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Expression of the Histamine H_4 Receptor in Human Tissue

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1. Introduction

The histamine H_4 receptor (H_4R) is the most recently identified of the four histamine receptors (H_1R–H_4R), and belongs to the same G-protein-coupled receptor (GPCR) family. The amino acid sequence of H_4R shares approximately 26%, 27%, and 58% homology with H_1R, H_2R, and H_3R, respectively, in the transmembrane regions (Nguyen et al., 2001). Furthermore, H_4R couples to Gi/o proteins and shows 10^3- to 10^4-fold higher ligand affinity than H_1R and H_2R (Thurmond et al., 2008).

Several organs express H_4R, and immune tissues such as the spleen, thymus, bone marrow, and leukocytes have a wide range of expression levels (Oda et al., 2000). It has been reported that chemotaxis of mast cells and eosinophils is stimulated by histamine via H_4R; the receptor is therefore attractive as a new target of research into allergic diseases (de Esch et al., 2005).

2. Expression of H_4R in synovial tissue in rheumatoid arthritis

A role for histamine has been implicated in rheumatoid arthritis (RA). RA consists mainly of synovial tissue inflammation that may be dispersed throughout the body, but its molecular etiology remains unclear. Macrophage infiltration and excessive formation of fibroblasts cause a variety of cytokines to be secreted from synovial membranes in patients with RA, and this in turn stimulates osteolytic activity (Sweeney & Firestein, 2004). There is evidence of a significant increase in histamine concentration in synovial samples from patients with RA (Frewin et al., 1986). These observations suggest a potentially significant role of H_4R in the cause, progression, and treatment of RA.

The presence of H_1R and H_2R in human synovial cell culture (HSCC) has been clearly shown by ligand-binding experiments (Nagata, 1991). However, there has been no definitive evidence or conclusive reports of the similar presence of H_3R or H_4R. Therefore, utilizing our expertise in reverse transcription polymerase chain reaction (RT-PCR) techniques, we examined the H_4R-specific mRNA expression in HSCC obtained from 11 RA patients who underwent artificial knee-replacement surgery (Ikawa et al., 2005).

After excising the synovial membrane specimen under aseptic conditions, the sample was treated with collagenase and trypsin solution to separate it into single cells. The cells were cultured for 2 weeks in medium containing fetal bovine serum. When the culture reached confluence, the cells were harvested and all RNA extracted. Analysis of the expression of the 4 subtypes of histamine receptor-specific mRNA in 2 patients with RA (RA1 and RA2) by RT-PCR showed that, under the experimental conditions, H_1R-, H_2R-, and H_4R-specific mRNAs were expressed, but H_3R-specific mRNA was not (Fig. 1). Expression of H_4R-specific mRNA was confirmed in all 11 samples (RA1–RA11; Fig. 2). Notably, the intensity of the separated H_4R-specific mRNA bands varied considerably from one sample to another, suggesting differences in cellular concentrations of H_4R between patients.

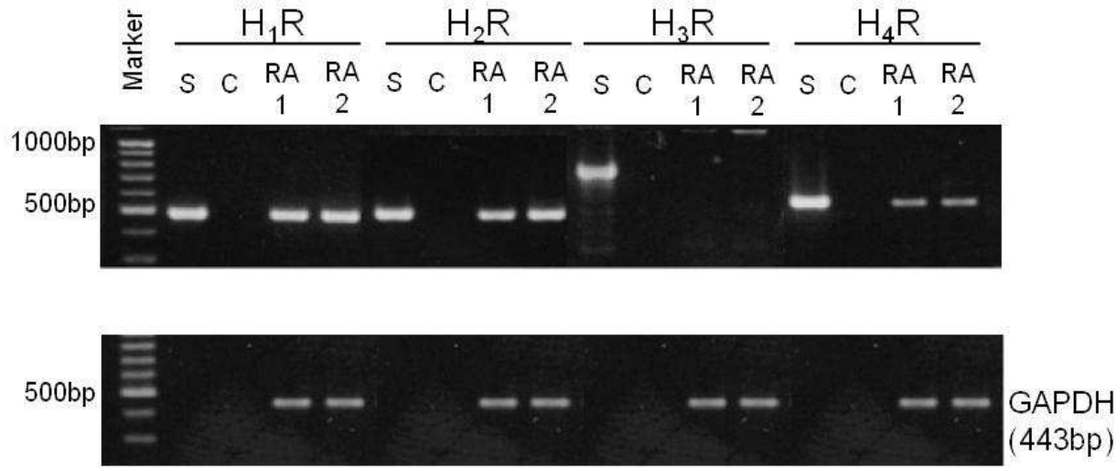

Fig. 1. Expression of mRNAs specific to 4 subtypes of histamine receptor (H_1R, H_2R, H_3R, and H_4R) in HSCC from 2 patients with RA. The gel was loaded with 5 L of amplified products. A 100-bp DNA ladder was used as a molecular weight marker. S: standard; C: control; samples from 2 RA patients, RA1 and RA2. Figure reprinted with permission from the Pharmaceutical Society of Japan.

Inflamed synoviocytes consist of 3 cell types: (1) macrophage-like cells; (2) fibroblast-like cells; and (3) dendritic cells (Tanaka, 2005). High levels of lymphocyte infiltration have been observed in RA compared to other types of arthritis (Fonseca et al., 2005). A variety of cell types such as macrophage-like cells, dendritic cells, and granulocytes have also been identified in the human RA synovium. As H_4R has been reported to be present in immune cells, expression of H_4R mRNA seems most likely to occur in cells derived from the hematopoietic system, such as macrophage-like or dendritic cells from synovial sites. Consequently, we examined the protein expression levels of H_4R in RA HSCC, and used fluorescence immunoassays (Ohki et al., 2007) to determine the types of the cells in which expression occurred by identifying co-expression of cell type-specific human proteins: PH and CD55 for fibroblast-like cells; CD68 and CD163 for macrophage-like cells; and CD1a and CD208 for dendritic cells.

First, we examined the expression patterns of prolyl-4-hydroxylase (PH) (red) and CD68 (green) using 2 morphologically distinct cell types we identified in our HSCC: fibroblast-like and macrophage-like cells. In similar experiments, no expression of human dendritic cell markers (either CD1a or CD208) was detectable. Subsequent assays for fibroblast and

macrophage markers showed that human H_4R protein is expressed in both fibroblast-like and macrophage-like cells in RA synovial tissues (Fig. 3 and Fig. 4). Others have also reported identification of H_4R in synovial tissue of patients with RA (Grzybowska-Kowalczyk et al., 2007).

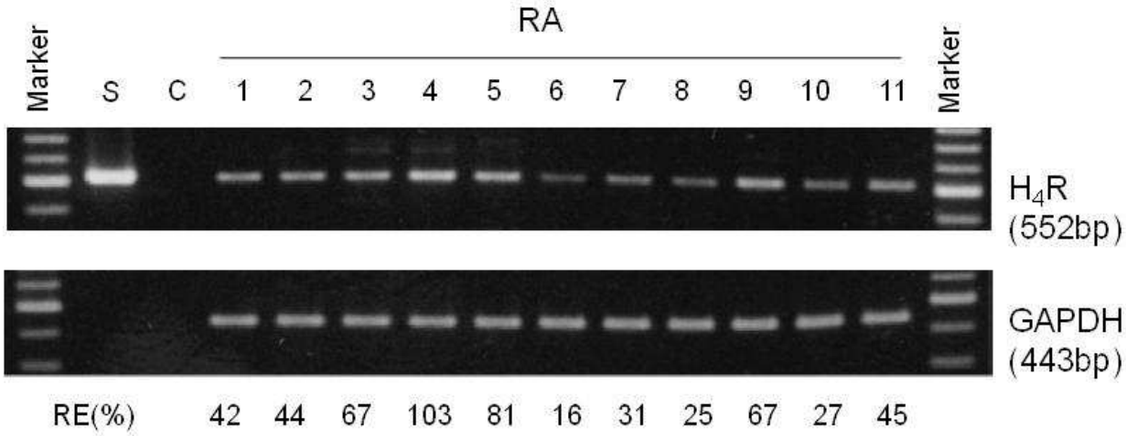

Fig. 2. Expression of H_4R-specific mRNA in HSCC from 11 RA patients. The gel was loaded with 5 L of amplified products. A 100-bp DNA ladder was used as a molecular weight marker. S: standard; C: control; RE: relative expression; sample from 11 different RA patients, RA1 to RA11. RE of mRNA was calculated by normalizing the separated sample intensity value, taking that of the corresponding internal control (GAPDH) as 100%. Intensity values were measured using an image analyzer (IX81, OLYMPUS). Figure reprinted with permission from the Pharmaceutical Society of Japan.

Next, we focused attention on the expression of H_4R mRNA in synovial tissues, and serum matrix metalloproteinase-3 (MMP-3) concentration in RA. We found a significant negative correlation between H_4R expression in synovial tissues and serum MMP-3 concentration, but no correlation between MMP-3 and H_1R or H_2R (Yamaura et al., 2011). These observations suggest that H_4R is a potential target of novel pharmacotherapeutic agents for RA, and H_4R functional analysis may be useful in developing such treatments.
In addition, we detected H_4R expression in human cartilage chondrocytes and in a murine chondrocytic cell line, ATDC5 (Yamaura et al., in press). Further work is needed to determine the expression mechanism and function of H_4R on chondrocytes.

3. Expression of H_4R in human skin

Following detection of H_4R expression in synovial tissue, we also analyzed H_4R expression in human epidermal tissue (Yamaura et al., 2009) and fibroblast cell cultures (Ikawa et al., 2008). Our immunoassays revealed that H_4R is expressed in both human epidermal tissues and dermal fibroblasts.
Keratinocytes are the major constituent of the epidermis. We found that immunohistochemical staining showed strong H_4R expression in keratin (K)10-positive differentiated keratinocytes in the prickle cell and granular layers of the epidermis (Fig. 5a). By contrast, H_4R was weakly expressed in K14-positive proliferating keratinocytes in the basal layer (Fig. 5b).

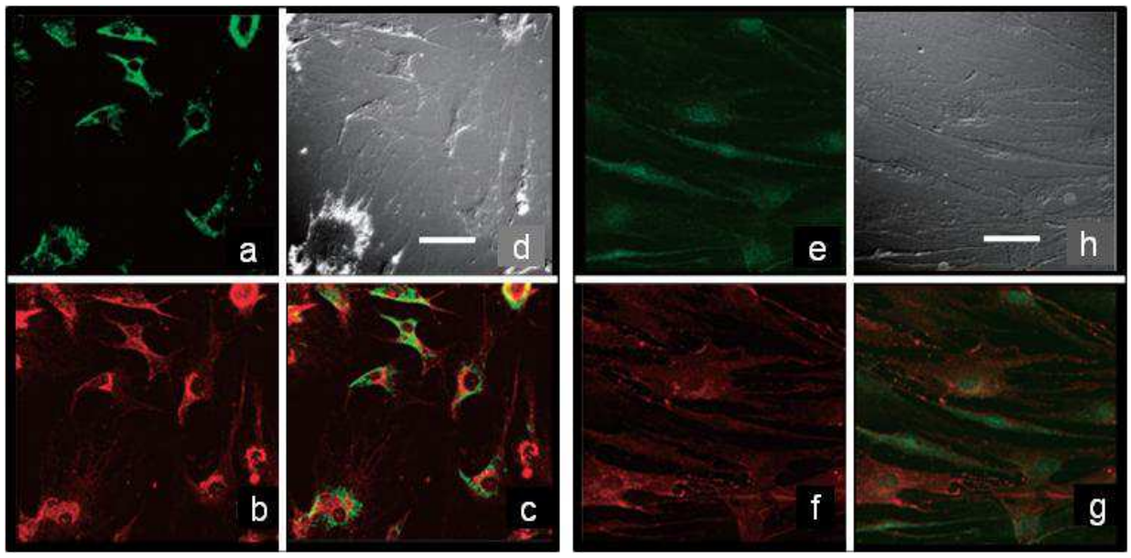

Fig. 3. Co-expression of H_4R protein with fibroblast-specific marker proteins. (a) Mouse anti-PH followed by Cy2-conjugated anti-mouse (green); (b) rabbit anti-H_4R followed by Cy3-conjugated anti-rabbit (red); (c) superposition of a on b; (d) Nomarski phase contrast microscopy image (NPCMI); (e) rabbit anti-H_4R followed by Cy2-conjugated anti-rabbit (green); (f) mouse anti-CD55 followed by Cy3-conjugated anti-mouse (red); (g) superposition of e on f; (h) NPCMI. Scale bar: 50 m. Figure reproduced with permission from the Pharmaceutical Society of Japan.

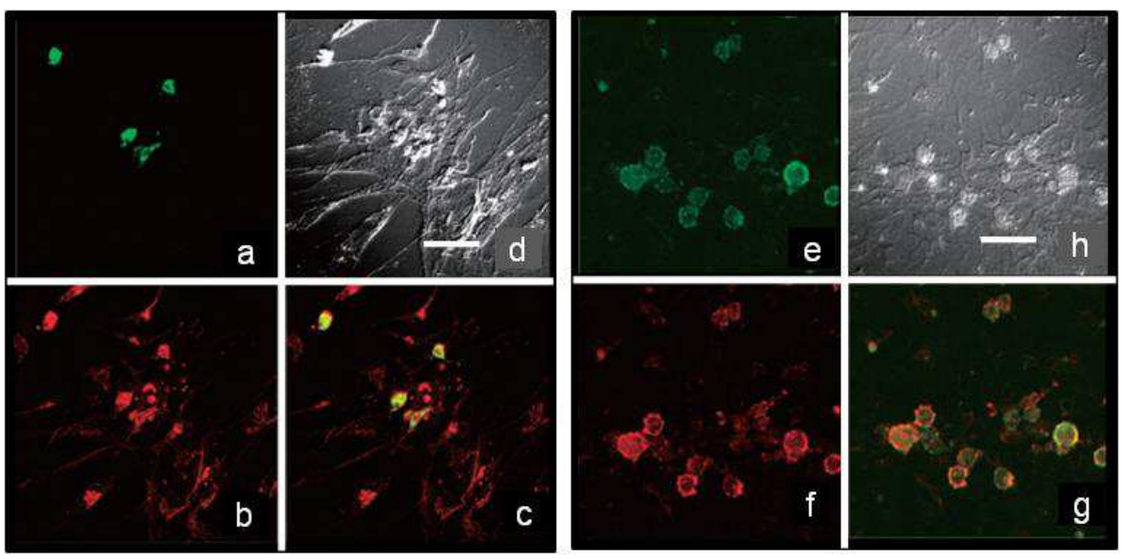

Fig. 4. Co-expression of H_4R protein with macrophage-specific marker proteins.(a) Mouse anti-CD68 followed by Cy2-conjugated anti-mouse (green); (b) rabbit anti-H_4R followed by Cy3-conjugated anti-rabbit (red); (c) superposition of a on b; (d) NPCMI; (e) mouse anti-CD163 followed by Cy2-conjugated anti-mouse (green); (f) rabbit anti-H_4R followed by Cy3-conjugated anti-rabbit (red); (g) superposition of e on f; (h) NPCMI. Scale bar: 50 m. Figure reproduced with permission from the Pharmaceutical Society of Japan.

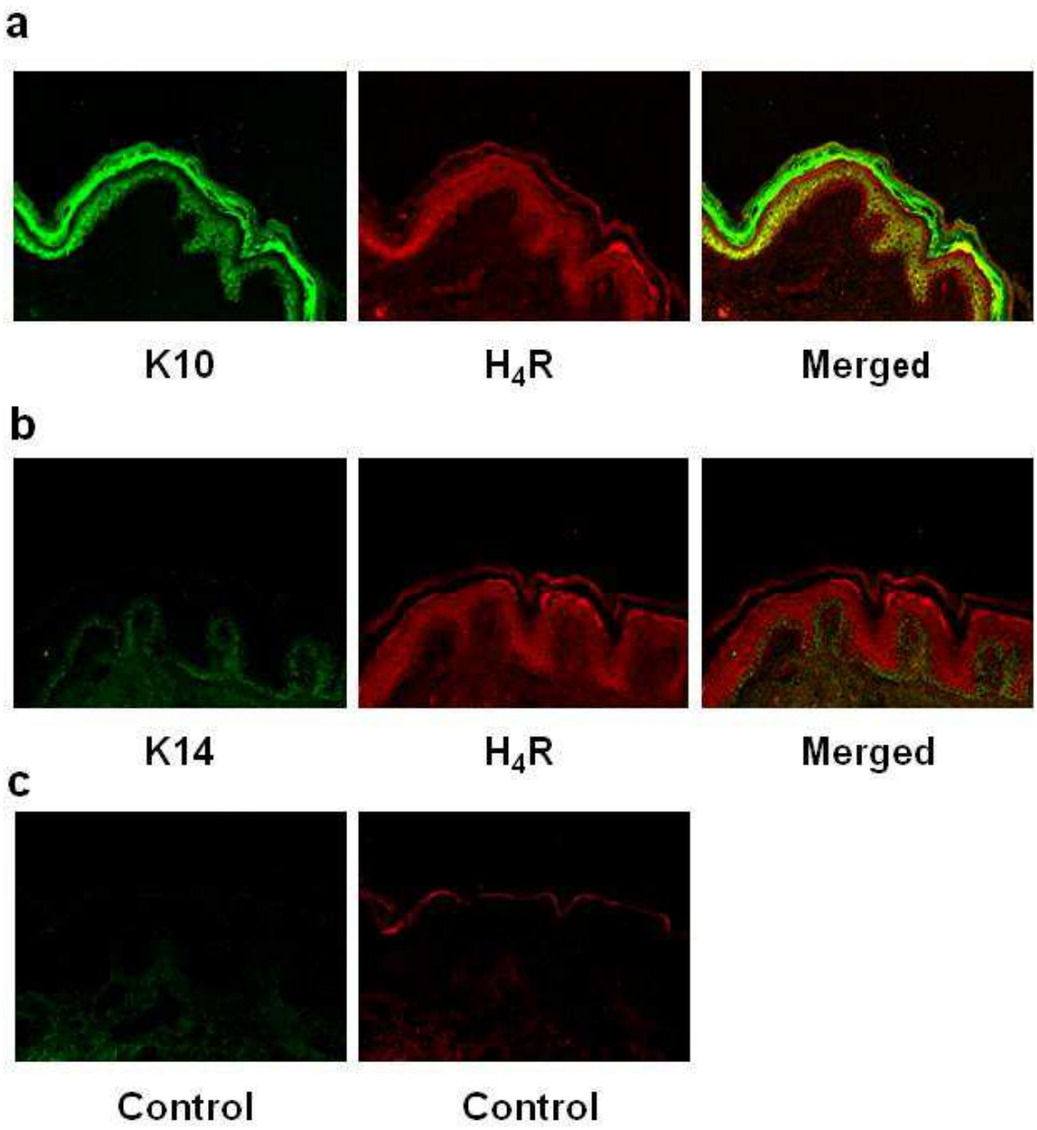

Fig. 5. H_4R expression in human epidermal tissues. Double immunofluorescence staining of human epidermal tissues with anti-human H_4R antibody followed by Cy2-conjugated anti-rabbit secondary antibody (red), and anti-K10 (a) or anti-K14 (b) antibody followed by Cy2-conjugated anti-mouse secondary antibody (green). (c) For the negative control, tissues were only exposed to the secondary antibody. Figure reprinted with permission from the Japanese Society of Toxicology.

Keratinization is the result of keratinocytes dividing in the basal lamina and moving to the upper layer as they mature. K10 is expressed in keratinocytes in the early stages following differentiation, whereas K14 is expressed in undifferentiated keratinocytes. Accordingly, our results suggest that keratinocytes increase expression of H_4R following differentiation; however, further work is necessary to determine the expression mechanism and the physiological role of the receptors.

Increased H_4R expression has been reported in $CD4^+$ T cells of patients with atopic dermatitis (Gutzmer et al., 2009), and skin mast cells have been shown to express H_4R (Lippert et al., 2004). These findings suggest that dermal cells may play an important role, via H_4R, in skin disorders. Dermal fibroblasts are a major component of the dermis. When the skin is damaged, they perform important roles including production of extracellular matrix molecules such as collagens. We have demonstrated the expression of H_4R in human dermal fibroblast cells using immunohistochemical staining (Fig. 6). Furthermore, Western blot analysis showed enhancement of the expression level of H_4R in dermal fibroblasts by stimulation with dexamethasone (Fig. 7).

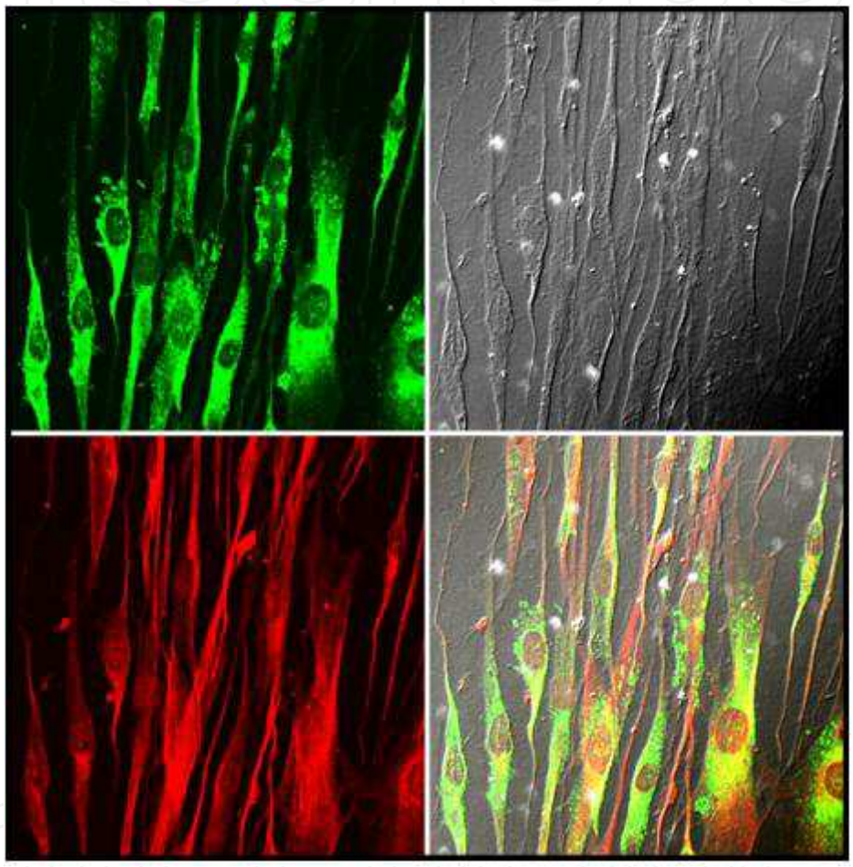

Fig. 6. Expression of H_4R on human dermal fibroblasts.Double immunofluorescence staining of dermal fibroblast cells treated with anti-human PH antibody followed by Cy2-conjugated anti-mouse secondary antibody (green), and anti-human H_4R antibody followed by Cy3-conjugated anti-rabbit secondary antibody (red). Magnification x 400. Figure reprinted with permission from the Japanese Society of Toxicology.

Ohnishi et al. (2008) reported that the levels of leukotriene B_4 receptor 1, which belongs to the GPCR family, were up-regulated by dexamethasone. This suggests that H_4R, which is part of the same family, may be up-regulated by dexamethasone too. This up-regulation of H_4R expression might be associated with itching that occurs as a rebound phenomenon after withdrawal of high-dose topical glucocorticoids. We confirmed that significant enhancement of pruritus occurred after chronic topical application of dexamethasone in

mice (Yamaura et al., 2011). However, further study is needed to investigate the relationship between the expression of H_4R in skin and pruritus, which are both enhanced by dexamethasone.

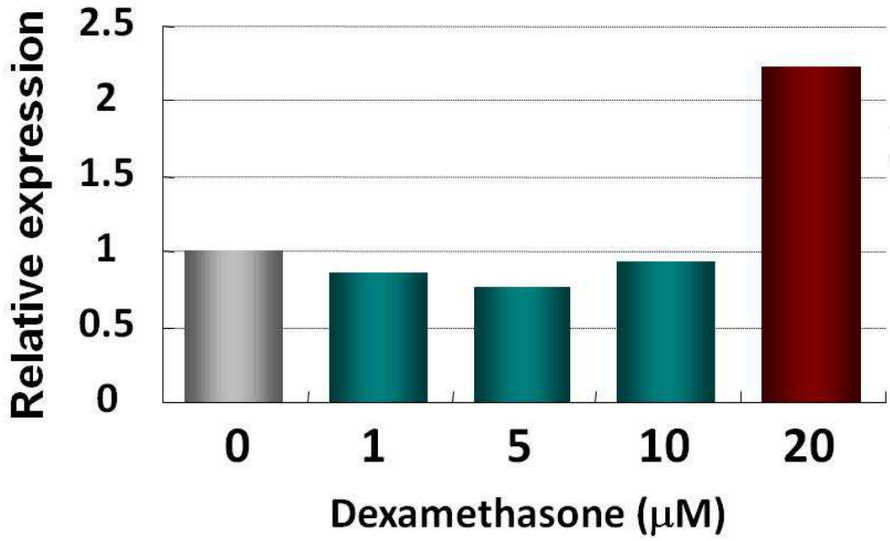

Fig. 7. Effects of dexamethasone on H_4R protein expression in human dermal fibroblast cell cultures. Expression of H_4R was analyzed by Western blot analysis. Protein levels of H_4R were normalized to the corresponding β-actin protein levels. The mean value of the non-treatment group was set to 1.0. Data are expressed as means (n=2–3). Figure reprinted with permission from the Japanese Society of Toxicology.

4. Effect of H_4R antagonists on pruritus model

Chronic pruritus, associated with chronic conditions such as skin, liver, and kidney diseases and metabolic disorders, is a major diagnostic and therapeutic problem and can have a profound impact on the quality of life of patients. Recent studies have raised the possibility that H_4R, in addition to the H_1R, may contribute to histamine-mediated pruritic responses in mice (Bell et al., 2004). Both specific H_4R agonists and histamine were shown to induce pruritic responses which could be blocked by pretreatment with H_4R antagonists; the response was also found to be markedly attenuated in H_4R-deficient mice. We thus examined the effectiveness of selective H_4R antagonists as antipruritic drugs by their effect on histamine H_1R antagonist-resistant acute pruritus induced by substance P in mice.

We investigated the effect of the H_1R antagonist fexofenadine and the H_4R antagonist JNJ7777120 on histamine-induced acute pruritus (Yamaura et al., 2009). Oral administration of fexofenadine caused a slight reduction in scratching, whereas JNJ7777120 showed a significant reduction (Fig. 8). We then examined the effect of these antagonists in substance P-mediated acute pruritus. Fexofenadine showed no reduction in substance P-induced scratching. By contrast, JNJ7777120 at 10 and 30 mg/kg doses reduced substance P-induced scratching in a dose-dependent manner (Fig. 9). Although JNJ7777120 crosses the blood–brain barrier, it does not cause sedation in rodents (Dunford et al., 2007); hence its antipruritic action is not a secondary effect of sedation. The results suggest that H_1R has only limited involvement in histamine-induced pruritus. By contrast, the significant effect of JNJ7777120 suggests that H_4R

has a much greater role. Substance P-induced pruritus is resistant to H_1R antagonists (Togashi et al., 2002); given its occurrence in mast cell-deficient mice (Andoh et al., 2001), histamine from mast cells is unlikely to be involved. The role of H_1R is also thought to be minor, with fexofenadine having no observable effect. However, the suppression of the pruritic response by JNJ7777120 suggests that histamine may act via H_4R rather than H_1R.

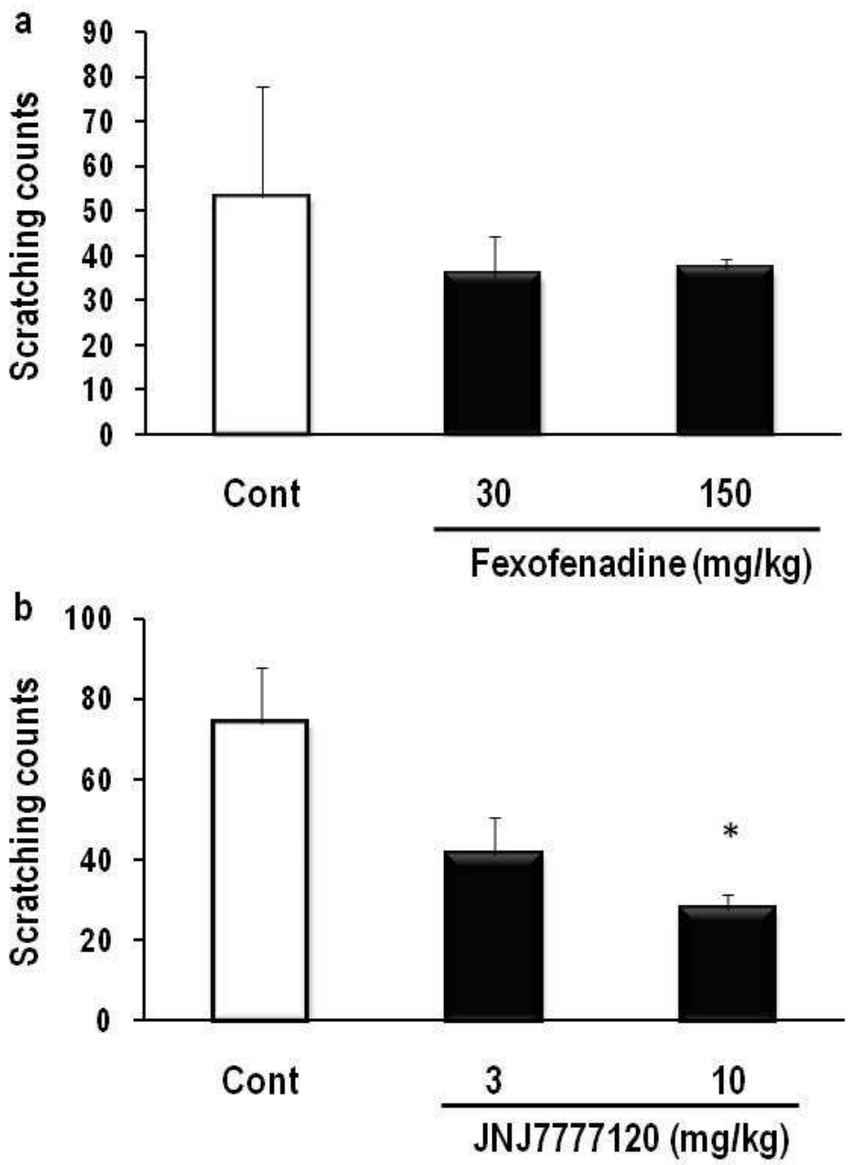

Fig. 8. Effect of H_4R on scratching behavior induced by histamine.Histamine (300 nmol) was injected intradermally into shaved skin on the back of each mouse. Immediately after the injection of pruritogen, scratching events were counted for 30 min using the MicroAct apparatus (Neuroscience Inc., Tokyo, Japan). Fexofenadine (a) or JNJ7777120 (b) was administered orally 20 min before the injection of pruritogen. Values represent the mean ± SEM of four mice. *$p<0.05$ vs. control (Dunnett's multiple comparisons). Figure reprinted with permission from the Japanese Society of Toxicology.

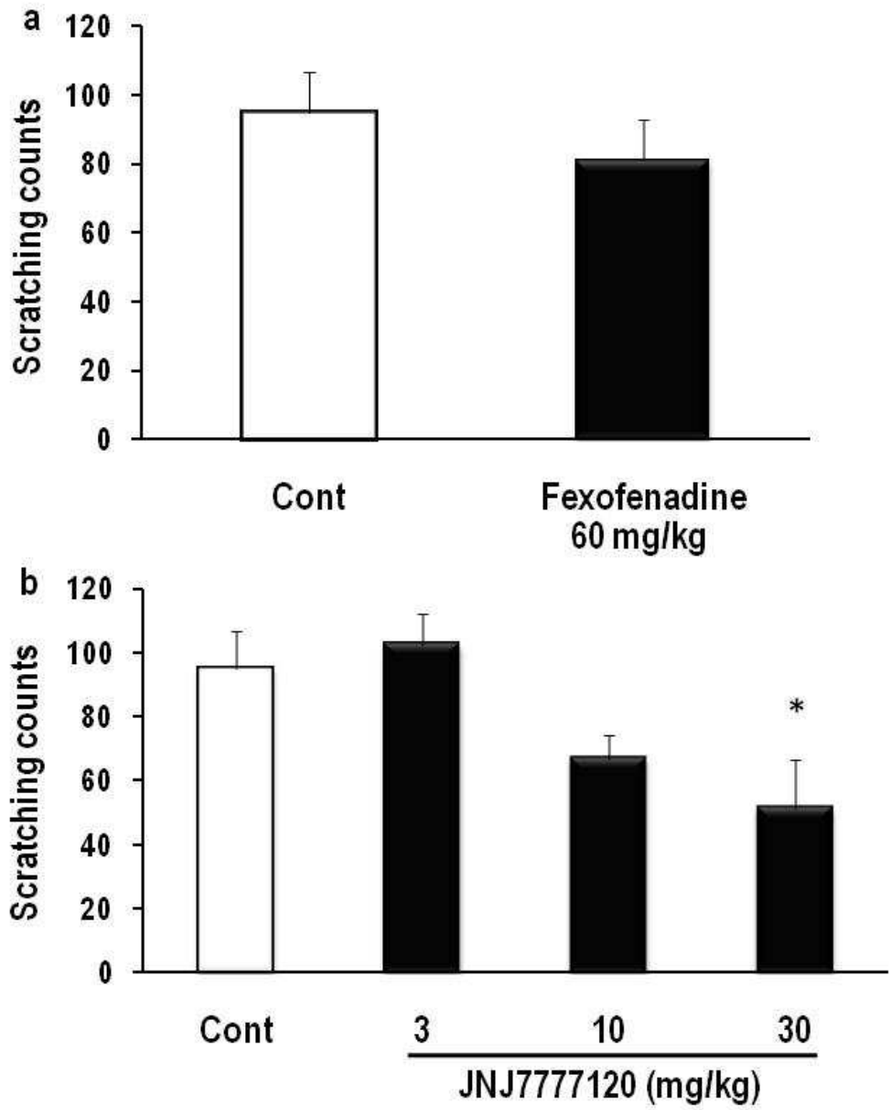

Fig. 9. Effect of H_4R on scratching behavior induced by substance P. Substance P (100 nmol) was injected intradermally into shaved skin on the back of each mouse. Immediately after the injection of pruritogen, scratching events were counted for 30 min using MicroAct. Fexofenadine (a) or JNJ7777120 (b) was administered orally 20 min before the injection of pruritogen. Values represent the mean ± SEM of four mice. *$p<0.05$ vs. control (Dunnett's multiple comparisons). Figure reprinted with permission from the Japanese Society of Toxicology.

Further, we created a chronic itch model in which repeated application of 2,4,6-trinitrochlorobenzene to the back skin of HR-1 mice was seen to elicit frequent scratching behavior at 24 h after challenge. JNJ7777120 at 10 and 30 mg/kg doses reduced this scratching behavior, whereas fexofenadine had no such effect (Suwa et al., 2011). These results suggest that H_4R antagonists may be useful for treatment of H_1R antagonist-resistant chronic pruritus such as atopic dermatitis.

5. Conclusion

We have demonstrated the expression of H_4R in human synovial cells from patients with RA and found a significant negative correlation between H_4R expression in RA synovial tissues and serum MMP-3 concentration. Furthermore, we have shown expression of H_4R in human skin and demonstrated that an H_4R antagonist ameliorates both H_1R antagonist-resistant acute and chronic pruritus. Taken together, these results suggest that histamine H_4R could be a new drug target for therapeutic use in RA or pruritic skin disorders such as atopic dermatitis.

6. Acknowledgment

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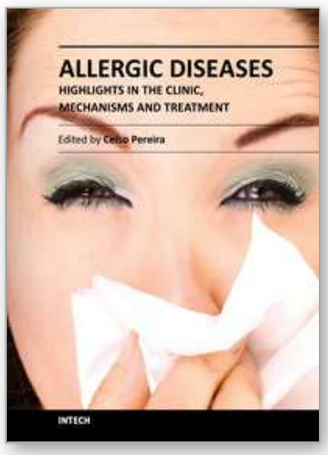

Allergic Diseases - Highlights in the Clinic, Mechanisms and Treatment

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The present Edition "Allergic diseases - highlights in the clinic, mechanisms and treatment" aims to present some recent aspects related to one of the most prevalent daily clinical expression disease. The effort of a group of outstanding experts from many countries reflects a set of scientific studies very promising for a better clinical care and also to the treatment and control of the allergy. This book provides a valuable reference text in several topics of the clinical allergy and basic issues related to the immune system response. The inflammatory reaction understanding in allergic disease is clearly evidenced, as well as new strategies for further researches.

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