Early View

Original research article

A double-blind randomised placebo-controlled trial investigating the effects of lesogaberan on the objective cough frequency and capsaicin evoked coughs in patients with refractory chronic cough

Huda Badri, Carmen Gibbard, Dimitra Denton, Imran Satia, Bashar Al-Sheklly, Rachel J Dockry, Kimberley Holt, Kevin McGuinness, Sam Treadway, Peter Whorwell, Lesley Houghton, Augustine Lee, K. Jane Escott, Theresa Lee, Greame Wilkinson, Alison Holt, Brendan J Canning, Jacky A Smith

Please cite this article as: Badri H, Gibbard C, Denton D, *et al.* A double-blind randomised placebo-controlled trial investigating the effects of lesogaberan on the objective cough frequency and capsaicin evoked coughs in patients with refractory chronic cough. *ERJ Open Res* 2022; in press (<https://doi.org/10.1183/23120541.00546-2021>).

This manuscript has recently been accepted for publication in the *ERJ Open Research*. It is published here in its accepted form prior to copyediting and typesetting by our production team. After these production processes are complete and the authors have approved the resulting proofs, the article will move to the latest issue of the ERJOR online.

A double-blind randomised placebo-controlled trial investigating the effects of lesogaberan on the objective cough frequency and capsaicin evoked coughs in patients with refractory chronic cough

Huda Badri^{1,2}, Carmen Gibbard¹, Dimitra Denton¹, Imran Satia^{1,3}, Bashar Al-Sheklly¹, Rachel J Dockry¹, Kimberley Holt¹, Kevin McGuinness⁴, Sam Treadway⁴, Peter Whorwell^{1,4}, Lesley Houghton^{1,5,6}, Augustine Lee⁷, K. Jane Escott⁸, Theresa Lee⁷, Greame Wilkinson⁹, Alison Holt⁹, Brendan J Canning¹⁰, Jacky A Smith^{1,4}.

Institutions

¹ The University of Manchester, UK. ² North Manchester General Hospital, Pennine Acute NHS trust, UK; ³ Department of Medicine, Division of Respiratory Medicine, McMaster University, Hamilton, Ontario, Canada; ⁴ Wythenshawe Hospital, Manchester University Foundation Trust, UK. ⁵ Division of Gastroenterology and Surgical Sciences, Leeds Institute of Medical Research at St James's Hospital, University of Leeds, UK. ⁶ Division of Gastroenterology and Hepatology, Mayo Clinic, Jacksonville, Florida, USA. ⁷ Pulmonary Medicine, Mayo Clinic, Jacksonville, Florida, USA. ⁸ Emerging Innovations, BioPharmaceuticals R&D, AstraZeneca, Cambridge, UK, Cambridge, United Kingdom. ⁹ Former employees at AstraZeneca, Alderley Park, UK. ¹⁰ Johns Hopkins Asthma and Allergy Centre, Baltimore, MD, USA.

Corresponding author: Dr Huda Badri.

Education and Research Centre 2nd floor,
University of Manchester,
Wythenshawe Hospital,
Southmoor Rd,
Manchester,
M23 9LT
UK
+44 (00)7968629781
huda.badri@manchester.ac.uk

Word count: 2819 (3000 max)

Conflict of interest statement:

JAS and KMG are inventor on a patent describing methods for detecting cough from sound recordings, licensed to Vitalograph Ltd. JAS has received funding for consultancy and research funds from Afferent Pharmaceuticals, Merck Inc., Bayer, Bellus, GSK, Xention Ltd, Ario Pharma Ltd, Glenmark, Almirall, AstraZeneca, Axalbion, Patara, Verona Pharma, NeRRe Pharmaceuticals, Menlo Pharmaceuticals, Attenua Inc. I.S. reports grants from ERS Respire 3 Marie Curie Fellowship, British Medical Association, North West Lung Centre Charity, grants and personal fees from Merck Canada, personal fees from GSK and AstraZeneca, outside the submitted work.

BJC is on the board of a pharmaceutical company.

JE is a current employee of AstraZeneca. GW and AH were previous employees of AstraZeneca.

HB, CLG, DD, BAS, RJD, KH, ST, PJW, LAH, AL, TL have no conflicts of interests.

Funding:

This study was funded by an MRC 'Mechanism of human disease' grant in collaboration with AstraZeneca MR/K015141/1.

JAS is funded by the NIHR Manchester Biomedical Research Centre, a Wellcome Investigator in Science Award and is an NIHR Senior Investigator. HB is funded by a North West Lung Centre

Pickering Fellowship. I.S is currently supported by the E.J Moran Campbell Early Career Award, Department of Medicine, McMaster University.

Author contribution to manuscript:

J.A.S, G.W, A.H., L.A.H and B.J.C, study concept. H.B., J.A.S., K.H., and R.J.D study design, protocol, set up of study, H.B., C.G., D.D., I.S., B.A.S, R.J.D, K.H., S.T., recruitment, and data collection, data analysis HB, JAS, AL, T.L. Manuscript written by H.B. and J.A.S but all authors contributed and reviewed the article.

ABSTRACT

Objective: Baclofen is a centrally acting Gamma-aminobutyric acid type B (GABA_B) receptor agonist which reduces gastro-oesophageal reflux and suppresses the cough reflex, however, central nervous system side effects limit its use. Lesogaberan is a novel peripherally acting GABA_B agonist but its effects on refractory chronic cough are unknown.

Design: We performed a single centre, placebo-controlled, double-blind randomised crossover study in patients with chronic cough, refractory to the treatment of underlying conditions. Patients were randomised to treatment with lesogaberan 120mg Modified Release (MR) twice daily (BD) or matched placebo for 2 weeks and then crossed over to the alternative therapy after a 2-week washout. The primary endpoint was 24-hour cough frequency measured with an acoustic monitoring system. Cough responses to capsaicin were also measured and gastro-oesophageal reflux assessed by 24h pH/impedance at screening.

Results: Twenty-two patients were randomised to receive lesogaberan/placebo or placebo/lesogaberan [female (73%), mean age 63.7 ±S.D.7.2 years, median cough duration 10.5 years (IQR 5.8-17.0), mean 45 (95%CI 29-67) reflux events in 24hrs and 2 patients had abnormal oesophageal acid exposure times]. Although lesogaberan reduced cough counts by 26% over placebo, this did not reach statistical significance (p=0.12). However, lesogaberan did significantly improve cough responses to capsaicin (p=0.04) and the number of cough bouts (p=0.04) compared with placebo. Lesogaberan was well-tolerated in this study.

Conclusions: Lesogaberan improved cough hyper-sensitivity and the number of bouts of coughing but not coughs per hour. This implies a possible role for peripheral GABA_B receptors in refractory chronic cough.

(Eudract number: 2014-005074-11, ISRCTN registration 77000698)

Introduction

Cough is the commonest presenting symptom in primary care consultations and an estimated 10% of adults worldwide suffer from a cough lasting greater than 8 weeks i.e., chronic cough (1, 2).

Patients presenting with chronic coughing are typically female, 50-70 years of age and report coughing on exposure to usually innocuous triggers such as changes in temperature, irritant aerosols such as cleaning products and use of their voice (3). They also have heightened responses to inhaled irritants known to activate airway nerves (4). Current management strategies focus on treating potential co-morbidities such as asthma, nasal disease and gastroesophageal disease which may improve coughing, but in some patients chronic coughing is refractory to treatment of associated co-morbidities or co-morbidities cannot be identified, known as refractory chronic cough (RCC). At present there are no effective licensed therapies for patients with refractory chronic cough.

Many studies report that gastroesophageal reflux disease (GORD) is commonly associated with chronic coughing. However, randomised controlled trials of antacid therapy in chronic cough patients have largely produced negative findings (5, 6), which has led to current cough consensus guidelines discouraging the routine prescribing of acid suppression therapy (7). The role of GORD in RCC is unclear. Various mechanisms have been suggested, including micro aspiration of refluxate, weakly/non-acid reflux and laryngopharyngeal reflux provoking coughing but little evidence is available. There is evidence however, to suggest temporal associations between reflux and cough events in patients with chronic cough, suggesting that reflux events may be capable of triggering coughing irrespective of their acidity (8-10). The oesophagus and the bronchial tree are both vagally innervated and share the same embryonic origins, thus, reflux episodes, even those confined to the oesophagus may provoke coughing through neuronal crosstalk. Therefore, therapies that reduce all reflux events (regardless of acidity) may have the greatest potential to be effective in patients with cough driven by reflux.

Gamma aminobutyric acid B (GABA_B) receptor agonists such as baclofen have been shown to reduce cough evoked experimentally by inhaling capsaicin, and therefore been proposed as a potential antitussive therapy (11, 12). GABA is the main inhibitory neurotransmitter in the nervous system and GABA_B receptors are widely distributed in the body including the airways and the lower oesophageal sphincter. Baclofen has also been shown to reduce the frequency of transient lower oesophageal relaxations (TLOS_R), the main mechanism mediating reflux events in GORD (13-15). However, baclofen is associated with central nervous system (CNS) side effects such as drowsiness and even seizures limiting its use in clinical practice.

Lesogaberan is a novel peripherally acting GABA_B receptor agonist devoid of CNS side effects as it is actively transported out of the central nervous system. Lesogaberan was developed as an add on treatment to proton pump inhibitors in patients with GORD. In initial trials, lesogaberan significantly reduced TLOS_Rs (16, 17) but was only marginally superior to placebo in achieving an improvement in typical symptoms of reflux disease (18). However patient selection for these studies has been questioned (19). Unlike baclofen, lesogaberan does not appear to suppress capsaicin-evoked cough in healthy controls, but this does not exclude an effect on the hypersensitised cough reflex responses exhibited by patients with RCC (20, 21).

We hypothesised that lesogaberan may be effective at reducing cough in RCC patients via two possible mechanisms i) by reducing TLOS_Rs and ii) by reducing the sensitivity of the cough reflex. We therefore performed a study investigating the effects lesogaberan on spontaneous cough frequency, capsaicin evoked cough responses in patients with RCC. We also explored whether patients with significant reflux-cough associations were more likely to benefit from lesogaberan.

Methods

The study was performed in accordance with the Declaration of Helsinki, Good Clinical Practice, and applicable regulatory requirements including local ethics board approval (Liverpool East, REC reference: 14/NW/1497).

Study subjects

Adults (>18yrs old) with RCC (> 8 weeks duration) attending a specialist cough clinic (between 23rd July 2015 and 10th September 2017) were recruited. All patients had previously undergone assessment and treatment trials to exclude other causes of RCC and manage any triggers, as per current guidelines (22). We did not select patients on the basis of typical GORD symptoms as these should be treated with standard therapies. Current smokers (<6 months abstinence), and ex-smokers with more than 20 pack- years history were excluded. Patients who had a respiratory tract infection or other significant illness within a 4-week period prior to starting the study were also excluded. Patients with significantly abnormal lung function (FEV1/FVC ratio <70%) or chest radiology were excluded. Patients taking ACE inhibitors or currently taking drugs thought to affect cough reflex sensitivity (opiates, pregabalin or gabapentin) were also excluded.

Study Design

This was a single centre, double-blind randomised, placebo-controlled, crossover study, comparing 2 weeks treatment with lesogabaran (120mg MR BD) vs. placebo in RCC patients. At screening, patients underwent High Resolution Impedance Manometry (HRIM) followed by simultaneous 24-hour cough and pH/impedance monitoring. Patients were then randomised to lesogabaran 120mg MR BD or matched placebo for 2 weeks followed by a 7–14-day washout period and then crossed over to the alternative therapy. For each treatment period, we measured capsaicin cough challenge and 24h cough frequency at baseline (pre-treatment) and on day 14 of therapy. Capsaicin cough challenge was performed at 2 hours post dose (corresponding to the C_{max}) on day 14. Leicester Cough Questionnaire (LCQ), Reflux Symptom Questionnaire 7-day recall (RESQ-7) and Visual Analogue Scores (VAS) were completed at the same time points.

STUDY PROCEDURES

Cough measurements

Cough frequency

Participants underwent 24-hour ambulatory acoustic cough monitoring using the VitaloJAK™ cough monitor (Vitalograph Ltd, Buckingham, UK). Cough sounds per hour were quantified by a semi-automated method using validated custom-written software (23-25). Cough “bouts”, defined as continuous coughing without a 2 second pause were also analysed to permit evaluation of reflux-cough associations (8, 26). Awake and night-time coughs were also analysed as secondary outcomes.

Capsaicin evoked cough responses

Participants inhaled four breaths of doubling concentrations of capsaicin (to total lung capacity, 0.97-1,000μM, Stockport Pharmaceuticals Ltd, Stockport, UK) at 30 second intervals using a dosimeter (KoKo dosimeter; DeVilbiss Healthcare Inc., Somerset, PA) from a nebuliser pot with flow limitation. Coughs evoked in the first 15 seconds post each inhalation were counted. The challenge continued until the maximum tolerated dose, or the final concentration was inhaled. The maximum number of coughs evoked at any concentration of capsaicin (Emax) and the concentration evoking 50% of the maximal response (ED50) were noted.

Patient reported cough measures

Cough specific quality of life was assessed using the Leicester Cough Questionnaire (LCQ) (27). Cough severity was also rated by the patients using a one-hundred-millimetre VAS.

Oesophageal studies

All patients underwent HRIM, (Sandhill Scientific INC. Highland ranch, CO, USA) and analysed according to the Chicago classification (version 3.0) (28). HRIM was carried out to assess oesophageal motility and accurately locate the lower oesophageal sphincter (LOS) to ensure the correct placement of the pH impedance catheter. 24-hour multi-channel intraluminal impedance-

pH monitoring, using a Sleuth® pH/impedance ambulatory system (Sandhill Scientific Inc. Highland Ranch, CO, USA) was then carried out. The distal pH sensor was placed 5cm above the LOS. Patients underwent simultaneous, synchronized 24hour cough recording using the VitaloJAK® ambulatory cough recorder (Vitalograph Ltd, UK). The purpose of the synchronised 24hr pH impedance and cough frequency measurement was to identify reflux-cough associations and thus analyse cough responses in the context of these associations. The 24hr pH impedance data were manually analysed for reflux episodes using the BioView Analysis system (Sandhill Scientific INC, Highlands Ranch, CO) and then reviewed by an experienced technician. Patients taking proton pump inhibitors and/or H2 antagonists were requested to stop their treatments for 2 weeks prior to the test. Data collected during mealtimes and drinks were excluded from the analysis.

Gastroesophageal reflux episodes were defined as sequential, orally progressing drops in impedance to less than 50% of the baseline values in 2 consecutive channels and propagating in retrograde direction to at least the next impedance segment. Acid and non-acid reflux episodes were analysed.

Symptom Association Probability (SAP)

The SAP was used to assess the probability of an association between cough bouts and reflux episodes (8). Each 24hr impedance trace was divided into 2-minute segments and each segment was assessed for the presence of reflux and/or cough. All cough bouts were then assessed for reflux occurring in the preceding 2 minutes. The SAP is calculated assuming reflux and cough events are linked if they fall within 2 minutes of one another. The Fishers exact test is used to assess whether the proportion of linked events exceeds that expected by chance alone. A SAP of 95% or greater within an individual patient is assumed to indicate a positive association. A validated, semi-automated, custom-written SAP analysis tool based on the Weusten et al method was used (29). Other measurements that were calculated were; i) the Symptom Index (SI) defined as the overall percentage of reflux related cough episodes [(calculated by number of reflux related cough episodes/ total number of cough episodes) x 100% and ii) the Symptom Sensitivity Score (SSI)

which is defined as the percentage of cough related reflux episodes [calculated by number of cough bouts associated reflux episodes/total number of reflux episodes) x100](30).

Patient reported reflux symptoms

The RESQ-7 was used to assess frequency and intensity of GORD symptoms. This is a validated 13 item questionnaire, with a higher score indicating higher symptom burden (31).

Dose Justification

We used a lesogaberan dose of 120mg MR bd for a two-week period. To date there are no data to determine the optimal dose of lesogaberan required to reduce or inhibit cough in humans.

Therefore, we based our dosing selection on safety data and the assumption that doses effective at inhibiting TLOSRS will likely also be effective for inhibiting cough. In a multiple dosing study of lesogaberan, MR preparation doses of 60mg, 120mg, 180mg and 240mg BD, for 4 weeks, in 661 patients with persistent GORD were used (18) with no apparent advantage of increasing the dose beyond 120mg. In addition to this there were no serious AEs at the 120mg BD dose; the proportion of AEs was similar to placebo (120mg 39% vs placebo 31%), whereas there were cases of altered liver function enzymes at 240mg (18).

Blinding and Randomisation

Randomisation was carried out using a computer-generated scheme by Almac, Craigvon, UK, who were independent of the study site and procedures. Blinded study medication was supplied to the pharmacy where it was dispensed. Un-blinding was by way of un-blinding scratch cards per treatment.

Patients were randomised to two sequences: lesogaberan/placebo or placebo/lesogaberan. The treatment assigned to a patient was determined by an Almac generated randomisation scheme (block size =10). Treatment numbers were allocated sequentially as the subjects were enrolled.

Outcomes

The primary outcome was the effect of lesogaberan on objective 24h cough frequency. Secondary outcomes were efficacy in SAP positive vs. SAP negative patients after 2 weeks treatment as

measured by cough frequency (24h cough recording), changes in capsaicin evoked cough responses (Emax/ED50) and patient reported outcomes capturing cough severity and reflux symptoms (VAS, LCQ, RESQ7). We also explored the effect of lesogaberan on cough bouts.

Sample size

Based upon previous cough frequency data in an unselected group of patients with RCC, with 22 patients this study had 80% power to detect a 43% reduction in cough with lesogaberan over placebo, assuming a standard deviation of log cough frequency of ± 0.42 . Significance was set at the standard level of $p < 0.05$ (32).

Statistical methods

Generalised Estimating Equation (GEE) models were used to analyse the data (IBM SPSS statistics version 22). The analysis used all the data from the intention to treat population, (all randomised patients who took at least one dose of the study drug). For cough frequency endpoints (24h, daytime and night-time cough rates), we calculated percentage change from baseline after natural log transformation of the data (0.1 was added to the entire night-time cough frequency data as it contained zero numbers). The GEE models assessed the influence of treatment, period and sequence adjusting for the period specific baseline. Potential carryover effects were examined by the sequence in the model. The influence of a positive/negative SAP on the treatment response was assessed as a factor in the model. Similar models were used to assess the effect of treatment and SAP status on cough severity VAS, LCQ, capsaicin cough responses and RESQ7 scores. We estimated the difference between treatments and calculated the percentage difference. Significance was set at $p < 0.05$.

RESULTS

Participants

Twenty-two patients were recruited (between 23rd July 2015 and 10th September 2017), two withdrew after randomisation and 20 completed the study, (Figure 1). Patients were predominantly female, middle-aged with normal lung function, and a median cough duration of 10.5 years. There were no current smokers, most (almost 75%) had never smoked and the rest had minimal smoking history. Ten patients were taking acid-suppression therapy (proton pump inhibitors and H2 antagonists) for typical reflux symptoms; of these 8 were able to withhold this therapy for the duration of the trial. SAP data were only available on 19 patients as 3 were missing (1 failed cough monitor, 1 failed pH/impedance monitor and 1 had oesophageal studies elsewhere). The mean number of reflux events were within normal limits (<70 events/24hr) similar to previous studies (8-10) (Table 1).

Spontaneous cough frequency

Lesogaberan reduced 24-hour cough frequency by 26% compared with placebo. ($p=0.12$, Figure 2 and Table 2). SAP category (Figure 3) and RESQ 7 (online supplement) did not significantly predict the response of cough to lesogaberan. Both daytime and night-time cough rates were reduced by lesogaberan compared with placebo but neither reached statistical significance ($p=0.18$ and $p=0.14$ respectively). We explored the effect of lesogaberan on cough bouts as this method of cough quantification is required for evaluating reflux-cough associations. Lesogaberan reduced 24-hour cough bouts by 31% over placebo, $p=0.04$, Figure 2). There were no significant period or sequence effects.

Capsaicin Evoked cough

16 out of 22 patients completed capsaicin cough challenge due to loss of the supply of capsaicin during the study. Lesogaberan reduced capsaicin evoked cough compared with placebo, ($p<0.001$).

The Emax was lower ($p=0.02$) and the ED50 higher ($p=0.02$) after lesogaberan treatment compared with placebo (Figure 4)

Gastro-oesophageal Reflux Measures

Complete impedance data was available in 19 patients. The mean number of reflux events was 45 (95% CI 29-67) with a median of 83 cough bouts (95% CI 46-191) during the 24-hour pH impedance study period, i.e., almost double the number of cough bouts compared to reflux events. The symptom index (SI) showed that 11.6% (95% CI 8.9-20.2) of these cough bouts were preceded by reflux within a 2-minute window. Even in the 58% of patients with a positive SAP analysis the proportion reflux episodes associated with cough bouts [the symptom sensitivity index (SSI)] was only 17.3% (95% CI 11.5-26.0), therefore the potential efficacy of blocking reflux events with lesogaberan in this study was highly limited by the low number of cough episodes temporally linked to reflux. Only 2 of the 19 patients with complete impedance data had a pathological AET (i.e. a pH of <4 for greater than 4% of the 24hour period. therefore the influence of this on the effect of lesogaberan was not explored.

Patient reported outcomes

Lesogaberan treatment had no effect on the LCQ (+0.4 [-0.5-1.3]; $p=0.36$), daytime cough severity VAS or the RESQ 7 (see Table 2 and online supplement).

Treatment emergent adverse events

Lesogaberan 120mg MR BD was well tolerated by the vast majority of patients (Table 3). There were no serious adverse events, in particular there were no significant changes in liver function tests (Table 3).

DISCUSSION

To the best of our knowledge, this is the first study to investigate the effects of a peripherally acting GABA_B receptor agonist in a group of patients with RCC. Lesogaberan reduced spontaneous cough frequency by 26% over placebo, but this reduction did not reach statistical significance. Similarly, the patient reported outcomes did not suggest a significant benefit from lesogaberan in cough or reflux symptoms. Interestingly, lesogaberan did reduce capsaicin evoked cough responses and exploratory analysis suggested that bouts of coughing were significantly reduced by 31% after lesogaberan over the effects of placebo.

The association between RCC and gastro-oesophageal reflux disease has long been described. Pooled data from trials of acid suppressing therapy suggests that patients with symptoms of heartburn and/or abnormal oesophageal acid exposure accompanying their RCC are most likely to gain benefit from acid suppressing therapies (6). The role of non-acid reflux (whether liquid or gaseous) remains controversial and is challenging to investigate in the absence of effective therapies. We used an unlicensed therapy, lesogaberan, to inhibit TLOSRS and thus reflux events (irrespective of acidity or nature) in this patient group and evaluated the effects on cough. Compared with our previous study of temporal reflux cough associations in unselected RCC patients (8), this refractory group had fewer reflux events, and a much smaller number of cough events preceded by reflux (11% versus 33%); despite this, a similar proportion were SAP positive(8). This may have contributed to the negative findings in this study, however, our previous work found that significant reflux-cough associations were independent of the number of reflux events.

In the normal physiological state TLOSRS are under inhibitory control of GABA_B. In clinical and pre-clinical studies, lesogaberan reduced the number of TLOSRS by preventing the loss of inhibition of these events (33-35). In patients with typical reflux and partial improvement in their symptoms with PPIs/H₂ antagonists, there was a clear dose response between lesogaberan therapy

and the reduction in reflux episodes, from 30mg to 240mg BD (17). At the 120mg MR dose of lesogaberan there was a reduction in reflux events by 45% (95% CI -35.to -53.1)(18). Even if lesogaberan was highly effective and blocked all reflux events in our participants, a maximum 11% reduction in cough bouts would be expected, as only this small proportion of coughs appeared to be triggered by reflux in this patient group. In fact, the reduction in cough bouts we observed was much greater (31% over placebo) suggesting an alternative mechanism independent of reflux maybe involved.

The effects of lesogaberan on capsaicin evoked coughs differed from our previous observations in healthy volunteers (36). In RCC patients lesogaberan significantly reduced capsaicin evoked cough suggesting an inhibition of the cough reflex, whereas there was no effect in healthy controls. RCC patients are known to have heightened cough responses to capsaicin inhalation relative to healthy volunteers (37). Lesogaberan attenuated this response, suggesting a possible role for peripheral GABA_B receptors in the pathophysiology of RCC. It is possible that the non-significant improvements in cough frequency and significant improvements in cough bouts could be a consequence of the inhibition of peripheral nerves. Although the dose of lesogaberan used in this study had comparable effects to higher doses on reflux events, whether this dose was optimal for suppressing cough reflex hypersensitivity is unknown.

This study demonstrated a reduction in cough bouts. Although this is not standard methodology for quantifying coughs, patients with RCC describe attacks of coughing as particularly troublesome (38, 39) and grouping cough events is necessary to allow the relationships between cough and reflux to be analysed. We speculate that the reason for this could be that GABA_B receptors may play a particular role in the triggering of cough bouts but has little influence on the subsequent bout duration.

The main limitation of this study was the sample size, which was insufficient for a 26% reduction in 24hr cough frequency to be statistically significant. Post hoc analysis from this study showed that we needed to recruit at least 70 patients (requiring a multicentre study) to achieve statistical

significance. Recent data has estimated that the minimal clinically important difference in objective cough counts in RCC patients is between 20-30% (40) suggesting the improvement in cough frequency in this study could be of clinical relevance. Additionally, objective reflux measurements were only carried out at baseline and not after treatment with drug/placebo. Thus, we had no objective measurement of the effect of lesogaberan on reflux events, or cough-reflux associations. We did not include repeated oesophageal studies in the protocol as this would have been a very significant burden of invasive monitoring for patients. Finally, we cannot exclude the possibility that lesogaberan might have had a more substantial effect in a subgroup of RCC patients selected for significant reflux-cough associations i.e., a positive SAP.

CONCLUSION

Lesogaberan demonstrated no effect on objective hourly cough frequency but reduced the number of cough bouts and capsaicin evoked cough responses, providing some suggestion of a potential role for peripheral GABA_B receptors in the mechanism of RCC. It is unclear whether the efficacy could be enhanced by selecting patients with greater cough-reflux associations.

Acknowledgement:

The delivery of this study was supported by the NIHR Manchester Clinical Research Facility.

Funding source: Medical Research Council; drug and placebo were provided by AstraZeneca, Cambridge UK.

Table 1: Patient demographics
N=22

Age, years	63±7.0
Female, n (%)	16 (73)
Body Mass Index, kg.m ⁻²	25.8±4.0
Smoking status, n (%)	
Never	16 (73)
Ex-smoker	6 (27)
Pack-Years	0 (0-2.4)
Cough duration, years	10.5 (5.8-17.0)
FEV ₁ , % predicted	95±14.6
FVC, % predicted	110±20.7
Baseline 24hr coughs, coughs/hour	24 (12-32)
LCQ score	14.2±3.8
VAS-day time, mm	43±25.6
VAS-night time, mm	17 (8-25)
SAP positive*, n (%)	11(58)
Symptom Index* %	11.6 (11.5-26.0)
Symptom Sensitivity Index** %	17.3 (11.5-26.0)
Acid Exposure Time* %	2.35 (0.65-3.83)
Total reflux episodes**	48.9 (23.5-68.8)
Patients with abnormal reflux events**, n (%)	3 (15)
Ineffective oesophageal motility**, n (%)	8 (40)
LOSP**† , mmHg	22.2 (6.7-26.2)

Data displayed as frequency (%), mean±SD or median (IQR). *n=19, **n=20, †normal pressure is 26 mmHg. FEV₁ forced expiratory volume in 1 second, FVC forced vital capacity, LCQ Leicester Cough Questionnaire (range 3-21), LOSP lower oesophageal sphincter pressure, SAP symptoms association probability, VAS visual analogue scale. AET acid exposure time is the percentage of a 24hr period where the pH in the oesophagus is <4 (normal value is pH,4 for 4% of 24hrs)

Table 2: Outcomes

	Lesogaberan		Placebo		Difference	P Value
	Week 0	Week 2	Week 0	Week 2		
24 hr cough frequency, coughs/hour	24.6 (13.8-38.0) N=21	22.7 (6.7-38.8) N=21	19.5 (11.1-32.3) N=22	22.6 (11.4-29.2) N=18	-26.1%	0.12
Day cough frequency, coughs/hour	34.7 (17.0-58.9) N=21	28.3 (8.9-55.0) N=21	28.8 (15.1-48.0) N=22	34.6 (14.7-42.5) N=18	-23.1%	0.18
Night cough frequency, coughs/hour	2.5 (0.7-7.1) N=21	1.3 (0.1-9.6) N=21	1.8 (0.2-5.0) N=22	1.02 (0.4-1.9) N=18	-28.1%	0.41
VAS – day, mm	53.5 (19.8-61.3) N=18	31.5 (22.4-61.9) N=18	45.5 (22.3-65.5) N=20	35.5(18.3-63.5) N=20	-3.1 (-12.1-5.92)	0.50
VAS – night, mm	16.0 (6.5-46.0) N=18	12.0 (0.9-38.1) N=18	17.5 (2.5-28.6) N=20	14.5 (2.0-36.3) N=20	1.0 (-5.8-7.8)	0.768
LCQ	14.0 ±3.5 N=21	14.9 ±3.0 N=21	14.7 ±3.8 N=21	14.7 ±3.5 N=20	+0.4 (-0.5-1.3) P=0.36	0.36
EMAX	25.5 (14.5-40.3)	26.5(15.3-33.8)	23 (14.3-34)	28 (17.5-46.8)		0.02
ED50	0.89 (-0.1- 0.8)	1.19(0.59-2.10)	1.19(0.29-1.80)	1.04(0.37-1.72)		0.02

Data expressed as mean ± SD or median (IQR). Statistical significance P<0.05. Leister Cough Questionnaire (LCQ), cough visual analogue scale (VAS).

Table 3: Treatment emergent adverse events.

Adverse Event	Lesogaberan (N=21)	Placebo (N=22)
Headache	1 (4.8)	4 (18.2)
Worsening cough	3 (14.3)	2 (9.1)
Diarrhoea	3 (14.3)	1 (4.5)
Heartburn	2 (9.5)	1 (4.5)
Back pain	2 (9.5)	1 (4.5)
Constipation	0	2 (9.1)
Light headed	2 (9.5)	1 (4.5)
Cold like symptoms/LRTI	0	2 (9.1)
Nausea	1 (4.8)	1 (4.5)
Rhinorrhoea/runny nose	2 (9.5)	0
Sore throat	1 (4.8)	1 (4.5)
Vomiting	2 (9.5)	0
Deranged urea and creatinine	1 (4.8)	0
Haematuria	1 (4.8)	0
Haemoptysis	0	1 (4.5)
Insomnia	0	1 (4.5)
Loss of Appetite	0	1 (4.5)
Low blood sugar (BM<xxx)	1 (4.8)	0
Nose bleed	0	1 (4.5)
Right eyelid soreness	1 (4.8)	0
Urinary frequency	0	1 (4.5)
Skin rash	0	1 (4.5)

References

1. Burt CW, Schappert SM. Ambulatory care visits to physician offices, hospital outpatient departments, and emergency departments: United States, 1999--2000. *Vital Health Stat* 13. 2004(157):1-70.
2. Song WJ, Chang YS, Faruqi S, Kim JY, Kang MG, Kim S, et al. The global epidemiology of chronic cough in adults: a systematic review and meta-analysis. *Eur Respir J*. 2015;45(5):1479-81.
3. Hilton E, Marsden P, Thurston A, Kennedy S, Decalmer S, Smith JA. Clinical features of the urge-to-cough in patients with chronic cough. *Respir Med*. 2015;109(6):701-7.
4. Belvisi MG, Birrell MA, Khalid S, Wortley MA, Dockry R, Coote J, et al. Neurophenotypes in Airway Diseases. Insights from Translational Cough Studies. *Am J Respir Crit Care Med*. 2016;193(12):1364-72.
5. Chang AB, Lasserson TJ, Gaffney J, Connor FL, Garske LA. Gastro-oesophageal reflux treatment for prolonged non-specific cough in children and adults. *Cochrane Database Syst Rev*. 2011(1):CD004823.
6. Kahrilas PJ, Howden CW, Hughes N, Molloy-Bland M. Response of chronic cough to acid-suppressive therapy in patients with gastroesophageal reflux disease. *Chest*. 2013;143(3):605-12.
7. Song WJ, Millqvist E, Morice AH. New ERS cough guidelines: A clinical framework for refining the patient management strategy. *Asia Pac Allergy*. 2019;9(4):e36.
8. Smith JA, Decalmer S, Kelsall A, McGuinness K, Jones H, Galloway S, et al. Acoustic cough-reflux associations in chronic cough: potential triggers and mechanisms. *Gastroenterology*. 2010;139(3):754-62.
9. Sifrim D, Dupont L, Blondeau K, Zhang X, Tack J, Janssens J. Weakly acidic reflux in patients with chronic unexplained cough during 24 hour pressure, pH, and impedance monitoring. *Gut*. 2005;54(4):449-54.
10. Blondeau K, Mertens V, Dupont L, Pauwels A, Farre R, Malfroot A, et al. The relationship between gastroesophageal reflux and cough in children with chronic unexplained cough using combined impedance-pH-manometry recordings. *Pediatr Pulmonol*. 2011;46(3):286-94.
11. Dicpinigaitis PV, Dobkin JB, Rauf K, Aldrich TK. Inhibition of capsaicin-induced cough by the gamma-aminobutyric acid agonist baclofen. *Journal of clinical pharmacology*. 1998;38(4):364-7.
12. Dicpinigaitis PV, Dobkin JB. Antitussive effect of the GABA-agonist baclofen. *Chest*. 1997;111(4):996-9.
13. Mittal RK, Holloway RH, Penagini R, Blackshaw LA, Dent J. Transient lower esophageal sphincter relaxation. *Gastroenterology*. 1995;109(2):601-10.
14. Lidums I, Lehmann A, Checklin H, Dent J, Holloway RH. Control of transient lower esophageal sphincter relaxations and reflux by the GABA(B) agonist baclofen in normal subjects. *Gastroenterology*. 2000;118(1):7-13.
15. Grossi L, Spezzaferro M, Sacco LF, Marzio L. Effect of baclofen on oesophageal motility and transient lower oesophageal sphincter relaxations in GORD patients: a 48-h manometric study. *Neurogastroenterol Motil*. 2008;20(7):760-6.
16. Boeckxstaens GE, Beaumont H, Mertens V, Denison H, Ruth M, Adler J, et al. Effects of lesogaberan on reflux and lower esophageal sphincter function in patients with gastroesophageal reflux disease. *Gastroenterology*. 2010;139(2):409-17.
17. Miner PB, Jr., Silberg DG, Ruth M, Miller F, Pandolfino J. Dose-dependent effects of lesogaberan on reflux measures in patients with refractory gastroesophageal reflux disease: a randomized, placebo-controlled study. *BMC gastroenterology*. 2014;14:188.
18. Shaheen NJ, Denison H, Bjorck K, Karlsson M, Silberg DG. Efficacy and safety of lesogaberan in gastro-oesophageal reflux disease: a randomised controlled trial. *Gut*. 2013;62(9):1248-55.
19. Kahrilas PJ, Boeckxstaens G. Failure of reflux inhibitors in clinical trials: bad drugs or wrong patients? *Gut*. 2012;61(10):1501-9.

20. Badri H, C LG, Denton D, Satia I, Dockry RJ, Holt K, et al. Effect of centrally and peripherally acting GABAB agonism on the healthy human cough reflex. *Pulm Pharmacol Ther.* 2021;71:102079.
21. Hilton EC, Baverel PG, Woodcock A, Van Der Graaf PH, Smith JA. Pharmacodynamic modeling of cough responses to capsaicin inhalation calls into question the utility of the C5 end point. *J Allergy Clin Immunol.* 2013;132(4):847-55 e1-5.
22. Morice AH, Millqvist E, Bieksiene K, Birring SS, Dicpinigaitis P, Domingo Ribas C, et al. ERS guidelines on the diagnosis and treatment of chronic cough in adults and children. *Eur Respir J.* 2020;55(1).
23. Khalid S, Murdoch R, Newlands A, Smart K, Kelsall A, Holt K, et al. Transient receptor potential vanilloid 1 (TRPV1) antagonism in patients with refractory chronic cough: a double-blind randomized controlled trial. *J Allergy Clin Immunol.* 2014;134(1):56-62.
24. Abdulqawi R, Dockry R, Holt K, Layton G, McCarthy BG, Ford AP, et al. P2X3 receptor antagonist (AF-219) in refractory chronic cough: a randomised, double-blind, placebo-controlled phase 2 study. *Lancet.* 2015;385(9974):1198-205.
25. Smith JA, Holt K, Dockry R, Sen S, Sheppard K, Turner P, et al. Performance of a digital signal processing algorithm for the accurate quantification of cough frequency. *Eur Respir J.* 2021.
26. Morice AH, Fontana GA, Belvisi MG, Birring SS, Chung KF, Dicpinigaitis PV, et al. ERS guidelines on the assessment of cough. *Eur Respir J.* 2007;29(6):1256-76.
27. Birring SS, Prudon B, Carr AJ, Singh SJ, Morgan MD, Pavord ID. Development of a symptom specific health status measure for patients with chronic cough: Leicester Cough Questionnaire (LCQ). *Thorax.* 2003;58(4):339-43.
28. Kahrilas PJ, Bredenoord AJ, Fox M, Gyawali CP, Roman S, Smout AJ, et al. The Chicago Classification of esophageal motility disorders, v3.0. *Neurogastroenterol Motil.* 2015;27(2):160-74.
29. Weusten BL, Roelofs JM, Akkermans LM, Van Berge-Henegouwen GP, Smout AJ. The symptom-association probability: an improved method for symptom analysis of 24-hour esophageal pH data. *Gastroenterology.* 1994;107(6):1741-5.
30. Bredenoord AJ, Weusten BL, Smout AJ. Symptom association analysis in ambulatory gastro-oesophageal reflux monitoring. *Gut.* 2005;54(12):1810-7.
31. Ryden A, Denison H, Karlsson M, Vakil N. Development and validation of a patient-reported outcome instrument in partial responders to proton pump inhibitors. *Scand J Gastroenterol.* 2013;48(9):1018-26.
32. Belvisi MG, Birrell MA, Wortley MA, Maher SA, Satia I, Badri H, et al. XEN-D0501, a Novel Transient Receptor Potential Vanilloid 1 Antagonist, Does Not Reduce Cough in Patients with Refractory Cough. *Am J Respir Crit Care Med.* 2017;196(10):1255-63.
33. Branden L, Fredriksson A, Harring E, Jensen J, Lehmann A. The novel, peripherally restricted GABAB receptor agonist lesogaberan (AZD3355) inhibits acid reflux and reduces esophageal acid exposure as measured with 24-h pHmetry in dogs. *Eur J Pharmacol.* 2010;634(1-3):138-41.
34. Lehmann A, Jensen JM, Boeckxstaens GE. GABAB receptor agonism as a novel therapeutic modality in the treatment of gastroesophageal reflux disease. *Adv Pharmacol.* 2010;58:287-313.
35. Boeckxstaens GE, Rydholm H, Lei A, Adler J, Ruth M. Effect of lesogaberan, a novel GABA(B)-receptor agonist, on transient lower oesophageal sphincter relaxations in male subjects. *Aliment Pharmacol Ther.* 2010;31(11):1208-17.
36. Badri HG, C.L., Satia, I., Valdramidou, D., Canning, B.J., Houghton, Holt, A., Wilkinson, G., Smith, J.A., editor The Role of GABAB Receptor Mechanisms in The Human Cough Reflex. British Thoracic Society Winter Meeting 2014; 2014 December 2014. London, UK2014.
37. Birring SS, Matos S, Patel RB, Prudon B, Evans DH, Pavord ID. Cough frequency, cough sensitivity and health status in patients with chronic cough. *Respir Med.* 2006;100(6):1105-9.
38. Kelsall A, Decalmer S, Webster D, Brown N, McGuinness K, Woodcock A, et al. How to quantify coughing: correlations with quality of life in chronic cough. *Eur Respir J.* 2008;32(1):175-9.
39. Vernon M, Leidy NK, Nacson A, Nelsen L. Measuring cough severity: Perspectives from the literature and from patients with chronic cough. *Cough.* 2009;5:5.

40. Nguyen AM, Muccino D, Birring SS, Bacci ED, Vernon M, Mines D, et al., editors. Defining Minimal Clinically Important Differences (MCID) in Chronic Cough: Analyses of Objective Cough Counts from a Phase 2 Randomised Controlled Trial. *Journal Of Allergy and Clinical Immunology*; 2019.

Figure 1: Consort diagram

Figure 2: Cough Frequency and cough bout data before and after 2 weeks placebo and lesogaberan treatment. Lesogaberan reduced 24-hour cough frequency by 26% (p=0.12) and cough bouts by 31% over placebo (p=0.04).

Figure 3: Reflux as a predictor of Lesogaberan effect on 24-hour cough counts

Figure 4. Capsaicin evoked coughs chronic cough patients after 2 weeks treatment of placebo vs lesogabaran

Supplementary data

The symptom association probability method for analysis of 24-hour oesophageal pH data.

Developed by Weusten et al 1994, this is a parameter that expresses the likelihood that cough is related to reflux. In order to calculate this parameter simultaneous, (synchronised) acoustic cough and combined pH-impedance reflux recording need to be carried out. Each 24 hour impedance trace was divided into 2 minute segments and each segment was assessed for the presence of reflux and/or cough. All cough epochs were then assessed for reflux occurring in the preceding 2 minutes. Data collected during mealtimes and drinks were excluded from the analysis because reflux could not be accurately evaluated during these periods. *Weusten BL, Roelofs JM, Akkermans LM, Van Berge-Henegouwen GP, Smout AJ. The symptom-association probability: an improved method for symptom analysis of 24-hour esophageal pH data. Gastroenterology. 1994 Dec;107(6):1741-5. doi: 10.1016/0016-5085(94)90815-x. PMID: 7958686.*

Table 4: Detailed outcome data

		Lesogaberan		Placebo		Difference (%)	P Value
		Week 0	Week 2	Week 0	Week 2		
VAS – day, mm		53.5 (19.8-61.3) N=18	31.5 (22.4-61.9) N=18	45.5 (22.3-65.5) N=20	35.5(18.3-63.5) N=20	-3.11 (-12.1-5.92)	0.50
VAS – night, mm		16.0 (6.5-46.0) N=18	12.0 (0.9-38.1) N=18	17.5 (2.5-28.6) N=20	14.5 (2.0-36.3) N=20	1.02 (-5.8-7.8)	0.768
LCQ		14.0 ±3.5 N=21	14.9 ±3.0 N=21	14.7 ±3.8 N=21	14.7 ±3.5 N=20	+0.40 (-0.46-1.26) P=0.36	0.36
RESQ7**							
i)	Heartburn frequency	0.0 (0.0-1.3) N=21	0.0 (0.0-1.0) N=21	0.0 (0.0-0.8) N=21	0.0 (0.0-0.7) N=21	-0.47 (-0.11-0.17)	0.15
ii)	Regurgitation frequency	0.0 (0.0-0.75) N=21	0.0 (0.0-0.8) N=21	0.0 (0.0-0.63) N=21	0.0 (0.0-0.1) N=21	-0.03 (-0.30-0.37)	0.85
iii)	Burp frequency	1.0 (0.0-2.8) N=21	2.0 (0.0-2.3) N=21	1.0 (0.0-3.5) N=21	2.0 (0.5-3.5) N=21	-0.35 (-0.95-0.25)	0.26
iv)	Cough frequency	2.3 (2.2-3.1) N=20	2.3 (2.3-2.8) N=21	2.3 (2.3-2.7) N=20	2.3 (2.3-3.0) N=20	-0.84 (-0.40-0.56)	0.73

v)	Heartburn Intensity	0.0 (0.0-0.9) N=21	0.0 (0.0-0.5) N=21	0.0 (0.0-1.0) N=21	0.0 (0.0-0.9) N=21	-0.3 (-0.13-0.69)	0.55
vi)	Regurgitation Intensity	0.0 (0.0-0.5) N=21	0.0(0.0-0.6) N=21	0.0(0.0-0.8) N=21	0.0(0.0-0.3) N=21	+0.07 (-0.88- 0.23)	0.39
vii)	Burp Intensity	1.0 (0.0-1.5) N=21	0.0(0.0-2.0) N=21	1.0(0.0-2.5) N=21	1.0(0.0-2.0) N=21	+0.05 (-0.11- 0.21) p=0.51	0.51
viii)	Cough Intensity	1.3 (1.2-1.5) N=21	1.3(1.0-1.5) N=21	1.3(0.9-1.7) N=21	1.3 (0.9-1.8) N=21	-0.11 (-0.27- 0.05)	0.12

Data expressed as mean±SD or median (IQR). Statistical significance P<0.05. Leicester Cough Questionnaire (LCQ), Reflux Symptoms Questionnaire, 7 day recall (RESQ7), cough visual analogue scale (VAS). ** RESQ7 questionnaire domains for cough, burp, heartburn, regurgitation frequency and intensity were analysed.