Available online at www.sciencedirect.com
SCIENCE DIRECT®
Vascular Pharmacology 43 (2005) 267 – 276
Vascular Pharmacology
www.elsevier.com/locate/vph

Evaluation of nasal barrier dysfunction at acute- and late-phase reactions in a guinea pig model of allergic rhinitis

Sofia Elovsson [a], Amir Smailagic [b], Ingrid Erjefalt [b], Catharina Zackrisson [b], Christina Eriksson [b], Xiangdong Wang [b,*]

[a] *Department of Clinical Study Management, AstraZeneca R&D Lund, Sweden*
[b] *Biological Sciences, AstraZeneca R&D Lund, S-221 87 Lund, Sweden*

Received 25 November 2004; received in revised form 10 May 2005; accepted 23 August 2005

Abstract

Allergic rhinitis is a common disease characterized by the symptoms of pruritus, sneezing, hypersecretion and nasal blockage. Increased mucosal barrier permeability has been suggested to be an indicator for the severity of allergic rhinitis. This study investigates the passage of radiolabelled albumin from the nasal mucosal circulation into the lumen in guinea pigs intraperitoneally sensitized and intranasally challenged with antigen. In order to characterize the allergic rhinitis model, we evaluated a number of potential influencing factors in nasal plasma exudation, including antigen doses, volumes of antigen solution used, and animal position during the nasal lavage, and the conditions of nasal lavage. The number of eosinophils and levels of histamine and leukotriene B_4 in the nasal lavage and eosinophils in the nasal mucosa were determined at the early and late phases after antigen challenge. We also compared the effects of topical nasal treatments for allergic rhinitis on nasal inflammatory responses. Our results demonstrate that, in the guinea pig nasal mucosa, topical challenge with antigens induces plasma exudation and histamine release at the acute-phase reaction, and plasma exudation and eosinophil infiltration at the late-phase reaction. These changes are similar to those reported in human allergic rhinitis. Alterations of nasal plasma exudation, histamine release and eosinophil influx were dependent upon the concentrations and volumes of antigens. An antihistamine inhibited the acute-phase reaction partially, whereas budesonide inhibited effects at the late-phase reaction. We suggest that this model of guinea pig allergic rhinitis with the early and late responses may be useful for high-throughout screening of new drugs.

Keywords: Rhinitis; Plasma exudation; Early response; Late response; Histamine; Eosinophils

1. Introduction

Allergic rhinitis, with an incidence about 20% of the world's population, is characterized by the symptoms of pruritus, sneezing, hypersecretion and nasal blockage (Bellanti and Wallerstedt, 2000; Naclerio, 1991). The factors involved in the development and formation of nasal symptoms include eosinophil infiltration of the nasal mucosa and cavity (Terada et al., 1994), release of histamine (Gruber, 1994; Persson, 1990; Greiff et al., 2003; Howarth et al., 2000), and dysfunction of the nasal mucosal epithelial and endothelial barrier (Kaise et al., 1995; Persson, 1990). Extravasation of plasma protein from postcapillary venules can be employed as an accessible index reflecting the severity of the airway mucosal inflammation (Greiff et al., 2003). The contraction-like deformation of endothelial cells to inflammatory stimuli results in the increased permeability of subepithelial microvessels and plasma exudation into the nasal tissue. Leaked plasma molecules then passes through the mucosal epithelial tight junctions, dependent upon the basolateral hydrostatic pressure load on the epithelial surface cells (Persson, 1990).

In order to characterize the guinea pig allergic rhinitis model, we evaluated a number of potential influencing factors in nasal plasma exudation, a passage of radiolabelled human serum albumin (HSA) from the nasal mucosal circulation into the lumen, including antigen doses, volumes of antigen solution used, and animal position during the nasal lavage, and the conditions of nasal lavage in guinea pigs intraper-

* Corresponding author. Tel.: +46 46 337 883; fax: +46 46 336 624.
E-mail address: xiangdong.wang@astrazeneca.com (X. Wang).

1537-1891/$ - see front matter © 2005 Published by Elsevier Inc.
doi:10.1016/j.vph.2005.08.016

itoneally sensitized and intranasally challenged with antigen. We characterized the response to challenge by measurement of plasma exudation, eosinophil influx and levels of histamine and leukotriene B_4 in the nasal lavage and mucosa at the early and late phases after antigen challenge. Furthermore, the present study compares the effects of topical nasal treatments for allergic rhinitis, azalestine, levocabastine and budesonide, on nasal inflammatory responses.

2. Materials and methods

2.1. Animals

Dunkin–Hartley male guinea pigs, weighing 250–300 g, were purchased from Möllegaard Breeding Center, Ejby, Denmark and housed in plastic cages with aspen bedding (4 guinea pigs/cage). The animal room was maintained at 20 °C with a daily light–dark cycle (0600–1800 light). Animals were kept with food and water ad libitum. The study was approved by the Malmö/Lund ethical committee for animal experiments.

2.2. Sensitization and challenge

Guinea pigs were sensitized to OVA (OVA) by an intraperitoneal injection of 0.5 ml saline containing 100 mg Al $(OH)_3$ (F2200; Anachemia, Montreal, Canada) and 2 μg OVA (grade III; Sigma, St. Louis, MO, USA) (Erjefält et al., 1993). Three weeks after sensitization, animals were anaesthetized by intramuscular administration (1 ml/kg) of a 2:3 ratio of Xylazine (Rompun, 20 mg/kg; Bayer, Leverkusen, Germany) and Ketamine (Ketalar, 50 mg/kg; Park Davies, Detroit, MI, USA) and placed in a head-down supine position with an angle 25°. The exposure of the nasal cavity to allergen was performed by dropping OVA solution at different doses into bilateral nasal cavities. For the negative control, animals received either sensitization and challenge with saline or sensitization with saline and challenge with OVA.

2.3. Plasma exudation

The nasal mucosal barrier permeability was assessed by measuring the passage of ^{125}I-labeled human serum albumin

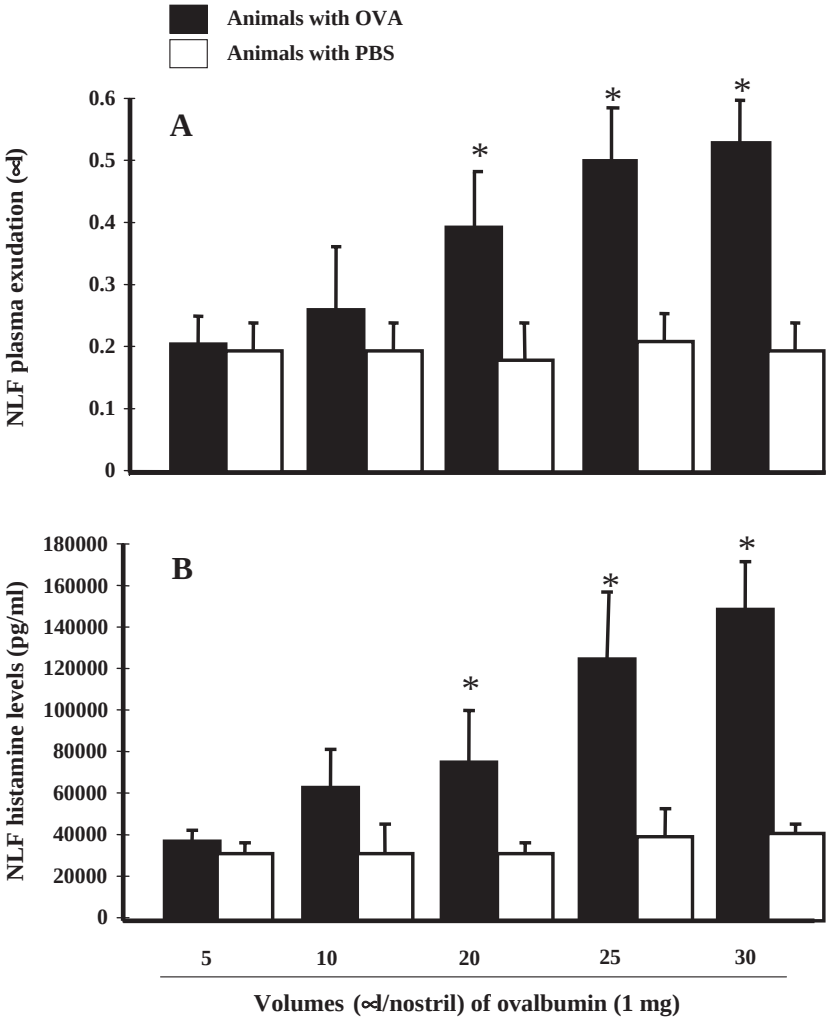

Fig. 1. Plasma exudation (A) and histamine release (B) in nasal lavage fluid harvested from OVA-sensitized guinea pigs intranasally challenged with PBS or OVA in different volumes with a fixed concentration. * Stands for p values less than 0.05, as compared with the respective group of OVA-sensitized guinea pigs intranasally challenged with PBS.

(HSA, Institute for engergiteknikk, Kjeller, Norway) from the circulation to the nasal cavity as a marker of plasma exudation. After the anaesthesia with the mixture of Xylazine and Ketamine, guinea pigs were injected intravenously with 0.1 ml of ^{125}I-labelled HSA (10^6 cpm/ml) via the ear marginal vein. After 20-min equilibration, animals were sacrificed by an over-dose of anaesthetic. Blood was harvested by cardiac puncture and centrifuged at 4000 *g*. The nasal lavage solution was collected from the nostrils into a plastic tube. The lavage fluid was centrifuged at 1200 *g*, 4 °C, and then separated into supernatant and cells. Levels of ^{125}I in supernatant and plasma were measured in a γ-counter (1272 Clinigamma, LKB, Wallac OY, Finland) after that the samples had been weighed. Plasma exudation (μl) was calculated by ^{125}I counts per gram supernatant / ^{125}I counts per milliliter of plasma.

2.4. Nasal lavage fluid (NLF)

In order to eliminate tissue blood from the circulation to avoid potential contamination, the abdominal wall was opened and the abdominal aorta was cut. After then, the trachea was exposed and opened at the third ring under the larynx. The cutting edge was cleaned and the trachea was cannulated with a catheter (a teflon sond, 5206, B and K, Norway) into the orientation to oropharynx. The animal was in a supine position of head-down on a board tilted at 30°. The catheter was connected to a peristaltic pump (Gelson miniplus 3, Pra-Tech Co., Stockhlom, Sweden) and the nasal cavity was gently rinsed with phosphate-buffered saline (PBS) containing 1% fetal bovine serum at the speed of 0.2 ml/min for 10 min.

2.5. NLF cytology

The cell pellet from the NLF was resuspended in PBS for counting total leukocyte number using a 15-parameter, semiautomated haematology analyzer (Sysmex F820, TOA Medical Electronics Co. Kobe, Japan). Cell differentiation was counted after the cytospin and staining with May Grynwald Giemsa.

2.6. Histamine measurement

Histamine was measured by a sensitive radioimmunoassay (RIA)-kit (Immunotech, KEMILA, Sollentuna, Sweden). The assay is based on the conversion of histamine to [^{3}H] methylhistamine in the presence of the enzyme histamine — *N*-methyltransferase using *S*-adenosyl-L-[methyl-^{3}H] methionine as the methyl donor.

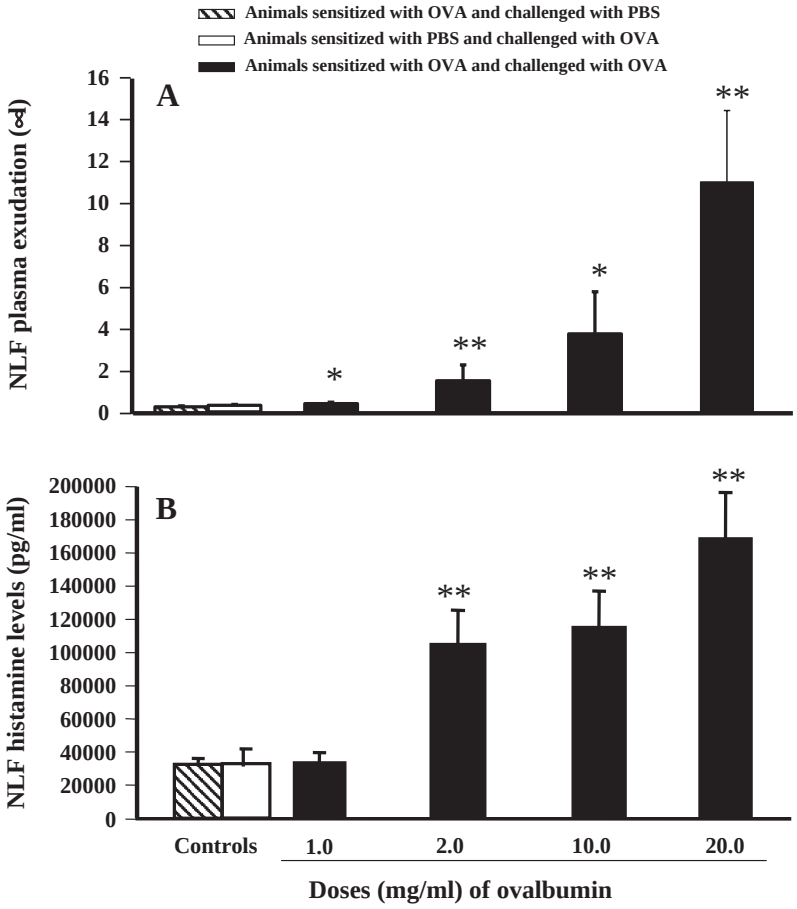

Fig. 2. Plasma exudation (A) and histamine release (B) in nasal lavage fluid harvested from OVA-sensitized guinea pigs intranasally challenged with PBS or OVA in different concentrations with a fixed volume (20 μl/nostril). * and ** stand for *p* values less than 0.05 and 0.01, respectively, as compared with OVA-sensitized guinea pigs intranasally challenged with PBS.

2.7. Light and electron microscopy

The nasal septum were removed and rinsed in 0.9% saline. Nasal mucosal specimens (0.5 mm cubes) were separated from the cartilage for evaluating either by light microscopy (5 animals/group) or electron microscopy (3 animals/group). The specimens were fixed in 5% formaldehyde solution buffered with phosphate sodium–calcium at pH 7.2 (Histofix, 300 mOsmol, HistoLab, Göteborg, Sweden) overnight. The mucosa were then dehydrated, cleared in xylene, and embedded in paraffin. Two 3-μm sections of each septum were stained with hematoxylin and eosin. Other specimens were immersed in 2% glutaraldehyde in 0.1 mol l^{-1} sodium phosphate buffer (pH 7.4) at room temperature overnight and postfixed for 1 h in 2% osmium tetroxide before being dehydrated in graded ethanol solutions, and embedded in epoxy resin (Ladd, Wien, Austria). Sections were cut at 60 nm on an ultramicrotome (Ultracut E; Reichert-Jung, Burlington, VT, U.S.A.), contrasted with uranyl acetate and lead citrate, and examined in a JEM-100 CX electron microscope (Jeol, Tokyo, Japan).

2.8. Staining of eosinophils and mast cells

A piece of harvested septum and turbinate was fixed in 4% paraformaldehyde or Carnoy's solution containing absolute ethanol, chloroform, acetic acid (6:3:0.5, pH 4.0), or MFAA solution containing methanol, formaldehyde, acetic acid (8.5:1:0.5, pH 3.42) (Tas and Berndsen, 1977). Tissue in 4% buffered parafomaldehyde was kept at 4 °C in PBS containing 20% sucrose and then incubated in PBS containing 20% sucrose and 5% glycerol for 2 h. The septum and the turbinate were stained for eosinophils with a solution containing 3,3-diaminobensidin (0.75%), sodium cyanide (0.5%), and 300 μl H_2O_2. With the reaction controlled under microscope, the eosinophils were visualized as brown or black spots.

2.9. Drug formulation

The vehicle consisted of the following ingredients: sodium chloride (8.5 mg/ml), EDTA (0.1 mg/ml), dried citric acid (0.15 mg/ml), sodium citrate (0.5 mg/ml), and polysorbate 80

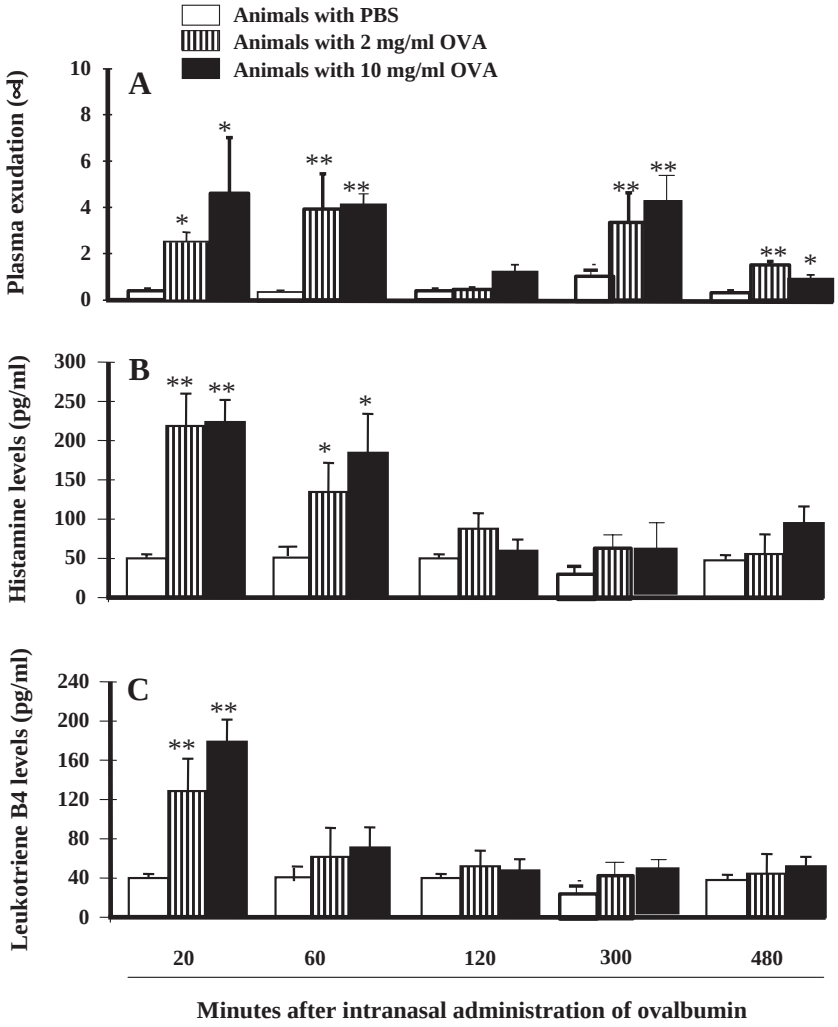

Fig. 3. Dynamic alterations in plasma exudation (A) and histamine release (B) in nasal lavage fluid harvested from OVA-sensitized guinea pigs intranasally challenged with PBS or OVA in concentrations (2 and 10 mg/ml) at the volume of 20 μl/nostril. * and ** stand for p values less than 0.05 and 0.01, respectively, as compared with the respective group of OVA-sensitized guinea pigs intranasally challenged with PBS.

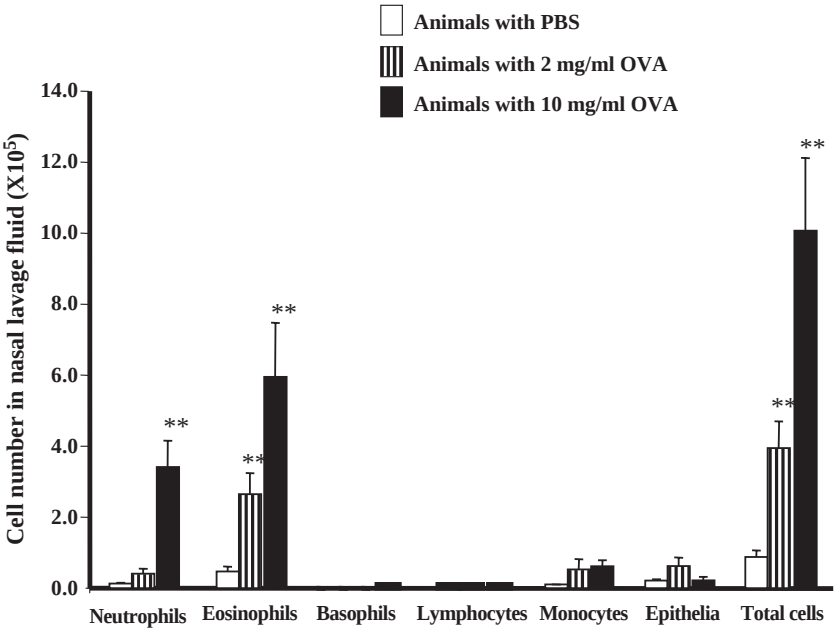

Fig. 4. Leukocyte infiltration in nasal lavage fluid harvested from OVA-sensitized guinea pigs 5 h after intranasal challenge with PBS or OVA in concentrations (2 and 10 mg/ml) at the volume of 20 μl/nostril. ** stands for p values less than 0.01, as compared with the respective group of OVA-sensitized guinea pigs intranasally challenged with PBS.

(0.2 mg/ml) in Milli-Q water. Budesonide (AstraZeneca, Lund, Sweden) was homogenised in Polysorbate 80 and water using a dispersing tool ("Ultra turrax"). The homogenised budesonide was added to the vehicle at concentrations of 1 mg/ml. Levocabastine, Lastin (Janssen-Cilag AB, Apoteksbolaget, Sweden) and Azelastine, Lastin (Santen Pharma AB, Apoteksbolaget, Sweden) were dissolved in PBS to 0.5 and 1 mg/ml, respectively. Vehicle or drugs were administered intranasally, 25 μl/nostril, 20 min before the intranasal challenge with OVA.

2.10. Experimental design

2.10.1. Evaluation of the model

The optimal delivery protocol of antigen challenge was evaluated by varying the volumes of OVA solution (5 to 30 μl per nostril), concentrations of OVA (1 to 40 mg/ml), and formulations of antigen dissolved in distilled water, saline, or PBS. The distribution of antigen in the nasal cavity, trachea, and lungs was monitored by following the trace of radiolabelled proteins after nasal delivery. Animals were placed in supine and stomach positions during the nasal lavage, in order to determine the optimal position to obtain maximal lavage within the nasal cavity. In order to study the response challenges, animals were administered intranasally with histamine, mast cell stimulator C48/80 compound, or OVA. Response duration of the nasal mucosa to OVA was evaluated 10 and 20 min, 1, 2, 4, 6, 8, 12, and 24 h after nasal challenge to clarify the acute and late phases of nasal inflammatory response. The nasal inflammation was characterized by plasma exudation, production of histamine and leukotriene B_4, eosinophil influx into the nasal cavity and the mucosa, and mast cells in the nasal mucosa. Additional animals were used for histological examination, in order to eliminate potential contamination of radioisotopes. Exclusion criteria in the present study include 1) animals with sensitization failure characterized by no detectable precipitates of adjuvant [$Al(OH)_3$] in the peritoneum; 2) animals with severe or fatal bronchoconstriction and dying before the termination; 3) animals with other infections characterized by signs of lavage fluid and lungs and microbiological analysis; and 4) technical failure, e.g. tracer injection and operation.

2.10.2. Model responses to drugs

In order to investigate the potential application of the rhinitis model for testing drug efficacy, effects of budesonide,

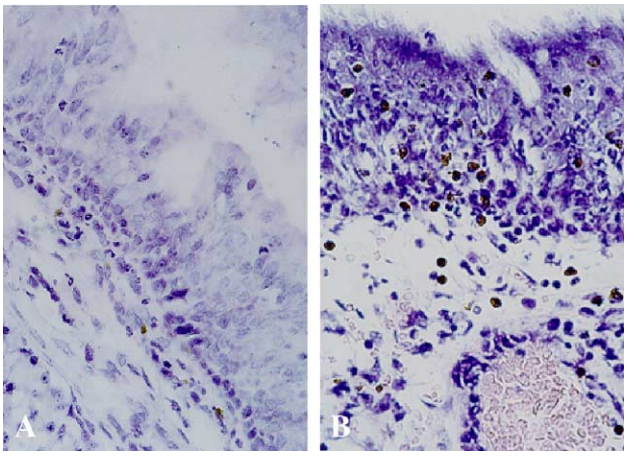

Fig. 5. Section through nasal mucosa region showing the migration of eosinophils with EPO-positive stainings from the capillary to the nasal lumen in OVA-sensitized guinea pigs 5 h after intranasal challenge with OVA (B), as compared with OVA-sensitized guinea pigs intranasally challenged with PBS (A). (Haematoxylin and eosin; magnification ×200).

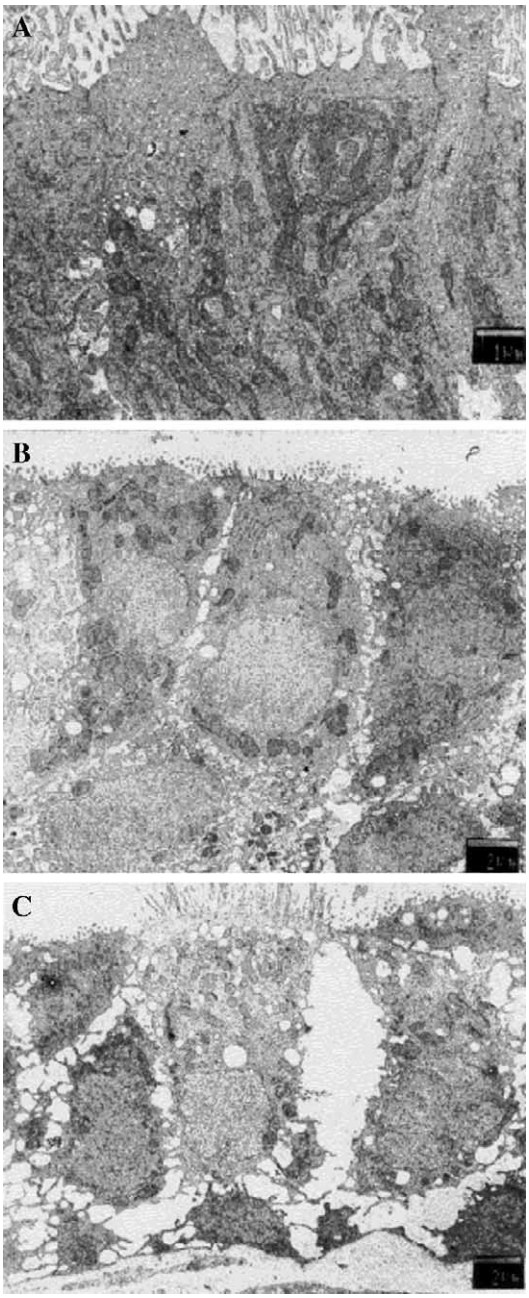

Fig. 6. Nasal mucosal ultrastructure (origin magnification: ×6000) from transmission electronic microscopy in OVA-sensitized guinea pigs 10 (B) and 30 min (C) after intranasal challenge with OVA, showing the increase in interepithelial space and latered epithelial cell shapes, as compared with OVA-sensitized guinea pigs intranasally challenged with PBS (A).

levocabastine, and azelastine on nasal plasma exudation and eosinophil infiltration were studied 20 min (acute-phase response) and 5 h (late-phase response) after the intranasal challenge with 25 μl of OVA solution at the concentration of 2.5 mg/ml PBS in OVA-sensitized guinea pigs. The animals were anaesthetized with an intramuscular administration of Xylazine and Ketamine and put in a supine position of head-up on a board tilted at 30°. Budesonide (1.0 mg/ml), levocabastine (0.5 mg/ml), azelastine (1.0 mg/ml) or vehicle was intranasally administered 1 h before challenge.

2.11. Statistical analysis

Data are presented as means ± standard error of the mean. The significance of the differences between groups and time points was calculated using Student *t*-test with two-tailed distribution and two-sample equal variance, after the analysis of one-way ANOVA. A p value of less than 0.05 was considered significant.

3. Results

3.1. Optimal volumes and doses for nasal challenge

The rate of sensitization failure was 5%, the rate of infection was 0.5%, and the mortality rate due to acute severe bronchoconstriction before the termination time point was 3% prior to the termination. Effects of different volumes of OVA solution were evaluated by measuring nasal plasma permeability and the release of histamine in the nasal lavage fluid, as shown in Fig. 1. One milligram of OVA was used in different volumes. Levels of nasal plasma exudation (Fig. 1A)

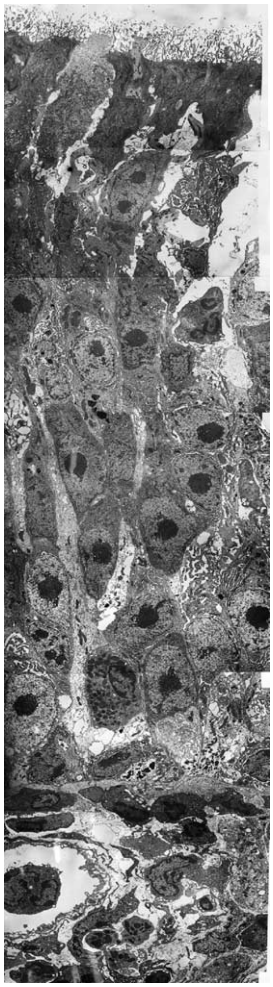

Fig. 7. Nasal mucosal ultrastructure (origin magnification: ×6000) from transmission electronic microscopy in OVA-sensitized guinea pigs 5 h after intranasal challenge with OVA, showing the eosinophil migration from the capillary to the tissue, degranulation, and epithelial changes.

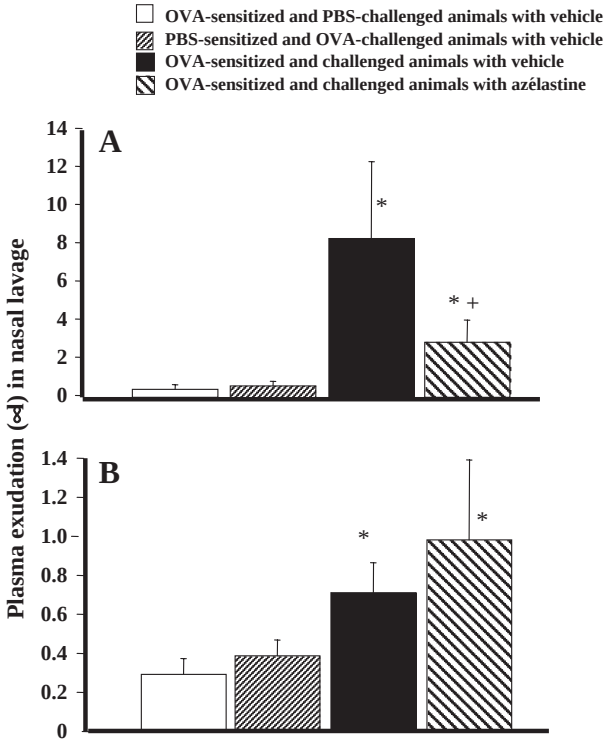

Fig. 8. Values of plasma exudation in nasal lavage fluid from OVA-sensitized guinea pigs pretreated with vehicle or azelastine 20 min (A) and 5 h (B) after intranasal challenge with PBS or OVA in concentrations (2 mg/ml) at the volume of 20 μl/nostril. * Stands for p values less than 0.05, as compared with the respective group of OVA-sensitized guinea pigs intranasally challenged with PBS. + Stands for p values less than 0.05, as compared with OVA-sensitized and challenged guinea pigs pretreated with vehicle.

and histamine (Fig. 1B) increased in animals intranasally receiving OVA 20 μl/nostril or more. A volume-related pattern was noted. Using a fix volume of solution (20 μl/nostril) and varied concentrations of OVA (1, 2, 10, and 20 mg/ml), animals exhibited a significant increase in plasma exudation (Fig. 2A) and histamine release (Fig. 2B) in nasal lavage fluid from 1 and 2 mg/ml, respectively ($p<0.05$ and 0.01, respectively). There was no statistical difference between animals with OVA sensitization and PBS challenge and with PBS sensitization and OVA challenge (Fig. 2). Formulations of OVA in water, saline or PBS did not stimulate significant increases in plasma exudation or histamine release. Nasal administrations of histamine and compound 48/80 induced a significant increase in plasma exudation with dose- and volume-dependent patterns (data not shown).

3.2. Optimal positioning of antigen

Study of antigen distribution demonstrated that a high volume (50 μl/nostril) of the antigen resulted in the rapid appearance of radiolabelled HSA in the lung. It took about 3 h to detect radiolabelled protein within the lung at the volume of 20 μl/nostril. To determine the optimum position for performing the nasal lavage, animals were placed either in supine or stomach position during the nasal lavage. The levels of radiolabelled proteins lavaged from the supine position was higher than those from the stomach laying position (data not shown). Correlation coefficient analyzed by a Pearson's correlation between plasma exudation to the nasal cavity and to the mucosal tissue was 0.89 ($p=0.1$).

3.3. Dynamics of nasal responses

Levels of plasma exudation to nasal cavity were increased significantly 20 and 60 min and 5 and 8 h after intranasal challenge with OVA at both 2 and 10 mg/ml, as compared to controls ($p<0.05$ or less; Fig. 3A). Nasal challenge with OVA induced a significant increase in levels of histamine at 20 and 60 min ($p<0.05$ or less; Fig. 3B), while the levels of leukotriene B_4 in nasal lavage fluid were significantly increased 20 min after OVA challenge, as compared to controls ($p<0.05$; Fig. 3C). There was no statistical difference in response between animals receiving 2 or 10 mg/ml of OVA. Fig. 4 shows the increased number of total cells, dominated by eosinophils and neutrophils, in nasal lavage fluid 5 h after intranasal challenge with OVA with a dose-dependent pattern. Histological analysis demonstrated that eosinophils appeared in the outside of the nasal mucosal capillary and the number of eosinophils between the nasal mucosal epithelial cells was

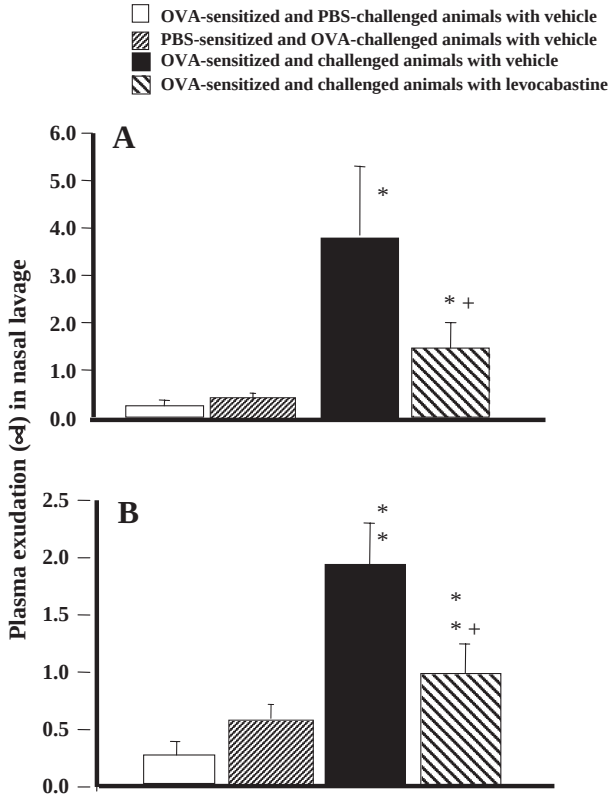

Fig. 9. Values of plasma exudation in nasal lavage fluid from OVA-sensitized guinea pigs pretreated with vehicle or levocabastine 20 min (A) and 5 h (B) after intranasal challenge with PBS or OVA in concentrations (2 mg/ml) at the volume of 20 μl/nostril. * and ** stand for p values less than 0.05 and 0.01, respectively, as compared with the respective group of OVA-sensitized guinea pigs intranasally challenged with PBS. + Stands for p values less than 0.05, as compared with OVA-sensitized and challenged guinea pigs pretreated with vehicle.

obviously higher in OVA-sensitized and challenged animals than controls, as shown in Fig. 5. The gaps between nasal epithelial cells became wider and the shape of epithelial cells was shortened from 10 min onwards after nasal challenge with OVA (Fig. 6). Furthermore, eosinophils filled with granules appeared out of the capillary, whereas eosinophil granules were diffuse in the submucosal tissue and between epithelial cells in OVA-sensitized and challenged animals (Fig. 7).

3.4. Model responses to drugs

After pretreatment with vehicle, there was no significant difference of plasma exudation into the nasal cavity between animals with OVA-sensitization and PBS-challenge and with PBS-sensitization and OVA-challenge, as shown in Figs. 8 and 9. Local pretreatment with azelastine and levocabastine reduced significantly OVA-induced plasma exudation to the nasal cavity at 20 min, as compared with OVA-sensitized and challenged animals pretreated with the vehicle, while the levels of plasma exudation in these animals pretreated with azelastine and levecabastin were significantly higher than in controls ($p<0.05$, respectively; Figs. 8A and 9A). At the 5-h time point, the inhibitory effect of azelastine disappeared (Fig. 8B), but the effect levocabastine was still evident (Fig. 9B; $p<0.05$), even though it was significantly higher than in controls ($p<0.01$). When animals were pretreated with budesonide, plasma exudation was significant when evaluated at 5 h ($p<0.05$; Fig. 10B). Pretreatment with azelastine or levecabastine had no inhibitory effects on OVA-induced increase in the number of neutrophils and eosinophils, while budesonide reduced significantly OVA-induced number of neutrophils, eosinophils and total cells at 5 h ($p<0.05$ or less, Fig. 10C).

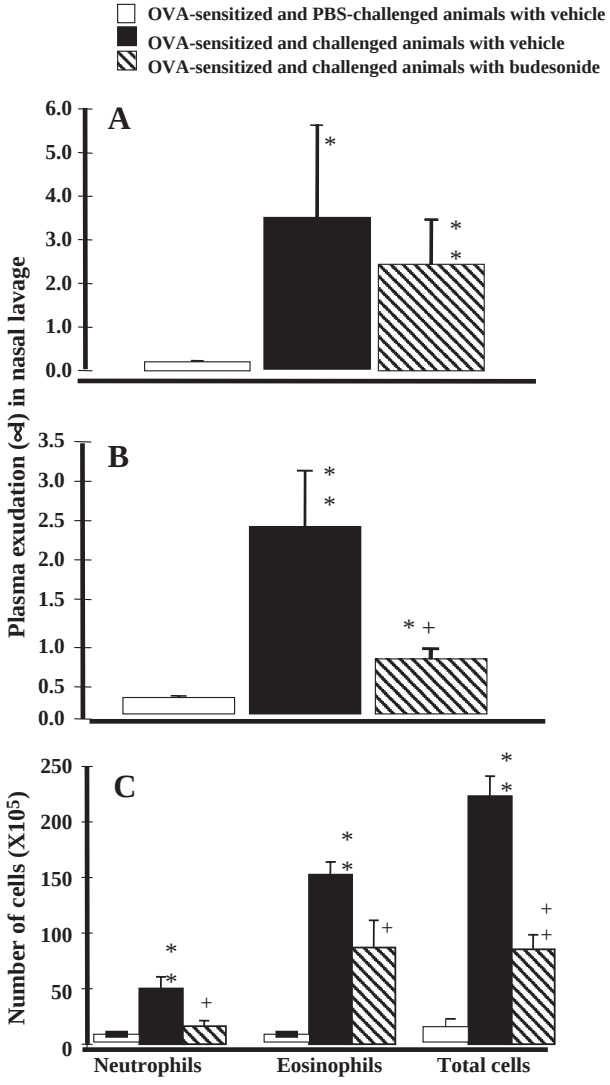

Fig. 10. Values of plasma exudation and leukocyte infiltration (C) in nasal lavage fluid from OVA-sensitized guinea pigs pretreated with vehicle or budesonide 20 min (A) and 5 h (B) after intranasal challenge with PBS or OVA in concentrations (2 mg/ml) at the volume of 20 μl/nostril. * and ** stand for p values less than 0.05 and 0.01, respectively, as compared with the respective group of OVA-sensitized guinea pigs intranasally challenged with PBS. + and ++stand for p values less than 0.05 and 0.01, respectively, as compared with OVA-sensitized and challenged guinea pigs pretreated with vehicle.

4. Discussion

This study evaluates a guinea pig model of allergic rhinitis induced by the sequence of intraperitoneal injection and nasal challenge with OVA. Animals with allergic rhinitis developed an increase in plasma exudation with acute (within 1 h) and late (during 5–8 h) responses to the antigen challenge, histamine release at acute phase, and leukocyte influx at the late phase, similar to findings in human allergic rhinitis (Yang et al., 2003; Greiff et al., 2002a). The severity of allergic rhinitis in guinea pigs was dependent upon nasal delivery of both antigen concentrations and volumes, and the accumulation of nasal plasma exudate was influenced by the animals position during lavage.

Plasma exudation in tracheobronchial and nasal airway has been considered as a first line mucosal defense system and becomes pathogenic in allergic disease (Persson et al., 1991). Nasal plasma exudation in guinea pigs develops by the same mechanism as in humans (Persson, 1990). Our study demonstrates that alterations in nasal mucosal barrier permeability can be a characteristic of local inflammatory responses to antigen in the guinea pig model of allergic rhinitis. The evidence to support this was that the severity of plasma exudation was related to the amount of antigen and the area of the nasal mucosa exposed to antigens. It seems that nasal plasma exudation in the acute phase may result from the local interaction between antigens and mast cells, since the increased permeability of labeled protein was noted in the nasal mucosa rather than in the tracheal mucosa (data not shown). The results from antigen distribution showed that antigens were only in the nasal cavity during the acute response. It is possible that plasma proteins may pass through interepithelial cells to the nasal lumen, since the space between nasal epithelia was increased from 10 min onwards after the local challenge with antigens, similar to the previous findings (Luts et al., 1990). Although the molecular mechanism by which the nasal barrier permeability was increased remains unclear, the findings from

TEM indicate that epithelial constriction and reshape or increased sub-epithelial pressure may be involved in the occurrence of plasma exudation. The evidence to support this was that epithelial cells became round and small and the interepithelial conjunction was broken 10–30 min after antigen challenge. Such alterations provide pathological fundaments for plasma protein leakage from paraepithelial pathways as previously found (Greiff et al., 2003; Erjefält et al., 1995).

Another potential is that antigens may enter the lower part of the airway and cause dysfunction of the airway mucosal capillary barrier, including nasal, tracheal and bronchial tissues. Our data indicate that acute-phase response in the nasal tissue is independent upon the inflammatory response from the lower airway, since the radiolabelled antigen reached the lower airway from 3 h and on after the nasal delivery. In order to investigate potential involvement of the lower airway reaction in nasal permeability, nasal plasma exudation was measured 20 min and 5 h after directly intratracheal instillation of 20 μg OVA in 0.2 ml PBS. Our preliminary results demonstrated that nasal permeability was not changed after intratracheal exposure to antigens, but the number of eosinophils in the nasal tissue significantly increased (data unshown). The OVA dose used for nasal challenge caused the death of animals immediately after intratracheal administration.

Histamine has been suggested to play an important role in the maintenance of nasal mucosa barrier integrity in both humans and guinea pigs, since topical administration of histamine caused increased permeability of the nasal mucosa (Yang et al., 2003; Greiff et al., 2002b) and antihistamine reduced acute allergen challenge-induced plasma exudation in allergic rhinitis (Greiff et al., 2002a). Our results demonstrated that histamine might be partially involved in the occurrence of nasal plasma exudation at the acute-phase reaction and has no effects on eosinophil recruitment from the circulation to nasal lumen at the late-phase reaction, even though that azelastine and levocabastine have a half-life of about 20–40 h (Simons and Simons, 1999). It has been proposed that multiple cells, mediators and molecules are responsible for the selective recruitment and activation of inflammatory cells at the late-phase reaction, such as T lymphocytes, eosinophils, leukocytes, cytokines, chemokines, and adhesion molecules (Ciprandi et al., 2003a,b; Ueda et al., 2003; Chantry and Burgess, 2002). This may be the reason why topical administration of the broad anti-inflammatory drugs, e.g. budesonide showed greater inhibitory effects on the total nasal symptoms and the inflammatory exudation evoked by allergen challenge in patients with rhinitis, as compared to antihistamine (Svensson et al., 1998; Yanez and Rodrigo, 2002). Similar to clinical findings, the present study found that local administration of budesonide also reduced the late-phase response induced by antigen challenge better than antihistamines.

In conclusion, the results of this study demonstrate that, in the guinea pig nasal mucosa, topical challenge with antigens induced plasma exudation and histamine release at the acute-phase reaction, and plasma exudation and eosinophil infiltration at the late-phase reaction, similar to that reported in human allergic rhinitis. Alterations of nasal plasma exudation, histamine release and eosinophil influx in nasal lavage fluid were dependent upon the concentrations and volumes of antigens. As the reference compounds, antihistamine had partial effects at the acute-phase reaction, while budesonide showed inhibitory effects at the late-phase reaction. We suggest that this model of guinea pig allergic rhinitis with the early and late responses may be useful for high-throughout screening of new drugs.

References

Bellanti, J.A., Wallerstedt, D.B., 2000. Allergic rhinitis update: epidemiology and natural history. Allergy Asthma Proc. 21, 367–370.

Chantry, D., Burgess, L.E., 2002. Chemokines in allergy. Curr. Drug Targets Inflamm. Allergy 1, 109–116.

Ciprandi, G., Tosca, M.A., Cirillo, I., Vizzaccaro, A., 2003a. The effect of budesonide on the cytokine pattern in patients with perennial allergic rhinitis. Ann. Allergy, Asthma, & Immun. 91, 467–471.

Ciprandi, G., Tosca, M.A., Cosentino, C., Riccio, A.M., Passalacqua, G., Canonica, G.W., 2003b. Effects of fexofenadine and other antihistamines on components of the allergic response: adhesion molecules. J. Allergy Clin. Immunol. 112, S78–S82.

Erjefält, I., Greiff, L., Alkner, U., Persson, C.G., 1993. Allergen-induced biphasic plasma exudation responses in guinea pig large airways. Am. Rev. Respir. Dis. 148, 695–701.

Erjefält, J.S., Erjefält, I., Sundler, F., Persson, C.G.A., 1995. Epithelial pathways from entry of bulk plasma. Clin. Exp. Allergy 25, 187–195.

Greiff, L., Andersson, M., Erjefält, J.S., Svensson, C., Persson, C.G.A., 2002a. Loss of size-selectivity at histamine-induced exudation of plasma proteins in atopic nasal airways. Clin. Physiol. Funct. Imaging 22, 28–31.

Greiff, L., Persson, C.G.A., Andersson, M., 2002b. Desloratadine reduces allergen challenge-induced mucinous secretion and plasma exudation in allergic rhinitis. Ann. Allergy, Asthma, & Immun. 89, 413–418.

Greiff, L., Andersson, M., Erjefalt, J.S., Persson, C.G., Wollmer, P., 2003. Airway microvascular extravasation and luminal entry of plasma. Clin. Physiol. Funct. Imaging 23, 301–306.

Gruber, B.L., 1994. Mast cells and basophils. In: Sigal, L.H., Ron, V. (Eds.), Immunology and Inflammation. McGraw-Hill, New York, pp. 337–351.

Howarth, P.H., Salagean, M., Dokic, D., 2000. Allergic rhinitis: not purely a histamine-related disease. Allergy 55 (Suppl 64), 7–16.

Kaise, T., Sakakura, Y., Ukai, K., 1995. Ability of histamine to increase nasal mucosal permeability to macromolecules in guinea pigs. Ann. Otol. Rhinol. Laryngol. 104, 969–973.

Luts, A., Sundler, F., Erjefalt, I., Persson, C.G., 1990. The airway epithelial lining in guinea pigs is intact promptly after the mucosal crossing of a large amount of plasma exudate. Int. Arch. Allergy Appl. Immunol. 91, 385–388.

Naclerio, R.M., 1991. Allergic rhinitis. N. Engl. J. Med. 325, 860–869.

Persson, C.G.A., 1990. Plasma exudation in tracheobronchial and nasal airways: a mucosal defence mechanism becomes pathogenic in asthma and rhinitis. Eur. Respir. J. 3, 652s–657s.

Persson, C.G., Erjefalt, I., Alkner, U., Baumgarten, C., Greiff, L., Gustafsson, B., Luts, A., Pipkorn, U., Sundler, F., Svensson, C., et al., 1991. Plasma exudation as a first line respiratory mucosal defence. Clin. Exp. Allergy 21 (1), 17–24 (Jan).

Simons, F.E., Simons, K.J., 1999. Clinical pharmacology of new histamine H1 receptor antagonists. Clin. Pharmacokinet. 36, 329–352.

Svensson, C., Andersson, M., Greiff, L., Blychert, L.O., Persson, C.G., 1998. Effects of topical budesonide and levocabastine on nasal symptoms and plasma exudation responses in seasonal allergic rhinitis. Allergy 53, 367–374.

Tas, J., Berndsen, R.G., 1977. Does heparin occur in mucosal mast cells of the rat small intestine? J. Histochem. Cytochem. 25, 1058–1062.

Terada, N., Konno, A., Togawa, K., 1994. Biochemical properties of eosinophils and their preferential accumulation mechanism in nasal allergy. J. Allergy Clin. Immunol. 94, 629–642.

Ueda, T., Takeno, S., Furukido, K., Hirakawa, K., Yajin, K., 2003. Leukotriene receptor antagonist pranlukast suppresses eosinophil infiltration and cytokine production in human nasal mucosa of perennial allergic rhinitis. Ann. Otol. Rhinol. Laryngol. 112, 955–961.

Yanez, A., Rodrigo, G.J., 2002. Intranasal corticosteroids versus topical H1 receptor antagonists for the treatment of allergic rhinitis: a systematic review with meta-analysis. Ann. Allergy, Asthma, & Immun. 89, 479–484.

Yang, T.Q., Takeuchi, K., Hayashi, S., Majima, Y., 2003. Effect of histamine on the permeability of the nasal mucosa in vivo. Acta Oto-laryngol. 123, 401–405.