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A new chronic itch model accompanied by skin lesions in hairless mice

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Abstract

The present study was performed to develop a new chronic itch model accompanied by skin lesions using hairless mice. The effects of some drugs on the itch response in this model were also studied. 2,4,6-Trinitrochlorobenzene (TNCB) was applied repeatedly on the rostral back of sensitized hairless mice every 2 days for 54 days, and the scratching behavior was observed on day 0, 18, 36 and 54. The skin symptoms and total IgE level were also observed. The number of scratches observed at 24 and 48 h after TNCB challenge was increased gradually from day 18 to day 54. An intimate relationship was observed between the number of scratches and the skin score at 48 h after TNCB on day 54. The skin symptoms and total IgE levels were also elevated gradually from day 18 to day 54. Chlorpheniramine, cyproheptadine and methysergide caused no effect on the scratching behavior accompanied by skin lesions at 48 h after TNCB challenge, even at a high dose. On the other hand, L-733,060, naloxone, naltrexone, prednisolone and dexamethasone caused a significant inhibition of the scratching behavior induced by TNCB. Therefore, this model may be useful to evaluate the effects of drugs on the itch response accompanied by skin lesions, such as atopic dermatitis.

Keywords: Hairless mice; Scratching behavior; Itch; TNCB; Atopic dermatitis

1. Introduction

Itch is an unpleasant cutaneous sensation that provokes the desire to scratch [1], and is the most common symptom of various skin diseases, such as atopic dermatitis, contact dermatitis and urticaria. In these diseases, scratch provoked by itch aggravates the lesions of the skin and makes the dermatitis worse [2]. However, the mechanisms of itch accompanied by skin lesions, such as atopic dermatitis, are not yet completely understood. It is necessary to establish appropriate animal models to understand the mechanism of itch and to develop effective drugs for itch in chronic skin disease.

In 1995, Kuraishi et al. [3] reported that injections of prutitogenic, but not algesiogenic, agents into the rostral back elicited hind-paw scratching at the site of injection in mice. Therefore, the scratching behavior in mice is usually employed as an index for physiological

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and pharmacological studies on itch and antipruritic agents [4–6]. Histamine, an important mediator for causing itch in humans, does not show frequent scratching behavior in ddY, BALB/c and NC/Nga mice. On the other hand, in ICR mice, histamine causes frequent scratching behavior. These findings suggest that the sensitivity to various pruritogenic agents differs according to the basis of the mouse strains [7]. Furthermore, Fuchibe et al. [8] reported that hairless mice are highly sensitive to pruritogens, and their skin can be easily examined without shaving, which causes lesions or stimulates the skin. In the present study, we employed hairless mice because of the advantage of two points, that is, high sensitively to pruritogens and suitability for time-course studies due to glabous skin.

In this study, we demonstrated that the new itch model, accompanied by skin lesions, could be produced by the repeated application of 2,4,6-trinitrochlorobenzene (TNCB) in hairless mice. Furthermore, we evaluated the effects of some antipruritic drugs on scratching behavior in this model.

2. Materials and methods

2.1. Animals

Female hairless mice (HOS: HR-1), 6 weeks of age, were obtained from Hoshino Experimental Animal Supply, Saitama, Japan. The animals were housed in an air-conditioned room with controlled temperature (24±2 °C) and humidity (55±15%). Food and water were provided ad libitum. All procedures involving animals were conducted in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center.

2.2. Reagents and drugs

The following drugs were obtained from the sources shown in parentheses: TNCB (Tokyo Chemical, Tokyo, Japan), D-chlorpheniramine maleate (Sigma, St. Louis, MO, USA), cyproheptadine hydrochloride (Sigma), methysergide maleate (Sigma), naloxone hydrochloride (Sigma), naltrexon hydrochloride (Sigma), prednisolone (Wako, Osaka, Japan), dexamethasone (Wako) and L-733,060 (Sigma). Prednisolone and dexamethasone were suspended in a 5% gum arabic solution (Kokusan Chemical, Tokyo, Japan) and the others were dissolved in saline.

2.3. Sensitization and challenge procedures

Hairless mice were sensitized by the single epicutaneous application of 100 μl of 1% TNCB in acetone to the entire dorsal back (designated as day −7) as described previously [9]. Seven days later (day 0), 50 μl of 1% TNCB in acetone/olive oil (3:1) was repeatedly applied to the dorsal back every 2 days for 54 days.

2.4. The measurement of TNCB-induced scratching behavior in sensitized hairless mice

Scratching behavior was automatically detected and objectively evaluated using MicroAct (Neuroscience, Tokyo, Japan), as reported previously [10]. A small magnet, 1 mm in diameter and 3 mm long, was inserted subcutaneously into the both hind paws of a mouse under ether anesthesia at least 1 day before the measurement of scratching behavior. The mouse was placed into an observation chamber (11 cm in diameter, 18 cm high), which surrounded by a round coil. The electric current induced in the coil by the movement of the hind limb implanted magnets was amplified and recorded. Then, characteristic waves reflecting the scratching event were detected by a computer. The number of scratching behaviors was counted for 60 min at 0, 24 and 48 h after TNCB challenge on day 0, 18, 36 and 54, respectively.

2.5. Dermatitis score

The skin symptoms were evaluated on day 0, 18, 36 and 54 after TNCB challenge according to the scoring method described previously [11] and the following symptoms were observed: 1, erythema/hemorrhage; 2, edema; 3, excoriation/erosion; 4, dryness. Respectively, the symptoms were classified as follows: 0, no sign; 1, mild; 2, moderate; 3, severe. The sum of the individual scores (maximum score: 12) was taken as the dermatitis score.

2.6. Determination of IgE level

Blood was collected from the tail vein on day 0, 18, 36 and 54. The serum was obtained by centrifugation 3000×*g* for 10 min at 4 °C and stored at −20 °C until use. Total IgE levels in the serum were measured by means of an enzyme immunoassay (Bethyl Laboratories Inc, Montgomery, TX, USA).

2.7. Effects of drugs on scratching behavior

Effects of drugs on scratching behavior at 48 h after TNCB challenge were evaluated on day 54. Histamine H_1 receptor antagonists, D-chlorpheniramine maleate (3, 10, 30 mg/kg), serotonin 5-HT_2 receptor antagonists, cyproheptadine hydrochloride (1, 3, 10 mg/kg) and methysergide maleate (1, 3, 10 mg/kg), the glucocorticoids, prednisolone (1, 3, 10 mg/kg) and dexamethasone (1, 3, 10 mg/kg) were orally administered 1 h before the measurement of scratching behavior. The μ-opioid receptor antagonists, naloxone hydrochloride (0.3, 1, 3 mg/kg) and naltrexone hydrochloride (0.3, 1, 3 mg/kg), and a tackykinin NK_1 receptor antagonist, L-733,060 (1, 3, 10 mg/kg), were subcutaneously administered 15 min or 30 min prior to measurement, respectively.