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Olopatadine hydrochloride suppresses hot flashes induced by topical treatment with tacrolimus ointment in rats

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ABSTRACT

Tacrolimus ointment is prescribed for patients with atopic dermatitis, although it is known to cause transient burning sensations and hot flashes in the applied skin. The aim of this study was to evaluate the effects of olopatadine hydrochloride (olopatadine), an antiallergic agent with a histamine H1 receptor (H1R) antagonistic activity, on the incidence of hot flashes induced by topical treatment with tacrolimus ointment in rats. Consequently, the skin temperature was increased by the topical application of tacrolimus ointment in rats, and the rise in skin temperature was inhibited by pretreatment with olopatadine in a dose-dependent manner. Inhibitory effect of olopatadine on tacrolimus-induced skin temperature elevation was significantly more potent than that of cetirizine hydrochloride, other antiallergic agent with H1R antagonistic activity, at doses in which both agents exhibit comparable H1R antagonistic activity in rats. These results suggest that H1R antagonistic activity-independent mechanism contribute to the inhibitory effect of olopatadine on tacrolimus-induced skin temperature elevation. Olopatadine also significantly inhibited increases in vascular permeability and nerve growth factor production in the skin induced by topical tacrolimus treatment. Thus, the onset of hot flashes in rats is quantitatively determined by measuring the skin temperature and olopatadine attenuates hot flashes induced by topical tacrolimus ointment in rats, suggesting that the combination application with olopatadine and tacrolimus ointment is useful for improving medication adherence with tacrolimus ointment treatment in patients with atopic dermatitis.

1. Introduction

Atopic dermatitis is a chronic relapsing eczematous skin disease characterized by pruritus and inflammation and accompanied by signs of cutaneous physiological dysfunction (dry, barrier-disrupted skin). Tacrolimus ointment is non-steroid topical calcineurin inhibitor and is known to inhibit activated T cell-dependent proinflammatory cytokine production (Williams, 2002). Therefore, tacrolimus ointment is frequently employed when the effects of existing treatments, such as topical corticosteroids are insufficient, or the physician hesitates to administer these drugs due to their topical side-effects (Saeki et al., 2009). However, tacrolimus often causes symptoms of stimulation, such as a transient burning sensation and hot flashes in the applied regions (Reitamo et al., 2000), which is the most common reason for premature study discontinuation (Reitamo et al., 2007). Therefore, achieving palliation of these symptoms is important for preventing the need to withdraw therapy with tacrolimus ointment.

Olopatadine hydrochloride (olopatadine, Kyowa Hakko Kirin Co., Ltd., Japan) is an antiallergic agent with a histamine H1 receptor (H1R) antagonistic action that is applied to treat the signs and symptoms of allergic rhinitis, chronic urticaria and eczema dermatitis (Ohmori et al., 2002). In the present study, we investigated the effects of olopatadine treatment on the incidence of hot flashes induced by the topical application of tacrolimus ointment. First, we demonstrated that the elevation of temperature induced by the administration of topical tacrolimus ointment to the rat abdominal skin can be measured using a thermometer. Next, olopatadine was administered orally to the rats, and the effects on the temperature changes induced by tacrolimus ointment were examined. In general, flare and heat sensations are the result of an increased blood flow induced by vasodilation, which subsequently enhances vascular permeability. It has been reported that nerve growth factor (NGF) stimulates plasma extravasation in the rat skin (Otten et al., 1984). Therefore, we investigated the effects of olopatadine on the enhanced vascular permeability and NGF production induced by topical treatment with tacrolimus ointment.

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2. Materials and methods

2.1. Experimental animals

Male 6-week-old Hairless Wister Yagi (HWY) rats were purchased from Japan SLC (Shizuoka, Japan). The rats were kept in a specific pathogen-free animal facility with a maintained temperature of 19–25 °C, humidity of 30–70% and 12-h day/night cycle and given access to food and water ad libitum. The experiments were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, and the experimental protocol used in this study was approved by the Committee for Animal Experiments at Kyowa Hakko Kirin Co., Ltd. (Shizuoka, Japan).

2.2. Materials

Olopatadine was synthesized at Yokkaichi Plant, Kyowa Yuka Co., Ltd. (Mie, Japan). Cetirizine hydrochloride (cetirizine) was purchased from Sigma-Aldrich. Olopatadine and cetirizine were dissolved in distilled water. Tacrolimus ointment (0.03% and 0.1%, Protopic®) was purchased from Astellas Pharmaceuticals (Tokyo, Japan).

2.3. Drug treatment

Olopatadine at doses of 0.01 and 0.1 mg/kg or cetirizine at a dose of 10 mg/kg was administered orally to the rats once daily for four consecutive days. Thirty min after the final administration of olopatadine or cetirizine, tacrolimus ointment (200 mg/body) was applied to the tape-stripped abdominal skin of rats.

2.4. Measurement of skin temperature

The skin temperature of the rats was measured using a ThermoTracer (TH5100, NEC avio) before and 30 min after the application of tacrolimus ointment. The temperature change was defined as the difference between the skin temperature before and after tacrolimus application.

2.5. Measurement of vascular permeability in the skin

One hour after the topical application of tacrolimus ointment to the abdominal skin, the anesthetized rats were intravenously injected with 1.0 w/v% Evans blue solution at a dose of 25 mg/kg. Thirty min later, the animals were killed, and the tacrolimus-treated skin was removed. Evans blue dye that had leaked into the tissue was extracted with formamide at 45 °C, and its absorbance at 625 nm was determined with a spectrophotometer (THERMOmaxTM; Molecular Devices, USA). The data were analyzed as per the protocol of each organization.

2.6. Measurement of NGF in the skin

One hour after the topical application of tacrolimus ointment, the tacrolimus-treated skin was collected. The skin samples were minced and homogenized in phosphate-buffered saline containing a protease inhibitor (CompleteTM; Roche Diagnostics) with a Polytron tissue homogenizer. The precipitate was removed via centrifugation, and the supernatant was collected. The NGF (Promega) and protein (Thermo Scientific) concentrations were analyzed with an assay kit according to the manufacturer's protocol.

2.7. Statistical analysis

The data are presented as the mean+S.E.M. Student's *t*-test or the Aspin–Welch test following the *F* test were used for the analysis of differences between two groups. Multiple comparisons among treatment groups were made using a one-way analysis of variance followed by Tukey test, Dunnett test or Williams test, or the Kruskal–Wallis followed by Steel–Dwass test. In all tests, a *P* value of <0.05 was considered to be statistically significant.

3. Results

3.1. Temperature changes in the skin induced by tacrolimus ointment

First, we investigated whether hot flashes are induced by topical treatment with tacrolimus ointment in rats. In order to mimic the skin condition of patients with atopic dermatitis, the skin barrier function of the abdominal skin in the rats was disrupted by tape stripping prior to the application of tacrolimus ointment. The incidence of hot flashes in the rats was evaluated by measuring the temperature at sites of treatment with tacrolimus ointment. As shown in Fig. 1, the skin temperature was significantly increased by a single application of tacrolimus ointment (0.03% and 0.1%), compared with single treatment with white petrolatum. No skin temperature changes were observed after the third treatment with tacrolimus ointment, indicating that the elevation of the skin temperature induced by tacrolimus treatment was transient.

3.2. Effects of olopatadine and cetirizine on the temperature changes in the skin induced by tacrolimus ointment

In order to investigate whether olopatadine has the potential to inhibit the elevation of skin temperature induced by topical treatment with tacrolimus ointment in rats, olopatadine was administered orally once daily for four consecutive days prior to tacrolimus ointment application. As shown in Fig. 2A, although olopatadine had no significant effect on skin temperature in the normal rats, the skin temperature elevation induced by topical treatment with tacrolimus ointment was significantly suppressed in the olopatadine-treated rats compared with that observed in the olopatadine-untreated rats. Olopatadine at doses of 0.01 and 0.1 mg/kg, which inhibited histamine-induced plasma leakage in rats by 63.4% and over 75% respectively (Tamura, 2011), suppressed the increase in the skin temperature induced by topical tacrolimus ointment dose dependently (Fig. 2B). Cetirizine at 10 mg/kg, which inhibited histamine-induced plasma leakage in

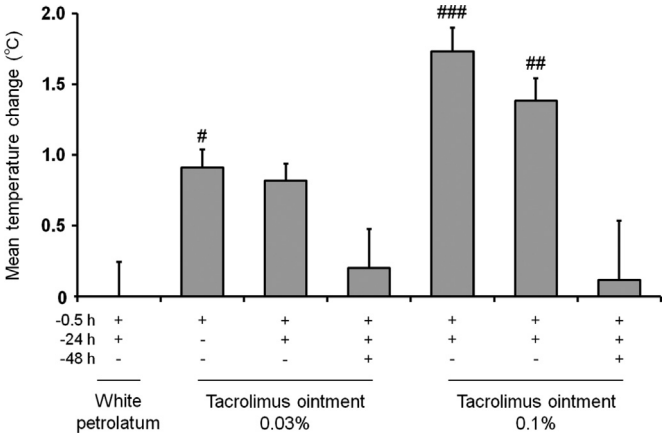

Fig. 1. Temperature changes in the abdominal skin induced by tacrolimus ointment in rat. Tacrolimus ointment (0.03% and 0.1%) was applied to the abdominal rat skin one (−0.5 h), two (−0.5, −24 h) or three (−0.5, −24, −48 h) consecutive times. The temperature changes in the abdominal skin were measured before and 0.5 h after the last treatment with tacrolimus ointment. Each column represents the mean+S.E.M. of six to 18 rats. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ compared to the white petrolatum group according to Dunnett test.