

Effect of Mucosal TRPV1 Inhibition in Allergic Rhinitis

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Abstract: Transient receptor potential vanilloid-1 (TRPV1) has been implicated as a mediator of itch in allergic rhinitis. To address this possibility, we synthesized a TRPV1 blocker (SB-705498) for nasal administration in patients with seasonal allergic rhinitis. The pharmacological activity of SB-705498 was confirmed on human TRPV1-expressing HEK293 cells, using fluorometric calcium imaging, and in patients with allergic rhinitis subjected to nasal capsaicin challenges. The effect of SB-705498 was studied in patients with seasonal allergic rhinitis subjected to daily allergen challenges for 7 days, using a double-blind, placebo-controlled, randomized and cross-over design. SB-705498 was delivered by nasal lavage 2 min. before each allergen challenge. Primary end-point was total nasal symptom score on days 5–7. Nasal peak inspiratory flow (nPIF) and eosinophil cationic protein (ECP) content in nasal lavages were also monitored. Daily topical applications of SB-705498 at a concentration that inhibited capsaicin-induced nasal symptoms had no effect on total symptom score, nPIF and ECP levels in allergen-challenged patients with seasonal allergic rhinitis. The individual symptoms, nasal itch or sneezes, were also not affected. These findings may indicate that TRPV1 is not a key mediator of the symptoms in allergic rhinitis. However, additional studies, using drug formulations with a prolonged duration of action, should be conducted before TRPV1 is ruled out as a drug target in allergic rhinitis.

Transient receptor potential vanilloid-1 (TRPV1) is an ion channel activated by heat, protons, capsaicin – the pungent compound in chilli pepper – and various membrane-derived lipids [1–4]. Notably, TRPV1-mediated responses are up-regulated in the setting of inflammation [5]. TRPV1 is expressed on primary sensory neurons, including those innervating the human nasal mucosa [6,7]. In addition, TRPV1 may be expressed on other structures in the nasal mucosa, such as submucosal glands, epithelial cells and vascular endothelium [6]. Interestingly, an increased number of nerve fibres was observed in patients with allergic rhinitis compared with healthy individuals [7].

We have recently proposed that TRPV1 mediates itch in allergic rhinitis [8]. Accordingly, patients with allergic rhinitis reported heightened itch responses to topical challenges with the TRPV1 activators capsaicin and olvanil at concomitant seasonal allergen exposure. This observation is of particular interest considering the recent finding that histamine, an itch-producing mediator in allergic rhinitis, produces less skin scratching in mice subjected to genetic or pharmacological inactivation of TRPV1 [9,10]. Moreover, desensitization or ablation of capsaicin-sensitive primary sensory neurons reduces responses to several pruritogens in animals and man, providing a clear link between itch and TRPV1-expressing primary afferents [10,11]. Thus, TRPV1 may represent a potential drug target in allergic rhinitis [8].

In this study, we examined the effects of the TRPV1 blocker SB-705498 [12–14], delivered as a high volume nasal

lavage to reach a large area of the nasal mucosa, in patients with seasonal allergic rhinitis. Initially, nasal capsaicin challenges were carried out and a concentration of SB-705498 that counteracted the sensory symptoms was identified. This concentration of the TRPV1 blocker was then given repeatedly to patients with seasonal allergic rhinitis. Concomitantly, these patients were subjected to daily nasal allergen challenges to mimic seasonal allergy exposure. Disease activity, as reflected by nasal symptoms and nasal peak inspiratory flow (nPIF), was monitored daily. Finally, eosinophil cationic protein (ECP) was measured in nasal lavage fluids as an index of eosinophil granulocyte activity, a hallmark of allergic inflammation.

Materials and Methods

Synthesis and in vitro characterization of SB-705498. The TRPV1 blocker SB-705498 was synthesized as described previously [12]. The molecular structure was confirmed by spectroscopic methods. The purity of the compound, as determined by nuclear magnetic resonance and high-pressure liquid chromatography, was found to be >99%. The pharmacological activity of SB-705498 was characterized on HEK293 cells expressing the human TRPV1, using fluorometric calcium imaging. The cells were plated in 96-well black-walled plates (Costar, Cambridge, MA, USA) and loaded with Fura 2-AM (1 μ M), probenecid (2 mM) and pluronic acid (20%) for 1 hr at 37°C. The cells were then washed with physiological buffer solution (PBS), containing 140 mM NaCl, 5 mM KCl, 10 mM glucose, 10 mM HEPES, 2 mM CaCl₂ and 1 mM MgCl₂, and allowed to equilibrate for a period of 30 min. in the dark before the start of the experiments. The intracellular calcium concentration ([Ca²⁺]_i) was determined at 25°C in a Flexstation 3 (Molecular Devices, Sunnyvale, CA, USA), and the maximum increase in dye fluorescence was determined after capsaicin application in the presence of various concentrations of SB-705498 or vehicle. These experiments were

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performed in triplicate on three independent occasions. The cells were incubated with various concentrations of SB-705498 for 1 min. (determination of $pIC_{50} \pm SE$) or with one concentration (1 μM) for 30 min. (determination of $pK_B \pm SE$) prior to capsaicin challenge. SB-705498 was dissolved in ethanol and dilutions further prepared in PBS buffer. The final concentration of ethanol did not exceed 1%.

Clinical trials. Patients with allergic rhinitis examined out of season were enrolled in two different clinical studies (Study 1 and 2). Nasal symptoms were used as primary outcome variable in both studies. The study patients all presented a history without indications of upper respiratory tract disease except for allergic rhinitis, a normal nasal inspection and a positive skin-prick test to either birch or grass pollen allergen. Specific exclusion criteria were perennial allergic rhinitis, structural nasal abnormalities, upper respiratory tract infections within 14 days before the first visit and pregnancy/lactation. No medication but the study drug was allowed during the course of the studies except for rescue medication (loratadine 10 mg once daily). The studies were approved by the regional ethics committee, and informed consent was obtained from all participants.

SB-705498 treatment during capsaicin challenge (Study 1). In the first study, patients were subjected to capsaicin challenges after exposure to SB-705498 or placebo in a double-blind, randomized and cross-over design. Twelve patients with a history of seasonal allergic rhinitis (median age, 26 years; range, 23–30 years; 10 men and two women) received nasal challenges with capsaicin (5 μM), delivered as single nasal spray actuations (100 μl) to both nasal cavities (Sigma-Aldrich, St. Louis, MO, USA) outside of pollen season. An interval of at least 5 days was allowed in between the two capsaicin challenges. SB-705498 (30 μM) was dissolved in warm isotonic saline (37°C), containing 4% ethanol, immediately before administration to the patients. This solution (without SB-705498) was used as placebo. SB-705498 or placebo (vehicle) was administered as nasal lavages, using a nasal pool device, containing 15 ml of the fluid [15]. The fluid was administered to each nostril in sequence and maintained in each nasal cavity for 2.5 min. Capsaicin was administered 2 min. after the treatment with SB-705498 or placebo (vehicle), and the symptoms were recorded 2 min. after the capsaicin challenge. Pain or smart, heat, sneezes or itch, secretion and blockage were recorded and assigned individual scores as follows: 0, no symptoms; 1, mild symptoms; 2, moderate symptoms; and 3, severe symptoms.

SB-705498 treatment during repeated allergen challenges (Study 2). In the second part of the study, patients with a history of seasonal allergic rhinitis were subjected to repeated allergen challenges after treatment with SB-705498 or placebo in a double-blind, randomized and cross-over design. A 2-week washout period was allowed in between the two allergen challenges. Twenty-six patients were included in the study, i.e., the 12 individuals who participated

in *Study 1* and 14 additional patients (median age, 25 years; range, 20–30 years; 22 men and four women).

To identify an individual symptom-producing yet tolerable dose of allergen to be used for repeated challenges, each patient was subjected to a titration procedure where increasing doses of either birch or timothy pollen allergen (Aquagen; ALK, Hørsholm, Denmark) were administered [16]. Doses of 100, 300, 1000 and 3000 standardized quantity units (SQ-Units) were given in sequence with 10-min. intervals until the patient responded with at least five sneezes or a symptom score of ≥ 2 on a scale from 0 to 3 for either of the symptoms secretion or nasal blockage. As described previously, the dose that produced this effect was chosen as the daily challenge dose for a 7 days' allergen challenge series [16].

A solution containing 30 μM SB-705498 (prepared as described previously) or placebo (vehicle) was administered consecutively to both nasal cavities once daily during the 7 days' allergen challenge series, using a pool device as described earlier [17]. A solution with a higher concentration of SB-705498 could not be prepared because of precipitation of the compound. The cumulative dose of SB-705498 did not exceed 2 mg, which is $<1/200$ of the oral dose previously given to human beings; this latter dose was well tolerated and associated with no or mild adverse effects [14]. The daily allergen challenge was given 2 min. after the exposure to SB-705498 with a spray device, delivering 100 μl per actuation in each nostril (for further details, see table 1).

The symptoms were recorded every morning and evening throughout the allergen challenge series as well as 10 min. after each allergen challenge. Accordingly, itch or sneezes, secretion and congestion, respectively, were assigned a score as follows: 0, no symptoms; 1, mild symptoms; 2, moderate symptoms; and 3, severe symptoms. The scores were added to a total nasal symptom score (TNSS) for each observation point (range, 0–9). Regarding the itch and sneeze symptoms, the more prominent of the two was used in the calculations. The mean value for the TNSS was calculated for the last 3 days of each allergen challenge series, for morning symptoms, evening symptoms and symptoms 10 min. after allergen challenge, respectively, and used in the analysis [16]. Note that the symptoms were also scored on the morning of the day before the start of the allergen challenge series, i.e., baseline observations.

Nasal peak inspiratory flow was monitored every morning and evening as well as 10 min. after allergen challenge, using a standard flow meter (Clement-Clark, Harlow, UK). The highest flow of three measurements was registered on each occasion. The assessments were carried out after scoring of nasal symptoms. Mean nPIF from observations made on the fifth, sixth and seventh challenge days were calculated in the same way as described above for symptoms and used in the analysis.

Nasal lavages were carried out the day before the first allergen challenge and the day after the last day of each allergen challenge series. Firstly, a 15-ml lavage with saline was performed over 5 min.

Table 1.

Schematic illustration of the study protocol for *Part 2* of the clinical trial in patients with seasonal allergic rhinitis ($n = 26$). Patients were subjected to allergen challenges once daily for 7 days (indicated as study day 1–7). Treatment with the Transient receptor potential vanilloid-1 blocker SB-705498 was given 2 min. prior to each allergen challenge. Symptoms and nasal peak inspiratory flow (nPIF) were scored in the morning, 10 min. post-challenge, and in the evening throughout the allergen challenge series. In addition, baseline symptoms were scored on the day before the challenge series. Nasal lavages were carried out on the day before the allergen challenge series and the day after the completed series. The study had a double-blind, placebo-controlled, randomized and cross-over design.

	Study day							
	–1	1	2	3	4	5	6	7
SB-705498		X	X	X	X	X	X	X
Allergen		X	X	X	X	X	X	X
Symptom		X	(X)	(X)	(X)	X	X	X
nPIF		(X)	(X)	(X)	(X)	X	X	X
Lavage	X							
								X

The symbol (X) indicates that symptoms and nPIF were monitored on these days but, not used in the analysis.

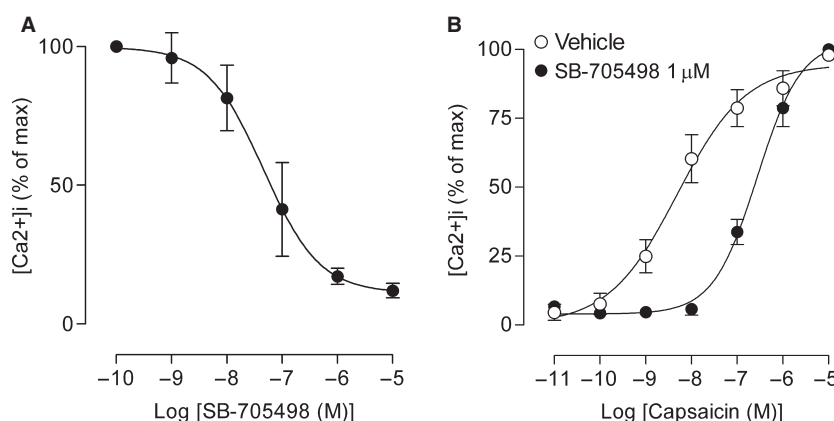


Fig. 1. Inhibitory effect of the transient receptor potential vanilloid-1 (TRPV1) blocker SB-705498 on capsaicin-induced calcium responses in HEK293 cells expressing human TRPV1, using fluorometric calcium imaging. (A) Inhibition of the calcium response to 20 nM capsaicin in the presence of various concentrations of SB-705498. (B) Concentration–response curve for capsaicin in the absence (vehicle) or presence of 1 μ M SB-705498. The results are depicted as relative fluorescence ratios, using the maximal response to capsaicin as internal standard in each experiment. Each value represents the mean $\pm$ SE of three (A) or four (B) independent experiments.

to remove any constituents that might have accumulated on the mucosal surface for an unknown period of time, and the lavage fluid was discarded. Briefly thereafter, a pool device containing 15 ml was used for a 5-min histamine lavage (0.4 mg/ml). This experimental measure was employed to produce plasma exudation and thereby force ECP out of the tissue and into the airway lumen [17]. These lavage fluids were centrifuged ($105 \times g$, 10 min., 4°C), and the samples were obtained from the supernatant and stored at -20°C until analysed. ECP was measured using a fluoroimmunoassay (Pharmacia-Diagnostics, Uppsala, Sweden) with a quantification limit of 2.0 ng/ml. For samples below the quantification limit values were set to 1.0 ng/ml and used for calculations.

Statistics. Data are presented as median levels with interquartile ranges, i.e., the difference between the third and first quartiles, within parenthesis. The data were subjected to analysis of variance (Friedman test), and paired comparisons were performed using the Wilcoxon signed rank test. P -values <0.05 were considered significant.

Results

In vitro effects of the TRPV1 blocker SB-705498.

As shown in calcium imaging experiments, SB-705498 produced a concentration-dependent and apparently competitive inhibition of capsaicin responses (fig. 1). The IC_{50} for the inhibition of 20 nM capsaicin-induced responses was estimated as 46 nM ($\text{pIC}_{50} = 7.3 \pm 0.2$, $n = 3$). The dissociation constant for SB-705498, calculated according to Van Rossum [18], was found to be 25 nM ($\text{pK}_B = 7.6 \pm 0.3$, $n = 4$), which is similar to that reported previously [13]. Assuming a similar interaction between capsaicin and SB-705498 at TRPV1 in the human nasal mucosa, a concentration of 30 μM should be expected to produce a substantial inhibition of the response to 1 μM capsaicin, which according to our earlier study evoked significant sensory symptoms, such as pain, smart and itch [8].

In vivo effects of SB-705498 (Study 1 and 2).

Capsaicin challenge. The dose of capsaicin employed in this study produced sensory symptoms dominated by a moderate

Table 2.

Effects of the transient receptor potential vanilloid-1 (TRPV1) blocker SB-705498 on nasal symptoms (range, 0–3) produced by capsaicin challenge in patients with seasonal allergic rhinitis ($n = 12$). The symptoms pain or smart and a sensation of heat were attenuated by the TRPV1 blocker.

	SB-705498	Placebo	p -value
Pain or smart	0.0 (0.0)	1.5 (1.0)	0.002
Heat	0.0 (0.0)	1.0 (1.0)	0.008
Sneezes or itch	0.0 (0.0)	0.0 (1.0)	0.180
Secretion	1.0 (1.0)	1.0 (0.0)	0.157
Blockage	0.5 (1.5)	1.0 (1.0)	0.480

Interquartile ranges are given within parenthesis.

sensation of pain or smart and heat (table 2). This effect was counteracted by pre-treatment with SB-705498 ($p < 0.01$).

Allergen challenge. The allergen titration procedure in patients with allergic rhinitis outside the pollen season resulted in allergen doses of 100, 300, 1000 and 3000 SQ-U in 4, 8, 11 and 3 individuals, respectively. Birch pollen allergen was used for 11 patients and grass pollen allergen for the remainder.

The repeated allergen challenges produced around-the-clock allergic rhinitis symptoms that reached a stable plateau during the last 3 days of the allergen challenge series (table 3). Symptoms recorded at morning and evening observations over challenge day 5, 6 and 7 indicated mild symptoms. Yet, the levels were significantly increased compared with baseline morning observations from a score of 0.0 (0.3) prior to the allergen challenge series to a mean score of 1.0 (1.3) over last 3 days of the challenge series ($p < 0.0001$). Rescue medication was not used by any of the study participants.

Pharmacological treatment with SB-705498 did affect neither the around-the-clock symptoms, i.e., morning and

Table 3.

Effects of the transient receptor potential vanilloid-1 (TRPV1) blocker SB-705498 on total nasal symptoms (TNSS; range, 0–9) in patients with seasonal allergic rhinitis ($n = 26$). When the patients received placebo, the allergen challenge series produced moderate to marked post-challenge symptoms and mild around-the-clock symptoms. The TRPV1 blocker did not affect these symptoms.

	SB-705498	Placebo	<i>p</i> -value
Evening symptoms	1.5 (1.7)	1.0 (1.3)	0.130
Morning symptoms	1.7 (1.3)	1.0 (1.3)	0.203
Post-challenge symptoms	4.8 (1.7)	5.0 (2.4)	0.370

Interquartile ranges are given within parenthesis.

Table 4.

Effects of the transient receptor potential vanilloid-1 (TRPV1) blocker SB-705498 on nasal itch or sneezes (range, 0–3) in patients with seasonal allergic rhinitis ($n = 26$). When the patients received placebo, the allergen challenge series produced moderate to marked post-challenge itch or sneezes and mild around-the-clock itch or sneezes. The TRPV1 blocker did not affect these symptoms.

	SB-705498	Placebo	<i>p</i> -value
Evening symptoms	0.3 (0.7)	0.0 (1.0)	0.845
Morning symptoms	0.3 (0.3)	0.0 (0.7)	0.679
Post-challenge symptoms	1.7 (1.2)	1.7 (0.7)	0.668

Interquartile ranges are given within parenthesis.

evening observations, nor those recorded 10 min. after the daily allergen challenges (table 3). Moreover, no significant effects were observed on sensory symptoms, i.e., the individual symptoms itch or sneeze (table 4). Furthermore, no significant effects were observed for secretion and blockage (data not shown), except morning nasal blockage that was 0.7 (1.0) for placebo and 1.0 (1.0) for SB-705498 ($p < 0.05$). SB-705498 did affect neither the post-challenge nPIF nor the evening nPIF, whereas the morning nPIF was somewhat reduced by the treatment (table 5).

In this study, nasal lavage fluid levels of ECP were below the level of quantification in 49 of 208 observations and one observation was missing. When the patients received placebo, ECP was increased 24 hr after the allergen challenge series compared with the baseline observation prior to the series ($p < 0.05$), indicating mild allergic inflammation. The med-

Table 5.

Effects of the transient receptor potential vanilloid-1 (TRPV1) blocker SB-705498 on nasal peak inspiratory flow (nPIF) in patients with seasonal allergic rhinitis ($n = 26$). When the patients received placebo, the allergen challenge series produced reductions in nPIF. The TRPV1 blocker did not counteract this effect. However, a somewhat smaller morning nPIF was noted in the treatment group compared with the placebo group.

	SB-705498	Placebo	<i>p</i> -value
Evening nPIF	170 (80)	163 (47)	0.605
Morning nPIF	137 (70)	150 (57)	0.005
Post-challenge nPIF	137 (60)	130 (68)	0.094

Interquartile ranges are given within parenthesis.

ian levels were 5.5 (6.3) ng/ml before the challenge series and 7.1 (21.2) ng/ml thereafter. No differences in ECP were observed between the treatments at the observation point after the allergen challenge series; median levels for placebo and the TRPV1 blocker were 7.1 (21.2) and 9.5 (27.2) ng/ml, respectively.

Discussion

In this study, we demonstrate that the TRPV1 blocker SB-705498, delivered by nasal lavage, inhibits the sensory symptoms evoked by a subsequent capsaicin challenge. In contrast, it failed to reduce allergen challenge-induced symptoms in patients with allergic rhinitis, including the sensory symptom, itch or sneezes. These findings are of relevance to the function of TRPV1 in the human nasal airway and to TRPV1 as a potential drug target in seasonal allergic rhinitis.

In the first part of this study, we characterized the pharmacological effects of SB-705498 on capsaicin-induced calcium responses *in vitro*. We then focused on the inhibition of capsaicin-induced sensory symptoms to demonstrate an effective TRPV1 blockade *in vivo*. The concentration of capsaicin used in these experiments was based on previous observations in man and likely represented a concentration in the low to moderate range on the concentration–response curve [15,19]. In agreement, the symptom scores recorded in this study reached 1.5 for pain or smart, and 1.0 for sensation of heat on a scale from 0 to 3. Our findings indicate that the employed concentration of SB-705498 is able to inhibit TRPV1 in the nasal mucosa and demonstrate for the first time that topical administration of a TRPV1 blocker can reduce TRPV1-mediated nasal symptoms in humans.

In this study, we employed a model in which symptom-producing allergen challenges were administered once daily for 7 days, using a nasal spray device [16], allowing symptoms to be scored in the morning, 10 min. post-challenge and in the evening. As shown previously, morning and evening symptoms are well established after five allergen exposures [16]. Therefore, we focused our analysis on the last 3 days of the challenge series. The symptoms observed 10 min. post-challenge were stable and similar to those reported previously [16]. Also, corresponding reductions in nPIF were observed. The around-the-clock symptoms were significantly increased compared with the baseline observation, although they were slightly milder than those reported previously [16]. It may be argued that the symptom levels around-the-clock were too low for a pharmacological effect to be detected. However, we have previously demonstrated clear effects of anti-allergic drugs in this experimental setting [16,20].

The present pharmacological intervention with a TRPV1 blocker in the allergen challenge model did affect neither the TNSSs recorded 10 min. post-challenge, nor the scores recorded in the morning or evening. Moreover, it did not reduce the individual itch or sneeze symptoms, which we previously suggested was in part mediated through TRPV1 [8].

Given the positive effect of the SB-705498 in the capsaicin challenge tests and the mode of administration, reaching a large mucosal surface area, these observations may indicate that TRPV1 is not a relevant treatment target in seasonal allergic rhinitis. However, several key considerations need to be made. Firstly, the dose of capsaicin in the initial capsaicin challenge experiments was relatively low, as reflected in the symptom score, potentially overestimating the inhibitory effect of SB-705498. Accordingly, it cannot be excluded that a higher concentration of TRPV1 blocker would have produced symptom relief. Secondly, the formulation of SB-705498 was not optimized with regard to its pharmacokinetic properties, such as tissue penetration, mucosal retention and duration of action. Taken the above issues into account, further studies are warranted before TRPV1 can be discarded as a drug target in allergic rhinitis.

Although neurogenic inflammation, typically demonstrated as capsaicin-induced plasma exudation in rodents [21], may not be a key disease mechanism in human airway conditions [15,19], sensory nerves are certainly involved in the symptoms of allergic rhinitis, including itch, sneezes and nasal secretion [22]. Given the methodological concerns, the result of this study cannot entirely exclude a role for TRPV1 in allergic rhinitis. However, neuronal TRPV1 might play a more prominent role in other airway diseases associated with marked sensory hyper-reactivity.

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