

Transient receptor potential vanilloid 1 (TRPV1) antagonism in patients with refractory chronic cough: A double-blind randomized controlled trial

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Background: Inhalation of capsaicin, the extract of hot chili peppers, induces coughing in both animals and human subjects through activation of transient receptor potential vanilloid 1 (TRPV1) on airway sensory nerves. Therefore the TRPV1 receptor is an attractive target for the development of antitussive agents.

Objective: We sought to assess the antitussive effect of TRPV1 antagonism in patients with refractory chronic cough.

Methods: Twenty-one subjects with refractory chronic cough (>8 weeks) attending a specialist clinic were recruited to a randomized, double-blind, placebo-controlled crossover trial assessing a TRPV1 antagonist (SB-705498). Cough reflex sensitivity to capsaicin (concentration of capsaicin inducing at least 5 coughs) and 24-hour cough frequency were coprimary end points assessed after a single dose of SB-705498 (600 mg) and matched placebo. Cough severity and urge to cough were reported on visual analog scales, and cough-specific quality of life data were also collected.

Results: Treatment with SB-705498 produced a significant improvement in cough reflex sensitivity to capsaicin at 2 hours and a borderline significant improvement at 24 hours compared with placebo (adjusted mean difference of +1.3 doubling doses at 2 hours [95% CI, +0.3 to +2.2; $P = .0049$] and +0.7 doubling doses at 24 hours [95% CI, +0.0 to +1.5; $P = .0259$]). However, 24-hour objective cough frequency was not improved compared with placebo. Patient-reported cough severity, urge to cough, and cough-specific quality of life similarly suggested no effect of SB-705498.

Conclusion: This study raises important questions about both the role of TRPV1-mediated mechanisms in patients with

refractory chronic cough and also the predictive value of capsaicin challenge testing in the assessment of novel antitussive agents. (*J Allergy Clin Immunol* 2014;■■■:■■■-■■■.)

Key words: Cough, transient receptor potential vanilloid 1, capsaicin, cough sounds, sensory nerves

Effective antitussive agents remain a significant unmet clinical need. It has been known for decades that inhaling aerosols of the hot chili pepper extract, capsaicin, induces coughing.¹⁻³ Furthermore, clinical studies have demonstrated that patients with chronic dry cough have heightened responses to inhaled capsaicin⁴⁻⁸ that improve with successful treatment.⁹ Consequently, capsaicin challenge has become an established tool in cough research,¹⁰ and the receptor responsible for transducing capsaicin responses, the transient receptor potential vanilloid 1 (TRPV1), is an attractive target for the development of antitussive agents.

First described in 1997,¹¹ TRPV1 is primarily expressed by unmyelinated sensory nerves (C-fibers)^{12,13} and, in addition to cough, is responsible for the burning pain associated with capsaicin exposure. It is a polymodal transducer, activated not only through vanilloids like capsaicin but also through a wide variety of other stimuli, such as heat,¹¹ acidity,¹⁴ inflammatory mediators (bradykinin, prostaglandin E₂, and leukotriene B₄),¹⁵⁻¹⁷ and lip-oxygenase products (12-hydroperoxyicosatetraenoic acid and 15-hydroperoxyicosatetraenoic acid).¹⁸

The mechanisms underlying enhanced capsaicin responses in chronic cough are not well understood, but responsiveness of unmyelinated airway fibers could be increased through decreasing the activation threshold of the TRPV1 channel, upregulation of TRPV1 expression, or phenotypic switching of myelinated fibers to also express TRPV1.¹⁹ Indeed, one study suggested that TRPV1 expression might be increased in the endobronchial mucosa of patients with chronic cough.²⁰ However, amplification of responses could also occur in the central nervous system through sensitization of central pathways, failure of inhibitory control mechanisms, or both.^{21,22}

Several TRPV1 antagonists have been developed²³; however, during early-phase clinical trials, some molecules caused hyperthermia²⁴ and impaired perception of noxious heat.^{25,26} SB-705498 is a highly selective and potent competitive antagonist of the human TRPV1 receptor,^{27,28} which does not exhibit significant disturbances in temperature perception or control.²⁹ It has shown activity in a human experimental model of skin hyperalgesia (UVB-evoked inflammation).²⁹ However, allergen-induced symptoms in patients with allergic rhinitis did not improve with intranasal SB-705498 alone or when combined with fluticasone propionate.³⁰ Similarly, in subjects with nonallergic rhinitis, it

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Abbreviations used

C2: Concentration of capsaicin inducing at least 2 coughs
 C5: Concentration of capsaicin inducing at least 5 coughs
 CQLQ: Cough Quality of Life Questionnaire
 HRT: Hormone replacement therapy
 IQR: Interquartile range
 $T_{\max}$: Time to maximum observed plasma concentration
 TRPV1: Transient receptor potential vanilloid 1
 VAS: Visual analog scale

did not improve symptoms after cold dry air exposure.³¹ SB-705498 is rapidly absorbed after oral administration (peak concentrations, 45 minutes to 2 hours) but has a long elimination half-life of 50 to 60 hours. In animals it crosses the blood-brain barrier and therefore potentially has activity in both the peripheral and central nervous systems.

Thus the aim of this study was to assess the antitussive effects of TRPV1 receptor antagonism by using SB-705498 in patients with refractory chronic cough. Capsaicin cough challenges were used to assess airway TRPV1 blockade and combined with 24-hour cough monitoring to assess the translation of this mechanism into reductions in spontaneous cough frequency. Some of these data have been published in abstract form.³²

METHODS

Detailed methods are provided in the [Methods](#) section in this article's Online Repository at www.jacionline.org.

Study design

A randomized, double-blind, placebo-controlled crossover trial was performed comparing a single dose of 600 mg of SB-705498 (6×100 mg) with matched placebo. The coprimary end points were cough responses to inhaled capsaicin (2 hours after dose) and objective cough frequency over the 24 hours after dosing. Subjects also rated cough severity and urge to cough on visual analog scales (VASs). Because of the long half-life of SB-705498 (50–60 hours), a 4-week washout separated the 2 treatment periods, and the Cough Quality of Life Questionnaire (CQLQ) was repeated at 14 days after dosing to assess for prolonged treatment effects. The study design is shown in [Fig 1](#).

Subjects

Twenty-one patients with refractory chronic cough (>8 weeks' duration) attending a specialist cough clinic were recruited into the study. All subjects had undergone full investigation and treatment trials for possible causes of cough. Current smokers and exsmokers with a greater than 5 pack-year smoking history were excluded, as were those who did not cough at least 5 times (concentration of capsaicin inducing at least [C5]) after capsaicin inhalation up to a concentration of 250 $\mu\text{mol/L}$. The study was approved by the research ethics committee (11/H1008/2), and the patients provided written informed consent.

Procedures

Capsaicin challenge. Subjects took single inhalations of doubling concentrations of capsaicin solution (0.49–1000 $\mu\text{mol/L}$; Stockport Pharmaceuticals Ltd, Stockport, United Kingdom) at 1-minute intervals from flow-regulated nebulizer pots controlled by a dosimeter (Koko dosimeter; DeVilbiss Health Care, Somerset, Pa). Three additional placebo pots (0.9% saline) were randomly placed in the challenge sequence. The concentration of capsaicin inducing at least 2 coughs (C2) and C5 values within 15 seconds of

inhalation were recorded. If C2/C5 occurred with placebo inhalation, analyses were performed by treating such data as missing and also imputing the next capsaicin concentration in the sequence.

Ambulatory cough recording. The VitaloJAK cough monitor (Vitalograph, Buckinghamshire, United Kingdom) was used to make 24-hour acoustic recordings from a free-field microphone and contact sensor. Silences and background noise were removed by using validated, custom-written software,³³ and cough sounds were counted with an audio editing package (Audition version 3; Adobe Systems, San Jose, Calif).

Additional end points. Blood samples were collected for pharmacokinetic testing up to 4 hours after dosing and at 24 hours. Subjects rated cough severity and urge to cough on separate 100-mm VASs before dosing and 2 and 24 hours after dosing. The CQLQ, a cough-specific quality-of-life questionnaire, was completed at baseline and 14 days after treatment.³⁴ Vital signs, electrocardiograms, body temperature, hematology, biochemistry, and adverse events were monitored throughout to assess safety.

Data analysis

Both capsaicin cough responses and 24-hour cough frequency were log-transformed before analysis, and the change from predose measures was calculated. The differences between SB-705498 and placebo were analyzed by using mixed-effects modeling (SAS version 9.2; SAS Institute, Cary, NC), with statistical significance set at the .025 level.

The study was powered for changes in capsaicin sensitivity. Assuming an SD of 0.50 for the difference in logC5 values between active and placebo treatments,³⁵ the power to detect a 1-sided 2.5% difference in logC5 of 0.35 (>1 doubling dose) is 93% with 24 subjects.

RESULTS**Subjects**

Patients' characteristics are summarized in [Table I](#). The majority of subjects were female and middle aged, as is typical of patients presenting with refractory cough. At screening, subjects were highly sensitive to capsaicin; the median capsaicin C5 value was 7.81 $\mu\text{mol/L}$ (range, 0.49–125 $\mu\text{mol/L}$). Of 21 patients screened and randomized, 2 withdrew after the first treatment period because of symptoms consistent with a viral upper respiratory tract infection. Both patients had received placebo treatment ([Fig 2](#)).

Capsaicin cough responses

Treatment with SB-705498 increased C5 values at 2 hours after dosing compared with baseline values (median, +2 doubling doses; interquartile range [IQR], 0 to +2 doubling doses); however, there was no change with placebo treatment (median, 0 doubling doses; range, –1 to +1 doubling doses; [Table II](#) and [Fig 3](#) summarize the raw data). The increase in C5 values was similar at 24 hours after dosing (median, +2 doubling doses; IQR, +1 to +2 doubling doses), and again, there was no change with placebo treatment (median, 0.0 doubling doses; IQR, 0 to +1 doubling doses).

The difference between SB-705498 and placebo in the log-transformed change from before dosing was investigated by using a repeated-measures mixed-effects model with fixed-effects terms for treatment, period, subject, and period baselines and interactions between period and time point and treatment and time point. Subject was treated as a random effect in the model, and time point (2 and 24 hours) was treated as a repeated effect. The improvement in C5 values with SB-705498 treatment was significantly greater at 2 hours and borderline significant at 24 hours compared with that after placebo treatment (adjusted mean difference of +1.3 doubling doses at 2 hours [95% CI, +0.3

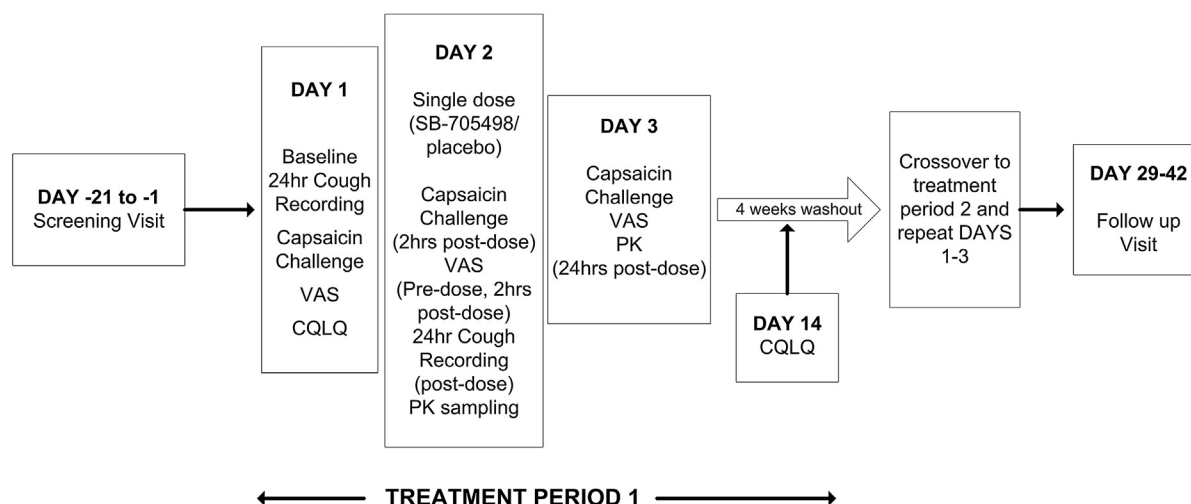


FIG 1. Summary of study design and procedures. VAS scores were recorded for both cough severity and urge to cough. PK, Pharmacokinetics sampling.

TABLE I. Baseline characteristics of randomized subjects

Demographics	
Age (y), mean (range)	53 (34-70)
Sex, no. (%)	21
Female	15 (71)
Male	6 (29)
White race, no. (%)	21 (100)
Height (cm), mean (range)	167.7 (152-184)
Weight (kg), mean (range)	75.4 (54.9-98.5)
BMI (kg/m ²)	26.6 (22.3-31.7)
FEV ₁ (% predicted), mean $\pm$ SD	96.7 $\pm$ 13.3
FVC (% predicted), mean $\pm$ SD	97.4 $\pm$ 11.1

BMI, Body mass index; FVC, forced vital capacity.

to +2.2 doubling doses; $P = .0049$] and +0.7 doubling doses at 24 hours [95% CI, +0.0 to +1.5 doubling doses; $P = .0259$].

C5 occurred on placebo inhalation on 1 occasion at baseline and 1 occasion at 2 hours but no occasions at 24 hours. Imputing the next capsaicin concentration for these missing data suggested a very similar effect for SB-705498 treatment on C5 values compared with placebo, an adjusted mean difference of +1.2 doubling doses at 2 hours (95% CI, +0.3 to +2.0 doubling doses; $P = .0042$) and +0.7 doubling doses at 24 hours (95% CI, +0.0 to +1.4 doubling doses; $P = .0212$).

For the C2 end point, treatment with SB-705498 produced borderline significant changes over placebo at 2 hours but no effect at 24 hours after dosing (adjusted mean difference of +0.7 doubling doses at 2 hours [95% CI, 0.0 to +1.3 doubling doses; $P = .0255$] and +0.4 doubling doses at 24 hours [95% CI, -0.4 to +1.2 doubling doses; $P = .1452$]). There were more missing data because of subjects coughing twice with placebo inhalation (1 occasion at baseline, 2 occasions at 2 hours, and 3 occasions at 24 hours). However, imputing the next concentration of capsaicin as C2 did not change the findings with no significant improvements in C2 values over those seen with placebo at either 2 hours (adjusted mean difference, +0.5 doubling doses [95% CI, -0.1 to 1.1 doubling doses; $P = .0483$]) or 24 hours (adjusted mean difference, +0.6 doubling doses [95% CI, -0.1 to 1.4 doubling doses; $P = .0436$]).

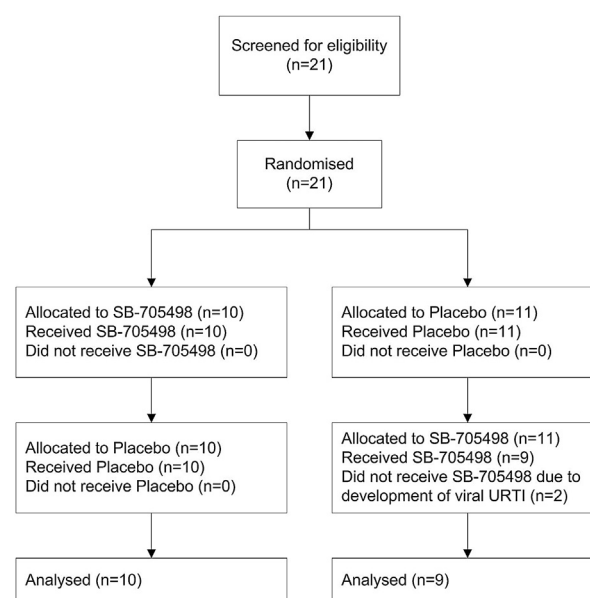


FIG 2. CONSORT flow diagram. URTI, Upper respiratory tract infection.

Objective cough frequency

There was no significant difference between hourly cough count profiles between placebo and SB-705498 based on a negative binomial model, with fixed-effects terms for subject and period baselines, treatment, period, and time after dosing (in hours) and a treatment by time after dosing (in hours) interaction and subject as a random effect ($P = .508$, Fig 4). There were no missing data for cough frequency. Twenty-four hour cough frequency decreased by a median of -12.9% (IQR, -29.3% to +12.1%) with SB-705498 relative to predose values and by a median of -23.0% (IQR, -42.4% to -2.6%) with placebo relative to predose values (Table II). Cough frequency was very stable despite the 1-month separation in treatment periods. For the daytime cough count analysis, there was a notable difference seen between the 2 periods. The means for placebo were higher than for SB-705498 in period 1 but lower than for SB-705498 in period 2,

TABLE II. Capsaicin challenge and cough frequency data for placebo and SB-705498 treatment

End point	Placebo			SB-705498		
	Before dose ($\mu\text{mol/L}$)	2 h After dose ($\mu\text{mol/L}$)	24 h After dose ($\mu\text{mol/L}$)	Before dose ($\mu\text{mol/L}$)	2 h After dose ($\mu\text{mol/L}$)	24 h After dose ($\mu\text{mol/L}$)
Capsaicin						
LogC5	0.89 (0.44 to 1.04)	0.59 (0.29 to 1.19)	0.89 (0.59 to 1.49)	0.29 (0.29 to 0.89)	1.19 (0.89 to 1.19)	0.89 (0.59 to 1.19)
LogC2	0.59 (−0.31 to 0.89)	0.29 (−0.01 to 0.74)	0.59 (−0.01 to 0.89)	0.29 (0.29 to 0.89)	0.89 (0.29 to 0.89)	0.59 (0.59 to 0.89)
	Before dose (c/h)		After dose (c/h)	Before dose (c/h)		After dose (c/h)
Cough frequency						
24 h	23.6 (12.0 to 37.0)		18.2 (8.9 to 25.8)	20.7 (11.4 to 32.3)		17.0 (9.6 to 34.2)
Day	31.6 (16.4 to 51.1)		26.7 (13.3 to 41.2)	29.1 (16.6 to 54.5)		27.7 (12.1 to 50.7)
Night	1.6 (0.3 to 2.8)		0.9 (0.1 to 2.6)	0.9 (0.1 to 4.3)		1.5 (0.0 to 4.5)

All data are medians and IQRs. c/h, Coughs per hour.

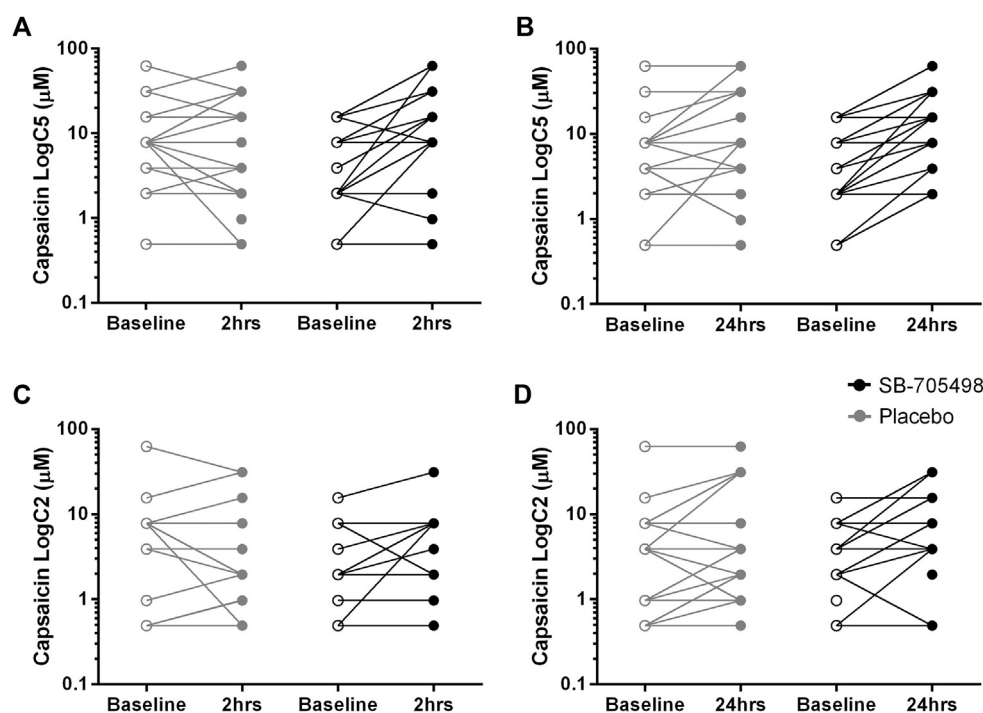


FIG 3. Cough reflex sensitivity logC5 and logC2 values at baseline and after SB-705498 and placebo treatment: 2 hours (A and C) and 24 hours (B and D). Note that symbols are unconnected when subjects coughed on placebo inhalation at one of the time points.

suggesting a possible treatment-by-period interaction. However, this was not formally tested.

Secondary end points

There appeared to be no significant differences in either cough severity or urge-to-cough VASs when comparing SB-705498 with placebo (Fig 5, A and B). Similarly, CQLQ scores suggested no significant improvement with SB-705498 compared with placebo (Fig 5, C).

Pharmacokinetics

Fig 6 shows the average receptor occupancy was estimated at approximately 45% at peak plasma concentrations (2 hours after dosing), with a gradual reduction in occupancy to 25% at 24 hours after dosing.

Adverse events

No subjects experienced serious adverse events during the study. Ten nonserious adverse events were reported on placebo treatment and 7 were reported on SB-705498 treatment. Headache was the most common adverse event reported in 3 patients during placebo treatment and 2 patients during SB-705498 treatment. In particular, there were no reports of fever and no significant changes in tympanic temperature (see Fig E1 in this article's Online Repository at www.jacionline.org) with SB-705498 treatment compared with placebo treatment.

DISCUSSION

To the best of our knowledge, this is the first study to assess a TRPV1 receptor antagonist as an antitussive agent in patients. Despite clear evidence of TRPV1 antagonism in the airways, with

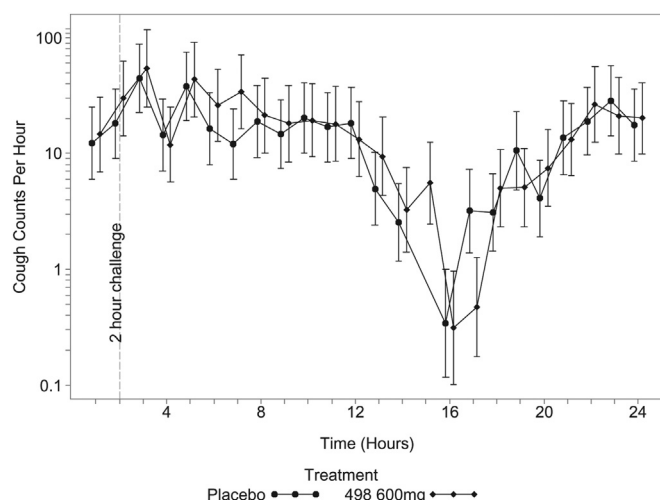


FIG 4. Objective cough frequency in the 24 hours after dosing for SB-705498 and placebo (adjusted geometric mean cough counts per hour with 95% CIs). Note the adjusted mean for placebo at 15 hours has not been plotted because of wide CIs (0.000002-4.785); this increased variability likely arises from differences in onset of sleep between patients.

a significant reduction in experimentally induced cough responses to capsaicin with SB-705498 treatment, this did not translate into any significant improvement in spontaneous cough frequency. Patient reports of cough severity, urge to cough, and cough-specific quality of life similarly suggested no effect of SB-705498 over placebo. Therefore this study raises important questions about the role of TRPV1-mediated mechanisms in patients with refractory chronic cough and also the predictive value of capsaicin challenge testing in the assessment of novel antitussive agents.

A significant body of evidence from both preclinical and clinical studies has suggested that activation of TRPV1 receptors plays an important role in evoking cough and in the sensitization of the cough reflex seen in a range of respiratory disorders. Therefore the finding in this study that significant shifts in cough responses to capsaicin were not associated with any improvements in spontaneous cough frequency is somewhat unexpected. However, enhanced cough responses to capsaicin are not implicitly a consequence of alterations in TRPV1 expression or function and might arise from neuroplastic change anywhere in the cough reflex pathway (central or peripheral). Indeed, there is some evidence that sensitization of central processes might be important in patients with chronic cough. For example, the observation that reflux events in the distal esophagus are temporally linked to and might therefore trigger coughing is best explained by cross-talk between esophageal and airway vagal afferents where they converge in the brainstem.³⁶

Furthermore, although TRPV1 receptors are clearly capable of responding to an array of stimuli, the extent to which this single receptor plays a key integrative role in mediating triggers of coughing remains to be determined. In the clinic patients with chronic cough are known to report coughing on trivial exposure to a very broad range of stimuli, including environmental irritants (perfumes, cigarette smoke, and dry air), mechanical stimuli to the larynx/pharynx (talking and eating), and temperature changes (both warm and cold),³⁷ which suggests the involvement of other receptors, including other TRP channels, such as TRPA1.³⁸ Such generalized sensitivity is perhaps more consistent with

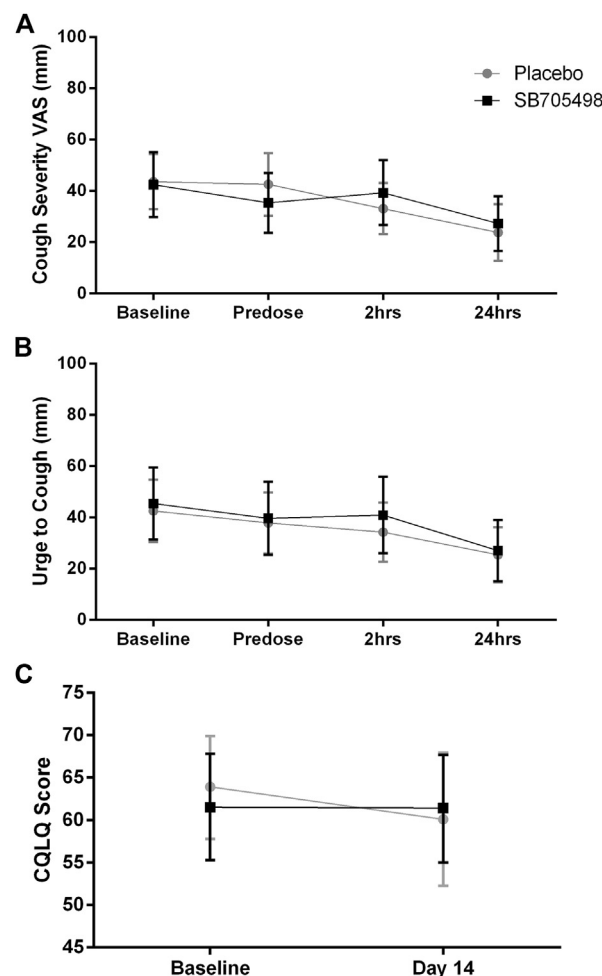


FIG 5. Cough severity (A) and urge-to-cough (B) VAS scores and CQLQ scores (C) before and after SB-705498 and placebo treatment (means and 95% CIs).

upregulation/sensitization of a variety of receptors on peripheral nerve terminals or a central nervous system process. Therefore blockade of any single ion channel responsible for stimulus transduction might not be the most effective strategy for treating chronic cough.

Although this study suggests that TRPV1-mediated mechanisms might not be important in patients with refractory chronic cough, some questions remain outstanding, such as whether a more potent antagonist, more prolonged dosing, or both might yield positive results. At 2 hours after treatment, SB-705498 receptor occupancy was estimated to be 45% and associated with more than a doubling dose shift in C5 values over placebo, and then by 24 hours, occupancy was 25% with a 0.7 doubling dose shift (significant when an approximation of C5 is imputed into the analysis). Although these levels of receptor occupancy might be inadequate to inhibit G protein-coupled receptor function, for ion channels, modest occupancy often produces maximal antagonism because large changes in threshold for neuronal firing can be brought about by small changes in current flow.^{39,40} Whether larger shifts in C5 values might be needed to alter the frequency of spontaneous coughing remains unclear, nonetheless, some authors have considered smaller changes in capsaicin response to be clinically important.⁴¹ Unfortunately preclinical toxicology

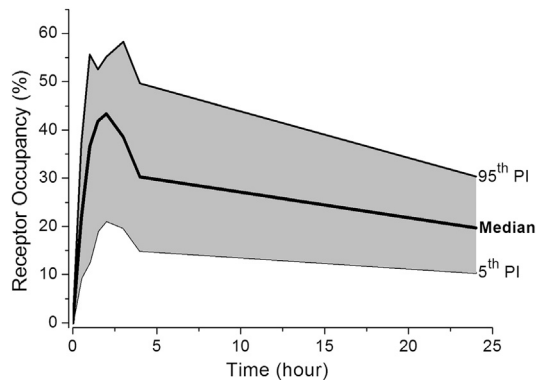


FIG 6. Estimated TRPV1 receptor occupancy of SB-705498 over a 240-hour period after oral administration of a 600-mg dose. *PI*, Prediction interval.

studies suggested SB-705498 was unsuitable for multiple oral dosing in human studies because of adrenal vacuolation. However, the relatively long half-life of SB-705498 did provide good exposure to therapy over 24 hours, and cough-specific quality of life was checked at 14 days to capture more prolonged action but still did not suggest any clinical effect. Finally, it is important to appreciate that failure of TRPV1 inhibition to modify coughing in patients with refractory chronic cough does not exclude an antitussive effect in other airway conditions. Heightened cough responses to capsaicin inhalation have been demonstrated in several other conditions associated with cough, including viral upper respiratory tract infections,⁴² asthma,⁴³ chronic obstructive pulmonary disease,⁴⁴ and idiopathic pulmonary fibrosis,⁴⁵ and further investigation will be required to understand the potential role of TRPV1 in patients with these conditions.

In addition to providing insights into the mechanisms underlying chronic cough, this study also has important implications for the design of future trials testing novel antitussive agents. Changes in capsaicin challenge have been used to imply antitussive effects of a variety of pharmacologic therapies for cough.^{35,41,46} This study clearly demonstrates that significant changes in C5 values are not inevitably associated with improvements in objectively measured cough frequency. Of note, recent studies of morphine and gabapentin in patients with refractory cough found no change in cough challenge responses, despite demonstrating improvements in cough-specific quality of life.^{47,48} Together, these observations draw into question the utility of cough challenges in testing novel antitussive therapies. The use of such challenges alone as a generic test of cough reflex sensitivity, without the accompaniment of other well-validated tools for the assessment of cough, has the potential to provide misleading results.

We have recently reported a modification of the standard capsaicin challenge methodology, extending the challenge beyond the C5 end point to include the full dose response and averaging cough responses over 4 inhalations at each concentration.⁴⁹ By using this novel challenge, the maximal cough response best differentiated patients with chronic cough from healthy control subjects and correlated strongly with spontaneous cough frequency. Standard challenges and the somewhat arbitrary C5 end point tend to correlate weakly with cough frequency,^{50,51} and there is very significant overlap of values obtained from healthy control subjects with those of patients with chronic cough.⁶ Although it would have been interesting to study the effect of

SB-705498 on maximal cough responses, which might better predict changes in cough frequency, the results were not available at the time of study design. Nonetheless, in this study the standard capsaicin challenge and C5 end point still provided important evidence of target engagement and future optimization of challenge methodology, and end points might improve their value in predicting antitussive effects.⁴⁹

In contrast, this study confirms the importance of ambulatory 24-hour objective cough monitoring in proof-of-concept studies of novel therapies for cough. Patients with refractory cough exhibit high cough frequencies, which remain stable over time, and as a consequence, the number of subjects required for proof-of-concept studies is relatively small.⁵² Some investigators have found cough studies confounded by large placebo effects (up to 85%), particularly when associated with upper respiratory tract infections and when cough is counted over short time periods in laboratory conditions.⁵³ Our experience with 24-hour ambulatory recording is that much smaller placebo effects are observed.⁵⁴

In conclusion, despite clear evidence of engagement of the TRPV1 receptor, as evidenced by the shift in the capsaicin threshold, there was no improvement in any clinical efficacy parameter with TRPV1 antagonism in patients with chronic cough. Therefore this study provides the first evidence that TRPV1 receptor activation might not be an important determinant of spontaneous cough frequency. Future studies are still required to understand whether more potent antagonists, more prolonged dosing, or both might still be effective and also whether TRPV1 antagonists are effective antitussive agents in other patient groups. However, it is clear that reductions in capsaicin responses in patients with chronic cough do not necessarily predict antitussive effects.

We thank all the patients who took part in this study.

Key messages

- Despite significantly reducing cough induced by inhaled capsaicin, an oral TRPV1 antagonist did not reduce objective cough frequency in patients with refractory chronic cough.
- These data question the role of TRPV1-mediated mechanisms in patients with refractory chronic cough and also the predictive value of capsaicin challenge testing in the assessment of novel antitussive agents.

REFERENCES

1. Forsberg K, Karlsson JA. Cough induced by stimulation of capsaicin-sensitive sensory neurons in conscious guinea-pigs. *Acta Physiol Scand* 1986;128: 319-20.
2. Mohammed SP, Higenbottam TW, Adcock JJ. Effects of aerosol-applied capsaicin, histamine and prostaglandin E2 on airway sensory receptors of anaesthetized cats. *J Physiol* 1993;469:51-66.
3. Fuller RW, Karlsson JA, Choudry NB, Pride NB. Effect of inhaled and systemic opiates on responses to inhaled capsaicin in humans. *J Appl Physiol* 1988;65: 1125-30.
4. Choudry NB, Fuller RW. Sensitivity of the cough reflex in patients with chronic cough. *Eur Respir J* 1992;5:296-300.
5. Ternesten-Haspeus E, Johansson K, Lowhagen O, Millqvist E. Inhalation method determines outcome of capsaicin inhalation in patients with chronic cough due to sensory hyperreactivity. *Pulm Pharmacol Ther* 2006;19:172-8.
6. Prudon B, Birring SS, Vara DD, Hall AP, Thompson JP, Pavord ID. Cough and glottic-stop reflex sensitivity in health and disease. *Chest* 2005;127:550-7.

7. Singapuri A, McKenna S, Brightling CE. The utility of the mannitol challenge in the assessment of chronic cough: a pilot study. *Cough* 2008;4:10.
8. Qiu Z, Yu L, Xu S, Liu B, Zhao T, Lu H. Cough reflex sensitivity and airway inflammation in patients with chronic cough due to non-acid gastroesophageal reflux. *Respirology* 2011;16:645-52.
9. O'Connell F, Thomas VE, Pride NB, Fuller RW. Capsaicin cough sensitivity decreases with successful treatment of chronic cough. *Am J Respir Crit Care Med* 1994;150:374-80.
10. 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;29:1256-76.
11. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 1997;389:816-24.
12. Mezey E, Toth ZE, Cortright DN, Arzubi MK, Krause JE, Elde R, et al. Distribution of mRNA for vanilloid receptor subtype 1 (VR1), and VR1-like immunoreactivity, in the central nervous system of the rat and human. *Proc Natl Acad Sci U S A* 2000;97:3655-60.
13. Sanchez JF, Krause JE, Cortright DN. The distribution and regulation of vanilloid receptor VR1 and VR1 5' splice variant RNA expression in rat. *Neuroscience* 2001;107:373-81.
14. Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, et al. The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron* 1998;21:531-43.
15. Grace M, Birrell MA, Dubuis E, Maher SA, Belvisi MG. Transient receptor potential channels mediate the tussive response to prostaglandin E2 and bradykinin. *Thorax* 2012;67:891-900.
16. Carr MJ, Kollarik M, Meeker SN, Undem BJ. A role for TRPV1 in bradykinin-induced excitation of vagal airway afferent nerve terminals. *J Pharmacol Exp Ther* 2003;304:1275-9.
17. Kollarik M, Undem BJ. Activation of bronchopulmonary vagal afferent nerves with bradykinin, acid and vanilloid receptor agonists in wild-type and TRPV1-/- mice. *J Physiol* 2004;555:115-23.
18. Hwang SW, Cho H, Kwak J, Lee SY, Kang CJ, Jung J, et al. Direct activation of capsaicin receptors by products of lipoxygenases: endogenous capsaicin-like substances. *Proc Natl Acad Sci U S A* 2000;97:6155-60.
19. Lieu TM, Myers AC, Meeker S, Undem BJ. TRPV1 induction in airway vagal low-threshold mechanosensory neurons by allergen challenge and neurotrophic factors. *Am J Physiol Lung Cell Mol Physiol* 2012;302:L941-8.
20. Groneberg DA, Niimi A, Dinh QT, Cosio B, Hew M, Fischer A, et al. Increased expression of transient receptor potential vanilloid-1 in airway nerves of chronic cough. *Am J Respir Crit Care Med* 2004;170:1276-80.
21. Sekizawa S, Chen CY, Bechtold AG, Tabor JM, Bric JM, Pinkerton KE, et al. Extended secondhand tobacco smoke exposure induces plasticity in nucleus tractus solitarius second-order lung afferent neurons in young guinea pigs. *Eur J Neurosci* 2008;28:771-81.
22. Joad JP, Munch PA, Bric JM, Evans SJ, Pinkerton KE, Chen CY, et al. Passive smoke effects on cough and airways in young guinea pigs: role of brainstem substance P. *Am J Respir Crit Care Med* 2004;169:499-504.
23. Szallasi A, Sheta M. Targeting TRPV1 for pain relief: limits, losers and laurels. *Expert Opin Investig Drugs* 2012;21:1351-69.
24. Gavva NR, Treanor JJ, Garami A, Fang L, Surapaneni S, Akrami A, et al. Pharmacological blockade of the vanilloid receptor TRPV1 elicits marked hyperthermia in humans. *Pain* 2008;136:202-10.
25. Eid SR. Therapeutic targeting of TRP channels—the TR(i)P to pain relief. *Curr Top Med Chem* 2011;11:2118-30.
26. Krarup AL, Ny L, Astrand M, Bajor A, Hvid-Jensen F, Hansen MB, et al. Randomised clinical trial: the efficacy of a transient receptor potential vanilloid 1 antagonist AZD1386 in human oesophageal pain. *Aliment Pharmacol Ther* 2011;33:1113-22.
27. Gunthorpe MJ, Hannan SL, Smart D, Jerman JC, Arpino S, Smith GD, et al. Characterization of SB-705498, a potent and selective vanilloid receptor-1 (VR1/TRPV1) antagonist that inhibits the capsaicin-, acid-, and heat-mediated activation of the receptor. *J Pharmacol Exp Ther* 2007;321:1183-92.
28. Rami HK, Thompson M, Stemp G, Fell S, Jerman JC, Stevens AJ, et al. Discovery of SB-705498: a potent, selective and orally bioavailable TRPV1 antagonist suitable for clinical development. *Bioorg Med Chem Lett* 2006;16:3287-91.
29. Chizh BA, O'Donnell MB, Napolitano A, Wang J, Brooke AC, Aylott MC, et al. The effects of the TRPV1 antagonist SB-705498 on TRPV1 receptor-mediated activity and inflammatory hyperalgesia in humans. *Pain* 2007;132:132-41.
30. Bareille P, Murdoch RD, Denyer J, Bentley J, Smart K, Yarnall K, et al. The effects of a TRPV1 antagonist, SB-705498, in the treatment of seasonal allergic rhinitis. *Int J Clin Pharmacol Ther* 2013;51:576-84.
31. Murdoch RD, Bareille P, Bentley J, Newlands A, Smart K, Patel D. TRPV1 is not a target for the treatment of non-allergic rhinitis: a clinical study [abstract]. *Thorax* 2012;67:A129.
32. Smith JA, Murdoch RD, Newlands A, Smart K, Khalid S, Kelsall A, et al. The impact of a selective oral TRPV1 antagonist in patients with chronic cough [abstract]. *Thorax* 2012;67:A128.
33. Sumner H, Kelsall A, Woodcock A, Smith JA, McGuinness K. A semi automatic method to reduce the time taken for manual cough counting [abstract]. *Am J Respir Crit Care Med* 2010;181:A5555.
34. French CT, Irwin RS, Fletcher KE, Adams TM. Evaluation of a cough-specific quality-of-life questionnaire. *Chest* 2002;121:1123-31.
35. Dicpinigaitis PV, Dobkin JB, Rauf K. Comparison of the antitussive effect of codeine and the GABA-agonist baclofen. *Clin Drug Invest* 1997;14:326-9.
36. Smith JA, Decalmer S, Kelsall A, McGuinness K, Jones H, Galloway S, et al. Acoustic cough-reflex associations in chronic cough: potential triggers and mechanisms. *Gastroenterology* 2010;139:754-62.
37. McGarvey L, McKeagney P, Polley L, MacMahon J, Costello RW. Are there clinical features of a sensitized cough reflex? *Pulm Pharmacol Ther* 2009;22:59-64.
38. Birrell MA, Belvisi MG, Grace M, Sadofsky L, Faruqi S, Hele DJ, et al. TRPA1 agonists evoke coughing in guinea pig and human volunteers. *Am J Respir Crit Care Med* 2009;180:1042-7.
39. Mathisen LC, Skjelbred P, Skoglund LA, Oye I. Effect of ketamine, an NMDA receptor inhibitor, in acute and chronic orofacial pain. *Pain* 1995;61:215-20.
40. Hollander-Jansen M, Dingemanse J, Langemeijer MW, Danhof M. Relationship between receptor occupancy at 37 degrees C and the anticonvulsant effect of flunitrazepam in rats. *Pharm Res* 1989;6:585-91.
41. Dicpinigaitis PV, Spinner L, Santhyadka G, Negassa A. Effect of tiotropium on cough reflex sensitivity in acute viral cough. *Lung* 2008;186:369-74.
42. O'Connell F, Thomas VE, Studham JM, Pride NB, Fuller RW. Capsaicin cough sensitivity increases during upper respiratory infection. *Respir Med* 1996;90:279-86.
43. Fujimura M, Kamio Y, Kasahara K, Bando T, Hashimoto T, Matsuda T. Prostanoids and cough response to capsaicin in asthma and chronic bronchitis. *Eur Respir J* 1995;8:1499-505.
44. Blanc FX, Macedo P, Hew M, Chung KF. Capsaicin cough sensitivity in smokers with and without airflow obstruction. *Respir Med* 2009;103:786-90.
45. Hope-Gill BD, Hilldrup S, Davies C, Newton RP, Harrison NK. A study of the cough reflex in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2003;168:995-1002.
46. Dicpinigaitis PV. Review: effect of drugs on human cough reflex sensitivity to inhaled capsaicin. *Cough* 2012;8:10.
47. Morice AH, Menon MS, Mulrennan SA, Everett CF, Wright C, Jackson J, et al. Opiate therapy in chronic cough. *Am J Respir Crit Care Med* 2007;175:312-5.
48. Ryan NM, Birring SS, Gibson PG. Gabapentin for refractory chronic cough: a randomised, double-blind, placebo-controlled trial. *Lancet* 2012;380:1583-9.
49. 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;132:847-55, e1-5.
50. Decalmer SC, Webster D, Kelsall AA, McGuinness K, Woodcock AA, Smith JA. Chronic cough: how do cough reflex sensitivity and subjective assessments correlate with objective cough counts during ambulatory monitoring? *Thorax* 2007;62:329-34.
51. Faruqi S, Thompson R, Wright C, Sheedy W, Morice AH. Quantifying chronic cough: objective versus subjective measurements. *Respirology* 2011;16:314-20.
52. Kelsall A, Houghton LA, Jones H, Decalmer S, McGuinness K, Smith JA. A novel approach to studying the relationship between subjective and objective measures of cough. *Chest* 2011;139:569-75.
53. Eccles R. Importance of placebo effect in cough clinical trials. *Lung* 2010;188(suppl 1):S53-61.
54. Smith JA. Assessing efficacy of therapy for cough. *Otolaryngol Clin North Am* 2010;43:157-66, xi.

METHODS

Subjects

Patients with refractory chronic cough for more than 8 weeks were recruited from a tertiary referral specialist cough clinic (University Hospital of South Manchester, Manchester, United Kingdom) from September 2011 to January 2012. These patients had undergone full investigation (lung function, bronchial challenge testing, nasendoscopy, computed tomographic scan, bronchoscopy, and 24-hour impedance/pH monitoring), and their symptoms had not improved with treatment trials for gastroesophageal reflux disease, asthma, eosinophilic bronchitis, and/or nasal disease, as indicated.

Study design

A randomized, double-blind, placebo-controlled crossover trial was performed, comparing a single dose of 600 mg of SB-705498 (6×100 mg) with a matched placebo. The study design is summarized in Fig 1. The coprimary end points were cough responses to inhaled capsaicin and objective cough frequency over the 24 hours after dosing. After screening, subjects underwent baseline 24-hour cough recording, capsaicin challenge, and subjective measures of cough and then were randomized to receive either SB-705498 or matched placebo in a double-blind fashion. On each treatment (day 2, Fig 1), capsaicin responses were measured at 2 hours after dosing (ie, at maximum observed plasma concentration) and again at 24 hours after dosing. Ambulatory sound recordings were commenced before dosing and continued for 24 hours. Coughing associated with the capsaicin challenge at 2 hours after dosing were excluded from the cough counts, and the 24-hour postdose capsaicin challenge was performed after the cough recording had been completed. Blood samples were also collected at regular time points after dosing up to 4 hours for pharmacokinetic analysis, after which subjects were allowed home to complete the 24-hour cough recording. They returned the next day for 24-hour assessments. Subjects also rated cough severity and urge to cough on VASs before dosing and 2 and 24 hours after dosing. Because of the long half-life of SB-705498 (50–60 hours), treatment effects could have extended well beyond the initial study period, and therefore the CQLQ score was measured at 14 days after dosing. After a 4-week washout period, subjects were crossed over to receive the alternative medication, and day 1 to 3 study procedures were repeated. After the second period of treatment, a follow-up visit was scheduled 29 to 42 days after the last dose of study medication.

Procedures

Capsaicin challenge. A stock concentration of 1000 $\mu\text{mol/L}$ capsaicin solution (Stockport Pharmaceuticals) was serially diluted with 0.9% normal saline to obtain a range of 12 concentrations from 0.49 to 1000 $\mu\text{mol/L}$. In addition to using these 12 concentrations for the cough challenge, 3 additional placebo pots containing 0.9% normal saline alone were placed in random positions in the cough challenge sequence. A pseudorandomization schedule was used to avoid adjacent placebo inhalations in the sequence. Dilution of capsaicin and placement of placebos were performed by a separate person who was not involved in the study to ensure that the person administering the test remained blind. The cough challenge was performed with DeVilbiss nebulizer pots incorporating a fixed baffle assembly and a flow regulator valve and was controlled by using a Koko dosimeter. The apparatus was calibrated to deliver 0.012 mL per inhalation. The cough challenge was performed as a single inhalation of doubling concentration of capsaicin at 1-minute intervals and stopped when the patients reached C5, which was defined as the concentration resulting in 5 or more coughs within 15 seconds of inhalation. Spirometry was performed before and after each challenge (Spirotrac IV, Vitalograph) according to standard criteria (American Thoracic Society/European Respiratory Society guidelines) to check for bronchoconstriction.

Ambulatory cough recording. Ambulatory cough-sound monitoring was performed with a custom-built device attached to a lapel microphone and a chest wall sensor positioned over the sternum (VitaloJAK, Vitalograph). Recordings were transferred to a personal computer; silences/background noise were removed by using validated, custom-written software; and cough sounds were counted by a trained observer using an audio editing

package (Adobe Audition 3.0, Adobe Systems). Previous studies found excellent agreement between trained observers performing manual cough counts^{E1,E2} and using this particular technique.^{E3}

VASs. Cough severity and urge to cough were rated by patients on 100-mm VASs. For cough severity, the extremes of the scale were labeled “no cough” and “maximum severity ever experienced.” For the urge to cough, the extremes were labeled “no urge to cough at all” and “maximum urge to cough ever experienced.” Patients rated cough severity and urge to cough at the baseline visit, before dosing, and 2 and 24 hours after dosing (before each capsaicin challenge).

CQLQ. The CQLQ is a 28-item survey in which each item is scored on a 4-point Likert scale (1, strongly disagree; 2, disagree; 3, agree; and 4, strongly agree). A total score was calculated for each subject, where 28 is the lowest score (good quality of life) and 112 is the maximum score (poor quality of life). The CQLQ was completed at baseline and at day 14.

Pharmacokinetics. Blood samples were drawn for pharmacokinetics analysis on each treatment day before dosing and then at 30 minutes, 1 hour, 1 hour and 30 minutes, 2 hours, 3 hours, 4 hours, and 24 hours after dosing.

Safety measures. In addition to the above procedures, all patients had a physical examination, routine hematology and biochemistry testing, 12-lead electrocardiograms, and tympanic temperature and vital sign measurements throughout the study. FEV₁ was also measured before and after each capsaicin challenge to check for bronchoconstriction.

Inclusion/exclusion criteria

Inclusion criteria. A subject was eligible for inclusion in this study only if all of the following criteria applied:

1. Aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and bilirubin levels were 1.5 times the upper limit of normal or less. An isolated bilirubin level of greater than 1.5 times the upper limit of normal was acceptable if bilirubin was fractionated and direct bilirubin was less than 35%.
2. The subject was considered healthy, as determined by a responsible and experienced physician based on a medical evaluation, including medical history, physical examination, and laboratory tests, with the exception of chronic cough. A subject with a clinical abnormality or laboratory parameters outside the reference range for the population being studied was included only if the investigator and the GlaxoSmithKline Medical Monitor agreed that the finding was unlikely to introduce additional risk factors and would not interfere with the study procedures.
3. The subject had idiopathic or chronic cough resistant to treatment targeted at potential triggers.
4. Subjects were male or female and aged 18 to 75 years inclusive at the time of signing the informed consent form.
5. A female subject was eligible to participate if the following were accurate:
 - I. She was of nonchildbearing potential, which was defined as female subjects with a documented hysterectomy, or postmenopausal, which was defined as 12 months of spontaneous amenorrhea. Female subjects receiving hormone replacement therapy (HRT) and whose menopausal status was in doubt were required to use one of the contraception methods in section 8.1 of the protocol if they wished to continue their HRT during the study. Otherwise, they were to discontinue HRT to allow confirmation of postmenopausal status before study enrollment. For most forms of HRT, at least 2 to 4 weeks will elapse between the cessation of therapy and the blood draw; this interval depends on the type and dosage of HRT. After confirmation of postmenopausal status, the subjects resumed the use of HRT during the study without use of a contraceptive method.
 - II. She was of child-bearing potential and agreed to use one of the contraception methods listed in section 8.1 of the protocol for an appropriate period of time (as determined by the product label or investigator) before the start of dosing to sufficiently minimize the risk of pregnancy at that point. Female subjects agreed to use contraception until 84 days after the last dose.

- III. She had undergone tubal ligation and agreed to perform a pregnancy test at time points specified in the protocol's "Time and events table."
6. Male subjects with female partners of child-bearing potential agreed to use one of the contraception methods listed in section 8.1 of the protocol. This criterion was followed from the time of the first dose of study medication until 84 days after the last dose.
7. The subject was a nonsmoker for at least 6 months with a pack history of 5 pack years or less.
8. The subject had a body weight of 50 kg or greater and a body mass index within the range 19 to 32.0 kg/m² (inclusive).
9. The subject was capable of providing written informed consent, which included compliance with the requirements and restrictions listed in the consent form.
10. The subject has a single ECG QT interval corrected for rate using Bazett's formula (QTcB) of less than 450 ms or a QTcB of less than 480 ms in those with bundle branch block.

Exclusion criteria. A subject was not eligible for inclusion in this study if any of the following criteria applied:

1. history of a positive prestudy hepatitis B surface antigen or positive hepatitis C antibody result within 3 months of screening or at screening;
2. current or chronic history of liver disease or known hepatic or biliary abnormalities (with the exception of Gilbert syndrome or asymptomatic gallstones);
3. history of confirmed positive prestudy drug/alcohol screen result;
4. history of regular alcohol consumption within 6 months of the study, which was defined as an average weekly intake of greater than 21 units for male subjects or greater than 14 units for female subjects;
5. participation in a clinical trial and receipt of an investigational product within the following time period before the first dosing day in the current study: 30 days, 5 half-lives, or twice the duration of the biological effect of the investigational product (whichever was longer);
6. exposure to more than 4 new chemical entities within 12 months before the first dosing day;
7. use of prescription or nonprescription drugs, including vitamins and herbal and dietary supplements (including St John's Wort), within 7 days (or 14 days if the drug was a potential enzyme inducer) or 5 half-lives (whichever was longer) before the first dose of study medication, unless it was the opinion of the investigator and GlaxoSmithKline Medical Monitor that the medication would not interfere with the study procedures or compromise subject safety;
8. known lung cancer or other active malignancy or history of such illness;
9. current treatment with oral corticosteroids or other immunosuppressive agents;
10. FEV₁ of less than 80% of predicted value before dosing;
11. failure to reach C5 after an oral inhalation challenge of capsaicin at a dose level of 250 μmol/L;
12. history of sensitivity to any of the study medications or components thereof or a history of drug or other allergy that, in the opinion of the investigator or GlaxoSmithKline Medical Monitor, contraindicated their participation;
13. participation in the study would result in donation of blood or blood products in excess of 500 mL within a 56-day period;
14. pregnant female subjects, as determined by positive human chorionic gonadotropin test results at screening or before dosing;
15. lactating female subjects;
16. unwillingness or inability to follow the procedures outlined in the protocol;
17. mental or legal incapacitation;
18. cotinine levels indicative of smoking or history of regular use of tobacco- or nicotine-containing products within 6 months before screening; and

19. consumption of red wine, Seville oranges, grapefruit, or grapefruit juice from 7 days before the first dose of study medication.

Blinding process. The study medication SB-705498 was matched with a placebo tablet provided by GlaxoSmithKline that was identical in appearance. All investigators and participants were blind to the treatments until all the study visits were completed and all the cough recordings were analyzed.

No subject's treatment was unblinded during the study.

Statistical analyses

Primary end points

Capsaicin challenge (C5). The primary end point was the capsaicin concentration required to achieve C5 after a single dose of SB-705498 at time to maximum observed plasma concentration (T_{max} ; 2 hours) compared with before dosing (day 1). The data were logged (base 2) for analysis and the difference from day 1 (equivalent to ratio on log scale). The difference between SB-705498 and placebo in log-transformed change from predose values was investigated by using a repeated-measures mixed-effects model with fixed-effects terms for treatment, period, subject, and period baselines and interactions between period and time point and treatment and time point. The subject was treated as a random effect in the model, and time point (2 and 24 hours) was treated as a repeated effect. The mean treatment difference and associated 95% CIs are reported on the log (base 2) scale (ie, the number of doubling doses).

Some subjects achieved C2 or C5 on inhalation of placebo (ie, 0.9% saline) during the cough challenge. As such, a result does provide some information (ie, the C2/C5 is known to be higher than the last actual capsaicin dose inhaled). For the *a priori* analysis, it was planned to impute the next highest concentration of capsaicin in the sequence (after the placebo). The analyses were also performed *post hoc*, treating such subjects as representing missing data, to ensure the significant changes were not dependent on the imputed data.

Objective cough frequency. Twenty-four-hour cough counts (coughs per hour) after a single dose of SB-705498 compared with placebo were analyzed by first log transforming the cough counts taken on day -1 and day 1 of each period in the 24-hour postdose period. The time period during which the challenge was conducted was excluded from the cough count, and cough counts were aligned relative to dosing time on each study day. The difference between SB-705498 and placebo in log-transformed count rates was investigated by using a negative binomial model with fixed-effects terms for subject and period baselines, treatment, period, and time after dosing (in hours) and a treatment-by-time interaction. The subject was treated as a random effect in the model. The mean treatment difference and associated 95% CIs were back-transformed to provide a treatment ratio and 95% CIs for the ratio.

Our hypothesis was to test whether the drug had a positive effect over placebo and not to determine whether it had a negative effect. We would only progress if we saw a positive treatment effect, and therefore the team decided to base decisions on a 1-sided *t* test. When assessing positive effects, note that a 1-sided *t* test at an α value of .025 is equivalent to a 2-sided *P* value at an α value of .05.

Secondary end points

Capsaicin challenge (C2). C2 after a single dose of SB-705498 at T_{max} (2 hours) was compared with baseline and was analyzed in an identical fashion to the C5 response variable. The C2 and C5 values from the 24-hour postdose challenge were also analyzed similarly to the analysis at T_{max} .

Subjective cough measures. Urge-to-cough and cough severity VASs after a single dose of SB-705498 compared with placebo were analyzed descriptively by treatment group. CQLQ results at 14 days after a single dose of SB-705498 compared with placebo were analyzed descriptively by treatment group.

Pharmacokinetic analysis. Plasma SB-705498 concentration-time data were analyzed by using noncompartmental methods (WinNonlin Professional, Version 5.2; Pharsight Corporation, Sunnyvale, Calif). Calculations were based on the actual sampling times recorded during the study. From the plasma concentration-time data, the following PK parameters were determined, as data permit: maximum observed plasma concentration, T_{max} , concentration at 24 hours after dosing (C24), and area under the plasma concentration-time curve (AUC_[0- t_{last}]).

The receptor occupancy of SB705498 was determined from plasma concentration data, assuming a 1:1 plasma/lung ratio (based on preclinical tissue homogenate data) by using the following formula:

$$Ro = 100 * \frac{C(t)}{C(t) + IC_{50}},$$

where $C(t)$ is the plasma concentration at time t and IC_{50} is the free fraction of the inhibitory concentration of 50% (1.4 $\mu\text{g/mL}$).

REFERENCES

- E1. Decalmer SC, Webster D, Kelsall AA, McGuinness K, Woodcock AA, Smith JA. Chronic cough: how do cough reflex sensitivity and subjective assessments correlate with objective cough counts during ambulatory monitoring? *Thorax* 2007;62:329-34.
- E2. Smith JA, Owen EC, Jones AM, Dodd ME, Webb AK, Woodcock A. Objective measurement of cough during pulmonary exacerbations in adults with cystic fibrosis. *Thorax* 2006;61:425-9.
- E3. Sumner H, Kelsall A, Woodcock A, Smith JA, McGuinness K. A semi automatic method to reduce the time taken for manual cough counting [abstract]. *Am J Respir Crit Care Med* 2010;181:A5555.

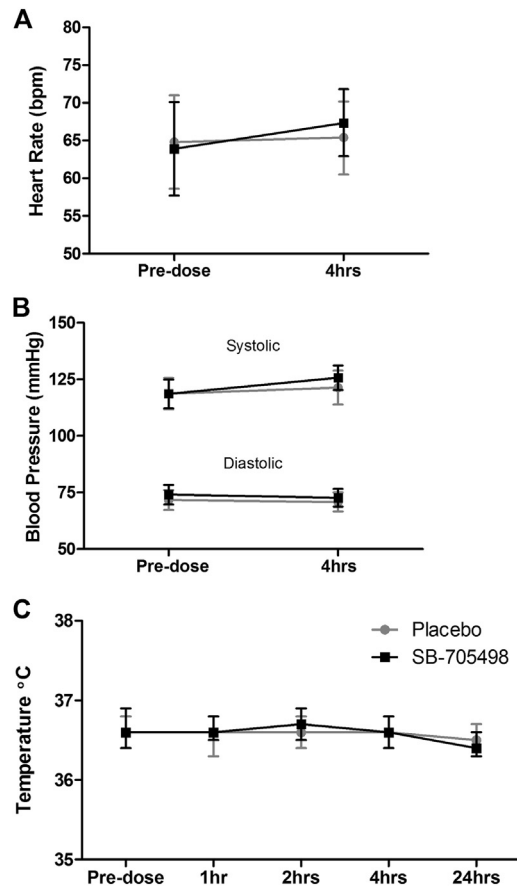


FIG E1. Vital signs before and after dosing with placebo and SB-705498. Heart rate (A), systolic and diastolic blood pressure (B), and temperature (C) are shown. All data are displayed as means and 95% CIs.