% CI: 2.6–4.8) to 3.3 L/s (95% CI: 2.4–4.3) (P < 0.05) but their PIF values did not change significantly.

Inhalation of 1 mL 1% menthol was not followed by any significant changes. None of the capsaicin challenges was followed by any significant changes of inspiratory flows.

Among the controls, the mean FIF₅₀ decreased significantly from 4.3 L/s (95% CI: 3.2–5.4) (P < 0.05) to 3.8 L/s (95% CI: 2.7–4.8) after the placebo inhalation (Fig. 2) but the PIF values did not change significantly. There were no significant changes after inhalation of 0.5% or 1% menthol. None of the capsaicin challenges was followed by any significant changes of inspiratory flows.

Discussion
The main finding of this study was that in patients with chronic cough, capsaicin cough thresholds were significantly

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Capsaicin concentration (μmol/L) causing C5 and C10 in 14 patients with SHR and 15 control subjects. Capsaicin provocations were preceded by either inhalation of 1 mL solution of 0.05% menthol, 0.5% menthol or 1% menthol. Data are presented as medians (μmol/L capsaicin) with ranges from the 25th to the 75th percentile.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>Controls</td>
</tr>
<tr>
<td>Pre-inhalation 0.05% menthol (placebo)</td>
<td></td>
</tr>
<tr>
<td>C5</td>
<td>4 (2–10)</td>
</tr>
<tr>
<td>C10</td>
<td>8 (3.5–32)</td>
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<tr>
<td>Pre-inhalation 0.5% menthol</td>
<td></td>
</tr>
<tr>
<td>C5</td>
<td>4 (1.8–8)</td>
</tr>
<tr>
<td>C10</td>
<td>6 (4–56)</td>
</tr>
<tr>
<td>Pre-inhalation 1% menthol</td>
<td></td>
</tr>
<tr>
<td>C5</td>
<td>4 (2–14)</td>
</tr>
<tr>
<td>C10</td>
<td>32 (8–224)</td>
</tr>
</tbody>
</table>

| Figure 1 | Box plot presentation showing the log capsaicin concentration eliciting 5 or 10 or more coughs in 14 patients with chronic cough after pre-inhalation of either 0.05% (placebo), 0.5% or 1% menthol. The horizontal line in centre of each box is the median. The top and bottom of the box represent the 25th and 75th percentiles, and whiskers indicate the 10th and 90th percentiles. |

| Figure 2 | Mean changes (L/s) of FIF₅₀ (±95% CI) after inhalation of placebo (1 mL 0.9% saline with 0.05% menthol) or 1 mL of 1% menthol in 14 patients with chronic cough and in 15 control subjects. |

<table>
<thead>
<tr>
<th>Placebo</th>
<th>Menthol 1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>Controls</td>
</tr>
</tbody>
</table>
higher after inhalation 1% of menthol in comparison to after placebo inhalation. The placebo inhalation was also followed by reduced inspiratory flows both in chronic cough patients and in control subjects whereas no comparable reduction of was seen after inhalation of 1% menthol solution.

Capsaicin (8-methyl-N-vanillyl-6-nonenamide) stimulates the nonmyelinated C-fibre endings of the sensory nervous system via the transient receptor potential vanilloid subtype 1 (TRPV1) and causes depolarisation across the nerve cell membrane and a local release of neuropeptides.24 There is mounting evidence that TRPV1 regulation is complex and that modulation of selected TRP ion channels may have beneficial effects at targeting key features of different respiratory diseases including airways inflammation, airways hyperreactivity, mucus secretion and cough.16,25 Menthol, acting via the TRPM8, might interfere with the TRPV1 and the cough outcome from capsaicin.16 Cough provocation with capsaicin has, in several studies, shown good reproducibility in healthy subjects and in patients with cough induced by environmental irritants26–28 which indicates that the influence of menthol on capsaicin cough sensitivity in this study is of significance. None of the capsaicin challenges was followed by any significant changes of inspiratory flows. There are only few reports of corresponding studies on inspiratory flows but this supports earlier findings of Ryan and Gibson29 but is different to what Cho et al. reported.30

Neuro-modulators for sensory neuropathic cough and new compounds to block TRP receptors hold promise for chronic cough and airway hypersensitivity.9,16 There is a rich choice of over-the-counter medications based on menthol oil. Not least, there are menthol-based preparations for different airway symptoms, though few scientific studies can confirm measurable effects. Sant’Ambrogio et al. showed in 1991 that menthol stimulated laryngeal cold receptors in the absence of cold air31 and in 1994 Morice et al. showed that in healthy subjects cough sensitivity to citric acid was reduced when the citric acid provocation was preceded by inhalation of menthol.32 Laude et al. demonstrated corresponding results in guinea pigs.33 Nishino et al. found that nasal inhalation of L-menthol reduced the sensation of respiratory discomfort associated with loaded breathing34 whereas on the other hand, Kenia et al. reported no difference in cough count in children after inhalation of menthol compared to after inhalation of a placebo.35 Inhalation of a 1% menthol solution in the premedication of fiberoptic bronchoscopy did not reduce the cough counts during the procedure but significantly increased the peak expiratory flows and reduced cough and dyspnoea reported by the patients on the day after the bronchoscopy.36 In a recent study Wise et al. report of increased capsaicin cough thresholds in healthy subjects after inhalation from an open bottle containing menthol.37 That study differs from the herein presented results where we found no effects on capsaicin cough sensitivity in the control group. Further, in the present study the menthol was nebulised and given in a randomized and double-blind order. To our knowledge, no comparable studies have been done regarding cough patients. There are reports on the menthol effects (or lack of effects) on nasal airway resistance35,38 where the findings do not indicate any objective changes but a subjective sensation of relief and increased nasal airflow after inhalation of menthol. The new findings that the TRPM8 receptor is signalling increased mucus secretion, which may be protective, could account for the decreased capsaicin sensitivity by preventing capsaicin access to the epithelium.39

The findings in this study that inhalation of 1% menthol was not followed by the same decrease of inspiratory flows provoked by physiologic saline inhalation may be one explanation of the benefit that menthol seems to induce in the airways. Earlier studies have shown a close connection between chronic cough, laryngeal dysfunction, and extra thoracic airway hyperresponsiveness induced by hypertonic saline challenge and resulting in decreased inspiratory flows.29,40 The current results, though assessed by isotonic saline, are in line with these reports but need to be confirmed in future studies with a greater number of participants. However, since there are no international established guidelines and predicted values for inspiratory flows, it may be problematic to interpret the outcome.

We conclude that in patients with chronic cough that is not caused by asthma, COPD or infections, pre-inhalation of menthol reduces cough sensitivity to inhaled capsaicin and influences inspiratory flows. The common practice of using menthol as a reliever for various airway discomforts may have scientific support in these findings but greater studies are needed to confirm the results. The use of menthol in different cigarette brands could be questioned since it could conceal the natural irritation following smoking.

Conflict of interest

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

Acknowledgement

This study was supported by grants from the Regional Health Care Authority of West Sweden, the Swedish Asthma and Allergy Association and the Swedish Heart and Lung Foundation. We are grateful to Margareta Brandt Gertmo and Marianne Wallgren for excellent help with the recordings and with the menthol and capsaicin preparations.

References


