

Characterization of Anti-Inflammatory Properties and Evidence for No Sedation Liability for the Novel Antihistamine SUN-1334H

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Abstract

Background: The anti-inflammatory potential of antihistamines has significant clinical utility. Long-term pharmacotherapy of so-called 'safe' antihistamines may be hampered by side effects in the central nervous system. In the present study, the new potential antihistamine SUN-1334H was compared with different antihistamines for anti-inflammatory effects, sedation potential and interaction with alcohol. **Meth-**
od: Nasal and skin allergy were induced in guinea pig and mice by ovalbumin sensitization and challenge. Neurogenic nasal inflammation was induced by capsaicin. Sedation potential and interaction with alcohol were assessed by i.v. and intracerebroventricular pentobarbital-induced sedation and alcohol-induced ataxia models. **Results:** Ovalbumin sensitization and challenge caused rhinitis pathology including inflammatory cell infiltration, IL-4, and protein leakage in the nasal lavage fluid (NLF) and presence of inflammatory cells in nasal epithelium. A 5-day treatment of antihistamines reduced these markers of inflammation. SUN-1334H, cetirizine and hydroxyzine caused comparable inhibition of NLF leukocytes, IL-4 and total protein concentrations. Fexofenadine and desloratadine showed moderate inhibition of NLF leuko-

cytes and had no significant effect on IL-4 concentration. While fexofenadine had no effect on total protein concentration, the effect of desloratadine was comparable with the other antihistamines. In neurogenic nasal inflammation induced by capsaicin, SUN-1334H and fexofenadine caused better inhibition at lower and middle dose levels than the other antihistamines. In skin allergy models, SUN-1334H showed potent reduction of passive and active cutaneous anaphylactic reactions. In central nervous system side effects models, SUN-1334H, desloratadine and fexofenadine were devoid of any significant effects. **Conclusions:** The results are suggestive of a high anti-inflammatory to sedation index of SUN-1334H among leading antihistamines.

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Introduction

Antihistamines are widely used in the treatment of allergic rhinitis, urticaria, hay fever, conjunctivitis and pruritis associated with these and several other conditions. They work by inhibiting the action of histamines by competitive binding to H₁ receptors. Histamine is a primary mediator of an early-phase response. However, histamine also modulates the late-phase response that appears several hours after the early-phase response and is characterized by cellular influx of eosinophils, neutrophils, baso-

phils, mononuclear cells, and T lymphocytes [1]. Of these cells, eosinophils are most invariably found infiltrated in the tissues during allergic inflammation and hence considered as hallmark of allergic inflammatory diseases [2–4]. Eosinophils are also the principal cells entering nasal secretions in the early-phase response [5]. Severity of symptoms of allergic rhinitis and asthma can be correlated with the degree of eosinophilia in the nose and sputum [6]. Eosinophils produce various inflammatory mediators including cytokines, chemokines, basic granule proteins, lipid mediators and growth factors capable of inducing airway mucosal damage [7]. In vivo, eosinophil accumulation in allergic diseases has been associated with increased expression of IL-4 and other Th2 cytokines [8–10]. There is direct and indirect evidence from a limited number of animal studies indicating that IL-4 is associated with eosinophil recruitment [11]. In this context, expression of the adhesion molecule VCAM-1 induced by IL-4, alone or together with other cytokines [12], has been associated with relatively selective recruitment of eosinophils and lymphocytes at the sites of allergic inflammation [13]. In vitro studies show that histamine released during the early-phase may act as a stimulatory signal for the production of cytokines and the expression of endothelial adhesion molecules [14, 15]. These effects are dose-dependent and can be affected by H₁ and H₂ receptor antagonists [16]. Hence, treatment of long-term ongoing allergic inflammation with H₁ receptor antagonists can be regarded as an attractive approach, in view of their prospective long-term safety in the clinic. There have been several studies addressed to identification of novel anti-inflammatory properties for this class of drugs [17, 18]. Anti-allergic and anti-inflammatory effects of antihistamines observed in vitro are generally recognized at concentrations that are several hundred-fold higher than would be expected to occur in vivo [19, 20]. However, some antihistamines may show anti-inflammatory effects at therapeutic concentrations [21, 22]. Thus, H₁ antihistamines differ with regard to their anti-allergic and anti-inflammatory potencies. This may help to differentiate the present drugs or newly discovered molecules. In the first part of this study we have compared different antihistamines in animal models of nasal and skin allergy.

In addition to cell-mediated inflammation, dysregulation of nasal afferent pathways leading to neurogenic inflammation may play an important role in pathogenesis of rhinitis and asthma [23, 24]. Activation of C-fibers located in the airway mucosa induces vasodilation, plasma extravasation and mucous secretion [25]. Neuropeptide mediators substance P, neurokinin A, nerve growth factor and CGRP have been implicated in the mediation

of neurogenic inflammation [26]. Antihistamines may modulate neurogenic inflammation as demonstrated in the animal studies [27]. As a part of the present study, different antihistamines were assessed in the capsaicin-induced rhinitis model.

Histamine as a neurotransmitter influences alertness or cortical activation and the sleep/waking cycles through activation of brain H₁ receptors [28], which are ubiquitous throughout the human frontal cortex [29]. All first-generation antihistamines induce sleepiness and impair cognitive performance. Second-generation antihistamines differ in their effects on the central nervous system (CNS) due to their different brain H₁ receptor occupancy [30–32]. In the subjects requiring antihistamine treatment, CNS side effects of these drugs may interfere with daily activities, and have significance due to potential interactions these drugs can have with other CNS drugs or alcohol consumption. For clinical assessment of CNS effects, subjective sleepiness and objective/psychomotor function testing as well as PET measurement of H₁ receptor occupancy by antihistamines has been recommended by the Consensus Group on New-Generation Antihistamines [33]. In preclinical setup, no good animal models are so far available to determine sedative potential of antihistamines. In the present study, we have used an i.v. and intracerebroventricular (i.c.v.) pentobarbital-induced sedation model to assess the sedative potential of antihistamines and an alcohol-induced ataxia model to assess antihistamine-alcohol interactions.

Recently, SUN-1334H was reported as a novel, potent, selective H₁ receptor blocker with no affinity for cholinergic, α -adrenergic or serotonergic receptors. Its fast onset and long duration of action along with excellent efficacy on oral administration in preclinical models of skin and nasal allergy, no interaction with CYP P450 enzymes and lack of CNS side effects make this molecule a valuable candidate for therapy of rhinitis and urticaria [34]. This comparative study showed that SUN-1334H possesses anti-inflammatory activity with no liability for inducing sedation. It is also notable that the present models of anti-inflammatory and sedative/ataxic activity can differentiate among antihistamines which are otherwise difficult to differentiate.

Materials and Methods

Animals

Male and female Dunkin Hartley guinea pigs (300–400 g), male CD1 mice (25–30 g) and female BALB/c mice (25–30 g), bred at the animal facility of Sun Pharma Advanced Research Com-

pany Limited (SPARC Ltd.; Vadodara, India), were used for different studies. During acclimatization and experiments, animals were housed in polypropylene cages, at an ambient temperature of $22 \pm 2^\circ\text{C}$ and relative humidity of 55–60%, air changes more than 12/h and an automatically controlled 12-hour/12-hour light/dark cycle (light intensity 275–300 lx). Animals were allowed free access to a pelleted standard diet (Harlan Teklad Global Diet, UK), except during experiments. Drinking water in water bottles was available ad libitum to all animals. The animal usage in individual studies was approved by the institutional animal ethics committee of SPARC Ltd. The laboratories where experiments were carried out had a low noise level ($<84\text{ dB}$) and the controlled environmental conditions were identical to those of the animal holding rooms.

Materials

Ovalbumin (chicken egg, grade V), aluminum hydroxide gel, Hanks' Balanced Salt Solution (HBSS), pentobarbital sodium, Bradford reagent and capsaicin were purchased from Sigma (St. Louis, Mo., USA). Ethanol was purchased from Spectrochem (Mumbai, India). Carboxymethylcellulose was purchased from Hi-Media Laboratories. SUN-1334H, cetirizine 2HCl, desloratadine, hydroxyzine HCl, fexofenadine HCl and dexamethasone were provided by the organic synthesis department of SPARC Ltd. Ovalbumin was dissolved in saline, SUN-1334H and cetirizine 2HCl were dissolved in distilled water, and all other drugs were suspended in 5 mg/ml carboxymethylcellulose at the time of administration. Capsaicin was dissolved in a mixture of 5% ethanol and 5% Tween 80 and was further diluted with saline to prepare a final concentration of 1 mM. Evans blue dye (Sigma) was dissolved in saline after grinding and sonication. Hematoxylin, Giemsa stain (Qualigens, India) and eosin (SD Fine Chem, India) were obtained as ready-to-use solutions.

Drug doses were based on clinical and experimental animal doses. Data of pilot studies carried out in our laboratories was also utilized in deciding the doses. Names of drugs or their salts are used interchangeably.

Sensitization Protocols

We used a procedure for active sensitization, as described previously [35]. Guinea pigs were randomized and placed in different groups and injected i.p. with 100 μg /animal ovalbumin plus 5 mg/animal aluminum hydroxide gel on days 1, 7, 14 and 21. Non-sensitized animals received suspension of aluminum hydroxide gel. After 7 days of systemic sensitization, animals were topically instilled with 60 mg/ml, 20 μl /nostril ovalbumin solution. A week after the last sensitization, animals were shaved on their dorsal skin area and challenged by the intradermal (i.d.) injection of ovalbumin solution (25 μl of 200 μg /ml). Successful sensitization was confirmed by edema and redness at the site of injection.

For sensitization of the female BALB/c mice, ovalbumin (25 μg /animal) and aluminum hydroxide gel (2.6 mg/animal) was administered on days 1, 7, and 14, followed by a booster dose of ovalbumin (100 μg /animal) and aluminum hydroxide gel (2.6 mg/animal) on day 21. Control animals received aluminum hydroxide gel without ovalbumin. Successful sensitization was confirmed in a few animals in each group by the formation of redness and wheal due to i.d. injection of ovalbumin solution (25 μg /animal). Experiments were carried out 7 days after confirmation of sensitization.

Leukocyte Count in Nasal Lavage of Sensitized Guinea Pigs

Sensitized guinea pigs were intranasally challenged with ovalbumin solution (60 mg/ml, 20 μl /nostril). 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 HBSS (500 μl , pH 7.4). Nasal lavage fluid was collected and centrifuged at 120 g for 10 min. The cell pellet was suspended in 100 μl of ice-cold HBSS, and the cell count was determined using hemocytometer. Aliquots of the cell suspension were transferred to slides and were stained with Giemsa. A differential cell count was performed, based on normal morphological criteria, to determine cell composition of the nasal lavage. Prophylactic treatment of SUN-1334H (0.3–10 mg/kg), cetirizine (0.3–10 mg/kg), desloratadine (0.3–10 mg/kg), fexofenadine (3–100 mg/kg), hydroxyzine (0.3–10 mg/kg), and dexamethasone (1 mg/kg) was performed orally once daily for 5 consecutive days. The last treatment was given 2 h before antigen challenge.

Estimation of Total Protein and IL-4 in Nasal Lavage Fluid

Total protein in the nasal lavage fluid (NLF) was estimated using Bradford reagent. Briefly, 10 μl of nasal lavage fluid was incubated with 250 μl of Bradford reagent for 15 min and intensity of color reaction was estimated at 595 nm for the quantification of protein concentration.

IL-4 estimation in the nasal lavage was performed using ELISA kit (USCN Life Sciences, China), as per the manufacturer's instructions. In brief, the individual samples were incubated with monoclonal antibody against IL-4 for 2 h. An enzyme-linked polyclonal antibody specific for IL-4 was added to the wells. Following wash to remove any unbound antibody-enzyme reagent, a substrate solution was added. The reaction was stopped after 30 min of addition of substrate and the color intensity was measured at 450 nm.

Histopathological Evaluation of Nasal Cavity

At the end of the nasal lavage collection, described above, animals were sacrificed by an overdose of anesthesia. Nasal cavities were removed for histopathological analysis. The head was removed and the lower jaw was discarded. The nasal cavity was separated from skin, muscle and soft tissues and immersed in the freshly prepared 10% neutral buffered formalin for 48 h. Following this, the tissue was rinsed in running tap water and decalcified in 10% formic 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 the incisive papillae, first and second palatal ridges, and stained with hematoxylin-eosin. Sections were examined under a light microscope to obtain a general impression of the histopathological features of the specimen and infiltration of cells in the epithelium and subepithelium. The degree of inflammatory cell recruitment in the nasal mucosa was graded as described in the literature [36]. Disruption of epithelium observed in individual slides was also noted. All slides were coded before analysis and read blind, to avoid observer bias.

Homologous Passive Cutaneous Anaphylaxis Reaction in Guinea Pigs

The passive cutaneous anaphylaxis (PCA) reactions were studied by a previously described method [37]. Blood was ob-

tained 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-, 32- and 64-fold dilutions of serum were injected i.d. into the shaved dorsal back area of nonsensitized guinea pigs. Four hours later, these animals were anesthetized by pentobarbital (50 mg/kg, i.p.) and infused with 1 ml of ovalbumin (10 mg/ml) containing Evans blue dye (5 mg/ml), through a catheter placed in the jugular vein. Areas of the skin reaction patch due to extravasation of dye at the site of serum injection were measured. Skin patches were removed and placed in a 3:7 mixture of sodium sulfate (5 mg/ml) and acetone overnight for extraction of dye. The extract was separated by centrifugation and the amount of dye in each skin reaction patch was quantified colorimetrically at 620 nm. SUN-1334H (1, 3, 10 mg/kg), cetirizine (1, 3, 10 mg/kg), desloratadine (1, 3, 10 mg/kg), fexofenadine (10, 30, 100 mg/kg), and hydroxyzine (1, 3, 10 mg/kg) were given by per oral (p.o.) route, 2 h prior to the ovalbumin challenge.

Active Cutaneous Reaction in BALB/c Mice

Abdominal skin of sensitized mice was shaved. Evans blue dye (2 mg/ml, 100 µl/animal) was injected i.v. and ovalbumin (500 µg/ml, 50 µl/animal) was administered i.d. into the shaved skin area [38]. Twenty minutes after the i.d. injection of ovalbumin, animals were sacrificed and the area of extravasated blue dye was measured. Two hours before ovalbumin skin challenge, all the drugs were administered orally to mice which had been deprived of food for 4h.

Capsaicin-Induced Sneezing in Guinea Pigs

Overnight-fasted guinea pigs were orally administered with SUN-1334H (0.3–10 mg/kg), cetirizine (0.3–10 mg/kg), desloratadine (0.3–10 mg/kg), fexofenadine (10–100 mg/kg) and hydroxyzine (0.3–10 mg/kg). Animals were acclimatized to Perspex observation boxes (26 × 26 × 26 cm) for about 30 min before induction of sneezing. One hour after drug treatment, capsaicin solution (1 mM, 10 µl/nostril) was instilled into both nostrils to induce sneezing and rubbing. The number of sneezes were counted continuously for 30 min. Sneezing was characterized by an explosive expiration just after a deep inspiration [39, 27]. The room where experiments were carried out was free of strong odors.

Sleeping Time by i.v. Pentobarbital in CD1 Mice

Mice were placed in Perspex observation boxes (13.5 × 13.5 cm) for about 30 min for acclimatization. SUN-1334H, cetirizine, desloratadine, fexofenadine and hydroxyzine at 25–150 mg/kg were given orally to animals which had fasted for 4 h. This was followed by a bolus i.v. injection of 35 mg/kg pentobarbital sodium, 1 h after the drug treatments. The sleeping time was assessed as the time period between the loss and recovery of righting reflex for each mouse. Thus, for some animals sleeping time was determined by the addition of several different periods during which there was loss of righting reflex.

Sleeping Time by i.c.v. Pentobarbital in CD1 Mice

One hour before pentobarbital administration, SUN-1334H, cetirizine, desloratadine and hydroxyzine at doses of 10–100 mg/kg and fexofenadine at 30–300 mg/kg were given orally to animals which had fasted for 4 h. Pentobarbital sodium (50 µg/animal)

dissolved in simulated cerebrospinal fluid was injected i.c.v. at 5 µl volume with a 10-µl Hamilton syringe to individual animals, using the direct injection method of Haley and McCormick [40]. Vehicle control animals received simulated cerebrospinal fluid. The sleeping time, determined as described above, was assessed for each animal individually. At the end of all i.c.v. experiments, dilute India ink was injected at the site of pentobarbital injection and the animals were killed immediately. Only the data from those animals showing distribution of ink into the ventricles was utilized for calculations.

Ethanol-Induced Ataxia in CD1 Mice

Mice were trained to ride on an accelerating (acceleration speed 4–40 rpm) rotarod (Ugo Basile, Model 7650) just before the experiments. Only those animals which were able to maintain their position on the rotarod for 300 s without falling off were selected for further experiments. The animals had fasted for 4 h. SUN-1334H, cetirizine, desloratadine (50 mg/kg, p.o.), hydroxyzine (25 mg/kg, p.o.) and fexofenadine (100 mg/kg, p.o.) were given orally and 1 h thereafter ethanol (31.7% v/v) at 10 ml/kg volume was administered i.p. Fall-off time latency on a 7-rpm rotating rod was assessed at 10, 30, 60 and 90 min after ethanol administration.

Statistical Analysis

The data of IL-4, total protein concentration in the NLF, and dye concentrations in the PCA were analyzed using 1-way ANOVA and Dunnett's test. All other data were analyzed using the non-parametric Kruskal-Wallis 1-way ANOVA followed by Mann-Whitney U test. The percent incidence data in i.c.v. pentobarbital-induced sedation experiments was compared with the vehicle control group by the χ^2 test. Significance was considered to be $p < 0.05$.

Results

Effect of Antihistamine Treatment on Ovalbumin-Induced Cellular Infiltration in NLF of Guinea Pigs

Antigen challenge evoked a potent infiltration of inflammatory cells in the nasal secretions. From the pilot studies carried out in our laboratory, it was concluded that infiltration peaked at 4 h of nasal antigen challenge. Abundant eosinophils, lymphocytes, neutrophils and macrophages were observed in the NLF of ovalbumin-sensitized animals. Eosinophils and lymphocytes together formed >60% cells in the NLF. Animals treated with antihistamines once daily for 5 days showed a reduction in eosinophil and lymphocyte infiltration in a dose-dependent manner. Overall, SUN-1334H, cetirizine and hydroxyzine caused a comparable inhibition of nasal leukocytes. Fexofenadine and desloratadine were moderately effective, with lowest doses showing no significant effect on leukocyte counts. None of the antihistamines were able to attenuate the nasal eosinophilia completely at the

highest dose levels. However, dexamethasone, an oral corticosteroid, completely abolished the eosinophilia and provided a good validation tool for the model. These results are presented in table 1.

Treatment Effect on Ovalbumin-Induced Increase in Total Protein and IL-4 in NLF of Guinea Pigs

Nasal ovalbumin challenge in ovalbumin-sensitized guinea pigs caused a greater than 4-fold increase in the total protein and a greater than 18-fold increase in IL-4 content of the NLF. Nasal lavage protein contains serum proteins, such as albumin, which provide the evidence that nasal vascular permeability in the animals challenged with ovalbumin was increased. Prophylactic once daily treatment of SUN-1334H, cetirizine, desloratadine and hydroxyzine at 1 mg/kg for 5 days caused a significant reduction in total protein content. This effect of SUN-1334H was in congruence with earlier reported potent reductions of vascular permeability by this compound [34]. The positive control dexamethasone also caused a marked inhibition of nasal lavage protein content. These results are presented in figure 1.

A significant reduction of NLF IL-4 content was caused by SUN-1334H, cetirizine and hydroxyzine at 10-mg/kg doses. The effect of desloratadine and fexofenadine was not statistically significant. SUN-1334H, cetirizine and hydroxyzine showed highest reduction of IL-4. The results of IL-4 estimation are presented in figure 2.

Treatment Effect on Leukocyte Infiltration in the Nasal Mucosa: Histopathological Evaluation

The kinetics of cellular infiltration in earlier work in our laboratory demonstrated that infiltrations reach a maximum between 4 and 8 h, when the above-mentioned protocol for ovalbumin sensitization and challenge is used [35]. Hence, in the present study a 4-hour time point was chosen for histopathological evaluation of nasal cavities. Ovalbumin challenge in sensitized guinea pigs resulted in extensive eosinophilic inflammation of the nasal mucosa. The response was characterized by presence of inflammatory cells in the interstitial tissues between the blood vessels and surface epithelium. Apart from eosinophils, a marked infiltration of lymphocytes, neutrophils and mast cells was noted in ovalbumin-challenged animals. Antihistamine treatment caused a dose-dependent reduction of eosinophilia. However, these effects were most prominent at 3 and 10 mg/kg doses. SUN-1334H, cetirizine, desloratadine and hydroxyzine were most effective in reducing the inflammatory cell influx. Nasal mucosa of antihistamine-treated animals also

Table 1. Effect of antihistamine treatments on inflammatory cell infiltration in NLF of guinea pigs

Group	Dose mg/kg	Cell number, $\times 10^3$ cells/ml NLF		
		eosinophils	lymphocytes	neutrophils
Nonsensitized		0.1 $\pm$ 0.0	0.2 $\pm$ 0.1	0.5 $\pm$ 0.2
Sensitized		34.8 $\pm$ 1.3 ^b	34.8 $\pm$ 3.7 ^b	11.9 $\pm$ 1.4 ^b
SUN-1334H	0.3	27.9 $\pm$ 1.3 ^a	15.5 $\pm$ 2.1 ^a	4.6 $\pm$ 0.9 ^a
	3	9.6 $\pm$ 0.4 ^a	9.1 $\pm$ 0.7 ^a	5.3 $\pm$ 0.9 ^a
	10	3.4 $\pm$ 0.4 ^a	1.4 $\pm$ 0.2 ^a	3.9 $\pm$ 0.7 ^a
Cetirizine	0.3	32.5 $\pm$ 0.9 ^a	13.0 $\pm$ 1.1 ^a	5.6 $\pm$ 1.7 ^a
	3	5.3 $\pm$ 0.4 ^a	8.9 $\pm$ 1.7 ^a	2.7 $\pm$ 1.0 ^a
	10	3.4 $\pm$ 0.3 ^a	5.2 $\pm$ 0.4 ^a	5.1 $\pm$ 0.8 ^a
Desloratadine	0.3	42.0 $\pm$ 15.2	16.4 $\pm$ 1.42	8.0 $\pm$ 5.2
	3	22.0 $\pm$ 2.5 ^a	12.5 $\pm$ 1.3 ^a	9.0 $\pm$ 0.8 ^a
	10	12.7 $\pm$ 3.4 ^a	8.4 $\pm$ 5.0 ^a	6.2 $\pm$ 2.2 ^a
Hydroxyzine	0.3	23.0 $\pm$ 1.6 ^a	13.4 $\pm$ 2.9 ^a	7.2 $\pm$ 0.8 ^a
	3	13.3 $\pm$ 0.6 ^a	11.5 $\pm$ 1.22 ^a	4.6 $\pm$ 0.8 ^a
	10	6.1 $\pm$ 2.5 ^a	3.0 $\pm$ 2.24 ^a	3.9 $\pm$ 1.05 ^a
Fexofenadine	3	32.5 $\pm$ 2.1	18.4 $\pm$ 4.3	13.4 $\pm$ 1.9
	30	16.9 $\pm$ 0.1 ^a	16.1 $\pm$ 2.2 ^a	5.7 $\pm$ 0.4 ^a
	100	10.7 $\pm$ 9.9 ^a	11.3 $\pm$ 2.8 ^a	3.4 $\pm$ 1.0 ^a
Dexamethasone	1	0.6 $\pm$ 0.3 ^a	14.70 $\pm$ 2.8 ^a	0.6 $\pm$ 0.4 ^a

Data are means $\pm$ SEM of 6 animals. Nonsensitized group was not sensitized to ovalbumin and were challenged. Sensitized control was ovalbumin sensitized and challenged. Different drugs were administered p.o. once daily for 5 days to ovalbumin-sensitized animals. Two hours after last treatment, animals were challenged with ovalbumin (60 mg/ml, 20 μ l/nostril). Nasal lavage was performed after 4 h of ovalbumin challenge.

^a p < 0.05 vs. sensitized controls; ^b p < 0.05 vs. nonsensitized controls. Kruskal-Wallis 1-way ANOVA followed by Mann-Whitney rank sum test.

showed a significant reduction in lymphocytes and mast cell infiltrations. Dexamethasone treatment caused a complete attenuation of cellular influx in the nasal mucosa of ovalbumin treated animals. The photomicrographs of sections of nasal mucosa are presented as figure 3.

Treatment Effect on Serum IgG Titer by Homologous PCA in Guinea Pigs

Ovalbumin sensitization and challenge is known to increase ovalbumin-specific IgG titers in plasma [35]. Animals injected i.d. with the serum presumably containing IgG antibody to ovalbumin showed a distinct dermal allergic reaction to i.v. ovalbumin leading to edema and plasma extravasation in the surrounding tissue. The overall reductions of dye concentration and area of dye extravasation at different dose levels and different se-

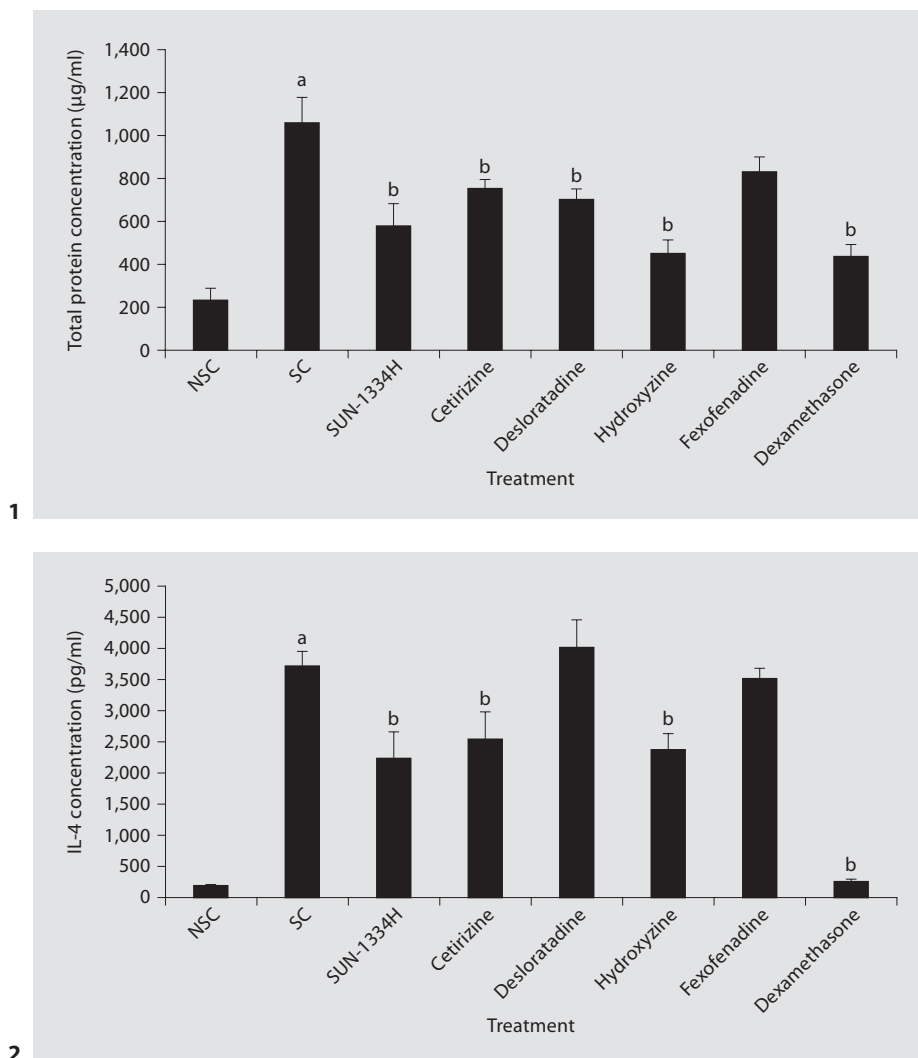


Fig. 1. Effect of antihistamine treatment on total protein concentration in nasal lavage of guinea pig. Nonsensitized control (NSC) = nonsensitized ovalbumin challenged. Sensitized control (SC) = ovalbumin-sensitized and challenged. SUN-1334H, cetirizine, desloratadine, hydroxyzine and dexamethasone at 1 mg/kg and fexofenadine at 10 mg/kg doses were administered orally once daily for 5 days to ovalbumin-sensitized animals. Two hours after the last treatment, ovalbumin challenge (60 mg/ml, 20 µl/nostril) was undertaken, and nasal lavage was collected 4 h after the challenge. Total protein was estimated using Bradford reagent. Each bar represents mean $\pm$ SEM of 6 animals. ^a $p < 0.05$ vs. nonsensitized control. ^b $p < 0.05$ vs. sensitized control. ANOVA followed by Dunnett's test.

Fig. 2. Effect of antihistamine treatment on IL-4 concentration in nasal lavage of guinea pig. Nonsensitized control (NSC) = nonsensitized ovalbumin challenged. Sensitized control (SC) = ovalbumin sensitized and challenged. SUN-1334H, cetirizine, desloratadine, hydroxyzine at 10 mg/kg, fexofenadine at 100 mg/kg, and dexamethasone at 1 mg/kg dose were administered orally once daily for 5 days to ovalbumin sensitized animals. Two hours after the last treatment, ovalbumin challenge (60 mg/ml, 20 µl/nostril) was undertaken, and nasal lavage was collected 4 h after the challenge. IL-4 content was estimated using an ELISA kit. Each bar represents mean $\pm$ SEM of 6 animals. ^a $p < 0.05$ vs. nonsensitized control. ^b $p < 0.05$ vs. sensitized control. ANOVA followed by Dunnett's test.

rum dilutions were calculated by addition of areas and dye concentrations. Antihistamine treatments caused a significant reduction in PCA reaction. All antihistamines showed significant reduction in dye extravasation compared to control. Among different antihistamines used in

this study, the highest overall reduction of skin extravasation was offered by SUN-1334H. At lower serum IgG concentrations, all antihistamines exhibited similar inhibition of dye extravasation. These results are presented in table 2.

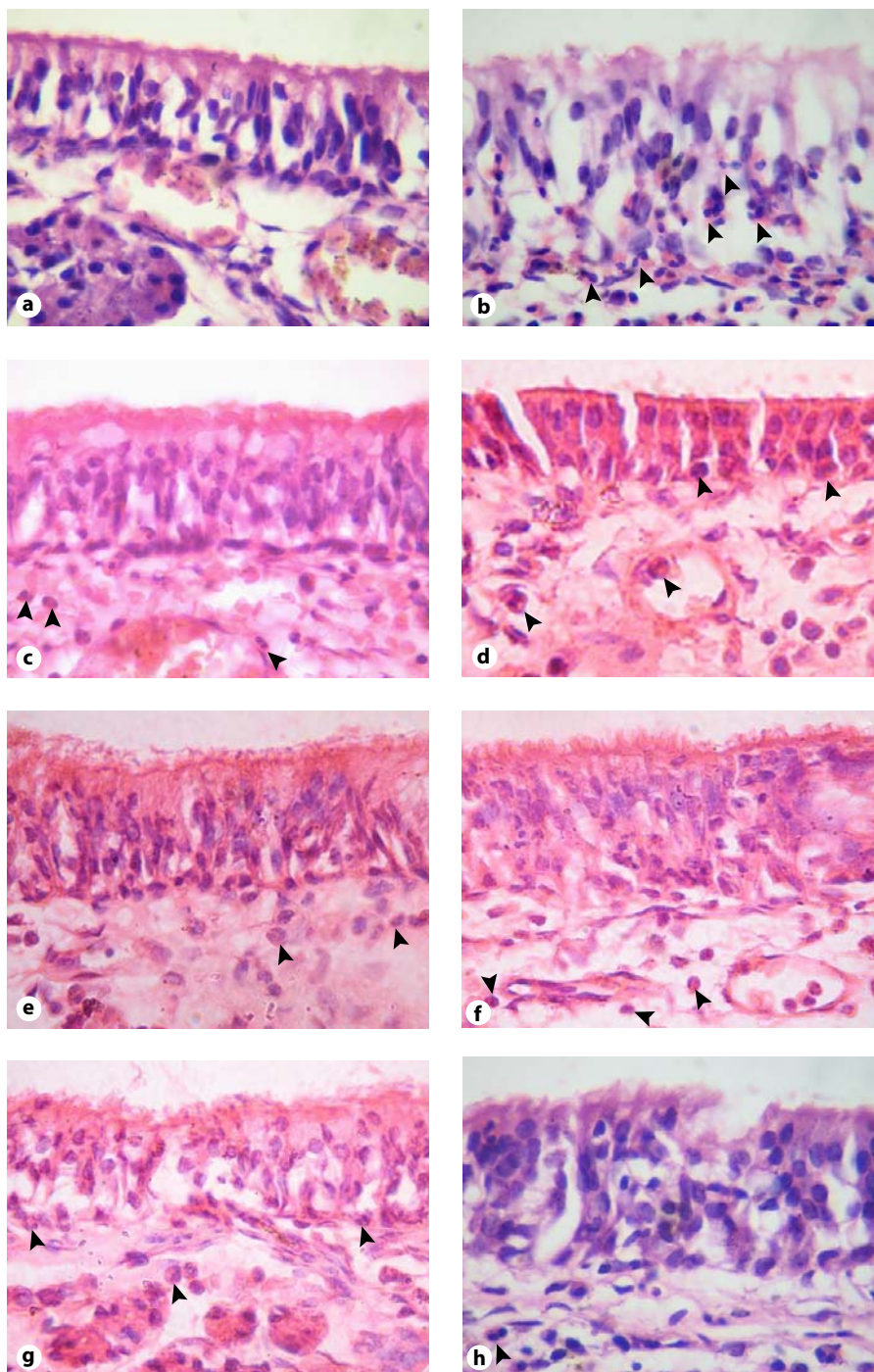


Fig. 3. Light photomicrographs of hematoxylin-eosin stained sections of nasal mucosa from the nonsensitized (**a**) and sensitized guinea pigs (**b**) at 4 h after i.n. ovalbumin challenge. No leukocytes were observed in nasal mucosa of nonsensitized animals while there was a marked infiltration of leukocytes, particularly eosinophils in sensitized animals at 4 h. Results shown in these images indicate reduced infiltration of eosinophils with SUN-1334H (**c**), cetirizine (**d**), desloratadine (**e**), hydroxyzine (**f**) 3 mg/kg, fexofenadine (**g**) 30 mg/kg, and dexamethasone (**h**) 1 mg/kg administered orally, once daily for 5 days. Original magnification $\times 100$.

Treatment Effect on Skin Reaction Induced by Ovalbumin in Actively Sensitized BALB/c Mice

Sensitized female BALB/c mice showed positive skin reaction when challenged i.d. with ovalbumin. Extravasation of Evans blue dye at the site of injection was observed within 5 min of antigen challenge. Distinct visu-

alization of the dye spot was observed after 15–20 min of antigen challenge. SUN-1334H and desloratadine showed inhibition of dye leakage at the site of antigen challenge, which was higher than cetirizine and fexofenadine. These results are presented in table 3.

Table 2. Effect of antihistamine treatment on ovalbumin-specific PCA reactions in guinea pigs

Group	Dose range mg/kg	Dye extracted from the skin μ g	Area of skin dye leakage mm^2
Vehicle	–	247.2 $\pm$ 12.3	3,737.7 $\pm$ 80.4
SUN-1334H	1–10	139.0 $\pm$ 9.6 ^a	2,109.4 $\pm$ 61.2 ^b
Cetirizine	1–10	152.7 $\pm$ 7.5 ^a	2,588.0 $\pm$ 66.8 ^b
Desloratadine	1–10	170.0 $\pm$ 6.2 ^a	2,423.7 $\pm$ 68.5 ^b
Hydroxyzine	1–10	197.4 $\pm$ 8.1 ^a	2,417.3 $\pm$ 59.0 ^b
Fexofenadine	10–100	160.8 $\pm$ 5.2 ^a	2,299.0 $\pm$ 84.2 ^b

Data are means $\pm$ SEM of 6 animals. Dilutions of serum (1:1, 1:16, 1:32 and 1:64) of ovalbumin-sensitized animals were injected i.d. (100 μ l/site) on dorsal surfaces of nonsensitized animals. Four hours after dermal sensitization, animals were challenged with ovalbumin solution (10 mg/ml, 1 ml/animal) containing Evans blue dye (5 mg/ml). SUN-1334H, cetirizine, desloratadine, hydroxyzine and fexofenadine were given p.o. 2 h before ovalbumin challenge. Sum of wheal diameters and amount of dye extravasations over the different dilutions and dose ranges were calculated.

^a $p < 0.05$ vs. vehicle control, ANOVA followed by Dunnett's test; ^b $p < 0.05$ vs. vehicle control, Kruskal-Wallis 1-way ANOVA followed by Mann-Whitney rank sum test.

Table 3. Effect of antihistamines on ovalbumin-induced active cutaneous anaphylaxis in female BALB/c mice

Treatment	Dose mg/kg	Wheal diameter mm
Nonsensitized	–	0.0 $\pm$ 0.0
Sensitized	–	109.6 $\pm$ 4.5
SUN-1334H	0.03	91.4 $\pm$ 17.3
	0.3	63.7 $\pm$ 6.4*
	0.6	51.7 $\pm$ 13.2*
	1.8	18.1 $\pm$ 2.0*
Cetirizine	0.03	98.5 $\pm$ 8.6
	0.3	80.1 $\pm$ 11.6*
	0.6	62.1 $\pm$ 5.0*
	1.8	29.3 $\pm$ 10.0*
Desloratadine	0.03	88.9 $\pm$ 1.1*
	0.3	92.3 $\pm$ 3.5*
	0.6	36.1 $\pm$ 6.7*
	1.8	14.7 $\pm$ 1.1*
Hydroxyzine	0.03	94.7 $\pm$ 3.5
	0.3	75.3 $\pm$ 7.8*
	0.6	54.2 $\pm$ 6.1*
	1.8	16.2 $\pm$ 5.4*
Fexofenadine	3	71.3 $\pm$ 7.0*
	6	73.6 $\pm$ 9.2*
	12	39.1 $\pm$ 11.1*
	18	20.3 $\pm$ 13.1*

Data are means $\pm$ SEM of 12–15 animals. Nonsensitized animals were not sensitized to ovalbumin and were challenged. Sensitized animals were ovalbumin sensitized and challenged. Cutaneous reaction was induced by i.d. challenge of ovalbumin (500 μ g/ml, 50 μ l/animal) and quantified by Evans blue dye leakage. All treatments were given p.o. 2 h before the ovalbumin challenge.

* $p < 0.05$ vs. sensitized control group, Kruskal-Wallis 1-way ANOVA followed by Mann-Whitney rank sum test.

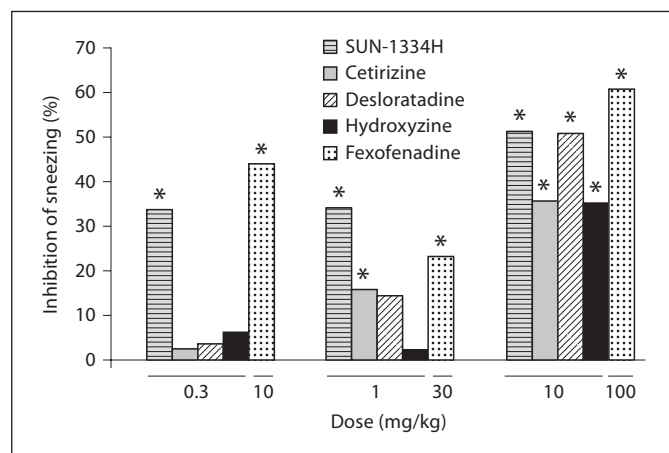
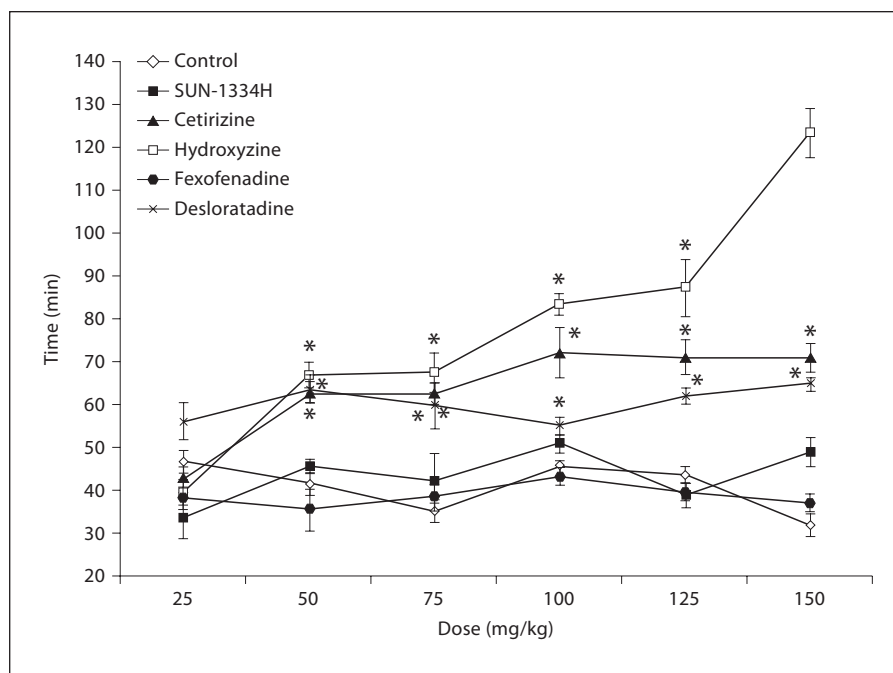


Fig. 4. Effect of antihistamine treatment on capsaicin-induced sneezing in guinea pigs. Intranasal instillation with capsaicin solution (1 mM, 10 μ l/nostril) induced sneezing in animals. Drugs were administered orally 1 h prior to intranasal capsaicin instillation. The number of sneezes were counted for 30 min. Each bar represents mean $\pm$ SEM of 6 animals. Data represented as percent inhibition of sneezing as compared to vehicle control group. * $p < 0.05$ vs. vehicle control group. Kruskal-Wallis 1-way ANOVA followed by Mann-Whitney rank sum test.

Inhibition of Capsaicin-Induced Sneezing in Guinea Pigs

Intranasal instillation of capsaicin solution (1 mM) caused a rapid induction of sneezing. This behavior reached to a peak within 3–4 min, receding thereafter and persisting for around 20–25 min. Acute antihistamine pretreatment caused a significant and dose-dependent reduction of sneezing. At 0.3- and 1-mg/kg doses, the inhibitory effect of SUN-1334H was higher than other antihistamines. Cetirizine showed significant effect at 1- and 10-mg/kg doses. While desloratadine and hydroxyzine showed significant inhibition of sneezing at 10-mg/kg dose. Lower doses of these drugs were not effective. Fexofenadine was effective at 10–100-mg/kg dose levels. These results are shown in figure 4.

Fig. 5. Effect of antihistamine treatment on intravenous pentobarbital-induced sedation in mice. Drugs were administered p.o. 1 h prior to pentobarbital bolus infusion. Sleeping time was assessed for an individual animal. Each point on the graph represents mean $\pm$ SEM of 8 different animals. * $p < 0.05$ vs. vehicle control. ANOVA followed by Mann-Whitney rank sum test.



Modulation of i.v. Pentobarbital-Induced Sedation in Mice

Intravenous administration of pentobarbital caused an immediate loss of the righting reflex, with control sleeping time latency ranging between 32 and 47 min. SUN-1334H and fexofenadine did not prolong pentobarbital-induced sleeping time at any of the doses studied. Hydroxyzine caused a dose-dependent increase in sleeping time. The potentiating effect of hydroxyzine was statistically significant at doses of 50–150 mg/kg. At these dose levels, cetirizine and desloratadine also caused a significant prolongation of sleeping time. However, cetirizine and desloratadine-induced potentiation of sedation was not dose-dependent. These results are shown in figure 5.

Modulation of i.c.v. Pentobarbital-Induced Sedation in Mice

Central administration of pentobarbital precludes its pharmacokinetic interaction with other drugs. Such a method has been successfully used to demonstrate potentiation of CNS depressant activity of other drugs. Intracerebroventricular administration of pentobarbital (50–175 μ g/animal) in mice caused a dose-dependent induction of sedation within a few seconds of its administration, as reported by the previous workers [41]. A dose of 50 μ g/animal was selected for interaction studies because this dose induced a short duration of sedation allowing

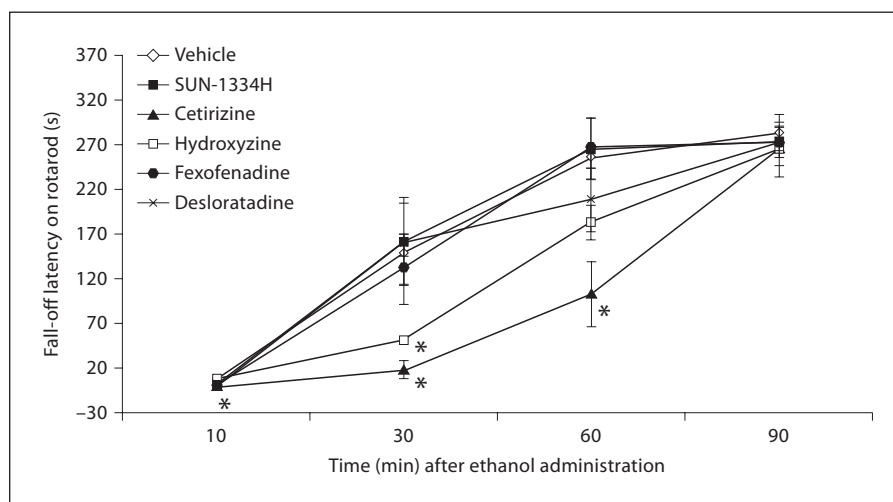
Table 4. Sedation potential of antihistamines assessed by potentiation of pentobarbital-induced sleeping time in mice

Treatment	Dose mg/kg	Sedation time, s	% incidence of sleeping ≥ 5 min
Control	–	226 $\pm$ 15	22.20
SUN-1334H	10	213 $\pm$ 16	20.00
	30	173 $\pm$ 23	0.00
	100	264 $\pm$ 42	35.7
Cetirizine	10	278 $\pm$ 17	40.00
	30	271 $\pm$ 56	25.00
	100	349 $\pm$ 40 ^b	69.23 ^a
Desloratadine	10	188 $\pm$ 41	14.29
	30	174 $\pm$ 49	12.50
	100	174 $\pm$ 36	12.50
Hydroxyzine	10	272 $\pm$ 24 ^b	20.00
	30	386 $\pm$ 58 ^b	75.00 ^a
	100	435 $\pm$ 138 ^b	66.67 ^a
Fexofenadine	30	207 $\pm$ 28	20.00
	100	213 $\pm$ 46	20.00
	300	217 $\pm$ 23	20.00

Data are means $\pm$ SEM. Drugs were administered p.o. 1 h prior to pentobarbital (50 μ g/animal i.c.v.). Latency period of sedation was assessed for each individual animal. Percentage incidence of sleeping time ≥ 5 min was calculated based on number of animals showing ≥ 5 min of sedation in each group.

^a $p < 0.05$ vs. respective control groups, χ^2 test; ^b $p < 0.05$ vs. respective control groups, Mann-Whitney rank sum test.

Fig. 6. Effect of antihistamines on ethanol-induced motor incoordination in mice. Mice trained to ride on a rotating rod were administered p.o. with drugs followed by ethanol (31.7% v/v, 10 ml/kg). Fall-off latencies of individual animals were measured after 10, 30, 60 and 90 min of ethanol administration. Each point on the graph represents mean $\pm$ SEM of 7 different animals. SUN-1334H, desloratadine, and cetirizine were dosed at 50 mg/kg, fexofenadine at 100 mg/kg and hydroxyzine at 25 mg/kg. Vehicle control was animals treated with distilled water as vehicle and ethanol. * $p < 0.05$ vs. control. ANOVA followed by Mann-Whitney rank sum test.



possible prolongation of sedation by antihistamines to be assessed. Pilot studies in our laboratory supported this notion.

The mean control sedation time latency for the vehicle control group was 226 s. SUN-1334H, fexofenadine and desloratadine had no effect on sedation induced by i.c.v. pentobarbital administration. Hydroxyzine at 10–100-mg/kg doses caused a significant increase in pentobarbital-induced sedation. Such an effect of cetirizine was significant only at the 100-mg/kg dose. Incidence-wise analysis of data also corroborated this conclusion. Thus, percentage of animals showing more than 300 s sedation (incidences of >300 s sedation) were highest in hydroxyzine- (30 and 100 mg/kg) and cetirizine- (100 mg/kg) treated groups. These results are presented in table 4.

Treatment Effect on Ethanol-Induced Ataxia in CD1 Mice

Intraperitoneal administration of ethanol induced muscular incoordination, as indicated by the inability of animals to stride on a rotating rod. There was a rapid onset of ataxia after ethanol administration. The falling off latency in the vehicle control group indicated a significant effect of ethanol up to 30 min after its administration. SUN-1334H, desloratadine and fexofenadine pretreatment did not affect ethanol-induced ataxia. Cetirizine and hydroxyzine caused a significant potentiation of ethanol-induced ataxia. The falling off latencies in the cetirizine- and hydroxyzine-treated groups were lower than control at 60 min, indicating that these drugs prolonged the effects of ethanol. These results are shown in figure 6.

Discussion

Antihistamines are widely used for the treatment of dermal and upper respiratory allergies. They work by acting as inverse agonists at histamine H_1 receptors, leading to inhibition of signaling pathways IP_3 , DAG, and Ca^{2+} ions. The pharmacological and safety profiles of antihistamines vary, although they appear similar in their clinical efficacy. Preclinical comparative studies to understand differentiating characteristics of these drugs are scanty. Such an understanding would be useful for development of newer drugs as well as correct usage of existing drugs.

The anti-inflammatory and anti-allergic properties of antihistamines can provide added advantage in terms of prophylactic and therapeutic use of these drugs. Currently marketed antihistamines show anti-inflammatory effects at varying dose levels. While levocetirizine and cetirizine show anti-inflammatory effects at therapeutically relevant concentrations [21, 22], effects of other drugs may be relevant only at concentrations that are not achieved normally with the highest approved therapeutic doses. Hence, antihistamines providing anti-inflammatory effects at therapeutic dose levels may be advantageous. Although higher doses may be required for anti-inflammatory effects, the dosing is limited by the side effects of these drugs on the CNS and cardiovascular system. Hence, drugs with high safety margins would be preferred.

SUN-1334H is a highly selective H_1 receptor antagonist with efficacy in allergic animal models on oral administration. The molecule has no potential for anticholinergic

linergic side effects as evidenced by lack of muscarinic or nicotinic receptor binding in recombinant receptor binding assays, and its inability to effect isolated tissue contractions induced by the cholinergic agonist carbachol [34]. It has high plasma protein binding in both animals and humans (83–92%) and a low volume of distribution (0.7–0.8 l/kg; unpubl. data), which may entail a lesser risk of toxicity. In cardiovascular system safety models, SUN-1334H shows no effect on hERG channels or in dog ECG, indicating its good cardiovascular safety profile [34].

In the present study, SUN-1334H, cetirizine, and hydroxyzine showed similar effects in reducing nasal infiltration of eosinophils and lymphocytes; fexofenadine, and desloratadine were less effective in these parameters. Histology of the nasal cavity also reflected similar findings. It has been proposed that some antihistamine-induced anti-inflammatory actions may be receptor dependent while others are receptor independent. Inhibition of mast cell and basophil release of histamine are independent of H_1 receptor antagonism, while adhesion protein expression and migration of inflammatory cells may be secondary to H_1 receptor inhibition [20]. Histamine by its action on H_1 receptors has been shown to activate NF- κ B, an important transcription factor that binds with promoter/enhancer regions of genes regulating production of a number of pro-inflammatory cytokines and adhesion molecules [42]. Antihistamines down-regulate activation of NF- κ B to inhibit inflammatory cell accumulation and activation [43]. Cetirizine and azelastine have been shown to down-regulate NF- κ B expression in parallel with inhibition of generation of the cytokines, IL-1 β , IL-6, IL-8, TNF- α and GM-CSF [44, 45]. Antihistamine-induced NF- κ B down-regulation may also lead to inhibition of expression of adhesion molecules such as ICAM-1 [46]. In clinical studies, conjunctival ICAM-1 expression was reduced by cetirizine, azelastine, loratadine, levocabastine [19, 47] and nasal ICAM-1 secretion was reduced by cetirizine and loratadine in seasonal allergic rhinitis patients [19].

Receptor-independent effects of antihistamines consists of inhibition of de novo generation of proinflammatory products such as superoxide radicals, the arachidonic acid products, LTB₄ and LTC₄, and the release of granule associated products such as neutrophil elastase and eosinophil cationic protein. These effects are suggested to be mediated by calcium mobilization and inhibition of the activity of membrane associated enzymes such as protein kinase C and NADPH oxidase [20]. However, these effects require high drug concentrations, which do not correspond to therapeutic dosages.

SUN-1334H, hydroxyzine and cetirizine induced a greater reduction of NLF IL-4 than other antihistamines. IL-4 is implicated in allergic inflammatory disorders such as allergic rhinitis and asthma in humans [48, 49] and in the induction of eosinophils in the allergic inflammatory response in guinea pigs and mice [35, 50]. IL-4 is the product of activated CD4⁺ T lymphocytes of the Th2 subset and is also released by human mast cells, basophils and eosinophils. It has been shown that cultured endothelial cells activated with IL-4 express VCAM-1, which is responsible for increased adherence of eosinophils, basophils and lymphocytes, but not neutrophils [51]. IL-4 has also been suggested to accumulate eosinophils in rat skin. In asthmatic and atopic subjects there is a correlation between tissue eosinophilia and the presence of cells expressing IL-4 mRNA. For NLF IL-4 inhibition, a 100-mg/kg dose of fexofenadine and 10-mg/kg dose of other antihistamines was used. These are indeed high doses compared to antihistamine doses. Therefore, it will be interesting to assess the anti-inflammatory potential of these drugs at lower doses and when administered for a prolonged period. Clinically, levocetirizine and desloratadine given for 2 weeks have been shown to cause reduction of IL-4 in human subjects [52]. Nevertheless, the present study indicates that SUN-1334H has more potential to reduce IL-4 concentration than has cetirizine. Our previous study showed that acute administration of antihistamines does not reduce IL-4 and eosinophil infiltration in the NLF, unlike corticosteroids [35]. Repeated administrations of antihistamines cause reduction of IL-4 and eosinophilia. These effects are most likely mediated through NF- κ B modulation by antihistamine treatment, as discussed above. Thus, a long-term treatment with safe antihistamines may be beneficial in reducing the inflammation through major pathways like NF- κ B. Indeed, the benefit of long-term clinical administration of antihistamines as prophylaxis for allergic diseases such as rhinitis, asthma and other atopic conditions has been proposed [53]. Since higher doses of antihistamines may be required to achieve anti-inflammatory effects, H_1 antagonists with the widest therapeutic window (i.e. with high anti-inflammatory activity and low potential for dose-limiting sedation and cognitive impairment) may offer greatest therapeutic potential.

Passive cutaneous anaphylaxis is a model of allergic inflammation characterized by formation of local edema at the site of antigen challenge. PCA reaction is mediated by the IgE and IgG antibodies. Passive sensitization and challenge of guinea pigs with serum containing IgG resulting in increased microvascular permeability is due to

locally generated mediators such as histamine, PAF, LTC₄, LTD₄, serotonin and several others [54]. Antihistamine produces varying degrees of inhibition of PCA reaction. In both dermal allergic models (PCA in guinea pig and active cutaneous anaphylaxis in mice), SUN-1334H showed a potent effect that was significantly better than desloratadine and fexofenadine. The efficacy of antihistamines in urticaria is attributed to their H₁ antagonistic activity on histamine receptors located on the skin nerve C-fibers and the endothelium of the postcapillary venules, which reduces itching and extravasation, respectively, causing reduction of wheal formation [55]. SUN-1334H has been shown to manifest potent reduction of histamine-induced wheal in beagle dogs. Inhibition of wheal in this model was better than the leading drugs in the therapeutic class in terms of duration and potency [34]. The present study extended these effects in allergic skin models, which are considered to be more disease-oriented models of this human condition. Better skin distribution of SUN-1334H may explain its excellent efficacy in skin models of allergy. SUN-1334H may prove an important therapeutic modality for the treatment of skin allergy.

Intranasal administration of capsaicin has been shown to induce rhinorrhea, congestion, sneezing and nasal burning in humans [56, 57], and sneezing, lacrimation, rhinorrhea and rubbing in guinea pigs [27]. Stimulation of capsaicin-sensitive sensory fibers is known to induce numerous peptide mediators which in turn act on mast cells, immune cells and vascular smooth muscle cells producing inflammation. Capsaicin activates ion channel VR1 receptors localized on small-diameter sensory nerves. This activation results in opening of nonselective cation channels, increasing tryptase, an enzyme localized to mast cells. Although numerous substances are released from capsaicin-sensitive sensory neurons, substance P and other tachykinins (e.g. CGRP) are the major mediators of neurogenic inflammation [58]. In this study, SUN-1334H potently inhibited capsaicin-induced sneezing. Olopatadine has been shown to reduce similar effects of capsaicin in guinea pig [27]. It is not possible to establish whether capsaicin-induced sneezing is mediated directly by sensory peptides or by histamine in this model. Hence, effects on tryptase, substance P or tachykinins needs to be evaluated. Capsaicin-sensitive neurons may participate in the pathogenesis of allergic/vasomotor rhinitis.

Histamine as a neurotransmitter in the brain interacts with histamine receptors and is implicated in the sleep/wake cycle, attention, learning memory and regulation of appetite [28]. These varied activities of histaminergic system are mediated by the brain H₁ receptors which have a

wide distribution throughout the CNS. Occupancy of central H₁ receptors by antihistamines depends on their ability to cross the blood-brain barrier. The central H₁ receptor antagonism leads to adverse effects in the CNS, such as sedation/drowsiness. Some second-generation antihistamines cause low sedation due to their low cerebral H₁ receptor occupancy. The Consensus Group on New-Generation Antihistamines states that H₁ receptor occupancy measured by PET (which should be under 20%) and objective and psychomotor functions are the most important approaches to label a compound as non-sedative, apart from more variable subjective sleepiness [33]. CNS-related side-effect assessment in clinical or nonclinical studies have complexities associated with subjectivity, limitation of techniques and disease characteristics. Rhinitis itself can induce drowsiness and may interfere with assessment of sedation by an antihistamine during the clinical trials.

SUN-1334H had no significant effect on pentobarbital-induced sedation. Pentobarbital induces sedation through modulation of opening of GABA-Cl⁻ channels [59]. In this model, hydroxyzine showed dose-dependent increase of sedation; potentiation of sedation by cetirizine and desloratadine did not increase with increasing dose. Fexofenadine was devoid of effect on pentobarbital-induced sedation. These results show correlation with sedation potential of these clinically available antihistamines. Hence, this model can be considered as reasonably predictable. Pentobarbital is a substrate for CYP2A6 and 3A4 and hence probability of PK interaction in the potentiation of its effects by antihistamines cannot be ruled out. Therefore, sedation potential was also evaluated in the i.c.v. pentobarbital model. In this model, hydroxyzine and cetirizine were the only antihistamines causing potentiation of sedation, supporting the results of peripheral pentobarbital administration. Percentage incidence of sedation was also highest in hydroxyzine followed by cetirizine treated groups.

This study provides evidence for a better tolerance profile of SUN-1334H, which may be due to its reduced CNS penetration. CNS penetration of a drug is determined by factors such as its lipophilicity, ionization and molecular weight. However, the key determinant of brain penetration of antihistamine may be the efflux pump mechanisms. In brain endothelium, P-glycoproteins, encoded by the multidrug resistance gene *mdr1* can pump drugs with diverse structures out of brain, reducing unwanted or undesired CNS effects [60]. There is evidence to indicate that loratadine, desloratadine, cetirizine and fexofenadine are substrates for brain P-glycoproteins [61,

62]. Whether such a mechanism operates in the non-sedative effect of SUN-1334H remains to be established.

At clinical dose, cetirizine per se is known to cause performance impairment in driving and psychometric test batteries. Cetirizine and alcohol appear to have additive effect on the performance in this test [63]. The present study also showed that SUN-1334H, fexofenadine and desloratadine did not affect ethanol-induced ataxia, whereas hydroxyzine and cetirizine potentiated these effects of ethanol. These results denote that SUN-1334H does not have potential to augment the reported effects of ethanol upon oculomotor coordination, cognitive function and driving. Thus, SUN-1334H is a safe molecule in terms of its CNS side-effect profile.

In conclusion, clinical anti-inflammatory effects of antihistamines may require higher doses and long-term treatment in contrast to short-term antihistamine effects. It is desirable therefore for the safety margin of these drugs to be higher in order to be able to administer higher doses of these drugs for significantly long durations. Such treatment may work as prophylaxis to onset of respiratory and skin allergy. The present study indicates that different antihistamines show different anti-inflammatory to sedation ratios. SUN-1334H showed high anti-inflammatory activity with low potential for sedative effects. The therapeutic window of this drug is thus high and could offer safe long-term pharmacotherapy of allergic inflammation of skin and nose.

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