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CLINICAL TRIAL REPORT

Decongestant Effect of "Coldamaris Akut", a Carrageenan- and Sorbitol-Containing Nasal Spray in Seasonal Allergic Rhinitis

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Purpose: This study aimed to develop a hyperosmolar, barrier-forming nasal spray based on carrageenan and sorbitol, and to demonstrate its decongestant effect in the context of allergic rhinitis (AR).

Methods: The efficacy of the nasal spray components was tested in vitro by barrier function, virus replication inhibition, and water absorption assays. The decongestant effectiveness was assessed in a randomized, controlled, crossover environmental chamber trial, where participants with a history of seasonal grass pollen AR were exposed to grass pollen allergens under controlled conditions. Forty-one adults were randomized to receive either carrageenan- and sorbitol-containing nasal spray (CS) or saline solution (SS). After 1 week, participants repeated the exposure with the treatment they had not received before. The primary efficacy endpoint was the mean change in nasal congestion symptom score (NCSS). Secondary efficacy endpoints were nasal airflow, nasal secretion, total nasal symptom score (TNSS), total ocular symptom score (TOSS) and total respiratory symptom score (TRSS).

Results: Preclinical assays demonstrated barrier-building, virus-blocking, and water-withdrawing properties of the CS components. In the clinical study, there was no significant difference in mean NCSS change from pre- to post-treatment between CS and SS. However, nasal airflow increased over time after treatment with CS, while it declined after SS, leading to a growing difference in airflow between CS and SS ($p = 0.04$ at 6:00 h). Mean nasal secretion over 2–6 h was reduced by ~25% after CS ($p = 0.003$) compared to pre-treatment, while it was reduced by only ~16% after SS ($p = 0.137$). No significant differences in TNSS, TOSS and TRSS were observed between CS and SS.

Conclusion: CS improves nasal airflow and reduces nasal secretion in adults with AR. We propose CS as a safe and effective adjuvant to baseline pharmacological treatments.

Trial Registration: NCT04532762.

Keywords: Allergic rhinitis, nonpharmacological, drug-free, barrier, Carragelose, carrageenan

Introduction

Nasal congestion, meaning a fullness, blockage, or obstruction of the nasal cavity, is a frequently described symptom in clinical practice. It can significantly impair quality of life (QOL), reduce daytime productivity at work or school, and negatively impact quality of night-time sleep.[1] Nasal congestion is usually treated with local decongestants like xylometazoline or oxymetazoline. Unfortunately, rebound swelling of the mucosa is observed upon prolonged use of these topical vasoconstrictors. This can lead to a gradual overuse and a vicious circle of self-treatment, which patients are often unaware of.[2,3]

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Nasal congestion is caused by airborne irritants like tobacco smoke or dust, or by viruses and allergens, which cause viral and allergic rhinitis and sinusitis, respectively. Allergic rhinitis is a type I allergic reaction where otherwise innocuous allergens such as pollen or animal dander crosslink receptor-bound IgE on mast cells.[4] This crosslinking results in a biphasic response. The early phase is characterized by the release of pre-formed mediators such as histamine which cause characteristic symptoms like pruritus, rhinorrhea, sneezing, and nasal congestion. The late phase is characterized by the release of newly synthesized mediators such as cytokines and chemokines, which strongly contribute to inflammation and thereby to a worsening of the disease. Seasonal allergic rhinitis (SAR) or hay fever is caused by seasonal peaks in the airborne load of pollens and is the most common type of allergic rhinitis. It is one of the most common chronic conditions in high-income countries,[5] and it is estimated that in Europe, up to 40% of the population suffer from pollen allergy.[6] In contrast to viral rhinitis, which is usually self-limiting with a symptom duration of about 1 to 2 weeks, symptoms of allergic rhinitis can continue over longer periods. Allergic patients using topical decongestants are therefore at higher risk of experiencing the rebound effect and would benefit from a decongestant that does not induce this habituation.

We have developed nasal sprays based on iota-carrageenan (Carragelose®), a naturally occurring polymer from red seaweed, which forms a protective layer on mucosal surfaces that prevents viruses and allergens from interacting with the mucosal surface. Carragelose® is approved for marketing in the EU and parts of Asia and Australia as a component of nasal sprays, throat sprays and lozenges. Previous pre-clinical and clinical studies by us and others have shown that carrageenan-containing nasal sprays have a broad, non-specific mode of action and prevent attachment of small particles like virus or pollen to mucosal cells.[7–16] Carrageenan-containing nasal sprays reduce the symptoms of the common cold and the viral load in nasal lavage.[14] Symptom duration is shorter, and viral titers in nasal fluids decrease faster in patients with common cold when treated with carrageenan-containing nasal spray compared to placebo.[12,13] We have recently demonstrated a clinical anti-allergic effect of carrageenan.[16] Since the virus-blocking effect of carrageenan is based on its physical barrier function, we hypothesized that carrageenan acts also against other small airborne particles by the same mechanism of action, resulting in the alleviation of AR symptoms.

To broaden the beneficial effect of our nasal spray, we wanted to add a decongestant activity by enhancing the osmolarity of the solution. This causes outflux of water from the nasal mucosa cells, thereby reducing mucosal swelling and hence nasal congestion. Cingi et al reported that a hyperosmolar xylitol-containing nasal spray improved the QOL score compared to pre-treatment in participants suffering from nasal obstruction.[17] Bergmann et al noticed an improvement in nasal breathing upon use of hypertonic saline nasal spray in 70% of the patients,[18] and a meta-analysis showed that hypertonic saline irrigation brings greater benefit than isotonic saline in improvement of nasal symptoms of rhinitis.[19] A hypertonic nasal spray containing mucoadhesive carrageenan combines sustainable decongestant and anti-viral activity. Hypertonicity could be achieved by addition of ionic and/or non-ionic osmolarity donors like sodium chloride (NaCl). However, carrageenans change their conformation depending on the ionic strength of the environment.[20,21] Enhancing osmolarity using NaCl might therefore affect their anti-viral properties. Alternatively, hypertonicity could be achieved by adding sorbitol, a water-soluble, membrane impermeant polyol (sugar alcohol) that is frequently used in food processing to preserve moisture and add sweetness and texture.

Here, we report preclinical in vitro and ex vivo data that are the basis for optimization of the decongestant nasal spray formulation. Furthermore, we show results of a randomized, controlled, crossover clinical trial on a decongestant effect of the carrageenan- and sorbitol-containing nasal spray (CS) in adults with a history of moderate-to-severe SAR. The primary objective of this trial was to demonstrate a decongestant effect on the nasal mucosa of the CS in comparison with 0.5% saline solution nasal spray (SS). The secondary objective was to demonstrate the clinical performance of the CS in comparison with SS by objective measurements of nasal airflow and nasal secretion as well as patient-reported nasal, ocular and respiratory symptoms.

Methods

Preclinical Assays

Material – Viruses and Cells

Human Rhinoviruses HRV1a and HRV8

The human cervical epithelial carcinoma cell line (HeLa) was obtained from the American Type Culture Collection

(ATCC, USA). The cells were cultivated in Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich, Austria) supplemented with 10% fetal bovine serum (Sigma-Aldrich, Austria) and 1% antibiotic-antimycotic mix (Sigma-Aldrich, Austria) in a 37°C incubator (Sanyo, Japan; CO_2: 5%; relative humidity: >95%). HRV1a and HRV8 serotypes were obtained from the ATCC and grown on HeLa cells. Virus stocks were frozen at −80°C, and virus titers were determined by 50% tissue culture infective dose ($TCID_{50}$) assays. In all experiments, a medium containing 2% fetal bovine serum and 1% antibiotic–antimycotic mix was used.

Human Coronavirus (hCoV) OC43

Vero (embryonic African green monkey kidney) cells were purchased from the ATCC. The cells were cultivated in OptiPro serum free medium (Thermo Fisher Scientific, USA) supplemented with 4 mm L-glutamine (Sigma-Aldrich, Austria) in a 37°C incubator (CO_2: 5%, relative humidity: >95%). hCoV OC43 was obtained from the ATCC and propagated in the same medium. The stocks were frozen at −80°C and virus titers were determined by $TCID_{50}$ assay.

Carrageenan

Iota- and kappa-carrageenan were purchased from Dupont former FMC Biopolymers. The dry polymer powders were dissolved in sterile water for medical use (B. Braun, Germany) to a final concentration of 2.4 mg/mL, containing 0.5% NaCl (Sigma-Aldrich, Austria). This stock solution was sterile filtered through a 0.22 µm filter (Sarstedt, Germany) and stored at 4°C until use.

In Vitro Viral Inhibition Assay

To test if osmolarity could be adjusted with NaCl without compromising the virus-blocking effectiveness of carrageenan, a series of formulations containing 1.2 mg/mL iota-carrageenan and 0.4 mg/mL kappa-carrageenan with NaCl concentrations between 0.5% and 2.3% were tested against human rhinoviruses HRV1a and HRV8. HeLa cells were seeded in 96-well plates (Techno Plastic Products – TPP, Switzerland). 4-fold concentrated serial dilutions of the test samples (carrageenan plus varying concentrations of NaCl) and 4-fold concentrated virus dilution were prepared. Equal volumes of virus and test sample dilutions were mixed and incubated at room temperature (RT) for 30 minutes (min). The mixture was diluted with an equal volume of medium with 4% fetal bovine serum and antibiotic-antimycotic before it was added to the cells for infection at a multiplicity of infection (MOI) of 0.7. After 48 hours (h) at 33°C, cells were washed, and viability was assessed with alamarBlue™ (resazurin sodium salt, Sigma-Aldrich, Austria) using a microplate reader (Omega, BMG Labtech, Germany). Viability was corrected for toxicity of increasing salt concentrations and normalized to the viability of non-infected cells. The same experimental set-up was used to test viral inhibition effectiveness of the final formulation of the commercial product, containing 1.2 mg/mL iota-carrageenan, 0.4 mg/mL kappa-carrageenan, 0.5% NaCl, and 7% sorbitol (Sigma-Aldrich, Austria) in McIlvaine buffer (citric acid monohydrate, Roth, Germany; disodium hydrogen phosphate, Sigma-Aldrich, Austria). Half-maximal inhibitory concentrations (IC_{50}) were calculated with XLfit® Excel add-in version 5.3.1. Results were normalized to toxicity and non-infected control.

All percentages referring to nasal spray components here and in the following subsections are % weight/volume if not mentioned otherwise.

Hemagglutination Assay

To assess anti-viral activity against hCoV OC43, a hemagglutination assay was performed as described previously.[22] On a 96-well plate (TPP), two hemagglutination units of hCoV OC43 per well are incubated with a semi-logarithmic dilution series of test or control samples for 10 min at RT (final concentrations: 0.002–3 µg/mL iota-carrageenan diluted in 0.5% to 2.6% NaCl with or without 7% sorbitol and McIlvaine buffer). A suspension of chicken red blood cells (1% v/v in phosphate buffered saline, modified, without calcium chloride and magnesium chloride, Sigma-Aldrich, Austria) is added to each well to allow hemagglutination of red blood cells (RBCs) by the virus for 1 h 30 min at 4°C. At the time point of assay evaluation, control RBCs in the absence of carrageenan are fully agglutinated by the virus, whereas inhibition of hemagglutination can be observed in samples treated with carrageenan up to a certain dilution factor. The minimal inhibitory concentration of each sample is noted for comparison of the anti-viral effectiveness of each sample. As an internal control, a specific batch of iota-carrageenan is used (assay reference).

Ex vivo Dehydration Assay

The swine nasal mucosa was received from the University Clinic for Swine at the University of Veterinary Medicine, Vienna. The nasal mucosa was excised from euthanized pigs and punched out into equal circular pieces with a diameter of 10 millimeter (mm). The mucosa pieces were weighed and put, with the mucosa-site upward, into 48-well plates, and 250 µL test solution were added to each well. Test solutions were iota- and kappa-carrageenan with 0.5% NaCl and 7% sorbitol; iota- and kappa-carrageenan with 0.5% NaCl without sorbitol; and a 2.4% NaCl solution. The plate was incubated for 60 min at 37°C, after which the mucosa pieces were weighed again.

In vitro Barrier Assay

A 1.25% agar solution (Sigma-Aldrich, Austria) was filled into the wells of a 96-deep-well plate (Biozym, Germany) and was left to solidify. 200 µL of CS and of negative control were added on top of the agar block. The negative control sample contained sorbitol and NaCl in the same concentrations as in CS, but did not contain carrageenan. Fluorescent particles (Thermo Fisher Scientific, USA) of 0.3 µm or 1.0 µm diameter, respectively, were added and incubated for 3 h at RT. Following multiple wash steps with 0.5% NaCl solution, particles were extracted from agar blocks using 0.1% Tween® 20 (BioRad, USA) in PBS over night at 4°C with 900 rpm shaking. Extraction supernatants were transferred into a 96-well black flat bottom plate and analyzed in a plate photometer (Omega, BMG Labtech, Germany) with an excitation and emission wavelength of 485 nm and 520 nm, respectively. Percent blocking was calculated relative to the fluorescence activity of the beads extracted from the negative control.

Clinical Study

Study Design

This was a prospective, controlled, double-blinded randomized two-way cross-over single-site study in adult female and male participants with moderate-to-severe grass pollen-induced SAR. The study evaluated two treatments, namely CS and SS nasal sprays. The study was conducted at the Vienna Challenge Chamber (VCC) in Vienna, Austria. The Ethics Committee of the City of Vienna oversaw trial conduct and documentation. The study included 5 visits. At visit 1 (screening visit), inclusion/exclusion criteria and medical and allergic history were assessed. At visit 2 (inclusion visit), participants were screened for appropriate allergic response in a 3 h allergen challenge qualification session in the VCC, and blood samples for safety lab were withdrawn. At visit 3, scheduled 7 days after visit 2, participants were randomized to one of the two treatment arms (CS or SS) in a fully blinded fashion (details of randomization see below) and underwent their first 6 h allergen challenge session. Approximately 1 h 45 min after start of allergen exposure, participants were dosed with the treatment they had been randomized to, and continued exposure for a total of 6 h (first treatment block). At visit 4, scheduled 7 days after visit 3 to allow complete symptom relief from the previous challenge, participants were exposed to the second allergen challenge and crossed over to the treatment they had not received before (second treatment block). A follow-up visit (end-of-study visit, visit 5) was scheduled at least one week after the second treatment block. Participants were asked to record adverse events (AEs) and the use of concomitant medication for the entire duration of the study.

Participants

Participants were female and male adults aged between 18 and 65 years of any ethnicity/race, with a documented clinically relevant history of moderate-to-severe SAR to grass pollen for the previous two years. Participants were selected from the VCC database and had to satisfy all inclusion and exclusion criteria to be enrolled into the study.

The key inclusion criterion was a moderate to severe response to standard grass pollen allergen mixture within the first 2 h of the allergen challenge qualification session in the VCC, defined as total nasal symptom score (TNSS) of at least 6 points (out of maximum 12), with the necessity to score at least moderate (2 points) for the single symptom nasal congestion. TNSS is the sum of nasal congestion, rhinorrhea, itchy nose and sneezing, each scored on a categorical scale from 0 to 3. Additional inclusion criteria were a positive Skin Prick Test (SPT) response (wheal diameter at least 3 mm larger than diluent control) to grass pollen solution (standard Allergopharma) at screening or within the last 12 months prior to study start; positive serum-specific IgE against recombinant major allergen components of the used grass pollen,

eg, g6 (specific CAP IgE ≥0.70 kU/L) at screening or within the last 12 months prior to study start; and a forced expiratory volume in 1 second (FEV1) of at least 80% of reference value[23] at screening. Asthma patients were allowed into the study only if the asthma condition was mild or intermittent, and if not treated with steroids.

Exclusion criteria comprised prior and ongoing conditions, diseases and treatments that may interfere with the study intervention and outcomes, in particular ongoing treatment with any allergen-specific immunotherapy product. Female participants of child-bearing potential were required to use birth control.

Randomization and Blinding

Randomization numbers were allocated to the study participants in ascending order of their screening numbers following their attendance at visit 3 (first treatment block). They were randomized using a cross-over randomization with balanced blocks. All personnel involved in the study, including investigators, site personnel, and sponsor's staff were blinded to the randomization codes. Persons responsible for labeling of investigational products were un-blinded, but not involved in other study activities. Unblinding occurred at the end of the study.

Interventions and Procedures

During each treatment block, sensitive participants were exposed to standard grass pollen allergen mixture in the VCC for 6 h using a validated method.[24,25] The VCC is a specially designed room that enables trial participants to be exposed to a controlled environment and to be challenged with a defined concentration of airborne allergens. During the challenge session, participants were under constant supervision and could communicate with medical staff outside the chamber. The chamber was filled with 100% fresh air, which was conditioned (filtered, heated, dried, cooled, and humidified) before being loaded with the challenge agent, a mixture of four grass pollen species (Timothy, Orchard, Perennial rye and Sweet vernal grass) (Allergon SB, Sweden). Air temperature (24°C), humidity (40%) and allergen load (1500 grains/m^3) were constantly monitored and maintained. During the 6-h challenge, subjective nasal symptoms (nasal congestion, rhinorrhea, itching, sneezing), ocular and respiratory symptoms were recorded every 15 min. Nasal airflow was measured by active anterior rhinomanometry (AAR) at a pressure difference of 150 Pa across the nasal passages (sum of the right and left nostril values). Nasal airflow was evaluated immediately before and every 30 min during exposure, with an additional assessment 2 h 15 min after start of exposure. Nasal secretion was evaluated by weighing paper tissues used by the participants during their stay in the chamber and collected every 30 min. Lung function was assessed before and every 2 h during the allergen challenge by measuring the Forced Expiratory Volume in 1 second (FEV_1) using a spirometer (PDD_301/sh, Piston Medical, UK) according to the site SOP. The normal range for FEV1 was: 1.5 L, 6.5 L (low, high). Vital signs (blood pressure, pulse rate, temperature and breathing frequency) were assessed at every visit, pre- and post-challenge.

1 h 45 min after entering the challenge chamber, ie, after developing pronounced allergic nasal symptoms including nasal congestion, participants applied 1 spray, corresponding to 140 µL per nostril, of either CS or SS. This resulted in a residual observation period of 4 h 15 min.

CS contained 1.2 mg/mL iota-carrageenan, 0.4 mg/mL kappa-carrageenan, 7% sorbitol, 0.5% NaCl, 1 mg/mL ethylene diamine tetra acetate, buffer and purified water. SS contained 0.5% NaCl in water. CS and SS were provided in identical 20 mL glass bottles (Schott) with APF spray pump (Aptar pharma).

Endpoints

The primary efficacy endpoint was the mean difference between CS and SS of the nasal congestion symptom score (NCSS) measured every 15 min during allergen exposure. Secondary efficacy endpoints were nasal airflow as assessed by active anterior rhinomanometry, total nasal symptom score (TNSS; sum of symptoms nasal congestion, rhinorrhea, itchy nose, and sneezing), total ocular symptom score (TOSS; sum of symptoms ocular itching, ocular redness, watery eyes), total respiratory symptom score (TRSS; sum of symptoms cough, wheeze, dyspnea), and nasal secretion. Each individual symptom of NCSS, TNSS, TOSS and TRSS was rated on a scale from 0 to 3, where 0 corresponded to no symptoms, 1 to mild symptoms (easy to tolerate), 2 to moderate symptoms (bothersome, but tolerable) and 3 to severe symptoms (hard to tolerate). Safety endpoints were i) frequency and severity of AE, related AE and serious AE (SAE)

throughout the study, ii) lung function and vital signs at screening and throughout the study, and iii) Electrocardiogram (ECG) results, physical examination findings and laboratory blood analysis at screening and at the follow-up visit.

Statistical Analysis

Sample size calculation was based on the expectation of a mean difference of 0.6 points with a standard deviation (SD) of 1.1 (SS = 2, CS = 1.4, effect size d = 0.56 and power = 90%), which was derived from previous studies. Thus, n = 36 participants were needed at an alpha level of p = 0.05. Considering the dropout rate of 10–15%, up to 50 participants needed to be screened in order to randomize about 42 participants and to get evaluable data from at least 36 participants.

Safety analyses including vital signs, laboratory data and AEs, were carried out in the safety population defined as all participants who started the allergen challenge qualification session.

Efficacy was analyzed in the Full Analysis Set (FAS) and in the Per-Protocol Set (PPS). The FAS comprised all participants who were randomized and was analyzed following the intention-to-treat principle, according to the treatment they had been assigned at randomization. The PPS comprised all participants in the FAS who did not have any clinically important protocol deviation.

The primary efficacy variable was analyzed in a confirmatory way between the two conditions CS and SS, assuming superiority for CS versus SS. The null hypothesis was defined as:

Mean NCSS [Delta pre-treatment (1 h 45 min) - post-treatment (mean 2–4 h)] {CS} ≤ Mean NCSS [Delta pre-treatment (1 h 45 min) - post-treatment (mean 2–4 h)] {SS}

The alternative hypothesis was defined as:

Mean NCSS [Delta pre-treatment (1 h 45 min) - post-treatment (mean 2–4 h)] {CS} > Mean NCSS [Delta pre-treatment (1 h 45 min) - post-treatment (mean 2–4 h)] {SS}

A 95% confidence interval (CI) for the mean difference of the two treatments was calculated. The superiority comparison of CS versus SS was performed using analysis of variance (ANOVA) appropriate for the cross-over design. Period (first or second treatment block) was included in the analysis model as a fixed effect to confirm the assumption of no period effect. Participant was included in the model as a random effect. Superiority was to be postulated if the lower bound of the 95% CI was > 0.

Secondary efficacy variables were analyzed in an explorative sense and are presented using descriptive methods. Exploratory efficacy analysis was performed for mean differences between the two treatments for consecutive intervals from 2 h onward to 6 h, analogous to the primary efficacy analysis. Respective statistical tests and p-values are to be regarded as descriptive and not as tests of hypotheses.

All attempts were made to collect all data per protocol. Missing or invalid data were neither replaced nor extrapolated. Outliers were not excluded from the primary analyses. Significance level was set to alpha = 5%. R version 4.0.3 was used for all statistical analyses.

Results

Carrageenan-containing nasal sprays are used to prevent and treat viral infections of the respiratory tract by blocking the viruses´ attachment to the mucosa. To enhance the benefit and broaden the applicability of the barrier-forming nasal spray, a decongestant effect should be added to the formulation. Usually, intranasally applied hyperosmotic saline solutions are used to withdraw water from the nasal mucosa, thereby reducing intranasal swelling. However, we found that increasing salt concentrations reduced the carrageenan's capacity to block the attachment of the human rhinoviruses HRV1a and HRV8 and of the human coronavirus OC43 to cells in a dose-dependent manner, as shown by IC_{50} values in Table 1. Therefore, the formulation was adjusted to 0.5% NaCl to preserve the carrageenan's beneficial virus-blocking effect. To achieve hyperosmotic activity, sorbitol was added to the formulation at a concentration of 7%, which increased the formulation's osmolarity, but, in contrast to high concentrations of NaCl, preserved the virus-blocking activity of carrageenan (also shown in Table 1).

After confirming that addition of buffer did not influence the antiviral activity of carrageenan (data not shown), the final product was formulated with 1.2 mg/mL iota-carrageenan, 0.4 mg/mL kappa-carrageenan, 0.5% NaCl, and 7% sorbitol in McIlvaine buffer. The osmolality was set to 787 mosmol/kg, corresponding to the osmolality of hyperosmolar

Table 1 In Vitro Assay – Virus-Blocking Effectiveness Against HRV1a, HRV8 and hCoV OC43.

Effectiveness of various formulations	IC_{50} / MIC [µg/mL]	IC_{50} 95% CI [µg/mL]
HRV1a		
Carrageenan + 0.5% NaCl	1.8	0.7; 3.0
Carrageenan + 0.9% NaCl	5.6	4.0; 7.1
Carrageenan + 2% NaCl	26.5	23.0; 30.0
Carrageenan + 2.3% NaCl	40.7	35.0; 46.6
Fold Change (Carrageenan + 0.5% NaCl) vs (Carrageenan + 2.3% NaCl)	22.3	
Carrageenan + 0.5% NaCl + 7% Sorbitol	3.7	2.2; 5.3
Carrageenan + 2.3% NaCl	104.5	82.8; 126.2
Fold Change (Carrageenan + 0.5% NaCl + 7% Sorbitol) vs (Carrageenan + 2.3% NaCl)	27.9	
HRV8		
Carrageenan + 0.5% NaCl	2.3	1.0; 3.6
Carrageenan + 0.9% NaCl	4.1	2.9; 5.3
Carrageenan + 2% NaCl	8.1	5.5; 10.7
Carrageenan + 2.3% NaCl	15.6	8.8; 22.4
Fold Change (Carrageenan + 0.5% NaCl) vs (Carrageenan + 2.3% NaCl)	6.9	
Carrageenan + Buffer + 0.5% NaCl + 7% Sorbitol	0.8	0.7; 1.0
Carrageenan + 2.3% NaCl	2.3	1.5; 3.1
Fold Change (Carrageenan + Buffer + 0.5% NaCl + 7% Sorbitol) vs (Carrageenan + 2.3% NaCl)	2.9	
hCoV OC43		
Carrageenan + 0.5% NaCl	0.007	N.a.
Carrageenan + 0.9% NaCl	0.007	N.a.
Carrageenan + 2.0% NaCl	0.080	N.a.
Carrageenan + 2.3% NaCl	0.080	N.a.
Carrageenan + 0.5% NaCl + 7% sorbitol	0.007	N.a.
Fold Change (Carrageenan + 0.5% NaCl) vs (Carrageenan + 2.3% NaCl)	11.4	
Fold Change (Carrageenan + 0.5% NaCl + 7% Sorbitol) vs (Carrageenan + 2.3% NaCl)	11.4	

Note: IC_{50} and MIC of the various formulations determined in a virus inhibition assay (two independent experiments each for HRV1a and HRV8) and a hemagglutination inhibition assay (for hCoV OC43), respectively.

Abbreviations: IC_{50}, inhibitory concentration neutralizing 50% of the virus; MIC, minimal inhibitory concentration inhibiting agglutination; CI, confidence interval; NaCl, sodium chloride; Carrageenan, 1.2 mg/mL iota-carrageenan and 0.4 mg/mL iota-carrageenan; n.a, not applicable.

saline solutions with concentrations of 2.3–3% salt. This formulation was then used for in vitro and ex vivo experiments as well as for the clinical study.

Ex vivo experiments showed that incubation of nasal porcine mucosa with CS or a 2.4% saline solution of similar osmolality withdrew considerable amounts of liquid from the mucosa, resulting in a weight loss of 21±5% and 14±8%, respectively. In comparison, the weight of the mucosa incubated with carrageenan in 0.5% NaCl remained equal (weight change of 1±6%), indicating that the hyperosmolality alone, and not the carrageenan, is responsible for the weight loss

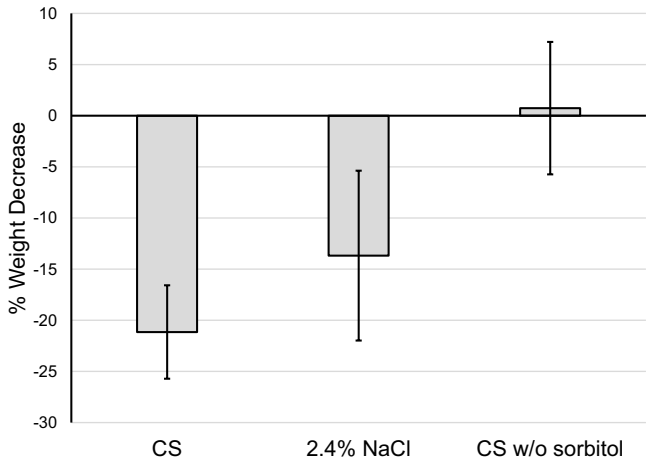

Figure 1 Ex vivo assay – Hyperosmolar effect of CS nasal spray with and without sorbitol. Weight decrease of ex-vivo porcine nasal mucosa after incubation for 1 h at 37°C in CS (carrageenan + 0.5% NaCl + 7% sorbitol in buffered aqueous solution), a 2.4% NaCl solution, or carrageenan + 0.5% NaCl in buffered aqueous solution without sorbitol (CS w/o sorbitol). Error bars represent SD of replicates.

(Figure 1). These results demonstrate a beneficial effect of sorbitol when added to the CS that could support nasal decongestion via its water draining properties.

A proof of principle for the barrier function of carrageenan in the formulation containing 7% sorbitol and 0.5% NaCl was demonstrated by a vertical in vitro barrier assay. The assay is based on previously reported protocols,[26,27] where fluorescent particles were used to evaluate the ability of a sample solution to inhibit diffusion of particulate matter into an agar block. As shown in Figure 2, CS nasal spray exhibited a blocking activity of 99±0% for particles of 0.3 µm diameter, and of 80±2% for particles of 1.0 µm diameter, allowing only 1% and 20%, respectively, of particles to reach the agar block, compared to the negative control. This indicates that the nasal spray can provide protection against external particles that might trigger or worsen allergic reactions.

The potential of the CS to alleviate nasal congestion in humans was examined in a clinical study in patients with allergic rhinitis. Figure 3, Panel A gives a graphical overview of the study, Panel B depicts the assessments carried out during each treatment block. Between September and October 2020, a total of 46 participants were screened after giving informed consent and were included in the safety population. Forty-one participants fulfilled all in/exclusion criteria,

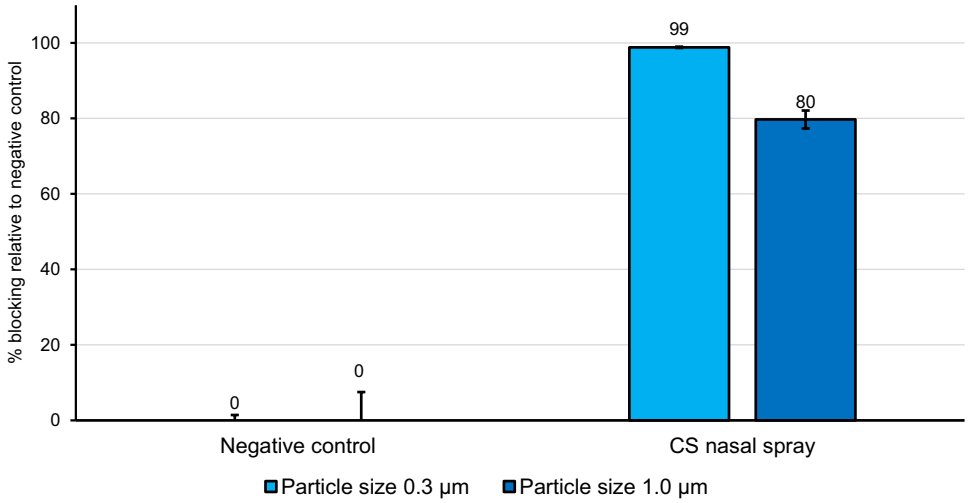

Figure 2 In vitro assay – Barrier function of CS nasal spray. Results of the percentage blocking activity of CS nasal spray relative to negative control (contains sorbitol and NaCl in same concentration as in CS but does not contain the barrier forming component carrageenan). Amounts of barrier-crossing particles were analyzed 3 h after application of beads. Cyan = % blocking activity for particle size of 0.3 µm; blue = % blocking activity for particle size of 1.0 µm. Error bars represent SD of replicates.

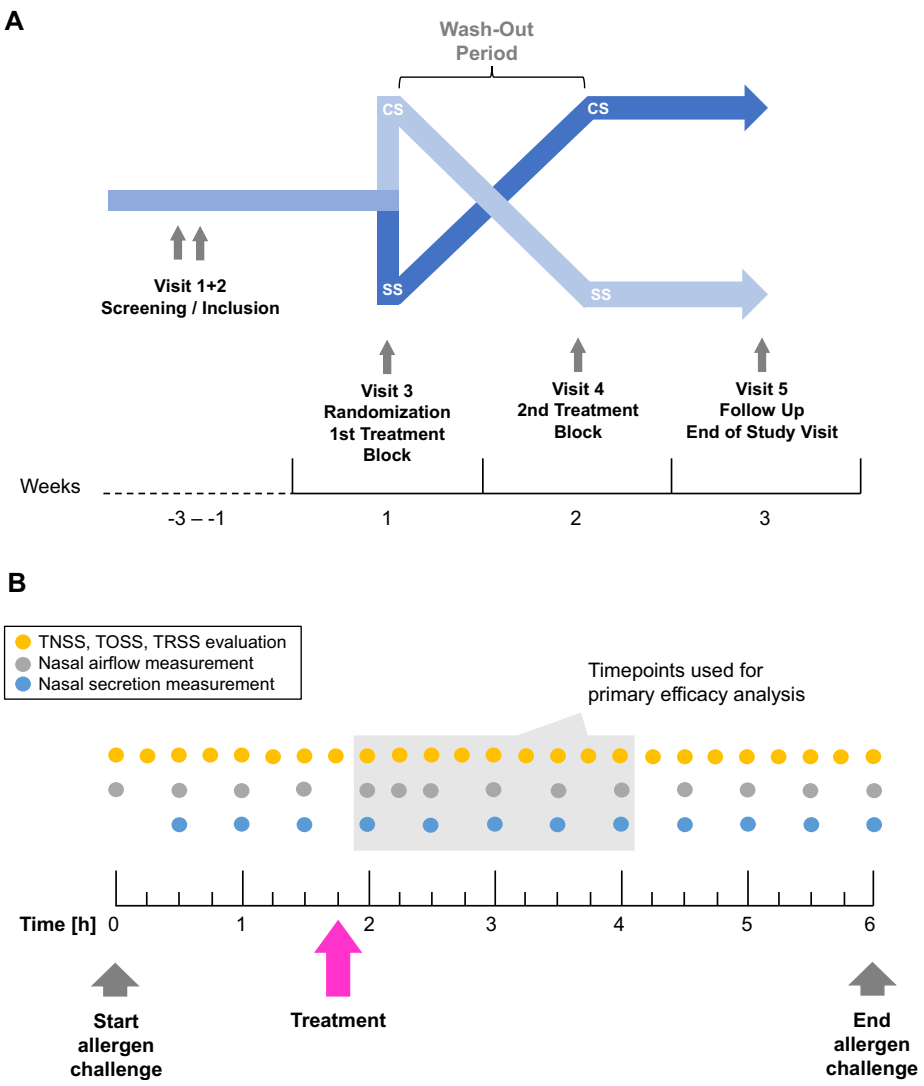

Figure 3 Clinical study – Graphical abstract. Panel (**A**) Study overview. Panel (**B**) Efficacy assessments carried out per treatment block.
Abbreviations: CS, carrageenan- and sorbitol-containing nasal spray; SS, saline solution; TNSS, total nasal symptom score; TOSS, total ocular symptom score; TRSS, total respiratory symptom score.

were randomized to one of the two possible treatment sequences, and hence constitute the FAS. Two participants missed visit 4 due to AEs. Four participants did not respond to either treatment with CS or SS and were excluded from the PPS based on the finding that hypertonic saline nasal spray has no effect on nasal congestion in approximately 30% of the population.[18] No other exclusionary protocol deviations, including use of prohibited medication, were reported. Hence, 35 participants constitute the PPS. Figure 4 shows the flow of participants through the study.

Demographic characteristics are summarized in Table 2. 27/46 (59%) of the participants were females, 19/46 (41%) were males. Participants were aged between 21 and 62 years, with a mean age of 34.6 years (SD 10.9). The mean BMI was 23.9 kg/m^2, all participants' BMIs were below 30, ie, none of the participants were obese. All participants had a history of moderate to severe SAR to grass pollen with a prior duration of between 8 and 43 years, on average 23.5 years.

In the following, all efficacy results are shown for the FAS, analyzed by intention-to-treat. Results for the PPS were similar as for the FAS.

All participants developed nasal congestion upon the start of the allergen challenge. The mean NCSS increased notably after 15 min, continued to rise until the 1 h 45 min timepoint, and then decreased following the intake of either CS or SS

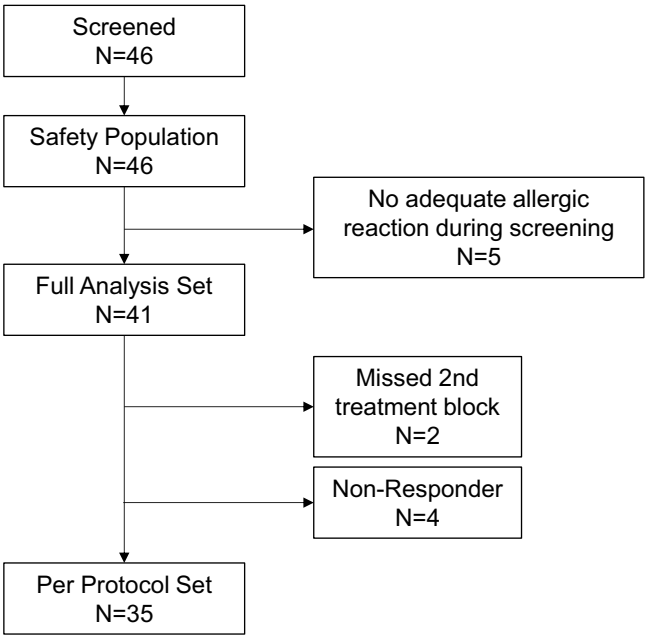

Figure 4 Clinical study – CONSORT flow chart.

(Figure 5A). The overall mean NCSS was 0.1 (SD 0.3) before starting the allergen challenge (timepoint 0 h 0 min), and it increased to 2.3 (SD 0.7) after CS treatment and to 2.2 (SD 0.5) after SS treatment at timepoint 1 h 45 min (Supplementary Table S1). However, only a small difference of 0.16 (SD 0.50) for CS and 0.11 (SD 0.53) for SS between pre-treatment NCSS (timepoint 1 h 45 min, ie, directly before the treatment), and the mean NCSS across the time interval 2–4 h could be detected (Supplementary Table S2). No phase-effect (p-value > 0.05, Wilcoxon test) and no carry-over effect (p-value > 0.05, ANOVA) was observed. The mean difference between CS [Pre-treatment - ø (2–4 h)] and SS [Pre-treatment - ø (2–4 h)] across all participants was 0.02, 95% CI [−0.19; 0.24], p > 0.05 (paired *t*-test) (Figure 5B). With the lower bound of the 95% CI < 0, superiority of CS versus SS in terms of NCSS could not be established.

Figure 6 shows the absolute nasal airflow in both treatments, measured before treatment (timepoint 1 h 30 min) and at the end of the allergen challenge period (6 h). Between these two timepoints, nasal airflow increased in CS-treated, but decreased

Table 2 Clinical Study – Demographic Characteristics at Baseline (Safety Population)

		All participants (N=46)
Sex		
Female	N (%)	27 (59%)
Male	N (%)	19 (41%)
Ethnicity		
Caucasian	N (%)	28 (61%)
Not specified	N (%)	18 (39%)
Age	Years (min/max)	34.6 (21/62)
BMI	Kg/m^2 (min/max)	23.9 (19.1/29.8)

Abbreviation: BMI, body mass index.

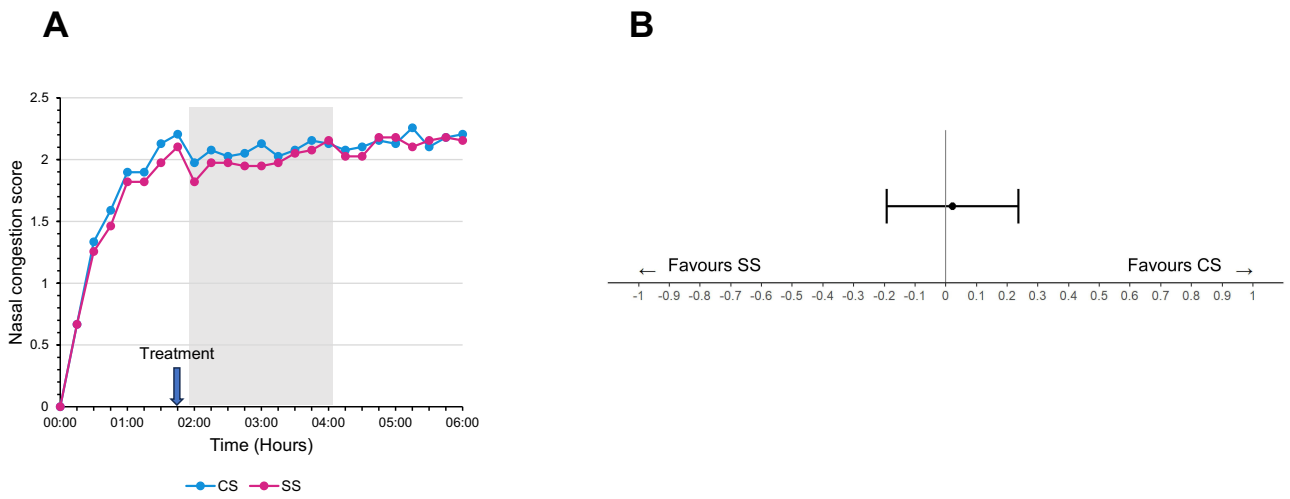

Figure 5 Clinical study – NCSS pre- and post-treatment during the grass pollen allergen exposure challenge for the FAS. Panel (**A**): Baseline corrected mean time course of NCSS. The gray square highlights the timepoints included in the primary efficacy analysis. (**B**): Primary efficacy analysis: Mean and 95% CI of the difference between treatments in terms of mean NCSS Δ [pre-treatment – ø (2–4 h)]) for the FAS. The mean difference of CS – SS = 0.02, 95% CI [−0.19;0.24], p > 0.05 (paired *t*-test).
Abbreviations: NCSS, Nasal Congestion Symptom Score; FAS, full analysis set; CS, carrageenan-sorbitol containing nasal spray; SS, saline solution; CI, confidence interval.

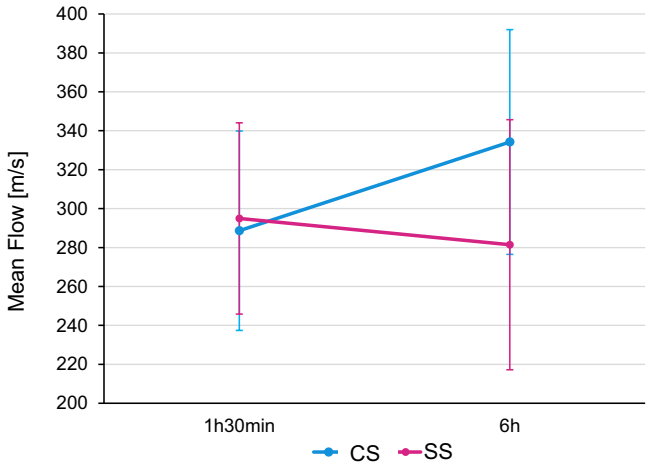

Figure 6 Clinical study – Anterior nasal airflow before and after treatment for the FAS. Mean airflow at timepoints 1 h 30 min (before treatment) and 6 h after start of allergen challenge. Error bars denote 95% CI. P=0.039 for comparison between treatments in difference from pre-treatment to timepoint 6 h.
Abbreviations: CS, carrageenan-sorbitol containing nasal spray; SS, saline solution; FAS, full analysis set; CI, confidence interval.

in SS-treated participants. In total, an improved anterior nasal airflow was measured in 23/38 (61%) of the participants following treatment with CS, compared to only 13/38 participants (34%) after SS treatment (Table 3). This difference between the treatments was statistically significant (p = 0.024, McNemar's test for paired nominal data).

Table 3 Clinical Study – Improvement/Worsening of Airflow After 6 h Compared to Pre-Treatment (1 h 30 min), Evaluated Within Treatment Groups for the FAS

		CS (6 h – 1 h 30 min)	
		Better or equal	**Worse**
SS (6 h – 1 h 30 min)	Better or equal	10	3
	Worse	13	12

Notes: p-value: 0.024 (McNemar's test for paired nominal data for comparison between treatments).
Abbreviations: FAS, full analysis set; CS, carrageenan-sorbitol containing nasal spray; SS, saline solution.

Table 4 Clinical Study – Tissue Weight Differences Between Pre-Treatment (Timepoint 1 h 30 min) and the Mean of All Post-Treatment Timepoints (2–6 h) for the FAS

Treatment	Mean Weight [g] ± SD			p-value
	Pre-treatment	Post-treatment	Difference post - pre	
CS	3.99 ± 3.24	2.99 ± 2.16	−1.00 ± 1.96	0.003*
SS	3.07 ± 2.59	2.57 ± 1.87	−0.50 ± 1.70	0.137**

Notes: Pre-treatment = mean at timepoint 1 h 30 min. Post-treatment = mean of all timepoints from 2-6 h. *t-test (Normality assumption confirmed). ** Wilcoxon signed rank test (Normality assumption rejected).
Abbreviations: CS, carrageenan-sorbitol containing nasal spray; SS, saline solution; SD, standard deviation.

In order to unravel the temporal dynamics that led to the post-treatment differences, we also followed nasal airflow changes over time by subtracting the mean pre-treatment value (timepoint 1 h 30 min) from the mean post-treatment value of varying post-treatment periods. Positive values indicate higher nasal airflow post-treatment compared to pre-treatment. As shown in Supplementary Figure S1, treatment with the CS led to an increase of nasal airflow over the course of the 4 h remaining observation time compared to pre-treatment, while it declined in the SS group. This led to a significantly higher airflow in the CS group compared to the SS group at the end of the 6 h treatment block. The difference between CS and SS in nasal airflow change from pre-treatment to the end of the 6 h treatment block in the FAS population was 54.29 mL/s (95% CI 2.92; 105.66). The difference was significantly in favor of the CS (p = 0.04, paired *t*-test) (Supplementary Table S3).

Changes in nasal secretion from pre- to post-treatment were calculated in an analogous manner. Nasal secretion declined in both groups post-treatment when compared to pre-treatment; however, the decline was stronger after CS than after SS treatment. For the CS, the weight of nasal secretion changed from 3.99 g during pre-treatment to 2.99 g averaged over the remaining observation time (2–6 h), representing a mean tissue weight difference of −1.00 g or −25% (p = 0.003, *t*-Test). After SS, the mean tissue weight difference from pre-treatment to the mean of the 2–6 h post-treatment period was only −0.50 g (p = 0.137, Wilcoxon signed rank test) (Table 4). Figure 7 shows the mean and 95% CI for the nasal secretion weight difference in gram between CS [pre-treatment – post-treatment] and SS [pre-treatment – post-

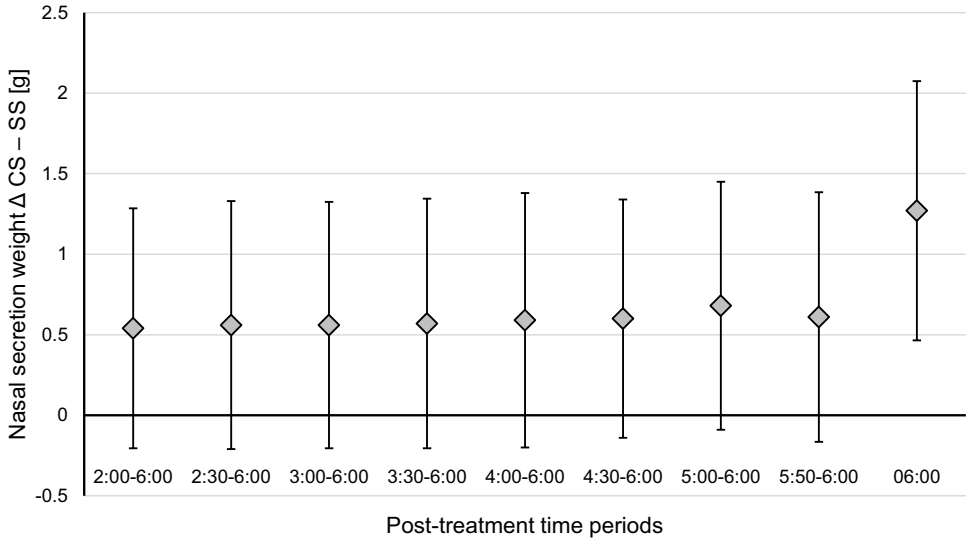

Figure 7 Clinical study – Mean and 95% CI of the difference between treatments in terms of nasal secretion weight change from pre-treatment to post-treatment for the indicated post-treatment periods for the FAS. Mean difference and 95% CI are given in gram (g). Error bars indicate the 95% CI.
Abbreviations: CS, carrageenan-sorbitol containing nasal spray; SS, saline solution; FAS, full analysis set; CI, confidence interval.

Table 5 Clinical Study – Adverse Events by System Organ Class/Preferred Term and Severity for the Safety Population (N=46).

SOC	PT	Mild	Moderate	Severe	Total
General disorders and administration site conditions		**1**	**0**	**0**	**1**
	Pyrexia	1	0	0	1
Infections and infestations		**0**	**1**	**1**	**2**
	Nasopharyngitis	0	1	0	1
	Pharyngitis	0	0	1	1

Notes: Bolded values indicate numbers of AEs in each severity category for the respective SOC, total of all PTs. Regular font indicates numbers of AEs in each severity category for the respective PT.
Abbreviations: SOC, system organ class; PT, preferred term.

treatment] for the indicated post-treatment periods. The difference between treatments becomes significant at the end of the observation period (6 h 0 min; lower bound of the 95% CI > 0).

TNSS, TOSS and TRSS over the 6 h treatment block did not show any pronounced differences between CS and SS group (data not shown).

In the safety population, a total of 3 AEs occurred in 2 participants during the trial: pyrexia (mild), nasopharyngitis (moderate) and pharyngitis (severe) (Table 5). Pharyngitis and pyrexia occurred in the same participants 4 days after the first treatment block with SS. Nasopharyngitis occurred 4 days after the first treatment block with CS. None of the AEs were considered related to the study treatment, none were classified as serious, and all were resolved by the end of the study. Both participants missed the second treatment block due to these AEs.

Vital signs and laboratory values showed no relevant differences between baseline and follow-up visit (data not shown), indicating good tolerability of both allergen challenge and treatment with CS and SS.

Discussion

This paper includes preclinical and clinical data demonstrating the safety and efficacy of a carrageenan- and sorbitol-containing (CS) nasal spray. The in vitro and ex vivo data indicate that the formulation is osmotically active while preserving the barrier-forming, virus- and particle-blocking capacity of the carrageenan. The results of the barrier assay suggest a tendency to better blockage of smaller particles; however, other parameters such as shape, charge or density might also contribute to differences in blocking effectiveness. We have previously shown the prophylactic anti-allergic clinical effectiveness mediated by this barrier function.[16] In contrast to the previous study, the present study primarily aimed to show the decongestant effect of the hyperosmotic formulation. Therefore, CS was applied 1 h 45 min after start of the allergen challenge, ie, when congestion had already fully developed. The clinical data show that the CS nasal spray is safe and well tolerable in participants with moderate-to-severe SAR. Although the primary endpoint based on the subjective rating of nasal congestion was not met, two objective parameters, nasal airflow and nasal secretion, showed a significant improvement upon treatment with CS nasal spray. Nasal airflow increased upon CS administration, but decreased upon administration of saline solution, leading to a significantly higher airflow in CS treated participants at the end of the challenge. The majority (61%) of participants had an increased nasal airflow after CS, but only 34% had an increased nasal airflow after SS administration. The amount of nasal secretion was reduced both after CS and SS administration, but this reduction was significant only after the CS. The low incidence of AEs, none of them considered treatment-related, suggested safety of CS nasal spray similar to saline solution used in this study and similar to carrageenan-only (no sorbitol) nasal spray as demonstrated in previous studies.[11,13–16,28,29]

The beneficial effect of the CS nasal spray is presumably achieved via multiple modes of action attributed to carrageenan and sorbitol. First, carrageenan has excellent mucoadhesive properties that are eg exploited for intranasal drug delivery.[30] We hypothesize that a hyperosmolar mucoadhesive layer of carrageenan allows a more sustainable drainage of water from the mucosa than other, carrageenan-free hyper-osmotic nasal sprays. Furthermore, it forms a protective barrier in the nasal mucosa

that prevents small particles like pollen and dust from entering the nasal mucosa and hinders further induction or aggravation of AR symptoms like nasal congestion and nasal secretion.[12] Second, polyols like sorbitol are known and widely used as humectants in the cosmetics and food industry based on their hygroscopic properties.[31] In the context of rhinitis, xylitol, another polyol with similar properties as sorbitol, was shown to keep the nasal passages and sinuses moist and clean for a longer time than saline alone. Five-days-use of a hyperosmolar xylitol-containing nasal spray led to a significant improvement of the overall QOL score compared to pre-treatment in participants suffering from nasal obstruction.[17] Moreover, a xylitol solution was as effective in rats as the glucocorticoid mometasone in the reversal of histopathological changes caused by long-term treatment with oxymetazoline.[32]

Strengths of this study include the cross-over design, in which each participant serves as their own control, the random assignment to minimize possible effects from the order of treatments, and the blinding of investigators, site personnel, and the sponsor's staff. Another strength is the use of an environmental challenge chamber to induce AR symptoms, which allows to control environmental conditions like temperature, humidity, and allergen type and concentration, and thus enables the performance of allergology studies out of allergy season and under uniform allergen exposure conditions. This limits variation and helps reduce the number of study participants. Moreover, use of the challenge chamber allows the study personnel to supervise the administration of medication and documentation of outcomes, thereby enhancing participant compliance.[33–39]

This study used the NCSS, a subjective scoring scale, as primary endpoint. The rationale for the selection of the primary endpoint was that nasal congestion comes with a significant impact upon patients' QOL, which is considered an important determinant of the severity of nasal diseases.[40,41] In fact, the degree of health-related QOL impairment has been demonstrated to drive patients' choice between treatment options.[42] Assessment of QOL in the form of patient reported outcome measures (PROMs) is regarded a standard outcome measure in clinical trials, acknowledging the fact that the classical, objective outcome variables may only partially characterize the disease of the patient. However, the focus on a PROM as primary endpoint also poses problems due to the low degree of correlation between subjective and objective outcomes assessing nasal symptoms, as systematically reviewed by Ta et al.[43] The authors consequently recommend using objective outcome measures to complement and confirm validated patient reported outcomes,[43] as we have done in the secondary endpoints.

The findings of our study support this conclusion, showing discrepancies between subjective and objective evaluations. As described in the results section, only very slight differences between groups and between timepoints were observed by NCSS that may reach significance only with a much larger sample size. In contrast, differences between CS and SS in nasal airflow improvement measured by AAR became significant towards the end of the allergen challenge, indicating that this sensitive method is able to pick up subtle changes that cannot possibly be detected by PROMs like the NCSS with the available number of participants. Rhinomanometry enables the objective and accurate measurement of nasal congestion, and is considered the gold standard for measuring nasal airway patency and resistance.[44] The method has been demonstrated to be sensitive in quantifying nasal patency after nasal provocation testing and to assess the efficacy of medications used to treat nasal congestion/obstruction.[45] The implementation of rhinomanometry as objective endpoint in addition to the subjective symptom scores is therefore a particular upside of this study. Analogously, objective determination of nasal secretion revealed a significant reduction after treatment compared to pre-treatment, which was not captured by the TNSS with sufficient sensitivity.

In this study, the analysis of the primary endpoint was based on the time window from 2 to 4 h after the start of allergen exposure, that is, starting 15 min after treatment administration and ending 2 h 15 min after treatment administration. This interval was selected based on the expectation that the most pronounced effect of the treatment would manifest shortly thereafter. The mean residence time of carrageenan at the mucosa of approximately 4 hours was determined in a prior study using nasal mucociliary clearance time assessment in healthy volunteers,[10] and we expected the most pronounced effect to manifest in the first half of this period. However, nasal airflow continuously increased from post-treatment until the end of the allergen challenge period.

In sum, based on our findings, we propose the CS as a meaningful adjuvant to baseline pharmacological treatments.

Conclusion

Coldamaris akut, a carrageenan- and sorbitol containing nasal spray, is a safe treatment option for adults with grass pollen allergy. Even though the primary, subjective efficacy endpoint was missed based on the available data, symptom relief was demonstrated based on objective endpoints.

Data Sharing Statement

The data supporting the findings of this study are available upon reasonable request from the corresponding author for 1 year after publication of this paper. The dataset includes experimental data collected during the in vitro and ex vivo experiments presented in this paper, individual de-identified participant data collected during the clinical study described in this paper, as well as the synopse of the clinical investigation report. Due to legitimate commercial interests, the data cannot be made publicly available in a repository. However, we encourage interested researchers to contact us for access to the data, which will be shared in accordance with ethical guidelines and company policies.

Ethics Statement

The study was conducted in Austria in accordance with the Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects, the International Council for Harmonisation Guideline on Good Clinical Practice, and all applicable local regulatory requirements and laws. The study was approved by the Ethics Committee of the City of Vienna (protocol code COA_19_03, EK 19/277/1219). Informed consent was obtained from all study participants.

Acknowledgments

This paper has been uploaded to medRXiV as a preprint: https://www.medrxiv.org/content/10.1101/2024.05.03.24306805v1. This research was sponsored by Marinomed Biotech AG.

Disclosure

NUM, MMK, HD, AR, PG, CK, CS, MKS and EPG are employees of Marinomed Biotech AG. CG is a former employee of Marinomed Biotech AG. MS received consulting fees from Marinomed Biotech AG. In addition, Dr Martina Morokutti-Kurz, Ms Christiane Koller, and Dr Eva Prieschl-Grassauer report a patent WO2017009351. The other authors have no competing interests in this work.

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