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Analysis of the spontaneous scratching behavior by NC/Nga mice: a possible approach to evaluate antipruritics for subjects with atopic dermatitis

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Abstract

We investigated the spontaneous scratching by NC/Nga mice to design a new method for evaluating the itch of subjects with atopic dermatitis. The numbers of scratchings in various strains of mice were classified based on the duration of the scratching. Prolonged scratching was frequent in skin-lesioned NC/Nga mice, but not in ICR, BALB/c and non-lesioned NC/Nga mice. Pretreatment with dexamethasone or tacrolimus significantly suppressed long-duration scratching in NC/Nga mice but did not suppress short-duration scratching induced by ovalbumin active cutaneous anaphylaxis in BALB/c mice and in ICR mice subcutaneously injected with histamine. In contrast, pretreatment with chlorpheniramine or ketotifen significantly suppressed short-duration scratching induced by ovalbumin active cutaneous anaphylaxis in BALB/c mice and in ICR mice subcutaneously injected with histamine, but not long-duration scratching seen in NC/Nga mice. These findings indicate that the mechanism of spontaneous scratching in NC/Nga mice differs from that induced by several pruritogen injections. This new method shows good correlation with the therapeutic activity of drugs in cases of atopic dermatitis in humans and may serve as a useful model for evaluating antipruritic drugs and for studying mechanisms involved in atopic dermatitis.

Keywords: Atopic dermatitis; NC/Nga, mouse; Scratching; Itch; MicroAct

1. Introduction

Itching is a characteristic symptom in various forms of dermatosis, such as atopic dermatitis, and, consequently, it constitutes a major diagnostic criterion (Hanifin and Rajka, 1980; Williams et al., 1994). It is well known that with the itch–scratch cycle, there is a strong desire to scratch, and this aggravates the skin lesions and more itching ensues (Kimura and Miyazawa, 1989; Wahlgren, 1999). Therefore, it is important to reduce the itching and scratching to prevent aggravation of the skin lesion in pruritic diseases and to upgrade the quality of life of patients (Caroline, 1999). Histamine causes itching in humans (Wahlgren, 1992). However, histamine H_1 receptor antagonists generally do not inhibit the itching and scratching of patients with atopic dermatitis, hence, histamine is considered not to be the major pruritogen in atopic dermatitis (Berth-Jones and Graham-Brown, 1989; Wahlgren et al., 1990; Hägermark and Wahlgren, 1996; Klein and Clark, 1999; Munday et al., 2002). Steroids and immunosuppressants have therapeutic effects on atopic dermatitis (Nakagawa et al., 1994; Hanifin and Tofte, 1999; Hiroi, 2001), but there are also side-effects such as skin atrophy and irritation (Smith, 1995; Assmann et al., 2001). To develop more effective medication and to elucidate the mechanisms related to itching in atopic dermatitis, it is necessary to establish a pertinent animal model.

NC/Nga mice were established as an inbred strain from Japanese fancy mice (Kondo et al., 1964). Those NC/Nga mice raised under conventional conditions developed skin lesions spontaneously with diagnostic characteristics of a high concentration of total immunoglobulin E in the plasma and invasion of inflammatory cells into the lesions

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(Matsuda et al., 1997; Suto et al., 1999). Furthermore, skin-lesioned NC/Nga mice frequently scratch their face, ears and rostral part of the back using their hind paws (Tohda et al., 1997). All these features are similar to events in patients with atopic dermatitis, hence, the NC/Nga mouse may be a suitable model of human atopic dermatitis. On the other hand, Kuraishi et al. (1995) reported that a subcutaneous injection of some pruritogenic agents into the rostral back of mice led to scratching of the injected site, using the hind paws. They indicated that pruritogenic stimuli elicit scratching of the stimulated region by the hind paws in a dose-dependent manner and that painful stimuli do not elicit scratching. These findings suggest that cutaneous itch sensation elicits hind paw scratching in the mouse and that the scratching is a possible index of itching. This method using itching induced by a pururitogen injection, is suitable for evaluating the itch seen in cases where mediators have been clarified, such as urticaria. However, this is not suitable for atopic dermatitis involving related mechanisms that are not fully understood.

At first, we analysed scratching behavior of NC/Nga mice based on ordinary observation; however, evaluation was difficult because of individual differences in frequency and difficulties of classifying the intensity of scratching behavior. Therefore, we observed spontaneous scratching by the NC/Nga mice and its time course in detail and compared the effects of several drugs with that for the scratching induced by pruritogen injection.

2. Materials and method

2.1. Animals

Male NC/Nga and ICR mice were purchased from SLC Japan (Shizuoka, Japan) and female BALB/c mice were from Charles River Japan (Kanagawa, Japan). These mice were all housed under conditions of controlled temperature (23 ± 3 °C), humidity (55 ± 15%) and lighting (lights on from 07:00 to 19:00 h), then used for study at age 6–7 weeks. NC/Nga mice with severe skin lesions (skin-lesioned NC/Nga mice) were used at 14–20 weeks of age. Food and tap water were provided ad libitum. All studies reported here have been reviewed by the Taisho Pharmaceutical Animal Care Committee and have met the Japanese Experimental Animal Research Association Standards as defined in the Guidelines for Animal Experiments (1987).

2.2. Materials

Histamine (Wako, Osaka, Japan), ovalbumin (Seikagaku Kogyo, Tokyo, Japan) and tacrolimus (Prograf®, purchased from Fujisawa, Japan) were dissolved in physiological saline. Chlorpheniramine maleate (Wako), ketotifen fumarate (Sigma, St. Louis, USA) and dexamethasone (Wako) were suspended in 1% (v/v) Tween 80 (Wako).

2.3. Sensitization

BALB/c mice were sensitized with ovalbumin. Ovalbumin, 2 μg, with 2 mg of aluminum hydroxide gel, was injected intraperitoneally, twice at 2-week intervals. Two weeks after the second sensitization, scratching behavior was elicited by ovalbumin injected intradermally.

2.4. Measurement of scratching behavior

Spontaneous scratching by NC/Nga, ICR and BALB/c mice was measured for 24 h (19:00–19:00). In evaluating the effects of some agents to stabilize the incidence of scratching behavior, NC/Nga mice were kept together with skin-lesioned mice. Spontaneous scratching of NC/Nga mice was measured for 24 h after vehicle administration, then the next day, the agents were administered, and scratching behavior was measured for 24 h.

To elicit scratching behavior, 50 μg of ovalbumin or 2 μg of histamine was injected intradermally into the rostral part of the back of sensitized or nonsensitized mice, as reported elsewhere (Inagaki et al., 1999). Immediately after the injection, the mice were placed in the observation chamber and scratching behavior was monitored for 30 min. Chlorpheniramine, ketotifen or tacrolimus was administered orally 1 h before and dexamethasone was administered orally 4 h before the injection.

Scratching behavior was automatically detected and objectively evaluated using MicroAct (Neuroscience, Tokyo, Japan), as reported elsewhere (Inagaki et al., 2002). A small magnet (1 mm in diameter, 3 mm long) was implanted subcutaneously into both hind paws of a mouse under ether anesthesia at least 6 h before the measurement of scratching. The mouse was placed in an observation chamber (11 cm in diameter, 18 cm high), which was surrounded by a round coil. The electric current induced in the coil by the movement of magnets attached to the hind paws was amplified and recorded.

2.5. Measurement of locomotor activity

Locomotor activity in BALB/c and skin-lesioned NC/Nga mice was measured using SUPERMEX (PAT. P, Muromachi Kikai, Tokyo, Japan) for 24 h (19:00–19:00, lights on from 07:00 to 19:00). The mice were placed individually in the observation cage connected to the sensor (PYS-001, Muromachi Kikai).

2.6. Data analysis

Experimental values are given as means and S.E.M. Statistical comparisons were made using Tukey's multiple comparison for three strains' spontaneous scratching, Student's unpaired *t* test or Dunnett's multiple comparison for

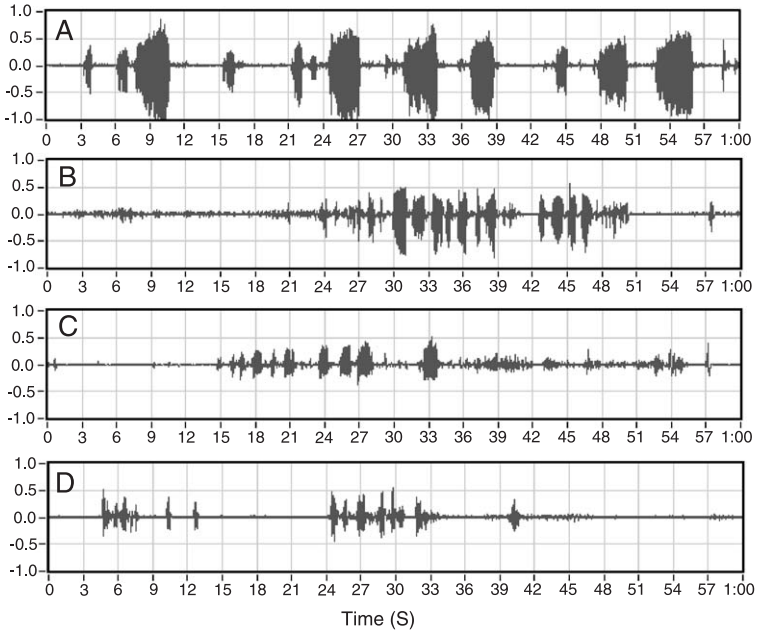

Fig. 1. Typical traces representing the scratching behavior in skin-lesioned NC/Nga mice (A), non-lesioned NC/Nga mice (B), BALB/c mice (C) and ICR mice (D). The movement of hind legs (small implanted magnets) was detected as signals from the chamber's coil. To measure the number of scratching behaviors, the signal was analysed using special software.

pruritogen-induced scratching, and Student's paired *t* test for spontaneous scratching of NC/Nga mice; a $P < 0.05$ value was considered as having statistical significance.

3. Results

3.1. Spontaneous scratching by ICR, BALB/c and NC/Nga mice

Spontaneous scratching was measured in mice with lesioned or normal skin (non-lesioned). Then, characteristic waves (Fig. 1) reflecting scratching behavior were recorded using a computer. The parameters used for the detection of consecutive scratching behavior shown in Table 1 were established on the basis of our repeated preliminary experiments. Mice generally showed several scratchings, so that a series of such behaviors was counted as one scratch event. Results for scratching behavior are given as numbers. Scratching behavior detected by MicroAct was classified into four groups of scratching duration (0.3–0.5, 0.5–1.0, 1.0–1.5 and over 1.5 s). Scratchings had a longer duration in skin-lesioned NC/Nga mice than in ICR, BALB/c and non-lesioned NC/Nga mice; especially scratchings with duration over 1.5 s were significantly frequent with the *P* value being less than 0.01 (Fig. 2).

Table 1
Analysis parameters of MicroAct for detecting waves corresponding to consecutive scratching behavior in mice

Threshold	0.10 V
Event gap	0.20 s
Minimum duration	0.30 or 1.50 s
Maximum frequency	20 Hz
Minimum frequency	2 Hz

3.2. Comparison of locomotor activity with spontaneous scratching in BALB/c and skin-lesioned NC/Nga mice

Locomotor activity and spontaneous scratching were measured simultaneously for 24 h (19:00–19:00). In BALB/c mice, locomotor activity was increased during the dark phase, but it was constantly low during the entire day in skin-lesioned NC/Nga mice (Fig. 3A). Spontaneous scratching of over 0.3-s duration had a pattern similar to that of the locomotor activity seen in each strain (Fig. 3B). On

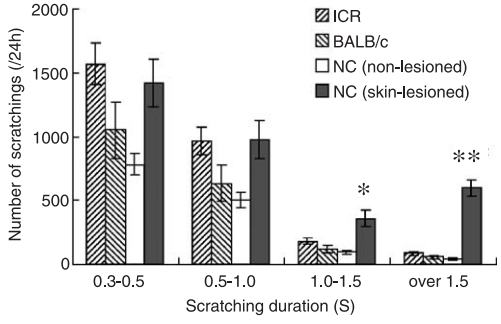

Fig. 2. Spontaneous scratching by ICR, BALB/c, skin-lesioned and non-lesioned NC/Nga mice, as measured for 24 h and classified according to the duration of scratching. Values are the means and S.E.M. for six mice. $*P < 0.05$, $**P < 0.01$ when compared with other groups.

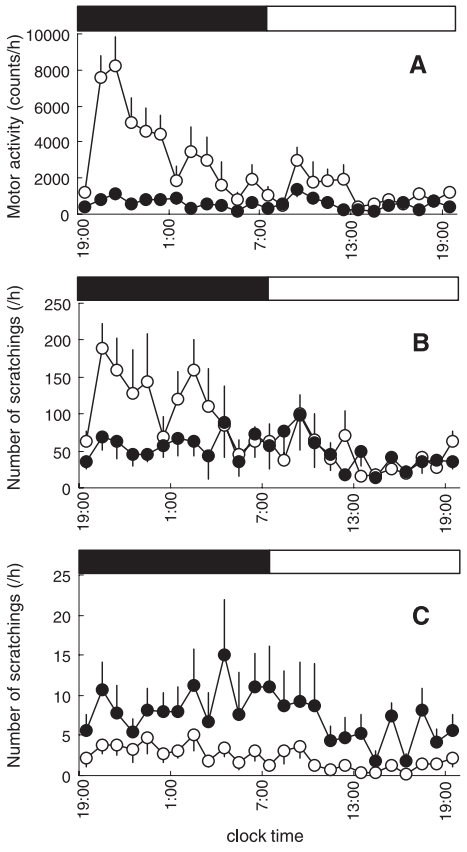

Fig. 3. Locomotor activity and spontaneous scratching in BALB/c and skin-lesioned NC/Nga mice during a 24-h (19:00–19:00) period. (A) Locomotor activity. (B) Spontaneous scratching with a duration over 0.3 s. (C) Spontaneous scratching with a duration over 1.5 s. (●) Male skin-lesioned NC/Nga mice; (○) female BALB/c mice. Values are the means and S.E.M. for eight mice.

the other hand, spontaneous scratching of over 1.5-s duration was increased during the entire day (relatively much at the dark phase) in skin-lesioned NC/Nga mice, but it was constantly low all day in BALB/c mice (Fig. 3C).

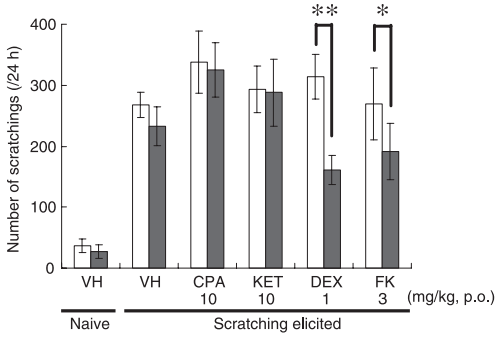

Fig. 4. Effects of drugs on spontaneous scratching behavior by NC/Nga mice. VH: vehicle, CPA: chlorpheniramine, KET: ketotifen, DEX: dexamethasone, FK: tacrolimus. Scratching elicited: NC/Nga mice were kept together with skin-lesioned mice to elicit stable scratching. Scratching behavior was measured for 24 h (□; PRE), then for 24 h after administration of drugs (■; POST). Values are the means and S.E.M. for five to seven mice. $^{*}P<0.05$, $^{**}P<0.01$ when compared with PRE.

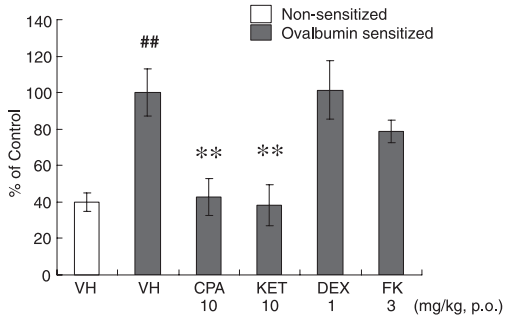

Fig. 5. Effects of drugs on ovalbumin-sensitized/ovalbumin-challenged scratching behavior by BALB/c mice. VH: vehicle, CPA: chlorpheniramine, KET: ketotifen, DEX: dexamethasone, FK: tacrolimus. Mice were sensitized with ovalbumin in the presence of hydroxide gel twice at 2-week intervals. Two weeks after the second sensitization, the mice received an intradermal injection of ovalbumin (50 μg/site). Scratching behavior was measured for 30 min. Values are the means and S.E.M. for 8–10 mice. $^{\#\#}P<0.01$ when compared with non-sensitized group, $^{**}P<0.01$ when compared with the vehicle and ovalbumin-sensitized group.

3.3. Effects of several drugs on spontaneous scratching by NC/Nga mice

The effects of chlorpheniramine, ketotifen, dexamethasone and tacrolimus on spontaneous scratching in NC/Nga mice were examined. The scratching behavior in NC/Nga mice, housed for 1 week with the same strain mice with severe dermatitis, was increased significantly compared with events in naïve mice. Pretreatment with dexamethasone or tacrolimus significantly suppressed the scratching of over 1.5-s duration, but ketotifen and chlorpheniramine did not do so (Fig. 4).

3.4. Effects of several drugs on the scratching behavior induced by ovalbumin active cutaneous anaphylaxis in BALB/c mice and histamine injection in ICR mice

The effects of chlorpheniramine, ketotifen, dexamethasone and tacrolimus on the scratching induced by ovalbu-

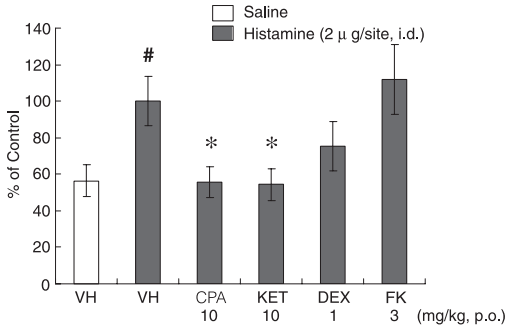

Fig. 6. Effects of drugs on histamine-induced scratching behavior by ICR mice. VH: vehicle, CPA: chlorpheniramine, KET: ketotifen, DEX: dexamethasone, FK: tacrolimus. The mice received an intradermal injection of saline or histamine (2 μg/site). Scratching behavior was measured for 30 min. Values are the means and S.E.M. for 8–10 mice. $^{\#}P<0.05$ when compared with findings in the saline-injected group, $^{*}P<0.05$ when compared with data for the vehicle- and histamine-injected groups.

min active cutaneous anaphylaxis in BALB/c mice and histamine injection in ICR mice were also examined. Pretreatment with chlorpheniramine or ketotifen significantly suppressed the scratching of over 0.3-s duration in each test, while dexamethasone and tacrolimus did not do so (Figs. 5 and 6).

4. Discussion

Histamine, serotonin and substance P are regarded as mediators causing itch in humans (Hägermark, 1992; Greaves and Wall, 1996). They also elicit scratching in mice following administration to the skin (Kuraishi et al., 1995; Andoh et al., 1998; Inagaki et al., 1999; Yamaguchi et al., 1999, 2001). However, after the administration of these pruritogens, skin lesions were not observed in the mice. Thus, it is considered that the scratching behavior induced by pruritogen injection has disappeared within a short period (approximately 30 min), and that the intensity of the scratching (e.g., duration and amplitude) is relatively weak. Continuous, intensive scratching may produce skin lesions in mice or humans. Although intensive scratching is observed in skin-lesioned mice, it is impossible to classify the intensity of scratching behavior, using the traditional method in which an observer counts the number of scratching behaviors of mice. We measured spontaneous scratching by ICR, BALB/c, skin-lesioned and non-lesioned NC/Nga mice for 24 h and scratching behaviors of various duration were recorded. However, there was no significant difference between skin-lesioned and non-lesioned mice. Therefore, for exact and specific data on intensive scratching, we classified spontaneous scratching of mice for each duration (0.3–0.5, 0.5–1.0, 1.0–1.5 and over 1.5 s) and compared findings among these mice. Consequently, there was a much longer duration of scratching in skin-lesioned NC/Nga mice, and there was a marked difference, especially in the scratching with duration over 1.5 s. Also, the circadian rhythms of locomotor activity and spontaneous scratching were observed simultaneously in BALB/c and skin-lesioned NC/Nga mice. Scratching with over 0.3-s duration was numerous in BALB/c mice, as also seen for locomotor activity, so that scratching was considered to be a reaction accompanying increases in locomotor activity. These results indicate that scratching of short duration is included as a general feature of behavior and did not participate in the occurrence of dermatitis. On the other hand, scratching with over 1.5-s duration was observed throughout the day, comparatively frequently in the dark period, in skin-lesioned NC/Nga mice. These results suggest that long-duration scratching is a characteristic behavior seen only in mice with symptoms of dermatitis.

In human atopic dermatitis, it is considered that steroids and immunosuppressants have therapeutic effects, e.g., on skin symptoms or itch sensation (Nakagawa et al., 1994; Hanifin and Tofte, 1999; Hiroi, 2001). They have anti-inflammatory effects in vitro and in vivo, but there are few reports on their antipruritic effects with short-term administration. It is therefore considered that there are no suitable models for evaluating itching in atopic dermatitis. In preliminary experiments, these drugs did not have effects on itching with short-term administration in traditional in vivo models of atopic dermatitis. Therefore, to establish a model that reflects clinical effects on atopic dermatitis, we carefully observed the time course of spontaneous scratching by NC/Nga mice.

The effects of an antihistamine, an antiallergic agent, a steroid and an immunosuppressant were also studied. In the itch model induced by pruritogen injection, the number of long-duration (over 1.5 s) scratchings were few (20.0 ± 3.7 times/30 min in ovalbumin-sensitized/ovalbumin-challenged mice, 4.4 ± 1.3 times/30 min in histamine-injected mice). Therefore, the scratching behavior of short-duration (over 0.3 s) was monitored using these models. Scratching behavior induced by intradermally administered histamine was inhibited by chlorpheniramine and ketotifen, but not by dexamethasone and tacrolimus. Therefore, this behavior was induced by the response through histamine receptors in sensory nerves. Moreover, similar effects of these drugs were observed for the scratching behavior induced by ovalbumin active cutaneous anaphylaxis in BALB/c mice. Therefore, this behavior was induced by the response to histamine released from mast cells. On the other hand, the spontaneous scratching (over 1.5-s duration) by NC/Nga mice was inhibited by dexamethasone and tacrolimus, but not by chlorpheniramine and ketotifen. From 4 h after the administration of dexamethasone, and from 2 h after the administration of tacrolimus, the spontaneous scratching was inhibited for several hours. This finding is consistent with effects in atopic dermatitis in the human. Steroids and immunosuppressants, but not antihistamines, are effective (Berth-Jones and Graham-Brown, 1989; Wahlgren et al., 1990; Nakagawa et al., 1994; Hägermark and Wahlgren, 1996; Hanifin and Tofte, 1999; Klein and Clark, 1999; Hiroi, 2001; Munday et al., 2002). The long-duration scratching by NC/Nga mice may not be mediated by histamine, and the mechanism may differ completely from that of the behavior seen in the two previous models. Because steroids and immunosuppressants inhibit cytokine production by T cells (Kino et al., 1987; Andersson et al., 1992), some cytokines from T cells may play an important role in causing the scratching behavior seen in NC/Nga mice. There is a report that interleukin-2 may play a role in causing pruritus and inflammation in the skin of atopic dermatitis patients (Wahlgren et al., 1995). We do not know yet if this cytokine is involved in causing the scratching behavior seen in NC/Nga mice. More studies are needed to elucidate the mechanism involved in scratching behavior in NC/Nga mice or humans.

In summary, we suggest that the long-duration scratching in NC/Nga mice is a characteristic behavior and is one index for evaluating the itching in cases of atopic dermatitis. This

new model, related to long-duration scratching in NC/Nga mice, is useful for evaluating medications prescribed for the itching which occurs in atopic dermatitis.

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