Dear Editor

**Histamine H1-Receptor Antagonistic Drug Olopatadine Suppresses TSLP in Atopic Dermatitis Model Mice**

Olopatadine hydrochloride (OLP) is as second-generation, anti-histamines, H1-receptor antagonistic drug that is used for treating allergic rhinitis, urticaria, and various eczemas. Some studies reported that OLP suppressed the productions by epithelial cells and mast cells of several chemical mediators and Th2 cytokines/chemokaines in vivo and vitro.1,2 In this study, we evaluated whether OLP suppressed thymic stromal lymphopoietin (TSLP), and evaluated the levels of Th2 cytokines in lesional skin of atopic dermatitis model mice with quantitative real-time RT-PCR. The DS non-hair (DS-Nh) mice are a model of human atopic dermatitis (AD), that spontaneously develops dermatitis under conventional conditions as described previously.3,6

Male DS-Nh mice were obtained as F1 (Nh/+) from male DS-Nh (Nh/Nh) × female DS (+/+) mice housed under specific pathogen-free (SPF) conditions for 5 weeks. The animals were then moved to conventional conditions. Subsequently, we subcutaneously injected saline into the abdominal region of mice and administered OLP (10 mg/kg/day) 5 days a week for a total of 11 weeks. The control mice were administered saline through the esophagus during the same period. First, tissues were obtained by biopsy from facial lesional skin at 10-16 weeks of age. Samples were then fixed in 10% formalin and prepared for hematoxylin and eosin (HE) staining. Clinically, erythema, edema, and slight erosions were observed on the faces of control DS-Nh mice. Histopathologically, hyperkeratosis, acanthosis, and infiltration of lymphocytes were apparent in skin lesions of control DS-Nh mice, and these changes were prominently attenuated by treatment with oral OLP. In this study, the histological findings were evaluated as reported previously.6 In brief, samples were scored for the severity and character of the inflammatory response using a subjective grading scale. Responses were graded as follows: 0, no response; 1, minimal response; 2, mild response; 3, moderate response; and 4, marked response. The slides were blinded, randomized, and read to determine the histology score. All of the slides were evaluated by the same dermatopathologist using the same subjective grading scale. The total histology score was calculated, where this included inflammation, neutrophils, mononuclear cells, edema, epithelial hyperplasia, and mast cells.

The tissues obtained by biopsies of half of the facial lesions of DS-Nh mice were frozen and stored at -80°C. Total RNA was extracted using RNeasy Mini Kits according to the manufacturer’s instructions (Qiagen, Valencia, CA, USA). Quantitative real-time RT-PCR was performed as described previously.6 Oligonucleotide primers and probes specific for IL-4, CCL24, and TSLP were used in this study. For each sample, triplicate test reactions and a control reaction lacking reverse transcriptase were analyzed for gene expression and the results were normalized to those of the ‘housekeeping’ glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) mRNA.

Histamine is a biogenic amine and an important mediator in various allergic and inflammatory conditions. It exerts the effects through the activation of four distinct histamine receptors that belong to the G-protein-coupled-receptor superfamilly.7 OLP has an antagonistic action against histamine H1 receptor and inhibits the release of various chemical mediators and Th2 cytokines/chemokaines.2

In this study, the total histologic score of the control DS-Nh mice groups showed more severe dermatitis compared with the OLP groups (Fig. 1a). In contrast previous report indicated that OLP suppressed IL-4 expressions in NC/Nga mice.2 However, in our experiments, the IL-4 expressions did not have a difference between control and OLP groups at only 10-12 weeks. This result suggested that the difference of IL-4 expressions seemed to be due to murine or environmental difference. The expression of TSLP mRNA in DS-Nh mice that received OLP was lower than the levels in control DS-Nh mice (Fig. 1b). Similarly, the expression of IL-4, CCL 24 and TSLP mRNA increased at 10-16 weeks in control DS-Nh mice (Fig. 1b). Recent studies have revealed that TSLP is not only expressed by epithelial cells and keratinocytes but also by mast cells, fibroblasts, dendritic cells, and various other cells. In contrast TSLP expression in

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**Fig. 1a** Histopathological effect of OLP on the skin lesions of DS-Nh mice. The total histology score is shown. Each point represents the mean value ± standard error of the mean (SEM) of 5 mice. **P < 0.01, and ***P < 0.001: significantly different from the control group (Mann-Whitney two-sided U test).
Fig. 1b OLP inhibits cytokine expressions in skin lesions of DS-Nh mice. Quantitative real-time PCR was performed to evaluate the expression levels of IL-4, CCL24, and thymic stromal lymphopoietin (TSLP) in the skin lesions of Ds-Nh mice with or without olopatadine treatment at ages 10-16 weeks. Glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) was used as a control to normalize for differences in each sample. Data are shown as the mean ± SEM, *P < 0.05, **P < 0.01, and ***P < 0.001 (Mann-Whitney two-sided U test).

the lesional skin is significantly elevated especially in the epidermis with acute and chronic AD, but not in uninvolved skin of the patients.8 Our data indicated that TSLP expressions were significantly suppressed as well as IL-4 and CCL24 expression by OLP. However, the target cells of OLP are unknown, and future studies in AD model mice are needed.

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