The effect of gefapixant, a P2X3 antagonist, on cough reflex sensitivity: a randomised placebo-controlled study

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Gefapixant reduces coughing in patients and blocks ATP- and distilled-water-induced cough, but not cough evoked by citric acid or capsaicin, suggesting a unique TRPV4/ATP pathway may underlie cough hypersensitivity seen in chronic refractory cough http://bit.ly/2Gcr9Lr

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ABSTRACT We evaluated the effect of gefapixant on cough reflex sensitivity to evoked tussive challenge.

In this phase 2, double-blind, two-period study, patients with chronic cough (CC) and healthy volunteers (HV) were randomised to single-dose gefapixant 100 mg or placebo in a crossover fashion. Sequential inhalational challenges with ATP, citric acid, capsaicin and distilled water were performed 1, 3 and 5 h after dosing. Mean concentrations evoking ⩾2 coughs (C2) and ⩾5 coughs (C5) post dose *versus* baseline were co-primary endpoints. Objective cough frequency (coughs·h^{-1}) over 24 h and a cough severity visual analogue scale (VAS) were assessed in CC patients. Adverse events were monitored.

24 CC patients and 12 HV were randomised (mean age 61 and 38 years, respectively). The cough challenge threshold increased for ATP by 4.7-fold (C2, p⩽0.001) and 3.7-fold (C5, p=0.007) for gefapixant *versus* placebo in CC patients; in HV, C2 and C5 increased 2.4-fold (C2, p=0.113; C5, p=0.003). The distilled water C2 and C5 thresholds increased significantly (p<0.001) by a factor of 1.4 and 1.3, respectively, in CC patients. Gefapixant had no effect on capsaicin or citric acid challenge. Median cough frequency was reduced by 42% and the least squares mean cough severity VAS was 18.0 mm lower for gefapixant *versus* placebo in CC patients. Dysgeusia was the most frequent adverse event (75% of HV and 67% of CC patients).

ATP-evoked cough was significantly inhibited by gefapixant 100 mg, demonstrating peripheral target engagement. Cough count and severity were reduced in CC patients. Distilled water may also evoke cough through a purinergic pathway.

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This study is registered as a clinical trial (NCT02476890). Merck & Co., Inc.'s data sharing policy, including restrictions, is available at http://engagezone.merck.com/ds_documentation.php Requests for access to the clinical study data can be submitted through the EngageZone site or *via* email to dataaccess@merck.com

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Introduction

Chronic cough (CC), *i.e.* cough lasting ⩾8 weeks, has been reported in up to 10% of the general population [1, 2]. Patients often experience physical, social and psychological effects from paroxysms of coughing that may be as frequent as hundreds or even thousands of times each day persisting for months or years [3–8].

CC patients may have underlying disorders, including asthma, pulmonary fibrosis, lung cancer, chronic obstructive pulmonary disease (COPD), rhinitis, gastro-oesophageal reflux or oesophageal dysmotility, or they may have unexplained CC for which no associated condition can be identified. In patients with refractory chronic cough (RCC), conventional treatment of underlying disorders is frustratingly inadequate in ameliorating bouts of coughing. Many patients also exhibit an unexplained hypersensitivity to external stimuli, such as a change in temperature, strong smells or aromatic compounds [9]. Cough hypersensitivity syndrome (CHS) is an overarching diagnosis for patients with exquisite sensitivity to otherwise innocuous stimuli [10].

Cough challenge with inhaled tussive agents has been used to assess cough reflex response for several decades [11]. The most common challenge agents include citric acid, capsaicin and fog (*i.e.* distilled water), which stimulate cough through various peripheral nerve receptors in the airways [12]. ATP also induces cough in conditions such as asthma and COPD [13–15]. Recently, ATP challenge has been characterised in patients with and without CC, and although patients do exhibit a heightened cough reflex, the difference in sensitivity is surprisingly small [16]. Thus, the role of ATP in cough reflex sensitivity remains to be fully elucidated.

P2X3 receptors are ligand-gated ion channels that respond to ATP. Medications targeting these receptors may treat patients through normalisation of afferent sensitivity, specifically afferents that innervate the upper and lower airways [17]. Gefapixant is a P2X3 receptor antagonist that has demonstrated efficacy in the treatment of RCC [18]. To further elucidate the role of purinergic mechanisms in the cough reflex, we conducted a study of gefapixant on cough reflex sensitivity to four inhaled challenge agents, ATP, distilled water, capsaicin and citric acid, in both healthy volunteers (HV) and CC patients. Our hypothesis was that gefapixant would differentially affect cough reflex sensitivity depending on the challenge agent used.

Methods

This double-blind, randomised, two-period, crossover study (protocol 014; ClinicalTrials.gov NCT02476890) in HV and CC patients was conducted at a single site (Hull York Medical School, Cottingham, UK) in accordance with principles of Good Clinical Practice and was approved by the Yorkshire and the Humber - Sheffield Research Ethics Committee (Jarrow, UK). Subjects provided informed consent prior to being enrolled in the study.

Subjects

Enrolled HV and CC patients were between 18 and 80 years of age, inclusive, and were non-smokers for at least 5 years. HV had a forced expiratory volume in 1 s (FEV_1) ⩾80% at screening. CC patients had refractory cough for ⩾1 year (cough unresponsive to ⩾8 weeks of treatment for underlying conditions including reflux disease, asthma and rhinitis) and demonstrated significant cough symptoms as determined by a score >20 out of 70 on the Hull Airway Reflux Questionnaire (HARQ). Additional exclusion criteria are provided in the supplementary material.

Study design

After screening, there was a baseline visit before each of two, 1-day treatment periods that were separated by a minimum 48-h washout period. Treatment consisted of gefapixant 100 mg (two gefapixant 50-mg tablets) and placebo (two matching placebo tablets). Treatments were administered in a double-blind fashion in which subjects and study personnel were blinded to treatment codes. Subjects were assigned to one of two treatment sequences based on a computer-generated randomisation schedule using a permuted block algorithm to allocate subjects' numbers. Stratification was used (HV *versus* CC patients). An equal number of subjects was randomly assigned to each sequence.

At baseline and during each treatment period, cough reflex sensitivity was measured by determining the lowest concentration of inhaled solution required to evoke ⩾2 coughs (C2) and the lowest concentration of inhaled solution required to evoke ⩾5 coughs (C5) for four separate cough challenges (ATP, capsaicin, citric acid and distilled water). The cough challenges were performed in the morning of each baseline visit and 1, 3 and 5 h after dosing during the treatment periods. Objective cough monitoring (from the end of the cough reflex sensitivity challenge to the following day (up to 24 h) was performed at baseline and during each of the two treatment periods in CC patients. Subjects returned 2 weeks after their last treatment visit for a follow-up visit.

The challenge agents were prepared by dilution of stock solutions with saline. The following pre-defined concentrations were used for each challenge agent: ATP: 0.1 mM, 0.3 mM, 1 mM, 3 mM, 10 mM, 30 mM, 100 mM, 300 mM; capsaicin: 0.3 μM, 1 μM, 3 μM, 10 μM, 30 μM, 100 μM, 300 μM, 1000 μM; citric acid: 1 mM, 3 mM, 10 mM, 30 mM, 100 mM, 300 mM, 1 M, 3 M; distilled water: 20%, 40%, 60%, 80%, 100% distilled water in 0.9% saline). Capsaicin and citric acid were obtained from the National Health Services manufacturing pharmacy (Stockport, UK). ATP was obtained from Sigma Aldrich (Gillingham, Dorset, UK).

Primary and secondary endpoints

The concentration of the challenge agents inducing C2 and C5 were assessed 1, 3 and 5 h after exposure; for distilled water, the number of coughs generated during 1 min of exposure was recorded. The co-primary endpoints were the concentrations inducing C2 and C5 for each challenge averaged across the three time points.

Secondary efficacy endpoints included a cough severity visual analogue scale (VAS), an urge-to-cough VAS, cough frequency and total HARQ score in CC patients. CC patients completed the two VAS (100-mm scale) at screening and 1 h after the final cough challenge during the treatment periods; cough severity was scored from "no cough" to "worst cough"; urge-to-cough was scored from "no urge-to-cough" to "worst urge-to-cough" during the previous 1 h.

An ambulatory recording device was used to measure cough frequency [19]. Change from baseline in objective cough frequency and urge-to-cough were measured during treatment periods 1 and 2 (up to 24 h for each measure). Recordings were started at the end of the cough challenge protocol and continued overnight until the following day. Each clock hour was compared across the 3 days of recording. A minimum of 5 h of synchronous and contiguous recording was required before data were considered eligible for analysis.

The HARQ (completed at screening and 1-h post dose during the treatment periods) comprises 14 items, each with a score ranging from "0" (no problem) to "5" (severe/frequent problem) [20]. The total HARQ score is the sum of these 14 item scores with a maximum total score of 70.

Safety evaluation

Safety was assessed through monitoring of adverse events (AEs) and serious AEs, physical examinations, vital signs, 12-lead ECGs and clinical laboratory tests (haematology, chemistry and urine analysis).

Statistical methods

For each challenge, CC patients and HV were analysed separately. C2 and C5 analyses were also performed separately.

Log transformation was used for the co-primary endpoints. A log C2 and C5 concentration was generally regarded as normally distributed within a population, so the treatment comparisons were performed using a mixed effect repeated measures (MMRM) model that included fixed effects for period, treatment group and all interaction terms of treatment, time point and period, with the baseline value (in log scale) as a

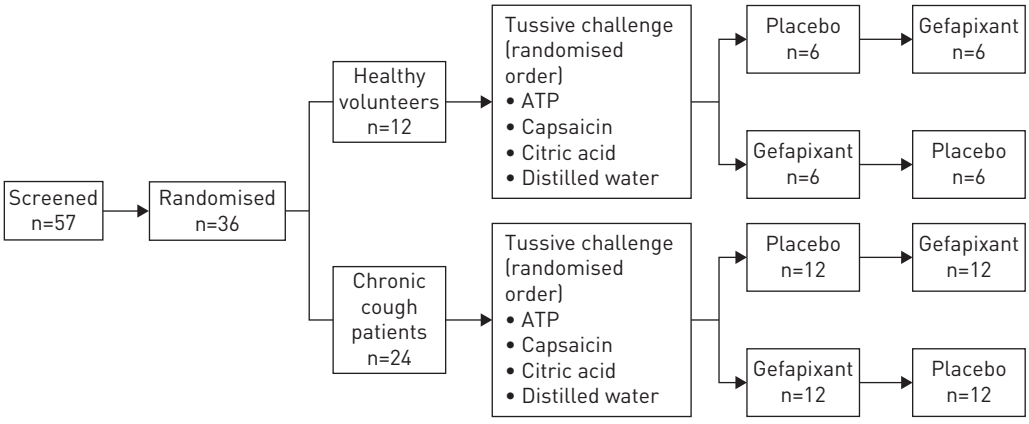

FIGURE 1 Disposition of subjects. Tussive challenges were administered 1, 3 and 5 h post dose. All randomised subjects were included in the full analysis set for efficacy analyses as well as the safety set for evaluation of safety. All randomised subjects completed the study.

covariate. The MMRM model used all available data at 1, 3 and 5 h post dose. An unstructured covariance matrix was applied for the MMRM.

For cough reflex sensitivity testing, if a subject did not achieve C2 or C5 at the maximum concentration of the challenge agent, 1.5 times that concentration was imputed.

Secondary endpoints for subjects with CC were analysed using a MMRM model that included fixed effects for period, treatment group and the interaction term of treatment and period, with the period-specific baseline value as a covariate.

TABLE 1 Baseline characteristics

	Healthy volunteers	Chronic cough patients
Subjects n	12	24
Female sex n (%)	11 (92)	21 (88)
Age years	37.8±8.65	61.1±8.69
Age range years	26–52	48–73
Weight kg	71.5±13.24	69.1±16.46
Duration of cough years	N/A	14.6±9.89
Duration range years	N/A	3–44
Cough severity VAS	N/A	68.6±17.45

Data are presented as mean±SD, unless otherwise stated. VAS: visual analogue scale; N/A: not applicable.

TABLE 2 Treatment comparison using a mixed effect repeated measures model for C2 and C5 based on natural log-transformed data for the full analysis set of chronic cough patients and healthy volunteers

	C2		C5	
	Gefapixant 100 mg	**Placebo**	**Gefapixant 100 mg**	**Placebo**
Chronic cough patients n	24	24	24	24
ATP mM				
Geometric mean	18.1*	3.9	33.9**	9.2
Ratio (95% CI)	4.7 (2.0–10.8)		3.7 (1.5–9.2)	
Distilled water %				
Geometric mean	83.4**	61.8	91.0**	69.1
Ratio (95% CI)	1.4 (1.1–1.6)		1.3 (1.1–1.6)	
Capsaicin µM				
Geometric mean	5.6	4.1	10.0	7.8
Ratio (95% CI)	1.4 (0.8–2.5)		1.3 (0.7–2.4)	
Citric acid mM				
Geometric mean	58.6	46.5	114.6	86.5
Ratio (95% CI)	1.3 (0.6–2.6)		1.3 (0.7–2.7)	
Healthy volunteers n	12	12	12	12
ATP mM				
Geometric mean	120.2	49.5	272.5**	113.5
Ratio (95% CI)	2.4 (0.8–7.4)		2.4 (1.4–4.0)	
Distilled water %				
Geometric mean	111.9*	76.4	127.1	100.7
Ratio (95% CI)	1.5 (1.3–1.7)		1.3 (0.9–1.8)	
Capsaicin µM				
Geometric mean	21.1	20.8	86.8	17.7
Ratio (95% CI)	1.0 (0.5–2.0)		0.8 (0.3–1.9)	
Citric acid mM				
Geometric mean	475.5	272.5	1232	914.6
Ratio (95% CI)	1.7 (0.8–4.0)		1.4 (0.5–3.8)	

Missing values for the minimum concentration required to induce ⩾2 coughs (C2) or ⩾5 coughs (C5) (unable to reach) were imputed using 1.5× maximum concentration level. The geometric mean was estimated by exponentiating the least squares (LS) mean (in log scale). The ratio of gefapixant to placebo was estimated by exponentiating the LS mean difference (in log scale). *: p-value (LS mean difference *versus* placebo) <0.01; **: p-value (LS mean difference *versus* placebo) <0.001.

Results

Subjects

In total, 24 CC patients and 12 HV were randomised; all subjects completed the study and were included in the primary efficacy population (full analysis set (FAS)) and the safety population (figure 1). Baseline characteristics were comparable between treatment sequences although mean age of HV was lower (38 years) that that of CC patients (63 years) and more women than men were enrolled. The median duration of CC was 12 years (table 1).

Primary endpoints

Gefapixant was associated with an increase in the concentration of ATP and distilled water required to induce C2 and C5 for both HV and CC patients *versus* placebo.

The ATP cough challenges in CC patients showed a 4.7-fold (p=0.0006) concentration increase to induce C2 and a 3.7-fold (p=0.0067) increase for C5 with gefapixant *versus* placebo. In HV, a 2.4-fold (p=0.0029) increase was seen for C5 (table 2, figure 2); the change at C2 was of a similar degree but did not achieve statistical significance (figure 3). The concentration of distilled water to induce C2 and C5 increased (p<0.05), but only by a factor of 1.4 to 1.3 in CC patients and 1.5 to 1.3 in HV. Capsaicin and citric acid concentrations did not increase with gefapixant for C2 and C5 in either HV or CC patients (table 2).

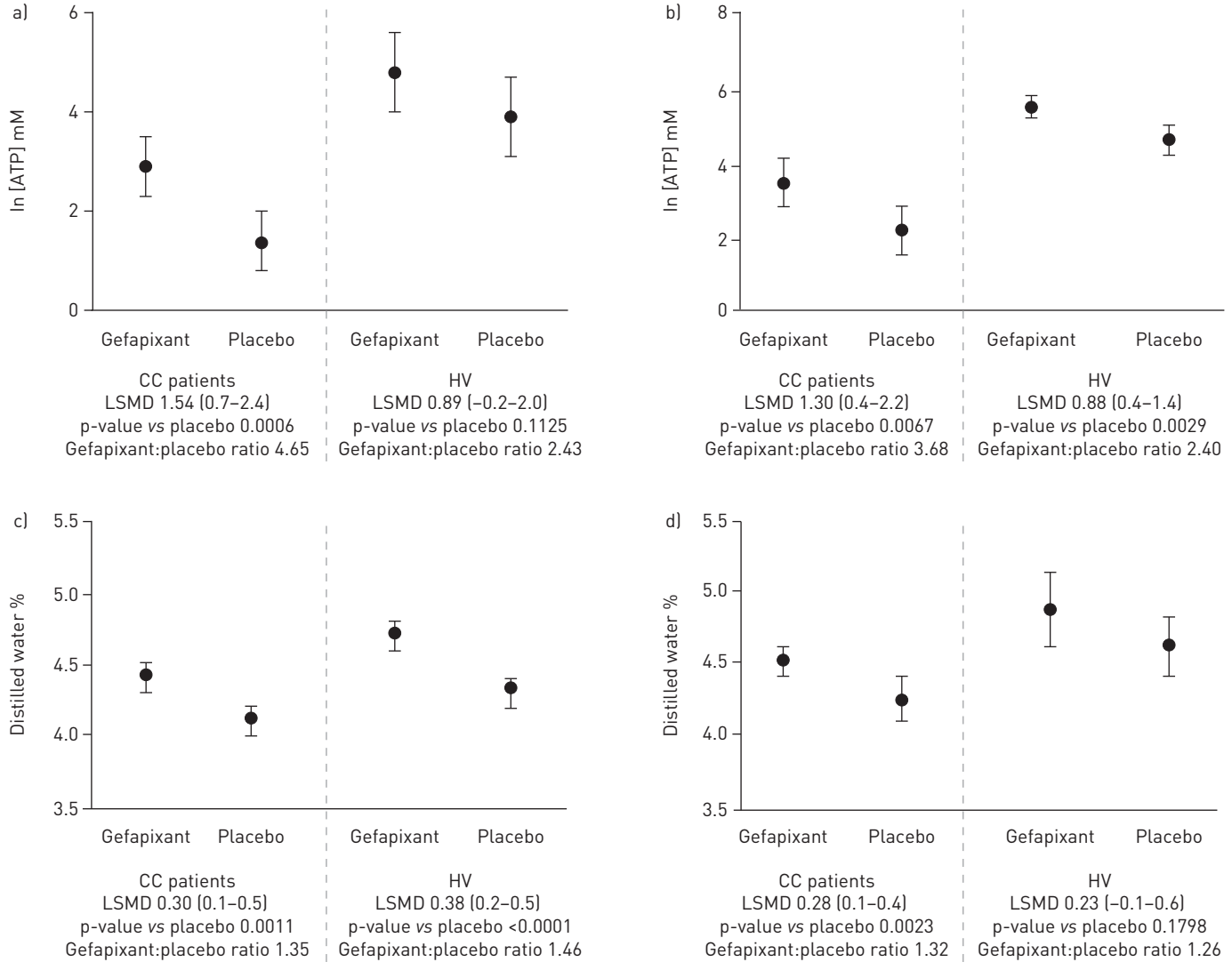

FIGURE 2 Mean concentrations evoking ⩾2 coughs (C2) and ⩾5 (C5) coughs for a, b) ATP and c, d) distilled water cough challenges. Mixed effect repeated measures analysis based on natural log-transformed data: full analysis set population (primary analysis of the mean post-dose response (hours 1, 3 and 5) *versus* baseline). Least squares mean difference (LSMD) presented with 95% confidence intervals. a) C2 mean response to ATP inhalation; b) C5 mean response to ATP inhalation; c) C2 mean response to distilled water; d) C5 mean response to distilled water. CC: chronic cough; HV: healthy volunteers.

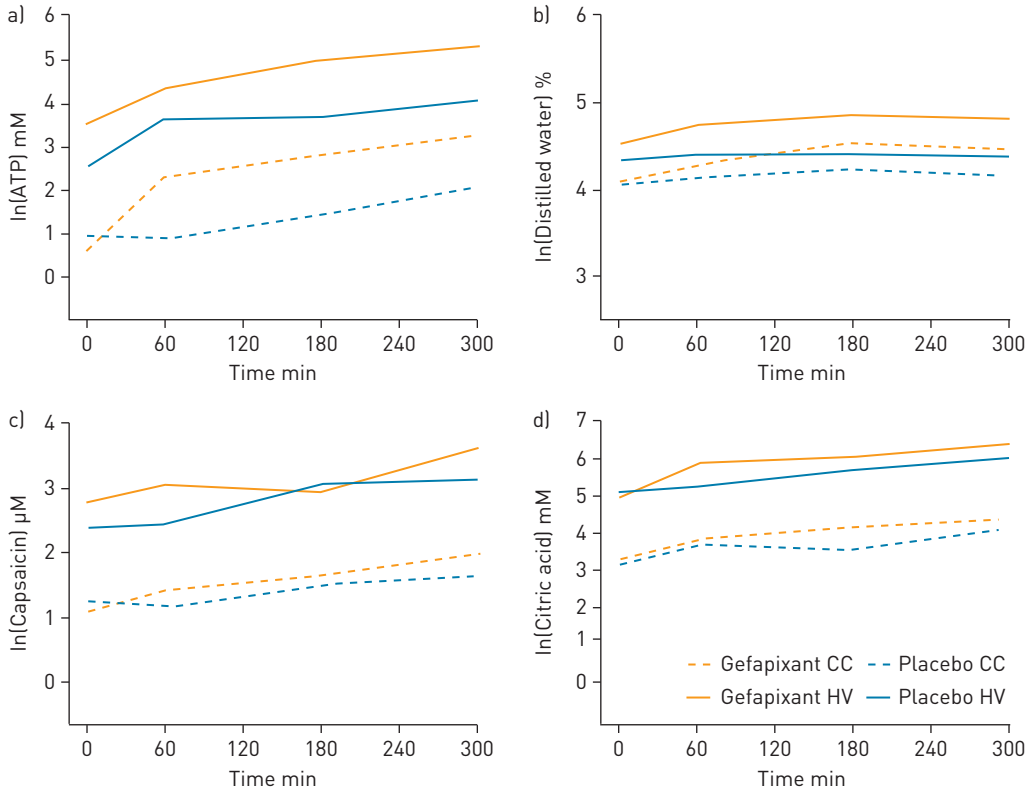

FIGURE 3 Cough challenges. Natural log-transformed mean concentrations evoking ⩾2 coughs (C2) over time (hours 0, 1, 3 and 5). a) ATP challenge; b) distilled water challenge; c) capsaicin challenge; d) citric acid challenge. CC: chronic cough patients; HV: healthy volunteers.

Secondary endpoints

Cough severity VAS

A greater reduction in change from baseline on the cough severity VAS was observed with gefapixant *versus* placebo (p=0.004, table 3).

Urge-to-cough VAS

A greater reduction in change from baseline on the urge-to-cough VAS was observed with gefapixant *versus* placebo (p=0.002, table 3).

HARQ

A significant reduction from baseline in HARQ total score was observed with gefapixant treatment although a reduction with placebo treatment was also observed and the difference for gefapixant *versus* placebo did not achieve significance (table 3).

Cough frequency

A greater reduction from baseline in cough frequency was observed with gefapixant treatment *versus* placebo (p=0.008, table 3).

Safety

There was an increased incidence of AEs with gefapixant *versus* placebo in both HV and CC patients (table 4). No subject had a serious AE or an AE leading to discontinuation from the study. The most common AEs were related to taste (*i.e.* ageusia or dysgeusia) (table 4).

Discussion

This trial demonstrated that a significant increase in ATP and distilled water concentrations were required to elicit C2 or C5 after dosing with gefapixant 100 mg. In contrast, no effect was observed in the capsaicin or citric acid challenges. Responses for all challenges in HV mimicked the responses of CC patients but to a lesser degree. Additionally, gefapixant 100 mg decreased cough severity and frequency among CC patients.

TABLE 3 Summary of endpoints assessing cough burden in chronic cough patients upon treatment with gefapixant and placebo

	Subjects n	LS mean (95% CI)	p-value
Cough severity VAS			
Gefapixant 100 mg	24	−26.2 (−36.2–−16.2)	
Placebo	24	−8.2 (−18.7–2.2)	
Gefapixant *versus* placebo		−18.0 (−29.8–−6.2)	0.004
Urge-to-cough VAS			
Gefapixant 100 mg	24	−29.8 (−38.9–−20.7)	
Placebo	24	−11.7 (−20.9–−2.6)	
Gefapixant *versus* placebo		−18 (−29.1–−7.0)	0.002
HARQ total score			
Gefapixant 100 mg	24	−16.2 (−22.1–−10.3)	
Placebo	24	−11.0 (−17.0–−5.1)	
Gefapixant *versus* placebo		−5.2 (−10.9–0.6)	0.077
Cough frequency over 24 h coughs·h^{-1}			
Gefapixant 100 mg	24	−7.7 (−10.1–−5.3)	
Placebo	22	−4.1 (−6.5–−1.7)	
Gefapixant *versus* placebo		−3.6 (−6.2–−1.0)	0.008

Mixed effect repeated measure model includes fixed effects for treatment group, period and the treatment-by-period interaction, with the baseline value as a covariate. VAS: visual analogue scale; HARQ: Hull Airway Reflux Questionnaire.

TABLE 4 Summary of AEs

	Healthy volunteers		Chronic cough patients	
	Gefapixant 100 mg	Placebo	Gefapixant 100 mg	Placebo
Subjects n	12	12	24	24
Any AEs	12 (100.0)	6 (50.0)	23 (95.8)	8 (33.3)
Serious AEs or AEs leading to discontinuation	0	0	0	0
Most frequent AEs				
Dysgeusia	9 (75.0)	1 (8.3)	16 (66.7)	0
Ageusia	6 (50.0)	1 (8.3)	7 (29.2)	0
Dry mouth	4 (33.3)	0	6 (25.0)	1 (4.2)
Hypoaesthesia (oral)	3 (25.0)	0	4 (16.7)	0
Headache	0	3 (25.0)	6 (25.0)	2 (8.3)
Paraesthesia (oral)	1 (8.3)	1 (8.3)	4 (16.7)	2 (8.3)

Data are presented as n (%), unless otherwise stated. AE: adverse event.

The primary function of the cough reflex is to prevent or minimise aspiration. People with conditions in which cough reflex sensitivity is diminished (*e.g.* stroke, parkinsonism or dementia) frequently experience such events [21, 22]. It is unsurprising, then, that a series of nociceptors located in the upper airways, attached to vagal afferents, have evolved to defend the airway against such insults. The investigation of cough reflex sensitivity by inhalational tussive challenge has been used for over 60 years as a tool to study the physiology and clinical pharmacology of this vital protective reflex. Citric acid was the first agent to be used and, although its precise mechanism of action is still unclear, this challenge is related to the buffered pH of the solution used [23]. Capsaicin acts through a specific nociceptor, transient receptor potential cation channel subfamily v member 1 (TRPV1), which is also acid sensitive but has different characteristics of adaptation [24]; evoked cough can be blocked by specific TRPV1 antagonists [25]. Distilled water again has different attributes, with very rapid adaptation and marked tachyphylaxis. It is thought to trigger cough *via* osmoreceptors. Finally, the most recently described tussive challenge agent, ATP, produces a concentration-dependent increase in coughing with a slightly greater response seen in those with CC [16]. This latter phenomenon of increased sensitivity to challenge agents is seen with all modalities of cough challenge, but the effect size is small, implying that increased peripheral nociceptor sensitivity may not be a fundamental mechanism in the profound hypersensitivity seen in CHS [26].

These challenge agents are thought to act in the immediate vicinity of the airway epithelium. Buffering will rapidly occur with the small droplets of distilled water fog and citric acid. ATP is rapidly metabolised to AMP and adenosine. Capsaicin is highly lipid soluble and avidly taken up into cell membranes. The more central pathways of the vagal afferents through the nodose and jugular ganglia to the solitary nucleus are extremely complex and varied, and exhibit marked plasticity and redundancy in disease [27]. In this environment, the interpretation of cough challenge studies must be undertaken with care.

Our finding that gefapixant led to increases in concentrations needed to induce multiple coughs upon ATP exposure is consistent with peripheral target engagement of the ATP-activated P2X3 receptors in the pathophysiology of CC [28]. It suggests that release of ATP by airway cells may directly stimulate afferent nerves, causing coughing. However, the rapid metabolism of ATP would imply continuous release of ATP to stimulate P2X3, a receptor with a purportedly rapid desensitisation [29]; this observation is compatible with the brief coughing bouts seen following ATP inhalation. A notable other finding in our study was the significant, although smaller, effect of gefapixant in the distilled water challenge. In a recent paper, Bonvini *et al.* [30] describe a mechanism whereby hypo-osmolarity could lead to ATP release. TRPV4 is a nociceptor widely located in the airways and activated by hypo-osmotic stimuli [31]. The authors show that activation of TRPV4 causes a release of ATP *via* pannexin channels and subsequent ATP activation of the neuron can be blocked by a P2X3 antagonist. Administration of a TRPV4 agonist produced prolonged firing of both guinea pig and human Aδ vagus nerve fibres. The antagonist had no effect on citric acid- or capsaicin-sensitive C fibres. As in the current study, P2X3 antagonism also had no effect on the cough sensitivity of guinea pigs to capsaicin challenge.

These findings suggest that there are at least two distinct pathways engendering the cough reflex: one, the TRPV4/ATP pathway responsible for cough hypersensitivity, and two, direct stimulation of nociceptors. Inhibition of TRPV1 and transient receptor potential cation channel subfamily a member 1 (TRPA1) by specific antagonists has no effect in CC [25, 32], whereas, as we show here, even a single dose of gefapixant inhibits ATP receptors to produce a significant improvement. We believe that this is the first demonstration in humans of two separate sensory pathways evoking cough, with TRPV4/ATP as the most likely candidate mechanism underlying cough hypersensitivity. However CC is most likely a heterogeneous phenomenon, triggered by a variety of peripheral mechanisms, thus explaining the significant subgroup of non-responders to gefapixant seen in phase 2 studies. Presumably cough in these patients is mediated *via* other, non-P2X2/3-related mechanisms.

Gefapixant has been evaluated in patients with CC at doses ranging from 7.5 mg to 600 mg twice daily [18, 33–35]. A proof-of-concept study demonstrated efficacy at the high dose of 600 mg twice daily [18] and subsequent dose-ranging studies demonstrated efficacy in doses from 15 mg to 50 mg twice daily with no apparent efficacy advantage with doses above 50 mg twice daily [33–35]. In this study, a single dose of gefapixant 100 mg demonstrated a significant reduction in objective cough frequency and positive improvements in patient-reported outcomes on cough severity, urge-to-cough and improved quality of life in CC patients. These effects after a single 100-mg dose are notable because patient-reported outcomes are often delayed in onset when compared with objective scores in studies of CC; these findings confirm the very rapid onset of action seen with a P2X3 receptor antagonist.

Results observed with ATP and, to a lesser extent, distilled water demonstrate their possible utility for assessing agents that target purinergic receptors such as P2X3, although their use as diagnostic tools for CHS appears to be limited. A previous study showed that although CC patients had significantly more coughing at lower concentrations of ATP, they did not appear to have an intrinsically heightened sensitivity to ATP [16]. Perhaps this indicates that although ATP may constitute the final common mediator for cough hypersensitivity, it may not be the excitatory cause of neural sensitisation. Our putative surrogate for TRPV4 activation, distilled water, had an even lesser response to P2X3 antagonism and has a greater degree of adaptation than ATP. Both agents were administered directly to the airways and it may be that the seat of pathological hypersensitivity is located more centrally.

Gefapixant was associated with taste disturbance AEs at the dose of 100 mg in this study. Although gefapixant has generally not been associated with serious AEs, taste disturbances are the most commonly reported AEs [18, 33]. Previous preclinical research has identified that P2X receptors, particularly P2X2/3 receptors, play an important role in the transmission of taste signals [36, 37]. Studies of purinergic P2X2/3 double-genetic knockout mice have demonstrated a loss of taste-evoked activity [38]. Previous studies with gefapixant suggest a mechanistic role in taste disturbance from P2X3 antagonism based on dose-related taste disturbance [33–35]. Effects on cough reduction were observed at lower doses for which taste disturbances were more limited or minimal; phase 3 studies are ongoing and will provide further evidence of whether positive improvements in the treatment of CC can be achieved with acceptable safety and tolerability [33–35].

There are several important limitations to this study. Taste disturbance may well have influenced the results by unblinding participants. A single dose may not represent effects that occur with chronic therapy.

A further important limitation for this study is its small sample size, which limited our ability to assess an impact from the testing order of tussive agents. However, for individual subjects, the order remained the same for each study day and randomisation was carried out between patients in a block design to minimise the risk of any order effect, which would be balanced by the crossover nature of the study. Previous studies have demonstrated significant cross tachyphylaxis between challenges and a tendency for a reduced response on repeated challenge. This latter phenomenon may account for the upward drift of the cough challenges with time seen in figure 3. However, a *post hoc* analysis of the effect of challenge order in this study found no evidence of carryover between different challenges [39].

In summary, we demonstrated that purine ATP-evoked cough was inhibited by gefapixant 100 mg in both HV and CC patients, although results were more limited in HV. To a smaller, but statistically significant degree, cough was also reduced following the distilled water challenge. An effect of gefapixant on capsaicin- or citric-acid-evoked cough for either HV or CC patients was not observed. Knowledge of the mechanism of drug action is required to understand the relevance of challenge agents in an antitussive drug-discovery model. In this experimental design we have been able to differentiate at least two separate pathways for evoked cough challenge in humans, with the TRPV4/ATP axis most likely to underlie cough hypersensitivity.

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