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Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on scratching behavior in mice

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ABSTRACT

The present study was performed to study the effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on scratching behavior in hairless mice, which are highly sensitive to pruritogens (mediators causing itching), except for histamine, and are suitable for time-course studies due to their hairless skin. TCDD is a well-known environmental pollutant that causes skin diseases with itching; therefore, we examined whether TCDD induced itching. Oral administration of TCDD caused no increase in scratching behavior when used alone, whereas TCDD in combination with distilled water or acetone/olive oil application caused a significant increase in scratching behavior. Furthermore, nerve growth factor (NGF) content in the skin increased significantly. A single administration of chlorpheniramine (histamine H1 receptor antagonist), tranilast (chemical mediator release inhibitor) and olopatadine (histamine H1 receptor antagonist) had no effect on scratching behavior induced by TCDD in combination with acetone/olive oil application. With repeated administration for 7 days, chlorpheniramine and tranilast had no effect on scratching behavior, whereas olopatadine significantly inhibited scratching behavior. In addition, only olopatadine significantly inhibited NGF content in the skin.

From these findings, it can be concluded that TCDD is not a pruritogen but causes alloknesis (itchy skin) with the simultaneous use of trivial external stimulation. In addition, it was found that drugs which decreased skin NGF contents may inhibit this scratching behavior.

1. Introduction

Itching is an unpleasant cutaneous sensation that provokes the desire to scratch [1], and is the most common symptom of various skin diseases, such as dry skin, atopic dermatitis and contact dermatitis. In these diseases, scratching provoked by itching aggravates skin lesions and worsens dermatitis [2]. It has been assumed that the important mediators for causing itching are histamine, prostaglandins, tryptase, IL-2 and substance P [3–7]. On the other hand, it has become clear that nerve growth factor (NGF) stimulates the extension and function of sensory nerve C fibers. An increase in sensory nerve C fibers in the epidermis is partly responsible for intense itching sensations [8]. In spite of a number of these findings, the mechanisms of itching are not yet completely understood; therefore, it is important to detect substances that trigger itching, and to elucidate the mechanism of itching in skin disease.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a well-known environmental pollutant that causes toxicity through the aryl hydrocarbon receptor (AhR) [9]. In humans, TCDD causes skin diseases with itching [10,11]. Furthermore, Tauchi et al. [11] reported that transgenic mice expressing the constitutive active form of AhR in keratinocytes developed skin lesions with itching. It is well known that type 2 helper T (Th 2) cells are important for itching in inflamed skin, but TCDD suppresses the Th2-type immune reaction by impairing of T-cell functions [12,13]; therefore, it seems that TCDD does not cause an itching sensation by Th2-type immune responses, and other mechanisms may be important.

In this study, we therefore investigated whether TCDD induces itching in hairless mice, which are highly sensitive to pruritogens and suitable for time-course studies due to their hairless skin [14]. To enhance the sensitivity of TCDD, distilled water or acetone/olive oil as a trivial external stimulation was used. Furthermore, we studied the effects of antipruritic drugs on scratching behavior in order to identify the characteristics of this itch model.

2. Materials and methods

2.1. Animals

Six-week-old male hairless mice (Hos:HR-1) 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. Hairless mice are suitable for time course studies due to their hairless skin. In addition, we have reported that hairless mice are comparatively more sensitive to pruritogens, except for histamine,

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than Nc/Nga, ICR and ddY mice [14]; therefore, hairless mice were used in this study. 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: TCDD (Wako, Osaka, Japan), D-chlorpheniramine maleate (Sigma, St. Louis, MO, USA), tranilast (Kissei Pharmaceuticals Co., Ltd., Nagano, Japan) and olopatadine hydrochloride (Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan). TCDD was dissolved in corn oil (Sigma) and administrated by gavage. The other drugs were suspended in 5% gum arabic solution (Kokusan Chemical, Tokyo, Japan) and administered by gavage.

2.3. Animal treatment

TCDD (0.3 or 1 ng/kg) was orally administered to mice in corn oil every day for 54 days, while control mice received the vehicle alone. In addition, 50 μL distilled water or acetone/olive oil (3:1) was repeatedly applied to the rostral part of the back of mice every 2 days for 54 days.

2.4. Scratching behavior

Scratching behavior was automatically detected and objectively evaluated using MicroAct (Neuroscience, Tokyo, Japan), as reported previously [15]. A small magnet, 1 mm in diameter and 3 mm long, was inserted subcutaneously into both hind paws of a mouse under ether anesthesia at least one day before the evaluation of scratching behavior. The mouse was placed into an observation chamber (11 cm in diameter, 18 cm high), surrounded by a round coil. The electric current induced in the coil by movement of the hind limbs implanted with magnets was amplified and recorded. Characteristic waves reflecting a scratching event were detected by a computer. The number of scratching behaviors was counted for 60 min on days 0, 18, 36 and 54.

2.5. Measurement of NGF contents in the skin of hairless mice

On day 54, skin samples were removed from the dorsum of hairless mice, and minced on ice. Five hundred microliters of lysis buffer (20 mM Tris, 137 mM NaCl, 1% Nonidet P-40, 10% glycerol, and protease and phosphatase inhibitors, pH 8) were added to 0.1 g of skin samples. Samples were gently homogenized and the collected supernatants were applied to ELISA after appropriate acid treatment using the NGF E_{max}™ immunoassay system (Promega Corp., Madison, WI, USA), according to the manufacturer's instructions.

2.6. Effects of drugs on scratching behavior

The effects of drugs on scratching behavior were evaluated immediately after acetone/olive oil application on day 54 (in single administration) and day 60 (in repeated administration). Histamine H1 receptor antagonist, chlorpheniramine (3, 10, 30 mg/kg) and olopatadine (3, 10, 30 mg/kg), and a chemical mediator release inhibitor, tranilast (30, 100, 300 mg/kg) were orally administered 1 h before counting scratching behaviors.

2.7. Effects of drugs on NGF contents

The effects of drugs on NGF contents were evaluated immediately after acetone/olive oil application on day 60 (repeated administration). Histamine H1 receptor antagonist, chlorpheniramine (30 mg/kg) and olopatadine (30 mg/kg), and a chemical mediator release inhibitor, tranilast (300 mg/kg) were orally administered 1 h before removal of skin samples.

2.8. Statistical analysis

All experimental 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. Effect of TCDD on scratching behavior in hairless mice

Fig. 1 shows the changes in scratching behavior induced by TCDD. The number of scratching behaviors was unchanged even at a dose of 1 ng/kg. TCDD in combination with the distilled water application (Fig. 2A) or acetone/olive oil application (Fig. 2B) significantly increased scratching behavior from day 36 with 1 ng/kg and on day 54 with 0.3 ng/kg. It was confirmed that an increase of scratching behavior was observed only at the application site of distilled water or acetone /olive oil.

3.2. Changes in NGF contents in the skin

We measured NGF contents in skin homogenates of hairless mice and the results are shown in Fig. 3. On day 54, TCDD in combination with the distilled water and acetone/olive oil application resulted in a significant increase of NGF contents compared with TCDD alone. On the other hand, corn oil in combination with the distilled water and acetone/olive oil application had no significant effect on NGF contents compared with the control. In addition, NGF content in the skin was increased significantly by chronic treatment with TCDD alone compared with corn oil-alone groups (control, distilled water, acetone/olive oil).

3.3. Effects of drugs on scratching behavior induced by TCDD in combination with repeated application of acetone/olive oil

Fig. 4 shows the effects of a single administration of drugs on scratching behavior after application of acetone/olive oil on day 54. A single administration of chlorpheniramine, tranilast and olopatadine had no significant effect ($P>0.05$) on scratching behavior at 30, 300 and 30 mg/kg, respectively. As shown in Fig. 5, repeated administration of chlorpheniramine and tranilast for 7 days had no significant effect on scratching behavior, whereas olopatadine at a dose of 30 mg/kg significantly inhibited scratching behavior.

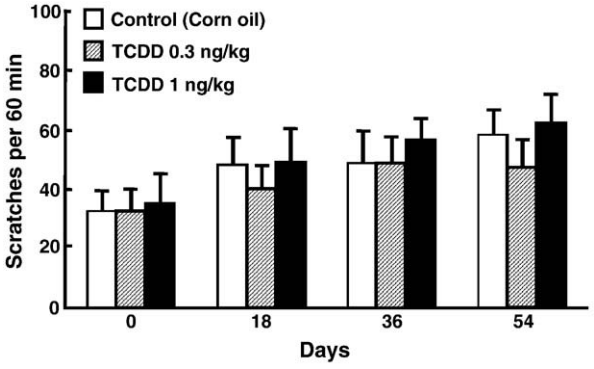

Fig. 1. Changes in scratching behavior induced by TCDD in hairless mice. Control group was administered corn oil (open column), and the TCDD group was administered 0.3 ng/kg (hatched column) or 1 ng/kg (closed column) TCDD. Each column and vertical bar shows the means ± S.E.M. ($n=10$).