

Results Capsaicin-evoked cough was increased in COPD patients (Fig.1A) and in CS-exposed guinea-pigs (Fig.1B) compared to controls. Capsaicin induced greater depolarisation in nerve tissue from CS-exposed guinea-pigs, and in human vagus nerves from smokers, compared to controls. Capsaicin also induced greater $[Ca^{2+}]_i$ increases in airway-terminating jugular and nodose (which are normally capsaicin-unresponsive) neurons from CS-exposed guinea-pigs. XEN-D0501 (i.p. 1 h before cough recording) almost completely inhibited the cough response to capsaicin in both air- and CS-exposed guinea-pigs (Fig.1B).

Conclusions CS-exposure evoked increased cough to capsaicin in guinea-pigs, mimicking the enhanced cough phenotype observed in COPD patients. This was paralleled by enhanced capsaicin responses in isolated vagus nerves and airway neurons from CS-exposed guinea-pigs and in human vagus from smokers suggesting the enhanced cough phenotype is due to increased TRPV1-mediated sensory nerve responsiveness. Inhibition of the CS-enhanced cough response by XEN-D0501 further implicated a role for TRPV1. This data, together with the finding that TRPV1 KO mice display less inflammation in a similar pre-clinical model of CS-exposure², indicates the potential utility of TRPV1 antagonists in the treatment of COPD, which is currently being evaluated in an ongoing COPD clinical trial.

REFERENCES

- 1 Round. *Br J Clin Pharmacol*. 2011;72:921–931
- 2 Baxter ATS. 2014;A2079

P5 LIPID-LADEN MACROPHAGES IN BRONCHOALVEOLAR LAVAGE FLUID ARE NOT DIAGNOSTIC OF AIRWAY REFLUX

YA Hayman, L Sadofsky, S Faruqi, SP Hart, AH Morice. *Department of Cardiovascular and Respiratory Studies, University of Hull and Hull York Medical School, Castle Hill Hospital, Cottingham, UK*

10.1136/thoraxjnl-2014-206260.155

Aims Demonstration of lipid-laden macrophages in respiratory secretions has been suggested to be a marker of reflux and aspiration. However studies looking at the diagnostic value of quantifying macrophage ingested lipids have been inconclusive. We wanted to look evaluate the utility of this technique in diagnosing airway reflux.

Methods In this prospective study bronchoalveolar lavage samples were collected from patient's undergoing flexible bronchoscopy for various indications (lung cancer, chronic cough, ILD etc). Cells collected were stained with Oil-Red-O. Lipid-laden macrophage index (LLMI) was used to quantify lipid accumulation. This is calculated by grading the amount of intracellular Oil-Red-O positive particles per 100 alveolar macrophages. A score of 0 (no opacification) to 4 (total opacification) is assigned to each macrophage. The sum of the scores yields the LLMI. Patients were asked to complete the Hull Airways Reflux Questionnaire (HARQ), a validated tool to diagnose airways reflux. One of the investigators, blind to the analysis, independently reviewed the clinical notes to establish a diagnosis of associated airway reflux. The investigator performing cell analysis was blind to the clinical details. The groups with and without a clinical diagnosis of airway reflux were compared. Correlations between the HARQ score and LLMI were sought.

Results Twenty nine patients (19 females, mean age 64 years) were included in the study. Of these in 11 a clinical diagnosis of associated airway reflux was made. The mean [SD] LLMI in the

group with airway reflux (95[105]) was not significantly different from those without airway reflux (90[75]). There was a weak correlation observed between the HARQ score and the LLMI (0.09) which was not statistically significant ($p = 0.69$).

Conclusions We fail to demonstrate significant association between LLMI and either a clinical diagnosis of airway reflux or the HARQ score. This could be due to the fact that macrophages scavenge both exogenous and endogenous material. However our study is limited by small numbers and disparate underlying clinical diagnoses. The small correlation of LLMI with HARQ scores merits further evaluation. Whether the proportion of macrophages phagocytising lipids or the degree of lipid ingestion by the macrophages is more important needs further study.

P6 MENTHOL HAS BENEFICIAL EFFECTS IN THE AIRWAYS THROUGH A TRPM8-INDEPENDENT MECHANISM

SA Maher, MA Birrell, SJ Bonvini, MA Wortley, ED Dubuis, F Shala, VC Jones, P Flajolet, Y Negreskul, Z Britton, L Hebib, MG Belvisi. *Imperial College, London, UK*

10.1136/thoraxjnl-2014-206260.156

Introduction Asthma is a debilitating disease of the airways characterised by symptoms such as bronchoconstriction, hyperresponsiveness and cough. Current therapies are associated with significant side effects and are ineffective in severe disease highlighting the need for novel treatments. Menthol, a cooling compound found in medicinal products, is commonly thought to activate the thermo-sensing cation-permeable Transient Receptor Potential Melastatin 8 (TRPM8) channel. Furthermore, menthol is known for its beneficial effects in the airways such as bronchodilation¹ and suppression of nerve activation and cough,² however the mechanism of action is unknown.

Aim To pharmacologically characterise the role of TRPM8 and menthol in the airways.

Methods TRPM8 gene expression was measured using Taqman real-time PCR. Mouse and guinea pig isolated vagal nerves were mounted in a grease-gap chamber and depolarisation (mV) recorded as an indicator of sensory nerve activity. Segments of murine and guinea pig trachea were attached to a force transducer in an organ bath and relaxation of carbachol-induced tone recorded (mg).

Results TRPM8 is expressed in mouse and guinea pig vagal ganglia. The selective TRPM8 agonist, WS3, caused activation of guinea pig and mouse isolated vagal nerves, which was inhibited by the TRPM8 antagonist, JNJ41876666. Furthermore, WS3-induced depolarisation was abolished in isolated vagal nerves from *Trpm8*^{-/-} mice. (-)-menthol (active form) caused a small depolarisation of mouse and guinea pig isolated vagal nerves, which was blocked by JNJ41876666. Interestingly, pre-incubation of (-)-menthol inhibited vagal nerve activation induced by the tussive stimulus, capsaicin, an effect that was not inhibited by JNJ41876666. WS3 and (-)-menthol caused a concentration-dependent relaxation of murine and guinea pig trachea, which was not abolished by JNJ41876666 nor in the *Trpm8*^{-/-} mouse airway. No expression of TRPM8 was detected in guinea pig or mouse airway smooth muscle.

Conclusions (-)-menthol caused a small TRPM8-dependent activation but a robust TRPM8-independent inhibition of vagal sensory nerve activity and relaxation of airway smooth muscle. Elucidating the mechanism behind the beneficial effects of (-)-menthol could lead to the development of promising new therapeutic targets for airway diseases such as asthma.

REFERENCES

- 1 Ito et al. *Pulm Pharmacol Ther* 2008;21(5):812–817
- 2 Plekova et al. *J Appl Physiol* 2013;115(2):268–274

P7 NEURONAL DYSFUNCTION IN ASTHMA; INSIGHTS FROM THE STUDY OF THE COUGH REFLEX

¹I Satia, ¹K Holt, ¹H Badri, ²M Woodhead, ³P O'Byrne, ¹P O'Byrne, ¹SJ Fowler, ¹JA Smith. ¹Centre for Respiratory and Allergy Research, University of Manchester, Manchester, UK; ²Central Manchester NHS Foundation Trust, Manchester, UK; ³McMaster University Medical Centre, Hamilton, Ontario, Canada

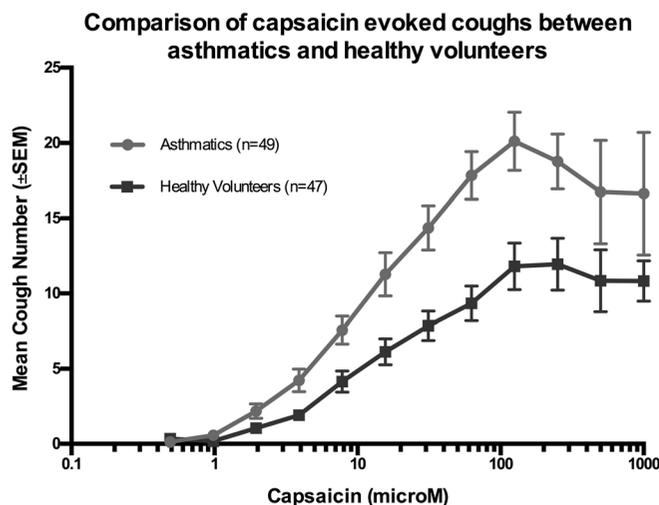
10.1136/thoraxjnl-2014-206260.157

Introduction Cough in asthma is common, troublesome, predicts severity and poor prognosis, yet remarkably little is understood about the underlying neuronal mechanism. Currently available asthma medications are not designed to directly treat cough, the archetypal airway neuronal reflex. Previous studies have commonly used the dose of capsaicin that evokes two coughs (C₂) or five coughs (C₅) as the standard measure to assess the sensitivity of the cough reflex. These measures poorly discriminate between health and disease, and correlate only weakly with objective cough rates.² A novel challenge methodology that uses the maximum number of evoked coughs (E_{max}) as an end point better discriminates between health and disease and correlates strongly with subjective cough measures.²

Objective To assess the differences in the maximum cough responses evoked by capsaicin (E_{max}) between asthmatics and healthy volunteers.

Method A capsaicin inhalational challenge (doubling doses 0.49 to 1000[micro]M) was performed. Four inhalations 30 seconds apart were performed at each concentration and the total coughs evoked at each dose were recorded and verified using a cough monitor. The highest total number of coughs evoked at any dose of capsaicin is denoted E_{max}.

Results Forty nine asthmatics were compared with 47 healthy volunteers. There was a significant difference in the median age between groups (asthmatics 22.9 (IQR 20–27), healthy volunteers 38.0 (29–47) p < 0.001). Equal ratios of females were recruited in both groups (31 in asthmatics and 30 in healthy volunteers). There were no significant differences in gender, body mass index, smoking history or lung function. Asthmatics were of the mild to moderate category (BTS steps 1/2/3, 45/39/16%). There was a significant difference in the E_{max} between asthmatics



Abstract P7 Figure 1

(mean coughs 20.5 (SD±10.1) and healthy volunteers 13.1 (±7.2) (p < 0.001). See Figure 1.

Conclusion Using this novel full dose response methodology, this data suggests that even during stability, asthmatics have an exaggerated cough response to capsaicin. This suggests that subgroups of asthmatics have neuronal dysfunction which can be identified by this capsaicin challenge.

REFERENCES

- 1 *J Allergy Clin Immunol*. 2008 Nov;122(5):903–7
- 2 *J Allergy Clin Immunol*. 2013 Oct;132(4):847–55

P8 OBJECTIVE COUGH FREQUENCY MONITORING IN BRONCHIECTASIS

¹A Spinou, ¹R Garrod, ²KK Lee, ²C Elston, ³MR Loebinger, ⁴KF Chung, ³R Wilson, ¹SS Biring. ¹Asthma, Allergy and Lung Biology, King's College London, London, UK; ²Adult Cystic Fibrosis Unit, King's College Hospital, London, UK; ³Host Defence Unit, Royal Brompton Hospital, London, UK; ⁴National Heart and Lung Institute, Imperial College London, London, UK

10.1136/thoraxjnl-2014-206260.158

Introduction and objectives Cough is a major symptom in bronchiectasis. Cough monitors are emerging as an important tool that assesses cough objectively. The aim of this cross-sectional study was to assess cough frequency in non-cystic fibrosis bronchiectasis, investigate its association with patient-reported symptoms and health-related quality of life (HRQOL), and investigate potential factors of cough frequency variability.

Methods Patients with non-cystic fibrosis bronchiectasis were recruited from 2 outpatient bronchiectasis clinics. All patients underwent 24-hour ambulatory cough monitoring with the Leicester Cough Monitor, and reported sleeping time in a diary. The patients also completed the Leicester Cough Questionnaire (HRQOL), and visual analogue score (VAS) for sputum and cough severity. Sputum bacteria colonisation status was assessed, and defined as at least 2 positive cultures, minimum 3 months apart and within one year.

Results 49 patients were recruited; median (IQR) age 65 (52, 70) years, 64% female. The aetiology of bronchiectasis were: idiopathic (45%), post infective (29%) and other (25%). The prevalence of sputum colonisation were: pseudomonas aeruginosa

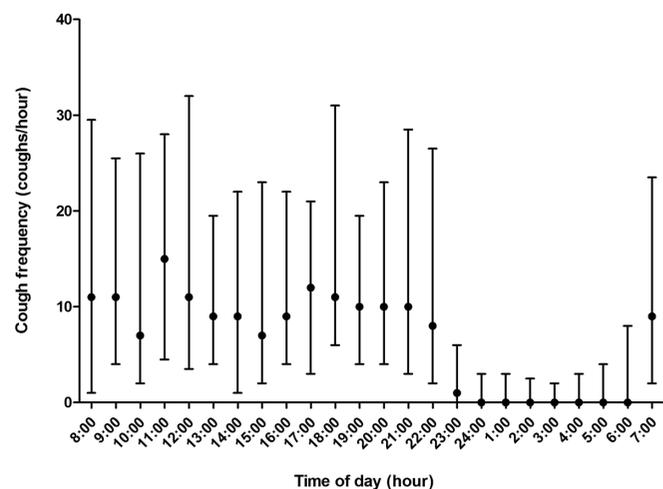


Figure 1. Cough frequency in patients with non-cystic fibrosis bronchiectasis. Data presented as median (IQR).

Abstract P8 Figure 1 Cough frequency in patients with non-cystic fibrosis bronchiectasis. Data presented as median (IQR)