

Oral Administration of Bepotastine Besilate Suppressed Scratching Behavior of Atopic Dermatitis Model NC/Nga Mice

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Key Words

Atopic dermatitis animal model • Antihistamines • Scratching behavior • NC/Nga mice • Bepotastine besilate • SCLABA system • Leukotriene B₄

Abstract

Background: Pruritus is the most severe problem in atopic dermatitis. Even though its mechanism is still not fully understood, antihistamines have been prescribed for atopic dermatitis. **Objective:** To evaluate the effect of antihistamine on atopic dermatitis, we analyzed the scratching behavior in atopic dermatitis model NC/Nga mice. **Methods:** BALB/c mice, in which scratching behavior was induced by intradermal injection of compound 48/80 (100 µg/100 µl/mouse), and NC/Nga mice, housed in a conventional environment and having developed spontaneous eczematous regions, were monitored with a SCLABA system after oral administration of bepotastine besilate. The number of eosinophils in the ear skin and the serum leukotriene B₄ (LTB₄) levels were also evaluated. **Results:** Bepotastine at doses of 3 and 10 mg/kg effectively inhibited the compound 48/80-induced scratching behavior of BALB/c mice 1 h after oral administration, comparable with the blood T_{max}, which was

reached within 0.8–1.6 h in humans. Bepotastine also significantly inhibited the scratching behavior of NC/Nga mice 1 h after oral administration. Even though 10 mg/kg bepotastine could not influence the number of tissue eosinophils, it effectively suppressed the serum LTB₄ levels, just comparable with the suppression of scratch behavior of NC/Nga mice. **Conclusion:** Bepotastine effectively suppressed the scratch behavior of atopic dermatitis model mice, which may not simply be explained by the suppression of histamine but also by the suppression of other mediators like LTB₄. Bepotastine could be useful in the treatment of pruritus, especially early after oral administration.

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Introduction

Atopic dermatitis is a chronically relapsing inflammatory skin disease with a high current prevalence rate of 10–15% in a number of developed countries, which has increased steadily over the last few decades. It is characterized by episodes of extreme pruritus and features of eczema, among which pruritus is the most severe problem bothering the patients. There is a vicious cycle of

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1018–2438/08/1454–0277\$24.50/0

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itching and scratching involved with atopic dermatitis that contributes to maintain symptoms and reduce patients' quality of life [1]. Even though the mechanism of itching evoked in atopic dermatitis is still not fully understood, antihistamines (histamine H₁ receptor antagonists) have long been prescribed for atopic dermatitis as adjuvant therapy with topical agents such as corticosteroids, in the attempt to reduce pruritus by blocking the action of histamine in the skin.

Dermatitis spontaneously developed in the conventionally housed NC/Nga mice has many similarities to human atopic dermatitis [2–5]. Dry, pruritic, lichenified skin lesions, which are histologically characterized by hyperkeratosis [6], acanthosis and parakeratosis, with a dermal infiltration of lymphocytes, eosinophils, mast cells and macrophages develop on the face, ears, neck and back. Serum immunoglobulin E levels are high and interferon- γ and interleukin 4 are expressed by mast cells and lymphocytes. Th1 cytokines exacerbate the dermatitis [4]. Steroid and tacrolimus ointment ameliorates the dermatitis when applied topically [7].

Bepotastine besilate (bepotastine) is one of the second-generation, nonsedating antihistamines that has been used as oral tablets in Japan [6] and shows suppressive action on eosinophil infiltration due to antigen stimulation in mice [8]. One of the characteristic features of bepotastine is its rapid absorption after oral administration, with the blood T_{max} reached within 0.8–1.6 h in humans [9]. Recently, Andoh and Kurahashi [10] reported that bepotastine effectively inhibited scratching behavior of male ICR mice induced by an intradermal injection of histamine, substance P and leukotriene B₄ (LTB₄), but not serotonin. Because an intradermal injection of substance P increased the cutaneous concentration of LTB₄, they concluded that the inhibition of the action of LTB₄ as well as histamine was involved in the antipruritic effect of bepotastine [10]. In this study, we first confirmed the antipruritic effect of bepotastine on the compound 48/80-induced scratching behavior of BALB/c mice and then analyzed the inhibitory effect on scratching behavior of atopic dermatitis model NC/Nga mice after oral administration of bepotastine.

Methods

Reagents

Bepotastine (TAU-284) was kindly provided by Tanabe Pharmaceutical (Tokyo, Japan). Compound 48/80 was purchased from Sigma Chemicals (St. Louis, Mo., USA).

Mice

Male BALB/c mice, 12 weeks old, were purchased from Japan SLC Inc. (Shizuoka, Japan). Male NC/NgaTnd mice were housed in our laboratory at Tokyo University of Agriculture and Technology, in a room of 12-hour light-dark cycles, at 23 $\pm$ 2°C, 60 $\pm$ 10% humidity, and were allowed laboratory chow and water ad libitum. Clinical skin severity scores for 12-week-old NC/Nga mice were defined as the sum of the individual scores graded as 0 (none), 1 (mild), 2 (moderate) and 3 (severe) for each of 5 signs and symptoms (itch, erythema/hemorrhage, edema, excoriation/erosion, and scaling/dryness) [3]. Animal experiments complied with the standards in the guidelines of the University Animal Care and Use Committee of Tokyo University of Agriculture and Technology.

Analysis of Scratching Behavior

Scratching behavior was assessed with a SCLABA system (Noveltec Inc., Kobe, Japan). Briefly, the nape of the neck and hind limbs were colored in blue and red, respectively, and each mouse was placed in a transparent acrylic observation cage. Scratching frequency and its duration were recorded on videotape for 20 min using a digital video camera (GR-DV700, JVC, Tokyo, Japan). Data were analyzed as previously described and validated in our laboratory [11].

Evaluation of Tissue Eosinophils

Ears were collected from the mice at the time point indicated. Tissue samples were fixed in 10% buffered formalin, embedded in paraffin, cut in 4- μ m sections and mounted on silane-coated slides. After hematoxylin-eosin staining, photos were taken using an Olympus BX41 microscope (Olympus, Tokyo, Japan) equipped with a 40 $\times$ /0.85 objective lens and an Olympus DP20 digital camera.

Enzyme Immunoassay for LTB₄

Peripheral blood was collected from the mice at the time point indicated, and serum was stored at -20°C. The serum level of LTB₄ was assayed using an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, Mich., USA) according to the manufacturer's protocol.

Statistical Analysis

All data are presented as means $\pm$ standard deviation. Statistical analysis was performed by paired t test, and p values <0.05 were considered statistically significant.

Results

Bepotastine Inhibited Scratching Behavior Induced by Compound 48/80

Compound 48/80 invokes degranulation in mast cells, releasing chemical mediators including histamine [12, 13]. Since scratching behavior begins a short time after administration of compound 48/80 to mice, pruritus induced by compound 48/80 may be histamine-induced. Because this scratching behavior continues for about 20 min, we observed the mice for 20 min and analyzed the

degree of itching by the frequency of scratching behavior and its duration.

Before bepotastine administration, the frequency of spontaneous scratching of 12-week-old male BALB/c mice during 20-min observation was 47.8 ± 62.0 ($n = 4$) (fig. 1a) and duration was 10.11 ± 12.99 s ($n = 4$) (fig. 1b). When scratching behavior was induced by intradermal injection of 100 μ l of compound 48/80 (1 mg/ml) to the upper back, scratching frequency was 192.3 ± 29.3 ($n = 4$) and duration was 82.41 ± 45.85 s ($n = 4$), both significantly increased. In contrast, in the group which was injected compound 48/80 1 h after oral administration of bepotastine (3 mg/kg), scratching was suppressed to a frequency of 59.0 ± 37.9 ($n = 4$) and a duration of 14.57 ± 10.37 s ($n = 4$), which are almost the same levels compared with the control. In the groups which only received bepotastine orally without compound 48/80 injection, the scratching frequency was 53.5 ± 45.4 ($n = 4$) and the duration was 16.84 ± 15.82 s ($n = 4$); no difference from the control was observed (fig. 1a, b). Therefore, bepotastine suppressed histamine-induced scratching behavior without behavioral suppression, such as sleepiness that is often experienced as a side effect of antihistamines.

Next, to evaluate the time course of the suppressive effect of itching by bepotastine, scratching behavior was induced by compound 48/80 in male BALB/c mice 1, 2, 4 and 8 h after oral administration of bepotastine (10 mg/kg). The results of the suppressive effect by oral administration, taking as 100% the scratching frequency and duration in the group in which scratching behavior was induced by compound 48/80 without oral administration of bepotastine ($n = 9$), are shown in figure 1c, d. Significant suppression of both scratching frequency and duration was observed 1 h after oral administration (scratching frequency $40.9 \pm 19.6\%$ and scratching duration $39.3 \pm 19.6\%$).

Bepotastine Inhibited Scratching Behavior of NC/Nga Mice

Since we were able to confirm that bepotastine significantly inhibited histamine-mediated scratching behavior in male BALB/c mice, we then observed the effect of bepotastine using atopic dermatitis model NC/Nga mice. Rash in the 12-week-old male NC/Nga mice used was score 9 (out of 15), which is comparable with previous reports [3]. Spontaneous scratching reflecting the rash was observed. When analyzed by a SCLABA system, the NC/Nga mice used in the study showed a scratching frequency of 113.8 ± 29.3 , with a duration of 23.91 ± 13.59 s during a 20-min observation ($n = 12$).

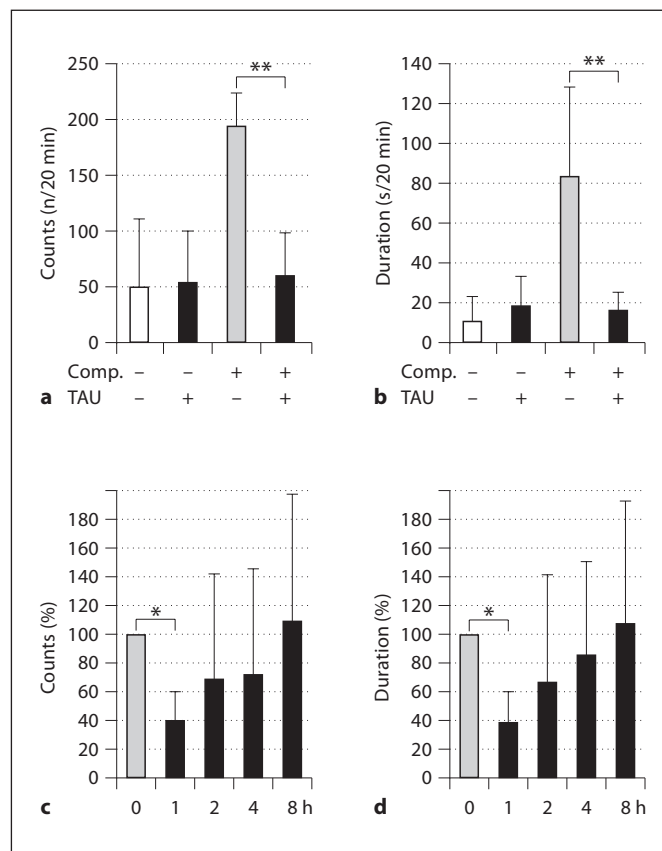


Fig. 1. Scratching behavior of BALB/c mice induced by compound 48/80. **a, b** Scratching behavior of 12-week-old male BALB/c mice was induced by intradermal injection of compound 48/80 1 h after oral administration of bepotastine besilate, and the scratching counts (**a**) and duration (**b**) during 20 min were evaluated ($n = 4$ in each group). Comp. = Compound 48/80 (100 μ g/100 μ l/mouse); TAU = bepotastine besilate (3 mg/kg body weight). ** $p < 0.01$. **c, d** Scratching behavior was induced by intradermal injection of 1 mg/ml (100 μ l/mouse) of compound 48/80 to 12-week-old male BALB/c mice 1, 2, 4 and 8 h after oral administration of bepotastine besilate (10 mg/kg body weight), and the scratching counts (**c**) and duration (**d**) during 20 min were evaluated ($n = 9$ in each group). Scratching counts and duration before bepotastine administration are shown as 100%. * $p < 0.05$, compared with the control mice without oral administration.

For each group (bepotastine 3 mg/kg, bepotastine 10 mg/kg and no administration), scratching frequency and duration were analyzed before oral administration as a control and 1, 2, 4 and 8 h after administration of bepotastine. The results of the suppressive effect by oral administration, taking the frequency and duration of spontaneous scratching without oral administration of bepotastine as 100%, are shown in figure 2. Significant suppression of scratching behavior was observed 1 h after adminis-

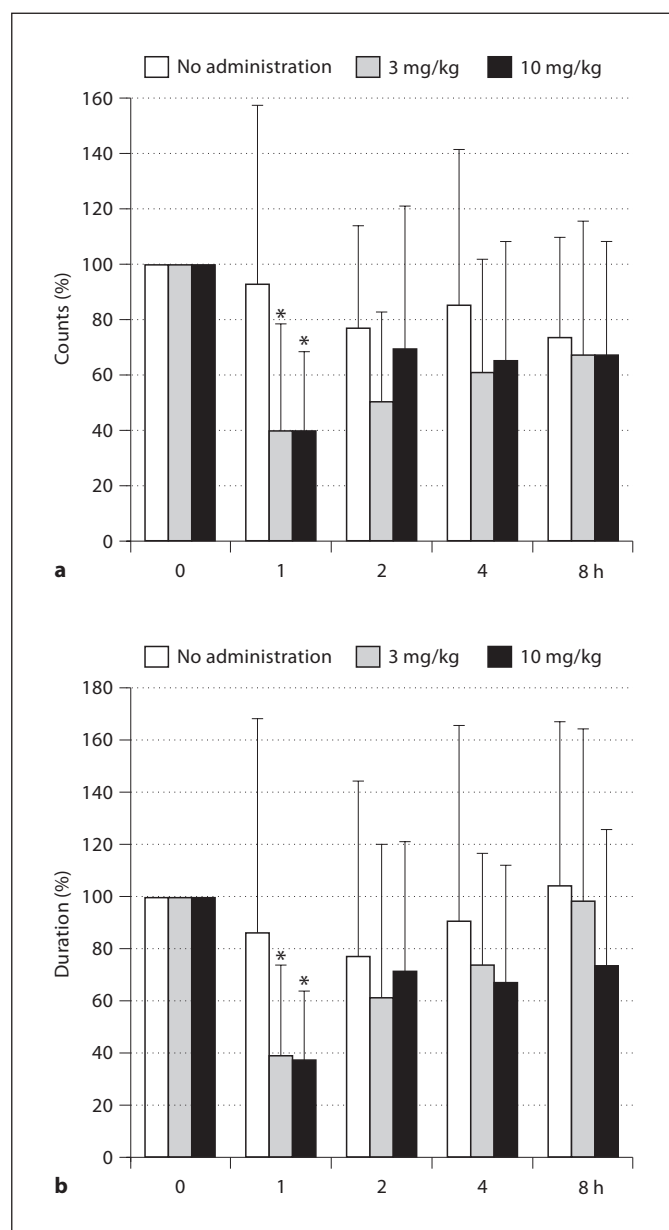


Fig. 2. Scratching behavior of 12-week-old male NC/Nga mice with a rash was observed 1, 2, 4 and 8 h after oral administration of bepotastine besilate (3 mg/kg body weight, 10 mg/kg body weight, and without administration), and the scratching counts (a) and duration (b) during 20 min were evaluated (n = 16 in each group). Scratching counts and duration before bepotastine administration are shown as 100%. * p < 0.05, compared with the mice without oral administration.

tration in both the bepotastine 3 mg/kg and bepotastine 10 mg/kg groups (scratching frequency 39.6 ± 38.7 and $39.8 \pm 28.7\%$, scratching duration of 38.0 ± 34.7 and $36.3 \pm 26.7\%$, respectively; n = 16 in each group).

Bepotastine Suppressed Serum LTB₄ Levels and Did Not Affect the Infiltration of Eosinophils in NC/Nga Mice

Because in human atopic dermatitis, antihistamines only have limited impact on itching and bepotastine was shown to act on LTB₄-induced pruritus, we analyzed the contribution of LTB₄ levels in NC/Nga mice. When compared with 12-week-old male BALB/c mice, same-aged male NC/Nga mice that were housed in conventional environment and had developed spontaneous skin eruption showed significant increased levels of LTB₄ (991.1 ± 833.7 pg/ml in BALB/c mice vs. $1,504.6 \pm 666.7$ pg/ml in NC/Nga mice; n = 6 in each group) (fig. 3a).

Although in the previous study [10] it was reported that pretreatment with bepotastine at 10 mg/kg did not affect substance-P-induced LTB₄ production in male ICR mice, our present study showed that oral administration of bepotastine (10 mg/kg) significantly suppressed serum LTB₄ levels in NC/Nga mice with a rash (711.3 ± 515.8 pg/ml 1 h after the oral intake and 858.8 ± 188.0 pg/ml 2 h after the intake, respectively; n = 6 in each group) (fig. 3b) almost to the levels seen in control BALB/c mice.

Because previous histopathological examination showed acanthosis (hyperplasia of keratinocyte layer) and infiltration of eosinophils and mononuclear cells in NC/Nga mice that were housed in conventional environment and had developed skin eruption [3], we evaluated the tissue eosinophil number in the ear skin with/without oral administration of bepotastine (10 mg/kg). Even though bepotastine was reported to have suppressive action on eosinophil infiltration due to antigen stimulation in mice [8], in our histopathological study, oral intake of bepotastine (1 h before the sample collection) did not affect the total eosinophil counts in 5 randomly selected fields of each ear (12 ears in each group). Figure 3c, d shows representative photos of each condition.

Discussion

Bepotastine is an antiallergenic drug which has suppressive action against type 1 allergy as a histamine H₁ receptor antagonist [6]. It clinically exerts a suppressive effect on pruritus accompanying urticaria or atopic dermatitis and shows excellent absorbability compared with other antipruritics, with a time to reach maximum concentration in plasma of 0.8–1.6 h in humans [9]. Its little interaction with other internal medicines is also a notable point for clinical practice.

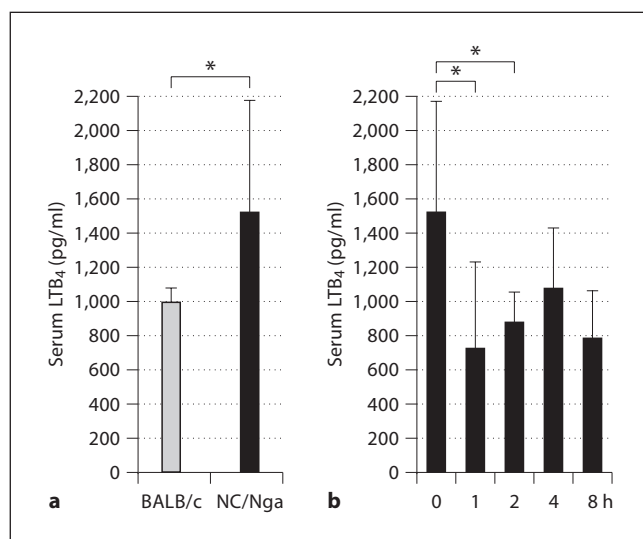


Fig. 3. Serum LTB₄ levels and tissue eosinophils. **a** Blood was collected from 12-week-old male BALB/c mice and 12-week-old male NC/Nga mice with a rash (n = 6 each), and serum LTB₄ levels were determined by the enzyme immunoassay. **b** At the time points indicated after the oral administration of 10 mg/kg body weight bepotastine besilate, blood samples were collected from 12-week-old male NC/Nga mice with a rash, and serum LTB₄ levels were determined (n = 6). **c, d** Ears obtained from 12-week-old male

NC/Nga mice with a rash were stained with hematoxylin-eosin, and the photos were taken using a microscope equipped with a 40×/0.85 objective lens and an Olympus DP20 digital camera. Infiltrated eosinophils in the skin tissue from NC/Nga mice without oral administration (**c**) and 1 h after administration of 10 mg/kg body weight bepotastine besilate (**d**) are shown with black arrows. Scale bars = 30 μm.

We assessed the suppressive effect of bepotastine on scratching behavior using atopic dermatitis model mice. Because scratching behavior in mice was noted as scratching the itching area by the hind legs, in the experiment with BALB/c mice, scratching behavior was monitored by continuously measuring the distance between the hind legs and the back where the itching sensation was induced by dorsal administration of compound 48/80 [14]. During the observation period, scratching behavior against histamine-induced itching by compound 48/80 injection was confirmed to be effectively inhibited by bepotastine without behavior suppression such as sleepiness. The significant inhibition of compound 48/80-induced scratching behavior 1 h after oral administration of bepotastine is consistent with the previous study performed by Andoh and Kurahashi [10], who used histamine, substance P and LTB₄, but not compound 48/80,

as pruritogen to induce scratching behavior in male ICR mice.

In the NC/Nga mice, used as models of atopic dermatitis [3], suppression of scratching behavior was observed at the fairly early stage of 1 h after oral administration. This suppression effect of bepotastine observed in NC/Nga mice was comparable with the suppression effect on compound 48/80-induced scratching behavior in BALB/c mice, and again the suppression effect on histamine, substance P and LTB₄-induced scratching behavior in ICR mice performed by Andoh and Kurahashi [10]. This leads us to expect that bepotastine can be effective on the point of quick action for atopic dermatitis patients with severe pruritus. In the nature of itching, the scratching behavior induced in BALB/c mice by compound 48/80 is histamine dependent, and the excellent antihistamine effects of bepotastine were confirmed in the present study by the sig-

nificant suppression of scratching behavior induced by compound 48/80. On the other hand, the spontaneous scratching behavior observed in the atopic dermatitis model NC/Nga mice cannot necessarily be explained as histamine mediated [15, 16]. Therefore, the significant suppression of spontaneous scratching behavior observed in NC/Nga mice 1 h after oral administration may suggest the possibility that this drug also has antipruritic action against causative substances other than histamine. In the previous study performed by Andoh and Kurahashi [10], the authors suggested that inhibition of the action of LTB₄ as well as histamine was involved in the antipruritic effect of bepotastine. They reported that substance P injection significantly increased the LTB₄ levels in the murine skin, which was slightly suppressed by pretreatment with bepotastine 1 h before the itching elicitation, but the suppression of scratching behavior could not reach statistical significance. However, in our present study with NC/Nga mice, oral administration of bepotastine can significantly suppress serum LTB₄ levels until 2 h before treatment, comparable with levels of control mice. Even though further studies may be required to determine how LTB₄ levels are regulated in NC/Nga mice and how bepotastine can suppress the extra production of LTB₄, it should be noted that bepotastine has the potential to suppress scratching behavior by the inhibition

of LTB₄-induced itch. On the other hand, even though bepotastine was reported to have a suppressive action on eosinophil infiltration due to antigen stimulation in mice [8], in our histopathological study, even 10 mg/kg bepotastine could not influence the number of tissue eosinophils in NC/Nga mice that already had developed skin eruption by housing in the conventional environment.

In summary, bepotastine quickly suppressed histamine-dependent scratching behavior induced by compound 48/80 in accordance with blood kinetics and also significantly suppressed spontaneous scratching behavior in atopic dermatitis model NC/Nga mice at the early stage of 1 h after oral administration. Even though the mechanism of itching evoked in atopic dermatitis is still not fully understood, the significant suppression of scratching behavior at an early stage after oral administration in this study suggests that taking bepotastine is effective in controlling the itching that occurs in atopic dermatitis, especially at the early time points when the patients feel itchy.

Acknowledgement

We thank Ai Kurabayashi for her excellent assistance in animal care.

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