

Efficacy of Repeated Pretreatment with Olopatadine Hydrochloride on Rhinitis Induced by Intranasal Instillation of Toluene-2,4-Diisocyanate in Rats

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Key Words

Olopatadine hydrochloride • Antihistamine • Rhinitis, animal model • Toluene-2,4-diisocyanate

Abstract

It is well known that starting treatment for cedar pollinosis therapy with second-generation antihistamines before the initial day of pollen scattering can relieve nasal symptom severity during the pollen season. Olopatadine hydrochloride (olopatadine) is an antiallergic agent with histamine H₁ receptor antagonistic action. We have evaluated the effects of repeated preadministration of olopatadine on the toluene-2,4-diisocyanate-induced rhinitis in rats. A single administration of olopatadine suppressed sneezing and the increases in histamine, nerve growth factor (NGF) and vascular endothelial growth factor (VEGF) production in nasal lavage fluid. When olopatadine was administered repeatedly once a day for 7 days before provocation, its inhibitory effects were enhanced compared to the effect of a single administration. Although the repeated administration of fexofenadine enhanced the inhibitory effects on sneezing, it did not inhibit the increases in NGF and VEGF production. These results show that the suppression of the increase in NGF and VEGF might partially be involved in the improvement of

nasal allergy signs by the treatment with olopatadine. It is expected that the early treatment with olopatadine may achieve stable therapeutic effects.

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Introduction

Clinically, histamine plays a key role in producing nasal symptoms. In the treatment of pollinosis, second-generation antihistamines are used as initial therapy in which treatment was started before the initial day of pollen scattering or from the early stage after the initial day of pollen scattering [1]. However, clear evidence in support of this therapy is poor.

Pollinosis is a seasonal allergic rhinitis (AR) caused by hypersensitivity to tree or grass pollens. The important mediators in the pathogenesis of AR are Th2 cytokines. Furthermore, neurogenic inflammation is central to the pathogenesis of AR. In the human nose, nerve growth factor (NGF), brain-derived neurotrophic factor and neuropeptides, such as substance P, neurokinin A and the calcitonin gene-related peptide, are upregulated and their release in nasal fluids has been demonstrated after allergen or hypertonic saline exposure [2–5]. Recently, vascu-

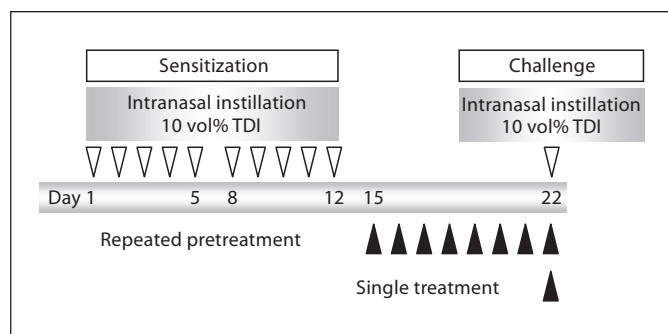


Fig. 1. Protocol for TDI sensitization and provocation. Rats were sensitized by instillation of 5 μ l of 10 vol% TDI solution into both nasal cavities once a day for 5 consecutive days. Three days later, the rats were sensitized again for a further 5 consecutive days. On day 22, 5 μ l of 10 vol% TDI solution or EA was instilled into both nasal cavities to induce nasal allergy signs in the sensitized rats. Drugs were orally administered 1 h before provocation or repeatedly once a day for 7 days before provocation with TDI.

lar endothelial growth factor (VEGF) and its receptor have been found to be increased in AR, as a result of the increase in nasal vascular permeability and congestion [6, 7]. VEGF participates in nasal mucosa swelling from increased blood vessel permeability in an immediate reaction of AR. VEGF may therefore play an important role in nasal mucosal inflammation in AR.

Toluene-2,4-diisocyanate (TDI) vapor can cause respiratory disorders such as asthma, rhinitis and nasal irritation [8]. TDI causes the release of neuropeptides from activated sensory nerves in the nasal cavity [9]. Activation of C fibers located in the airway mucosa produces neurogenic inflammation characterized by vasodilation, plasma extravasation, mucous secretion and neutrophil chemotaxis resulting from the release of neuropeptides [2].

Olopatadine hydrochloride (olopatadine, Kyowa Hakko Kirin Co. Ltd., Tokyo, Japan) is an antiallergic agent with histamine H_1 receptor (H_1R) antagonistic action, prescribed for patients with signs and symptoms of AR, chronic urticaria, eczema dermatitis, prurigo, pruritus cutaneus, psoriasis vulgaris and erythema exsudativum multiforme. Olopatadine ophthalmic solution, which inhibits the proinflammatory activity of conjunctival mast cells, is an effective therapy for allergic conjunctivitis. Recently, olopatadine nasal spray was launched for treatment of nasal allergy symptoms. We reported that single treatment with olopatadine inhibited the TDI-induced sneezing [10]. In the present study, we examined whether

repeated preadministration of olopatadine and fexofenadine hydrochloride before provocation were more effective than the single administration in reducing TDI-induced sneezing and increased NGF and VEGF levels in the nasal lavage fluid (NALF) in TDI-sensitized rats.

Materials and Methods

Experimental Animals

Male 6-week-old Brown Norway rats, which were purchased from Charles River Japan (Kanagawa, Japan), were maintained in a specific pathogen-free environment in closed racks with free access to food and water in the Institute of Laboratory Animals, Kyowa Hakko Kirin (Shizuoka, Japan). The study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals formulated by the Japanese Pharmacological Society, and the experimental protocol was approved by the Committee for Animal Experiments in Kyowa Hakko Kirin.

Reagents and Drugs

TDI and ethyl acetate (EA) were purchased from Wako Pure Chemical Industries (Osaka, Japan). TDI solution was diluted in EA. Olopatadine hydrochloride was synthesized in Yokkaichi Plant (Mie, Japan). Fexofenadine hydrochloride (fexofenadine) was synthesized or extracted from the commercially available tablets in our institute. Olopatadine was dissolved in distilled water. Fexofenadine was suspended in 0.5% methylcellulose solution.

TDI Sensitization and Provocation

Rats were sensitized and challenged with TDI, according to the method of our previous study [10] (fig. 1). Briefly, rats were sensitized by instillation of 5 μ l of 10 vol% TDI solution into both nasal cavities once a day for 5 consecutive days. This sensitization procedure was then repeated after a 2-day interval. Ten days after the second sensitization, 5 μ l of 10 vol% TDI solution was instilled into both nasal cavities to induce nasal allergy signs in the sensitized rats. Olopatadine (1 or 3 mg/kg) and fexofenadine (60 mg/kg) were orally administered 1 h before provocation or repeatedly once a day for 7 days before provocation with TDI.

Observation of Nasal Symptoms

Rats were placed into an observation cage divided into compartments (14 $\times$ 20 $\times$ 15 cm) for 60 min of habituation. After the intranasal TDI challenge, the number of sneezing episodes, a sign of nasal allergy, was counted in a blinded manner during a 10-min period just after exposure.

Measurement of Histamine, NGF and VEGF

At 6 h after the TDI challenge, rats were anesthetized with sodium pentobarbital. Nasal lavages were done using a catheter. The catheter with rubber tubing covering the head of the tube was inserted into the tracheal opening in the direction of the upper airway. Both sides of the nasal cavity were lavaged with phosphate-buffered saline (pH 7.4; ICN Biomedicals, Aurora, Ohio, USA) containing a protease inhibitor (Roche Diagnostics, Mannheim, Germany). The NALF was centrifuged. Each supernatant was fro-

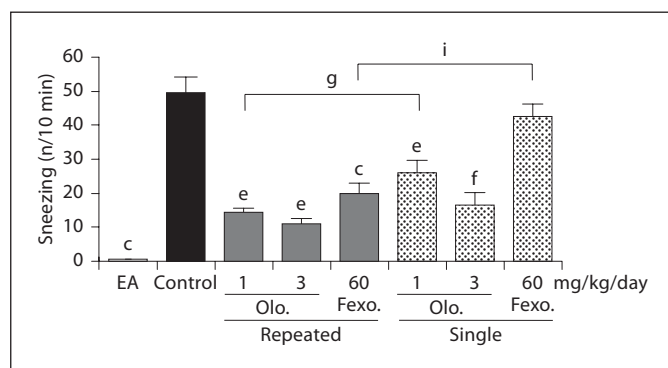


Fig. 2. Effects of repeated pretreatment with olopatadine (Olo.) and fexofenadine (Fexo.) on the number of sneezing episodes after the TDI challenge. Olopatadine and fexofenadine were orally administered 1 h before provocation or repeatedly once a day for 7 days before provocation with TDI. The number of sneezing episodes was counted for 10 min. Each column represents the mean $\pm$ SE of 6–8 rats. EA = TDI-sensitized rats were challenged with EA; control = rats were sensitized and challenged with TDI. ^c $p < 0.001$ compared to the control group by Student's t test or Aspin-Welch test; ^e $p < 0.01$, ^f $p < 0.001$ compared to the control group by Dunnett's test or Steel test; ^g $p < 0.05$, ⁱ $p < 0.001$, comparison between repeated and single administration.

zen at -35°C for subsequent assays. The amount of histamine (SPI-Bio, Montigny-le-Bretonneux, France), NGF (Promega, Madison, Wisc., USA) and VEGF (R&D Systems, Minneapolis, Minn., USA) were measured by commercially available ELISA kits.

Statistical Analysis

Data are shown as means $\pm$ SE. Student's t test following the F test was used for analysis of differences between 2 groups. Multiple comparisons among treatment groups were assessed by one-way analysis of variance, followed by Dunnett's test. Differences were considered to be significant for p values less than 0.05.

Results

Effect on Sneezing in the TDI-Induced Rat Rhinitis Model

Intranasal application of TDI induced an increase in the number of sneezing episodes compared to EA-challenged animals (fig. 2). The administration of olopatadine (1 and 3 mg/kg) once 1 h before provocation significantly suppressed the increased frequency of sneezing, respectively. Repeated preadministration of olopatadine (1 and 3 mg/kg/day) for 7 days before provocation also significantly suppressed the increased frequency of sneezing. The frequency of sneezing in rats after repeated pre-

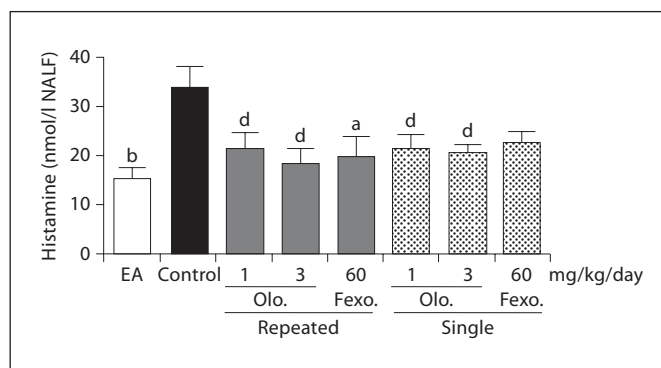


Fig. 3. Effects of repeated pretreatment with olopatadine (Olo.) and fexofenadine (Fexo.) on histamine levels in the NALF at 6 h after the TDI challenge. Each column represents the mean $\pm$ SE of 6–8 rats. EA = TDI-sensitized rats were challenged with EA; control = rats were sensitized and challenged with TDI. ^a $p < 0.05$, ^b $p < 0.01$ compared to the control group by Student's t test; ^d $p < 0.05$ compared to the control group by Dunnett's test.

administration of olopatadine at 1 mg/kg/day was significantly less than that after its administration once.

The administration of fexofenadine (60 mg/kg) once 1 h before the TDI challenge did not suppress the increased frequency of sneezing. Repeated preadministration of fexofenadine (60 mg/kg/day) significantly suppressed the increased frequency of sneezing, and the frequency of sneezing after repeated preadministration was significantly less than that after its administration once (fig. 2).

Effect of Olopatadine on Histamine Production in the NALF

We examined whether olopatadine had an effect on the levels of histamine in the NALF. At 6 h after the TDI challenge, the levels of histamine were significantly increased compared to EA-challenged animals (fig. 3). The single administration of olopatadine or its repeated preadministration significantly inhibited the increased levels of histamine. Repeated preadministration of fexofenadine significantly inhibited the increased levels of histamine.

Effect of Olopatadine on NGF Production in the NALF

At 6 h after the TDI challenge, the levels of NGF were significantly increased compared to EA-challenged animals (fig. 4). The single administration of olopatadine or its repeated preadministration significantly inhibited the

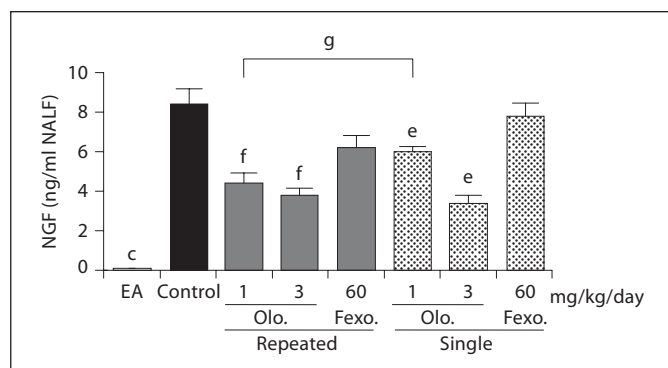


Fig. 4. Effects of repeated pretreatment with olopatadine (Olo.) and fexofenadine (Fexo.) on NGF levels in the NALF. Each column represents the mean $\pm$ SE of 6–8 rats. EA = TDI-sensitized rats were challenged with EA; control = rats were sensitized and challenged with TDI. ^c $p < 0.001$ compared to the control group by Aspin-Welch test; ^e $p < 0.01$, ^f $p < 0.001$ compared to the control group by Dunnett's test or Steel test. ^g $p < 0.05$, comparison between repeated and single administration.

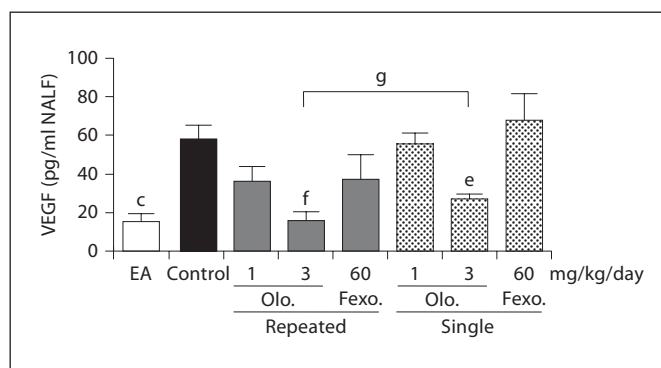


Fig. 5. Effects of repeated pretreatment with olopatadine (Olo.) and fexofenadine (Fexo.) on VEGF levels in the NALF. Each column represents the mean $\pm$ SE of 6–8 rats. EA = TDI-sensitized rats were challenged with EA; control = rats were sensitized and challenged with TDI. ^c $p < 0.001$ compared to the control group by Student's t test; ^e $p < 0.01$, ^f $p < 0.001$ compared to the control group by Dunnett's test; ^g $p < 0.05$, comparison between repeated and single administration.

increased levels of NGF. The increased level of NGF after repeated preadministration of olopatadine at 1 mg/kg/day was significantly less than that after its administration once. On the other hand, fexofenadine did not inhibit the increased levels of NGF even by repeated preadministration.

Effect of Olopatadine on VEGF Production in the NALF

As shown in figure 5, the level of VEGF after the TDI challenge was significantly increased compared to EA-challenged animals. The single administration of olopatadine significantly inhibited the increased levels of VEGF. The increased level of VEGF after repeated preadministration of olopatadine at 3 mg/kg/day was significantly less than that after its administration once. On the other hand, fexofenadine did not inhibit the increased levels of VEGF even by repeated preadministration.

Discussion

In the present study, single and repeated preadministration of olopatadine significantly reduced sneezing after the intranasal application of TDI in TDI-sensitized rats. Single administration of fexofenadine did not suppress the increased frequency of sneezing, but repeated preadministration did so. These findings suggested that repeated pretreatment with antihistamines is more effective

than their single administration on nasal allergy signs in rats. Clinically, histamine plays a key role in producing nasal symptoms, and second-generation antihistamines are used as initial therapy in AR. Kitamura et al. [11] reported that histamine content, histidine decarboxylase activity and gene expression in the nasal mucosa were significantly increased after the TDI challenge in rats. The present study demonstrated that intranasal application of TDI induced the increased levels of histamine in the NALF. Histamine, which stimulates substance-P-positive sensory nerve fibers, may lead to hyperresponsiveness. Clinical doses of olopatadine and fexofenadine are 5 and 60 mg. Pharmacokinetic profiles in rats are unknown. The used doses of olopatadine and fexofenadine are enough for the antihistamine activity in rats [12]. Single and repeated preadministration of olopatadine inhibited TDI-induced increased levels of histamine, NGF and VEGF. And its effects of repeated preadministration were enhanced compared to the effects after its single administration. On the other hand, single administration of fexofenadine did not suppress the increased levels of histamine, NGF and VEGF; however, repeated preadministration inhibited the increased levels of histamine.

Nasal fluids contain NGF, more so in patients with AR compared to healthy control subjects, and allergen challenge to patients with AR leads to acute release of NGF [3]. Neurogenic-peptide-positive sensory nerves fibers increased in the nasal mucosa. In the present study, TDI induced an increase in NGF in the NALF. Animals over-

expressing NGF augment allergic airway inflammation via a neuropeptide-dependent mechanism [13]. Therefore, a link between AR and NGF clearly exists, and the hypothesis that NGF might be responsible for sensorineural hyperresponsiveness, at least in AR, has drawn some support. NGF may induce hyperresponsiveness for the sneeze reflex, plus induce neurogenic inflammation, characterized by plasma extravasation and mucus secretion. Neuropeptide-positive nasal nerve fibers increased along with the exacerbation of reactions to TDI, therefore this response is likely to underlie the increase in sneezing. These results suggest the possibility that olopatadine reduces TDI-induced increases in the number of sneezing episodes by inhibiting the increase in NGF.

Increased vessel number and permeability are important features of the nasal mucosa in AR and are mediated in part by VEGF. TDI induced an increase in VEGF in NALF. VEGF is produced from mast cells and is thought to participate in nasal mucosa swelling by increased blood vessel permeability as an immediate reaction of AR [14]. VEGF in nasal mucus immediately increased when allergen was applied intranasally to subjects using cedar pollen [15]. Therefore, VEGF may play an important role in nasal mucosal inflammation in AR. In the present study, marked edema of skeletal muscle and desquamation of respiratory epithelium is observed in the pathological examination after provocation (data not shown). Single administration of olopatadine reduced the grade of this pathological change. On the other hand, fexofenadine did not reduce the edema. These findings suggest that VEGF expression was suppressed by olopatadine, resulting in amelioration of nasal symptoms in this model.

TDI-induced inflammation of the nasal mucosa was associated with a mixed Th1/Th2 immune response [16]. Recently, repeated pretreatment with antihistamines including olopatadine was reported to suppress TDI-induced upregulations of H₁R and IL-4 mRNAs in nasal mucosa [17]. Repeated pretreatment of antihistamines is

more effective than their single administration on the increased frequency of sneezing. Olopatadine showed stronger suppression of the elevation of NGF and VEGF compared to fexofenadine. Though the differences of effects between antihistamines may be depending on the length of treatment or the distribution of antihistamines into the nasal mucosa, pharmacokinetic profiles are unknown. Olopatadine appears to exert additional biological effects besides its blockade of H₁R. Airway epithelium not only functions as a physical barrier to the external environment, but also synthesizes and secretes a variety of cytokines, chemokines and mucus, which play important roles in the inflammation response [18]. It has been shown that H₁R participates in the upregulation of expression of cytokines, chemokines and adhesion molecules, suggesting that H₁R is first involved in inflammation, followed by acute allergic responses. Indeed, olopatadine inhibited histamine-stimulated production of CC chemokines in human nasal epithelial cells [19]. The potent antihistamine effect of olopatadine may be related to the specific antagonistic activity of the drug. It has been reported that the inhibitory effect of olopatadine on histamine receptors is hardly reduced in the presence of high levels of histamine because of noncompetitive antagonism [20]. The strong suppressive effect of olopatadine may be due to its strong antihistamine activity in vitro and in vivo. Alternatively, olopatadine might inhibit the production of NGF and VEGF by mechanisms other than a potent H₁R-antagonistic effect.

Therefore, the suppression of the increase in NGF and VEGF might partially be involved in the improvement of allergy-like behavior by the treatment with olopatadine unlike other antihistamines. It is suggested that the action of olopatadine has a rapid onset and its therapeutic effects are better than the treatment of fexofenadine even if olopatadine is taken at the time of symptom onset. In addition, it is expected that the early treatment with olopatadine may achieve stable therapeutic effects.

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