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Olopatadine hydrochloride suppresses the rebound phenomenon after discontinuation of treatment with a topical steroid in mice with chronic contact hypersensitivity

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Summary

Background Olopatadine hydrochloride (olopatadine; Allelock®) is one of the second-generation antihistamines that are treated for allergic disorders such as rhinitis, urticaria and eczema dermatitis. Olopatadine has recently been shown to have inhibitory effects on the chronic contact hypersensitivity induced by repeated application of oxazolone in mice. Although topical steroids have widely been prescribed for atopic dermatitis, a relapse often occurs within several days after discontinuation of their prolonged use.

Objectives We investigated the possible efficacy of olopatadine against the relapse after discontinuation of prolonged use of topical prednisolone in the Balb/c mice with oxazolone-induced chronic contact hypersensitivity.

Methods Mice with the chronic contact hypersensitivity induced by repeated application of oxazolone were treated with olopatadine as a sequential therapeutic agent. The effects of olopatadine were quantified by measurements of ear-swelling, and levels of cytokines and histamine in the lesioned ear.

Results Topical prednisolone (0.05 mg/ear/day) significantly inhibited the increases in ear swelling and production of IL-1β, IL-4, IL-18, granulocyte-macrophage colony-stimulating factor (GM-CSF) and histamine. However, after discontinuation of the treatment with topical prednisolone, the inflammation relapsed and the IL-4 level exceeded the control one. The sequential treatment with olopatadine (10 mg/kg/day) after discontinuation of the treatment with topical prednisolone alone, or topical prednisolone with olopatadine, significantly inhibited the increases in ear swelling and levels of IL-1β, IL-4, IL-18, GM-CSF, nerve growth factor and histamine.

Conclusions These results indicate that olopatadine is an antihistamine agent having inhibitory activities against the rebound phenomenon following the discontinuation of topical steroid therapy. Olopatadine is thus expected to be a sequential therapeutic agent after discontinuation of the chronic treatment with a topical steroid.

Keywords animal model, antihistamine, atopic dermatitis, olopatadine hydrochloride, rebound phenomenon, topical steroid

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Introduction

Atopic dermatitis (AD), allergic contact dermatitis and psoriasis vulgaris are the most common skin diseases. AD is a chronically 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. Topical steroids and emollients have widely been prescribed for the eczematous lesions of AD [1, 2]. Antihistamines have long been treated for AD as an adjunct therapy with topical agents, in the belief that they reduce pruritus by blocking the action of histamine in the skin [3]. The treatment with topical steroids of AD and other skin disorders is based on their potent anti-inflammatory effects. However, their topical use can cause intense skin atrophy, one of the side effects limiting their chronic use for skin diseases. Moreover, the exacerbated relapse often occurs after discontinuation of the prolonged use of topical steroids. This exacerbation of AD, sometimes associated with facial swelling, is called 'rebound phenomenon' [4–6]. The mechanism underlying the rebound phenomenon has not been fully clarified.

Genetic, environmental, skin barrier, pharmacological, psychological and immunological factors are involved in the pathogenesis of AD [7]. In particular, the T-helper (Th) 2 cells accumulated in the lesioned skin of AD are thought to play a major role in the pathogenesis of the dermatitis [8]. Some investigators have suggested that the lesioned skin in

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inflammatory dermatitis hyperexpresses several pro-inflammatory cytokines and chemokines, including IFNγ, IL-4, IL-1, IL-18, granulocyte-macrophage colony-stimulating factor (GM-CSF), TNF-α, IL-8 and eotaxin [9–13]. Moreover, in the lesioned skin of AD, the number of mast cells increases and the activated mast cells also produce the inflammatory mediators, such as histamine, IL-4, GM-CSF and nerve growth factor (NGF), and substance P [14–16]. Webb et al. [17] and Kitagaki et al. [18] have examined the cutaneous cytokine profile in mice exposed to repeated application of hapten, and observed the shift in the local cytokine pattern from a Th1- to a Th2-type profile. As most of these findings are also observed in patients with AD, the mouse models appear to mimic many, if not all, events occurring within the lesioned skin of patients with AD.

Olopatadine hydrochloride (olopatadine: 11-[(*Z*)-3-(dimethylamino)propylidene]-6,11-dihydrodibenz[*b,e*]-oxepin-2-acetic acid monohydrochloride) (Allelock® Kyowa Hakko Kogyo Co, Ltd, Shizuoka, Japan) is an anti-allergic agent with histamine H_1-receptor antagonistic action that is indicated for the signs and symptoms of allergic rhinitis, chronic urticaria, eczema dermatitis, prurigo, pruritis cutaneous, psoriasis vulgaris and erythema exsudativum multiform [19]. We have recently reported that olopatadine mitigates the cutaneous inflammation in a mouse model of chronic inflammatory dermatitis induced by repeated challenge of hapten to the ear [20]. Olopatadine attenuated the increased levels of cytokines and suppressed the expression of IFNγ and IL-4 mRNAs in the lesioned site. Indeed, olopatadine has been shown to inhibit the expression of IL-4 mRNA in rat basophilic leukemia (RBL)-2 mast cells [21]. In addition, olopatadine inhibits pruritus by reducing the tachykinin release from peripheral sensory nerve endings [22] as well as by blocking the action of histamine on its receptors in the skin.

Kimata [23] described the increased expression of Th2 cytokine mRNA in peripheral blood mononuclear cells obtained from patients with AD during the rebound phenomenon after discontinuation of the treatment with a topical steroid. Thus, in light of the efficacy of olopatadine mentioned above, we assumed that olopatadine could be effective against the rebound phenomenon following withdrawal of topical steroid therapy. The purposes of the present study were, first, to examine whether the relapse would occur after discontinuation of prolonged use of olopatadine or topical prednisolone in a mouse model of chronic inflammatory dermatitis induced by repeated challenge of oxazolone to the skin and, second, if the relapse occurred, to investigate the effect of switching from topical prednisolone to olopatadine on the relapse after discontinuation of prolonged use of a topical steroid.

Materials and methods

Materials

Male 6-week-old Balb/c mice were purchased from Charles River Japan (Kanagawa, Japan). The animals were kept in the specific pathogen-free animal facility that maintained a 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).

Drugs and materials

Olopatadine hydrochloride (olopatadine) was synthesized in Yokkaichi Plant, Kyowa Yuka Co, Ltd (Mie, Japan). Prednisolone and 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone) were purchased from Sigma Chemical (St Louis, MO, USA). Olopatadine was dissolved in distilled water at 1 mg/mL. Prednisolone and oxazolone were dissolved in acetone at 5 mg/mL, respectively.

The oxazolone-induced chronic contact hypersensitivity response

The Balb/c mice were sensitized and challenged with oxazolone as described previously [20]. Using eight mice in each group, the same skin site of the right ear was sensitized by a single application of 10 μL of 0.5 w/v% oxazolone in acetone 7 days before the first challenge (day 0), and 10 μL of 0.5 w/v% oxazolone in acetone was repeatedly applied to the sensitized right ear three times per week. In the non-sensitized animals, acetone alone was applied to the right ear. Olopatadine was orally administered at a volume of 1 mL/100 g body weight. Prednisolone was applied at a volume of 10 μL/ear. Each drug was administered once daily. On the day of oxazolone challenge, each drug was administered at 1 h before the challenge.

Experimental protocol

Experimental protocols was illustrated in Fig. 1. The challenge with oxazolone three times a week was continued throughout the experimental period from day 0 to day 37.

For assessment of the possible relapse after discontinuation of the treatment with olopatadine, topical prednisolone or their combination, olopatadine was orally administered at 10 mg/kg/day, and/or prednisolone was topically applied at 0.05 mg/ear/day to the right ear, once daily from day 0 to 17. In the other groups, the treatment with olopatadine, prednisolone or their combination was continued once daily from day 0 to 37. To evaluate the relapse, the ear thickness was measured with a dial thickness gauge (PEACOCK; Model G-1A, Ozaki Corp., Tokyo, Japan) just before the oxazolone challenge. At 24 h after the final challenge, the ear thickness was measured and blood was collected for measurements of serum IgE and the ears were removed.

For assessment of effects of the switching from topical prednisolone, or its combination with oral olopatadine, to the sequential olopatadine treatment on the relapse after the prednisolone discontinuation, topical prednisolone at 0.05 mg/ear/day, or its combination with oral olopatadine at 10 mg/kg, was treated once daily from day 0 to 17. After discontinuation of the treatment, olopatadine was orally administered at 10 mg/kg/day from day 18 to 37. The ear thickness was measured with the dial thickness gauge just before the oxazolone challenge. In addition, the ear thickness

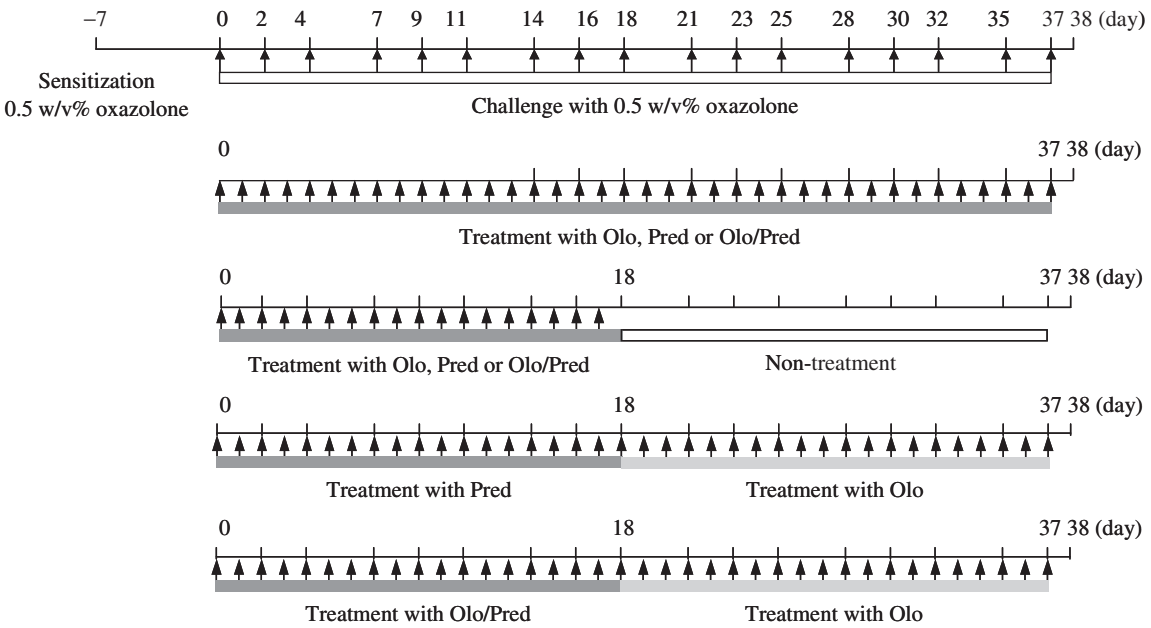

Fig. 1. Schedule of the elicitation of chronic contact hypersensitivity response and administration of drugs. Olo, olopatadine at 10 mg/kg/day; Pred, prednisolone at 0.05 mg/ear/day; Olo/Pred, olopatadine at 10 mg/kg/day and prednisolone at 0.05 mg/ear/day.

was measured and blood was collected, and the ears were removed at 24 h after the final challenge.

Measurements of IFNγ, IL-4, IL-1β, IL-18, GM-CSF and NGF 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 IFNγ, IL-4, IL-1β, IL-18, GM-CSF and NGF by specific ELISA. IFNγ and IL-4 levels were determined using the commercial kits of sandwich ELISA from Amersham Biosciences UK Limited (Little Chalfont, Buckinghamshire, England). IL-1β, IL-18 and GM-CSF levels were determined using the commercial kits of sandwich ELISA from R&D SYSTEMS (Minneapolis, MN, USA). NGF levels were determined using the commercial kit of sandwich ELISA 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).

Measurement of histamine in ears

Individual mouse ears were homogenized and centrifuged, and then the supernatant was used for the measurement of histamine by the method of Shore [24]. The fluorescence of each well was subsequently measured at 485 nm excitation and 530 nm emission wavelengths by using the microplate reader CytoFluor™ II (Perseptive Biosystems, Framingham, MA, USA).

Measurement of serum IgE

Serum harvested from each blood sample was used for IgE quantification. Serum IgE levels were determined using the commercial kit of sandwich ELISA from BD Science (San Diego, CA, USA) according to the manufacturer's instruction.

Statistical analysis

Data were presented as means ± SEM. The Aspin–Welch test or Student's t-test following the *F*-test was used for analysis of differences between two groups. Values of $P<0.05$ were considered statistically significant. All statistical calculations were performed with the Statistical Analysis System (SAS: Release 8.2, SAS Institute, Cary, NC, USA).

Results

Relapses of ear swelling following discontinuation of the treatment with olopatadine or prednisolone

Figure 2 shows the effects of olopatadine, topical prednisolone and their combination on the ear thickness response to repeated challenge with oxazolone when the treatment was continued or discontinued. In the oxazolone challenge (control) group, the ear thickness significantly increased from day 2 throughout the experimental period.

Olopatadine, when treated at 10 mg/kg/day during the whole experimental period, continuously and significantly suppressed the increase in ear thickness (Fig. 2a). Upon discontinuation of the treatment on day 18, the ear thickness began to increase while the significant suppression continued up to day 35. The discontinuation of olopatadine did not cause the surpassing flare ear swelling.

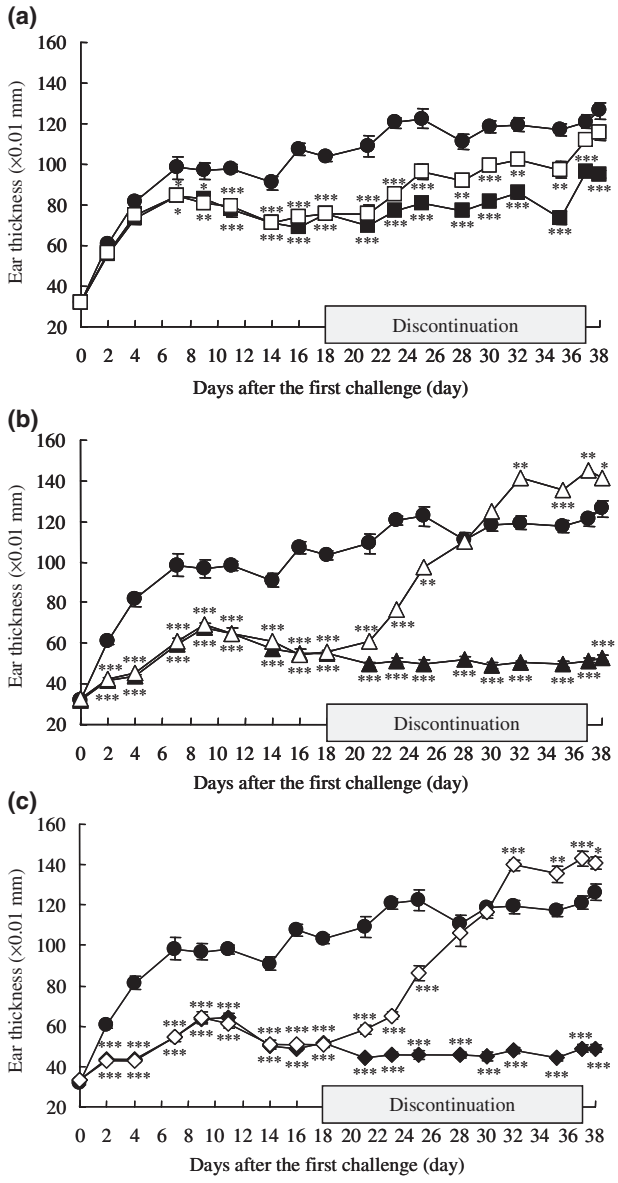

Fig. 2. Changes in the ear thickness after discontinuation of the treatment with olopatadine hydrochloride (olopatadine) or prednisolone. Mice were sensitized on the ear with oxazolone 7 days before the first challenge, and were repeatedly challenged on the sensitized ear with oxazolone three times per week until the end of the experimental period. Olopatadine was orally administered and prednisolone was applied topically, and the treatment was discontinued from day 18 to 37 as described in Materials and methods. (a) ●, control; ■, olopatadine at 10 mg/kg/day; □, discontinuation of olopatadine. (b) ●, control; ▲, prednisolone at 0.05 mg/ear/day; △, discontinuation of prednisolone. (c) ●, control; ◆, olopatadine at 10 mg/kg/day and prednisolone at 0.05 mg/ear/day; ◇, discontinuation of olopatadine and prednisolone. Each point represents the mean ± SEM. of eight mice. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, significantly different from the control group.

Topical prednisolone (0.05 mg/ear/day) also significantly inhibited the increase in ear thickness when treated during the whole experimental period (Fig. 2b). However, when the treatment with topical prednisolone was discontinued, the ear thickness increased gradually from day 18 to 37 and its inhibitory effect continued up to day 25. After day 32, its ear thickness exceeded that in the control group, showing a rebound phenomenon.

The combined therapy with topical prednisolone and olopatadine also significantly inhibited the increase in ear thickness; however, the discontinuation of the treatment from day 18 to 37 caused the recurrence of ear swelling whereas its inhibitory effect continued up to day 25. After day 32, its ear thickness exceeded that in the control group, and as was the case with the treatment and discontinuation of topical prednisolone alone (Fig. 2c).

Effects of olopatadine on the relapse of the ear swelling following discontinuation of the treatment with topical prednisolone

An exacerbated relapse of the ear swelling was observed following discontinuation of the treatment with topical prednisolone alone or its combination with olopatadine. To investigate the effect of olopatadine against this exacerbation, i.e., the rebound phenomenon, we administered olopatadine at 10 mg/kg/day after discontinuation of the treatment with topical prednisolone. Figure 3a shows the inhibitory effect of olopatadine on the recurrence of the ear swelling. Administration of olopatadine after discontinuation of the treatment with topical prednisolone significantly suppressed the increase in ear thickness. Figure 3b shows the sequential therapeutic effect of the treatment with olopatadine alone after discontinuation of the combined treatment with olopatadine and topical prednisolone. The treatment with olopatadine at 10 mg/kg/day alone significantly suppressed the increase in ear thickness after the discontinuation.

Effects on the levels of IFNγ, IL-4, IL-1β, IL-18, GM-CSF and NGF in the lesioned ear

To examine the mechanism by which olopatadine inhibits the development of ear swelling, we determined its effect on the levels of IFNγ, IL-4, IL-1β, IL-18, GM-CSF and NGF in the lesioned ear. As shown in Fig. 4, the levels of IL-4, IL-1β, IL-18, GM-CSF and NGF 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; 1057.6 ± 46.8 pg/site, oxazolone challenge; 561.2 ± 43.9 pg/site), resulting in Th2 dominance. Olopatadine significantly inhibited the increased levels of IL-4, IL-1β, IL-18, GM-CSF and NGF by 36.7%, 25.7%, 41.3%, 26.0% and 80.8%, respectively, and statistically significant inhibition was observed for IL-4, IL-18 and NGF. Prednisolone significantly inhibited the increased levels of IL-4, IL-1β, IL-18 and GM-CSF by 65.7%, 86.7%, 86.1% and 80.3%, respectively.

The level of IL-4 in the lesioned ear after discontinuation of the treatment with topical prednisolone significantly exceeded and those of IL-1β, IL-18, GM-CSF and NGF recovered to that in the oxazolone-challenged ear. Upon discontinuation of the treatment with olopatadine, those levels recovered to but did not elevate beyond the control levels.

The switching therapy with olopatadine, administered daily from day 18 to 37, after discontinuation of the treatment with topical prednisolone, significantly reduced the elevation of IL-4, IL-1β, GM-CSF and NGF levels by 38.8%, 27.3%, 38.2% and 62.3%, respectively. Similarly, the sequential therapy with olopatadine after discontinuation of the

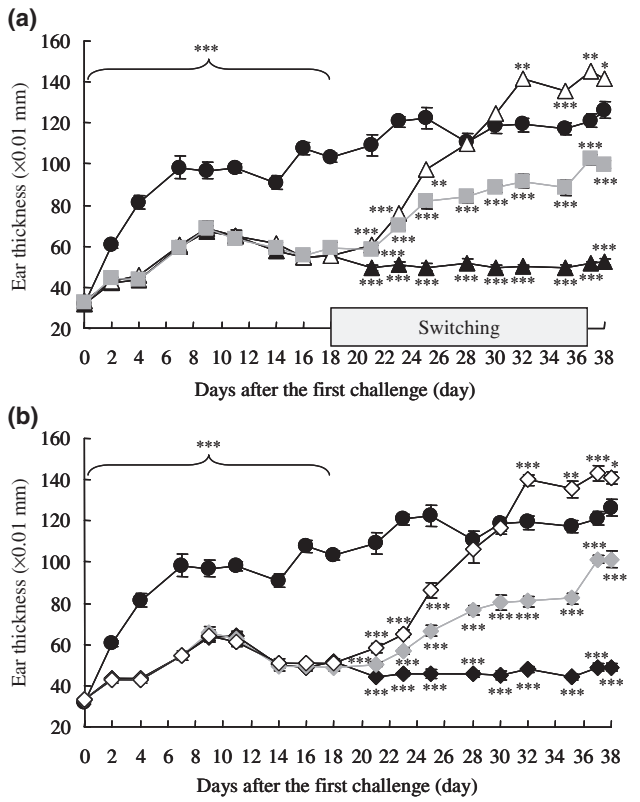

Fig. 3. Changes in the ear swelling after switching to the treatment with olopatadine hydrochloride (olopatadine). Mice were sensitized on the ear with oxazolone 7 days before the first challenge, and were repeatedly challenged on the sensitized ear with oxazolone three times per week until the end of the experimental period. After discontinuation of the treatment with topical prednisolone, olopatadine was sequentially treated from day 18 to 37. Olopatadine was orally administered and prednisolone was applied topically as described in Materials and methods. (a) ●, control; ▲, prednisolone at 0.05 mg/ear/day; △, discontinuation of prednisolone; ■, switching to the treatment with olopatadine at 10 mg/kg/day. (b) ●, control; ◆, olopatadine at 10 mg/kg/day and prednisolone at 0.05 mg/ear/day; ◇, discontinuation of olopatadine and prednisolone; ◆, switching to the treatment with olopatadine at 10 mg/kg/day. Each point represents the mean ± SEM. of 8 mice. Each column represents the mean+SE of eight mice. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, significantly different from the control group.

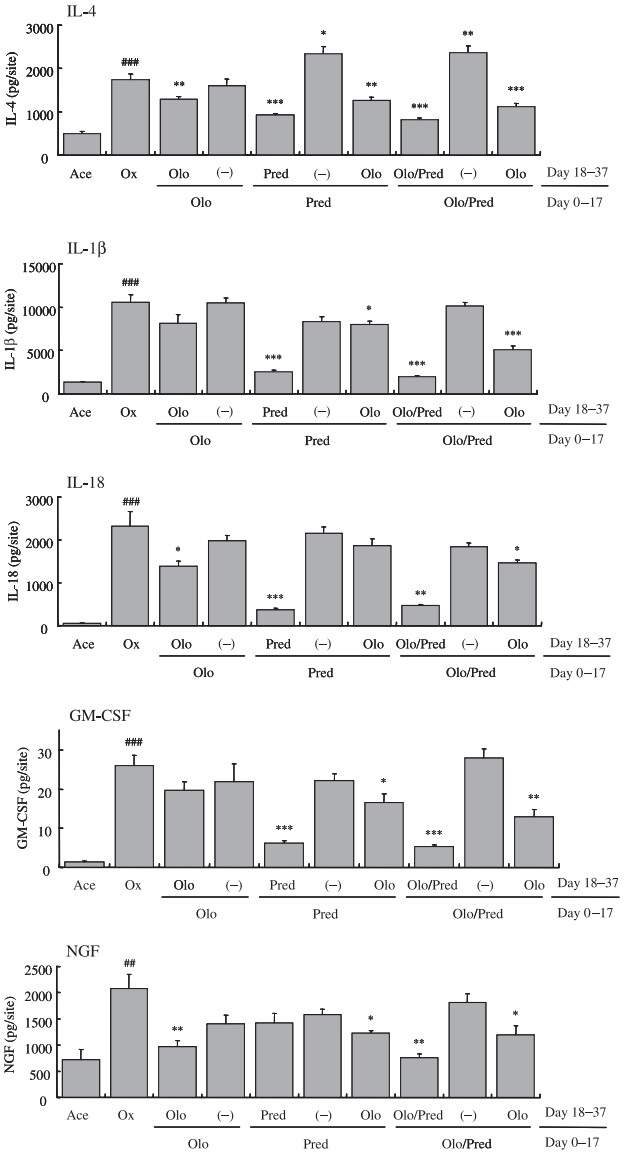

Fig. 4. Production of IL-4, IL-1β, IL-18, granulocyte-macrophage colony-stimulating factor (GM-CSF) and nerve growth factor (NGF) in the lesioned skin. IL-4, IL-1β, IL-18, GM-CSF and NGF levels in the homogenized ear tissue were measured 24 h after the final oxazolone challenge as described in Materials and methods. Ace, acetone; Ox, oxazolone challenge; Olo, olopatadine at 10 mg/kg/day; Pred, prednisolone at 0.05 mg/ear/day; Olo/Pred, olopatadine at 10 mg/kg/day and prednisolone at 0.05 mg/ear/day (−),discontinuation. Each column represents the mean+SEM. of 8 mice. $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$, significantly different from the acetone group. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, significantly different from the control group.

combined therapy with topical prednisolone and olopatadine significantly reduced the elevation of IL-4, IL-1β, IL-18, GM-CSF and NGF levels by 49.4%, 59.1%, 37.8%, 53.7% and 64.6%, respectively.

Effects on the levels of histamine in the lesioned ear

As shown in Fig. 5, the level of histamine in the lesioned ear taken at 24 h after the final challenge was significantly increased compared with that in the acetone-treated ear. Olopatadine significantly inhibited the increased levels of histamine by 27.3%. Prednisolone also significantly inhibited the increased levels of histamine. On the other hand, the levels of histamine in the lesioned ear after discontinuation of the treatment with olopatadine or topical prednisolone were not significantly different from those in the control group.

After discontinuation of the treatment with topical prednisolone, the switching therapy with olopatadine significantly reduced the elevation of histamine level by 32.0%. Moreover, the sequential therapy with olopatadine after discontinuation of the combination therapy significantly reduced the elevation of this level by 32.0%.

Effects on the serum IgE level

As shown in Fig. 6, the total serum IgE level was significantly increased by repeated challenge with oxazolone. Olopatadine did not affect the increase in total serum IgE levels. On the other hand, topical prednisolone significantly augmented the elevation of total serum IgE level in spite of their inhibiting the development of ear swelling and its level was further increased after discontinuation of the treatment with topical

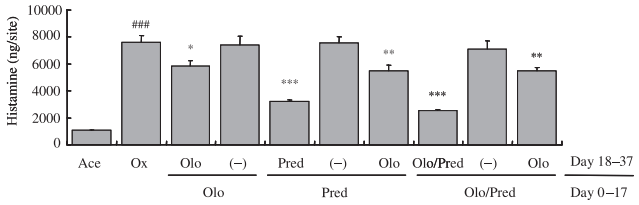

Fig. 5. Effects of olopatadine hydrochloride (olopatadine) and prednisolone on the production of histamine in the lesioned skin. Histamine levels in the homogenized ear tissue were measured 24 h after the final oxazolone challenge as described in Materials and methods. Ace, acetone; Ox, oxazolone challenge; Olo,olopatadine at 10 mg/kg/day; Pred, prednisolone at 0.05 mg/ear/day; Olo/Pred, olopatadine at 10 mg/kg/day and prednisolone at 0.05 mg/ear/day (–),discontinuation. Each column represents the mean+SEM. of eight mice. $^{###}P<0.001$, significantly different from the acetone group. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, significantly different from the control group.

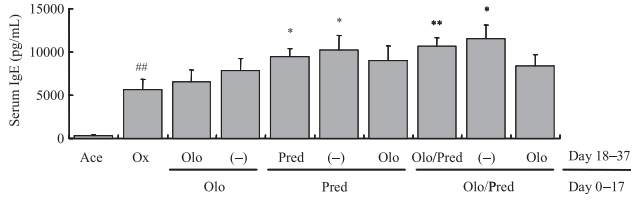

Fig. 6. Effects of olopatadine hydrochloride (olopatadine) and prednisolone on the increase of total serum IgE levels induced by repeated application of oxazolone. Blood was collected by orbital bleeding under ether anaesthesia 24 h after the final oxazolone challenge, and serum was harvested from each blood sample. Total serum IgE levels were determined as described in Materials and methods. Ace, acetone; Ox, oxazolone challenge; Olo, olopatadine at 10 mg/kg/day; Pred, prednisolone at 0.05 mg/ear/day; Olo/Pred, olopatadine at 10 mg/kg/day and prednisolone at 0.05 mg/ear/day, (–),discontinuation. Each column represents the mean+SEM. of 8 mice. $^{##}P<0.01$,significantly different from the acetone group. $^{*}P<0.05$, $^{**}P<0.01$, significantly different from the control group.

prednisolone. When olopatadine was administered after discontinuation of the treatment with topical prednisolone or its combination with olopatadine, the increase in total serum IgE levels was not statistically significant from that in the control group.

Discussion

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 [20], which showed that Th2 cytokines were abundant in the lesioned skin in this model. The Th2 cells accumulated in the lesioned skin are thought to be involved in the pathogenesis of dermatitis. 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 AD [25, 26]. These observations suggest that the Th2 cytokines, especially IL-4, play major roles in the development of dermatitis in the present mouse model. The present study confirmed that olopatadine mitigated the cutaneous inflammation in a mouse model of chronic inflammatory dermatitis induced by repeated challenge of oxazolone to the ear. Thus, olopatadine may offer a novel regimen for treating chronic inflammatory dermatitis such as AD by inhibiting the inflammatory cytokines.

In the present study, discontinuation of topical prednisolone, but not that of olopatadine, induced a relapse of ear swelling. We further showed that sequentially administered olopatadine after discontinuation of the treatment with prednisolone prevented this relapse phenomenon; olopatadine reduced the enhanced ear swelling response and the increased levels of IL-4, IL-1β, GM-CSF and NGF. The current first-line treatment regimen for the eczematous lesions of AD is the topical application of steroids and emollients. Although the topical steroid is commonly used for the treatment of AD and other skin disorders based on its potent anti-inflammatory effects, the relapse often occurs after discontinuation of the prolonged use of topical steroids [4–6]. Inoue et al. [27] reported that the discontinuation of the treatment with a topical prednisolone induced the relapse of skin inflammation in the mouse ear. In the present model of dermatitis, indeed, the discontinuation of the treatment with topical prednisolone caused the relapse of skin inflammation,the so-called 'rebound phenomenon', whereas the potent anti-inflammatory effect of topical prednisolone was confirmed during its treatment. In contrast, the discontinuation of olopatadine did not cause the relapse of skin inflammation, suggesting that it has a better safety profile than topical steroids. Accordingly, olopatadine may be a sequential therapeutic agent after discontinuation of the prolonged treatment with topical steroids.

The mechanism of the rebound phenomenon occurring after discontinuation of the prolonged use of topical steroids is not fully clarified. Kimata [23] reported that peripheral blood mononuclear cells taken from patients with AD during the rebound phenomenon after discontinuation of the prolonged treatment with topical steroid spontaneously produced Th2 cytokines. Inoue et al. [27] reported that Th2 cytokine mRNAs were abundantly detected in the lesioned skin. In addition, Almawi et al. [28] reported that steroids upregulated the cytokine receptor expression and enhanced cytokine effects. In this study, the level of IL-4 in the ear after discontinuation of the treatment with topical prednisolone was significantly elevated compared with that in the control ear during the rebound phenomenon. Moreover, the levels of IL-1β, IL-18, GM-CSF and histamine were also elevated nearly to the control level. Thus, the several cytokines, especially IL-4, and histamine are likely to act synergistically to cause the rebound phenomenon in this model. On the other hand, enhanced production of IgE is reported for the peripheral blood obtained from patients with AD during the rebound phenomenon after discontinuation of the treatment with topical steroids [23]. In the present study, topical prednisolone significantly augmented the increase in total serum IgE levels in spite of their inhibiting the development of ear swelling response, and this augmented increase continued during the rebound phenomenon after discontinuation of the treatment with topical prednisolone. Thus, these findings suggest that the rebound phenomenon may also involve the augmented increase of serum IgE levels.

The inhibition by olopatadine of the rebound phenomenon after discontinuation of the treatment with a topical steroid may be mediated by its suppressing effects on the increased levels of cytokines and histamine after discontinuation of steroid therapy. Our present data indeed indicated that sequentially administrated olopatadine inhibited the en-

hanced production of IL-4, other cytokines, NGF and histamine in the lesioned ear, when it prevented the rebound phenomenon after discontinuation of the treatment with a topical steroid. Keratinocytes are one of the important sources of cytokines, and express the receptor of histamine [29]. Accordingly, the blockade by olopatadine of histamine receptors of keratinocytes may be involved in the suppressed cytokines after discontinuation of a steroid. Mast cells also serve as an abundant source of Th1 and Th2 cytokines [14–16]. Olopatadine has been shown to inhibit the expression of IL-4 mRNA in RBL-2 mast cells [21], suggesting that the inhibition of the IL-4 mRNA may play a role. It is reported that the production of IL-4, a cytokine promoting IgE production, is enhanced in skin biopsy specimens of patients with AD [7, 23, 26]. In the present study, olopatadine inhibited the augmented increases of IL-4 and IgE levels in the lesioned ear following discontinuation of steroid therapy. The inhibition by olopatadine of the augmented IgE level may be ascribed to the suppressed increase of IL-4 level. Thus the inhibition of IgE production may have also contributed to the inhibited rebound phenomenon. Further investigation, however, is necessary to elucidate the exact mechanism of the inhibition by olopatadine of the rebound phenomenon.

In conclusion, olopatadine was demonstrated to be an antihistamine agent having inhibitory activities against the rebound phenomenon following discontinuation of the treatment with a topical steroid, possibly resulting from its diminishing effect on the elevated cytokines and histamine in the lesioned skin, and the elevated serum IgE level. Thus, olopatadine may be useful as a therapy in combination with a topical steroid, or a sequential therapeutic agent after discontinuation of the treatment with topical steroids for management of patients with recurring skin diseases.

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