Letter to the Editor

Olopatadine hydrochloride inhibits scratching behavior induced by a proteinase-activated receptor 2 agonist in mice

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Proteinase-activated receptors (PARs), a group of G protein-coupled receptors, are activated by the cleavage of their N-terminal domain by serine proteinases [1, 2]. The proteolytic cleavage of the N-terminal region of PARs unmasks a new N-terminal sequence that acts as a tethered ligand that binds and activates the receptor itself [1]. The PAR_2 subtype is highly expressed in the skin, is activated by trypsin and mast cell tryptase, and can be activated without the need for proteolysis by synthetic PAR_2 agonists, such as SLIGRL-NH_2 [1, 3]. Tryptase and PAR_2 are up-regulated on sensory nerves in the skin from atopic dermatitis patients [2]. Indeed, intradermal injections of SLIGRL-NH_2 evoke dose-dependent scratching in mice [4]. In addition, PAR_2 agonists induce the release of neuropeptide, such as substance P, from primary afferent neuron [5]. Pretreatment of mice with a histamine H_1 receptor antagonist, pyrilamine, has no effect on PAR_2-mediated scratching [4]. These results indicate that PAR_2 signaling induces itching independently of histamine H_1 receptor signaling.

Olopatadine hydrochloride ((Z)-11-(3-dimethylaminopropylidene)-6,11-dihydrodinenz [b,e]oxepin-2-acetic acid monohydrochloride) is a second-generation antihistamine. Olopatadine inhibits ear swelling and cytokine production in a murine chronic contact hypersensitivity model, while other antihistamines do not suppress them, suggesting that olopatadine exerts additional biological effects besides its blockade of histamine H_1 receptor [6]. In this study, we examined the effect of olopatadine on PAR_2 agonist-induced scratching behavior in mice.

Scratching behavior with a PAR_2 agonist was induced on the shaved neck of 6-8 weeks old C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME, USA) by subcutaneously injecting 50 μl of a PAR_2 agonist 2f-LIGRL-amide (1 mg/ml in phosphate-buffered saline (PBS); Calbiochem, Dermstadt, Germany). Injected mice were individually transferred to cages for observation from one hour before subcutaneous injection for acclimatization. Room temperature was maintained at 22 to 25°C. Scratching behavior was counted as once when a hind leg of the animal touched the shaved area and returned to the ground, as previously described [4]. The number of scratching behavior was counted from -10 to 40 minutes

defining the time of administering the PAR_2 agonist as 0 minutes. The total number of scratching was the sum of scratching behavior from 0 to 30 minutes. Subcutaneous administration of PBS alone induced no scratching behavior in mice (data not shown). The skin sections stained with hematoxylin and eosin which was excised from shaved neck after 30 minutes of treatment with the PAR_2 agonist or PBS were used for histological evaluation. We also examined expression levels of preprotachykinin A mRNA, the precursor for substance P, in the skin using quantitative real time polymerase chain reaction according to the manufacturer's instructions (Applied Biosystems, Forester City, CA, USA). In addition, immunohistochemical analysis of substance P using anti-substance P monoclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was done. Plasma levels of substance P were measured by a specific ELISA (R&D Systems, Mineapolis, MN, USA).

Olopatadine was orally given once at 1, 3, 10, or 30 mg/kg through a tube together with 1 ml of PBS 30 minutes before subcutaneous injection of the PAR_2 agonist. Pyrilamine (Sigma-Aldrich, St. Louis, MO, USA), a histamine H_1 receptor antagonist, was used to confirm that the PAR_2 agonist-induced scratching behavior is not mediated by histamine H_1 receptor. Pyrilamine (10 mg/kg) was subcutaneously injected into the hip of mice 30 minutes before the administration of the PAR_2 agonist. Furthermore, we examined the effect of olopatadine on the itch evoked by 150 μg of substance P (Peptide Institute, Osaka) treatment as the method for the PAR_2 agonist treatment. Mice orally given only PBS served as a control group.

The PAR_2 agonist induced scratching behavior just after the administration and its effect continued until 30 minutes after the administration (Figure 1A). The number of scratching behavior reached the maximum 5 minutes after the PAR_2 agonist administration at all groups and then decreased with time. Pretreatment with olopatadine significantly decreased the number of scratching behavior in mice treated at all doses compared with that in control mice. In addition, inhibition of the number of scratching behavior was almost dependent on the dose of olopatadine. According to the skin hematoxylin and eosin staining pictures, the PAR_2

agonist treatment induced substantial cutaneous edema compared with PBS treatment, which was inhibited by olopatadine administration (Figure 1C). By contrast, the number of scratching behavior in the pyrilamine-pretreated group was comparable to that in the control group, suggesting that the inhibitory effect of olopatadine on the PAR_2 agonist-induced scratching behavior is not mediated by histamine H_1 receptor. Similar results were obtained for the total number of scratching that was the sum of scratching behavior from 0 to 30 minutes (Figure 1B).

Plasma levels of substance P increased by the PAR_2 agonist treatment (Figure 2B), though there were no significant differences in the skin preprotachykinin A mRNA expression levels (Figure 2A). However, secreted substance P could not be detected in the PAR_2 agonist- or PBS-treated skin. Although this discrepancy is unclear, it might be due to short half-life of substance P and/or high solubility of substance P in organic solvent, such as formaldehyde, and water which were used for the fixation and staining of skin samples [7]. Olopatadine preadministration inhibited elevation of plasma substance P levels induced by the PAR_2 treatment (Figure 2B). Furthermore, substance P treatment induced scratching behavior, which was inhibited by olopatadine pretreatment (Figure 2C).

Histamine is the important mediator of itching in allergic skin diseases [2]. However, it is well recognized in the routine clinical setting that itching of atopic dermatitis, nasal congestion in allergic rhinitis, and bronchial asthma do not always respond to H_1 antihistamines. Our finding that H_1 antihistamine, pyrilamine did not inhibit scratching induced by the PAR_2 agonist suggests that H_1 antihistamine-resistant itching is mediated in part by PAR_2 signaling. Furthermore, the significant inhibitory effect on PAR_2-induced itching by olopatadine suggests that olopatadine may be effective for H_1 antihistamine-resistant itching.

Many studies have shown that olopatadine has various pharmacological and biological activities besides its histamine H_1 receptor antagonistic activity [6]. In particular, olopatadine suppresses release of neuropeptides, including substance P and calcitonin gene-related peptide,

from afferent neurons. Increased expression of substance P in the spinal dorsal horn following repeated hapten application is inhibited by olopatadine [8]. Olopatadine also attenuates the enhancement of capsaicin-induced substance P release by bradykinin from cultured dorsal root ganglion neurons [9]. Moreover, olopatadine decreases plasma levels of substance P in patients with atopic dermatitis, while cetiridine and fexofenadine do not decrease them [10]. In this study, olopatadine inhibited the PAR_2 agonist-induced substance P secretion. Furthermore, olopatadine decreased substance P-induced scratching behavior. Collectively, the inhibitory effect on PAR_2-induced itching by olopatadine may be related to the inhibition of substance P release and substance P-induced itching by olopatadine, which is downstream of PAR_2 signaling.

Conflict of interest

None.

References

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Figure legends

Figure 1. The time course (A) and total number (B) of the PAR_2 agonist-induced scratching behavior in either olopatadine- or pyrilamine-pretreated group. Thirty minutes before the PAR_2 agonist administration, olopatadine was orally administered once at each dose and pyrilamine was subcutaneously injected into the hip of mice. The time of administering the PAR_2 agonist was defined as 0 minutes. Mice orally given only PBS served as a control group. Representative histological sections stained with hematoxylin and eosin are shown (C). The skin sections were excised from shaved neck after 30 minutes of treatment with the PAR_2 agonist or PBS. Each group consisted of 6 mice. Values are expressed as mean ± SD. Original magnifications, x100.

Figure 2. Levels of preprotachykinin A (PPT-A) mRNA expression in the skin (A) and substance P in the plasma samples (B) from the PAR_2 agonist- or PBS-treated mice. The skin and plasma samples were obtained after 30 minutes of treatment with the PAR_2 agonist or PBS. Effect of olopatadine on total number of scratching behavior induced by substance P. Thirty minutes before substance P administration, olopatadine was orally administrated once at each dose. Total number of scratching behavior was counted from 0 to 30 minutes defining the time of administering substance P as 0 minutes. Mice orally given only PBS served as a control group. Each group consisted of 6 mice. Values are expressed as mean ± SD.

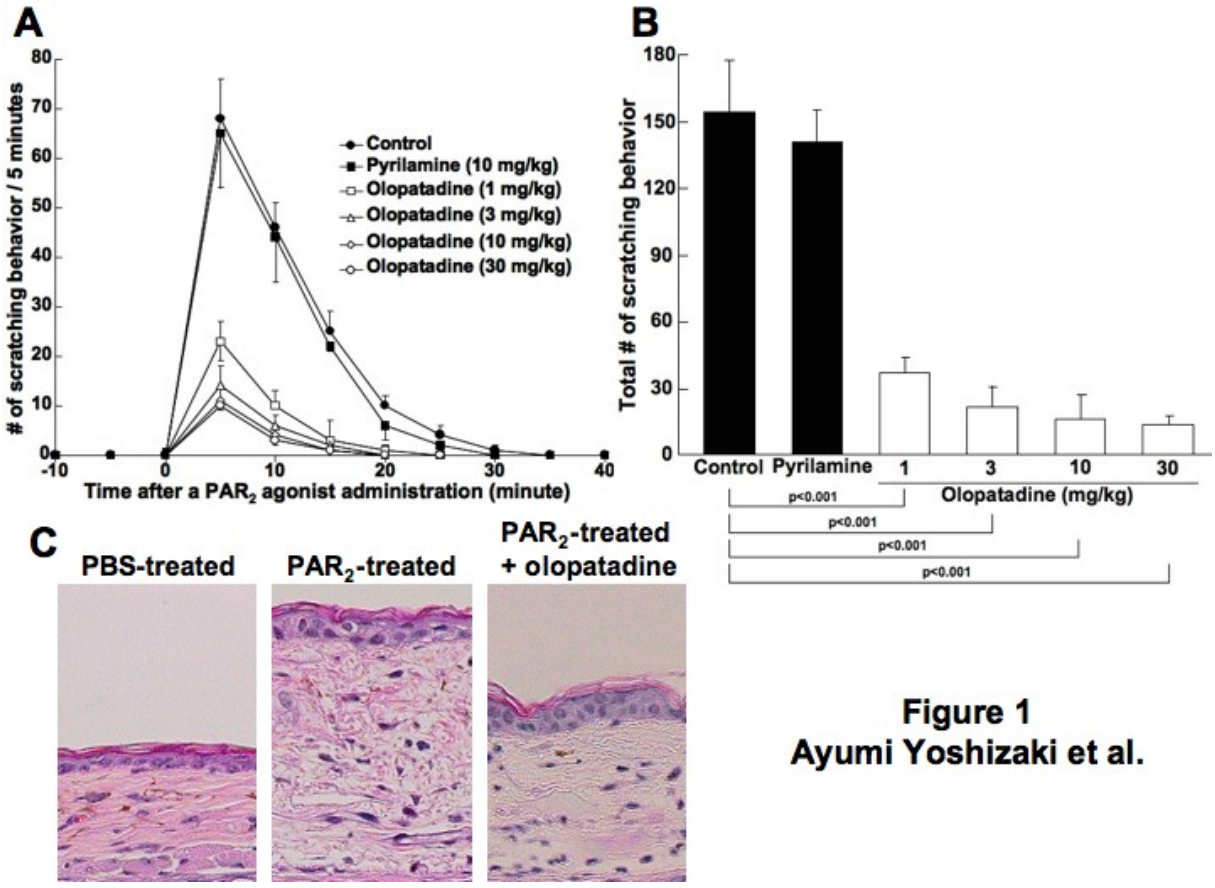

Figure 1
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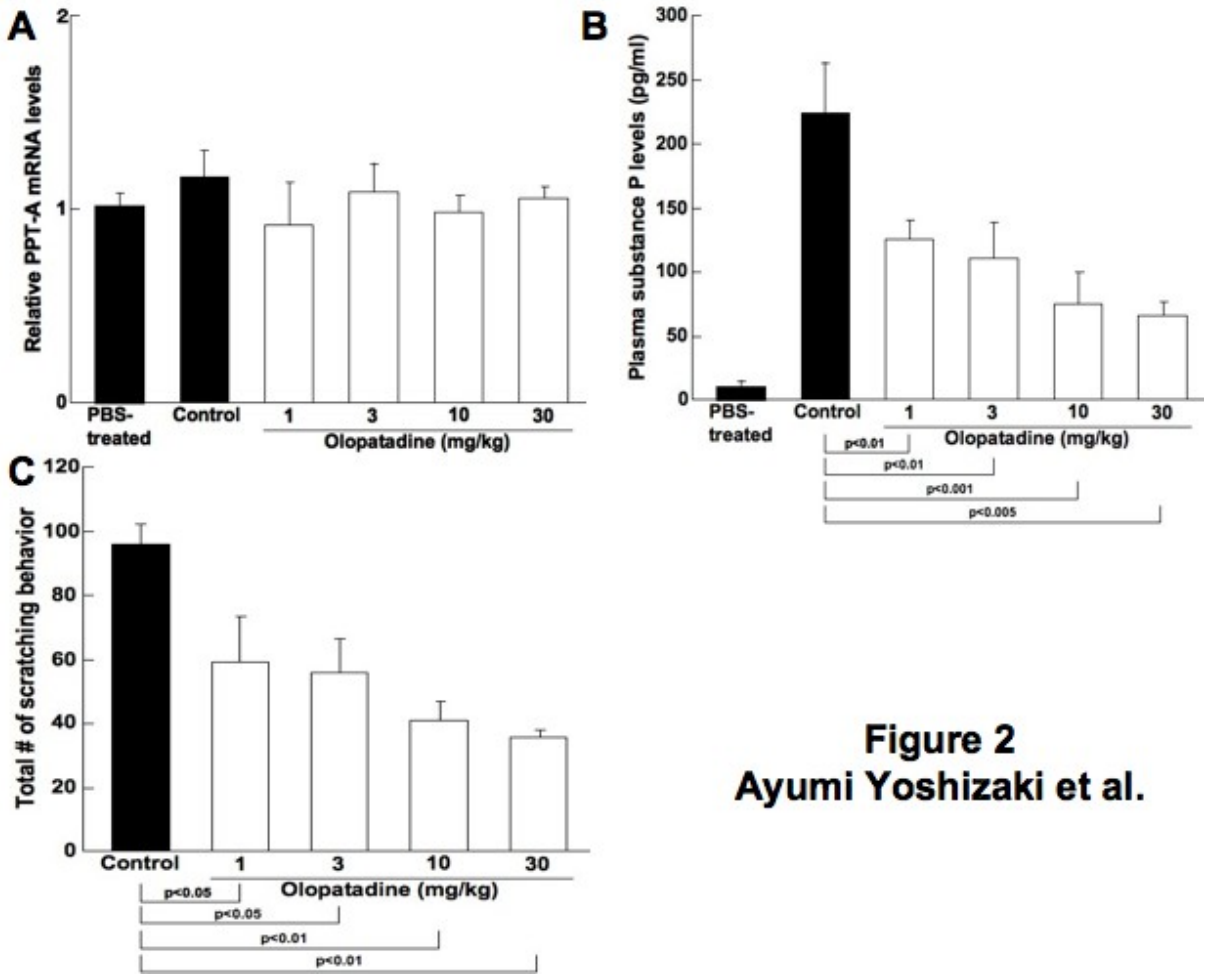

Figure 2
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