



Validation of guinea pig model of allergic rhinitis by oral and topical drugs

Prashant C. Bahekar, Jigar H. Shah, Upendra B. Ayer,
Sanjay N. Mandhane *, Rajamannar Thennati

Sun Pharma Advanced Research Company Limited, Atladara, Tandalja Road, Vadodara, Gujarat, 390 020, India

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Abstract

Ovalbumin-induced guinea pig model of rhinitis was assessed for its utility in the studies of rhinitis. Systemic sensitization and challenge with ovalbumin-induced rhinitis symptoms and an increase in anti-OVA-IgE and IgG titers, positive skin reactions and nasal lavage IL-4 concentration. Histopathology of nasal mucosa showed infiltration of eosinophils and other inflammatory cells consistent with the symptoms. Topical sensitization of ovalbumin yielded inconsistent symptoms of rhinitis. In systemic sensitization model, repeated challenge of ovalbumin caused similar response for at least 3 consecutive challenges. The symptoms were affected by relative humidity in the air and dosing volume of topical drugs. Sneezing and lacrimation were reduced by acute oral administration of the H₁ receptor antagonists and steroids or the prophylactic oral administration of cysteinyl leukotriene (CysLT₁) receptor antagonist montelukast or acute topical antihistamines, mast cell stabilizer sodium cromoglycate and anticholinergic agent ipratropium bromide, but not by a topical steroid. Nose rubbing was reduced significantly by some oral and topical antihistamines. Oral steroids offered excellent protection against all symptoms. Dexamethasone and montelukast also inhibited nasal lavage IL-4 concentration and inflammatory cell infiltration. Treatment with topical steroid fluticasone for 2 weeks had no effect on sneezing or rubbing. However, it caused complete inhibition of congestion. The cyclooxygenase inhibitor indomethacin had no effect on symptoms of rhinitis. The adrenergic α receptor agonist-decongestant oxymetazoline caused reduction in congestion. These results suggest that differential responsiveness to symptoms of rhinitis by a new agent can be very well profiled in the model in congruence with the mediation pathways and mechanism of action of drugs. The model provides complete symptomatic characterization of rhinitis and is a good tool for its study.

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* Corresponding author. Tel.: +91 265 6635500/6635600; fax: +91 265 2354897.

E-mail address: sanjay.mandhane@sunpharma.com (S.N. Mandhane).

1. Introduction

Allergic rhinitis is an IgE-mediated inflammatory disease of the nasal mucosal membrane characterized mainly by the early phase response exhibiting symptoms like sneezing, nasal rubbing, rhinorrhea, lacrimation and the late phase response primarily exhibiting nasal congestion/obstruction and less frequently cough [1]. Rhinitis can be classified as seasonal or perennial depending upon whether the symptoms occur in response to seasonal allergen exposure or round the year, respectively. Common seasonal allergens are derived from pollens of trees, grasses and weeds while perennial allergens are derived from dust mites, molds, animal danders and other sources of occupational origin. Often patients with perennial rhinitis show exacerbations of their symptoms on seasonal pollen exposure [2].

Elucidating the pathophysiology of rhinitis has helped immensely in its pharmacotherapy. In the sensitive individual, exposure to allergen initiates cascade of biochemical and cellular events resulting in synthesis and release of IgE. Subsequent allergen exposure leads to mast cell degranulation causing release of mediators including histamine, tryptase, cysteinyl leukotrienes (CysLT), cytokines (IL-4, IL-5, TNF- α etc.), platelet-activating factor (PAF) and prostaglandins [3]. The late phase response is primarily a cellular event involving a rush of eosinophils, basophils and lymphocytes which in turn become activated and release their mediators promoting nasal congestion, local edema and tissue damage. Pharmacotherapy of rhinitis is multivariate due to the fact that variety of interactive pathways at the base of this disease needs to be targeted. Currently, antihistamines, corticosteroids, mast cell stabilizers, decongestants, anti-leukotrienes and anticholinergics are being successfully used to treat different symptoms of rhinitis.

Due to the complexities of molecular/cellular pathways involved in rhinitis and its differential species symptomatology, it is difficult to model the disease in the laboratory animals. Guinea pigs are usually preferred for studying rhinitis because they mimic human rhinitis, although rats also seem to exhibit rhinitis symptoms on their topical sensitization with allergen [4]. Nabe et al. developed a model of allergic rhinitis using Japanese cedar pollens as a natural allergen and assessed classical H₁-receptor antagonists in passively and actively sensitized guinea pigs [5,6]. Different mediation mechanisms can be attributed to different symptoms in rhinitis model e.g. thromboxane A₂ and peptide leukotrienes in early and late phase nasal blockage [7], histamine H₁ receptors in sneezing and itching [8], histaminergic and cholinergic mechanisms in nasal secretion [9] and tachykinins in sneezing [10]. The molecular mechanisms in animal model of rhinitis or human rhinitis and the parallels between the two are incompletely understood, however, some symptoms of rhinitis such as sneezing, nose rubbing, nasal blockage and rhinorrhea can be modeled in experimental animals [11].

Although sensitization by topical exposure to allergens mimics the human situation more closely, study of rhinitis in animal model by this approach takes time to obtain an appropriate response [12]. In contrast, a prominent response is observed after systemic antigen exposure [4]. Systemic administration of antigen may accelerate the onset of disease in animals.

In the present study, we assessed different variables in ovalbumin-induced rhinitis like dose of ovalbumin, its route of administration or repeated challenge and related other parameters. The effects of oral and topical medications-histamine H₁ receptor antagonists, steroids, mast cell stabilizer, leukotriene antagonist, prostaglandin inhibitor, anticholinergic and nasal decongestant were also studied. The results indicate that the model is symptomatically and pathologically relevant to human rhinitis. It is possible to study modulation of individual disease symptoms in this model to predict the usefulness of new treatments in human rhinitis.

2. Materials and methods

2.1. Animals

Male and female Dunkin Hartley guinea pigs used for the study were provided by Animal Facility of Sun Pharma Advanced Research Company Limited (SPARCL), India. The animals weighed 250–300 g during the sensitization period (age: 4–6 weeks) and 400–450 g at the time of experiments. Animals were group housed (3 animal/cage) in an air-conditioned room at an ambient temperature of 23 $\pm$ 2 °C, 55 $\pm$ 5% relative humidity and room air changes >12/h. The room was maintained with automatic 12 h light/dark cycles, lights on at 7.00 a.m. Animals were provided with a standard laboratory diet (Harland Teklad, UK) and water *ad libitum*. All experiments were performed in accordance with the guidelines of Institutional Animal Ethics Committee of SPARCL.

2.2. Materials

Ovalbumin (chicken egg, Grade V) was purchased from Sigma, St. Louis, USA. Aluminium hydroxide and carboxymethylcellulose were purchased from S.D. Fine Chem Limited and Hi-media Laboratories, respectively. Cetirizine 2HCl, desloratadine, mizolastine HCl, fexofenadine HCl, epinastine HCl, rupatadine HCl, dexamethasone, prednisolone acetate, montelukast sodium and indomethacin were provided by the Organic Synthesis Department of SPARCL. Ovalbumin was dissolved in saline, cetirizine 2HCl was dissolved in distilled water and all other drugs were suspended in 5 mg/ml carboxymethylcellulose at the time of administration. Following drugs were formulated at SPARCL for their topical administrations: azelastine HCl, olopatadine HCl, fluticasone propionate and sodium cromoglycate. Oxymetazoline HCl (Nasivion®, Merck Ltd, India) and ipratropium bromide (Ipramist®, German Remedies Ltd, India) were obtained as marketed preparations. All the formulations were used in their original strengths.

2.3. Sensitization protocol

A procedure for active sensitization as described by Underwood et al. [13] was used with some modifications. Guinea pigs were randomized and made into different groups and sensitized on days 1, 7, 14 and 21 by intraperitoneal injection of ovalbumin (100 μ g/animal) and aluminium hydroxide (5 mg/animal) dissolved and suspended, respectively in 0.9% saline solution. Nonsensitized animals received suspension of aluminium hydroxide in saline. After 7 days of systemic

sensitization, animals were topically instilled with 60 mg/ml, 20 μ l/nostril ovalbumin solution. A week after last sensitization, animals were shaved on dorsal skin area and challenged by intradermal injection of ovalbumin solution (25 μ l of 200 μ g/ml). Successful sensitization was confirmed by edema and redness at the site of injection. To induce rhinitis ovalbumin challenge with 60 mg/ml (20 μ l/nostril) was done one week after topical sensitization in all experiments unless specified.

2.4. Induction of rhinitis

The room where experiments were carried out was free of noise and strong odors and was provided with controlled temperature of 23 ± 2 °C and $55 \pm 5\%$ relative humidity. Animals were placed in Perspex observation boxes (26 $\times$ 26 $\times$ 26 cm) for about 30 min for acclimatization. Observation boxes were thoroughly cleaned for every new animal. The drugs or vehicles were administered orally to overnight fasted animals or intranasally to non-fasted animals, 1 or 3 h before intranasal ovalbumin challenge. The local ovalbumin challenge was performed by instilling ovalbumin solution into both the nostrils using micropipette. Symptoms of rhinitis were observed and scored for a period of 2 h.

Number of sneezing and nose rubbing were counted continuously. Lacrimation and difficulty in breathing were scored. Sneezing was characterized by an explosive expiration just after a deep inspiration [14] and nose rubbing was characterized by an external perinasal scratch with either one or both fore limbs of animal. Lacrimation was scored on a 3-point scale as follows: +—hazy eyes; ++—lacrimation; +++—lacrimation with manifested conjunctivitis. Nasal acoustic phenomenon reflecting nasal congestion/obstruction was evaluated as +—impaired inspiration; ++—nasal crackles; +++—severe breathing impairment [15]. The nasal secretion (watery fluid) discharged through nostrils or spraying on the walls of observation chamber during sneezing was considered as the sign of rhinorrhea. Although it could not be scored, the gross observation was made for each animal.

2.5. Dose—response of ovalbumin-induced sneezing in sensitized guinea pigs

In one set of experiment, different groups of ovalbumin sensitized guinea pigs (systemic sensitization protocol as described above), were challenged with 5–100 mg/ml of ovalbumin solution (20 μ l) by instillation of ovalbumin into both the nostrils with the help of micropipette. The number of sneezing was counted for a period of 2 h. The dose—response of ovalbumin for induction of sneezing was plotted.

2.6. Effect of repeated nasal ovalbumin challenge on sneezing

In order to assess the sneezing behavior in the same animal on frequent challenge with ovalbumin, sensitized animals were challenged with ovalbumin (60 mg/ml, 20 μ l/nostril) repeatedly for 3 times at an interval of 7 days between each challenge. The sneezing response in an individual animal was evaluated.

2.7. Effect of humidity on ovalbumin-induced rhinitis symptoms

To assess the effect of humidity on the induction of rhinitis symptoms, guinea pigs sensitized to ovalbumin were divided into two groups of 6 animals each. Ovalbumin challenge was carried out in two different experimental rooms with a room temperature of 23 ± 2 °C in both the rooms and relative humidity of 50–60% in one room and 80–90% in the other. Rhinitis symptoms were assessed for 2 h.

2.8. Effect of topical sensitization with ovalbumin on sneezing and rubbing frequency

To evaluate the effect of topical sensitization, a group of guinea pigs ($n=8$) was sensitized with ovalbumin 2 times in a week (i.e. day 1 and 4), for two weeks. At every sensitization, number of sneezing and rubbing were counted for a period of 2 h after ovalbumin instillations.

2.9. Evaluation of efficacy of different drugs

Different groups of overnight fasted guinea pigs were orally administered with the histamine H_1 -receptor antagonists cetirizine (10 mg/kg), desloratadine (10 mg/kg), mizolastine (10 mg/kg), fexofenadine (50 mg/kg), epinastine (20 mg/kg) and rupatadine (10 mg/kg); the $CysLT_1$ receptor antagonist, montelukast (10 mg/kg); the prostaglandin inhibitor indomethacin (10 mg/kg) and the glucocorticoid receptor agonists dexamethasone (5 mg/kg) and prednisolone (5 mg/kg). These animals were challenged with intranasal ovalbumin 3 h after dexamethasone and prednisolone or 1 h after other drug treatments. The different symptoms of rhinitis were assessed.

In separate groups of animals, effects of topical treatments were evaluated. Azelastine (1 mg/ml, 10 μ l/nostril), olopatadine (6.65 mg/ml, 10 μ l/nostril), sodium cromoglycate (100 mg/ml, 20 μ l/nostril), oxymetazoline (0.5 mg/ml, 20 μ l/nostril), ipratropium bromide (0.25 mg/ml, 20 μ l/nostril) were administered 1 h before, whereas fluticasone (0.5 mg/ml, 20 μ l/nostril) was given 3 h before ovalbumin challenge.

Prophylactic treatment with some of the drugs was done as follows: intranasal fluticasone for 2 weeks, intranasal sodium cromoglycate for 3 days, twice daily and oral montelukast was for 3 days, once daily. Ovalbumin-induced rhinitis symptoms were assessed 2 h after the last treatment.

2.10. Effect of dosing volume of topical medication on ovalbumin-induced sneezing and rubbing

Systemically sensitized guinea pigs were divided into four different groups ($n=6$). Two groups were instilled intranasally with 10 and 40 μ l solution of olopatadine (6.65 mg/ml), whereas other two groups were instilled intranasally with placebo, 1 h prior to ovalbumin challenge (60 mg/ml, 20 μ l/nostril). Sneezing and nose rubbing were counted in individual animals for a period of 2 h after ovalbumin challenge.

2.11. Measurement of ovalbumin-specific serum IgE titers

On 7th and 14th day after the last booster sensitization with ovalbumin, blood was collected by cardiac puncture. After

coagulation, the serum was separated by centrifugation (7000 rpm for 10 min at 4 °C) and assayed for ovalbumin-specific IgE titers using ELISA assay (AbD seroTec, UK), according to the procedure described by the manufacturer of the kit.

2.12. Measurement of serum IgG titers by homologous passive cutaneous anaphylaxis (PCA)

The PCA reactions were studied by a previously described method [16]. Blood was obtained from ovalbumin sensitized guinea pigs by cardiac puncture. After coagulation, the serum was separated and heated at 56 °C for 4 h to denature the IgE antibodies. A 100 µl of 1, 16 and 64 fold dilutions of serum were injected intradermally into the shaved dorsal back area of nonsensitized guinea pigs. Four hr later, these animals were anesthetized by pentobarbital (50 mg/kg, i.p.) and infused with 1 ml of ovalbumin (10 mg/ml) containing Evan's blue dye (5 mg/ml), through a catheter placed in jugular vein. The skin reaction patch due to extravasation of dye at the site of serum injection were removed and placed in a 3:7 mixture of sodium sulphate (5 mg/ml) and acetone overnight for extraction of dye. The amount of dye in the skin patch of each reaction site was estimated colorimetrically at 620 nm. IgG titers were correlated with extravasation of the dye in the skin. Representative drugs cetirizine (10 mg/kg), montelukast (10 mg/kg) and dexamethasone (5 mg/kg) were given by per oral route 2 h prior to the ovalbumin challenge.

2.13. Measurement of IL-4 concentration in nasal lavage

Guinea pigs sensitized to ovalbumin were intranasally challenged with ovalbumin. Four hours later, animals were anesthetized by pentobarbital (50 mg/kg, i.p.). A cannula attached to the nasopharynx was connected to a perfusion pump (kd Scientific, New Hope, PA, USA) set to deliver a rate of 0.25 ml/min and perfused with Hank's Balanced Salt Solution (500 µl, pH 7.4). Nasal lavage was collected and IL-4 level in the nasal fluid were estimated using ELISA kit (USCN Life Sciences, China), as per the manufacturers instructions. All drugs were administered 2 h prior to ovalbumin challenge.

2.14. Histopathological evaluation of nasal cavity

Guinea pigs were euthanized with high dose of pentobarbital and exsanguinations after 2, 4, 8 and 24 h of ovalbumin challenge. The head was removed and lower jaw was discarded. The nasal cavity separated from skin, muscle and soft tissues was immersed in the freshly prepared 10% neutral buffered formalin for a period of 48 h. Following this, the tissue was rinsed in running tap water and decalcified in 10% nitric acid solution for 5 days. After rinsing in tap water, the tissue was processed for dehydration through graded alcohols and embedded in paraffin. Sections (4 µm) were cut at the level of incisive papillae, first and second palatal ridges and stained with haematoxylin-eosin stains. Sections were examined under a light microscope to obtain a general impression of the histopathologic features of specimen and infiltration of cells in epithelium and subepithelium. The

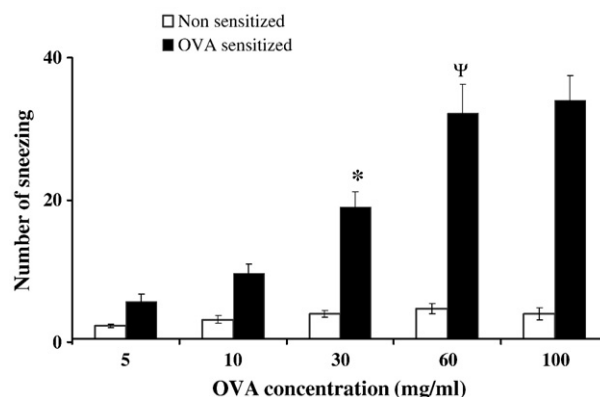


Figure 1 Dose–response of ovalbumin-induced sneezing in actively sensitized guinea pigs after nasal challenge with different ovalbumin concentrations (20 µl/nostril). OVA sensitized: sensitized and challenged with ovalbumin, Nonsensitized: nonsensitized ovalbumin challenged. The numbers of sneezes were assessed for a period of 2 h. Each bar represents mean ± SEM. *Significant compared to 5 mg/ml and 10 mg/ml ovalbumin and ^Ψsignificant compared to 30 mg/ml ovalbumin, $p < 0.05$, ANOVA followed by SNK test.

intensity of inflammatory cell recruitment in the nasal mucosa was graded using an arbitrary scale: Grade 0; not present or very slight: Grade 1; mild: Grade 2; moderate: Grade 3; severe, as described in the literature [17]. Disruption of epithelium observed in individual slides was also noted. All slides were coded before analysis and read blind, to avoid observer bias.

Effects of following treatments on histopathology of nasal mucosa were assessed: dexamethasone (5 mg/kg), fluticasone (20 µl/nostril) administered 3 h prior to ovalbumin challenge, montelukast (10 mg/kg), indomethacin (10 mg/kg), cetirizine (10 mg/kg) and azelastine (10 µl/nostril) administered 1 h prior to ovalbumin challenge.

2.15. Statistical analysis

Mean number of sneezing and rubbing were calculated from individual values of 6 or more number of animals. The data of symptom scores, cytokine, IgG and IgE assays was analyzed for statistical significance by one-way ANOVA and compared with vehicle control or a treatment group by Dunnett's test or SNK (Student–Newman–Keuls) test, respectively. Score of leukocytes infiltration in the nasal mucosa was analyzed by nonparametric Kruskal–Wallis ANOVA and Mann–Whitney *U*-test. The data of effect of humidity on rhinitis symptoms was analyzed by Student's 't' test. Significance was considered at $p < 0.05$. Lacrimation and congestion were rated according to their severity; however these scores were not analyzed for their statistical significance.

3. Results

3.1. Dose–response of ovalbumin-induced sneezing in guinea pigs

In ovalbumin sensitized guinea pigs, topical administration of ovalbumin produced characteristic symptoms of rhinitis. All

Table 1 Effect of repeated ovalbumin challenge on sneezing in actively sensitized guinea pigs

	Number of sneezes in a group of 6 animals					
	1	2	3	4	5	6
1st challenge	32	35	30	32	31	34
2nd challenge	34	36	34	30	36	32
3rd challenge	32	38	32	34	34	37
Mean	32.7	36.3	32.0	32.0	33.7	34.3
SEM	0.7	0.9	1.2	0.8	1.5	1.5
%CV	3.5	4.2	6.3	4.4	7.5	7.3

Animals were intranasally challenged with ovalbumin 60 mg/ml, 20 μ l/nostril at an interval of seven days for three times and number of sneezes were counted for a period of 2 h after each challenge. The intra-individual changes in sneezing response were calculated as percent coefficient of variation.

animals presented with rhinitis symptoms within 5 min of ovalbumin challenge; symptoms peaking between 10 and 30 min, receding thereafter. However, mild reactions were still apparent upto 2 h. Rhinitis was characterized by sneezing (sudden expiration), itching (nose rubbing), lacrimation and congestion (difficulty in breathing). The intensity of symptoms was ranked and the data was analyzed considering twelve intervals of 10 min during 2 h observation period. There was a dose-dependent increase in intensity and frequency of all symptoms at 5–60 mg/ml ovalbumin concentrations. At higher concentration (100 mg/ml) few animals also exhibited anaphylactic reactions such as asphyxia and mild convulsions. Number of sneezes as an index of rhinitis is presented as Fig. 1.

3.2. Effect of repeated nasal ovalbumin challenge on sneezing response

Guinea pigs sensitized to ovalbumin were challenged with ovalbumin 3 times at an interval of 7 days and rhinitis symptoms were rated on every occasion. There was no significant change in intensity, frequency and time-course of the symptoms from first

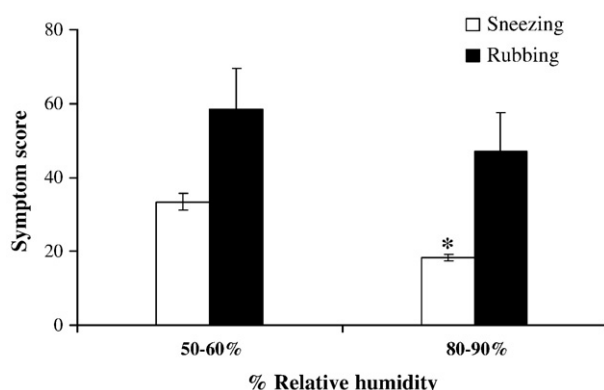


Figure 2 Effect of humidity on ovalbumin-induced rhinitis symptoms in actively sensitized guinea pigs were assessed at varied humidity 50–60% and 80–90% in two different groups. Sensitized animals were challenged with ovalbumin (60 mg/ml, 20 μ l/nostril) and number of sneezing and rubbing were noted for a period of 2 h. Each bar represents mean $\pm$ SEM of 6 animals. *Significant compared to the group kept at a relative humidity of 50–60%, $p < 0.05$, Student's t test.

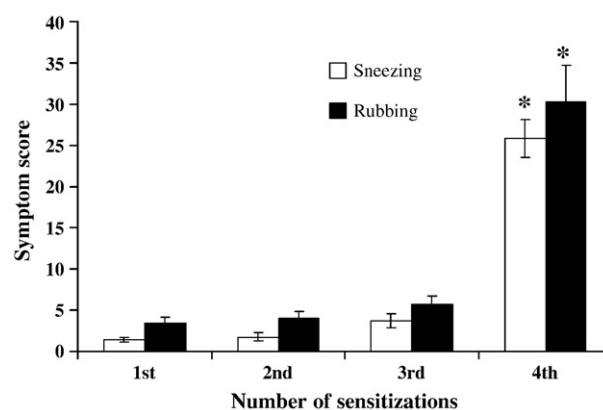


Figure 3 Effect of topical sensitization with ovalbumin on sneezing and rubbing frequency in guinea pigs. Animals were sensitized by intranasal ovalbumin administration (60 mg/ml, 20 μ l/nostril), twice in a week for two weeks and rhinitis symptoms (number of sneezing and rubbing) were assessed for 2 h at every ovalbumin instillation. Each bar represents mean $\pm$ SEM of 6 animals. *Significant compared to 1st, 2nd and 3rd sensitizations, $p < 0.05$, ANOVA, followed by Dunnett's test.

challenge to third challenge, indicating that the response to ovalbumin did not change significantly on 3 repeated administrations. The mean sneezing frequency in different groups was 33.50 and the mean intra-individual coefficient of variation was 5.53% (Table 1). Hence, it is possible to use the same animal for induction of rhinitis at least thrice for the study.

3.3. Effect of humidity on ovalbumin-induced rhinitis symptoms

Increase in humidity during induction of rhinitis (30% increases in relative humidity) caused reduction in the sneezing. The impact of humidity on the induction of sneezing was very significant, although the time-course of response was not altered significantly. In contrast to sneezing, increase in humidity had no effect on nose rubbing (Fig. 2).

3.4. Effect of topical sensitization with ovalbumin on sneezing and rubbing frequency

In one group of guinea pigs, topical sensitization was done twice in a week for two weeks. At third sensitization, weak and inconsistent rhinitis symptoms appeared. However, on the fourth instillation of ovalbumin, all the animals showed significant induction of rhinitis symptoms. Majority of the animals showed anaphylactic reaction as manifested by asphyxia and convulsions. Out of 8 animals in a group, 2 died after an episode of severe anaphylaxis. This data is represented as Fig. 3. Low dose of ovalbumin produced very mild symptoms. The inter-animal variability in the rhinitis response was quite high in low dose sensitization group.

3.5. Effect of oral drug treatments on rhinitis symptoms

Dose–response study of ovalbumin-induced rhinitis showed that 60 mg/ml (20 μ l/nostril) ovalbumin cause optimal induction of rhinitis symptoms. This dose of ovalbumin was selected for studies evaluating the effect of different drug treatments.

Table 2 Effect of oral drug treatments on ovalbumin-induced rhinitis symptoms in actively sensitized guinea pigs

Group	Dose (mg/kg, p.o.)	Frequency/severity of symptoms in 2 h			
		Sneezing	Rubbing	Lacrimation	Congestion
Nonsensitized	—	5.3±1.5	13.8±3.6	+	0
OVA sensitized	—	34.5±2.7 ^ψ	66.7±10.8 ^ψ	+++	+++
Cetirizine 2HCl	10	11.0±2.3*	57.0±14.7	++	++
Desloratadine	10	7.0±2.0*	36.5±0.5*	++	++
Mizolastine HCl	10	11.5±0.5*	69.5±10.5	+	+
Fexofenadine HCl	50	18.5±2.5*	35.5±5.5*	+	0
Epinastine HCl	20	10.3±1.4*	23.3±0.8*	+	0
Rupatadine HCl	10	12.3±2.1*	53.3±10.4	++	+
Dexamethasone	5	6.0±0.7*	38.0±17.6*	0	0
Prednisolone acetate	5	11.0±2.1*	34.3±8.4*	0	0
Indomethacin	10	29.7±2.3	118.0±15.5	+++	+++
Montelukast sodium	10	30.3±8.7	68.5±6.2	++	++
Montelukast sodium (prophylactic)	10	17.0±2.4*	52.0±5.6	0	0

Nonsensitized: nonsensitized ovalbumin challenged, OVA sensitized: sensitized and challenged with ovalbumin (60 mg/ml, 20 µl/nostril). Dexamethasone and prednisolone were administered orally 3 h and other drugs 1 h before ovalbumin challenge and rhinitis symptoms were assessed for 2 h. Prophylactic treatment of montelukast was given for three days (once daily) before challenge. Each value represents mean ± S.E.M. of 10 animals. *Significant compared to OVA sensitized and ^ψsignificant compared to nonsensitized animals, $p < 0.05$, ANOVA followed by Dunnett's test.

Selection of doses of different drugs was based on pilot studies done in our laboratory.

In general, all the H₁ receptor antagonists caused significant inhibition of rhinitis symptoms; however, they differed in the characteristics of their inhibitory effect. Sneezing and lacrimation were significantly inhibited by all antihistamines used in this study. Among different antihistamines, desloratadine, epinastine and fexofenadine were strikingly better effective in reducing nose rubbing. Nasal congestion was reduced to a significant extent by fexofenadine, epinastine, rupatadine and mizolastine. It should be noted that the clinical dose of fexofenadine is higher than other antihistamines.

Acute oral administration of the CysLT₁ antagonist, montelukast (10 mg/kg) and the cyclooxygenase inhibitor indomethacin (10 mg/kg) had only a slight effect on overall symptoms of rhinitis. Both these drugs had moderate effect on sneezing, while

they had no effect on rubbing. Prophylactic treatment of montelukast for a period of three days prior to challenge with ovalbumin caused significant inhibition of sneezing, lacrimation and congestion. However, this treatment had no effect on nose rubbing. Acute oral administration of dexamethasone and prednisolone at 5 mg/kg dose promptly inhibited all the symptoms of rhinitis including sneezing, itching, lacrimation and nasal congestion. This data is represented as Table 2.

3.6. Effect of topical drug treatments on ovalbumin-induced rhinitis

Treatment with topical histamine H₁ antagonists azelastine and olopatadine were effective in inhibiting some symptoms of rhinitis. Azelastine caused significant reduction of sneezing and rubbing. There was a considerable decrease in nasal secretion

Table 3 Effect of topical treatment on ovalbumin-induced rhinitis symptoms in actively sensitized guinea pigs

Group	Dose (µl/nostril)	Frequency/severity of symptoms in 2 h			
		Sneezing	Rubbing	Lacrimation	Congestion
Nonsensitized	—	5.0±1.5	15.5±3.6	+	0
OVA sensitized	—	34.5±2.7 ^ψ	66.7±10.8 ^ψ	+++	+++
Azelastine HCl (1 mg/ml)	10	13.2±3.4*	34.0±6.8*	+	+++
Olopatadine HCl (6.65 mg/ml)	10	8.7±3.7*	33.0±5.8*	+	+
Fluticasone propionate (0.5 mg/ml)	20	31.0±10.0	73.0±3.0	++	0
Sodium cromoglycate (100 mg/ml)	20	13.0±2.9*	68.0±8.4	++	0
Oxymetazoline HCl (0.5 mg/ml)	20	26.8±7.3	57.5±8.5	++	0
Ipratropium bromide (0.25 mg/ml)	20	17.8±3.6*	47.2±5.6	0	0

Nonsensitized: nonsensitized ovalbumin challenged, OVA sensitized: sensitized and challenged with ovalbumin. Fluticasone propionate and sodium cromoglycate were administered (twice a day) prophylactically for 14 and 3 days respectively. Last dose of fluticasone propionate was given 3 h prior, while other drugs were administered 1 h prior to ovalbumin (60 mg/ml, 20 µl/nostril) challenge and rhinitis symptoms were assessed for 2 h. Each value represents the mean ± S.E.M. of 10 animals. *Significant compared to OVA sensitized and ^ψsignificant compared to nonsensitized animals, $p < 0.05$, ANOVA followed by Dunnett's test.

compared to vehicle control animals, however, nasal congestion persisted in all animals. Olopatadine showed superior activity in relieving all symptoms of rhinitis than azelastine.

Prophylactic treatment of fluticasone 14 days prior to ovalbumin challenge had no significant effect on any of the rhinitis symptoms except congestion. Oxymetazoline administered 1 h before ovalbumin challenge showed better inhibition of nasal congestion but had no effect on any other symptoms of rhinitis. Acute intranasal instillation of ipratropium bromide and prophylactic administration of sodium cromoglycate significantly reduced sneezing and congestion. Ipratropium bromide, in addition, decreased lacrimation and nasal secretion (Table 3).

3.7. Effect of dosing volume of topical medication

Olopatadine at 10 μ l/nostril dose caused better inhibition of sneezing and rubbing compared to its 40 μ l/nostril dose. Placebo at both the dose volumes showed similar sneezing and rubbing response. These results are presented in Fig. 4.

3.8. Anti-ovalbumin IgE titers

Sensitized guinea pigs showed marked increase in ovalbumin-specific serum IgE antibody concentration on day 7 and day 14 after the last sensitization, compared to nonsensitized guinea pigs (Fig. 5).

3.9. Serum IgG titers by homologous passive cutaneous anaphylaxis

IgG antibody to ovalbumin was quantified by homologous PCA. Intradermal injection of serum, presumably containing IgG antibody to ovalbumin, induced a distinct skin reaction to intravenous ovalbumin. Extravasations of dye in the skin were inversely proportional to the dilution of serum. Representative drugs from different pharmacological classes caused potent inhibition of dye leakage (Fig. 6). Intradermal injection of serum obtained from nonsensitized animal did not cause any dye leakage. These results along with rise in IgE titers shown by the

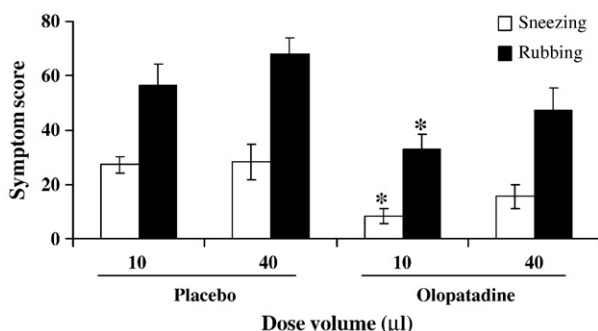


Figure 4 Effect of dosing volume on ovalbumin-induced sneezing and rubbing in actively sensitized guinea pigs. Placebo and olopatadine formulation were intranasally instilled at different dosing volumes—10 and 40 μ l/nostril, 1 hr before ovalbumin (60 mg/ml, 20 μ l/nostril) challenge and sneezing and rubbing response were assessed for 2 h. Each bar represents mean $\pm$ SEM of 6 animals. *Significant compared to same symptom scores of placebo for corresponding volume of administration, $p < 0.05$, ANOVA followed by SNK test.

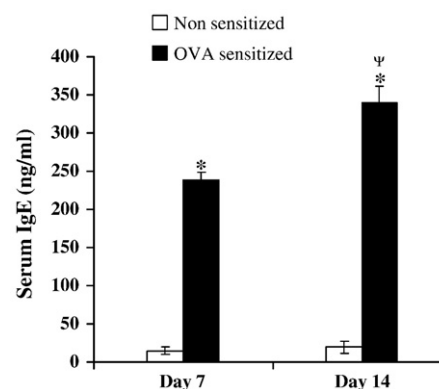


Figure 5 Changes in serum OVA-specific IgE concentration in actively sensitized guinea pigs on day 7 and 14 after last intranasal instillation of ovalbumin. Nonsensitized animals were intranasally treated with saline. Each bar represents mean $\pm$ SEM of 6 animals. *Significant compared to nonsensitized and ψ significant compared to 7-day sensitized animals, $p < 0.05$, ANOVA followed by SNK test.

ELISA confirm that in ovalbumin sensitized animals, the rhinitis is mediated by anti-OVA IgG and IgE antibodies.

3.10. IL-4 concentration in nasal lavage of sensitized guinea pig

IL-4 concentration in nasal lavage of ovalbumin sensitized guinea pigs was significantly higher compared to nonsensitized guinea

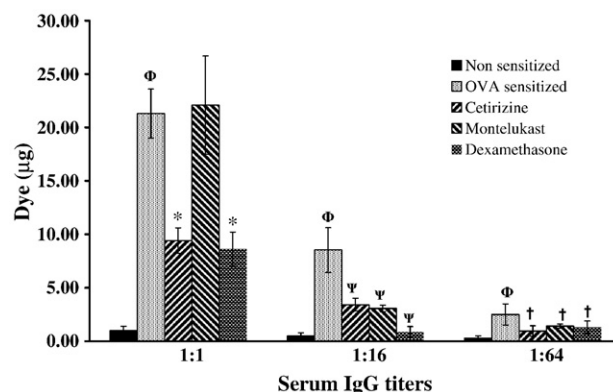


Figure 6 Effect of oral drug treatments on ovalbumin-specific IgG titers in guinea pigs by PCA. A 100 μ l of 1, 16 and 64 fold dilutions of serum collected from sensitized or nonsensitized animals were injected intradermally into the shaved dorsal back area of nonsensitized guinea pigs. Four hr later, these animals were anesthetized by pentobarbital (50 mg/kg, i.p.) and infused with 1 ml of ovalbumin (10 mg/ml) containing Evan's blue dye (5 mg/ml), through a catheter placed in jugular vein. The extravasated dye in the skin patch of each reaction site was estimated colorimetrically at 620 nm. Cetirizine (10 mg/kg), montelukast (10 mg/kg) and dexamethasone (5 mg/kg) were given by per oral route 2 h before the ovalbumin challenge. Each bar represents the mean $\pm$ S.E.M. of 6 animals. Φ Significant compared to nonsensitized animal at respective serum dilutions. Significant compared to OVA sensitized animal at 1:1*, 1:16 ψ and 1:64 $\dagger$ titers, $p < 0.05$, ANOVA followed by SNK test.

pigs at 4 h of antigen challenge. Treatments with intranasal fluticasone and oral dexamethasone and montelukast significantly reduced the level of IL-4 in nasal lavage, when given 2 h prior to challenge. At the same dosing schedule, H₁ receptor antagonist cetirizine and mast cell stabilizer sodium cromoglycate had no significant effect on the IL-4 levels. These results are presented as Fig. 7.

3.11. Time-course of histological changes and effect of drug treatment

Ovalbumin challenge in sensitized guinea pigs induced a marked infiltration of inflammatory cells in the nasal mucosa. Predominant presence of eosinophils at 2, 4, 8 and 24 h of ovalbumin challenge was noted in nasal mucosal epithelium. Apart from eosinophils, mast cells, neutrophils and lymphocytes were also seen infiltrated into the nasal mucosa of ovalbumin treated animals as compared to vehicle control animals. The analysis of time-course revealed that eosinophil infiltration peaked at 4–8 h. A marked disruption of mucosal epithelium was noted at 24 h of ovalbumin challenge. At 2 h of ovalbumin challenge, significant number of mast cells appeared in the epithelium, whereas at 8 and 24 h time point eosinophils were the major infiltrating cells. Oral administration of dexamethasone and montelukast caused potent inhibition of eosinophil infiltration at all time points of ovalbumin challenge; the effect of cetirizine in this regard was marginal at 2 h and even less at later time points. Indomethacin was ineffective in reducing cellular infiltrations. Topical administration of fluticasone caused a mild reduction of eosinophil infiltration, whereas topical ad-

Table 4 Effect of drug treatments on kinetics of histological changes in the nasal mucosa of guinea pig after ovalbumin challenge

Group	Scores of leukocyte infiltration at different time periods after nasal ovalbumin challenge		
	2 h	8 h	24 h
Nonsensitized	0.1±0.1	0.1±0.1	0.3±0.2
Sensitized nonchallenged	0.3±0.2	0.32±0.2	0.29±0.1
OVA sensitized	2.7±0.2 ^ψ	3.0±0.0 ^ψ	2.8±0.2 ^ψ
Cetirizine 2HCl	1.8±0.2*	2.5±0.2	2.8±0.3
Dexamethasone	1.1±0.2*	1.2±0.1*	1.3±0.2*
Indomethacin	2.8±0.1	2.7±0.2	2.8±0.1
Montelukast sodium	1.2±0.1*	1.1±0.1*	1.5±0.2*
Azelastine HCl	2.6±0.2	2.8±0.1	2.8±0.2
Fluticasone propionate	1.8±0.2*	2.4±0.1	2.6±0.2

Nonsensitized: nonsensitized animal challenged with saline, Sensitized nonchallenged: sensitized animal challenged with saline, Sensitized control: sensitized animal challenged with ovalbumin. Dexamethasone (5 mg/kg) and fluticasone (20 µl/nostril) were administered 12 and 3 h prior to ovalbumin challenge. Montelukast (10 mg/kg), indomethacin (10 mg/kg), cetirizine (10 mg/kg) and azelastine (10 µl/nostril) were administered 12 and 1 h prior to ovalbumin challenge. The intensity of inflammatory cell recruitment in the nasal mucosa was graded using an arbitrary scale: Grade 0; not present or very slight: Grade 1; mild: Grade 2; moderate: Grade 3; severe. Each value represents the mean ± S.E.M. of 6 animals. *Significant compared to OVA sensitized and ^ψsignificant compared to nonsensitized animals with respective time points, $p < 0.05$, Mann–Whitney *U*-test.

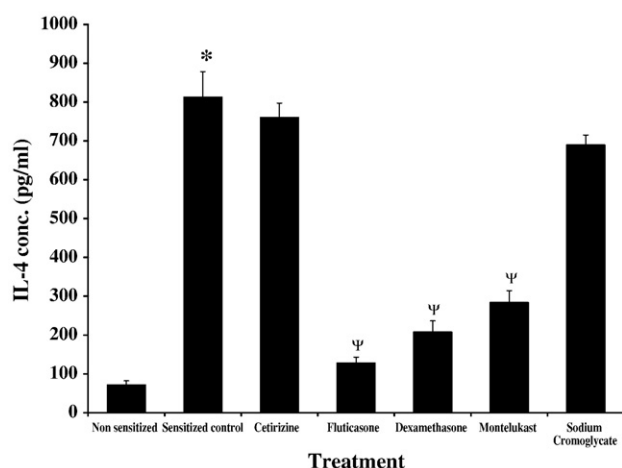


Figure 7 Effect of drug treatments on ovalbumin-induced IL-4 level in nasal lavage fluid. Nasal lavage was collected 4 h after ovalbumin (60 mg/ml, 20 µl/nostril) challenge. Nonsensitized: nonsensitized ovalbumin challenged, OVA sensitized: ovalbumin sensitized and challenged. Fluticasone propionate (0.5 mg/ml) and sodium cromoglycate (100 mg/ml) instilled topically at a dose volume 20 µl/nostril. Dexamethasone (5 mg/kg), cetirizine (10 mg/kg) and montelukast (10 mg/kg) were administered orally 2 h prior to ovalbumin challenge. Each bar represents mean ± S.E.M. of 6 animals. *Significant compared to nonsensitized and ^ψsignificant compared to sensitized animals, $p < 0.05$, ANOVA followed by SNK test.

ministration of azelastine did not reduce eosinophil infiltration. These results are summarized in Table 4 and Fig. 8.

4. Discussion

Allergic rhinitis is characterized by symptoms such as sneezing, itchy nose, rhinorrhea, lacrimation and nasal congestion, which are presumed to be triggered by multiple mediators released from mast cells and other inflammatory cells [18]. Treatment strategies aim either to reduce the effect of local release of mediators from the activated cells or end organ effects of released mediators. The most important pharmacological agents used to treat rhinitis include oral or intranasal H₁ receptor antagonists, intranasal corticosteroids, oral anti-leukotrienes, intranasal mast cell stabilizers, intranasal and oral decongestants and rarely oral corticosteroids and intranasal anticholinergic agents. Several novel drugs are under development for pharmacotherapy of rhinitis. However, studies on animal model of rhinitis are scanty in the literature. Most of the reported studies have attempted sensitization with antigen by topical route, wherein inhalation of antigen is done once or twice in a week for a long duration to induce symptoms. For instance, sensitization with inhalation of ovalbumin may require nine weeks to induce symptoms [15]. In the present study, intranasal dose of ovalbumin did not induce significant response whereas, its high dose led to severe reactions including anaphylaxis on fourth instillation. Systemic sensitization with ovalbumin has been shown to increase IgE levels

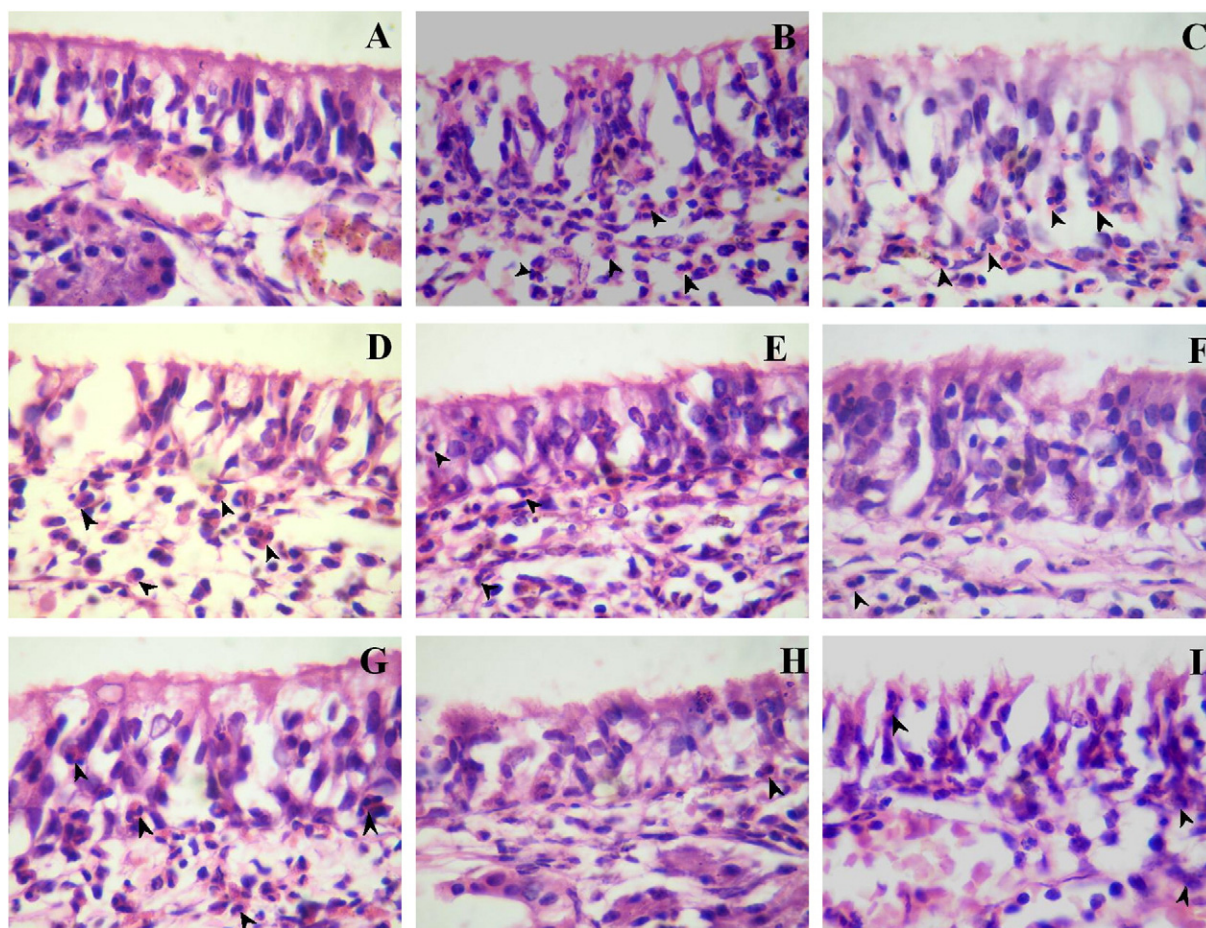


Figure 8 Light photomicrographs of haematoxylin-eosin stained sections of nasal mucosa from the nonsensitized (A) or sensitized guinea pigs at 2 h (B), 4 h (C), 8 h (D) and 24 h (E), after intranasal ovalbumin challenge. No leukocytes were observed in nasal mucosa of nonsensitized animals while there was a marked infiltration of leukocytes, particularly eosinophils in sensitized animals starting from 2 h which peaked at 8 h. Dexamethasone (5 mg/kg), fluticasone (20 μ l/nostril) administered 12 and 3 h prior to ovalbumin challenge. Montelukast (10 mg/kg), cetirizine (10 mg/kg) dosed 12 and 1 h prior to ovalbumin challenge. Dexamethasone (F) and montelukast sodium (H) strongly inhibited eosinophil infiltration at all time points of ovalbumin sensitization. Fluticasone propionate (G) showed a marginal effect. Cetirizine dihydrochloride (I) caused a very mild reduction in eosinophils infiltration at 2 h of ovalbumin administration. Original magnification $\times 100$.

in circulation [5]. Parenteral administration of antigen adsorbed on adjuvant leads to sustained delivery of antigen and more consistent and rapid response. We could induce positive skin reaction as well as increase in OVA-specific IgE on 4 doses of intraperitoneal ovalbumin administration and optimal level of symptoms in the 5th week of sensitization. The response to ovalbumin was dose-dependent at a concentration range of 5–60 mg/ml. These results indicate that availability of antigen in the systemic circulation leads to more prominent reactions and consistent manifestation of symptoms. During sensitization, antigen processing via antigen-presenting cells such as dendritic cells results in T-cell activation and elaboration of specific cytokines. Isotype switching and specific IgE production from B cells mediated mainly by IL-4 is a critical step in the allergic cascade. Although the temporal events in the cascade of inflammation are complex, it is known that within minutes of allergen exposure, sensitized mast cells release preformed and newly synthesized mediators. This early phase response is manifested by sneezing, itching and rhinorrhea. This is followed by

infiltration of other inflammatory cells into the nasal mucosa. Release of additional mediators from the infiltrated cells create a sustained inflammatory reaction with continued inflammatory cell recruitment and constitutes the late phase response. Ovalbumin sensitization and challenge in the present model led to an increase in ovalbumin-specific IgG and IgE titers in plasma, increase in IL-4 concentration in nasal lavage and infiltration of inflammatory cells in epithelium and subepithelium of the nasal mucosa. Hence, the model presented characteristic pathology of rhinitis. Three successive nasal challenges with ovalbumin at one week interval induced sneezing response of similar intensity, frequency and time-course indicating that once sensitized with ovalbumin, the same animal may be utilized for the induction of rhinitis repeatedly.

Environmental variables like temperature, humidity and air pollutants may influence rhinitis considerably. Humid air (95% relative humidity) has been shown to cause inhibition of allergen-induced sneezing, congestion and nasal secretion in patients with rhinitis [19,20]. Increased humidity in nostrils

decrease mast cell activation in early nasal response to an antigen [21]. On the other hand, dry air may irritate the nasal mucosa leading to exacerbation of symptoms [22]. In rabbits sensitized to antigen, dry air regardless of its temperature has been shown to cause marked bronchoconstriction suggesting importance of humidity in the inspired air [23]. Our observation of significant inhibition of sneezing when relative humidity in the room was increased by 30% indicates the sensitivity of the model to recognized variable in human situation.

In the subject sensitized to specific antigen, subsequent challenge with the same antigen leads to cross linking of IgE resulting in release of histamine and other preformed and synthesized mediators from mast cells and basophils. Histamine, by stimulation of the H_1 receptor on trigeminal nerve endings in the nasal mucosa contributes to the induction of sneezing, nasal itching, rhinorrhea and watery eyes [10,24]. Therefore, antihistamines are effectively used to relieve these symptoms. In the present study, acute oral and nasal antihistamine administration primarily caused inhibition of sneezing and to some extent lacrimation. Nasal rubbing was reduced only by desloratadine, epinastine and fexofenadine. It is believed that rubbing (itching) is mediated not only by histamine but also by prostaglandins (PGD_2), leukotrienes and trypase from basophils and mast cells [10]. Desloratadine and epinastine are known to inhibit some of these mediators [25]. Clinically, antihistamines used to manage rhinitis are administered over a period of few weeks to months. However, the present study employed single dose treatment, which may not be adequate to effectively reduce all the symptoms.

Of different antihistamines used in the present study, fexofenadine, mizolastine, epinastine and rupatadine were able to reduce nasal congestion. Apart from histamine, leukotrienes, thromboxane A_2 , PAF and cytokines or other unknown mediators might take part in arbitrating the increase in permeability of nasal mucosal blood vessels leading to nasal congestion [26]. Given that, antihistamines with selective antagonism of only H_1 receptors would not relieve nasal congestion [27], the ability of fexofenadine, mizolastine, epinastine and rupatadine to counter nasal congestion indicates that these drugs might interfere with other mediators apart from H_1 receptors. Rupatadine is orally active dual antagonist of histamine and PAF [28], whereas epinastine also inhibits release of inflammatory mediators from mast cells [29]. Clinical correlation of these effects might, however, need consideration of normally prescribed doses of these agents which may or may not be effective in relieving congestion. Indeed, some studies report that antihistamines have no effect on congestion [27]; other suggests they may reduce nasal congestion significantly [30] but incompletely [31]. More precise evaluation of nasal congestion in the experimental animals can be done by measuring specific airway resistance (sRaw). The present model provides preliminary scoring for undertaking refined studies on this parameter. In congruence with the understanding that antihistamines work mainly on early phase reactions, acute administration of cetirizine did not affect nasal IL-4 levels or eosinophil number.

Sodium cromoglycate acts by preventing extracellular calcium influx into the cytoplasm of mast cells and maintain their physiological integrity, thereby preventing release of different mediators including histamine. In the present study,

prophylactic intranasal administration of sodium cromoglycate significantly inhibited sneezing, lacrimation and congestion, but not nasal rubbing. These results are in agreement with previous studies showing that sodium cromoglycate has weak effect on compound 48/80-induced scratching behavior in mice or antigen-induced rhinitis symptoms [32].

Dexamethasone and prednisolone caused complete attenuation of rhinitis symptoms. Corticosteroids bind to cytoplasmic glucocorticoid receptors which translocate to the nucleus and affect gene expression positively (transactivation) or negatively (transrepression). These effects of corticosteroids may have significance in regulation of inflammatory proteins, leading to reduced survival of eosinophils, T-lymphocytes, macrophages and dendritic cells [33]. However, several other mechanisms for the action of steroids are proposed e.g. treatment with dexamethasone has been shown to increase the activity of neural endopeptidase which degrades the tachykinins that are involved in inducing sneezing and coughing in rhinitis [34,35]. In allergic inflammation, steroids are believed to work on late phase reactions. When used prophylactically, however, steroids can inhibit both early and late phase response to allergen provocation [36]. They are more effective than antihistamines in reducing nasal blockade, sneezing, rubbing and rhinorrhea in humans [37] and inhibit airway resistance and nasal obstruction in animals [7]. Hence, our results on efficacy of oral steroids are in complete agreement with the reported clinical and animal data.

The present model was also suitable to evaluate topical formulations. Whereas, azelastine caused reduction in rhinitis symptoms, except nasal congestion, olopatadine was very effective in overall reduction of symptoms. Azelastine reportedly has no effect on antigen-induced nasal permeability and airway resistance which is considered as an index of nasal congestion [38]. Olopatadine is reported to inhibit rhinitis symptoms [39], prevent the increase of histamine and peptide leukotrienes in nasal lavage fluid in actively sensitized guinea pigs [40], inhibit capsaicin-induced sneezing by blocking tachykinins released from sensory nerves [10] and inhibit nasal blockade in human rhinitis [41]. Inhibition of release of histamine and leukotrienes by olopatadine might play a role in relieving nasal congestion [42,43]. Although both these antihistamines were applied topically, their systemic absorption at the dose level used in this study cannot be ruled out. Incidentally, topical application of fluticasone formulation did not inhibit rhinitis symptoms, except congestion. It has been previously reported that, topical instillation of fluticasone inhibits dye leakage and also shows significant inhibition of sneezing and nasal rubbing after antigen challenge in actively sensitized rats [44,45]. Taken together, results of oral and topical steroids may signify the fact that fluticasone at the low dose levels may have very effect on early phase reaction on its topical treatment. Fluticasone caused inhibition of IL-4 in nasal lavage and inflammatory cell recruitment to nasal mucosa indicating that different pathways needs to be targeted to inhibit early and late phase reactions. Perhaps, polytherapy or combination therapy may tackle the disease more completely.

Dosing frequency, route and volume of administration of different medications may affect nasal responses to an antigen challenge. While designing the protocol for allergic rhinitis model, it is important to note that the intranasal

delivery of medication should be restricted to the nose and should not reach the lungs [46]. Drug substance or excipients used in the topical medications may cause irritation leading to exposure of an antigen or drug to the lamina propria and hence aggravation of response to allergen challenge. In the present study, a volume of 10 μ l/ nostril of olopatadine (6.65 mg/ml) gave better inhibition of sneezing and rubbing compared to its 40 μ l/nostril volume. Therefore, optimization of dose volume of drug for topical use is very important, since the drug formulations inducing irritation of nasal mucosa may show different outcome at higher doses. These parameters can be assessed using the present model.

Acute oral administration of montelukast did not affect rhinitis symptoms, whereas its prophylactic administration caused significant reduction of sneezing, lacrimation, congestion and moderate reduction in nose rubbing. Leukotrienes are known to increase nasal secretion, airway resistance and eosinophilic infiltration which contribute to induction of rhinitis symptoms [47,48]. Accordingly, CysLT₁ receptor antagonists have been reported to modify nasal obstruction in rhinitis and asthma in humans [49] and also chemically-induced coughing in guinea pigs [15]. In the present study, montelukast also decreased IL-4 levels and eosinophil infiltrations. The effects of montelukast on eosinophil recruitment are in agreement with the clinical observations [50].

Prostaglandins (primarily PGD₂) have been widely recognized in variety of inflammatory diseases. Prostaglandins are some of the important products of mast cell degranulation [51]. In this study, the prostaglandin synthesis inhibitor indomethacin had no effect on rhinitis symptoms. It is likely that, inhibition of cyclooxygenase enzyme produced shift of arachidonic acid metabolism to 5-lipoxygenase pathways with consequent increase in leukotriene C₄, D₄ and E₄. In fact, 5-lipoxygenase products such as 5-hydroxyeicosatetraenoic acid can increase preformed mediator (like histamine) from basophils. In any case, inhibition of prostaglandin alone is not adequate to relieve rhinitis symptoms.

Interference with autonomic outflow in the nasal mucosa is important in rhinitis pharmacotherapy, for relief of symptoms like congestion and secretions. While stimulation of α -adrenergic receptors causes vasoconstriction, thus decreasing blood flow and relieving nasal congestion, antagonism of muscarinic receptors may provide reduction in watery secretions. Current therapeutic regimens comprise occasional use of nasal decongestants and rarely anticholinergic agents. In the present study, acute topical treatment of oxymetazoline reduced nasal congestion, without affecting sneezing, rubbing and lacrimation. Oxymetazoline induces vasoconstriction by acting at postsynaptic α adrenergic receptors. Ipratropium bromide, an antimuscarinic agent significantly reduced sneezing, lacrimation, nasal secretion and congestion. Intranasal ipratropium bromide has been shown to reduce rhinorrhea in patient with allergic rhinitis [52]. Decrease of cholinergic outflow causes potent inhibition of symptoms, unfortunately with debilitating side effects.

To summarize, induction of rhinitis in guinea pigs by systemic ovalbumin administration provides with symptoms similar to actual human disease. The model is pathologically relevant to human rhinitis and is also sensitive to clinical pharmacotherapy of rhinitis. The quantification of the individual symptoms is possible in this model due to distinct manifestation of symptoms. Predictability of results, how-

ever, requires close and careful observations and analysis of different parameters. Study of symptoms, cellular and biochemical markers may have differential significance, hence, the data of all parameters needs to be considered in totality for interpretation of results.

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