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The antagonism of histamine H1 and H4 receptors ameliorates chronic allergic dermatitis via anti-pruritic and anti-inflammatory effects in NC/Nga mice

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Keywords
atopic dermatitis; histamine H1 receptor; histamine H4 receptor; keratinocytes; mast cells.

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Abstract

Background: Although histamine H1 receptor (H1R) antagonists are commonly used to treat atopic dermatitis, the treatment is not always effective. The histamine H4 receptor (H4R) was recently described as important to the pruritus in dermatitis. Here, we investigated whether the combination of a H1R antagonist plus a H4R antagonist attenuates chronic dermatitis in NC/Nga mice.
Methods: Chronic dermatitis was developed by repeated challenges with picryl chloride on the dorsal back and ear lobes. The therapeutic effects of the H1R antagonist olopatadine and H4R antagonist JNJ7777120 on scratching and the severity of dermatitis were evaluated. In addition, the mechanisms responsible for the anti-allergic effects of H1R and/or H4R antagonism were examined using bone marrow-derived mast cells (BMMC) and keratinocytes.
Results: JNJ7777120 attenuated scratching behavior after a single administration and improved dermatitis, as assessed with clinical scores, pathology, and cytokine levels in skin lesions when administered repeatedly. These effects were augmented by combined treatment with olopatadine, having a similar therapeutic efficacy to prednisolone. JNJ7777120 inhibited dose-dependently the production of thymus and activation-regulated chemokine/CCL17 and macrophage-derived chemokine/CCL22 from antigen-stimulated BMMC. In addition, olopatadine reversed the histamine-induced reduction of semaphorin 3A mRNA in keratinocytes.
Conclusion: Combined treatment with H1R and H4R antagonists may have a significant therapeutic effect on chronic dermatitis through the synergistic inhibition of pruritus and skin inflammation.

Atopic dermatitis (AD) is an allergic inflammatory disease characterized by intense pruritus, chronic eczematous plaques, and relapsing inflammation induced by repeated exposure to an antigen. The inflamed skin contains mast cells, eosinophils, and Th2 cells and exhibits histological abnormalities such as scaling, crusting, and lichenoid papules (1, 2). The cytokine milieu of the inflamed skin shows Th2-dominant responses indicated by production of IL-4, IL-5, and IL-13 (2). These responses are triggered by thymic stromal lymphopoietin (TSLP) produced by keratinocytes (3).

Vigorous pruritus is the most important issue for a therapeutic strategy in patients with AD, and the pruritus associated with AD is poorly controlled clinically and affects the quality of life of patients. Histamine has been extensively studied for its pruritogenic effects (4, 5). It has been shown to be a potent pruritogen when applied to both human normal skin (6) and diseased skin (7). However, there is no clear evidence that histamine H1 receptor (H1R) antagonists inhibit it.

Four types of histamine receptors have been identified to date. The fourth, the histamine H4 receptor (H4R), which

Abbreviations
AD, atopic dermatitis; BMMC, bone marrow-derived mast cells; H1R, histamine H1 receptor; H4R, histamine H4 receptor; HDC, histidine decarboxylase; MDC, macrophage-derived chemokine; NGF, nerve growth factor; PiCl, picryl chloride; Sema3A, semaphorin 3A; TARC, thymus and activation-regulated chemokine; TSLP, thymic stromal lymphopoietin.

was cloned in 2000, is expressed on several hematopoietic cells and plays important roles in the activation of mast cells, eosinophils, monocytes, dendritic cells, and T cells (8–11). Thus, H4R is considered a new therapeutic target for allergic inflammation in case of AD, asthma, and rhinitis (12–15). Using H4R-deficient mice or a H4R antagonist, it has been indicated that H4R plays roles in pruritus and acute inflammation (12, 13, 16–19). Previously, we examined the inhibitory effects of anti-histamines, which include pyrilamine, an H1R antagonist, cimetidine, an H2R antagonist, and thioperamide, an H3R/H4R antagonist on TPA-enhanced picryl chloride (PiCl)-induced allergic dermatitis in mice (12). As the results, we found that the H2R antagonist showed only a slight inhibition. In contrast, the H1R antagonist significantly inhibited the increase in the ear thickness in the early phase and the H3R/H4R antagonist significantly inhibited the delayed phase responses such as eosinophil infiltration, and the combination of the H1R antagonist and the H3R/H4R antagonist showed additive effects.

As far, there were several trials of the combination of H1R antagonist and H2R antagonist on AD, which indicated no benefit (20). Here, we investigated whether or not a H4R antagonist or H4R plus H1R antagonist inhibits chronic allergic dermatitis in NC/Nga mice. The mechanisms of anti-allergic function via the inhibition of H4R and/or H1R were also verified *in vitro*.

Materials and methods

Animals

Male NC/Nga mice (specific pathogen-free) aged 6 weeks were purchased from Japan SLC. All mice were treated in accordance with procedures approved by the local Animal Ethics Committee.

Sensitization and repeated challenge

For sensitization, 150 μl of a 5% (w/v) picryl chloride (Nacalai tesque, Kyoto, Japan) solution (3 : 1 in acetone/ethanol) was applied using a micropipette to the thoracic and abdominal areas. Five days later, the first challenge was performed by applying 200 μl of a 1.2% (w/v) PiCl solution in olive oil, to the back and to the left and right ears. This procedure was repeated once a week for up to 10 weeks.

Drugs

Olopatadine (3 mg/kg; AK scientific, Union, CA, USA), JNJ7777120 (30 mg/kg; provided by Johnson & Johnson Pharmaceutical Research & Development, L.L.C., San Diego, CA, USA), and prednisolone (3 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) were suspended in 0.5% carboxyl methylcellulose and administered orally in a volume of 5 ml/kg every other day from 5 to 10 weeks after the sensitization. The doses of these histamine antagonists were selected according to the previous reports (16, 18, 21, 22). For *in vitro* experiments, histamine antagonists were dissolved in DMSO and the final concentration of DMSO was adjusted to 0.1–0.2%.

Real-time PCR for histidine decarboxylase (HDC) and measurement of plasma levels of histamine

Forty-eight hours after the fifth challenge at 5 weeks, blood and the ear lobes were collected. The level of histamine in plasma was determined using a Histamine EIA kit (SPI-Bio, Montigny le Bretonneux, France), and total RNA of the tissues was extracted using an RNeasy Fibrous Tissue Kit (Qiagen, Hilden, Germany). The primers for real-time PCR were (forward) 5′-TGTGTCCGTCGTGGATCTGA-3′ and (Reverse) 5′-TTGCTGTTGAAGTCGCAGGAG-3′ for mouse GAPDH, and (forward) 5′-TCCATTAAGCTGTGGTTTGTGATTC-3′ and (reverse) 5′-CGCTTCTGACCAGAGATTCAAAGTA-3′ for mouse HDC.

Counting of scratching and scoring of dermatitis

Three days after the fifth challenge, the number of times the mice scratched during 2 h after receiving a single drug dose was counted and then the severity of the dermatitis was evaluated once a week from week 5 to 10 following repeated treatment. The observation items were (I) flare and hemorrhage, (II) edema, (III) excoriation and erosion, and (IV) incrustation and xerosis. Evaluation items were scored as follows: 0 = no sign; 1 = mild; 2 = moderate; 3 = severe. The sum of the scores for each evaluation item (maximum score: 12) was taken as the dermatitis score.

Histological analysis

Ten weeks after the sensitization, the ear lobe was excised and paraffin-embedded sections were prepared. Serial sections were stained with hematoxylin–eosin or 0.05% toluidine blue. $CD4^+$ helper T cells were immunostained with anti-mouse CD4 antibody (R&D systems, Minneapolis, MN, USA).

Measurement of levels of cytokines in skin lesions and IgE in plasma

Ten weeks after the sensitization, the ear lobe was immersed in liquid nitrogen, crushed with an SK Mill (Tokken, Kashiwa, Japan), and suspended in 1 ml of PBS containing a protease inhibitor (Complete™; Roche Diagnostics, Mannheim, Germany). The suspension was centrifuged at 1200 ***g*** for 10 min, and the supernatants of tissue homogenates and the plasma were used to analyze the levels of IL-4, IL-5, TSLP (Biolegend, San Diego, CA, USA), thymus and activation-regulated chemokine (TARC) (R&D systems), nerve growth factor (NGF) (Promega, Madison, WI, USA), and plasma IgE (Biolegend) with ELISA kits.

Preparation of bone marrow-derived mast cells

Bone marrow-derived mast cells (BMMC) were prepared from the bone marrow cells aspirated from NC/Nga mice (6–8 weeks old). The bone marrow cells were cultured in RPMI1640 medium that contained 10% FBS, HEPES (25 mM), penicillin (100 units/ml), streptomycin (100 μg/ml),

and recombinant mIL-3 (300 pg/ml; R&D systems) for 9 days. More than 80% of bone marrow cells were positive for FcεRI and c-kit, as assessed by flow cytometric analysis with PE-labeled anti-mouse FcεRI antibody (Biolegend) and FITC-labeled anti-mouse c-kit antibody (Biolegend).

Stimulation of BMMC with anti-DNP IgE and DNP-BSA, and measurement of levels of thymus and activation-regulated chemokine (TARC/CCL17) and macrophage-derived chemokine (MDC/CCL22)

Bone marrow-derived mast cells were primed overnight with anti-DNP IgE (0.5 μg/ml; Sigma-Aldrich). The cells were treated with olopatadine and/or JNJ7777120 for 30 min and then stimulated with DNP-BSA (1–25 ng/ml; LSL, Japan) for 24 h. The amount of histamine in the culture medium was determined by EIA, and TARC and MDC levels were measured with ELISA kits (R&D systems).

Change of semaphorin 3A (Sema3A) mRNA levels after histamine treatment in PAM212 cells

PAM 212, a murine keratinocyte cell line, kindly supplied by Dr. S. H. Yuspa, NCI, NIH, USA, was cultured in RPMI1640 medium containing 10% FBS, penicillin (100 units/ml), streptomycin (100 μg/ml), and HEPES (25 mM). The cells were incubated for 30 min in medium containing olopatadine and then stimulated with histamine at various concentrations. The level of mRNA for Sema3A was evaluated by real-time PCR. The primers for PCR were (forward) 5′-AGATGCTCCATTCCAGTTTGTTCAC-3′ and (Reverse) 5′-ACATAAGCCACCGCATCACTTGTA-3′ for mouse Sema3A.

Statistical significance

Values are presented as the standard error of the mean. The statistical significance of the results was analyzed with the Student's *t*-test, Dunnett's test for parametric analysis, or corresponding to score for nonparametric analysis.

Results

Increased histamine production and release in mice with PiCl-induced chronic dermatitis

The NC/Nga mice treated with PiCl for 5 weeks developed moderate dermatitis with erythema, crusting and skin erosion on the treated skin and ear lobes (Fig. 1B) compared with intact mice (Fig. 1A). Forty-eight hours after the fifth treatment, the level of histamine in plasma (Fig. 1C) and the level of HDC mRNA in skin lesions (Fig. 1D) were significantly increased.

Effects of single administration of olopatadine and JNJ7777120 on scratching behavior

Three days after the fifth challenge, drugs were administered and scratching counts were determined. As shown in Fig. 1E, the number of times the mice scratched during 2 h was significantly increased. Administration of olopatadine or JNJ7777120 apparently reduced the counts, and combined treatment significantly decreased them. The anti-pruritus effect of the combined treatment was as potent as that of prednisolone.

Amelioration of dermatitis by repeated administration of olopatadine and JNJ7777120

Olopatadine and/or JNJ7777120 were administered every other day after the fifth treatment with PiCl, and the dermatitis score was evaluated until week 10. The dermatitis score progressively increased dependent on the number of challenges with PiCl. JNJ7777120, but not olopatadine, prevented this increase (Fig. 1F). Interestingly, combined treatment had a significant effect from 8 weeks (Fig. 1F), and the severity of the dermatitis was reduced about 50% compared with the control group at 10 weeks. Consistent with the anti-pruritic effects, dual inhibition of H1R and H4R was as effective as prednisolone.

Effects of repeated administration of olopatadine and JNJ7777120 on histological changes and the number of mast cells

Histological examination revealed that the repeated treatment with PiCl caused severe acanthosis and the infiltration by inflammatory cells including lymphocytes and eosinophils of the ear lobe (Fig. 2B, E). The number of mast cells also increased significantly (Fig. 2G, I). Combined treatment with olopatadine and JNJ7777120 improved the hyperepidermis (Fig. 2C, F). JNJ7777120 decreased the number of mast cells (Fig. 2H, I). Olopatadine alone did not decrease but augmented the inhibitory effects of JNJ7777120 (Fig. 2I).

Effects of repeated administration of olopatadine and JNJ7777120 on levels of cytokines in tissue and IgE in plasma

Next, we assessed the levels of IL-4, IL-5, TSLP, TARC, and NGF in tissue, and IgE in plasma at 10 weeks after the sensitization. Olopatadine decreased the level of NGF but not IL-4, IL-5, TSLP, TARC, or IgE (Fig. 3). In contrast, JNJ7777120 not only significantly decreased the NGF levels but also markedly inhibited the increases in IL-4, IL-5, TSLP, and TARC (Fig. 3). Although neither olopatadine nor JNJ7777120 alone reduced the plasma IgE level, in combination they inhibited the increase in IgE level similar to prednisolone (Fig. 3F). The production of MDC in tissue was not significantly inhibited by olopatadine, JNJ7777120, and prednisolone (data not shown).

Effects of olopatadine and JNJ7777120 on production of TARC and MDC in BMMC

To analyze the mechanism behind the anti-inflammatory effects of H1R and H4R antagonists, we tested whether olopatadine and/or JNJ7777120 inhibit TARC and MDC

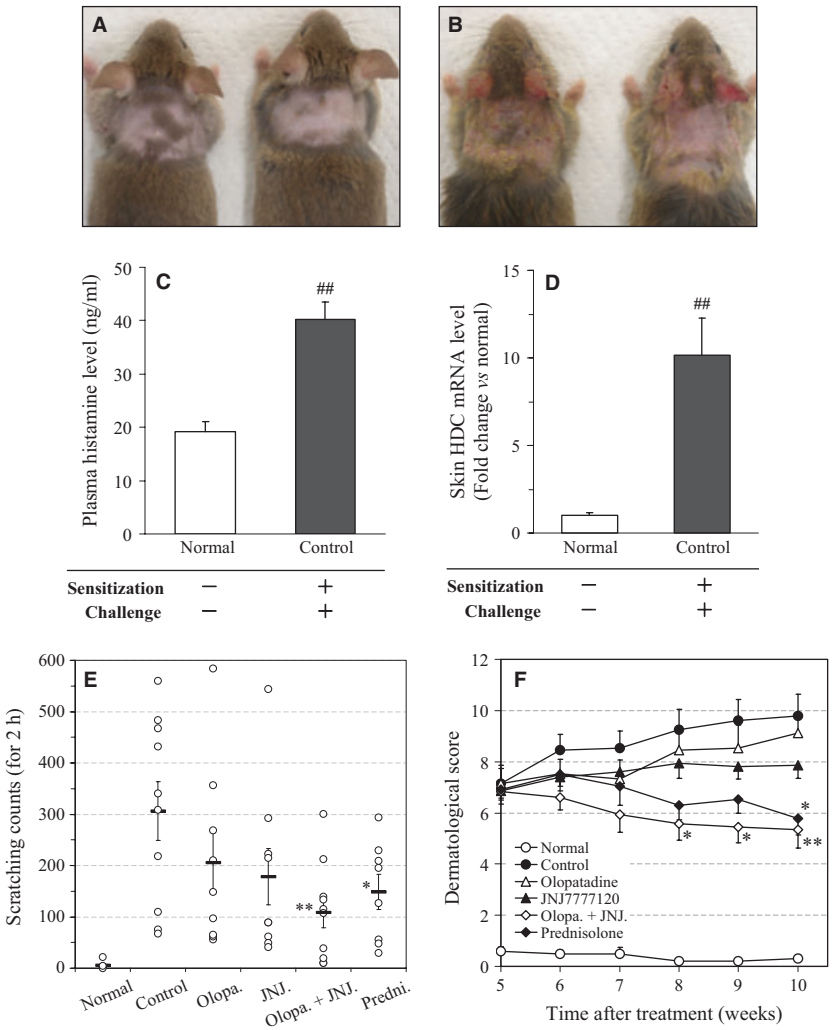

Figure 1 Increased histamine levels in picryl chloride (PiCl)-induced chronic allergic dermatitis and effects of olopatadine and JNJ7777120 on scratching counts and dermatological scores. NC/Nga mice were sensitized and challenged as described in 'Materials and Methods'. Forty-eight hours after the challenge, mice of the normal group (intact mice, A) and control group (PiCl-sensitized and challenged mice, (B) were photographed. The levels of histamine in plasma (C) and histidine decarboxylase (HDC) mRNA in the inflamed skin (D) were determined. Three days after the fifth challenge, mice were orally administered olopatadine (3 mg/kg), JNJ7777120 (30 mg/kg), olopatadine plus JNJ7777120, or prednisolone (3 mg/kg), and then, scratching counts for 2 h were determined (E). The severity of the dermatitis was scored once a week from week 5 to 10 during repeated administration (F). Statistical significance; $^{\#\#}P < 0.01$ *vs* normal group ($n = 4$), $^{*}P < 0.05$ and $^{**}P < 0.01$ *vs* control group ($n = 6$–10).

produced by BMMC. Bone marrow cells prepared from NC/Nga mice were induced to differentiate into FcεRI^{+}/c-kit^{+} mast cells (more than 80%) over 9 days (Fig. 4A). The stimulation of BMMC with the antigen increased the levels of histamine (Fig. 4B), TARC, and MDC (Fig. 4C) in the medium collected at 24 h. The histamine release was slightly inhibited by olopatadine at 10 μM (about 16% inhibition) but not JNJ7777120 at 30 μM (data not shown). The production of TARC and MDC was inhibited slightly by olopatadine at 10 μM and significantly by JNJ7777120 in a dose-dependent manner (30–100 μM) (Fig. 4C). The H3R/H4R antagonist thioperamide (100 μM) also suppressed the production of TARC (Fig. 4D). Furthermore, JNJ7777120 plus olopatadine inhibited additively TARC production (Fig. 4D). It was unlikely that JNJ7777120 reduced the production of these cytokines via toxic effects because JNJ7777120 did not reduce the antigen-induced release of IL-13 (data not shown).

Consistent with the reduction in TARC and MDC production in BMMC, the infiltration of CD4-positive cells in the skin 10 weeks after the sensitization was decreased by the combined treatment with olopatadine and JNJ7777120 (Fig. 4E, F).

Effects of olopatadine on histamine-induced down-regulation of Sema3A expression in PAM212 cells

Finally, we tested whether olopatadine and/or JNJ7777120 affect the level of mRNA for Sema3A, the regulatory

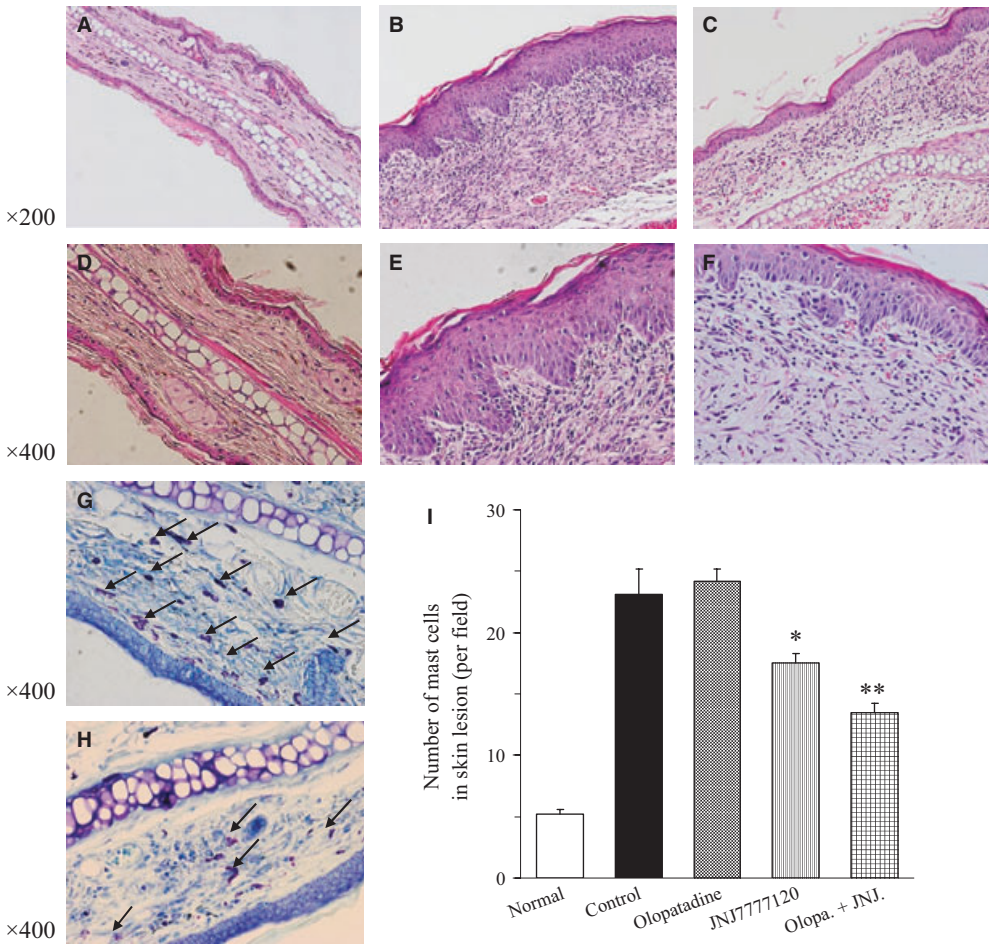

Figure 2 Effects of olopatadine and JNJ7777120 on histological changes in mice treated repeatedly with picryl chloride (PiCl). Ten weeks after the sensitization, skin specimens prepared from intact mice (normal group; A, D), PiCl-treated mice (control group; B, E), and PiCl-treated mice administered olopatadine plus JNJ7777120 (C, F) were stained with hematoxylin and eosin and observed with a light microscope at magnification ×200 (A, B, C) and ×400 (D, E, F). The skin specimens prepared from PiCl-treated mice (G) and PiCl-treated mice administered olopatadine plus JNJ7777120 (H) were stained with toluidine blue, and the numbers of mast cells were counted from four fields with a light microscope ×400 (I). Statistical significance; $*P < 0.05$ and $**P < 0.01$ *vs* control group ($n = 4$–5).

factor of neuronal elongation. The expression of H1R was detected by real-time PCR, but H4R was not detected in unstimulated and histamine-stimulated PAM212 cells (data not shown). The level of mRNA for Sema3A was decreased by histamine at 10 μM (Fig. 5A). The level reached a minimum at 3 h and slowly recovered over the next 9 h (Fig. 5B). The histamine-induced reduction in the level of Sema3A mRNA was antagonized by olopatadine (Fig. 5C) but not by JNJ7777120 at 30 μM (data not shown).

Discussion

In this study, we demonstrated that the H1R antagonist olopatadine and H4R antagonist JNJ7777120 improved scratching behavior and skin inflammation in a model of chronic allergic dermatitis established in NC/Nga mice. The effectiveness of the combined treatment against the dermatitis was almost equal to that of prednisolone.

Skin aberrations such as erythema, lichenification, and erosion accompanied the scratching behavior. The findings that histamine level in plasma and mRNA level for HDC in skin tissue were elevated in mice with chronic inflammation suggested histamine to be involved in the development of chronic allergic dermatitis in this model. As plasma histamine levels were reported to be significantly higher in patients with AD than controls (23, 24), this NC/Nga-based model of chronic dermatitis is an important tool for studying the roles of histamine in AD.

Recently, the anti-allergic effects of H4R antagonists were mainly evaluated in dermatitis model in mice (13, 17–19). Rossbach et al. (17) reported that a combination of H4R and H1R antagonism has prophylactic effects on acute hapten-induced scratching, but not chronic dermatitis. Only the study by Suwa et al. (19) was evaluated therapeutic effects for a H4R antagonist, but is not evaluated about benefits on combined treatment of a H1R and a H4R

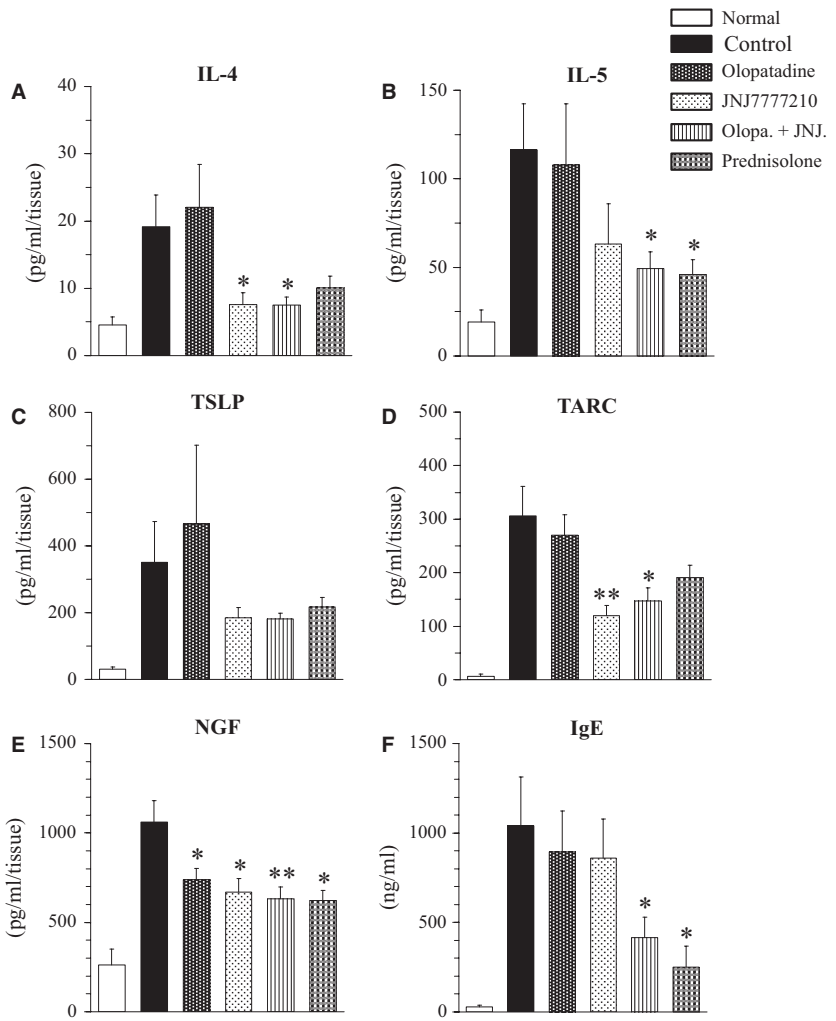

Figure 3 Effects of olopatadine and JNJ7777120 on tissue cytokines and plasma IgE levels in picryl chloride (PiCl)-induced chronic allergic dermatitis. Ten weeks after the sensitization, the levels of cytokines (A–D), nerve growth factor (NGF) (E) in inflamed skin and total IgE in plasma (F) were determined. Statistical significance; $*P < 0.05$ and $**P < 0.01$ *vs* control group ($n = 6$–10).

antagonist. In our study, we first clarified with the therapeutic efficacies in chronic dermatitis, administered both H1R and H4R antagonists. In our model of chronic dermatitis, a H4R antagonist suppressed the PiCl-induced scratching behavior and, combined with a H1R antagonist, had an additive effect as reported in an acute inflammatory model by Rossbach et al. and chemical-induced pruritus model by Dunford et al. (16). As Thurmond et al. (25) suggested that both H1R and H4R are expressed on C-afferent fiber terminals, these drugs might inhibit directly the transmission of itching responses from the peripheral to central nervous system. In addition, we found that both olopatadine and JNJ7777120 reduced the level of NGF in skin lesions. It has been reported that the level of NGF reflected the severity of itching and eruptions in AD (26, 27) and changed the correlation with clinical conditions in olopatadine-treated patients (27). Histamine also induced the production of NGF via H1R in human keratinocytes (28). Our findings suggested that H4R as well as H1R was involved in the production of NGF, resulting in the increase in innervation in the epidermis.

Conversely, Sema3A expression was found to be decreased at the horn layer with immunohistological staining in the skin lesions of patients with AD (29). The decrease in the expression of Sema3A in the inflamed skin was also observed in our model (data not shown). In addition, decrease in Sema3A mRNA in the epidermis from *Dermatophagoides farinae*-induced chronic dermatitis in NC/Nga mice was recovered by olopatadine (30). In this study, we found that histamine decreased the level of Sema3A and olopatadine blocked this reduction in mouse keratinocytes. Because human keratinocytes also express H4R (31), the expression of Sema3A in human keratinocytes might possibly be regulated by H4R as well as H1R. To confirm this possibility, we have now begun studying it using mouse and human primary keratinocytes. These findings indicated that histamine acting via H1R and H4R induces the pruritus in chronic skin inflammation through

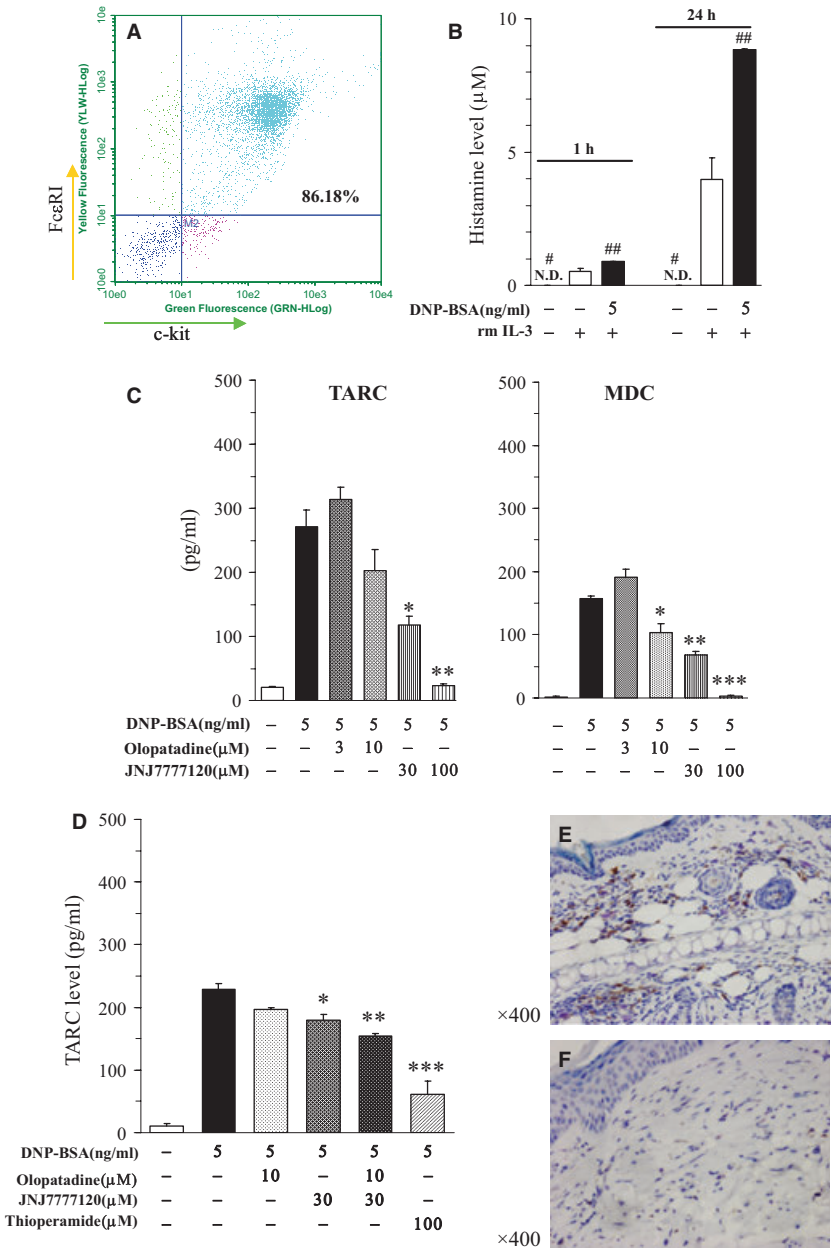

Figure 4 Effects of olopatadine and JNJ7777120 on production of thymus and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC) by antigen-stimulated bone marrow-derived mast cells (BMMC) and infiltration of CD4-positive cells in the skin lesions. (A) Bone marrow cells freshly prepared from NC/Nga mice were cultured with IL-3 (300 pg/ml) for 9 days and the expression of FcεRI and c-kit was confirmed by flow cytometry. (B) BMMC were primed overnight with 0.5 μg/ml of DNP IgE and then stimulated with DNP-BSA at the indicated concentrations. The level of histamine (1 and 24 h) in the supernatant was determined. Statistical significance; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *vs* unstimulated. (C, D) The cells were treated with olopatadine, JNJ7777120 and thioperamide for 30 min, and then stimulated with DNP-BSA for 24 h. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs* DNP-BSA stimulation. (E, F) Using the tissue specimens in Fig. 2, the CD4-positive T cells that had infiltrated the skin lesions in picryl chloride (PiCl)-treated mice were revealed by immunohistological staining (control group, E; olopatadine and JNJ7777120-administered group, F).

multiple mechanisms and that the combination of H1R and H4R antagonists has additive anti-pruritus effects.

Previously, we reported that the H3R/H4R antagonist thioperamide ameliorated ear swelling in mice with TPA-modified hapten-induced allergic dermatitis, and in combination with the H1R antagonist pyrilamine suppressed the biphasic allergic response: the early phase in which vascular permeability is increased by the degranulation of

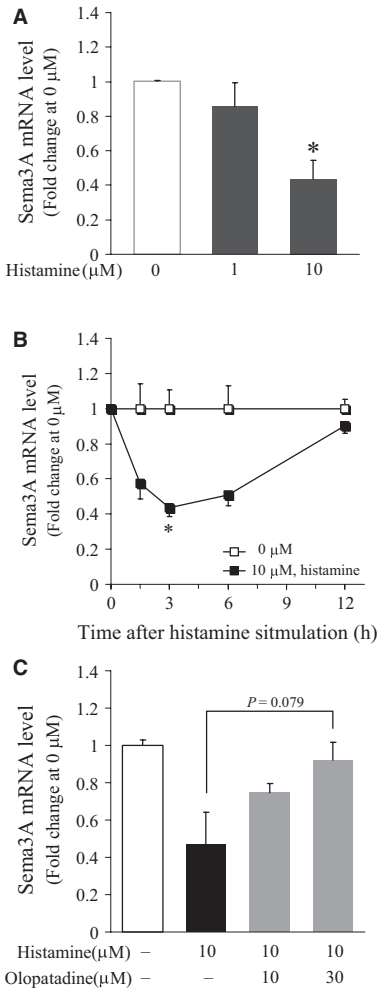

Figure 5 Effects of olopatadine on histamine-induced down-regulation of semaphorin 3A (Sema3A) expression in PAM212 cells. PAM212 cells were cultured with various concentrations of histamine for 3 h, and the level of mRNA for Sema3A was determined by real-time PCR (A). Changes in the level of Sema3A mRNA after the histamine treatment were also measured (B). The cells were cultured for 30 min in medium containing olopatadine and stimulated with histamine at 10 μM for 3 h (C). The level of mRNA is indicated as the fold difference normalized to the unstimulated control. Statistical significance; $*P < 0.05$ *vs* histamine at 0 μM.

mast cells and the late phase which is associated with infiltration by eosinophils and T cells (12). Here, we indicated that chronic allergic dermatitis was also clearly improved by a H1R antagonist plus H4R antagonist (Figs 1 and 2) with almost the same effect as prednisolone (Fig. 1). The effect of the combined treatment on the number of mast cells in skin lesion was also similar to that of prednisolone, which significantly decreased the infiltration of mast cells in skin lesion treated with PiCl repeatedly (32).

JNJ7777120 alone significantly inhibited the production of Th2 cytokines in the skin lesions (Fig. 3). We found that JNJ7777120 markedly inhibited the production of TARC and MDC by antigen-stimulated BMMC. BMMC significantly secreted histamine on stimulation with IgE and then produced TARC and MDC, which are chemokines acting through the CCR4 mainly expressed by Th2 cells. JNJ7777120 inhibited the production of TARC and MDC without inhibiting histamine release. Because BMMC have H4R but not H3R (8), the inhibitory actions of thioperamide could be attributed to H4R antagonism. The inhibition of TARC and MDC production by olopatadine, at least a part, is assumed to be due to the inhibition of degranulation. In one such example, it has been reported olopatadine inhibits IgE-mediated histamine release from human conjunctival mast cells (33). Thus, the histamine released by antigen-IgE stimulation could induce the production of TARC and MDC via H4R.

Because TARC levels in patients with AD correlate with the scoring of the AD index and eosinophil numbers (34, 35), an extracorporeal diagnostic agent, serum TARC (Shionogi & Co., Osaka, Japan), is used as a biomarker against AD in Japan. Our findings suggested that JNJ7777120 inhibited the infiltration of $CD4^+$ T cells in the skin lesions probably by inhibiting the production of TARC (Fig. 4), resulting in the alleviation of chronic dermatitis.

Prednisolone is used externally in the treatment of AD. In our model, prednisolone inhibited scratching count, dermatological score, the production of several cytokines, and the increase in serum IgE. Importantly, the combined treatment of olopatadine and JNJ7777120 could inhibit these parameters to almost similar extent. Glucocorticoids containing prednisolone frequently causes atrophy of skin and elicit side-effects by systemic administration. On the other hand, a new H4R antagonist from Palau Pharma is currently in Phase II clinical trial and has shown that the H4R antagonist is safe and very well tolerated. Therefore, our findings suggest the combined treatment of H1R and H4R antagonists might be a potent and safer therapeutic strategy to allergic diseases.

In conclusion, we clarified that histamine is involved in pruritus and the development of dermatitis in a model of chronic allergic dermatitis established in NC/Nga mice. In addition to the direct involvement of histamine in pruritus via H1R and H4R, we clarified that histamine was also associated with the regulation of Sema3A in keratinocytes and production of Th2 chemokines in BMMC, via H1R and H4R respectively. Thus, our findings indicated that combined treatment with a H1R antagonist plus H4R antagonist potentiated both anti-pruritic and anti-inflammatory effects, and had a pharmaceutical benefit equivalent to prednisolone. Our understanding of the roles of histamine in chronic dermatitis indicate that a blockade of the H4R antagonist as well as H1R antagonist might have more potential benefit for patients with AD.

Conflict of interest

The authors declare no financial or commercial conflict of interest.

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