

RESEARCH ARTICLE

Characteristics of scratching behavior in ADJM mice (atopic dermatitis from Japanese mice)

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Abstract

In order to elucidate the characteristics of scratching behavior in atopic dermatitis from Japanese mice (ADJM) mice, the effects of some antagonists of pruritogens on this behavior were studied. Both male and female ADJM mice showed frequent scratching behavior around the face, abdomen and back. The number of scratching behavior around the face was greater than on the abdomen and back, and scratching behavior in female mice was significantly more frequent than in male mice. Histamine H₁ antagonist, chlorpheniramine, p.o., inhibited this behavior potently and dose-dependently. Histamine H₁ antagonist with serotonin 5-HT(5-hydroxytryptamine)₂ antagonist, cyproheptadine, also inhibited this behavior. However, NK₁ antagonist, aprepitant, p.o., had no significant inhibitory effect even at a dose of 100 mg/kg, p.o., Mu antagonist, naloxone, and kappa agonist, nalfurafine, significantly inhibited this behavior at doses of 0.3 mg/kg, s.c., and 0.01 mg/kg, p.o., respectively. Histamine contents in the skin of ADJM mice were significantly higher than in BALB/c mice. These results strongly indicate that scratching behavior in ADJM mice is related with histamine H₁, opioid mu and opioid kappa receptors.

Keywords

ADJM mice, chlorpheniramine, cyproheptadine, nalfurafine, naloxone

History

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Introduction

Itching is defined as an uncomfortable, irritating sensation that provokes the urge to scratch and is one of the most important symptoms of allergic skin diseases including atopic dermatitis¹. Scratching behavior by the hind paws in mice was thought to be due to itch sensation, and this behavior has been reported by Kuraishi et al.², Sugimoto et al.³ and Inagaki et al.⁴. They described that subcutaneous injection or intradermal injection of histamine, serotonin, compound 48/80 and morphine caused frequent scratching with the hind paws at the injected site of these compounds. On the other hand, it is well known that NC/Nga (NC) mice easily develop spontaneous skin lesions on the face, neck, ears and dorsal skin with a high concentration of total IgE in the plasma⁵. Tohda et al.⁶ also described that NC mice frequently showed scratching around the face, ears and rostral part of the back under conventional conditions, and thus NC mice have been considered a useful model of human atopic dermatitis.

Recently, Matsushima⁷ reported that mutant mice showing high levels of serum IgE and an atopic dermatitis-like skin disease were found in a colony of the Koriyama (KOR) inbred strain at the Research Institute for Clinical Oncology in

Saitama Cancer Center (Saitama, Japan). The mutants were crossed with BALB/c mice for 12 generations followed by sibling mating and were maintained as atopic dermatitis from Japanese mice (ADJM) mice (Traf3ip2/Act 1 deficient mice, ADJM). In addition, scratching behavior of the face and ears with hind paws, most likely due to itching, was observed in all of the ADJM mice⁸.

Traf3ip2/Act1 is important for inflammatory responses and autoimmunity through its dual functions in CD40- and B-cell-activating factor-mediated pathway and IL-17 signaling in mammals⁹. From these findings, Matsushima et al.⁸ demonstrated that TRAF3IP2 protein plays an important role in atopic dermatitis suggesting the protein as a therapeutic target to treat atopic dermatitis and ADJM mice as a new model for atopic dermatitis.

This study was undertaken to elucidate the characteristics of scratching behavior of ADJM mice by investigating the effects of some antagonists of pruritogens such as chlorpheniramine, cyproheptadine, aprepitant, naloxone and nalfurafine on this scratching behavior.

Materials and methods

Animals

Male and female ADJM mice and BALB/c mice at 8–10 weeks of age were provided by the Research Institute of Clinical Oncology, Saitama Cancer Center. The animals were

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housed in an air-conditioned room maintained at $22 \pm 2^\circ\text{C}$ with relative humidity of $50 \pm 10\%$, and 12 h/12 h light/dark schedule. They were given standard laboratory rodent chow (F-2; Funahashi Farm, Chiba, Japan) and water *ad libitum*. All procedures involving animals were conducted in accordance with the Guidelines for Animal Experiments at Nihon Pharmaceutical University Advance Science Research Center.

Drugs

The drugs in the experiments were obtained from the sources shown in parentheses: D-chlorpheniramine maleate (Wako, Tokyo, Japan), cyproheptadine hydrochloride hydrate (Nichiko, Toyama, Japan), naloxone hydrochloride (Sigma-Aldrich, St Louis, MO), aprepitant (Ono, Osaka, Japan) and nalfurafine hydrochloride (Tore-Torii, Tokyo, Japan). All drugs except for naloxone were suspended in 5% gum arabic solution for oral administration. Naloxone was dissolved in saline and injected subcutaneously.

Scratching behavior

Scratching behavior was measured according to the method of Kuraishi et al.². Briefly, 10 min before testing, the mouse was placed in an observation cage for acclimation. A camcorder (Sony, HDR-CX180, Tokyo, Japan) was positioned above the mouse to record the behavior. The behavior of the mouse was recorded for 60 min. ADJM mice displayed scratching around the face (including ears), abdomen and back. The number of scratching episodes was counted by laboratory assistants who were blinded to the experimental conditions. Mice generally caused displayed scratching for about 1 s, and a series of these movements was counted as one episode of scratching.

Histamine contents

Skin tissue (approximately 100 mg) was homogenized in 2 ml of 0.4N perchloric acid with Polytron, and the homogenate was placed in an ice-bath for 1 h. After centrifugation at $400 \times g$ for 10 min at 4°C , histamine contents in the supernatant were determined by HPLC¹⁰.

Statistical analysis

Statistical significance was determined by one-way analysis of variance followed by Dunnett's test. A probability value less than 0.05 was considered significant. The data represent the mean $\pm$ SEM. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical interface for R (The R Foundation for Statistical Computing, Vienna Austria)¹¹. Effective dose (ED)50 value was calculated by the Probit method.

Results

Scratching behavior in ADJM mice

Results are shown in Figure 1. Both male and female ADJM mice showed frequent scratching behavior of the face, abdomen and back. Scratching of the face was more frequent than on the abdomen and back. The number of scratching episodes in female mice was greater than that in male mice.

Therefore, female mice were used, and the scratching episodes around the face were measured for the subsequent studies.

Effects of chlorpheniramine, cyproheptadine and aprepitant

Chlorpheniramine, histamine H_1 antagonist caused a dose-related inhibitory effect on scratching behavior, and significant effects were observed at doses of 3 and 10 mg/kg, p.o. (Figure 2). The ED50 value was 4.11 (1.77–7.81) mg/kg, p.o. Cyproheptadine, histamine H_1 with serotonin 5-HT₂ receptor antagonist, also caused an inhibitory effect on this behavior. Significant effects were observed at doses of 3 and 10 mg/kg, p.o. (Figure 3). The ED50 value was 4.45 (2.30–8.01) mg/kg, p.o. In contrast, aprepitant, a NK₁ antagonist, had no significant effect on scratching behavior even at a dose of 100 mg/kg, p.o. (Figure 4).

Effects of naloxone and nalfurafine

Naloxone, a representative mu antagonist, had a dose-related inhibitory effect on this behavior of ADJM mice, and significant effects were observed at doses of 0.3 and 1 mg/kg, s.c. (Figure 5). The ED50 value was 0.52

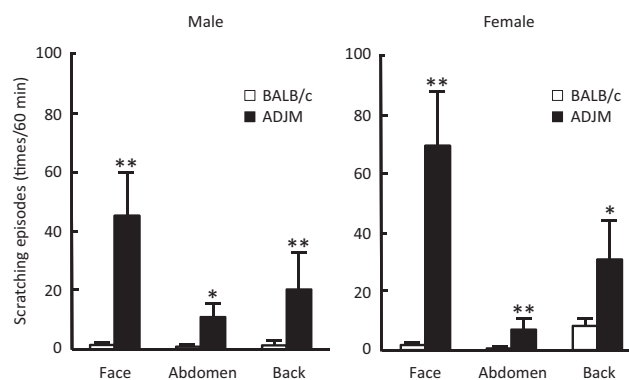


Figure 1. Scratching episodes in male and female ADJM mice. *, **Significantly different from the control group at $p < 0.01$ and $p < 0.01$ ($n = 7$).

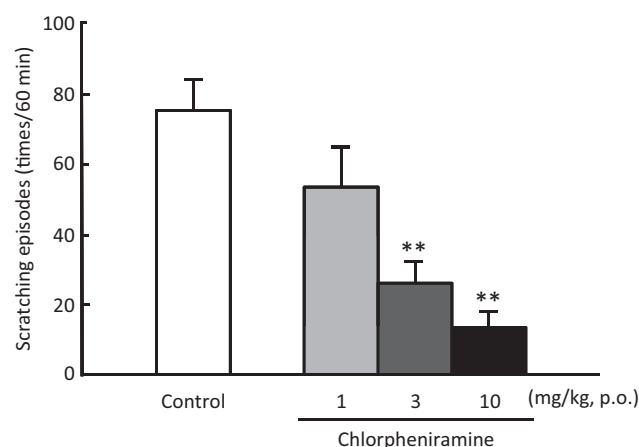


Figure 2. Effect of chlorpheniramine on scratching episodes in female ADJM mice. Chlorpheniramine was administered orally. **Significantly different from the control group at $p < 0.01$ ($n = 8-9$).

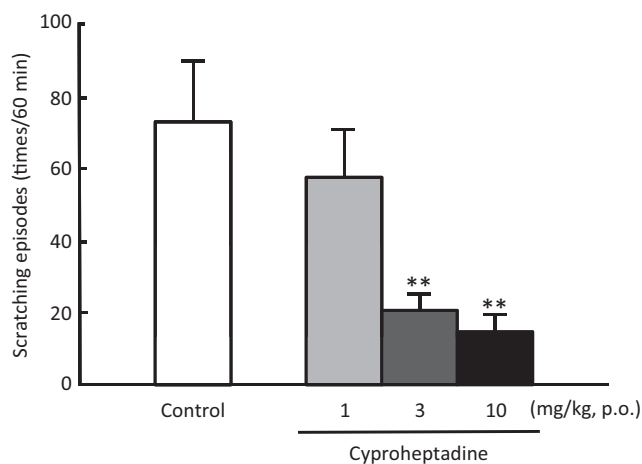


Figure 3. Effect of cyproheptadine on scratching episodes in ADJM mice. Cyproheptadine was administered orally. **Significantly different from the control group at $p < 0.01$ ($n = 7-9$).

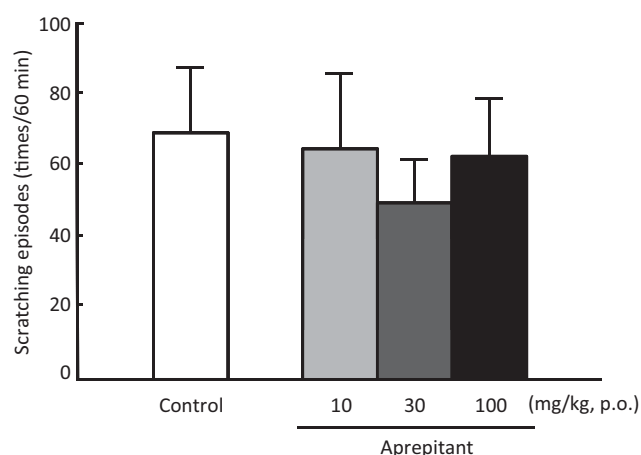


Figure 4. Effect of aprepitant on scratching episodes in ADJM mice ($n = 7-10$). Aprepitant was administered orally.

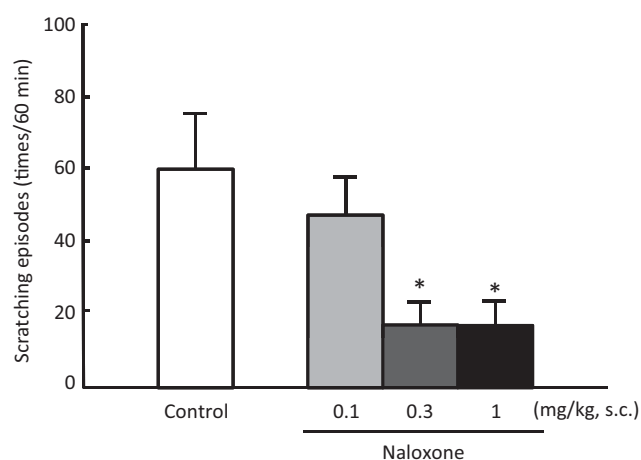


Figure 5. Effect of naloxone on scratching episodes in ADJM mice. Naloxone was administered subcutaneously. *Significantly different from the control group at $p < 0.05$ ($n = 8-9$).

(0.26–1.04) mg/kg, s.c. Nalfurafine, a kappa agonist, also showed significant inhibitory effects at doses of 0.01 and 0.05 mg/kg, p.o. (Figure 6), and the ED50 value was 0.02 (0.01–0.04) mg/kg, p.o.

Histamine content in the skin of ADJM mice

Results are shown in Figure 7. Histamine content in the skin of ADJM mice was significantly higher than that of BALB/c mice.

Discussion

This study revealed that ADJM mice showed a frequent scratching with the hind paws, especially around the face. The number of scratching episodes around the face was about 70 within 60 min. It is well known that NC mice display scratching behavior around the face, abdomen and back⁵. The property of scratching behavior in ADJM mice was similar to that of NC mice.

Chlorpheniramine at doses of 3 and 10 mg/kg, p.o., significantly inhibited the scratching behavior of ADJM mice, and the ED50 was 4.11 (1.77–7.81) mg/kg, p.o. Togashi et al.¹³ demonstrated that chlorpheniramine inhibited

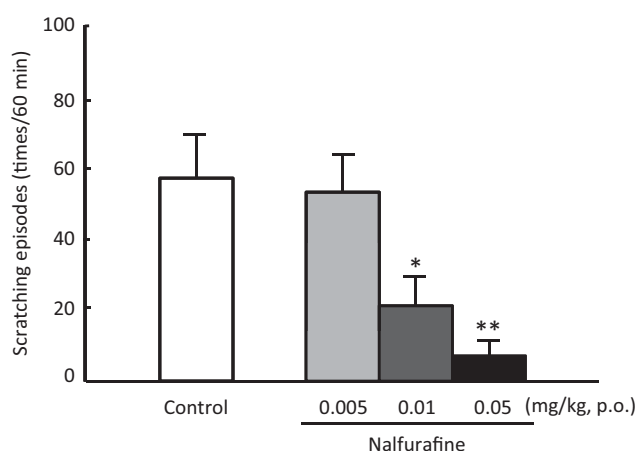


Figure 6. Effect of nalfurafine on scratching episodes in ADJM mice. Nalfurafine was administered intraperitoneally. *, **Significantly different from the control group at $p < 0.05$ and $p < 0.01$ ($n = 10$).

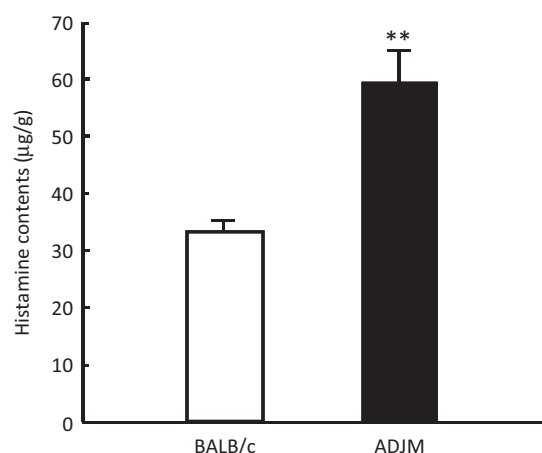


Figure 7. Histamine contents in the skin of ADJM mice. **Significantly different from the control group at $p < 0.01$ ($n = 5$).

histamine-induced scratching with ED50 value of 8.5 (1.7–25.5) mg/kg, p.o. In addition, Sugimoto et al.³ reported that scratching induced by a representative histamine releaser, compound 48/80, was inhibited by chlorpheniramine, and significant effects were observed at doses of 3 and 10 mg/kg, p.o. The ED50 value was 2.43 (1.37–4.47) mg/kg, p.o. The potency of chlorpheniramine on scratching of ADJM mice and histamine- or compound 48/80-induced scratching was found to be almost the same. We thought that the scratching behavior associated with itching of ADJM mice may be responsible for histamine, and thus we measured the histamine content in the skin of ADJM mice. We observed that histamine content in the skin of ADJM mice was significantly higher than that of control mice. These findings strongly suggest that the itching of ADJM mice was mainly due to histamine. Cyproheptadine also inhibited the scratching behavior with a significant effects at doses of 3 and 10 mg/kg, p.o. It is well recognized that cyproheptadine shows not only histamine H₁ receptor but also serotonin 5-HT₂ receptor antagonistic activity¹⁴. Therefore, it seems likely that the scratching behavior in ADJM mice may also be related to the serotonergic mechanism. In addition, it is well known that mast cells in rodents contain not only histamine but also serotonin and release them by mast cell activation¹⁵.

Scratching behavior in NC mice was not inhibited by chlorpheniramine even at a dose of 10 mg/kg, p.o.¹⁶. From these findings, the mechanism of scratching behavior in ADJM mice was somewhat different from that of NC mice. Substance P is well known to be an important mediator in the occurrence and maintenance of itching, and substance P is a tachykinin, which binds to neurokinin receptor; mainly neurokinin receptor 1¹⁷. Stander et al.¹⁸ described that aprepitant, a neurokinin receptor 1 antagonist, caused a considerable reduction of itch intensity, as assessed on a visual scale in human beings. As shown in the text, however, aprepitant caused no inhibition even at a dose of 100 mg/kg, p.o. suggesting that substance P is not involved in itching of ADJM mice.

It is well known that morphine sometimes causes itching in human beings¹⁹, and that opioid antagonists attenuate itching²⁰. For instance, Bergasa et al.²¹ and Monroe²² described that naloxone and nalmefene were effective in reducing itching in patients suffering from pruritic diseases, such as chronic urticaria and atopic dermatitis. In this study, naloxone significantly reduced the number of scratching episodes at doses of 0.3 and 1 mg/kg, and the ED50 value was 0.52 (0.26–1.04) mg/kg, s.c. Yamamoto et al.²³ reported that naloxone at doses of 0.03 and 0.1 mg/kg, s.c., inhibited the scratching behavior induced by morphine (100 nmol/site, i.d.). Although they did not calculate an ED50 value, the ED50 value was estimated to be about 0.2 mg/kg. These results suggest that the potencies of naloxone on itching of ADJM mice and morphine-induced scratching behavior are almost the same. Morphine has long been known to cause histamine release from mast cells, resulting in pruritus²⁴. In addition, Yamamoto et al.²³ demonstrated that scratching behavior induced by morphine (100 nmol/site, i.d.) was significantly antagonized by histamine H₁ antagonists, chlorpheniramine at a dose of 10 mg/kg, p.o. However, the dose of chlorpheniramine that showed an inhibitory effect against morphine-induced scratching was higher than that

against histamine-induced scratching behavior. In addition, fentanyl showed no histamine release²⁵, although the drug caused scratching behavior²³. From these findings, it is reasonable to presume that the involvement of histamine in opioid-induced scratching is relatively sparse. Therefore, the inhibitory effect of naloxone on itching of ADJM mice is mainly responsible for mu opioid receptor.

In this study, nalfurafine inhibited the itching of ADJM mice; a significant effects were observed at doses of 0.01 and 0.05 mg/kg, p.o., and the ED50 value was 0.02 (0.01–0.04) mg/kg, p.o. The dose was almost the same as the dose-inhibiting histamine-induced scratching behavior as reported by Togashi et al.¹³. In the past 10 years, there have been many reports that kappa opioid receptor is also relevant in itching. Nalfurafine is a selective kappa receptor agonist and has been confirmed to be effective for pruritus in hemodialysis patients^{26,27}. Togashi et al.¹³ reported that not only histamine H₁ antagonist, chlorpheniramine, but also nalfurafine reduced the number of scratching episodes induced by histamine. These findings suggest that there is some relationship between histamine H₁ receptor and opioid kappa receptor.

From the above findings, it can be concluded that the scratching behavior associated with itching in ADJM mice is related with histamine H₁, opioid mu and kappa receptors.

Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the article.

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