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Histamine H_4 receptor antagonist reduces dermal inflammation and pruritus in a hapten-induced experimental model

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ABSTRACT

Effects of the histamine H_4 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 H_1 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 H_4 receptor antagonist may be effective for amelioration of both skin inflammation and pruritus in patients with allergic dermatitis such as atopic dermatitis.

1. Introduction

The histamine H_4 receptor (H_4 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 H_4 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 H_4 receptor in immunity and inflammation, and in fact a number of in vivo studies have shown that the H_4 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 H_4 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 H_4 receptor. In addition, recent in vivo studies have shown that the H_4 receptor may contribute to pruritic responses (Dunford et al., 2007). Therefore, it is expected that the H_4 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 H_4 receptor likely participates, few drugs are effective for control of intractable pruritic dermatosis, including atopic dermatitis. Therefore the anti-itch effect of H_4 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 H_4 receptor antagonist 1-[(5-chloro-1H-indol-2-yl)carbonyl]-4-methylpiperazine (JNJ7777120) on this model to clarify whether such a H_4 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

JNJ7777120 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 sensititized 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

JNJ7777120 (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: CCAGCTAGTTGTCATCCTGCTCTTCTTTCTCG and CAGTGATGTGGACTTGGACTCATTCATGGTGC; mouse GAPDH: AACGACCCCTTCATTGAC 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

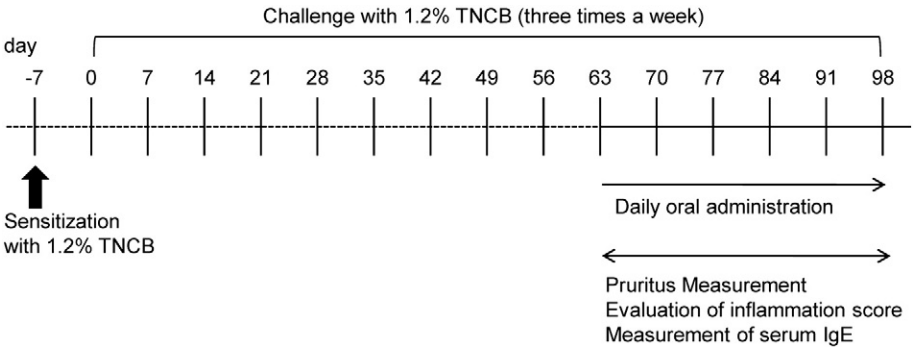

Fig. 1. Experimental schedule used for elicitation of atopic dermatitis-like skin lesions and administration of drugs.