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Nasal corticosteroid treatment reduces substance P levels in tear fluid in allergic rhinoconjunctivitis

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ARTICLE INFO

Article history:

Received for publication February 17, 2012.

Received in revised form May 22, 2012.

Accepted for publication June 8, 2012.

ABSTRACT

Background: The mechanisms underlying conjunctival symptom reduction by nasal corticosteroids in allergic rhinoconjunctivitis are unknown. A naso-ocular reflex may be present.

Objective: To study the effects of nasal fluticasone furoate (FF) on conjunctival symptoms and substance P and histamine levels in tear fluid after nasal grass pollen provocation (GPP).

Methods: A double-blind placebo-controlled study was performed in 26 grass pollen-allergic patients. A selective GPP was performed during the grass pollen season after 2 weeks of FF or placebo treatment. Nasal and conjunctival symptoms were scored using a visual analog scale (VAS), and tear fluid was collected for measuring substance P and histamine using an enzyme-linked immunosorbent assay.

Results: Compared with placebo, FF reduced conjunctival symptom scores during the pollen season (-1.75 [$-2.75, 0.20$] vs 0.0 [$0.0, 0.0$]; $P = .01$) and after GPP at 15 minutes (0.05 [$-0.42, 1.52$] vs 2.05 [$0.62, 3.62$]; $P < .001$) and 1 hour (-0.45 [$-1.75, 0.1$] vs 0.05 [$-0.97, 1.85$]; $P < .01$). Treatment with FF decreased substance P levels in tear fluid (44.11 [$32.81, 61.02$] vs 65.26 [$48.62, 79.73$] pg/mg protein; $P = .0098$). Histamine levels in tear fluid showed a GPP-induced increase in the placebo group (7.26 [$3.12, 9.69$] vs 5.71 [$2.05, 7.00$] ng/mg protein; $P = .02$), but not in the FF group (6.77 [$3.43, 13.00$] vs 5.24 [$3.18, 7.06$] ng/mg protein; $P = .08$).

Conclusion: FF nasal spray reduced conjunctival symptoms in grass pollen-allergic patients in parallel with lower substance P levels in tear fluid. These data help in understanding the reduction of conjunctival symptoms by intranasal anti-inflammatory therapy.

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Introduction

The naso-ocular interaction in allergic rhinoconjunctivitis is well recognized from an epidemiological point of view, with up to 70% of patients with allergic rhinitis suffering from conjunctival symptoms.¹ In practice, conjunctival symptoms are often overlooked, as evidenced by the diagnostic neglect of conjunctival symptoms in patients with allergic rhinitis.^{2,3} However, 15 % of patients suffering from allergic rhinoconjunctivitis state that their

ocular symptoms are the most bothersome of their condition,⁴ and 70% of allergic patients report their conjunctival symptoms are at least as severe as their rhinitis symptoms.³ We recently reported that nasal instillation of grass pollen leads to conjunctival hyperemia, itching, or tearing in allergic individuals along with the classical nasal symptoms.⁵ Mediators such as substance P and histamine were most likely involved, because their tear fluid levels correlated with the severity of conjunctival symptoms and inversely correlated with nasal flow after nasal grass pollen provocation (GPP).⁵ Furthermore, a unilateral nasal allergen provocation had been shown to result in nasal histamine release and bilateral ocular pruritus and lacrimation.⁶ As a consequence, one may speculate that allergic inflammation in the nose contributes to the conjunctival immune response in patients with rhinoconjunctivitis. A proof of this concept was provided by a study in which prevention of nasal allergen deposition by nasal filters reduced not only nasal but also conjunctival symptoms in patients with allergic rhinoconjunctivitis.⁷

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Disclosures: Authors have nothing to disclose.

Funding Sources: E.V. and V.H. are the recipients of a PhD fellowship of the Fonds voor Wetenschappelijk Onderzoek (F.W.O.) Vlaanderen. I.S. and P.H. are both fundamental clinical researchers of the F.W.O. Vlaanderen. S.B. was the recipient of a PhD fellowship from the Research Council of the KU Leuven. This work was supported by an unrestricted educational grant by GlaxoSmithKline.

Effective treatment options are available for allergic conjunctivitis, but better symptom control is needed in a subgroup of patients with severe symptoms.^{8–13} Recent studies demonstrated beneficial effects of intranasal steroids on conjunctival inflammation in allergic rhinoconjunctivitis.^{2,8–11} However, the potential mechanisms linking anti-inflammatory nasal treatment to a reduction of conjunctival symptoms need to be investigated, because it is unclear how intranasal corticosteroids may reduce conjunctival symptoms in patients with allergic rhinoconjunctivitis, and what mediators are involved in the therapeutic effects. To build further on the hypothesis that a naso-ocular reflex mechanism involving substance P is involved, we studied the effects of a nasal corticosteroid spray (fluticasone furoate [FF]) on conjunctival symptoms and neuro-inflammatory mediators such as substance P and histamine in tear fluid during the grass pollen season and after a nasal GPP.

Methods

Patient population

Twenty-six grass pollen-allergic patients with rhinoconjunctivitis symptoms during the last 2 grass pollen seasons were recruited before the pollen season of 2010 and asked to participate in a double-blind placebo-controlled study at the Department of Otorhinolaryngology, Head and Neck Surgery of the University Hospitals Leuven. An informed consent was signed before enrollment, and grass pollen sensitization was confirmed with a standard skin prick test. A nasal GPP was performed at baseline (week 0, Fig 1), and patients were included if they showed an induction of nasal and conjunctival symptoms with a visual analog scale (VAS) score of 5 or greater for at least 2 of the nasal and conjunctival symptoms, concomitant with a drop in peak nasal inspiratory flow (PNIF) values of more than 30% 15 minutes after GPP.^{14–16}

The following exclusion criteria were used: present or past wearing of contact lenses, ocular pathological condition or current ocular treatment, and asthma. Other exclusion criteria were the use of nasal or oral steroid treatment for 6 weeks or less and the use of nasal or oral antihistamine treatment for 4 weeks or less before the study, the use of leukotriene receptor antagonists for 6 weeks or less before the study, and past or ongoing immunotherapy for grass pollen.

Study design

Nasal provocations were performed during the grass pollen season to evaluate the effects of FF or placebo nasal spray on GPP-induced symptoms and mediators. At the start of the grass pollen season (visit 0), patients underwent a GPP with mixed grass pollen at 333 AU/mL (HAL Allergy, Leiden, The Netherlands) as described previously.⁵ Using a micropipette, 250 μ L of the provo-

cation solution was equally applied onto the nasal mucosa of the medial and lateral wall of both nostrils.⁵ Direct contact of the pipette with the nasal mucosa was avoided to exclude mechanical stimulation. Analyses were carried out before and at 15 minutes, 1 hour, and 24 hours after the GPP (Fig 1). The following parameters were investigated: PNIF, nasal and conjunctival symptoms, and histamine and substance P levels in tear fluid. Patients were randomly ($n = 13$ per group) assigned to receive either FF or placebo nasal spray. Twenty-four hours after the first GPP on visit 0, patients received treatment with FF nasal spray 110 μ g once daily or a placebo nasal spray once daily for 2 weeks.

After 1 week (visit 1), patients were asked to attend the outpatient clinic for evaluation of symptom severity and evaluation of compliance to treatment and correct medication use. A second GPP was performed at 2 weeks (visit 2), and the same parameters were evaluated as during visit 0.

The experimental protocol was approved by the Medical Ethics Committee of the University Hospital of Leuven.

Grass pollen counts

To schedule the treatment at the beginning of the grass pollen season of 2010, grass pollen counts in the air by the Belgian Scientific Institute of Public Health were consulted. The presence of grass pollen in the air was evaluated by a "Burkard Volumetric Spore Sampler" unit, collecting grass pollen in the air. Air is sucked into the sampler through a calibrated slit and is captured on a prepared adhesive tape at a set rate corresponding to the human respiration rate (10 L/minute). Results of these measurement were published daily on the website www.airallergy.be, and this was the guide to call patients for visit 0.

Evaluation of nasal and conjunctival symptoms

Major nasal and conjunctival symptoms were evaluated using a VAS score, with 0 indicating no symptom at all, and 10 indicating the most severe symptom. The following 4 nasal symptoms were asked for: sneezing, runny, blocked, and itchy nose. The 2 conjunctival symptoms comprised ocular pruritus and lacrimation. Total nasal and conjunctival symptoms were calculated. All values were expressed as mean change from the baseline, before the GPP.

Peak nasal inspiratory flow (PNIF)

Nasal congestion was evaluated by the measuring the PNIF, using the PNIF device of Clement Clark International, Essex, United Kingdom.⁴ PNIF is a valid means of evaluation of nasal air flow through the nose.¹⁷ Nasal peak flow has been shown to correlate well with the subjective feeling of obstruction¹⁸ and is a user-friendly and rapid technique for monitoring nasal flow in clinical

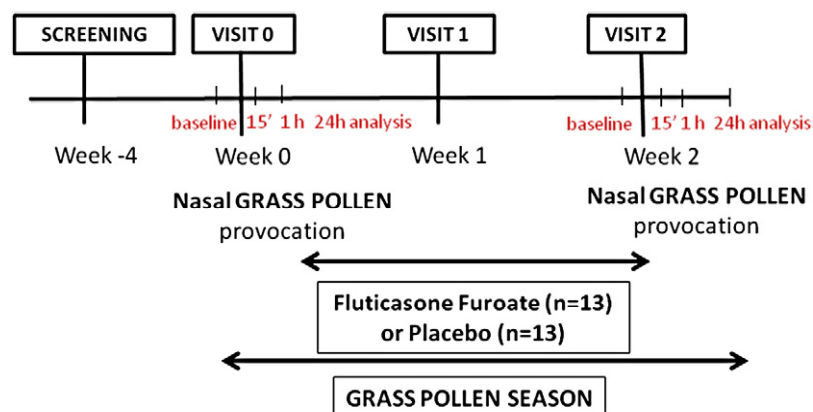


Fig. 1. Study design with different time points of the course of the study and start and stop of FF or placebo treatment.

trials and after nasal provocation. An anesthesia mask was placed over the mouth and nose of the patients after expiration, and patients were asked to close their lips and forcefully inspire air through the nose. The best value out of 3 consecutive measurements with a maximal inter-measurement variability of 10% was used as the objective evaluation of nasal peak flow.

Collection of tear fluid and measurement of histamine and substance P levels

Tear fluid was collected from the left ocular surface using paper Schirmer strips (Haag-Streit, Harlow, Essex, United Kingdom) as described previously.⁵ A Schirmer strip was placed at the level of the inferior palpebral conjunctiva, and the subject was asked to close the eyes for the duration of the collection. This technique is painless and atraumatic for the patient and leads to the rapid collection of approximately 30 μ L of tear fluid without the need for local anesthesia. The Schirmer strip with the tear fluid was then removed and placed in an Eppendorf tube and incubated for 24 h with 300 μ L of saline at 4°C for elution of the secretions. After 24 hours, the tubes were vortexed, the Schirmer strips were squeezed to the bottom of the tubes, and the supernatants were aliquoted to Eppendorf tubes and frozen at -20°C for subsequent analysis. Histamine levels in the collected tear fluid were measured using a commercially available competitive enzyme-linked immunosorbent assay sensitive to 1 ng/mL (Labor Diagnostike Nord GmbH & Co, Nordhorn, Germany). Substance P levels were measured in tear fluid by using a commercially available competitive enzyme-linked immunosorbent assay sensitive to 3.9 pg/mL (Cayman, Michigan). All measured concentrations were divided by the protein content measured in the tear fluid at every time point of each individual patient to exclude differences attributable to dilution.

Statistical analysis

All statistical analyses were performed with the GRAPH PAD PRISM program (Prism 4, version 4.03, 1992–2005 Graph-Pad Software Inc., San Diego, California). Comparisons between the different time points before and after nasal provocation were calculated using a nonparametric paired Wilcoxon ranked test or using a paired *t* test, depending on normality of the data. Comparisons between the different groups were made by a nonparametric Mann-Whitney test or a *t* test. Correlations were calculated using a Spearman coefficient. A difference was considered to be significant when $P < .05$. Data are represented as means $\pm$ standard error of the mean in case of normality, or when nonparametric statistics were used as medians (25%, 75%).

Results

Patient characteristics

Patients in both study arms were matched for age (placebo: 24.9 ± 1.9 years and FF: 24.7 ± 0.7 years) and 12 out of 13 patients in each group showed poly-sensitization, with house dust mite (placebo: 9/13 and FF: 8/13) and birch (placebo: 7/13 and FF: 8/13) being the most prominent allergens. All patients showed an induction of nasal and conjunctival symptoms after the GPP at week 0 (VAS score of >5 for at least 2 of the nasal and conjunctival symptoms). In the placebo- and FF-treated groups, the male/female ratio was 9/13 and 4/13, respectively. No differences in symptoms and mediator levels in tear fluid were seen between males and females (data not shown). In the placebo group, 1 patient was not subjected to a second GPP because he suffered from a vagal reaction after the GPP at visit 0.

Grass pollen counts during the study period

The study was performed during the grass pollen season of 2010, with the timing depending on the grass pollen counts reported by the Belgian Scientific Institute of Public Health. The screening of the patients was performed several weeks before the start of the grass pollen season. Forty-six and 283 pollen grains per cubic meter were counted in weeks 21 and 22, respectively. Our study started in week 23, when 343 grains per cubic meter were counted. The study continued in weeks 24, 25, and 26 where 439, 444, and 490 grains/ m^3 , respectively, were recorded. After the study, in week 27, 293 grains/ m^3 and in week 28, 45 grains/ m^3 were counted, indicating that this study took place during the peak of the grass pollen season.

Effects of FF nasal spray on total conjunctival and nasal symptoms during the grass pollen season

In the FF-treated group, patients showed lower conjunctival VAS scores at 1 week ($-1.75 [-2.75, 0.20]$ vs $0.0 [0.0, 0.0]$; $P = .01$) and at 2 weeks ($-1.85 [-3.80, 0.05]$ vs $0.0 [0.0, 0.0]$; $P < .01$) of treatment compared with baseline, and at 1 week ($-1.75 [-2.75, 0.20]$ vs $0.0 [-1.15, 1.30]$; $P = .04$) and 2 weeks ($-1.85 [-3.80, 0.05]$ vs $0.10 [-1.45, 1.25]$; $P = .01$) compared with placebo (Fig 2A).

In the FF-treated group, patients reported fewer nasal symptoms as reflected by lower VAS scores after 1 week ($-2.25 [-5.40, -0.45]$ vs $0.0 [0.0, 0.0]$; $P < .0001$) and 2 weeks ($-2.10 [-4.80, -0.40]$ vs $0.0 [0.0, 0.0]$; $P < .0001$) of treatment compared with baseline and at 1 week ($-2.25 [-5.40, -0.45]$ vs $0.10 [-1.47, 2.62]$; $P < .0001$) and 2 weeks ($-2.10 [-4.80, -0.40]$ vs $-0.30 [-2.02, 1.07]$; $P < .0001$) compared with the placebo group (Fig 2B). Patients in the placebo group showed no reduction in conjunctival

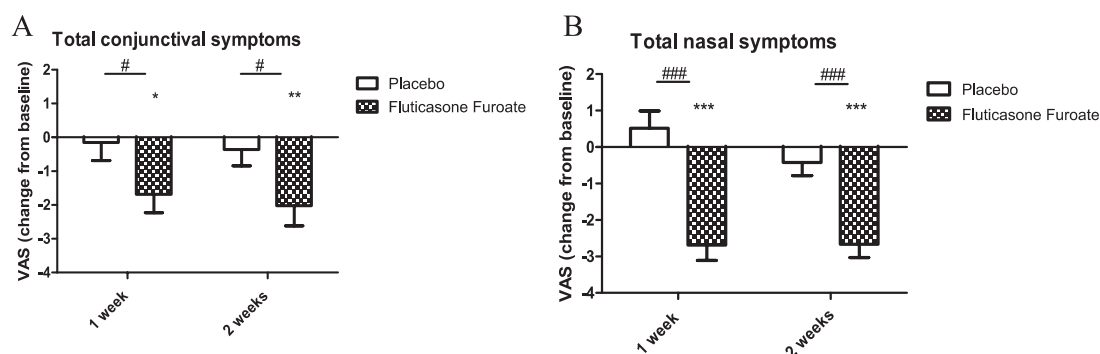


Fig. 2. Effects of placebo and fluticasone furoate treatment during the grass pollen season after 1 and 2 weeks of treatment on the total conjunctival (A) and total nasal (B) symptoms compared to baseline. * $P < .05$; ** $P < .01$; *** $P < .0001$; # $P < .05$; ### $P < .0001$.

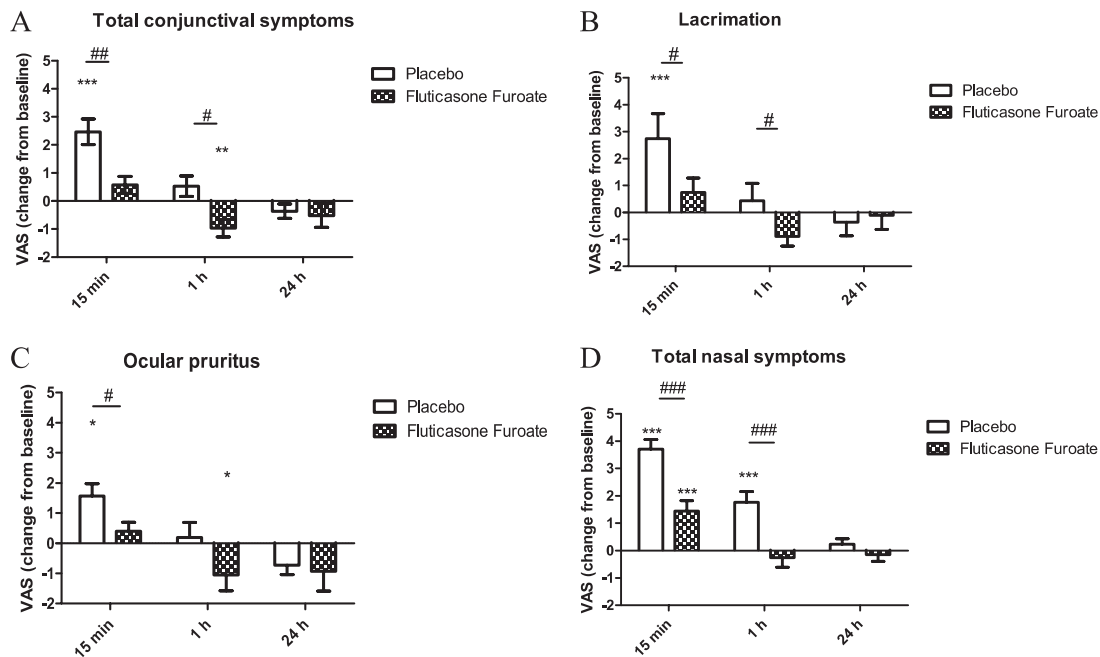


Fig. 3. The mean change from baseline for total conjunctival symptoms (A), lacrimation (B), ocular pruritus (C), and total nasal symptoms (D) were compared at every time point after a nasal grass pollen provocation, performed after 2 weeks of treatment with FF or placebo. * $P < .05$; ** $P < .01$; *** $P < .0001$; # $P < .05$; ## $P < .01$; ### $P < .0001$.

and nasal VAS scores after 1 week and 2 weeks of treatment compared with baseline (Fig 2A and B).

Effects of FF on the reduction of conjunctival and nasal symptoms after GPP

Fifteen minutes after GPP, patients in the placebo group had higher VAS scores for conjunctival symptoms compared with baseline (2.05 [0.62, 3.62] vs 0.0 [0.0, 0.0]; $P < .0001$), whereas this was not the case in the FF group (0.05 [−0.42, 1.52] vs 0.0 [0.0, 0.0]; $P = .12$). The VAS scores for conjunctival symptoms were lower in the FF-treated patients at 15 minutes (0.05 [−0.42, 1.52] vs 2.05 [0.62, 3.62]; $P = .0013$) and at 1 hour (−0.4 [−1.75, 0.77] vs 0.05 [−0.97, 1.85]; $P = .03$) after GPP compared with placebo (Fig 3A).

Patients in the placebo group showed higher VAS scores for lacrimation at 15 minutes after GPP compared with baseline (3.35 [0.67, 5.52] vs 0.0 [0.0, 0.0]; $P < .01$, Fig 3B). FF treatment reduced the VAS scores for lacrimation at 15 minutes (0.1 [−0.75, 2.45] vs 3.35 [0.67, 5.25]; $P = .0083$) and 1 hour (0.0 [−1.95, 0.1] vs 0.25 [−0.25, 2.75]; $P = .04$) after GPP compared with placebo treatment. Patients in the placebo group showed higher VAS scores for ocular pruritus 15 minutes after GPP compared with baseline (1.80 [0.40, 2.67] vs 0.0 [0.0, 0.0]; $P < .01$). FF treatment reduced VAS scores for ocular pruritus at 15 minutes (0.0 [−0.4, 1.35] vs 1.8 [0.4, 2.67]; $P = .03$) after GPP than placebo (Fig 3C).

Patients in the placebo group marked significantly higher nasal VAS scores at 15 minutes (3.40 [2.12, 4.37] vs 0.0 [0.0, 0.0]; $P < .0001$) and at 1 hour (1.55 [0.20, 3.32] vs 0.0 [0.0, 0.0] score; $P < .0001$) after a GPP compared with baseline. Patients in the FF group reported more nasal symptoms at 15 minutes after GPP compared with baseline (1.05 [0.0, 2.37] vs 0.0 [0.0, 0.0], $P < .0001$). However, FF treatment was associated with lower VAS scores for nasal symptoms at 15 minutes (1.05 [0.0, 2.37] vs 3.4 [2.12, 4.37]; $P < .0001$) and at 1 hour (0.0 [−0.92, 0.3] vs 1.55 [0.2, 3.32]; $P < .0001$) after GPP than placebo (Fig 3D).

Effects of FF nasal spray on PNIF after GPP

Grass pollen provocation induced a significant decrease in PNIF in the placebo group at 15 minutes and 1 hour after GPP (15

minutes: 52.50 ± 8.36 ; $P < .0001$ and 1 hour: 65.83 ± 11.31 ; $P < .01$) and in the FF group only at 15 minutes (96.92 ± 15.41 vs 153.8 ± 12.84 L/minute, $P < .0001$). Of note, higher PNIF values were found in the FF group at 15 minutes (96.92 ± 15.41 vs 52.50 ± 8.36 L/min; $P = .02$) and at 1 hour (133.5 ± 18.72 vs 65.83 ± 11.31 L/minute, $P < .01$) after GPP compared with placebo.

Effect of FF nasal spray on substance P and histamine levels in tear fluid after GPP

Nasal FF treatment was associated with lower substance P levels in tear fluid of FF-treated patients compared with placebo at 15 minutes after GPP (44.11 [32.81, 61.02] vs 65.26 [48.62, 79.73] pg/mg protein; $P = .0098$; Fig 4A). In the FF group, lower substance P levels were found at 15 minutes (44.11 [32.81, 61.02]; $P = .0005$), 1 hour (41.96 [26.64, 68.22]; $P = .0005$), and 24 hours (51.44 [32.06, 73.06] vs 63.25 [39.72, 91.08] pg/mg protein, $P = .0061$) after GPP compared with baseline (Fig 4A). In the placebo group, no significant changes in substance P levels were measured at 15 minutes (65.26 [48.62, 79.73] pg/mg protein, $P = .91$), 1 hour (58.75 [40.70, 93.08] pg/mg protein, $P = .67$) and 24 hours (71.50 [47.01, 86.29]; $P = .96$) after the GPP compared with baseline (67.09 [42.15, 110.6] pg/mg protein; Fig 4A). Although no increase in histamine levels were found at 15 minutes after GPP (6.20 [4.08, 8.81] vs 5.71 [2.05, 7.00] ng/mg protein; $P = .42$) and at 24 h (5.95 [2.62, 9.51] vs 5.71 [2.05, 7.00] ng/mg protein; $P = .20$) in the placebo group, significant higher levels were found at 1 hour (7.26 [3.12, 9.69] vs 5.71 [2.05, 7.00] ng/mg protein; $P = .02$) after GPP. Histamine levels did not show any alteration in the tear fluid of FF-treated patients at different time points after GPP (15 minutes: 5.49 [2.83, 10.45], $P = .08$; 1 hour: 6.77 [3.43, 13.00], $P = .08$; and 24 hours: 6.81 [3.17, 9.05] vs 5.24 [3.18, 7.06] ng/mg protein; $P = .11$) (Fig 4B).

After 2 weeks of FF and placebo treatment, a positive correlation was found between the substance P values and the VAS scores for ocular pruritus ($r = 0.40$, $P = .04$; Fig 5A). Additionally, a correlation between the substance P values and the VAS scores for lacrimation was found ($r = 0.35$, $P = .09$; Fig 5B) at 24 hours after the nasal GPP, reaching borderline significance.

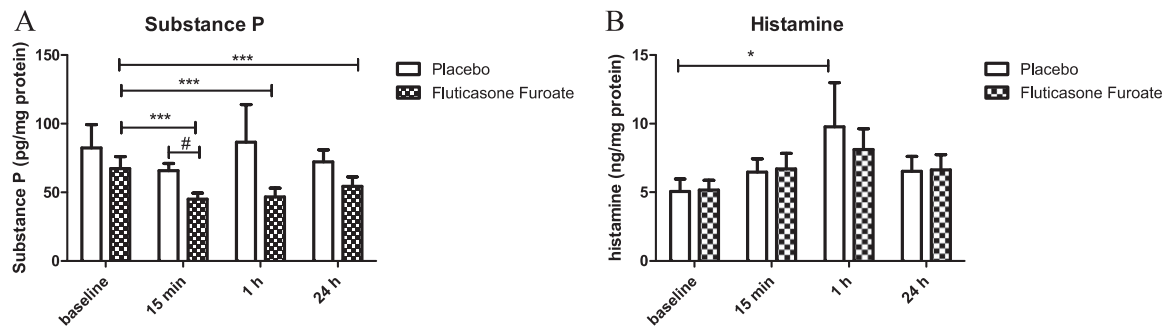


Fig. 4. Substance P (A) and histamine levels (B) in the tear fluid were compared at every time point after a nasal grass pollen provocation, performed after 2 weeks of treatment with FF or placebo. * $P < .05$; ** $P < .01$; *** $P < .0001$; # $P < .05$.

Discussion

In this study, we show an improvement of nasal and conjunctival symptoms after intranasal FF treatment in concordance with other pollen provocation studies.^{7–10} Moreover, we show for the first time that improvement in conjunctival symptoms by nasal FF therapy was associated with reduced levels of substance P in the tear fluid in allergic rhinoconjunctivitis during the grass pollen season and after a nasal GPP. So far, most studies on intranasal corticosteroids in allergic rhinoconjunctivitis have focused on nasal and conjunctival symptom reduction as the outcome parameter without evaluation of conjunctival mediators in relation to nasal treatment.^{8–11} The association between the reduction of conjunctival symptoms and lower levels of substance P in tear fluid suggests a potential role of substance P in the naso-ocular interaction in allergic rhinoconjunctivitis.

Substance P represents an important mediator in the neurogenic pathway involved in allergic inflammation and has already been reported to be present in the tear fluid of patients with allergic conjunctivitis and vernal conjunctivitis.^{18,19} Substance P has been shown to play an inflammatory role by enhancing cytokine secretion from and by being a chemoattractant for inflammatory cells.²⁰ As such, substance P can initiate an inflammatory cascade by the activation of mast cells, resulting in the release of histamine and chemokines such as regulated upon activation, normal T-cell expressed, and secreted and interleukin 8.²¹ The conjunctiva is highly innervated, and substance P as well as other neural mediators such as calcitonine-gene related peptide, vasoactive intestinal peptide, neuropeptide Y, and nerve growth factor are widespread in the conjunctiva.²² In contrast to substance P, levels of neurokinin A, neurokinin B, or vasoactive intestinal peptide were not detectable in the tear fluid after allergen provocation in our study (data not shown).

At present, we can only speculate on the precise mechanisms by which nasal FF spray reduced conjunctival substance P levels. In the light of the low systemic bioavailability of FF nasal spray,²³ we assume that the reduction in conjunctival symptoms and substance P levels are not the result of a systemic anti-inflammatory effect of FF. In general, nasal corticosteroids exert a potent anti-inflammatory effect with reduction of the immune response in the nose, resulting in less production of cytokines and infiltration of inflammatory cells in the upper airway mucosa.²⁴ These cells and mediators might influence afferent sensory nerve endings^{25,26} in the nose projecting to the trigeminal ganglion.²⁷ We speculate that reduced activation of these nasal sensory nerves will lead to reduced efferent responses via the efferent nerves innervating the conjunctival surface, resulting in a dampened conjunctival reaction with limited release of pro-inflammatory neural mediators such as substance P.²² Moreover, because of the anti-inflammatory effects of nasal corticosteroids and reduction of the local allergic response in the nose, the reduced number of inflammatory mediators and cells in the nose gives rise to fewer mediators and cells that can enter the systemic circulation and can home to other tissues, in this case the eyes.^{15,16} Additionally, corticosteroids can down-regulate tachykinin receptors and neuropeptide synthesis in neurons and in other immune cells,²⁸ and, they are able to up-regulate the synthesis of neuropeptide-degrading enzymes.²⁹ Although there is little or no systemic uptake of nasal corticosteroids, this might have an effect on the reduction of local inflammation in the nose.

In spite of the fact that human studies are less suitable to provide the ultimate proof of substance P being important for the development or severity of conjunctival symptoms, we here add evidence for an important role for substance P in allergic rhinoconjunctivitis. Besides the lower levels of substance P found in the tear fluid of FF-treated patients compared with placebo, positive corre-

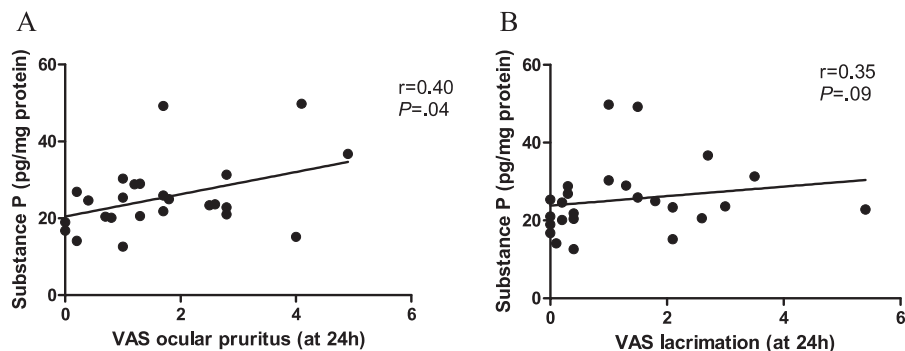


Fig. 5. Correlations between the substance P levels in tear fluid and the VAS scores reported for ocular pruritus (A) and the VAS scores reported for lacrimation (B) after 2 weeks of FF and placebo treatment and 24 h after a nasal grass pollen provocation.

lations were found between the substance P values in tear fluid and the VAS scores for ocular pruritus and lacrimation at 24 hours after GPP, indicating that lower conjunctival symptoms by nasal FF treatment are associated with lower substance P levels in tear fluid.

Although we did find increased levels of histamine in the tear fluid of the placebo group 1 hour after GPP compared with baseline, no differences in histamine levels in the tear fluid of the FF-treated patients were found at all time points after GPP. This observation can suggest a reduced mast cell activation at conjunctival level in the FF group; however, other mast cell mediators were not measured in this study. This observation is in line with the study performed by Baroody et al, in which no elevation of histamine and albumin was found in ocular secretions after a unilateral nasal provocation.⁵

In conclusion, FF nasal spray reduced conjunctival symptoms in grass pollen-allergic patients after a selective nasal grass pollen provocation and during the grass pollen season. These data point toward a naso-ocular interaction in allergic rhinoconjunctivitis, with involvement of substance P.

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