



Short communication

Re-evaluation of over-the-counter histamine H1-receptor antagonists based on their effects on murine models of allergen-induced nasal hyperresponsiveness

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ARTICLE INFO

Article history:

Received 3 August 2022

Received in revised form

26 September 2022

Accepted 12 October 2022

Available online 14 October 2022

Keywords:

CD4⁺ T cell

Mouse

Over-the-counter medicine

ABSTRACT

T cells play an essential role in the development of allergen-induced nasal hyperresponsiveness (NHR), a pathophysiological response in allergic rhinitis. The effects of histamine H1-receptor antagonists (antihistamines) on murine NHR models were investigated. Intragastric epinastine, fexofenadine, and loratadine administration suppressed allergen-induced immediate nasal response but not NHR in immunized mice. Regardless of the alleviation of stimulation-induced Th2 cytokine expression by loratadine and desloratadine *in vitro*, allergen-induced NHR and nasal eosinophil infiltration in Th2 cell-transferred mice were unaffected by loratadine *in vivo*. This influence on T cell-mediated NHR was excluded from the pharmacological effects of antihistamines.

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Allergic rhinitis (AR) is characterized by various nasal symptoms such as sneezing, rhinorrhea, and nasal conjunctiva along with inflammatory responses of the nasal mucosa.¹ Furthermore, many AR patients develop nasal hyperresponsiveness (NHR), a pathophysiological feature by which the AR symptoms are augmented.² We have proven the essential role of T cells in NHR development using murine AR models.³

Various histamine H1-receptor antagonists (antihistamines) are used to treat AR, partly as over-the-counter (OTC) medicines. Besides their strong inhibitory activity on histamine-mediated AR symptoms, several antihistamines have been reported to affect T

cell-related responses. For example, Th2 cytokine production by human T cells was suppressed by epinastine (EPN).⁴ Allergen-induced bronchial hyperresponsiveness (BHR) accompanied by lung eosinophil accumulation and Th2 cytokine production in murine models of bronchial asthma was attenuated by treatment with fexofenadine (FXF)⁵ and desloratadine (DES), an active metabolite of loratadine (LRT).⁶ However, the efficacy of these OTC antihistamines on NHR development has not been investigated. Here, we re-evaluated the usefulness of antihistamines by examining their effects on allergen-induced NHR in an immunized mouse model, as previously reported.^{3,7}

All animal experiments were approved by the Animal Care and Use Committee of Hiroshima University (A22-41). BALB/c mice immunized with ovalbumin (OVA) were challenged via intranasal injection of OVA for five consecutive days (Fig. 1A). These mice developed an immediate nasal response (INR), detected by an increase in the number of sneezes after the fourth OVA challenge. This response was primarily caused by histamine released from mast cells because of IgE-mediated degranulation.⁷ Consistently, daily intragastric administration of 20 mg/kg EPN, 120 mg/kg FXF, or 10 mg/kg LRT, which was 60 times the daily clinical doses, almost completely suppressed allergen-induced INR, probably via their

Abbreviations: antihistamine, histamine H1-receptor antagonist; AR, allergic rhinitis; BHR, bronchial hyperresponsiveness; DES, desloratadine; EPN, epinastine; FXF, fexofenadine; INR, immediate nasal response; i.g., intragastric; i.n., intranasal; LRT, loratadine; NALF, nasal lavage fluid; NHR, nasal hyperresponsiveness; OTC, over-the-counter; OVA, ovalbumin.

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Peer review under responsibility of Japanese Pharmacological Society.

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<https://doi.org/10.1016/j.jphs.2022.10.004>

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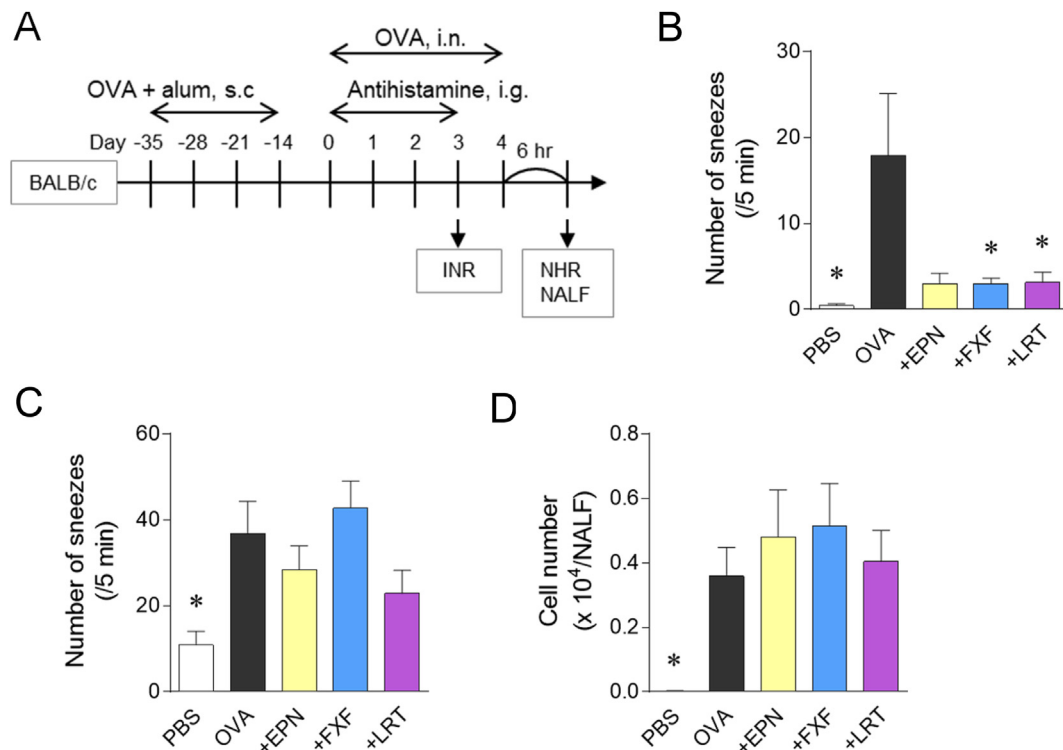


Fig. 1. Effects of histamine H1-receptor antagonists (antihistamines) on allergen-induced nasal responses in ovalbumin (OVA)-immunized mice. (A) Procedural overview of the experiments. Six-week-old female BALB/c mice (Jackson Laboratory Japan, Inc., Yokohama, Japan) were immunized four times with the weekly intraperitoneal injection of 20 μ g ovalbumin (OVA, Sigma–Aldrich, St. Louis, MO, USA), emulsified in 2 mg alum (Inject Alum; Thermo Fisher Scientific, Chicago, IL, USA). Two weeks after the last immunization, the mice were challenged once per day by the intranasal (i.n.) injection of 5 μ l PBS or OVA solution (30 mg/ml in PBS) without anesthesia for four consecutive days. These mice were treated with intragastric (i.g.) administration of 20 mg/kg epinastine (EPN), 120 mg/kg fexofenadine (FXF), or 10 mg/kg loratadine (LRT), that were purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan), 30 min before daily OVA challenge. (B) As an immediate nasal response (NHR), the number of sneezes was counted for 5 min immediately after the fourth OVA challenge. (C) Six hours after the last OVA challenge, that was performed without pretreatment of antihistamines, the nasal hyperresponsiveness (NHR) was evaluated by counting the number of sneezes for 5 min immediately following the i.n. administration of 10 μ l histamine (100 mM). (D) Subsequently, the number of eosinophils in the nasal lavage fluid (NALF) was assessed as described previously.³ Data are expressed as means $\pm$ SEM of 5–8 mice and analyzed using one-way ANOVA with Dunnett's multiple comparison test. * $p < 0.05$, compared with OVA-challenged control mice.

antagonistic activity for histamine-H1 receptor, although the effect of EPN was not statistically significant (Fig. 1B).

These mice also developed significant NHR compared with that seen in PBS-challenged mice (Fig. 1C). To avoid direct influence on histamine-evoked response, an additional allergen challenge without antihistamine administration was performed 24 h after the fourth challenge, and NHR was assessed 6 h after the final challenge by counting the number of sneezes evoked by histamine. Antihistamines did not significantly affect allergen-induced NHR, although LRT reduced it by 54% (Fig. 1C). Allergen-induced nasal eosinophil accumulation, evaluated by the nasal lavage immediately after NHR assessment, was not affected by antihistamines (Fig. 1D). These results contradict those of previous studies, at least demonstrating the alleviation of allergen-induced BHR and lung eosinophilia by FXF⁵ and nasal eosinophil accumulation by EPN.⁸ The reason for this discrepancy is currently unknown, although several experimental conditions were different. The effect of FXF was evaluated in the lung, but not in the nasal mucosa, with its higher dose and longer administration compared with that used in our current study. C57BL/6 mice were used for the EPN experiments. As further comparative studies are required, substantial FXF and EPN effectiveness on allergic nasal inflammation, including NHR development, may not be expected at least at their clinical doses.

We have demonstrated that allergen-induced NHR in our AR model largely depended on CD4⁺ T cells.³ DES was reported to

suppress T cell function via the reduction of DNA-binding activity of signal transducer and activator of transcription family transcription factors.⁹ Therefore, the effects of LRT and DES on stimulation-induced cytokine expression in Th2 cells were comparatively investigated. Th2 cells, differentiated from splenic naive CD4⁺ T cells of DO11.10 $\times$ RAG-2^{-/-} mice, as described previously,³ were stimulated with anti-CD3/CD28 antibodies for 6 h. Cytokine mRNA expression was assessed via quantitative real-time PCR. Th2 cells significantly expressed *Il4*, *Il5*, and *Il13* upon stimulation (Fig. 2A). At concentrations of 10 μ M or more, LRT and DES suppressed the expression of these cytokines in a dose-dependent manner (Fig. 2B). Since cytochrome P450 enzymes in the liver principally contribute to metabolize LRT into DES,¹⁰ both drugs were most likely to exhibit their effects in a direct manner at least in this experimental condition.

These results prompted us to investigate the effect of LRT on a murine model of T cell-dependent NHR (Fig. 3A).³ OVA challenge to Th2 cell-transferred mice evoked significant NHR and nasal eosinophil accumulation (Fig. 3B and C). These responses were not affected by LRT administration. The findings were contradictory to the results of the *in vitro* experiments (Fig. 2) and those of a previous study demonstrating the suppression of T cell-related allergic inflammation by DES.⁶ We further need to clarify the reason for the contradiction. NHR, accompanied by eosinophil accumulation developed in this model, solely depends on the

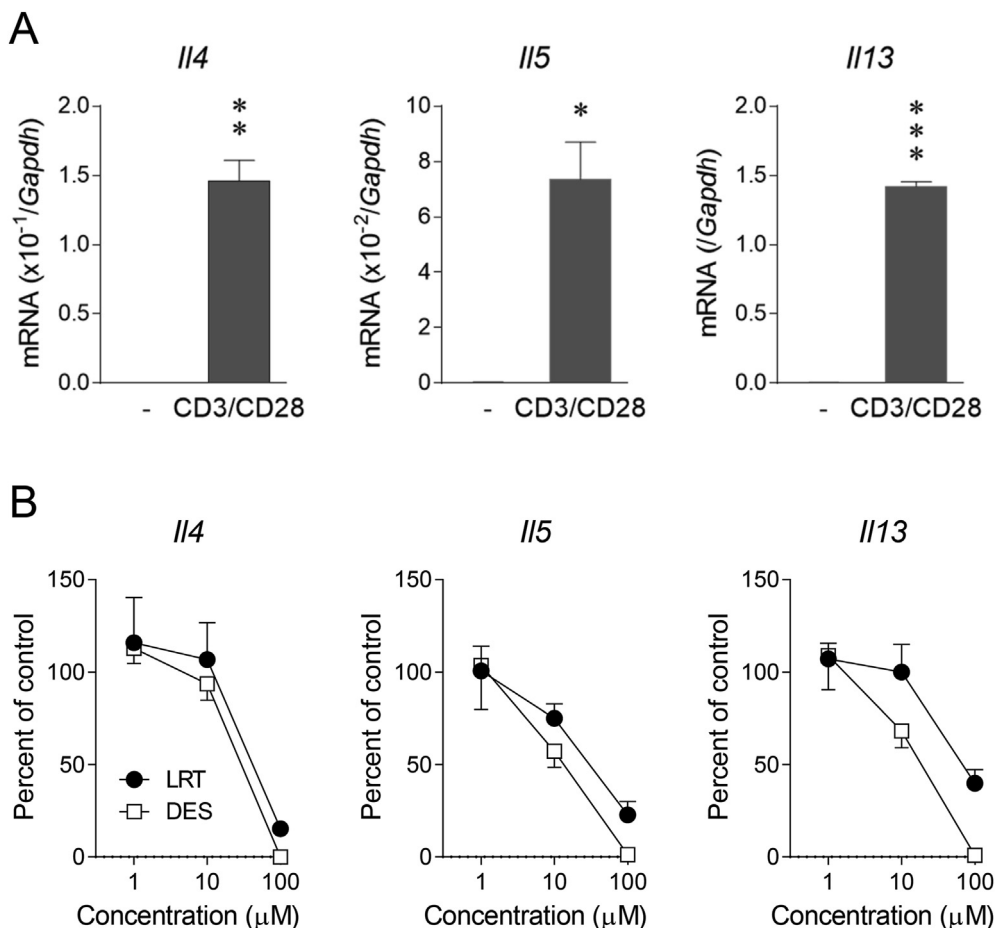


Fig. 2. Effects of antihistamines on cytokine production by Th2 cells. Naïve CD4⁺ T cells were purified from splenocytes of DO11.10 $\times$ RAG-2^{-/-} mice by an EasySep Mouse CD4⁺ T Cell Isolation Kit (Veritas, Santa Clara, CA, USA). The cells were then cultured with X-ray-irradiated splenocytes in AIM-V medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum, 0.3 μM synthetic OVA323–339 peptide, 10 U/ml recombinant murine IL-4 (PeproTech, Rocky Hill, NJ, USA), 10 U/ml recombinant IL-2 (Shionogi, Osaka, Japan), and 10 $\mu\text{g}/\text{ml}$ anti-IFN- γ monoclonal antibody (R4-6A2, eBioscience, San Diego, CA, USA). After 7-day culture, cells were stimulated with anti-CD3/CD28 antibodies (Dynabeads Mouse T-Activator CD3/CD28, Thermo Fisher Scientific) in the (A) absence or (B) presence of loratadine (LRT) or desloratadine (DES) for 6 h. Then, total RNA was extracted using an RNeasy mini kit (Qiagen, Hilden, Germany). Following the reverse transcription using random primers with SuperScript VILO cDNA Synthesis Kit (Thermo Fisher Scientific), quantitative real-time PCR for *Il4* (Mm00439618_m1), *Il5* (Mm00439646_m1), and *Il13* (Mm00434204_m1) was carried out using TaqMan gene expression probes (Thermo Fisher Scientific) on the ABI StepOnePlus Real-Time PCR System (Thermo Fisher Scientific). Data are expressed as means $\pm$ SEM of quadruplicate cultures as (A) relative transcript levels normalized with *Gapdh* (4351309) expression and (B) the percentage of stimulated (100%) and unstimulated (0%) control cells. The statistical analysis was performed using the Student's *t*-test. **p* < 0.05, ***p* < 0.01, and ****p* < 0.001, compared with unstimulated control.

transplanted allergen-specific Th2 cells.³ In clinical use, the serum LRT concentration reaches 10 nM after 10 mg of oral medication in adult subjects. Even considering that LRT was administered 60 times the clinical dose in this study, the serum LRT concentration was not likely to be 10 μM , in which LRT and DES exhibited their suppressive activity against T cell cytokine expression (Fig. 2). Therefore, the effectiveness of LRT and DES on T cell cytokine production and subsequent T cell-mediated NHR could not be expected at least at their clinical doses.

The ineffectiveness of LRT on NHR in Th2 transfer model clearly suggest that tendency toward NHR suppression in immunized mice was not caused by the antagonistic activity of LRT or DES remained in the mouse body against histamine used for NHR evaluation. The difference in the results between these models might be related with the effect on histamine-H1 receptor expression. Since the expression of histamine-H1 receptor is upregulated by histamine,¹¹ LRT potentially suppressed its expression, that might be augmented by mast cell-derived histamine, and consequently inhibited histamine-evoked sneezing response in allergen immunization model.

Relevantly, histamine-H1 receptor is constitutively activated even without histamine stimulation. Several antihistamines act

as inverse agonists and stabilize the receptor in an inactive form. Mizuguchi et al. compared the inverse agonistic activity of antihistamines, including EPN, FXF, and LRT, in HeLa cells.¹² EPN and FXF but not LRT exhibited the inverse agonistic activity and resultingly suppressed spontaneous histamine H1 receptor expression, whereas the expression evoked by histamine was abrogated by all of them. Although these findings are not consistent, at least in part, with the result of our present study, involvement of spontaneous and histamine-mediated expression and activation of histamine-H1 receptor in the effect of antihistamines on NHR deserves further investigation.

In conclusion, EPN, FXF, and LRT strongly suppressed allergen-induced INR probably by antagonizing mast cell-derived histamine. Additional effects of the antihistamines on T cell-mediated nasal inflammatory responses, including NHR development and eosinophil accumulation, are not expected in their clinical dose ranges. To control NHR in AR patients, topical steroids, by which we demonstrated the significant suppression of NHR in essentially the same model employed in this study,⁷ are probably needed, as approved by the Japanese guidelines for AR.¹

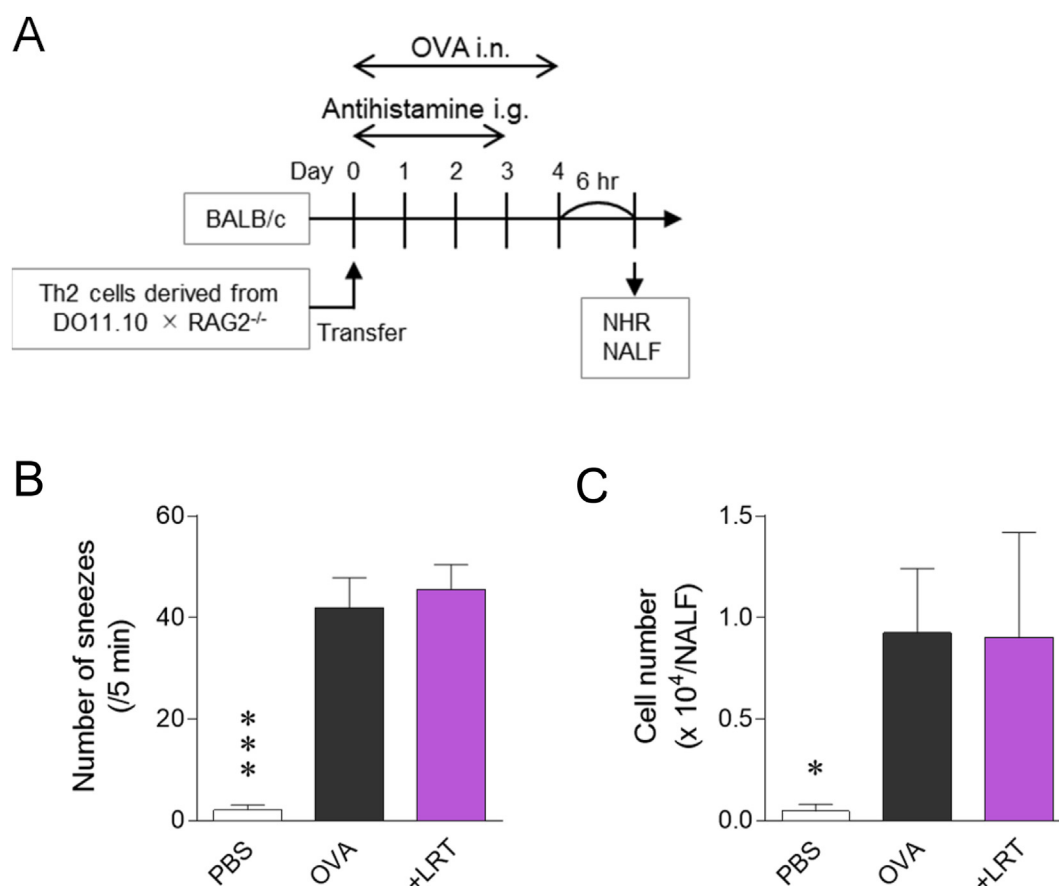


Fig. 3. Effects of antihistamines on allergen-induced nasal responses in Th2 cell-transferred mice. (A) Procedural overview of the experiments. Th2 cells were intravenously injected into BALB/c mice (2×10^7 cells/head). From 6 h after the cell transfer, repeated intragastric (i.g.) treatment with epinastine (EPN), fexofenadine (FXF), or loratadine (LRT) and intranasal (i.n.) ovalbumin (OVA) challenge were performed as shown in Fig. 1A. (B) Six hours after the last challenge, that was performed without antihistamine pretreatment, nasal hyperresponsiveness (NHR) was evaluated by counting the number of sneezes for 5 min immediately following the i.n. nasal histamine administration. (C) Subsequently, the number of eosinophils in the nasal lavage fluid (NALF) was assessed. Data are expressed as means $\pm$ SEM of 4–7 mice and analyzed using one-way ANOVA with Dunnett's multiple comparison test. * $p < 0.05$ and *** $p < 0.001$, compared with OVA-challenged control mice.

Funding

This study was supported in part by research grants from Japan Society for the Promotion of Science, Japan (JSPS KAKENHI, grant number 22H00398, 19H03145, and 18K19561 to O.K., 21K08486 and 18K08422 to A.M., and 19K09901 to M.G.), OTC Self-Medication Promotion Foundation (11-11 to O.K.) and Nipponham Foundation for the Future of Food to O.K.

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgment

The authors thank Drs. Zhou Qian, Shuhei Yamashita, and Anna Matsuura for their technical assistances during preparation of the manuscript.

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