



Immunopharmacology and Inflammation

Histamine H₄ receptor antagonist reduces dermal inflammation and pruritus in a hapten-induced experimental modelEriko Suwa^{a,1}, Katsunori Yamaura^{a,*}, Manabu Oda^a, Takao Namiki^b, Koichi Ueno^{a,c}^a Department of Geriatric Pharmacology and Therapeutics, Graduate School of Pharmaceutical Sciences, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260–8675, Japan^b Department of Frontier Japanese Oriental “Kampo” Medicine, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260–8670, Japan^c Center for Preventive Medical Science, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260–8670, Japan

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ABSTRACT

Effects of the histamine H₄ receptor antagonist 1-[(5-chloro-1H-indol-2-yl)carbonyl]-4-methylpiperazine (JNJ7777120) were examined for 99 days in a long-term experimental model of pruritic dermatitis induced by repeated challenge with 2,4,6-trinitrochlorobenzene (TNCB) in HR-1 mice. Repeated application of TNCB to the back skin of mice elicited frequent scratching behavior and skin lesions at 24 h after challenge and beyond. JNJ7777120 (10 and 30 mg/kg) reduced this scratching behavior and ameliorated the skin lesions in a dose-dependent manner, whereas the histamine H₁ receptor antagonist fexofenadine had no such effect and did not reduce the inflammation score, even though dexamethasone reduced the scratching bouts. Each of the three agents reduced the increase in the serum IgE concentration induced by TNCB, but only JNJ7777120 reduced the number of mast cells in the skin lesions elicited by repeated application of TNCB. These results indicate that treatment with a H₄ receptor antagonist may be effective for amelioration of both skin inflammation and pruritus in patients with allergic dermatitis such as atopic dermatitis.

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1. Introduction

The histamine H₄ receptor (H₄ receptor), which was cloned in 2000 (Oda et al., 2000), is expressed on several hematopoietic cells and has been shown to be important for the function of mast cells, eosinophils, monocytes, dendritic cells and T cells (de Esch et al., 2005; Fung-Leung et al., 2004). In addition, it has been reported that the H₄ receptor is involved in mediating the chemotactic response of mast cells, eosinophils, dendritic cells, monocytes, and NK cells to histamine (Damaj et al., 2007; O'Reilly et al., 2002; Thurmond et al., 2004). These findings support the participation of the H₄ receptor in immunity and inflammation, and in fact a number of in vivo studies have shown that the H₄ receptor has a role in allergic and inflammatory disease, for example in models of neutrophilia (Takeshita et al., 2003), allergic conjunctivitis (Varga et al., 2005), allergic rhinitis (Nakano et al., 2009), asthma and allergic dermatitis (Takahashi et al., 2009).

Because of the apparent relationship between the H₄ receptor and the number of circulating immune cells and cytokine production, histamine is thought to participate in not only the classical mechanism of inflammation but also the maintenance of inflammation via the H₄ receptor. In addition, recent in vivo studies have shown that the H₄ receptor may contribute to pruritic responses (Dunford

et al., 2007). Therefore, it is expected that the H₄ receptor could become a novel therapeutic target of anti-itch, anti-inflammatory and anti-allergic drugs (Zampeli and Tiligada, 2009). For diseases in which the H₄ receptor likely participates, few drugs are effective for control of intractable pruritic dermatosis, including atopic dermatitis. Therefore the anti-itch effect of H₄ receptor antagonists has been highlighted.

Atopic dermatitis is a chronic, pruritic, inflammatory skin disease with a wide range of severity (Bieber, 2008). Scratching induced by the strong itching exacerbates skin inflammation, and the resulting “itch scratch cycle” leads to chronic atopic dermatitis. Therefore, for effective treatment of atopic dermatitis, reduction of itching, and thus scratching, is important.

In the present study, we created a model of atopic dermatitis by repeated application of 2,4,6-trinitrochlorobenzene (TNCB) to the back skin of sensitized hairless mice, and then examined the effect of the H₄ receptor antagonist 1-[(5-chloro-1H-indol-2-yl)carbonyl]-4-methylpiperazine (JNJ7777120) on this model to clarify whether such a H₄ receptor antagonist would have therapeutic potential for atopic dermatitis.

2. Materials and methods

2.1. Animals

All experiments and procedures were approved by the Chiba University Institutional Animal Care and Use Committee. Female

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hairless mice (HOS: HR-1), 6 weeks of age, were obtained from Japan SLC Inc. (Hamamatsu, Japan), and housed under a controlled lighting (0700–1900 h) and temperature (24 °C) regime with food and water available ad libitum.

2.2. Reagents and drugs

JNJ777120 was kindly provided by Johnson & Johnson Pharmaceutical Research & Development (San Diego, CA, USA). Fexofenadine was extracted from commercially available tablets (Allegra, Sanofi Aventis, Tokyo, Japan) at our institution. The Allegra tablets were powdered before extraction with methanol under constant stirring. After centrifugation at 5000×g for 5 min, the supernatant was collected and the pellets were re-extracted. The supernatants from three such extractions were pooled, and then evaporated to dryness at 40 °C. The dry powder, which showed a single spectrophotometric peak upon HPLC, was used as fexofenadine. Dexamethasone was obtained from Sigma Chemical (St. Louis, MO, USA). TNCB was obtained from Tokyo Chemical (Tokyo, Japan), dissolved in acetone/olive oil (3:1) as a 1.2% (w/v) solution, and used for both sensitization and elicitation.

2.3. Sensitization and challenge procedure

The experimental protocols employed are illustrated in Fig. 1. Hairless mice were sensitized by application of 100 µl of 1.2% TNCB to the entire back skin (designated as day-7), and then 50 µl/10 g body weight of 1.2% TNCB solution (as a positive control) or acetone/olive oil (3:1) (as a negative control) was repeatedly applied to the same site three times a week, from day 0 to day 98.

2.4. Drug treatment

JNJ777120 (10 and 30 mg/kg/day), fexofenadine (60 mg/kg/day) and dexamethasone (3 mg/kg/day) were dissolved in a vehicle comprising 20% hydroxypropyl-β-cyclodextrin/water. Each drug was administered orally once daily from day 63 to day 99. On the day of TNCB challenge, each drug was administered at 20 min before the challenge.

2.5. Evaluation of inflammation score

The severity of dermatitis was evaluated on days 57, 64, 71, 78, 85, 92 and 99 in accordance with the scoring criteria described below. The inflammation score (minimum 0; maximum 12) was defined as the sum of individual scores, graded as 0, no symptoms; 1, mild; 2, moderate; 3, severe, for each of following four symptoms: (1) edema, (2) erythema/hemorrhage, (3) excoriation/erosion and (4) scaling/dryness.

2.6. Evaluation of pruritus

The number of bouts of scratching behavior was counted for 2 h at 24 h after TNCB challenge on days 64, 78, 92 and 99. Pruritus was evaluated by automatic counting of the bouts of scratching behavior using MicroAct (Neuroscience Inc., Tokyo, Japan) as reported previously (Inagaki et al., 2003).

2.7. Measurement of serum IgE

Blood was collected by retro-orbital bleeding on days 57 and 99. The serum was obtained by centrifugation at 1000×g for 20 min at 4 °C and stored at –30 °C until use. Serum IgE levels were determined using a commercial sandwich ELISA assay (Bethyl Laboratories Inc., Montgomery, TX, USA).

2.8. Histological assessment

After evaluation of pruritus on day 99, skin specimens were fixed in 10% buffered formalin and immersed in a series of 10%, 20% and 30% sucrose, followed by embedding in Optimal Cutting Temperature (OCT) compound (Sakura Fine Technical Co., Ltd., Tokyo, Japan) for histological examination. Frozen sections 10 µm thick were then cut, thoroughly rinsed, and stained with hematoxylin-eosin for standard histopathological observation, or with toluidine blue (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for assessment of mast cells, which were counted in two random fields for each mouse.

2.9. Expression of cytokine mRNA in skin

After evaluation of pruritus on day 99, the back skin of mice was sampled. Each specimen was homogenized, and the total RNA was extracted using an RNeasy Mini Kit (QIAGEN, Hilden, Germany). cDNA was prepared from the RNA by reverse transcription using PrimeScript RT reagent Kit (TAKARA Bio INC., Shiga, Japan) and then amplified by polymerase chain reaction (PCR) (mouse IL-4: 35 cycles of 95 °C for 45 s, 60 °C for 30 s, and 72 °C for 30 s; mouse GAPDH: 35 cycles of 94 °C for 45 s, 58 °C for 30 s, and 72 °C for 30 s). The following primers were used, mouse IL-4: CCAGCTAGTTGTCATCTGCTCTTCTTCTCG and CAGT-GATGTGGACTTGGACTCATTATGGTGC; mouse GAPDH: AAC-GACCCCTTCATTGAC and TCCACGACATACTCAGCAC. The PCR products were run on a 3% agarose gel, and the DNA was stained with ethidium bromide and visualized under UV. mRNA for GAPDH was used as an internal control.

2.10. Statistical analysis

All data are presented as mean ± S.E.M. Statistical significance was analyzed using Dunnett's method and the Mann-Whitney test for

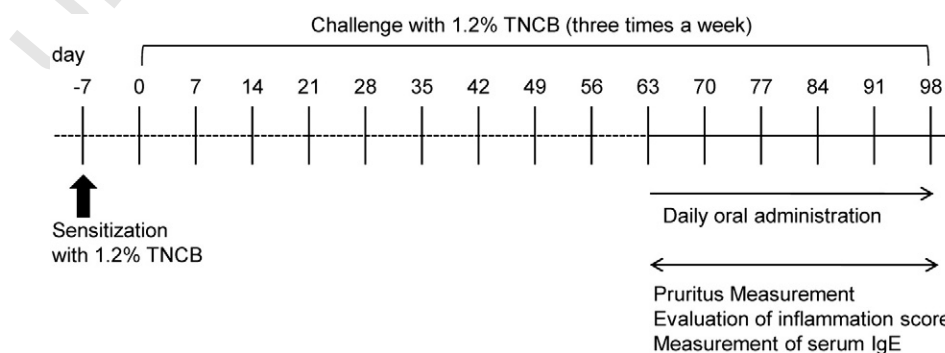


Fig. 1. Experimental schedule used for elicitation of atopic dermatitis-like skin lesions and administration of drugs.

multiple comparisons, Mann–Whitney test or Student's *t* test. Differences at $p < 0.05$ were considered statistically significant. All statistical analyses were conducted using StatLight software (Yukms Co., Ltd., Tokyo, Japan).

3. Results

3.1. Effect of H_4 receptor antagonist on inflammation score

Repeated application of TNCB induced atopic dermatitis-like skin lesions on the back skin of the treated mice (Fig. 2). The severity of inflammation increased gradually with repeated TNCB challenge (data not shown). Oral administration of JNJ777120 (10 and 30 mg/kg) from day 63 decreased the inflammation score from day 78 until the final day, in a dose-dependent manner. On the other hand, administration of dexamethasone and fexofenadine did not obviously decrease the inflammation score (Figs. 2, 3).

3.2. Effect of H_4 receptor antagonist on number of scratching bouts

Repeated application of TNCB evoked a significant increase in the number of scratching bouts (data not shown). Oral administration of JNJ777120 (30 mg/kg) suppressed this increase, and significant suppression was observed from day 78 to day 99. Administration of dexamethasone (3 mg/kg) also significantly suppressed the number of scratching bouts at days 92 and 99. On the other hand, fexofenadine (60 mg/kg) showed no suppressive effect on the number of scratching bouts (Fig. 4).

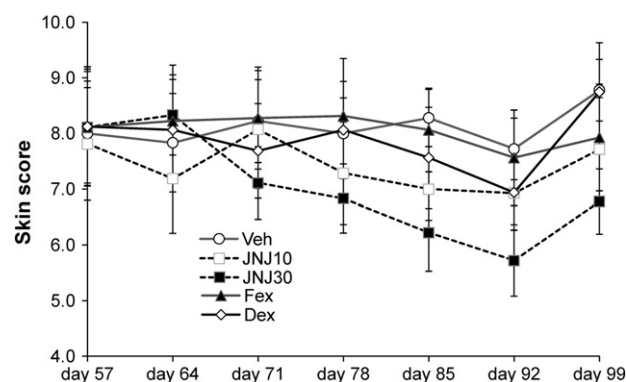


Fig. 3. Effects of JNJ777120, fexofenadine and dexamethasone on clinical score of skin symptoms induced by repeated application of TNCB. Each drug was administered orally once daily from day 63 to day 99. Skin score was determined at 24 h after application of TNCB. The score for the Nil group remained 0. Nil; acetone/olive oil, Veh; TNCB-treated (Veh), JNJ10; JNJ777120 (10 mg/kg), JNJ30; JNJ777120 (30 mg/kg), Fex; fexofenadine (60 mg/kg) and Dex; dexamethasone (3 mg/kg). Values represent the mean $\pm$ S.E.M. ($n = 7-9$).

3.3. Effect of H_4 receptor antagonist on total serum IgE concentration

Repeated application of TNCB caused an apparent elevation of the total serum IgE level. JNJ777120 (10 and 30 mg/kg), fexofenadine (60 mg/kg) and dexamethasone (3 mg/kg) attenuated the increase in

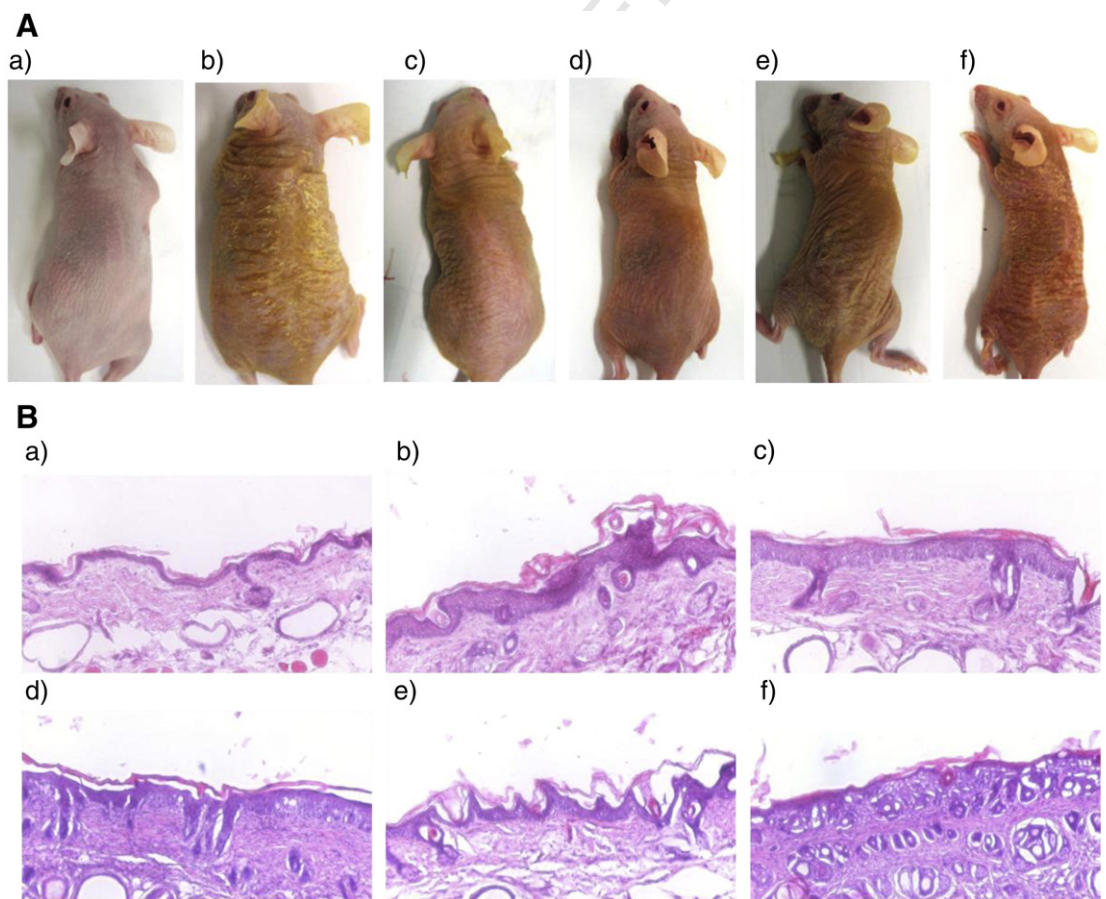


Fig. 2. Surface features (A) and a section of back skin stained with HE (B). Hairless mice were sensitized with 100 μ l of 1.2% TNCB over the entire back skin, and then 50 μ l/10 g body weight of 1.2% TNCB solution or acetone/olive oil (3:1) (as a negative control) was repeatedly applied to the same site three times a week, from day 0 to day 98. Each drug was administered orally once daily from day 63 to day 99. This photograph of the mice was taken on day 99. (a); acetone/olive oil (Nil), (b); TNCB-treated (Veh), (c); JNJ777120 (10 mg/kg), (d); JNJ777120 (30 mg/kg), (e); fexofenadine (60 mg/kg) and (f); dexamethasone (3 mg/kg).

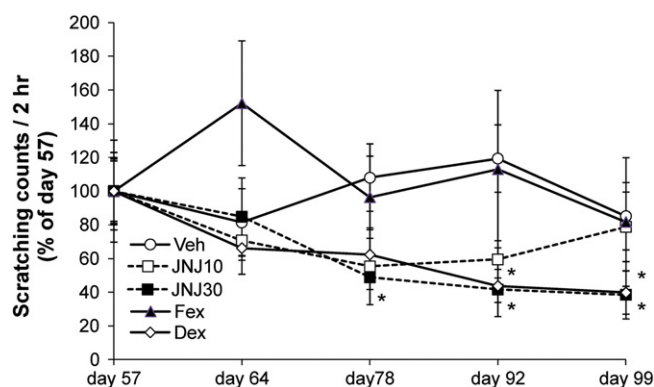


Fig. 4. Effects of JNJ777120, fexofenadine and dexamethasone on scratching behavior induced by repeated application of TNCB. Each drug was administered orally once daily from day 63 to day 99. The number of bouts of scratching behavior was counted for 2 h at 24 h after TNCB challenge. Veh; TNCB-treated (Veh), JNJ10; JNJ777120 (10 mg/kg), JNJ30; JNJ777120 (30 mg/kg), Fex; fexofenadine (60 mg/kg) and Dex; dexamethasone (3 mg/kg). Values represent the mean $\pm$ S.E.M. ($n = 7-9$). * $p < 0.05$ compared with each corresponding value on day 57 (Mann–Whitney test).

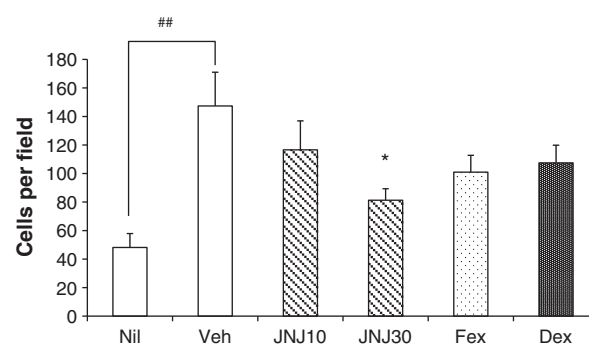


Fig. 6. Effects of JNJ777120, fexofenadine and dexamethasone on the number of mast cells in the skin. Nil; acetone/olive oil, Veh; TNCB-treated (Veh), JNJ10; JNJ777120 (10 mg/kg), JNJ30; JNJ777120 (30 mg/kg), Fex; fexofenadine (60 mg/kg) and Dex; dexamethasone (3 mg/kg). Values represent the mean $\pm$ S.E.M. (Nil $n = 4$, Veh, JNJ, Fex, Dex $n = 7-9$). ## $p < 0.01$ compared with the Nil group, * $p < 0.05$ compared with the Vehicle group (Dunnett's test).

4. Discussion

Atopic dermatitis is a common, inflammatory, chronically relapsing and pruritic skin disorder for which there is little effective medication. Therefore there is a considerable medical need for an effective treatment for this condition. Pruritus (itch) is a major characteristic of atopic dermatitis and one of its most debilitating symptoms, which exacerbates the associated cutaneous inflammation as a result of scratching. However, it continues to be contentious whether scratching itself precedes the induction of atopic dermatitis, or whether itching and scratching are a consequence of the presence of eczema (Buddenkotte and Steinhoff, 2010). Up to now, H_1 receptor antagonists as antihistaminic agents have been used for treatment of atopic dermatitis, but they often fail to ameliorate the pruritus. On the other hand, a number of in vivo studies have demonstrated involvement of the H_4 receptor in pruritus and skin inflammation. For example, histamine and selective H_4 receptor agonists cause scratching responses in mice, and these responses are almost completely attenuated in H_4 receptor-knockout mice or by pretreatment with the selective H_4 receptor antagonist JNJ777120 (Bell et al., 2004; Dunford et al., 2007). JNJ777120 significantly reduces the level of substance P (Yamaura et al., 2009), and the degree of hapten-induced scratching behavior (Rossbach et al., 2009). JNJ777120 has also been shown to ameliorate chronic allergic contact dermatitis induced by repeated challenge with TNCB (Seike et al., 2010). Treatment with two specific H_4 receptor antagonists before challenge with FITC reportedly led to a significant reduction of ear edema, inflammation, and mast cell and eosinophil infiltration, and JNJ777120 also significantly inhibited pruritus in the same model (Cowden et al., 2010). The evidence derived from these studies

the total IgE level in comparison with the Vehicle group at day 99 (Fig. 5).

3.4. Effect of H_4 receptor antagonist on skin mast cell count

Repeated application of TNCB led to a significant increase in the number of mast cells in the back skin of the treated mice. This increase was consistent with a Th2 response in this model. In the group treated with JNJ777120 (30 mg/kg), the number of mast cells in the dorsal skin decreased significantly, whereas the groups treated with fexofenadine (60 mg/kg) and dexamethasone (3 mg/kg) showed no obvious decrease (Fig. 6).

3.5. Effect of H_4 receptor antagonist on IL-4 mRNA level

The level of IL-4 mRNA in the TNCB-treated (Vehicle) group was significantly higher than that in the TNCB-non-treated (Nil) group. This increase was consistent with a Th2 response in this model. JNJ777120 (30 mg/kg) and dexamethasone (3 mg/kg) attenuated the increase in the level of IL-4 mRNA compared with the Vehicle group (Fig. 7).

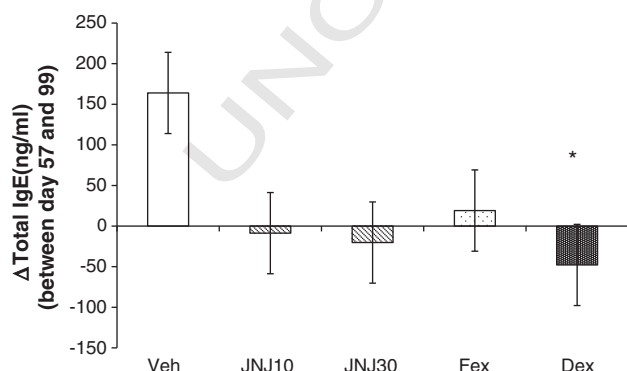


Fig. 5. Effects of JNJ777120, fexofenadine and dexamethasone on total serum IgE level. Blood was collected on days 57 and 99. Veh; TNCB-treated (Veh), JNJ10; JNJ777120 (10 mg/kg), JNJ30; JNJ777120 (30 mg/kg), Fex; fexofenadine (60 mg/kg) and Dex; dexamethasone (3 mg/kg). Values represent the mean $\pm$ S.E.M. ($n = 7-9$). * $p < 0.05$ compared with the Vehicle group (Dunnett's test).

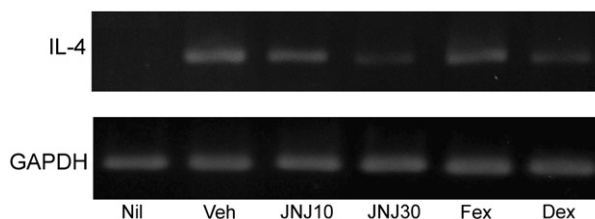


Fig. 7. Cytokine mRNA expression in the skin. Expression of IL-4 mRNA was determined by RT-PCR. The 3% agarose gel was loaded with 10 μ l of amplified product. Nil; acetone/olive oil, Veh; TNCB-treated (Veh), JNJ10; JNJ777120 (10 mg/kg), JNJ30; JNJ777120 (30 mg/kg), Fex; fexofenadine (60 mg/kg) and Dex; dexamethasone (3 mg/kg) ($n = 1$).

indicates involvement of the H₄ receptor in pruritus-associated diseases such as atopic dermatitis.

In the present study, to examine the role of the H₄ receptor in the pruritic response, we created a chronic disease model in hairless mice by long-term (i.e. 99 days) treatment with TNCB. Repeated topical application of TNCB caused an increase in dermal T lymphocytes, mast cells and the total serum total IgE level in hairless mice, as has also been demonstrated in human atopic dermatitis patients (Matsumoto et al., 2004), and also induced scratching behavior accompanied by skin lesions (Ueda et al., 2006). We then examined the effect of the H₄ receptor antagonist JNJ7777120 on this model.

Oral administration of JNJ7777120 (30 mg/kg) suppressed the increase in the number of scratching bouts, and significant suppression was observed from day 78 to day 99. As JNJ7777120 significantly reduces histamine-induced pruritus (Dunford et al., 2007), it is thought that in this model, JNJ7777120 ameliorated the itching induced by histamine. However, the H₁ receptor antagonist fexofenadine (60 mg/kg) showed no suppressive effect on the number of scratching bouts. This lack of effect on pruritus is consistent with the previously reported ineffectiveness of chlorpheniramine, cyproheptadine and methysergide in the TNCB model (Ueda et al., 2006). In this regard, the scratching behavior induced by TNCB is likely regulated by other histamine receptors, presumably the H₄ receptor. In relation to chemical mediators other than histamine, it has been reported that a NK1 receptor antagonist was able to significantly control itching in the TNCB model (Ueda et al., 2006), suggesting the potentially important role of substance P in our model. We have previously reported that JNJ7777120 significantly reduced scratching in response to substance P-induced (Yamaura et al., 2009), and therefore we considered that JNJ7777120 might control itching induced by substance P produced in the skin. It has also been reported that μ -opioid antagonist significantly inhibit itching in the TNCB model (Ueda et al., 2006), and also in animal experiments (Kuraishi et al., 2000; Yamamoto et al., 2010). These results suggest that opioid receptors may be involved in the itching sensation. The H₄ receptor is reported to affect the function of neurons in the mammalian central nervous system (Connelly et al., 2009), and the existence of an itch transmission mechanism acting via the H₄ receptor in the thalamus has been proposed (Thurmond et al., 2008). Therefore, in the present study, JNJ7777120 may have exerted such a centrally mediated anti-itch effect.

The symptoms of atopic dermatitis are exacerbated by a vicious cycle that develops when inflamed skin is scratched, making the skin even more inflamed and itchy, and causing patients to scratch more. Therefore it is thought that improvement of itching or skin inflammation would lead to improvement of the other. Oral administration of JNJ7777120 also decreased the dermal inflammation score in a dose-dependent manner. A decrease in the number of scratching bouts might have led to a decrease of skin excoriation, hemorrhage and scaling, and a reduction of mast cell infiltration. It has been reported that repeated application of TNCB induces a shift in the cutaneous cytokine milieu from a Th1 to a Th2 profile (Kitagaki et al., 1997). In the present study, the level of cutaneous IL-4 mRNA in TNCB-treated mice was significantly higher than that in TNCB-non-treated mice, and both JNJ7777120 and dexamethasone attenuated this increase. On the other hand, neither TNCB application nor drug treatment affected the level of mRNA for IFN- γ , which is a Th1 cytokine (data not shown).

Studies using other dermatitis models have shown that the levels of Th2 cytokines such as IL-4, -5 and 6 are decreased, whereas those of Th1 cytokines tend to be increased, by treatment with H₄ receptor antagonists (Seike et al., 2009; Cowden et al., 2010), suggesting that the H₄ receptor may modulate Th1–Th2 balance in the skin. It has also been reported that in peripheral blood mononuclear cells and Th2 cells H4 receptor stimulation upregulates the level of mRNA for IL-31, a cytokine that has been associated with Th2 cells and the induction of pruritus in atopic dermatitis (Gutzmer et al., 2009). In the present

study, it was thought that administration of JNJ7777120 led to amelioration of skin inflammation by limiting the shift towards a Th2-predominant milieu.

We found that the number of mast cells in the skin was increased by repeated application of TNCB, and that the extent of the increase was attenuated by treatment with JNJ7777120. It was thought that such reduction of mast cells led to a decrease in the burst size of allergic mediators such as histamine and IL-4, and that this may have contributed to the reduction of itching and inflammation in our model.

Additionally, JNJ7777120, fexofenadine and dexamethasone attenuated the increase in the total serum IgE level caused by repeated application of TNCB. As IL-4 is known to play a central role in IgE induction, reduction of the IL-4 level by drugs may have been at least partly responsible for the reduction of IgE we observed. In the JNJ7777120-treated group, improvement of skin barrier function resulting from reduction of scratching behavior may also have contributed to reduction of the IgE level.

On the other hand, dexamethasone had no effect on the inflammation score. This may have been attributable to the development of skin atrophy as a side effect of long-term steroid application. Thinning of the epidermis would have decreased the edema score, but symptoms such as easy bleeding and erythema of the dermis would have increased the scores for erythema/hemorrhage, excoriation/erosion and scaling/dryness. Furthermore, immunotoxicity attributable to steroid, such as a decrease in the weight of the spleen and thymus, was observed upon necropsy on day 99. In contrast, no such effects were observed for JNJ7777120 and fexofenadine (data not shown).

In conclusion, we have shown that the histamine H₄ receptor antagonist JNJ7777120 reduced pruritus and ameliorated skin inflammation, without any immunotoxicity, in a 99-day hapten-induced experimental model of atopic dermatitis. This effect was superior to that of the H₁ receptor antagonist fexofenadine or dexamethasone. As the conditions in human skin are much more complicated than those in the present murine model, further study will be needed. However, our results indicate that H₄ receptor antagonist might have therapeutic effectiveness for both skin inflammation and pruritus in allergic dermatitides such as atopic dermatitis.

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