



Effect of olopatadine hydrochloride, an anti-histamine drug, on rhinitis induced by intranasal instillation of toluene-2,4-diisocyanate in rats

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Abstract

The main symptoms of allergic rhinitis (AR) are sneezing, rhinorrhea and nasal obstruction. In patients with AR, levels of nerve growth factor (NGF) and vascular endothelial growth factor (VEGF) increase. Olopatadine hydrochloride (olopatadine) is an anti-allergic agent with histamine H₁ receptor antagonistic action. To investigate whether olopatadine has an effect on inflammatory reactions, toluene-2,4-diisocyanate (TDI)-sensitized rats were used as an animal model of nasal allergy. Nasal allergy signs (sneezing, rhinorrhea and inflammation) were induced after TDI challenge. Amounts of NGF and VEGF in the nasal lavage fluid increased. Olopatadine reduced nasal allergy signs and inhibited increases in NGF and VEGF. These findings suggest that the increases in NGF and VEGF production are involved in the mechanism responsible for nasal allergy signs in TDI-challenged rats. Other histamine H₁ receptor antagonists did not inhibit and instillation of histamine did not increase TDI-induced NGF and VEGF production. Therefore, olopatadine appears to exert additional biological effects other than its blockade of the histamine H₁ receptor. These results suggest that suppression of neurogenic inflammatory reactions might be partially involved in the improvement of allergy signs after treatment with olopatadine.

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1. Introduction

Symptoms of allergic rhinitis (AR) are sneezing, nasal rubbing, nasal blockage and rhinorrhea, caused by the interaction between chemical mediators and sensory nerves through activa-

tion of specific receptors. Neurogenic inflammation is central to the pathophysiology of AR and accounts for symptoms induced by irritants, a very common problem in this disease. In the human nose, nerve growth factor (NGF), and neuropeptides, such as substance P (SP), neurokinin A, and the calcitonin gene-related peptide, are up-regulated and their release in nasal fluids has been demonstrated after allergen or hypertonic saline exposure [1–4]. NGF is a neurotrophin that targets nociceptor fibers, leading to up-regulated activity, increased neuropeptide content, and dendrite sprouting [5–7]. Recently,

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angiogenic factor vascular endothelial growth factor (VEGF) has also been shown to increase in the nasal mucosa of patients with AR, as a result of the increase in nasal vascular permeability and congestion [8–10]. VEGF participates in nasal mucosa swelling from increased blood vessel permeability in an immediate reaction of AR [11]. VEGF may therefore play an important role in nasal mucosal inflammation in AR.

Toluene-2, 4-diisocyanate (TDI) is commonly used in plastics manufacture. TDI vapor can cause respiratory disorders such as asthma, rhinitis, and nasal irritation [12]. TDI causes the release of neuropeptides from activated sensory nerves in the nasal cavity and bronchi [13,14]. 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]. In a recent study, histamine content, histidine decarboxylase (HDC) activity and gene expression in the nasal mucosa were significantly increased after TDI exposure in sensitized rats [15]. Those studies showed that repeated exposure to TDI triggered a neurogenic inflammatory reaction in the nasal mucosa that correlated with the release of histamine.

Olopatadine hydrochloride (olopatadine, Kyowa Hakko Kogyo Co., Ltd. Japan) is an anti-allergic agent with histamine H₁ receptor antagonistic action, prescribed for patients with signs and symptoms of AR, chronic urticaria, eczema dermatitis, prurigo, pruritis cutaneous, psoriasis vulgaris and erythema exsudativum multiform. Olopatadine ophthalmic solution, which inhibits the proinflammatory activity of conjunctival mast cells, is an effective therapy for allergic conjunctivitis. Olopatadine inhibits the capsaicin-induced sneezing response [16]. We previously reported that olopatadine mitigates cutaneous inflammation in a mouse model of chronic inflammatory dermatitis, accompanied by decreased cytokine production [17]. In addition, olopatadine inhibits the increased number of scratching episodes by reducing elevated levels of NGF and SP at the lesioned site [18]. Recent studies suggest that NGF may be a key mediator in up-regulation of SP levels in sensory neurons. In the present study, TDI-sensitized rats were used as an animal model of nasal hypersensitivity to investigate changes in nasal symptoms, and NGF levels in the NALF. Effect of olopatadine on the above mentioned parameters in the nasal mucosa after a neurogenic inflammatory reaction induced by TDI was also examined.

2. Materials and methods

2.1. Animals

Male 6-week-old Brown Norway (BN) rats and Sprague–Dawley (SD) 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 Kogyo Co., Ltd. (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 Kogyo Co., Ltd.

2.2. Reagents and drugs

Toluene-2,4-diisocyanate (TDI) and ethyl acetate (EA) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). TDI solution was diluted in EA. Histamine dihydrochloride (histamine) was purchased from Sigma Chemical (St. Louis, MO). Olopatadine hydrochloride (olopatadine) was synthesized in Yokkaichi Plant,

Kyowa Yuka Co., Ltd. (Mie, Japan). Cetirizine hydrochloride (cetirizine) and fexofenadine hydrochloride (fexofenadine) were synthesized or extracted from the commercially available tablets in our institute. Loratadine was purchased from Pharm Chemical Shanghai Lansheng (Shanghai, China), and prednisolone was purchased from Sigma Chemical. Olopatadine and cetirizine were dissolved in distilled water. Loratadine, fexofenadine and prednisolone were suspended in 0.5% methylcellulose solution.

2.3. TDI sensitization and provocation

BN rats were sensitized and challenged with TDI, according to the method of Kitamura et al. [15] with slight modification (Fig. 1). Briefly, rats were sensitized by instillation of 5 μ L of 10% 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% TDI solution was instilled into both nasal cavities to induce nasal allergy signs in the sensitized rats. Non-sensitized rats were treated with EA in the same manner. Olopatadine (1, 3, and 10 mg/kg) and prednisolone (10 mg/kg) were orally administered 1 h before TDI challenge.

2.4. Observation of nasal symptoms

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

2.5. Effects of H1 receptor antagonists on NGF and VEGF production in NALF

SD rats were challenged with TDI, according to the method of Wilfong and Dey [19] with slight modifications. Briefly, 5 μ L of 10% TDI solution was instilled into both nasal cavities in normal rats. Non-stimulated rats were treated with EA in the same manner. The dose of each drug was determined according to our preliminary experiments. Olopatadine (3 and 10 mg/kg), loratadine (10 mg/kg), cetirizine (10 mg/kg), fexofenadine (60 mg/kg) and prednisolone (10 mg/kg) were orally administered 1 h before TDI challenge.

2.6. Histamine-induced NGF and VEGF production in NALF

Five μ L of histamine (0.1, 1, 10 μ M) solution was instilled into both nasal cavities in normal rats. At 6 h after the intranasal challenge, rats were anesthetized with sodium pentobarbital. Nasal lavages were done using catheter.

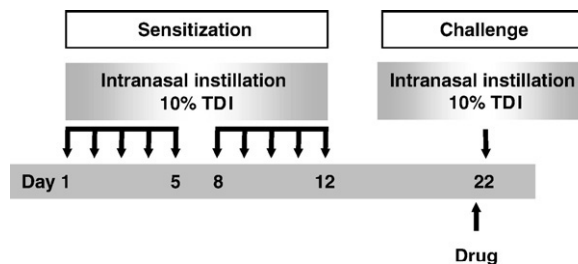


Figure 1 Protocol for TDI sensitization and challenge. Rats were sensitized by instillation of 5 μ L of 10% TDI solution into both nasal cavities once a day for 5 consecutive days. Three days later, rats were sensitized again for a further 5 consecutive days. On day 22, 5 μ L of 10% TDI solution was instilled into both nasal cavities to induce nasal allergy signs in the sensitized rats. Non-sensitized rats were treated with EA in the same manner. Drugs were orally administered 1 h before TDI challenge.

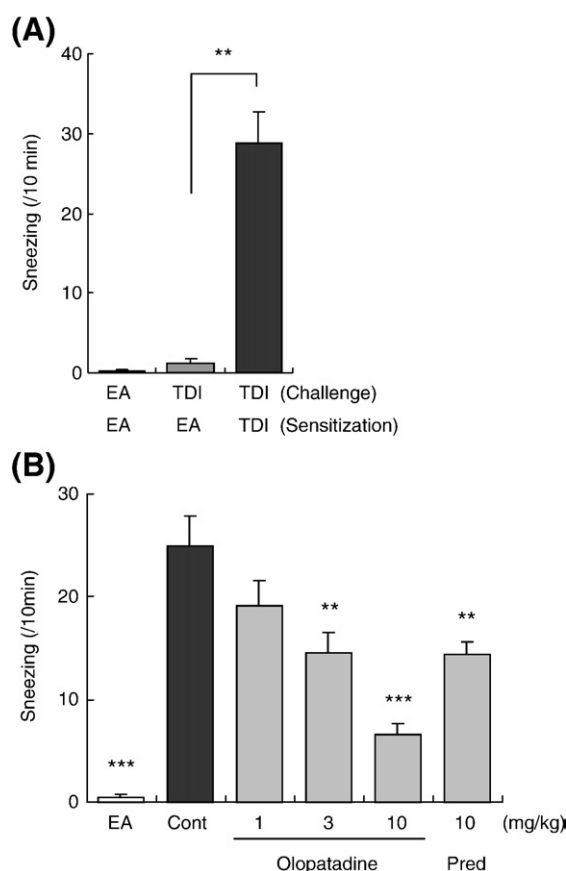


Figure 2 Effect of olopatadine on the number of sneezing episodes after TDI challenge. (A): TDI-sensitized or non-sensitized rats had 10% TDI applied intranasally. (B): Olopatadine and prednisolone were orally administered 1 h before TDI instillation in sensitized rats. Number of sneezing episodes was counted for 10 min. Each column represents mean $\pm$ S.E.M. of 6–8 rats. EA: ethyl acetate challenge, Cont: TDI challenge, Pred: prednisolone. Significant difference compared to the TDI-challenged group. ** $P < 0.01$, *** $P < 0.001$.

2.7. Measurement of NGF, SP, CGRP and VEGF

At 6 h after the intranasal challenge, rats were anesthetized with sodium pentobarbital. Nasal lavages were done using catheter. The catheter with rubber tubing covering the head of tube was inserted into the tracheal opening the direction of upper airway. Both sides of the nasal cavity were lavaged with phosphate-buffered saline (PBS; pH 7.4, ICN Biomedicals, Aurora, OH) containing a protease inhibitor (Complete™; Roche Diagnostics, Mannheim, Germany). The nasal lavage fluid (NALF) was centrifuged. Nasal mucosa was removed from the nasal septum and individual mucosa sample was homogenized in PBS containing the protease inhibitor and centrifuged. Each supernatant was aliquoted and frozen at -35°C for subsequent assays. ELISA for NGF (Promega, Madison, WI), SP (Assay designs, Ann Arbor, MI), CGRP enzyme immunoassay kit (SPI-BIO, Paris, France) and VEGF (R&D Systems, Minneapolis, MN) were conducted according to the manufacturer's instructions. The optical density of each well was determined using a microplate reader (THERMOmax™, Molecular Devices, Sunnyvale, CA).

2.8. Statistical analysis

Data are presented as means $\pm$ S.E.M. The Student's *t*-test following the *F*-test was used for analysis of differences between two groups.

Multiple comparisons among treatment groups were assessed by one-way analysis of variance, followed by the Dunnett's test. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Effect on sneezing in TDI-induced rat rhinitis model

Intranasal application of TDI-induced nasal allergy signs, such as sneezing and watery rhinorrhea, in TDI-sensitized rats. In TDI-sensitized rats challenged with TDI, there was an increase in the number of sneezing episodes compared to non-sensitized ($P = 0.0010$) and EA-challenged animals ($P = 0.0001$) (Fig. 2A). Olopatadine (3 and 10 mg/kg) significantly suppressed the increased frequency of sneezing by 46.2% and 74.9% ($P = 0.0075$, $P < 0.0001$), respectively (Fig. 2B). Prednisolone also significantly suppressed the increased number of sneezing ($P = 0.0074$).

3.2. Effect of olopatadine on NGF in a TDI-induced rat rhinitis model

As shown in Fig. 3, the level of NGF in the NALF and nasal mucosa at 6 h after intranasal challenge with TDI was significantly increased compared to EA-treated animals ($P < 0.0001$, $P = 0.0001$).

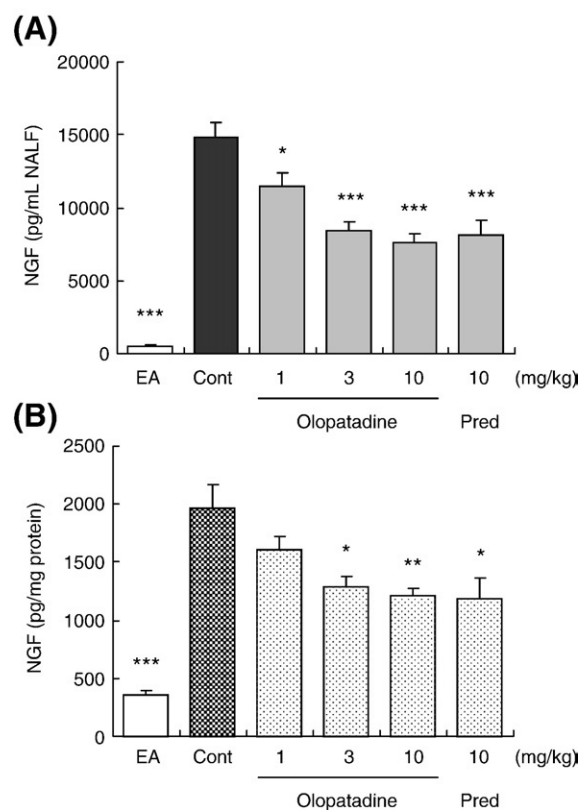


Figure 3 Effect of olopatadine on NGF level in the NALF (A) and mucosa (B) after TDI challenge in sensitized rats. Olopatadine and prednisolone were orally administered 1 h before TDI instillation. NGF levels in the NALF and nasal mucosa were measured 6 h after TDI challenge. EA: ethyl acetate challenge, Cont: TDI challenge, Pred: prednisolone. Each column represents mean $\pm$ S.E.M. of 8 rats. Significant difference compared to the TDI-challenged group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

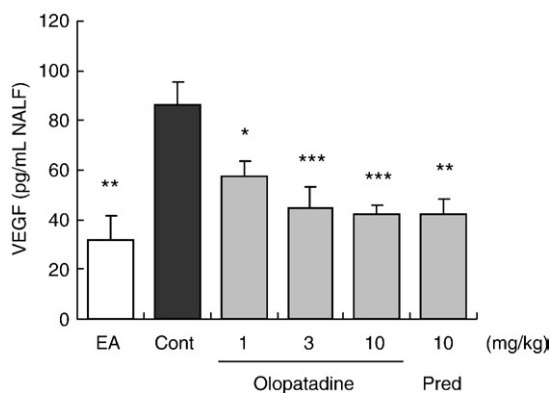


Figure 4 Effect of olopatadine on VEGF level in the NALF after TDI challenge in sensitized rats. Olopatadine and prednisolone were orally administered 1 h before TDI instillation. VEGF level in NALF was measured 6 h after TDI challenge. EA: ethyl acetate challenge, Cont: TDI challenge, Pred: prednisolone. Each column represents mean + S.E.M. of 6 rats. Significant difference compared to the TDI-challenged group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Olopatadine (1, 3, and 10 mg/kg) significantly inhibited increase in NGF in the NALF by 23.0%, 44.7%, and 50.5% ($P = 0.0194$, $P < 0.0001$, $P < 0.0001$), respectively (Fig. 3A). In the nasal mucosa, olopatadine (3 and 10 mg/kg) also significantly inhibited increase in NGF by 43.1% and 47.9% ($P = 0.0128$, $P = 0.0047$), respectively (Fig. 3B). Prednisolone significantly inhibited increased levels of NGF in both the NALF and nasal mucosa.

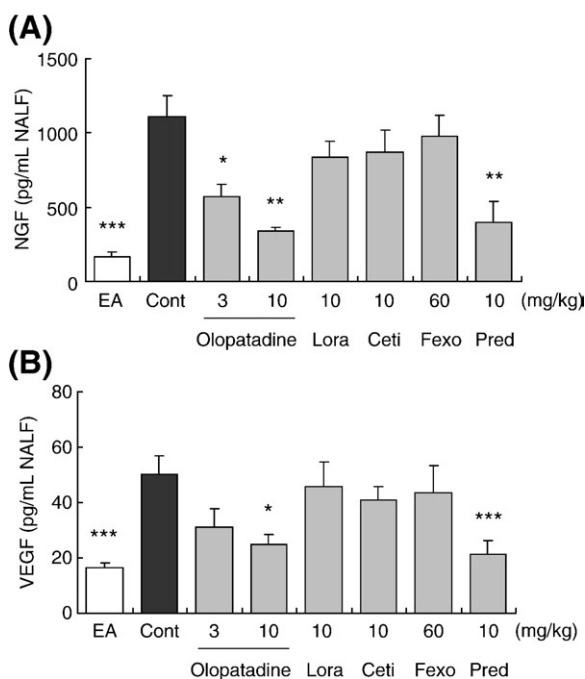


Figure 5 Effect of olopatadine, loratadine, cetirizine and fexofenadine on production of NGF (A) and VEGF (B) in the NALF induced by application of TDI. Drugs were orally administered 1 h before TDI instillation in non-sensitized rats. EA: ethyl acetate challenge, TDI: TDI challenge, Lora: loratadine, Ceti: cetirizine, Fexo: fexofenadine, Pred: prednisolone. Each column represents mean + S.E.M. of 7 rats. Significant difference compared to the TDI-challenged group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

3.3. Effect of olopatadine on VEGF in a TDI-induced rat rhinitis model

As shown in Fig. 4, the level of VEGF in the NALF at 6 h after intranasal challenge with TDI was significantly increased compared to EA-treated animals ($P = 0.0014$).

Olopatadine (1, 3, and 10 mg/kg) significantly inhibited the increase in VEGF in the NALF by 46.0%, 66.1%, and 70.7% ($P = 0.0207$, $P = 0.0009$, $P = 0.0004$), respectively. Increased levels of VEGF in the NALF were also significantly inhibited by prednisolone ($P = 0.0011$).

3.4. Effect of olopatadine and other histamine H_1 receptor antagonists on NGF and VEGF production in NALF after intranasal instillation of TDI in normal rats

To further investigate the mechanism of NGF and VEGF production, olopatadine inhibition, and the potential roles of histamine and histamine H_1 receptor, other histamine H_1 receptor antagonists were examined. Instillation of TDI induced an increase in NGF and VEGF production in the NALF, reaching a maximum at 6 h. As shown in Fig. 5(A), the level of NGF in the NALF at 6 h after intranasal challenge with TDI was significantly increased compared to EA-treated animals ($P < 0.0001$). Olopatadine (3 and 10 mg/kg) significantly inhibited the increase in NGF by 48.4% and 62.1% ($P = 0.0119$,

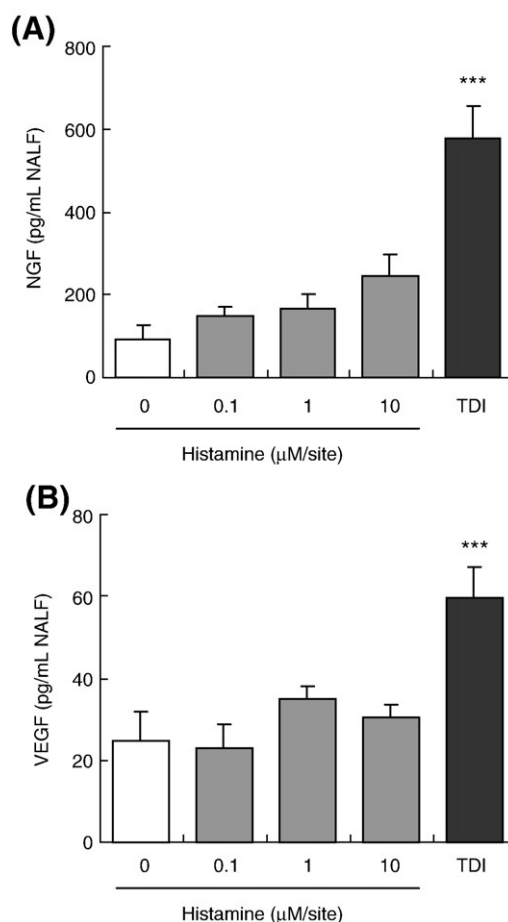


Figure 6 Concentration of NGF (A) and VEGF (B) in the NALF after histamine challenge. NGF and VEGF levels in the NALF were measured 6 h after histamine or TDI challenge. Each column represents mean + S.E.M. of 5–6 rats. Significant difference compared to the normal rat group. *** $P < 0.001$.

$P=0.0015$), respectively. In contrast, loratadine (10 mg/kg), cetirizine (10 mg/kg) and fexofenadine (60 mg/kg) did not inhibit increases in NGF. Prednisolone significantly inhibited increased levels of NGF ($P=0.0005$).

As shown in Fig. 5(B), the levels of VEGF in the NALF at 6 h after intranasal challenge with TDI were significantly increased compared to EA-treated animals ($P=0.0024$). Olopatadine (10 mg/kg) significantly inhibited the increase in VEGF by 74.8% ($P=0.0104$). In contrast, loratadine, cetirizine and fexofenadine did not inhibit the increase in VEGF. Prednisolone significantly inhibited an increase in VEGF ($P=0.0042$).

Instillation of histamine did not induce an increase of NGF and VEGF production in the NALF (Fig. 6).

4. Discussion

The present study demonstrated that intranasal application of TDI-induced nasal allergy signs, such as sneezing and watery rhinorrhea, in TDI-sensitized rats, plus increased levels of NGF and VEGF in the NALF. Olopatadine inhibited TDI-induced increases in the number of sneezing episodes and increases in NGF and VEGF, consistent with results obtained in a cutaneous model [18]. The NGF increase suggests that TDI-induced nasal allergy signs involve the sensory nerves.

In the human nose, NGF is highly expressed in the glandular and respiratory epithelium [4]. TDI causes the release of NGF and SP from activated sensory nerves. 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]. Sensory nerve fibers in the nasal mucosa, positive neurogenic peptides, increased. In the present study, TDI induced an increase in NGF in the NALF and mucosa in TDI-sensitized compared to non-sensitized rats. SP and calcitonin gene-related peptide production increased, however, not to significant levels (data not shown). These findings suggest that sensitization caused the increase in neuropeptide-positive nasal nerve fibers and exacerbation of nasal allergy-like signs. Animals overexpressing NGF augment allergic airway inflammation via a neuropeptide-dependent mechanism [20]. 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 mucous secretion. Neuropeptide positive nasal nerve fibers increased along with 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.

TDI induced an increase in VEGF in NALF in TDI-sensitized compared to non-sensitized rats. Increased vascular dilatation and permeability characterize AR. VEGF is produced from mast cells, and is thought to participate in nasal mucosa swelling through increased blood vessel permeability as an immediate reaction of AR [11]. VEGF in nasal mucus immediately increase when allergen is applied intranasally to subjects using cedar pollen [21]. Therefore, VEGF may play an important role in nasal mucosal inflammation in AR. Expression of VEGF in nasal polyps was significantly stronger than in specimens of healthy nasal mucosa. Thus, VEGF may play a significant role in the

induction of heavy tissue edema [22]. These findings suggest that VEGF expression was suppressed by olopatadine, resulting in amelioration of nasal symptoms in this model.

Olopatadine is a selective and potent histamine H_1 receptor antagonist and its clinical effect is believed to be mediated by its antagonistic action so far. Olopatadine significantly inhibited antigen-induced sneeze and nasal rubbing responses in both wild-type and H_1 receptor-deficient mice. However, cetirizine, a histamine H_1 receptor antagonist, failed to completely inhibit these responses in H_1 receptor-deficient mice, suggesting the involvement of other receptors in these responses [23,24].

Histamine content, HDC activity and gene expression in the nasal mucosa were significantly increased after TDI exposure in sensitized rats [15]. Histamine, that stimulates SP-positive sensory nerve fibers, may lead to hyperresponsiveness. Olopatadine reduced up-regulation of HDC mRNA, HDC activity and histamine content induced by TDI. In contrast, chlorpheniramine had no effect [25]. The inhibitory effect of olopatadine on NGF and VEGF production in rhinitis is not attributable to the histamine H_1 receptor antagonistic effect as no other histamine H_1 receptor antagonist tested exerted an inhibitory effect on TDI-induced NGF and VEGF production. Furthermore, levels of NGF and VEGF in the NALF did not increase after intranasal challenge with histamine. Clinical doses of olopatadine, loratadine, cetirizine and fexofenadine are 5, 10, 10, 60 mg. Pharmacokinetic profiles in rats are unknown. The used doses of olopatadine and the other H_1 antagonists have enough the anti-histamine activity in rats [26]. The used doses of olopatadine are increased about 1 to 5 times of the clinical blood concentration in rats. Thus, olopatadine has inhibitory activity against allergic rhinitis, possibly resulting from its more potently diminishing effect on elevated NGF than other histamine H_1 receptor antagonists. TDI-induced inflammation of the nasal mucosa was associated with a mixed Th1/Th2 immune response [27]. 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 [28]. It has recently been shown that H1R participates in up-regulation 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 [29]. These reports imply that olopatadine might inhibit production of cytokines through mechanisms other than a histamine H_1 receptor antagonistic effect. Further detailed characterization of the mechanism and site of action by which olopatadine inhibits cytokine production was required.

Therefore, TDI-sensitized rats were used as an animal model of nasal hypersensitivity induced by neurogenic inflammation, showing that levels of NGF and VEGF in nasal mucosa play an important role in the development of nasal hypersensitivity. Olopatadine significantly reduced nasal allergy signs, and inhibited up-regulation of NGF and VEGF content induced by TDI. These results suggest that suppression of the up-regulation of NGF and VEGF by olopatadine may underlie its therapeutic effect in the treatment of allergic inflammation. In conclusion, olopatadine appears to exert additional biological effects besides its blockade of the histamine H_1 receptor unlike other histamine H_1 receptor antagonists.

References

- [1] Baraniuk JN, Lundgren JD, Okayama M, Goff J, Mullol J, Merida M, et al. Substance P and neurokinin A in human nasal mucosa. *Am J Respir Cell Mol Biol* 1991;4:228–36.
- [2] Fang SY, Shen CL. Neuropeptide innervation and neuroendocrine cells in allergic rhinitis and chronic hypertrophic rhinitis. *Clin Exp Allergy* 1998;28:228–32.
- [3] Sanico AM, Stanisiz AM, Gleeson TD, Bora S, Proud D, Bienenstock J, et al. Nerve growth factor expression and release in allergic inflammatory disease of the upper airways. *Am J Respir Crit Care Med* 2000;161:1631–5.
- [4] Wu X, Myers AC, Goldstone AC, Togias A, Sanico AM. Localization of nerve growth factor and its receptors in the human nasal mucosa. *J Allergy Clin Immunol* 2006;118:428–33.
- [5] Levi-Montalcini R. The nerve growth factor 35 years later. *Science* 1987;237:1154–61.
- [6] Lewin G, Barde Y. Physiology of the neurotrophins. *Annu Rev Neurosci* 1996;19:289–317.
- [7] Mapp CE, Corona PC, De Marzo N, Fabbri L. Persistent asthma due to isocyanates. A follow-up study of subjects with occupational asthma due to toluene diisocyanate (TDI). *Am Rev Respir Dis* 1988;137:1326–9.
- [8] Benson M, Carlsson B, Carlsson LM, Wennergren G, Cardell LO. Increased expression of vascular endothelial growth factor-A in seasonal allergic rhinitis. *Cytokine*. 2002; 20: 268–273. Sarin S, Udem B, Sanico A, Togias A. The role of the nervous system in rhinitis. *J Allergy Clin Immunol* 2006;118:999–1016.
- [9] Kirmaz C, Ozbilgin K, Yuksel H, Bayrak P, Unlu H, Giray G, et al. Increased expression of angiogenic markers in patients with seasonal allergic rhinitis. *Eur Cytokine Netw* 2004;15:317–22.
- [10] Yuksel H, Kose C, Yilmaz O, Ozbilgin K, Degirmenci PB, Pinar E, et al. Increased expression of tissue vascular endothelial growth factor and foetal liver kinase-1 receptor in seasonal allergic rhinitis and relevance to asthma component. *Clin Exp Allergy* 2007;37:1183–8.
- [11] Boesiger J, Tsai M, Maurer M, Yamaguchi M, Brown LF, Claffey KP, et al. Mast cells can secrete vascular permeability factor/vascular endothelial cell growth factor and exhibit enhanced release after immunoglobulin E-dependent upregulation of Fce receptor I expression. *J Exp Med* 1998;188:1135–45.
- [12] Abe Y, Takeda N, Irifune M, Ogino S, Kalubi B, Imamura I, et al. Effects of capsaicin desensitization on nasal allergy-like symptoms and histamine release in the nose induced by toluene diisocyanate in guinea pigs. *Acta Otolaryngol* 1992;112:703–9.
- [13] Takeda N, Kalubi B, Abe Y, Irifune M, Ogino S, Matsunaga T. Neurogenic inflammation in nasal allergy: histochemical and pharmacological studies in guinea pigs. A review. *Acta Otolaryngol Suppl* 1993;501:21–4.
- [14] Wilfong ER, Dey RD. Nerve growth factor and substance P regulation in nasal sensory neurons after toluene diisocyanate exposure. *Am J Respir Cell Mol Biol* 2004;30:793–800.
- [15] Kitamura Y, Miyoshi A, Murata Y, Kalubi B, Fukui H, Takeda N. Effect of glucocorticoid on upregulation of histamine H₁ receptor mRNA in nasal mucosa of rats sensitized by exposure to toluene diisocyanate. *Acta Otolaryngol* 2004;124:1053–8.
- [16] Kaise T, Akamatsu Y, Ohmori K, Ishii A, Karasawa A. Inhibitory effect of olopatadine hydrochloride on the sneezing response induced by intranasal capsaicin challenge in guinea pigs. *Jpn J Pharmacol* 2001;86:258–61.
- [17] Tamura T, Matsubara M, Takada C, Hasegawa K, Suzuki K, Ohmori K, et al. Effects of olopatadine hydrochloride, an antihistamine drug, on skin inflammation induced by repeated topical application of oxazolone in mice. *Br J Dermatol* 2004;151:1133–42.
- [18] Tamura T, Amano T, Ohmori K, Manabe H. The effects of olopatadine hydrochloride on the number of scratching induced by repeated application of oxazolone in mice. *Eur J Pharmacol* 2005;524:149–54.
- [19] Wilfong ER, Dey RD. The release of nerve growth factor from the nasal mucosa following toluene diisocyanate. *J Toxicol Environ Health A* 2005;68:1337–48.
- [20] Quarcoo D, Schulte-Herbruggen O, Lommatzsch M, Schierhorn K, Hoyle GW, Renz H, et al. Nerve growth factor induces increased airway inflammation via a neuropeptide-dependent mechanism in a transgenic animal model of allergic airway inflammation. *Clin Exp Allergy* 2004;34:1146–51.
- [21] Yamashita T, Terada N, Hamano N, Kishi H, Kobayashi N, Kotani Y, et al. Involvement of vascular endothelial growth factor in nasal obstruction in patients with nasal allergy. *Allergol Intern* 2000;49:183–8.
- [22] Gosepath J, Brieger J, Lehr HA, Mann WJ. Expression, localization, and significance of vascular permeability/vascular endothelial growth factor in nasal polyps. *Am J Rhinol* 2005;1:7–13.
- [23] Sugimoto Y, Ogasawara R, Watanabe T, Kamei C. Inhibitory effect of olopatadine on nasal rubbing induced by capsaicin in histamine H₁ receptor-deficient and mast cell-deficient mice. *J Pharmacol Sci* 2004;94(Suppl):203.
- [24] Kayasuga R, Sugimoto Y, Watanabe T, Kamei C. Participation of chemical mediators other than histamine in nasal allergy signs: a study using mice lacking histamine H₁ receptors. *Eur J Pharmacol* 2002;449:287–91.
- [25] Kitamura Y, Das AK, Murata Y, Maeyama K, Dev S, Wakayama Y, et al. Dexamethasone suppresses histamine synthesis by repressing both transcription and activity of HDC in allergic rats. *Allergol Intern* 2006;55:279–86.
- [26] Tamura T, Sato H, Miki I, Suzuki K, Ohmori K, Karasawa A. Effects of orally administered olopatadine hydrochloride on the ocular allergic reaction in rats. *Allergol Intern* 2003;52:77–83.
- [27] Johnson VJ, Yucesoy B, Reynolds JS, Fluharty K, Wang W, Richardson D, et al. Inhalation of toluene diisocyanate vapor induces allergic rhinitis in mice. *J Immunol* 2007;179:1864–71.
- [28] Pawankar R. Epithelial cells as immunoregulators in allergic airway diseases. *Curr Opin Allergy Clin Immunol* 2002;2:1–5.
- [29] Yamauchi Y, Fujikura T, Shimomura T. The effect of H₁ antagonists carebastine and olopatadine on histamine induced expression of CC chemokines in cultured human nasal epithelial cells. *Allergol Intern* 2007;56:171–7.