Letter

Expression of histamine H4 receptor in human epidermal tissues and attenuation of experimental pruritus using H4 receptor antagonist

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ABSTRACT — Many medicines exist which can cause pruritus (itching) as "serious adverse events." Many severe pruritic conditions respond poorly to histamine H1 receptor antagonists; there is no generally accepted antipruritic treatment. Recently described histamine H4 receptors are expressed in haematopoietic cells and have been linked to the pathology of allergy and asthma. We previously reported their expression in human dermal fibroblasts; in this study we have investigated H4 receptor expression in human epidermal tissue and found it to be greater in keratinocytes in the epidermal upper layer than in the lower layer. We have also investigated the effect of histamine H4 receptor antagonists on histamine H1 receptor antagonist-resistant pruritus using a mouse model. Scratching behavior was induced by histamine (300 nmol) or substance P (100 nmol) injected intradermally into the rostral part of the back of each mouse. Fexofenadine, a histamine H1 receptor antagonist, reduced scratching induced by histamine but not by substance P, whereas JNJ7777120, a histamine H4 receptor antagonist, significantly reduced both histamine- and substance P-induced scratching. These results suggest that H4 receptor antagonists may be useful for treatment of H1 receptor antagonist-resistant pruritus.

Key words: Histamine H4 receptor, Substance P, JNJ7777120, Pruritus, Keratinocytes

INTRODUCTION

Pruritus is experienced as a sensation arising in the skin causing the desire to scratch. Many medicines have pruritus as a side-effect. Severe pruritus side effects are described in the package inserts of 687 out of 17,577 approved drugs in Japan. Pruritus is perhaps the most common symptom associated with numerous skin diseases; pruritus associated with chronic diseases such as atopic dermatitis is poorly controlled clinically and has a major effect on the quality of life of patients. Histamine H1 receptor (H1R) antagonists are commonly prescribed despite any clear evidence of their effectiveness (Wahlgren, 1991). However, the mechanisms underlying pruritus are complex and still poorly understood despite approximately a century of research (Graham *et al.*, 1951).

In 2000, a new histamine receptor subtype (H4) was cloned (Oda *et al.*, 2000). This H4 receptor (H4R) is expressed in several haematopoietic cells and has been shown to be important to the function of mast cells, eosinophils, monocytes, dendritic cells and T-cells, and also involved in allergic inflammation *in vivo* (Dijkstra *et al.*, 2007, 2008; Dunford *et al.*, 2006). We previously reported the expression of H4R in synovial cell cultures of rheumatoid arthritis patients (Ohki *et al.*, 2007) and dermal fibroblasts of osteoarthritis patients (Ikawa *et al.*, 2008).

Recent studies have raised the possibility that H4R may be an additional histamine receptor contributing to histamine-mediated pruritic responses in mice (Dunford *et al.*, 2007). Specific H4R agonists were shown to induce pruritus, which along with histamine-induced pruritus was blocked by pretreatment with H4R antagonists. Similarly,

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pruritus induced by histamine or H4R agonists was markedly attenuated in H4R-deficient mice. However, further study is required to elucidate the relationship between histamine H4R and pruritus.

The purpose of this study is to examine the effectiveness of H4R antagonists as antipruritic drugs. We first investigated H4R expression in human epidermal tissue, and then examined the effect of selective histamine H4R antagonist JNJ7777120 on histamine H1R antagonist-resistant pruritus in mice induced by substance P.

MATERIALS AND METHODS

Mice

All experiments and procedures were approved by the Chiba University Institutional Animal Care and Use Committee. Male ICR mice (6 weeks old) were obtained from Japan SLC Inc. (Shizuoka, Japan), and housed under controlled light (7am - 7pm) and temperature (24°C) with food and water available *ad libitum*. Hair on the rostral part of the back was clipped at least 1 day before the experiment.

Reagents

JNJ7777120 was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and fexofenadine was extracted from commercially available tablets (Allegra, Sanofi Aventis, Tokyo, Japan) in our institute. Allegra was powdered before extraction with methanol under constant stirring. After centrifugation at 5,000 g for 5 min the supernatants were collected and the pellets were re-extracted. The three supernatants were pooled. Supernatants were evaporated to dryness at 40°C and the dry powder was used as fexofenadine. Substance P (Peptide Institute, Osaka, Japan) and histamine dihydrochloride (Wako Pure Chemical Industries Ltd., Osaka, Japan) were dissolved in physiological saline and a volume of 20 µl injected intradermally into the rostral part of the back. JNJ7777120 and fexofenadine were suspended in 0.5% carboxymethyl cellulose solution (Sigma). JNJ7777120 (3/10/30 mg/kg) and fexofenadine (30/60/150 mg/kg) were administered *per os* (p.o.) to 4 to 5 mice 20 minutes before pruritogen injection.

Immunostaining

Dermal tissue specimens were obtained from osteoarthritis patients during surgery at Chiba University Hospital. Each patient provided informed consent before participation in the study. This research was performed in compliance with the procedures established by the University Bioethical Committee.

For fluorescence immunoassays, tissues were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (PB) overnight at 4°C. Tissues were then treated with the following anti-human primary antibodies: mouse anti-keratin 10 (Chemicon International Inc., Temecula, CA, USA), mouse anti-keratin 14 (Chemicon) and rabbit anti-H4R antibodies (Chemicon). Cy2-conjugated anti-mouse antibodies (Jackson ImmunoResearch, Laboratories, Inc., West Grove, PA, USA) and Cy3-conjugated anti-rabbit antibodies (Jackson ImmunoResearch) were used for secondary immunofluorescence assays of anti-keratin and anti-H4R antibodies, respectively. Tissues were then treated with 1% bovine serum albumin in PB as a blocking step and thereafter incubated with the primary antibodies, followed by incubation with the secondary antibodies. As negative control experiments, the primary antibodies were replaced with bovine serum albumin. After washing, a cover slip was mounted on the glass slide containing the tissues, which were then photomicrographed using an Olympus FV500 laser scanning confocal microscope.

Pruritus measurement

Immediately after the injection of histamine (300 nmol) or substance P (100 nmol), the mice were individually returned to their cages, to which they had been acclimated for at least 1 hr before the experiment, for observation of pruritic responses. Scratching behavior was evaluated for 30 min according to the method described by Kuraishi *et al.* (1995) using MicroAct.

Statistical analysis

All data are presented in the form (mean ± S.E.M.). Statistical significance was analyzed using Dunnett's method of multiple comparisons or Student's *t*-tests. Values of *p* < 0.05 were considered statistically significant. All statistical analyses were conducted using StatLight software (Yukms Co., Ltd., Tokyo, Japan).

RESULTS

Expression of histamine H4R in human epidermal tissues

Immunoassays were performed to ascertain whether H4R is expressed in human epidermal tissues. Immunohistochemical staining showed that K10-positive differentiated keratinocytes in the prickle cell layer and granular layer of epidermis strongly expressed H4R (Fig. 1A). In contrast, H4R expression was less in K14-positive proliferating keratinocytes in the basal layer (Fig. 1B).

Expression of histamine H4 receptor in human epidermal tissues

Fig. 1. H4R expression in human epidermal tissues. Double immunofluorescence staining of human epidermal tissues treated with anti-human H4R antibody followed by Cy3-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) Negative control tissues were only exposed to the secondary antibody. Magnification x 100.

Histamine-mediated pruritus

Pruritic responses to histamine were examined in ICR mice. Oral dosing of fexofenadine, a selective H1R antagonist, caused a slight (30%) reduction in scratching (Fig. 2A), whereas the selective H4R antagonist, JNJ7777120, showed a significant (63%) reduction (Fig. 2B).

Substance P-mediated pruritus

Pruritic responses to substance P were examined in ICR mice. The non-centrally acting H1R antagonist, fexofenadine, showed no reduction in substance P-induced scratching (Fig. 3A). In contrast, oral dosing of JNJ7777120, a selective H4R antagonist, at 10 and 30 mg/kg, reduced substance P-induced scratching in a dose-dependent man-

ner (Fig. 3B). Maximal inhibition of 46% was observed at 30 mg/kg ($p < 0.05$ vs vehicle-treated control).

DISCUSSION

Results of this study using fexofenadine suggest that H1 receptors have only limited involvement in histamine-induced pruritus; in contrast, the significant effects of the H4 receptor antagonist JNJ7777120 suggest that H4 receptors have a large involvement. Substance P-induced pruritus is resistant to H1 receptor antagonists (Togashi *et al.*, 2002); given its occurrence in mast cell deficient mice (Andoh *et al.*, 1998), histamine was thought not to be involved. The role of H1 receptors is also found to be

Fig. 2. Effect of H4R 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 MicroAct. Fexofenadine (A) or JNJ7777120 (B) was administered orally 20 min before the pruritogen injection. Values represent the mean $\pm$ S.E.M. of four mice. * $p < 0.05$ vs control (Dunnett's multiple comparisons).

Fig. 3. Effect of H4R on scratching behavior induced by substance P. Substance P (100 nmol) was injected intradermally into the 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 pruritogen injection. Values represent the mean $\pm$ S.E.M. of five mice. * $p < 0.05$ vs control (Dunnett's multiple comparisons).

small, with fexofenadine having no observable effect. However, the suppression of the pruritic response by JNJ7777120 suggests that histamine may have an involvement via the H4 rather than the H1 receptor. Although JNJ7777120 crosses the blood-brain barrier, it does not cause sedation in rodents (Dunford *et al.*, 2007); hence its pruritic inhibitory action is not a secondary effect of sedation.

Increased H4 receptor expression has been reported in CD4⁺T cells of patients with atopic dermatitis (Gutzmer *et al.*, 2009). The pruritogenic action of H4 receptor agonists administered intradermally suggests the existence of a pruritic mechanism acting via the H4 receptor and originating in the periphery (Dunford *et al.*, 2007). This study has demonstrated the expression of H4 receptors in human epidermal tissues. Keratinization proceeds by 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, whilst K14 is the keratin expressed in undifferentiated keratinocytes. Correspondingly, results of this study suggest that keratinocytes also increase expression of H4 receptors following differentiation; further work is necessary to determine the expression mechanism and the receptors' physiological role. It has been reported that keratinocytes play a major role in substance P-induced pruritus in producing mediators such as leukotriene B4 (Andoh *et al.*, 2001) and enhancers such as nitric oxide (Andoh and Kuraishi, 2003). However, since the H4 receptor antagonist JNJ7777120 has been shown here to be effective against the substance P-induced pruritic model, it is thought that a previously unknown pruritic mechanism may exist, driven by the response of H4 receptors expressed in keratinocytes and regulating their production of leukotriene B4 and nitric oxide.

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