EFFECT OF CENTRALLY AND PERIPHERALLY ACTING GABAB AGONISM ON THE COUGH REFLEX

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Abstract

Background: Currently there are no effective licensed anti-tussive therapies. Understanding how the neuronal mechanisms mediating the cough reflex in animal models translate to humans is important for the development of effective therapies. Preclinical studies suggest that the activation of GABAB receptors in both the peripheral and central nervous systems inhibit cough. Objective: To compare the effect of central and peripherally acting GABAB agonists (lesogaberan and baclofen) on the cough reflex in healthy volunteers. Methods: We performed a single center, double-blind, double-dummy, three-way crossover trial in healthy controls comparing single doses of lesogaberan (120mg MR), with baclofen (40mg) and placebos. Cough responses to inhaled capsaicin were assessed at screening and 2h post-dose on each study day. The primary endpoint was the maximum number of coughs evoked at any concentration of capsaicin (Emax) and the secondary endpoint was the concentration evoking 50% of the maximal response (ED50). Results: Fifteen patients enrolled onto the study (median age 29 (IQR 25-44) years; 7 females, mean BMI 24.6(±3.0). Lesogaberan treatment produced a small, statistically significant increase in Emax compared with placebo [mean 13.4coughs (95%CI 10.1-17.9) vs. 11.8coughs (8.8-15.9), p=0.04], but had no effect on ED50 [geometric mean 47.4μM (95%CI 24.4-91.7) vs 37.6 μM (95%CI 19.2-73.5), p=0.37]. In contrast, baclofen had no significant effect on Emax (11.1, 95%CI 8.1-15.4) (p=0.23), but significantly increased ED50 compared with placebo (geometric mean 75.2μM (95%CI 37.2-151.8), p=0.002). Conclusion: This data suggests the anti-tussive actions of GABAB agonists, in healthy volunteers, occur in the central rather than the peripheral nervous system.

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

ABSTRACT *(250 words)*

Background and Purpose : There are no effective, licensed, anti-tussive therapies. Understanding how the neuronal mechanisms mediating the cough reflex in animal models translate to humans is important for the development of effective therapies. Pre-clinical studies suggest that the activation of $GABA_B$ receptors in both the peripheral and central nervous systems inhibit cough. This study compares the effect of central and peripherally acting $GABA_B$ agonists (lesogaberan and baclofen) on the cough reflex in healthy volunteers (HV).

Experimental Approach: Single center, double-blind, double-dummy, three-way crossover trial in HV comparing single doses of lesogaberan, with baclofen and placebos. Cough responses to inhaled capsaicin were assessed at screening and 2h post-dose on each study day. The primary endpoint was the maximum number of coughs evoked at any concentration of capsaicin (Emax) and the secondary endpoint was the concentration evoking 50% of the maximal response (ED50).

Key Results: Fifteen HV were enrolled (median age 29 (IQR 25-44) years; 7 females, mean BMI 24.6($\pm$3.0). Lesogaberan (120mg MR) treatment produced a small, statistically significant increase in Emax compared

with placebo [mean 13.4coughs (95%CI 10.1-17.9) vs. 11.8coughs (8.8-15.9), p=0.04], but had no effect on ED50 [geometric mean 47.4μM (95%CI 24.4-91.7) vs 37.6 μM (95%CI 19.2-73.5), p=0.37]. In contrast, baclofen (40mg) had no significant effect on Emax (11.1, 95%CI 8.1-15.4) (p=0.23), but significantly increased ED50 compared with placebo (geometric mean 75.2μM (95%CI 37.2-151.8), p=0.002).

Conclusion and Implications: This data suggests the anti-tussive actions of $GABA_B$ agonists, in HV, occur in the central rather than the peripheral nervous system.

Key Words:

$GABA_B$, Lesogaberan, Baclofen, Cough, Capsaicin.

Abbreviations:

GABA: Gamma aminobutyric acid

CNS: Central Nervous system

PNS: peripheral Nervous System

ED50: capsaicin dose inducing half-maximal response

Emax: maximum cough response evoked by any concentration of capsaicin

INTRODUCTION

Cough is the commonest presenting symptom in physician consultations, yet effective licensed therapies remain an unmet clinical need (1). Most consultations for cough are likely to be a consequence of coughing associated with viral respiratory infections which typically resolve spontaneously however it is estimated that around 10% of the general population suffer from chronic coughing defined as a persistent cough of more than 8 weeks duration (1).

Gamma aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the nervous system, with $GABA_B$ receptors found in the brain, airways and the gastrointestinal tract. Animal studies have demonstrated the presence of peripheral $GABA_B$ receptors in the upper airways, the trachea and the lower oesophageal sphincter (2). Pre-clinical studies of central and peripheral $GABA_B$ agonists suggest that they are potent antitussive agents (3, 4). Interestingly $GABA_B$ agonists i.e. baclofen have also been shown to reduce cough responses to inhaled irritants such as capsaicin (5). It is unclear whether this is a central nervous system (CNS) or peripheral nervous system (PNS) effect and whether this change in experimentally evoked cough responses results in reduction of cough frequency in patients. Human data exits on the use of central $GABA_B$ agonists (baclofen) as an antitussive agents (5-8). However, CNS side effects such as drowsiness and even seizures have limited the use of baclofen in clinical practice. Lesogaberan is a novel predominantly peripherally acting $GABA_B$ receptor agonist, which is devoid of CNS side effects due to its uptake by GABA transporters (GAT) in the CNS, its effect on the human cough reflex is unknown.

We hypothesized that $GABA_B$ receptors were capable of inhibiting cough in both the central and peripheral nervous system, and therefore predicted that both a peripherally acting $GABA_B$ receptor agonist (lesogaberan), and a centrally acting $GABA_B$ receptor agonist (baclofen) would both significantly inhibit cough responses to inhaled capsaicin compared to matched placebo therapy. We therefore performed a trial in healthy controls comparing the effect of single doses of lesogaberan (120mg MR), with baclofen (40mg) and matched placebos on cough responses to inhaled capsaicin.

METHODS

The study was performed in accordance with the Declaration of Helsinki, Good Clinical Practice, and applicable regulatory requirements. The local ethics board approved this study (Liverpool East, REC reference: 14/NW/1497), registered in the ISRCTN registry (ISRCTN82391675)and patients provided written consent.

Study design

We carried out a single center, randomized double-blind, placebo-controlled, double dummy, three-way crossover trial in healthy volunteers comparing the effects of a single dose of lesogaberan 120mg Modified Release (MR), baclofen 40mg and matched placebos on evoked cough responses to capsaicin (Figure1; Study overview). Participants were dosed with either lesogaberan plus baclofen placebo, lesogaberan placebo plus baclofen or lesogaberan placebo plus baclofen placebo. Capsaicin cough challenge was performed at screening and then repeated on each study day at 2h post dosing which was the peak serum concertation (C_{max}) for both therapies. The washout period between doses was [?]7 days.

Participants

Volunteers with any clinically significant disease or diagnosis (including a history of lower or upper respiratory tract infection within the preceding 4 weeks) were excluded. Current smokers or ex-smokers with >10 pack year history and volunteers with <6 months abstinence were excluded. Pregnant or breastfeeding women were also excluded. We recruited 15 healthy volunteers from a database of previous volunteers.

Capsaicin evoked cough responses

Participants inhaled four breaths of doubling concentrations of capsaicin (0.97-1,000μM: Stockport Pharmaceuticals Ltd, Stockport, UK) at 30 second intervals using a dosimeter (KoKo dosimeter; DeVilbiss Healthcare Inc., Somerset, PA). Only the 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 (Emax) and the concentration evoking 50% of the maximal response (ED50) were calculated.

Blinding and Randomization

Randomization of participants was carried out using a computer generated scheme (Almac, Craigvon, UK) 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. Matched placebos for both Lesogaberan and Baclofen were manufactured and thus each study treatment consisted of 2 capsules Lesogaberan 120mg/matched Lesogaberan placebo plus Baclofen 40mg/matched Baclofen placebo. Each subject received (in a random, double-blinded fashion) (i) Lesogaberan plus Baclofen Placebo, (ii) Lesogaberan Placebo plus Baclofen and (ii) Lesogaberan Placebo plus Baclofen Placebo.

Outcomes

The primary outcome was the effect of lesogaberan vs baclofen vs placebo on cough responses (Emax), with the secondary outcome being the effect on cough sensitivity (ED50) to inhaled capsaicin.

Statistical methods

Generalized Estimating Equation (GEE) models were used to analyze the data (IBM SPSS statistics version 22). The GEE model was generated using a Poisson distribution for Emax and linear for logED50. The exchangeable correlation matrix was then selected and Wald's estimate for mean and 95% Confidence intervals generated.

The power calculation was based on previous cough challenge data, where one doubling dose of the challenge agent is generally accepted to be a clinically relevant difference in cough reflex sensitivity. Thus, with 15 participants treated, this study had approximately 90% power to detect a one doubling dose change in cough reflex sensitivity of 0.3 for lesogaberan compared with placebo, assuming a standard deviation of ± 0.29 (9).

RESULTS

Participants

Nineteen healthy volunteers were recruited, 4 of which failed screening (see consort, Figure2) leaving 15 completing the study. There were 8 males in the study, the median age was 29 years (IQR 25-44 years),

mean BMI was 24.6 kg/m^2 (SD±3.0). Median FEV1 percent predicted was 96% (IQR 89-117). All the volunteers were never smokers.

Capsaicin evoked cough responses

Rather than reducing cough responses, lesogaberan treatment was associated with a small, increase in the maximal cough response (Emax mean 13.4 coughs, 95% CI 10.1-17.9) compared with placebo (11.8 coughs, 95%CI 8.8-15.9; p= 0.04), but had no effect on ED50 (geometric mean 47.4 μM CI 24.4-91.7 vs placebo 37.6 CI 19.2-73.5 p=0.37). In contrast, baclofen had no significant effect on Emax (11.1, CI8.1-15.4; p=0.23), but ED50 was significantly increased compared with placebo (geometric mean 75.2μM 95%CI 37.2-151.8 p=0.002), i.e. approximately 1 doubling dose higher, indicating that the volunteers tolerated higher doses of capsaicin without coughing. There was no evidence of any period effect for either Emax (p=0.39) or ED50 (p=0.41) in these GEE models. There were too few subjects in this study for sequence effect to be assessed as in some cases only 2 subjects had the same sequence of treatment periods.

Adverse events

There were very few adverse events in this study, and all were considered minor. The most common adverse events were dizziness, headache and light headedness. (Table1) These observations were consistent with the known side effect profile of baclofen. In addition, there was no significant change in the subject's spirometry pre/ post dosing and pre/post capsaicin cough challenge.

DISCUSSION

This is the first human study to determine whether both peripherally acting and centrally acting $GABA_B$ agonists modulate cough responses to inhaled capsaicin compared with placebo. The data suggests that the actions of $GABA_B$ agonists, in healthy volunteers occur in the central rather than the peripheral nervous system. Lesogaberan (peripherally acting $GABA_B$ agonist) had no significant inhibitory effect on the cough reflex whereas baclofen (centrally acting $GABA_B$ agonist) increased the ED50. Interestingly, lesogaberan had a small but significant increase on Emax i.e. the subjects coughed, slightly, more on this drug.

The dose response curve from our data (Figure 3) demonstrates a subtle but significant right shift of the baclofen curve, suggesting a stronger stimulus is required to evoke coughing when on baclofen treatment, consistent with the existing literature. Dicpinigaitis et al, studied a similar group pf subjects to those in this study (20 healthy volunteers, mean age 30) who were dosed chronically with baclofen 10mg three times a day (6) and following that study he went on to study 20mg dosing in a similar group (7). In both of these studies the baclofen group demonstrated a significant increase in the log C5 capsaicin concentration. This is the concentration of capsaicin that induces 5 coughs which is broadly comparable to the ED50 in our study. There are two small case series in the literature which have suggested baclofen might also treat chronic cough. One was an open-label study evaluating a 4-week course of low-dose, oral baclofen against ACE inhibitor-induced cough (8) suggesting that baclofen treatment caused a prolonged antitussive effect. The other study reported suppression of cough and capsaicin cough responses in two chronic cough patients after a 14-day course of baclofen, 10mg; three times a day (5). Participants in both of these studies did not report significant adverse effects, however, in clinical practice patients report significant dizziness (up to 28% of patients) and drowsiness (up to 10% of patients) which limits its use (10).

It is worth noting that changes in experimentally evoked cough do not necessarily equate to clinical anti-tussive effects on spontaneous coughing. This was demonstrated by Belvisi et al., 2017 who studied the effect of a potent TRPV1 antagonist (XEN-D0501) vs placebo on capsaicin evoked cough and spontaneous 24-hour cough frequency (11). Despite XEN-D0501 leading to significant reduction in the capsaicin Emax, there was no improvement in objective cough frequency. In addition to this some agents (such as morphine and gabapentin) that have been shown to improve cough specific quality of life, and are currently used off-license in a clinical setting, do not appear to modulate the C5 endpoint in cough challenge (12). However, it remains unclear which challenge agents, methodology and endpoints are optimal at present. Thus the main role of cough challenge seems to be in confirming target engagement and understanding whether mechanisms

translate from animal studies to humans.

Interestingly, the data from this study suggests a difference between the effects of lesogaberan in humans compared with a guinea pig model. Canning et al., found that both lesogaberan and baclofen significantly reduced citric acid evoked coughs in guinea-pigs (4). Possible explanations for this include differences in the blood brain barrier between species and also relatively higher levels of dosing in the guinea pig, which may have saturated the GATs responsible for inhibiting the CNS effects of lesogaberan.

Despite the relatively small number of subjects in this study, statistically significant changes between the treatments were teased out by the dose response inhalational capsaicin challenge, suggesting the utility and power of this particular method and these endpoints when assessing cough reflex responses. The small but statistically significant increase in Emax with lesogaberan therapy is interesting, paradoxical in the context of the inhibitory nature of $GABA_B$ and difficult to explain. Our prediction was that Emax would have reduced with lesogaberan, mirroring the changes in animal studies. One potential explanation for the lack of reduction in Emax with lesogaberan in healthy volunteers is that $GABA_B$ is involved in endogenous inhibitory mechanisms controlling cough. In the healthy state, these control mechanisms are intact and thus additional $GABA_B$ agonism may have no effect on cough responses, whereas in patients with chronic cough there is increasing evidence that these descending inhibitory mechanisms may be deficient (13-15) and in such circumstances $GABA_B$agonism could have an effect in chronic cough patients not seen in healthy controls.

This study has some limitations. Firstly we used single doses of lesogaberan and baclofen, as opposed to multiple dosing. However, we were still able to replicate previous findings with baclofen after two weeks of dosing. Another possible limitation of this study is that capsaicin was used for the cough challenge, whereas the animal studies used citric acid. This choice was made in light of the fact that all previous human studies suggesting an effect of baclofen on cough responses had all employed capsaicin challenge, furthermore this particular challenge methodology has not been performed using citric acid. An effect of lesogaberan on citric acid but not capsaicin evoked cough cannot be excluded.

In conclusion, this data suggests $GABA_B$ receptor agonists are inhibitory of the human cough reflex in health, but only through their activity in the central nervous system. It remains to be seen whether peripheral $GABA_B$ agonists have any effect on the sensitized cough reflex in chronic cough patients.

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FIGURE LEGENDS

Figure 1. Three-way crossover study randomizing participants to receive lesogaberan, baclofen and their matched placebos in a double-bind, double dummy design.

Figure 2. Consort diagram showing patient flow through the study.

Figure 3. Cough responses to capsaicin challenge showing dose response curves when subjects were treated with lesogaberan= lesogaberan plus baclofen placebo, baclofen= baclofen plus lesogaberan placebo and placebo=lesogaberan placebo and baclofen placebo.

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