EUROPEAN RESPIRATORY journal

FLAGSHIP SCIENTIFIC JOURNAL OF ERS

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Original article

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Please cite this article as: Morice AH, Kitt MM, Ford AP, *et al*. The Effect of Gefapixant, a P2X3 antagonist, on Cough Reflex Sensitivity: A randomised placebo-controlled study. *Eur Respir J* 2019; in press (https://doi.org/10.1183/13993003.00439-2019).

The Effect of Gefapixant, a P2X3 antagonist, on Cough Reflex Sensitivity: A randomized placebo-controlled study

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Keywords: chronic cough, cough hypersensitivity syndrome, P2X3, ATP

Summary

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

Abstract

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

Methods: In this Phase 2, double-blind, 2-period study, chronic cough subjects (CC) and healthy volunteers (HV) were randomized to single-dose gefapixant 100 mg or placebo in crossover fashion. Sequential inhalational challenges with ATP, citric acid, capsaicin, and distilled water were performed 1, 3 and 5 hours after dosing. Mean concentrations evoking $\geqslant$2 (C2) and $\geqslant$5 (C5) coughs post dose vs. baseline were co-primary endpoints. Objective cough frequency (coughs/hr) over 24 hours, and Cough Severity Visual Analogue Scale (VAS) were assessed in CC. Adverse events (AE) were monitored.

Results: 24 CC and 12 HV were randomized (mean age 61 and 38 years, respectively). Cough challenge threshold increased for ATP by 4.7-fold (C2, p=<0.001) and 3.7-fold (C5, p=0.007) for gefapixant vs placebo in CC; in HV, C2 and C5 increased 2.4-fold (C2, p=0.113; C5, p=0.003). Distilled water C2 and C5 were increased significantly (p<0.001), by a factor of 1.4 and 1.3, respectively, in CC. Gefapixant had no effect on capsaicin or citric acid challenge. Median cough frequency was reduced by 42% and least-squares (LS) mean Cough Severity VAS was 18.0 mm lower for gefapixant vs. placebo in CC subjects. Dysgeusia was the most frequent AE (75% HV and 67% CC).

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

Clinical Trials Registry: NCT02476890

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, gastroesophageal reflux or oesophageal dysmotility or they may have unexplained chronic cough (UCC) where no associated condition can be identified. In refractory chronic cough (RCC) patients, conventional treatment of underlying disorders are frustratingly inadequate in ameliorating bouts of coughing. Many patients also exhibit a hypersensitivity to external stimuli such as a change in temperature, strong smells, and aromatics which are mysterious to both the patient and their doctor [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]. Adenosine triphosphate (ATP) has also been shown to induce cough in conditions such as asthma and COPD [13-15]. More recently, ATP challenge has been characterized in normal and chronic cough patients, 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 normalization 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 subjects. Our hypothesis was at that gefapixant would differentially affect cough reflex sensitivity dependent on the modality of challenge agent used.

Methods

This double-blind, randomized, 2-period, crossover study (Protocol 014; Clinical Trials Registry NCT02476890) in HV and CC subjects 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 Humber, Sheffield Research Ethics Committee (Jarrow, UK). Subjects provided informed consent prior to being enrolled in the study.

Subjects

Enrolled HV and CC subjects were between 18 and 80 years of age, inclusive, and were non-smokers for at least 5 years. Healthy subjects had a forced expiratory volume in 1 second (FEV_1) $\geqslant$80% at Screening. CC subjects had refractory cough for $\geqslant$ 1 year (cough unresponsive to $\geqslant$ 8 weeks of treatment for underlying conditions including reflux disease, asthma, or rhinitis) and demonstrated significant cough symptoms by a score > 20/70 on the Hull Airway Reflux Questionnaire (HARQ). Additional exclusion criteria are provided in the Supplementary Appendix.

Study Design

After screening, there was a baseline visit before each of two, 1-day treatment periods that were separated by a minimum 48-hour washout period. Treatment consisted of gefapixant 100 mg (2

gefapixant 50-mg tablets) and placebo (2 matching placebo tablets). Treatments were administered in a double-blind fashion where subjects and study personnel were blinded to treatment codes. Subjects were assigned to one of two treatment sequences based on a computer-generated randomization schedule using a permuted block algorithm to allocate subjects' numbers. Stratification was used (HV vs. CC subjects). An equal number of subjects were randomly assigned to each sequence.

At Baseline and during each Treatment Period, cough reflex sensitivity was measured by determining C2 (lowest concentration of inhaled solution required to evoke $\geqslant$2 coughs) and C5 (lowest concentration of inhaled solution required to evoke $\geqslant$5 coughs) 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 hours 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 hours) was performed at Baseline and during each of the two Treatment Periods in subjects with chronic cough. Subjects returned two 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 concentration ranges 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, 1M, 3M); Distilled Water: 20%, 40%, 60%, 80%, of 100% of distilled water in 0.9% saline). Capsaicin and citric acid were obtained from the NHS manufacturing pharmacy, Stockport, UK. ATP was obtained from Sigma Aldrich Gillingham, Dorset, UK.

Primary and Secondary Endpoints

The concentration of the challenge agents inducing ⩾2 (C2) and ⩾5 coughs (C5) were assessed at 1, 3, and 5 hours after exposure; for distilled water, the number of coughs generated during 1 minute of exposure was recorded. The co-primary endpoints were C2 and C5 for each challenge averaged across the 3 time points.

Secondary efficacy endpoints included cough severity visual analogue scale (VAS), urge-to-cough VAS, cough frequency, and total HARQ score in CC subjects. CC subjects completed the two VAS (100-mm scale) at Screening and one hour 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 hour.

To measure cough frequency, an ambulatory recording device was utilized [19]. Change from Baseline in objective cough frequency and urge-to-cough was measured during Treatment Periods 1 and 2 (up to 24 hours for each measure). Recordings were started at the end of cough challenge protocol and continued until the following day. Each clock hour was compared across the three days of recording. A minimum of 5 hours synchronous and contiguous recording was required before data was considered eligible for analysis.

The HARQ (completed at Screening and 1-hour 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 (AE)/serious AEs, physical examinations, vital signs, 12-lead ECGs, and clinical laboratory tests (hematology, chemistry, and urinalysis).

Statistical Methods

For each challenge, CC subjects and HV were analyzed separately. C2 and C5 analyses were also performed separately.

Log transformation was used for the co-primary endpoints. A log C2 and C5 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, period, and the baseline value (in log scale) as a covariate. The MMRM model used all available data at 1, 3, and 5 hours 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 chronic cough were analyzed using a MMRM model that included fixed effects for period, treatment group, and the interaction term of treatment and period, and the period-specific baseline value as a covariate.

Results

Subjects

Twenty-four CC subjects and 12 HV were randomized; all subjects completed the study and were included the primary efficacy population (Full analysis set [FAS]) and the safety population. Baseline characteristics were comparable between treatment sequences although mean age of HV was younger (38 years) compared with mean age for CC subjects (63 years) and more women than men were enrolled. The median duration of chronic cough 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 healthy and chronic cough subjects, versus placebo.

The ATP cough challenges in CC subjects 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 at C5 (Table 2; Figure 2) and whilst the change at C2 was of similar degree, it did not achieve statistical significance (Figure 3). Distilled Water C2 and C5 increased ($p<0.05$), but only by a factor of 1.4 to 1.3 in CC subjects and 1.5 ($p<0.05$) to 1.3 in HV. Capsaicin and citric acid concentrations did not increase with gefapixant for C2 and C5 in either HV or CC subjects (Table 2).

Secondary endpoints

Cough Severity VAS

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

Urge-to-Cough Visual Analogue Scale

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

Hull Airway Reflux Questionnaire (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 subjects (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 two or five coughs after dosing with gefapixant 100 mg. In contrast, no effect was observed on capsaicin or citric acid challenge. Responses for all challenges in HV mimicked responses of CC, but to a lesser degree. Additionally, gefapixant 100 mg improved cough severity and frequency among chronic cough subjects.

The primary function of the cough reflex is to prevent or minimize aspiration. Those with conditions where cough reflex sensitivity is diminished (e.g., stroke, Parkinsonism, or dementia), frequently succumb to such events [21, 22]. It is unsurprising then that a series of nociceptors located in 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, it is a challenge that is related to the buffered pH of the solution used [23]. Capsaicin acts through a specific nociceptor, TRPV1, which is also acid sensitive, but has different

characteristics of adaptation[24] and evoked cough can be blocked by specific TRPV1 antagonists [25]. Distilled water again has different attributes with very rapid adaption and marked tachyphylaxis. It is thought to trigger cough via osmoreceptors. Finally, the most recently described tussive challenge, ATP, produces a concentration-dependent increase in coughing with a slightly greater response seen in those with chronic cough [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 metabolized 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, 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 chronic cough[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 desensitization [29]; an observation that 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 with distilled water challenge. In a recent paper, Bonvini and colleagues [30] describe a mechanism whereby hypo-osmolarity could lead to ATP release. TRPV4 is a nociceptor widely located in the airways and is known to be activated by hypo-osmotic stimuli [31]. They show

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 fibers. There was no effect of the antagonist on citric acid or capsaicin-sensitive C fibers. As in the current study, there was also no effect of P2X3 antagonism on the cough sensitivity of guinea pigs to capsaicin challenge.

This suggests that there are at least two distinct pathways engendering the cough reflex. One, the TRPV4/ATP pathway responsible for cough hypersensitivity, and a second, by direct stimulation of nociceptors. Inhibition of TRPV1 and TRPA1 by specific antagonists has no effect in chronic cough [25, 32] whereas, as we show here, even after a single dose of gefapixant, inhibition of ATP receptors produces a significant improvement. We believe that this is the first demonstration in man of two separate sensory pathways evoking cough with TRPV4/ATP as the most likely candidate mechanism underlying cough hypersensitivity. However chronic cough 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 two studies. Presumably cough in these patients is mediated via other, non-P2X2/3-related mechanisms.

Gefapixant has been evaluated in patients with RCC at doses ranging from 7.5 mg to 600 mg twice daily (bid) [18, 33-35]. A proof-of-concept study demonstrated efficacy at the high dose of 600 mg bid[18] and subsequent dose-ranging studies demonstrated efficacy in doses from 15 mg to 50 mg bid with no apparent efficacy advantage with doses above 50 mg bid[33-35]. In this study, a single dose of gefapixant 100 mg demonstrated 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 chronic cough subjects. These effects after a single 100-mg dose are notable as patient-reported outcomes are often delayed in onset when compared with objective

scores in studies of chronic cough; 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 a diagnostic tool for CHS appears to be limited. A previous study showed that although CC subjects 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 sensitization. Our putative surrogate for TRPV4 activation, distilled water, had an even lesser response to P2X3 antagonism and has a greater degree of adaption than ATP. Both agents were administered direct to the airways and it may be speculated that the seat of pathological hypersensitivity may be 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 pre-clinical research has identified P2X receptors, particularly P2X2/3 receptors, as playing 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 in lower doses where 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 RCC 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 which occur with chronic therapy. A further important limitation for this study was 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 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 has 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 subjects, although results were more limited in HV. To a smaller, but statistically significant degree, coughs were also reduced following the distilled water challenge. An effect of gefapixant on capsaicin- or citric acid-evoked cough for either HV or CC subjects 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 man with the TRPV4/ATP axis most likely to underlie cough hypersensitivity.

Acknowledgements:

The authors thank Susan Lu (Merck & Co., Inc., Kenilworth, NJ, USA) for contributions to the interpretation of the study and scientific review of the manuscript. Anish Mehta (Merck & Co., Inc., Kenilworth, NJ, USA) provided writing and editorial support for the manuscript. Jennifer Pawlowski (Merck & Co., Inc., Kenilworth, NJ, USA) assisted with editorial and administrative support.

Funding:

This study was funded by Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA (the study was originally conducted and funded by Afferent Pharmaceuticals, which was acquired by Merck & Co., Inc., Kenilworth, NJ, USA).

Disclosures:

Alyn Morice has received consulting fees from Afferent, Merck Sharp & Dohme, Boehringer Ingelheim, Pfizer, and Proctor & Gamble, lecture fees from Boehringer Ingelheim and AstraZeneca, and grant support from Proctor & Gamble.

Michael M Kitt and Anthony P Ford are former employees of Afferent Pharmaceuticals and Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA and may own stock in the company. Andrew M Tershakovec is a former employee of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA and may own stock in the company.

Kayleigh Brindle, Rachel Thompson, Susannah Thackray-Nocera, and Caroline Wright have no financial conflicts of interest to disclose.

Author Contributions:

AHM: Conception/design/planning of the study, acquisition of data, analysis of data, interpretation of results, drafting the manuscript, and critical review/revision of the manuscript; MMK: Conception/design/planning of the study, acquisition of data, analysis of data, interpretation of results, and critical review/revision of the manuscript; APF: Conception/design/planning of the study, interpretation of results, and critical review/revision of the manuscript; AMT: Interpretation of results and critical review/revision of the manuscript; WCW: Analysis of data and critical review/revision of the manuscript; KB: Acquisition of data and critical review/revision of the manuscript; RT: Acquisition of data and critical review/revision of the manuscript; STN: Acquisition of data and critical review/revision of the manuscript; CW: Acquisition of data and critical review/revision of the manuscript.

Data Sharing:

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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Figure Legend

Figure 1: Disposition of Subjects. Tussive challenges were administered at 1, 3, and 5 hours post dose. All randomized subjects were included in the Full Analysis Set for efficacy analyses as well as the Safety Set for evaluation of safety. All randomized subjects completed the study.

Figure 2: C2 and C5 for ATP and Distilled Water Cough Challenges - Mixed Model Repeated Measures Analysis based on natural log-transformed data - FAS population (Primary Analysis of the mean post-dose response (Hour 1, 3 and 5) vs. baseline]

Figure 3: Cough Challenges [Natural log-transformed C2 response over time (Hours 0, 1, 3, and 5)]

Table 1 – Baseline Characteristics

	Healthy Subjects N=12	**Chronic Cough Subjects** N=24
N (%) Female	11 (92%)	21 (88%)
Mean Age in Years (SD)	37.8 (8.65)	61.1 (8.69)
Age Range (Years)	26-52	48-73
Mean Weight in kg (SD)	71.5 (13.24)	69.1 (16.46)
Mean Duration of Cough in Years (SD)	N/A	14.6 (9.89)
Duration Range (Years)	N/A	3-44
Mean Cough Severity VAS (SD)	N/A	68.6 (17.45)

Table 2a - Mixed Model Repeated Measures Analysis for C2 and C5 Based on Natural Log-Transformed Data – Chronic Cough Subjects (FAS Set)

	C2		C5	
	Gefapixant 100 mg (N=24)	Placebo (N=24)	Gefapixant 100 mg (N=24)	Placebo (N=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)	

Table 2b - Mixed Model Repeated Measures Analysis for C2 and C5 Based on Natural Log-Transformed Data – Healthy Subjects (FAS Set)

	C2		C5	
	Gefapixant 100 mg (N=12)	Placebo (N=12)	Gefapixant 100 mg (N=12)	Placebo (N=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)	

Treatment comparison was performed using a mixed effect repeated measures (MMRM) model. Baseline refers to the baseline on the log scale. Missing C2 or C5 (unable to reach) values were imputed using 1.5 x maximum concentration level. The geometric mean was estimated by exponentiating the 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 vs. Placebo) < 0.001; ** P value (LS Mean Difference vs. Placebo) < 0.01*

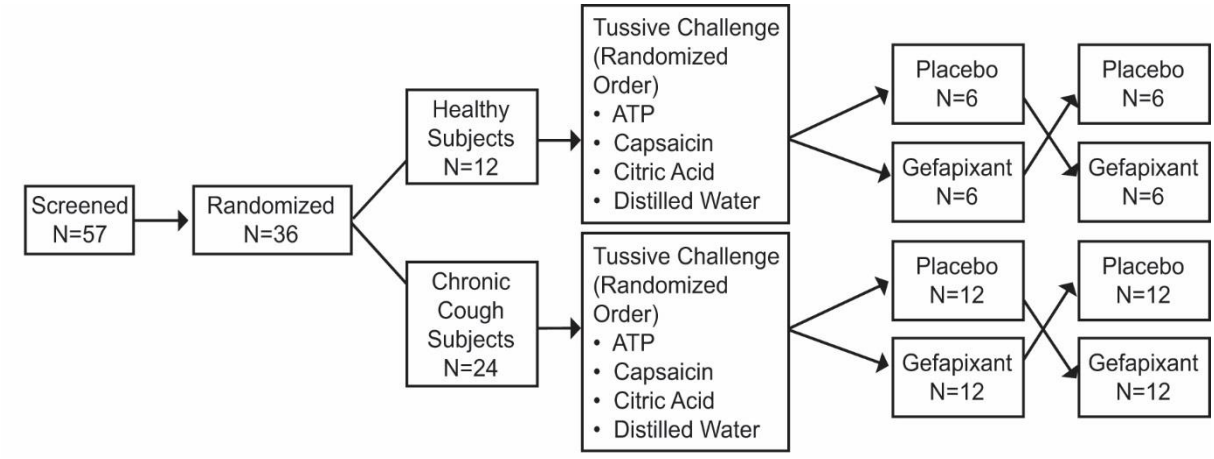
Table 3 – Summary of Endpoints Assessing Cough Burden in Chronic Cough Subjects upon Treatment with Gefapixant and Placebo

	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 vs. Placebo	--	-18.0 (-29.8, -6.2)	0.0037
Urge-to-Cough VAS			
Gefapixant 100 mg	24	-29.8 (-38.9, -20.7)	
Placebo	24	-11.7 (-20.9, -2.6)	
Gefapixant vs. Placebo	--	-18 (-29.1, -7.0)	0.0020
HARQ Total Score			
Gefapixant 100 mg	24	-16.2 (-22.1, -10.3)	
Placebo	24	-11.0 (-17.0, -5.1)	
Gefapixant vs. Placebo	--	-5.2 (-10.9, 0.6)	0.0766
Cough Frequency over 24 Hours (coughs/hr)			
Gefapixant 100 mg	24	-7.7 (-10.1, -5.3)	
Placebo	22	-4.1 (-6.5, -1.7)	
Gefapixant vs. Placebo	--	-3.6 (-6.2, -1.0)	0.0075

Mixed effect model includes fixed effects for treatment group, period, the treatment-by-period interaction, and the baseline value as a covariate

Table 4 – Summary of Adverse Events

	Healthy Subjects		Chronic Cough Subjects	
	Gefapixant 100 mg (n=12)	**Placebo (n=12)**	**Gefapixant 100 mg (n=24)**	**Placebo (n=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%)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Randomized
N=36
Healthy
Subjects
N=12
Chronic
Cough
Subjects
N=24
Tussive Challenge
(Randomized
Order)
• ATP
• Capsaicin
• Citric Acid
• Distilled Water
Tussive Challenge
(Randomized
Order)
• ATP
• Capsaicin
• Citric Acid
• Distilled Water
Placebo
N=6
Gefapixant
N=6
Placebo
N=6
Gefapixant
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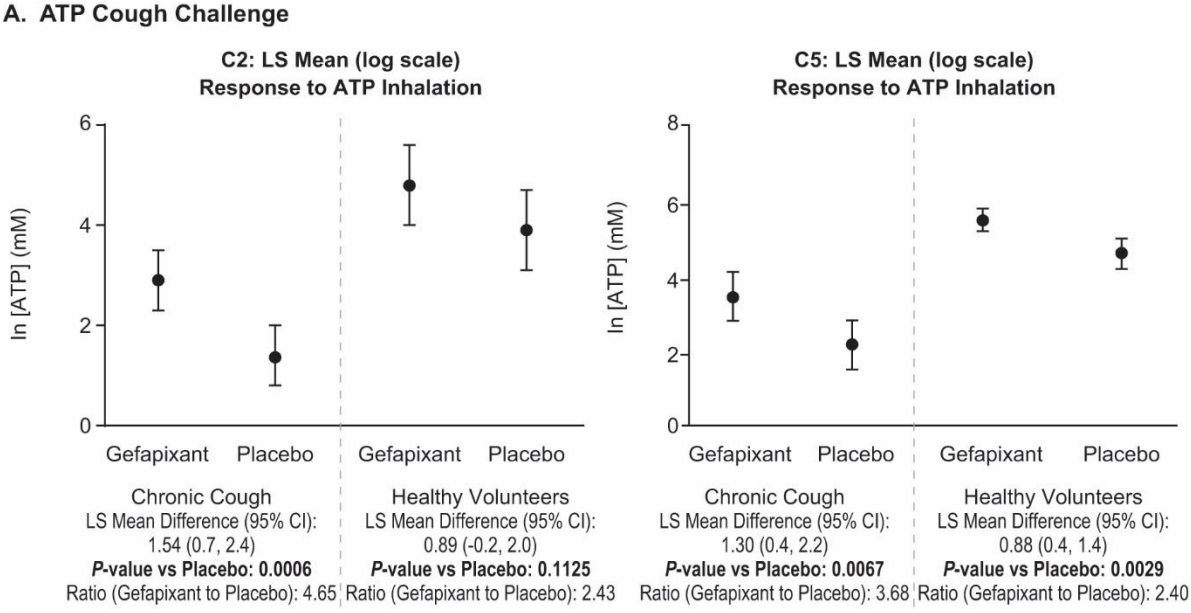
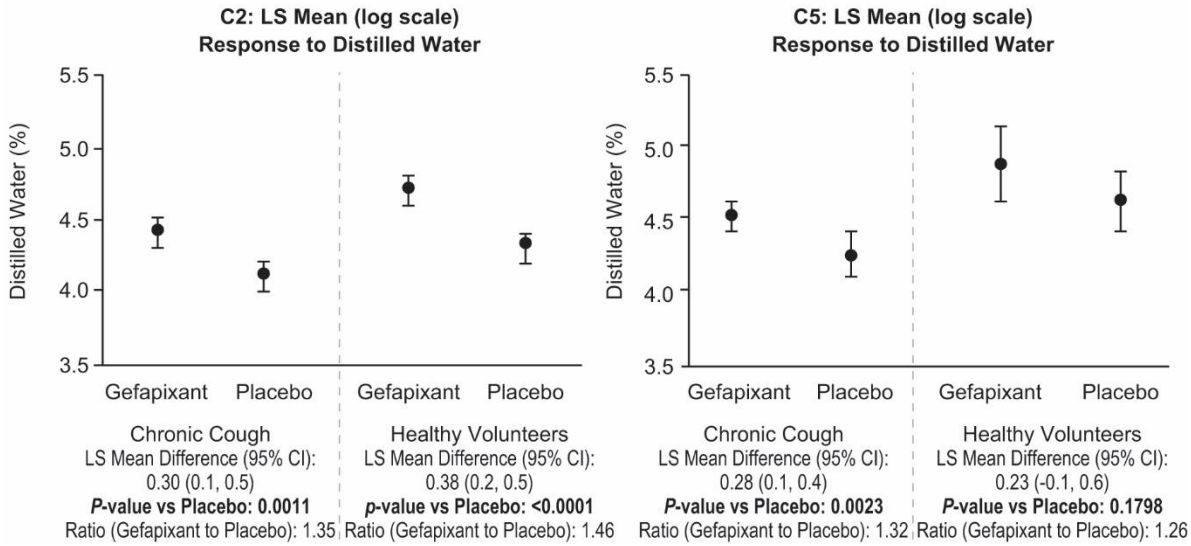
Placebo
N=12
Gefapixant
N=12

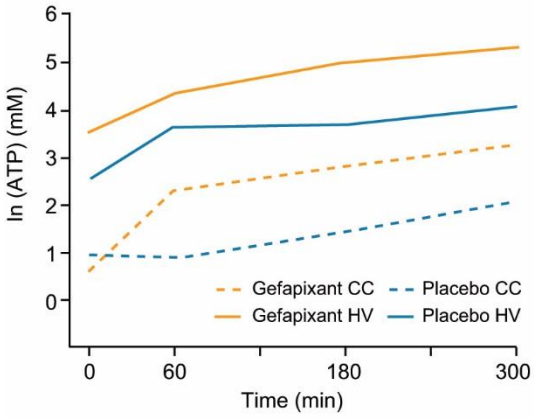
A. ATP Cough Challenge



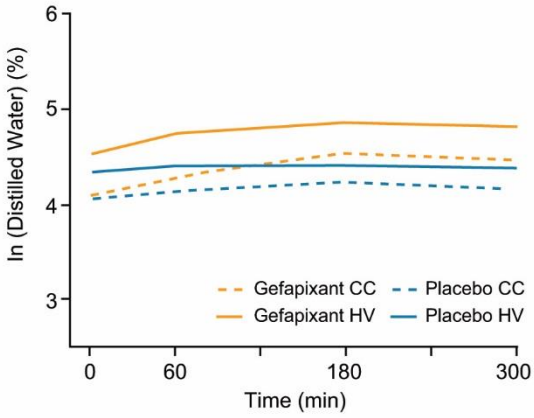
B. Distilled Water Cough Challenge



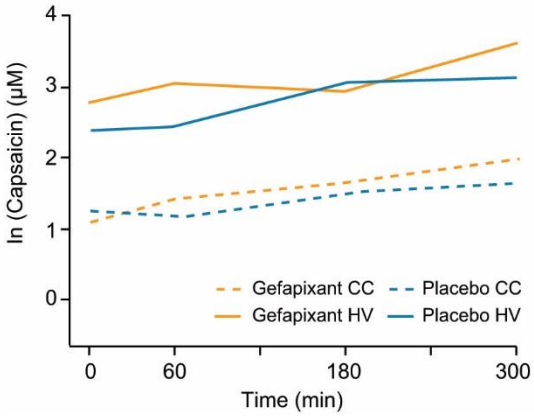
A. ATP Challenge



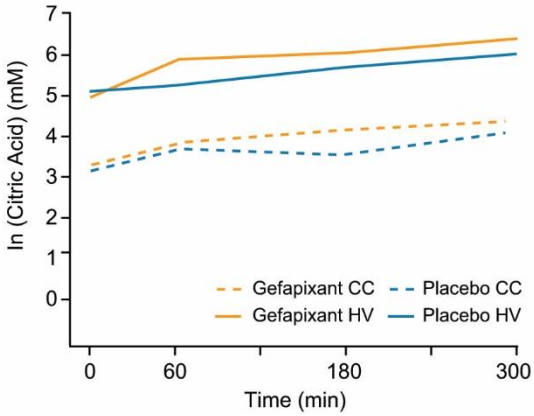
B. Distilled Water Challenge



C. Capsaicin Challenge



D. Citric Acid Challenge



Supplementary Appendix

Exclusion Criteria

Subjects were not eligible if they had a history of upper respiratory tract infection (URTI) or recent significant change in pulmonary status within 4 weeks of the Baseline Visit (Day 0); had acute worsening of asthma; did not cough during the ATP, capsaicin, or citric acid challenge at screening or only coughed twice at the 2 highest concentrations of the test solution; or demonstrated > 2 coughs to inhalation of the normal saline solution during the baseline challenge. Subjects were not eligible for this study if they had a history or symptoms of renal disease or renal obstructive disease. Angiotensin converting enzyme (ACE)-inhibitors within 4 weeks of the study or opioid use within 1 week of the baseline visit were not permitted.