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The effects of olopatadine hydrochloride on the number of scratching induced by repeated application of oxazolone in mice

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Abstract

It is suggested that atopic dermatitis is a skin disease associated with itching as subjective symptoms, and histamine H_1 receptor antagonists are used in order to prevent the itching, and the deterioration for scratch by itching. Histamine H_1 receptor selective anti-histamine olopatadine hydrochloride (olopatadine; Allelock®) shows consistent efficacy and safety in the treatment of allergic disorders. We investigated the possible efficacy of olopatadine on the number of scratching induced by repeated application of oxazolone in BALB/c mice. The repeated treatment of olopatadine significantly inhibited the ear swelling and the increased number of scratching. It significantly inhibited the increased production of interleukin (IL)-4, IL-1β and granulocyte–macrophage colony-stimulating factor (GM-CSF) in the lesioned ear. Moreover, it significantly inhibited the increased production of nerve growth factor (NGF) and substance P. On the other hand, loratadine, bepotastine and chlorpheniramine did not inhibit the ear swelling and the increased number of scratching. These results indicate that olopatadine inhibited not only the increased production of cytokines but also NGF and substance P unlike other histamine H_1 receptor antagonists. It was suggested that olopatadine suppressed the increased number of scratching by the anti-inflammatory effects. Therefore, olopatadine appears to exert additional biological effects besides its blockade of a histamine H_1 receptor.

Keywords: Olopatadine hydrochloride; Histamine H_1 receptor antagonist; Scratching; Atopic dermatitis; Animal model

1. Introduction

Itching is a characteristic symptom in various forms of dermatitis, such as atopic dermatitis and psoriasis. Atopic dermatitis is a chronic and relapsing inflammatory skin disease characterized by episodes of intense pruritus, multiple lesions with erythema, excoriation, erosions, lichenification, papules, dry skin, and susceptibility to cutaneous infection. In patients with atopic dermatitis, itch-associated scratching damages the skin and increases the inflammation, which in turn increases the itching further (Wahlgren, 1999). It is therefore important to reduce the itching and scratching to prevent aggravation of the skin lesion in pruritic diseases and to upgrade the quality of life of patients (Koblenzer, 1999).

There are a variety of known itch-associated mediators, including histamine, neuropeptides (substance P, calcitonin gene-related peptide, etc.), opioids, growth factors, cytokines, etc. (Rossi and Johansson, 1998; Stander et al., 2003). Histamine H_1 receptor antagonists have long been treated for atopic dermatitis as an adjunct therapy with topical agents, in the belief that they reduce pruritus by blocking the action of histamine in the skin.

Immunological analyses of the pathogenesis of atopic dermatitis have revealed that activated mast cells and eosinophils and an excess of differentiated T-helper (Th) 2 cells might play important roles in the development of dermatitis (Grewe et al., 1998). On the other hand, clinical evidence suggests that Th1 cells may also play an important role in atopic dermatitis pathology (Grewe et al., 1994). Together, these findings may indicate that Th1 and Th2 cells are not mutually exclusive, and that both Th cell subsets may contribute to the pathology of atopic dermatitis.

Olopatadine hydrochloride (olopatadine: (*Z*)-11-(3-Dimethylaminopropylidene)-6,11-dihydrodibenz [*b*,*e*]oxepin-2-acetic acid monohydrochloride, ALLELOCK®, Kyowa Hakko Kogyo Co., Ltd., Japan) is an anti-allergic agent with histamine

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H_1 receptor antagonistic action that is treated for the signs and symptoms of allergic rhinitis, chronic urticaria and eczema dermatitis (Ohmori et al., 2002). Olopatadine is also applied for diseases not related to allergy such as prurigo, pruritis cutaneous, psoriasis vulgaris and erythema exsudativum multiform. We have recently reported that olopatadine mitigates the cutaneous inflammation in a mouse model of chronic inflammatory dermatitis (Tamura et al., 2004), which observed the shift in the local cytokine pattern from a Th1 to a Th2 type profile in the mice exposed to repeated application of hapten (Webb et al., 1998; Kitagaki et al., 1997). Clinical effects of olopatadine are believed to be mediated by potent histamine H_1 receptor antagonistic action or by reducing the tachykinin release from peripheral sensory nerve endings (Hayashi et al., 2001) as well as by blocking the action of histamine on its histamine receptors in the skin. The purposes of the present study were to examine the possible efficacy of olopatadine on the number of scratching induced by repeated application of oxazolone in BALB/c mice.

2. Materials and methods

2.1. Materials

Male 6-week-old BALB/c mice (Charles River Japan, Kanagawa, Japan) were kept in the specific pathogen-free animal facility that maintained temperature of 19–25 °C, humidity of 30–70%, and a 12-h day/night cycle, and were given access to food and water ad libitum. The experiments were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, and the experimental protocol used in this study was approved by the Committee for Animal Experiments in Kyowa Hakko Kogyo Co., Ltd. (Shizuoka, Japan).

2.2. Drugs and materials

Olopatadine hydrochloride (olopatadine) was synthesized in Yokkaichi Plant, Kyowa Yuka Co., Ltd. (Mie, Japan). Loratadine was purchased from Pharm Chemical Shanghai Lansheng (Shanghai, China). Bepotastine besilate (bepotastine: TALION®, Tanabe Seiyaku, Osaka, Japan) was extracted from the commercially available tablets in our institute. Chlorpheniramine maleate salt (chlorpheniramine), prednisolone and 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone) were purchased from Sigma Chemical (St. Louis, MO, USA). Olopatadine, bepotastine and chlorpheniramine were dissolved in distilled water. Loratadine was suspended in 0.5% methylcellulose solution. Prednisolone and oxazolone were dissolved in acetone.

2.3. The oxazolone-induced chronic contact hypersensitivity response

Experimental protocols were illustrated in Fig. 1. The BALB/c mice were sensitized and challenged with oxazolone as described previously (Tamura et al., 2004). Using 12 mice in each group, the same skin site of the right ear was sensitized by a single application of 10 μl (each 5 μl for inner and outer of ear) of 0.5% oxazolone in acetone 7 days before the first challenge (day 0), and 10 μl of 0.5% oxazolone in acetone was repeatedly applied to the sensitized right ear 3 times per week. In the non-sensitized animals, acetone alone was applied to the right ear. Olopatadine was orally administered at 1 or 10 mg/kg/day, bepotastine was orally administered at 10 mg/kg/day, loratadine and chlorpheniramine were orally administered at 15 mg/kg/day and prednisolone was topically applied at 0.05 mg/ear/day to the right ear. Olopatadine, loratadine, bepotastine and chlorpheniramine were orally administered at a volume of 1 ml/100 g body weight. Prednisolone was applied at a volume of 10 μl/ear (each 5 μl for inner and outer of an ear). Each drug was administered once daily from days 18 to 30. On the day of oxazolone challenge, each drug was administered at 1 h before the challenge. The changes in the ear thickness were measured with a dial thickness gauge (PEACOCK; Model G-1A). At 24 h after the final challenge, blood was collected for measurement of serum IgE, and the animals in each group were sacrificed to remove the ears. Each ear sample was homogenized and centrifuged, and then the supernatant was stored at −80 °C until cytokine assays.

2.3.1. Observation of scratching

On days 0, 18 and 30, mice were put into an acrylic cage divided into compartments (7 × 7 × 15 cm) for 60 min

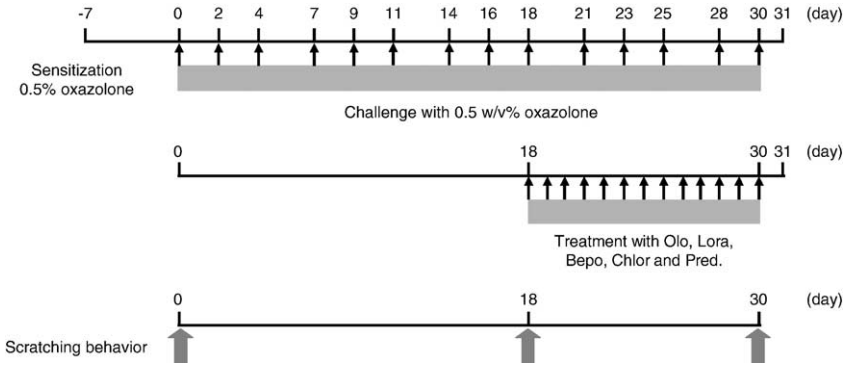

Fig. 1. Schedule of the elicitation of chronic contact hypersensitivity response and administration of drugs. Olo: olopatadine at 1, 10 mg/kg/day, Lora: loratadine at 15 mg/kg/day, Bepo: bepotastine at 10 mg/kg/day and Chlor: chlorpheniramine at 15 mg/kg/day were orally administered and Pred: prednisolone at 0.05 mg/ear/day was applied topically according to the protocol as described in Materials and methods.

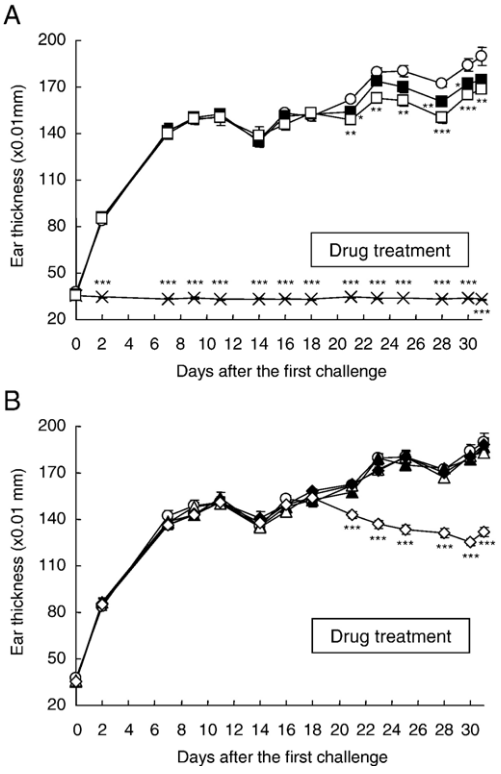

Fig. 2. Effects of olopatadine, other histamine H_1 receptor antagonists and prednisolone on the ear thickness induced by repeated application of oxazolone. Mice were sensitized on the ear with oxazolone 7 days before the first challenge (day 0), and were repeatedly challenged on the sensitized ear with oxazolone 3 times per week until day 30. Olopatadine, loratadine, bepotastine and chlorpheniramine were orally administered and prednisolone was applied topically according to the protocol as described in Materials and methods. Ear thickness was measured 24 h after each oxazolone challenge. Each point represents mean±S.E.M. of 12 mice. (A) ×: acetone, ○: control, ■: olopatadine at 1 mg/kg/day, □: olopatadine at 10 mg/kg/day. (B) ○: control, ▲: loratadine, △: bepotastine, ◆: chlorpheniramine, ◇: prednisolone. *$P<0.05$, **$P<0.01$, ***$P<0.001$: significantly different from the control group.

of habituation. Their behavior was then recorded using an unmanned digital video camera (Handycam, SONY, Tokyo, Japan) for 60 min. The videotapes were played back and the number of scratching episodes was counted. A series of scratching movements by the hind paw was taken as one scratching episode.

2.3.2. Measurements of cytokines and neural factors in ears

Individual mouse ears were homogenized in phosphate buffered saline (PBS; pH 7.4, ICN Biomedicals, Aurora, OH, USA) containing the protease inhibitor (Complete™, Roche Diagnostics, Mannheim, Germany) and centrifuged, and then the supernatant was used for the measurement of interferon (IFN) γ, interleukin (IL)-4, IL-1β, granulocyte–macrophage colony-stimulating factor (GM-CSF), nerve growth factor (NGF) and substance P by enzyme-linked immunosorbent assay (ELISA). IL-4 levels were determined using BIOTRAK™ kits from Amersham Biosciences UK Limited (Little Chalfont, Buckinghamshire, England). IFNγ, IL-1β, GM-CSF and substance P levels were determined using DuoSet® for IFNγ and IL-1β, GM-CSF Immunoassay Quantikine® M and substance P Immunoassay from R&D SYSTEMS (Minneapolis, MN, USA). NGF levels were determined using NGF E_{max}® kit from Promega (Madison, WI, USA). The assays were performed according to the manufacturer's instructions. The optical density of each well was determined by using the microplate reader THERMOmax™ (Molecular Devices, Sunnyvale, CA, USA).

2.3.3. Measurement of serum IgE

IgE levels were determined in serum using a commercial sandwich ELISA assay from BD Science (San Diego, CA, USA) according to the manufacturer's instruction.

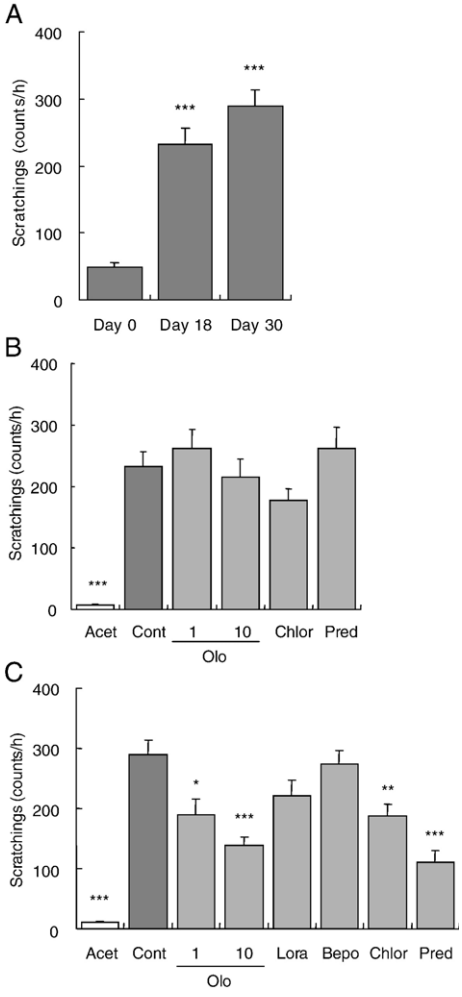

Fig. 3. Effects of olopatadine, other histamine H_1 receptor antagonists and prednisolone on the number of scratching induced by repeated application of oxazolone. Mice were sensitized on the ear with oxazolone 7 days before the first challenge, and were repeatedly challenged on the sensitized ear with oxazolone 3 times per week until day 30. Olopatadine, loratadine, bepotastine and chlorpheniramine were orally administered and prednisolone was applied topically according to the protocol as described in Materials and methods. The number of scratching was measured for 1 h after the oxazolone challenge. (A) Time course. ***$P<0.001$: significantly different from the control group on day 0. (B) Scratching on day 18; Acet: acetone, Cont: control, Olo: olopatadine, Chlor: chlorpheniramine, Pred: prednisolone. (C) Scratching on day 30; Acet: acetone, Cont: control, Olo: olopatadine, Lora: loratadine, Bepo: bepotastine, Chlor: chlorpheniramine, Pred: prednisolone. Each column represents mean±S.E.M. of 12 mice. *$P<0.05$, **$P<0.01$, ***$P<0.001$: significantly different from the control group.

2.4. Statistical analysis

Data were presented as means ± S.E.M. The Aspin–Welch test or 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 or the Steel test. In the number of scratching, Wilcoxon rank sum test was used for analysis of differences between two groups. Multiple comparisons among treatment groups were assessed by the Steel test. Values of $P<0.05$ were considered statistically significant. All statistical calculations were performed with the Statistical Analysis System (SAS Institute, Cary, NC, USA).

3. Results

3.1. Effects on the ear thickness after repeated challenge with oxazolone

In the oxazolone challenged group, the ear thickness significantly increased from day 2 throughout the experimental period. Olopatadine at 1 and 10 mg/kg/day significantly suppressed the increase in ear thickness (1 mg/kg/day; at days 21, 28 and 30, 10 mg/kg/day; at days 21–31, respectively), but it was slight (Fig. 2A). Topical prednisolone significantly inhibited the increase in ear thickness from day 20 throughout the experimental period (Fig. 2B). On the other hand, loratadine, bepotastine and chlorpheniramine did not significantly suppress the increase in ear thickness (Fig. 2B).

3.2. Effects on the number of scratching after repeated challenge with oxazolone

Repeated challenge with oxazolone evoked the increases in number of scratching (Fig. 3A). When olopatadine, chlorpheniramine or prednisolone was administered at day 18, single administration of olopatadine and prednisolone did not suppress the increased number of scratching but chlorpheniramine showed a tendency to suppress ($P=0.0690$) (Fig. 3B).

In contrast, repeated administration of olopatadine at 1 and 10 mg/kg/day significantly suppressed the increased number of scratching at day 30 by 35.6% and 54.2%, respectively (Fig. 3C). Chlorpheniramine and prednisolone also significantly suppressed the increased number of scratching at day 30 by 36.3% and 63.9%, respectively. On the other hand, loratadine and bepotastine did not affect the number of scratching at day 30 even after repeated treatment from day 18.

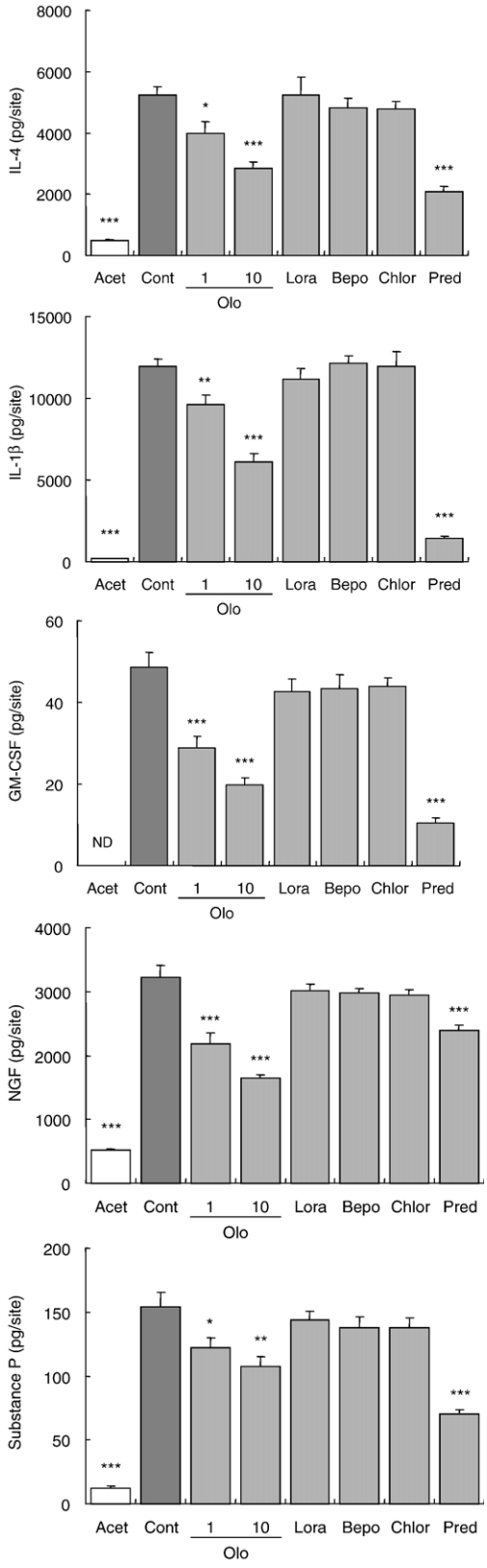

Fig. 4. Effects of olopatadine, other histamine H_1 receptor antagonists and prednisolone on the production of IL-4, IL-1β, GM-CSF, NGF and substance P in the lesioned skin induced by repeated application of oxazolone. Olopatadine, loratadine, bepotastine and chlorpheniramine were orally administered and prednisolone was applied topically according to the protocol as described in Materials and methods. IL-4, IL-1β, GM-CSF, NGF and substance P levels in the homogenized ear tissues were measured 24 h after the final oxazolone challenge as described in Materials and methods. Acet: acetone, Cont: control, Olo: olopatadine, Lora: loratadine, Bepo: bepotastine, Chlor: chlorpheniramine, Pred: prednisolone. Each column represents mean ± S.E.M. of 12 mice. $*P<0.05$, $**P<0.01$, $***P<0.001$: significantly different from the control group.

3.3. Effects on the levels of cytokines and neural factors in lesioned ears

To elucidate the mechanism by which olopatadine inhibited the development of ear swelling, we investigated its effect on the levels of cytokines and neural factors in the lesioned ear. As shown in Fig. 4, the levels of IL-4, IL-1β, GM-CSF, NGF and substance P in the lesioned ear taken at 24 h after the final challenge were significantly increased compared with those in the acetone-treated ear; in contrast, the IFNγ level was significantly decreased (acetone; 139.0±6.2 pg/site, oxazolone challenge; 42.1±3.1 pg/site).

Olopatadine at 1 mg/kg/day significantly inhibited the increased levels of IL-4, IL-1β, GM-CSF, NGF and substance P by 26.2%, 19.8%, 40.9%, 38.5% and 22.3%, and at 10 mg/kg/day significantly inhibited the increased levels of IL-4, IL-1β, GM-CSF, NGF and substance P by 50.2%, 49.5%, 59.7%, 58.6% and 33.3%, respectively. Topical prednisolone significantly inhibited the increased levels of IL-4, IL-1β, GM-CSF, NGF and substance P (66.2%, 89.2%, 78.9%, 30.7% and 59.5%, respectively). On the other hand, loratadine, bepotastine and chlorpheniramine did not affect them.

3.4. Effect on the serum IgE levels

The total serum IgE level was significantly increased by repeated challenge with oxazolone (acetone; 378.5±83.8 pg/ml, oxazolone challenge; 7992.8±434.2 pg/ml). Neither olopatadine nor other drugs affect the increase in total serum IgE levels (data not shown).

4. Discussion

The mechanisms underlying the development of atopic dermatitis remain to be clear. In general, various histamine H_1 receptor antagonists are prescribed for atopic dermatitis patients. However, patients often complain that the itching does not cease. The present study demonstrated that the repeated challenge with oxazolone evoked the increases in number of scratching. The repeated administration of olopatadine after the establishment of dermatitis significantly suppressed the increases in the number of scratching and ear swelling. On the other hand, the other second-generation histamine H_1 receptor antagonists tested (loratadine and bepotastine) failed to affect, not only the cutaneous inflammation, but also the scratching behavior. Chlorpheniramine, a classical histamine H_1 receptor antagonist, reduced the scratching behavior, but its effect was related to a sedative effect. Therefore, it was indicated that olopatadine suppressed the increased number of scratching by the anti-inflammatory effects and might inhibit the responses by a mechanism independent of histamine H_1 receptor antagonism.

The present study demonstrated that the oxazolone-repeated challenge increased the level of Th2 cytokines and decreased that of a Th1 cytokine in the lesioned skin. This is in agreement with the previous studies (Tamura et al., 2004), which showed that Th2 cytokines were abundant in the lesioned skin in this model. The Th2 cytokine IL-4 affects a broad spectrum of different cell types and regulates the immune response in a number of ways, thus suggesting a crucial role of IL-4 in the pathogenesis of atopic dermatitis (Kay et al., 1991; Bos et al., 1992). These observations suggest that the Th2 cytokines, especially IL-4, play major roles in the development of dermatitis in the present mouse model. Our present data indeed indicated that olopatadine inhibited the increased levels of IL-4 and other cytokines in the lesioned ear. Some investigators have reported that keratinocytes and mast cells produce the inflammatory mediators in the lesioned skin of atopic dermatitis (Horsmanheimo et al., 1994; Uchi et al., 2000; Giustizieri et al., 2004). Olopatadine inhibited the expression of IL-4 mRNA in mast cells or keratinocytes more potently than the other histamine H_1 receptor antagonists (Matsubara et al., 2005). Olopatadine, loratadine, cetirizine and fexofenadine reduce the production of cytokines (Yanni et al., 1999; Cook et al., 2000; Lippert et al., 2000; Jin et al., 2002; Abdelaziz et al., 1998). In this study, however, it is particularly interesting when ineffectiveness of loratadine is taken into account. Thus, olopatadine may offer a new regimen for treating chronic inflammatory dermatitis by inhibiting the inflammatory cytokines.

There are a variety of known itch-associated mediators. Histamine H_1 receptor antagonists have long been treated for atopic dermatitis to reduce pruritus by blocking the action of histamine in the skin. Olopatadine is a selective and potent histamine H_1 receptor antagonist, and it seems that the olopatadine reduced pruritus by blocking the action of histamine and by reducing the tachykinin release. Clinical effects of olopatadine are believed to be mediated by potent histamine H_1 receptor antagonistic action or by reducing the tachykinin release from peripheral sensory nerve endings (Hayashi et al., 2001).

NGF and substance P are released from keratinocytes, and these neuropeptides have widely played a role in skin diseases by the augmentation of the secretion of neuropeptides, such as neurokines (Scholzen et al., 1998; Burbach et al., 2001; Andoh et al., 2001). Moreover, NGF stimulates the sprouting of nerve fibers (Bull et al., 1998; Pincelli, 2000) and the proliferations of sensory neurons, resulting in augmented and itch sensation in skin diseases (Kinkelin et al., 2000). Toyoda et al. reported that atopic dermatitis patients have significant increases in the plasma levels of NGF (Toyoda et al., 2003) and substance P (Toyoda et al., 2002), and that these plasma levels in atopic dermatitis patients significantly correlate with the disease severity of atopic dermatitis. Our present data indeed indicated that NGF and substance P productions were increased in the lesioned ear. Olopatadine but not other histamine H_1 receptor antagonists suppressed the increased scratching partly because olopatadine might attenuate the increased neurogenic factors production, unlike other histamine H_1 receptor antagonists.

It is reported that olopatadine produced the inhibition of nasal rubbing in the both H_1 receptor-deficient mice and mast cell-deficient mice (Sugimoto et al., 2003). In addition, olopatadine has been shown to suppress the increased substance P levels in the skin lesions induced by repeated application of 2,4,6-trinitrochlorbenzane in DS-Nh mice (Kojima et al., 2004).

These observations therefore suggest that the suppression of NGF and substance P, in addition to the previously postulated mechanisms, is involved in the amelioration by olopatadine of pruritis and itch sensation in this model.

In conclusion, olopatadine inhibited the increases in the production of not only cytokines but also NGF and substance P unlike other histamine H_1 receptor antagonists. Thus, olopatadine appears to exert some additional biological effects besides its blockade of a histamine H_1 receptor.

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