



Effect of histamine H₄ receptor antagonist on allergic rhinitis in mice

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ABSTRACT

The aim of this study was to clarify the effect of histamine H₄ receptor antagonist, JNJ777120 (1-[(5-Chloro-1H-indol-2-yl)carbonyl]-4-methyl-piperazine) on allergic rhinitis in mice. We measured allergic symptoms (sneezing and nasal rubbing), serum total IgE and the levels of cytokines in nasal lavage fluid. Histamine H₄ receptor antagonist, JNJ777120, caused the dose-dependent inhibition of nasal symptoms by single and repeated intranasal administrations; however, JNJ777120 caused no inhibition of serum total IgE by single and repeated intranasal administrations. Therefore, we investigated the effect of JNJ777120 by oral administration. JNJ777120 also caused a significant inhibition of nasal symptoms by both single and repeated oral administrations. In addition, repeated oral administration of JNJ777120 caused significant inhibition of serum total IgE. Furthermore, JNJ777120 caused a significant decrease in the levels of IL-4 and a significant increase in the levels of IFN- γ in nasal lavage fluid. These results indicated that histamine H₄ receptor is closely related with allergic rhinitis and is important in the pathogenesis of allergic rhinitis. From these results, it can be concluded that histamine H₄ receptor antagonist might be a new strategy to treat allergic rhinitis with immunomodulatory function.

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1. Introduction

Allergic rhinitis is the most common immune-mediated disease, and the main symptoms are sneezing, pruritis, rhinorrhea and nasal obstruction. The pathogenesis of the nasal allergic reaction initially involves the interaction of allergens with a specific IgE antibody bound to the surface of mast cells and basophils on the nasal mucosa [1,2]. As a result, many symptoms associated with allergic rhinitis are caused by the release of mediators, including histamine, leukotriene, thromboxane A₂ and cytokines, from mast cells [3,4].

Histamine is one of the most important chemical mediators of allergy and inflammation [5,6], and plays an important role in eliciting nasal symptoms of allergic rhinitis, such as sneezing, itch, rhinorrhea and nasal obstruction [7,8]; therefore, histamine H₁ receptor antagonists, such as chlorpheniramine and ketotifen, have been used as the first choice in the treatment of nasal allergy. We have reported that histamine H₁ receptor antagonists inhibited nasal symptoms induced by antigen-antibody reaction in rats and mice [9,10]; however, it has been pointed out that these histamine H₁ receptor antagonists did not completely inhibit nasal symptoms [11].

Recently, a novel histamine receptor, histamine H₄ receptor has been identified [12–16]. Histamine H₄ receptor is expressed by immunologically relevant tissues, such as spleen and thymus, and mast cells and

leukocytes, such as eosinophils; therefore, histamine H₄ receptor is considered to exhibit immunomodulatory functions [14–17]. Chemotaxis and calcium mobilization of mast cells were considered important mechanisms for the action of histamine H₄ receptor [18]. In *in vivo* studies, histamine H₄ receptor antagonist, JNJ777120, has also been shown to be important in allergic inflammation [19,20]. Consequently, clarification of the relationship between histamine H₄ receptor and allergic disease, such as allergic rhinitis is eagerly anticipated; however, the role of histamine H₄ receptor in allergic rhinitis is still uncertain. The present study was therefore undertaken to clarify the effect of histamine H₄ receptor antagonist, JNJ777120, using a mouse allergic rhinitis model.

2. Materials and methods

2.1. Animals

Five-week-old female BALB/c mice were obtained from Japan SLC (Shizuoka, Japan). The animals were housed in an air-conditioned room with controlled temperature (24 ± 2 °C) and humidity (50 ± 15%). Food and water were given *ad libitum*. The number of animals for nasal symptoms and serum total IgE tests was 8 animals, and the number of animals for cytokine production test was 6 animals. All procedures involving animals were conducted in accordance with the guidelines for Animal Experiments at Okayama University Advanced Science Research Center and all procedures were licensed by the Animal Research Control Committee of Okayama University.

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2.2. Materials

The following reagents were used: ovalbumin (Grade VII; Sigma, St. Louis, MO, USA), aluminum hydroxide hydrate gel (Cosmo Bio; LSL, Tokyo, Japan) and pertussis toxin (Sigma). These reagents were dissolved in phosphate-buffered saline (PBS). The following agents were also used: ketotifen fumarate (Sigma) and JNJ777120 (1-[(5-Chloro-1H-indol-2-yl) carbonyl]-4-methyl-piperazine) (Sigma). Ketotifen was dissolved in PBS and administrated intranasally 15 min before antigen instillation or dissolved in distilled water and administrated orally 1 h before antigen instillation. JNJ777120 was suspended in PBS and administrated intranasally 15 min before antigen instillation or suspended in 0.5 w/v% methyl cellulose and administrated orally 1 h before antigen instillation.

2.3. Evaluation of nasal symptoms in mice

Before the experiment, the animals were placed into an observation cage (31 × 25 × 18 cm) for about 10 min for acclimatization. After nasal instillation of 2 µl of the drugs into the bilateral nasal cavities using a micropipette, the animals were placed back into the observation cage (one animal/cage), and the frequency of sneezing and nasal rubbing was counted for 30 min, and the frequency of sneezing and nasal rubbing are widely recognized as inflammatory parameters of rhinitis [21]. Nasal symptoms were observed on day 25 and 32. Ketotifen and JNJ777120 were administrated every day from day 25 to day 32.

2.4. Sensitization

Systemic sensitization by ovalbumin was performed as described by Kayasuga et al. [10]. The experimental procedure for the animal model of allergic rhinitis is summarized in Fig. 1. Briefly, the mice were given an intraperitoneal injection of ovalbumin (100 µg), alum (1 mg) and pertussis toxin (300 ng) on the first day. Five days later, they received a booster injection of 50 µg ovalbumin alone subcutaneously in the back. Local immunization was performed once a day from day 18 to day 32 after the first systemic immunization.

2.5. Measurement of serum total IgE

Blood specimens were collected from the ophthalmic veins on days 26 and 33. Serum was obtained by centrifugation 8350 ×g for 10 min at 4 °C and stored at −20 °C until measurement. Total IgE in the serum was measured using a mouse IgE enzyme-linked immunosorbent assay (ELISA) quantitation kit (Bethyl Laboratories Inc., Montgomery, TX, USA).

2.6. Collection of nasal lavage fluid (NLF)

Nasal lavage fluid (NLF) was collected 24 h after the last intranasal provocation with OVA. After euthanasia, the trachea at the upper level was ligated and then a catheter was guided into the nasopharynx. The nasal passages were gently perfused with 1 ml cold PBS and collected in a tube. NLF was centrifuged at 8350 ×g for 10 min at 4 °C to separate cells and supernatants.

2.7. Measurement of IL-4 and IFN-γ in NLF

The levels of IL-4 and IFN-γ in NLF were measured using a mouse IL-4 and IFN-γ enzyme-linked immunosorbent assay (ELISA) quantitation kit (BioLegend, Inc., San Diego, CA, USA).

2.8. Statistical analysis

All data are presented as the means ± S.E.M. Statistical analysis was performed by one-way analysis of variance with Dunnett's test or Student's unpaired *t* test. A probability value of less than 0.05 was considered significant.

3. Results

3.1. Effects of JNJ777120 and ketotifen on nasal symptoms by intranasal administration

Fig. 2 shows the effects of histamine H₄ receptor antagonist, JNJ777120, and histamine H₁ receptor antagonist, ketotifen, by intranasal administration on nasal symptoms induced by antigen. Sneezing and nasal rubbing in the control group increased gradually depending on daily intranasal sensitization. In addition, JNJ777120 dose-dependently inhibited nasal symptoms by single administration and repeated administration for a week. Significant effects were observed at a dose of 10 nmol/site by single administration and at doses 1, 3 and 10 nmol/site by repeated administration. Ketotifen significantly inhibited nasal symptoms at a dose of 100 nmol/site by single and repeated administrations.

3.2. Effect of JNJ777120 on serum total IgE by intranasal administration

Fig. 3 shows the effect of JNJ777120 on serum total IgE by intranasal administration. JNJ777120 caused no significant inhibition of serum total IgE by single and repeated administrations.

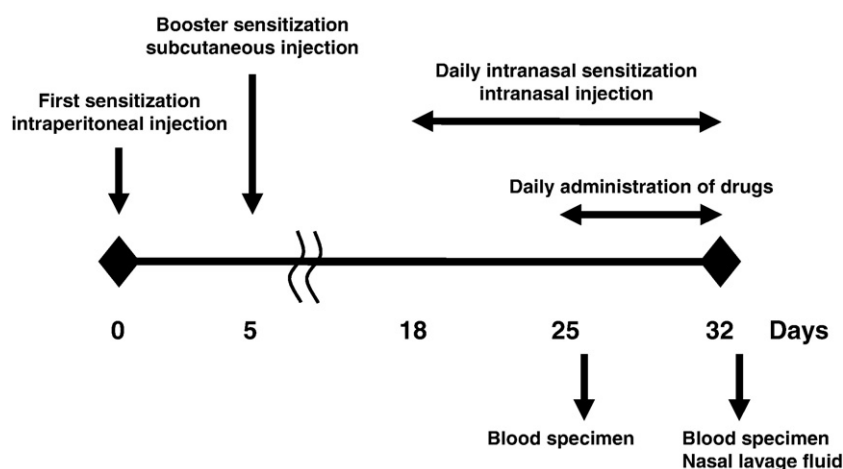


Fig. 1. Schedule of sensitization of mice, administration of the histamine H₁ and H₄ receptor antagonists, and collection of blood specimen and nasal lavage fluid.

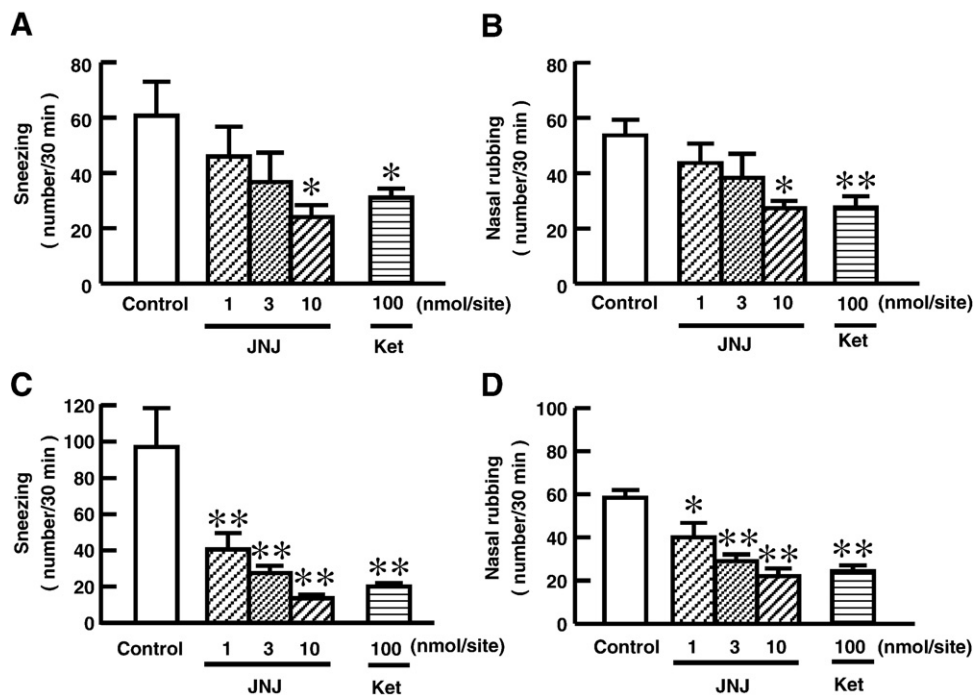


Fig. 2. Effects of JNJ777120 and ketotifen on nasal symptoms by intranasal administration. A, B: Single administration, C, D: repeated administration. Each column and vertical bar represents the means $\pm$ S.E.M. Statistical analysis was performed using Dunnett's test for multiple comparisons. JNJ: JNJ777120, ket: ketotifen. *, **: Significantly different from the control group at $P < 0.05$ and $P < 0.01$ ($n = 8$).

3.3. Effects of JNJ777120 and ketotifen on nasal symptoms by oral administration

Fig. 4 shows the effects of JNJ777120 and ketotifen by oral administration on nasal symptoms induced by antigen. JNJ777120 significantly inhibited nasal symptoms at a dose of 10 mg/kg by single and repeated administrations. Ketotifen significantly inhibited nasal symptoms at a dose of 10 mg/kg by single and repeated administrations.

3.4. Effect of JNJ777120 on serum total IgE by oral administration

Fig. 5 shows the effect of JNJ777120 on serum total IgE by oral administration. JNJ777120 caused no inhibition of serum total IgE by single administration; however, JNJ777120 significantly inhibited serum total IgE by repeated administration.

3.5. Effect of JNJ777120 on the levels of IL-4 and IFN- γ in NLF by repeated oral administration

Fig. 6 shows the effect of JNJ777120 on IL-4 and IFN- γ in NLF. The levels of IL-4 and IFN- γ in NLF in sensitized mice (control) were increased significantly compared with non-sensitized mice (PBS). JNJ777120 significantly decreased levels of IL-4 in NLF. On the other hand, JNJ777120 significantly increased levels of IFN- γ in NLF.

4. Discussion

It was found that a histamine H_4 receptor antagonist, JNJ777120, significantly inhibited nasal symptoms induced by antigen application by single intranasal administration. Dunford et al. [22] also reported that oral administration of JNJ777120 inhibited scratching behavior induced by histamine and histamine H_4 receptor agonist. From these findings, it is reasonable to presume that JNJ777120 is effective in an immediate allergic reaction mediated mainly by histamine. JNJ777120 is reported to be a selective histamine H_4 receptor antagonist from the findings that the compound showed at least 1000-fold selectivity over other

histamine receptors [23]; therefore, it is considered that these effects observed in JNJ777120 are not responsible for histamine H_1 receptor. Nakaya et al. [24] reported that histamine H_1 , H_3 and H_4 receptors were

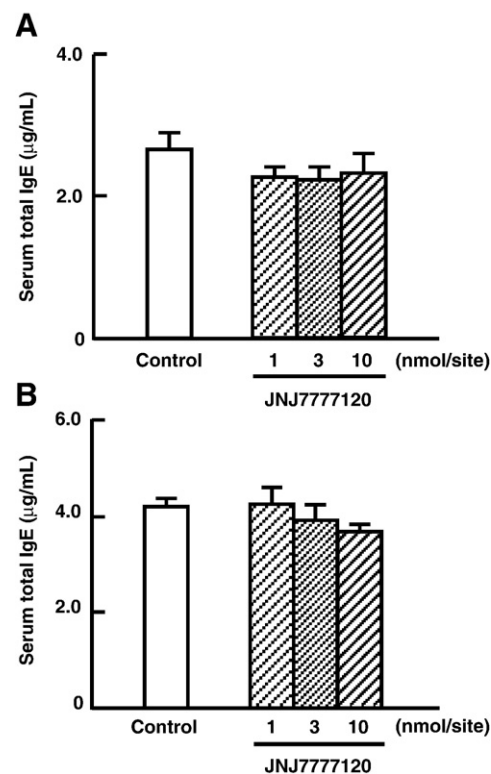


Fig. 3. Effect of JNJ777120 on serum total IgE by intranasal administration. A: Single administration, B: repeated administration. Each column and vertical bar represents the means $\pm$ S.E.M. Statistical analysis was performed using Dunnett's test for multiple comparisons. ($n = 8$).

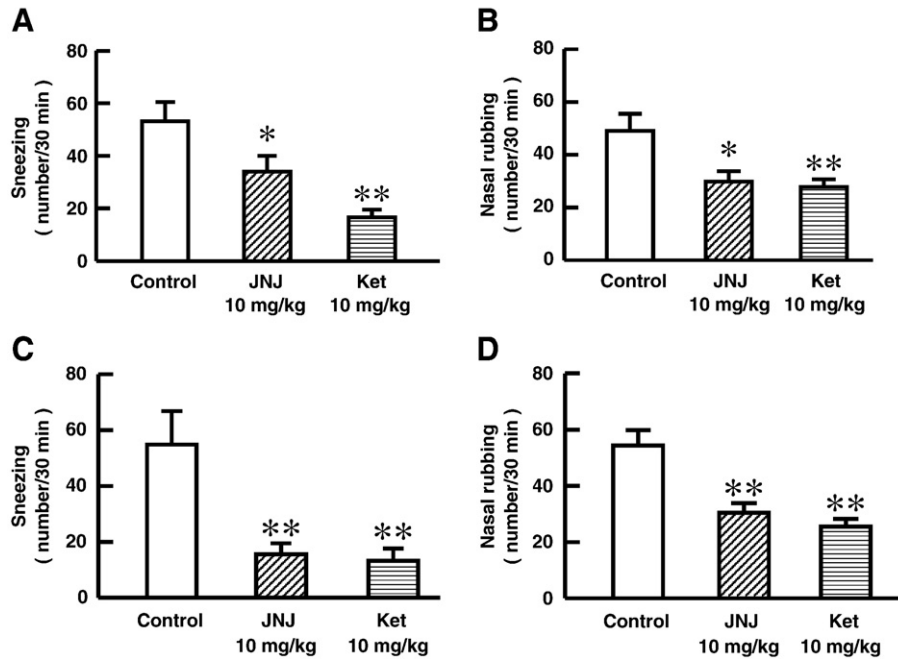


Fig. 4. Effects of JNJ777120 and ketotifen on nasal symptoms by oral administration. A, B: Single administration, C, D: repeated administration. Each column and vertical bar represents the means $\pm$ S.E.M. Statistical analysis was performed using Dunnett's test for multiple comparisons. JNJ: JNJ777120, ket: ketotifen. *, **: Significantly different from the control group at $P < 0.05$ and $P < 0.01$ ($n = 8$).

clearly localized in the same sensory nerve of human nasal mucosa. Moreover, it has been reported that histamine H_4 receptor is not involved in the control of IgE-mediated mast cell degranulation [20]; therefore, it is thought that JNJ777120 blocked histamine H_4 receptor expressed by the nasal sensory nerve, resulting in the inhibition of nasal symptoms such as sneezing and nasal rubbing.

Kitamura et al. [25] described that the level of histamine H_1 receptor mRNA expression was significantly increased in sensitized rat nasal mucosa. In addition, chlorpheniramine inhibited histamine H_1 receptor mRNA up-regulation strongly in sensitized rat nasal mucosa by repeated administration [26]. From these results, a similar mechanism may be applicable to histamine H_4 receptor, and repeated administration of JNJ777120 would inhibit not only histamine H_4 receptor but also histamine H_4 receptor mRNA up-regulation in nasal mucosa, which explains why JNJ777120 significantly inhibited nasal symptoms by repeated intranasal administration even at a smaller dose, which showed no inhibition when used as a single administration.

On the other hand, as shown in the text, JNJ777120 did not inhibit serum total IgE when used intranasally. Then, we investigated the effect

of JNJ777120 on both nasal symptoms and serum total IgE by oral administration. As a result, JNJ777120 significantly inhibited nasal symptoms by single and repeated oral administrations. Furthermore, JNJ777120 significantly decreased serum total IgE by repeated oral administration. Shapira et al. [27] reported that IL-4 induced B cells to switch to IgE production. On the other hand, IFN- γ is known to inhibit the switching of B cells to IgE production [28]. In the present study, therefore, we measured the levels of IL-4 and IFN- γ in NLF. JNJ777120 significantly decreased levels of IL-4 and significantly increased levels of IFN- γ in NLF when administered orally. Consequently, it is assumed that the decreased levels of IL-4 and increased levels of IFN- γ resulted in a significant decrease of serum total IgE.

Dunford et al. [29] reported that histamine H_4 receptor modulated CD4 $^{+}$ T-lymphocyte activation and Th2 responses in a mice asthma model. In addition, Gutzmer et al. [30] reported that histamine H_4 receptor stimulation suppressed IL-12p70 production, and mediated chemotaxis in human monocyte-derived dendritic cells. It is well known that IL-12 induces the differentiation of Th0 to Th1 and inhibits Th2

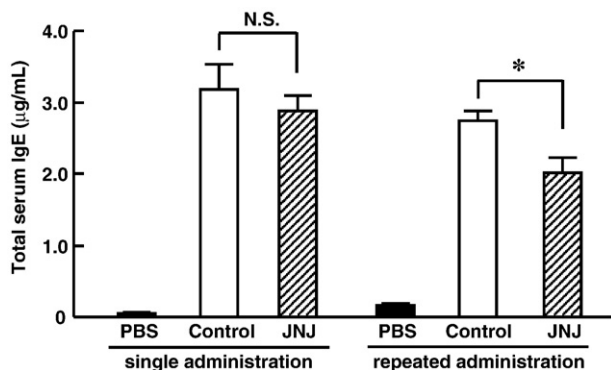


Fig. 5. Effect of JNJ777120 on serum total IgE by oral administration. Each column and vertical bar represents the means $\pm$ S.E.M. Statistical analysis was performed using Student's unpaired t test. PBS: Phosphate-buffered saline, JNJ: JNJ777120, ket: ketotifen. *: Significantly different from the control group at $P < 0.05$ ($n = 8$). N.S.: Not significant.

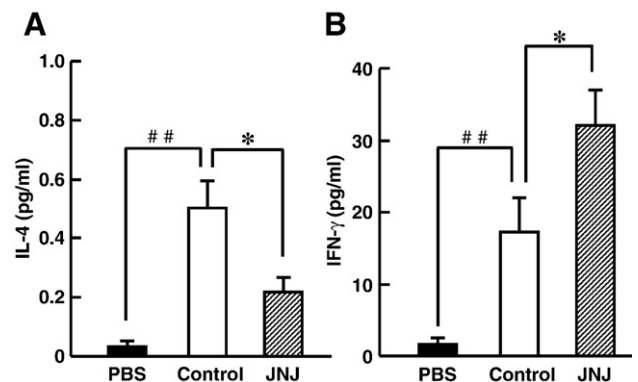


Fig. 6. Effect of JNJ777120 on cytokine production in nasal lavage fluid by repeated oral administration. A: IL-4, B: IFN- γ . Each column and vertical bar represents the means $\pm$ S.E.M. Statistical analysis was performed using Student's unpaired t test. PBS: Phosphate-buffered saline, JNJ: JNJ777120. #: Significantly different from the PBS group at $P < 0.01$ ($n = 6$). *: Significantly different from the control group at $P < 0.05$ ($n = 6$).

cytokine production [31,32]; therefore, it seems likely that Th1–Th2 polarization is mediated by histamine H₄ receptor. Furthermore, it is reported that JNJ777120 decreased the levels of IL-17 in a mice asthma model [29]. IL-17 is produced by Th17, and has been associated with many inflammatory diseases such as rheumatoid arthritis and asthma [33–35]. From these findings, it is expected that JNJ777120 influences cytokine and IgE production by controlling each cell in which it took part in the pathogenesis of allergy rhinitis.

In conclusion, it is clearly indicated that histamine H₄ receptor antagonist not only inhibited nasal symptoms but also exhibited immunomodulatory functions in a mice allergic rhinitis model; therefore, it is anticipated that histamine H₄ receptor antagonist can be developed as a new treatment for allergy rhinitis.

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