

SHORT COMMUNICATION

Olopatadine Hydrochloride Decreases Tissue Interleukin-31 Levels in an Atopic Dermatitis Mouse Model

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Atopic dermatitis (AD) is an inflammatory skin disease characterized by an intensely pruritic skin rash (1). A variety of mediators, including histamine and neuropeptides, are involved in pruritus. We previously reported that olopatadine hydrochloride (olopatadine), a histamine H1 receptor antagonist, significantly suppresses the number of scratching events associated with a decreasing number of intraepidermal nerve fibres via increased semaphorin 3A expression and decreased nerve growth factor (NGF) levels in NC/Nga mice (2). Oral olopatadine (Kyowa Hakko Kirin, Tokyo, Japan) has been prescribed in Japan and Korea for treatment of allergic rhinitis, urticarial, pruritus, eczema, prurigo, psoriasis vulgaris, and erythema multiforme, which was covered by insurance.

Recently, interleukin (IL)-31 was found to play a role in pruritus and skin barrier function in AD (3–5). It was reported that transgenic mice overexpressing IL-31 exhibit spontaneous pruritus and develop severe dermatitis (6). Moreover, serum and tissue IL-31 levels in patients with AD were increased compared with levels in control subjects, and IL-31 levels correlated with both disease activity and severity of AD (3, 7–9). Thus, we evaluated the effect of olopatadine on tissue IL-31 levels in an AD model using NC/Nga mice.

MATERIALS AND METHODS

AD-like dermatitis was induced by the topical application of *Dermatophagoides farinae* body (Dfb, 100 mg/mouse/application) ointment 3 h after barrier disruption by sodium dodecyl sulphate on shaved dorsal skin of NC/Nga mice. These proce-

dures were repeated twice per week for 4 weeks. After 2 weeks of Dfb application, mice were treated orally with either distilled deionized water (control) or olopatadine (3 or 10 mg/kg/day) daily, whereas another group of mice received 0.1% (w/w) topical tacrolimus (100 mg/mice/application) applied to the back skin twice per week for 2 weeks. Mice treated with only sodium dodecyl sulphate served as the sham group. Skin samples from the lesional skin were homogenized as previously reported (2). Subsequently, the tissue concentrations of IL-31, NGF, E-selectin, and amphiregulin were determined by enzyme-linked immunoassay (ELISA) according to the manufacturer's protocol (CSB-E13660m: Cusabio Biotech, Wuhan, Hubei Province, China, NGF Emax Immunoassay system: Promega, Madison, WI, USA, CD62E Quantikine ELISA kit and mouse amphiregulin Duoset: R&D systems, Minneapolis, MN, USA, respectively). The number of scratching episodes was determined by taking video images for 90 min. Both the F-test and Aspin-Welch test were used for analysis of differences between sham and control groups. Multiple comparisons among treatment groups were made using the Kruskal–Wallis test, followed by the Steel test.

RESULTS

As shown in Fig. 1A, IL-31 levels were significantly increased in mice that received Dfb application ($n=10$) compared with sham-treated mice ($n=6$). Olopatadine at 3 and 10 mg/kg/day ($n=10$ each) significantly suppressed this increase in IL-31 levels by 88.1% and 94.5%, respectively. Tacrolimus ointment also significantly suppressed the increase in IL-31 production by 94.3%.

In the sham, control, and olopatadine-treated groups, IL-31 correlated positively with the tissue concentra-

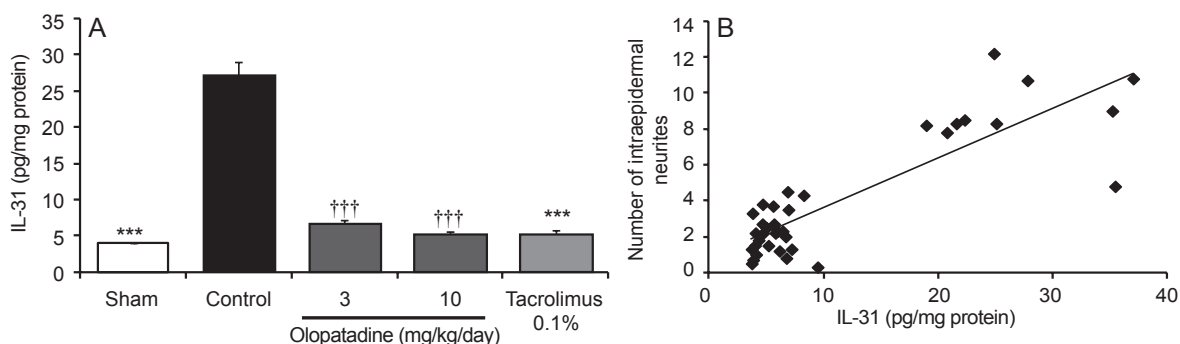


Fig. 1. (A) Effects of olopatadine and tacrolimus ointment on interleukin (IL)-31 levels in lesioned skin of NC/Nga mice. Each column represents the mean \pm standard error of 6–10 mice. *** $p < 0.001$, significant difference from the control group (Aspin-Welch test). ††† $p < 0.001$, significant difference from the control group (Steel test). (B) Relationship between IL-31 levels and the number of intraepidermal neurites. A correlation was assessed by Pearson's correlation analysis. Each square represents individual data-points from individual mice.

tions of several inflammatory and pruritus mediators, including NGF, IL-1b, E-selectin, and amphiregulin ($r=0.7574$, $r=0.7324$, $r=0.8368$, and $r=0.6970$, respectively). In particular, the correlation between IL-31 levels and the number of intraepidermal nerve fibres was strong (Pearson's correlation analysis: $r=0.8523$, $p<0.0001$; Fig. 1B), whereas olopatadine decreased IL-31 levels, as well as the number of scratching events, with a weak correlation ($r=0.5426$).

DISCUSSION

It has been reported that an increased number of peripheral nerve fibres may contribute to a reduction in the itch sensation threshold (alloknesis) in human AD patients and in NC/Nga mice (8, 9). The current study suggests that IL-31, as well as NGF, may increase scratching events by reducing the itch sensation threshold of NC/Nga mice. The weak association between scratch number and IL-31 may arise from the time restriction in recording the video image. Although neither the source of IL-31 in this model nor the mechanism of olopatadine on reducing IL-31 are clear, our study suggests that olopatadine may affect local skin lesions by reducing pruritus via decreasing tissue levels of IL-31.

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