ORIGINAL

Effects of corticosteroid on mRNA levels of histamine H_1 receptor in nasal mucosa of healthy participants and HeLa cells

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Abstract : The purpose of this study is to examine the effect of intranasal corticosteroid (INCS) administration on histamine H_1 receptor (H1R) gene expression in the nasal mucosa of healthy participants and the effects of dexamethasone on basal and histamine-induced H1R mRNA expression, and histamine-induced phosphorylation of extracellular signal-regulated kinase (ERK) in HeLa cells. Sixteen healthy participants were given INCS once daily for a week. After pretreatment of dexamethasone, HeLa cells were treated with histamine. Levels of H1R mRNA and phosphorylation of ERK were measured using real time PCR and immunoblot analysis, respectively. Levels of H1R mRNA in the nasal mucosa of healthy participants receiving INCS was significantly decreased. Dexamethasone suppressed basal levels of H1R mRNA, and histamine-induced up-regulation of H1R mRNA and ERK phosphorylation in HeLa cells. These data suggested that corticosteroid inhibited both basal transcription and histamine-induced transcriptional activation of H1R through its suppression of ERK phosphorylation in the signaling pathway involved in H1R gene transcription. It is further suggested that pre-seasonal prophylactic administration of INCS suppresses both basal and pollen-induced upregulation of H1R gene expression in the nasal mucosa of patients with pollinosis, leading to prevention of the exacerbation of nasal symptoms during peak pollen season. J. Med. Invest. 67 : 311-314, August, 2020

Keywords **:** *corticosteroids, extracellular signal-regulated kinase, healthy participants, histamine H_1 receptor, intranasal administration*

INTRODUCTION

Histamine is a major chemical mediator that induces nasal allergy symptoms through its binding to histamine H_1 receptor (H1R) in the development of allergic rhinitis (1). Antihistamines, H1R antagonists, are effective for the treatments of allergic rhinitis, including pollinosis (2). In Japan, prophylactic administration of antihistamines before the onset of pollen season is recommended for pollinosis treatment (1), because the pre-seasonal administration of antihistamines is more effective than post-onset administration in patients with pollinosis (3). In our previous study, we used environmental exposure units and demonstrated that pre-administration of ebastine, an antihistamine down-regulated H1R gene expression before pollen exposure and then inhibited pollen-induced nasal symptoms and pollen-induced up-regulation of H1R gene expression in the nasal mucosa of patients with pollinosis (4). Because, histamine signaling is regulated by the levels of H1R expression (5, 6), it is suggested that prophylactic administration of antihistamines inhibited both basal transcription and histamine-induced transcriptional activation of H1R in the nasal mucosa, resulting in summative suppression of nasal symptoms during peak pollen season in patients with pollinosis.

Intranasal corticosteroid (INCS) is also effective for the treatment of allergic rhinitis (7). A recent randomized placebo-controlled trial demonstrated that pre-seasonal prophylactic administration of INCS prevented the worsening of nasal symptoms during peak pollen season in patients with pollinosis (8). Accordingly, in the present study, we examined whether INCS down-regulates H1R gene expression in the nasal mucosa of healthy participants in vivo. We then examined whether dexamethasone, a corticosteroid inhibits basal and histamine-induced up-regulation of H1R mRNA, and histamine-induced phosphorylation of protein kinase Cδ (PKCδ) and extracellular signal-regulated kinase (ERK) in HeLa cells in vitro.

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PARTICIPANTS AND METHODS

Participants

We enrolled 16 healthy participants with no history of allergic rhinitis (10 males, 6 females ; 22-26 years old ; mean age : 24.2 years). Participants received intranasal doses of 200 μg of mometasone furoate in the right nostril using a nasal spray device once daily for a week. Nasal mucosa samples were obtained under local anesthesia with 4% lidocaine by scraping the surface of the inferior nasal concha with a small spatula before and after INCS administration for 7 days, as previously described (6). This study was approved by the Ethical Committee of Tokushima University Hospital (UMIN6094), and written informed consent was obtained from each patient before inclusion in the study.

Real-time quantitative RT-PCR

Nasal mucosa samples of participants were frozen in RNAlater® (Applied Biosystems, Foster City, CA, USA) and stored at -80℃ until use. Total RNA was isolated using the RNAqueous-Micro Kit (Applied Biosystems) following the manufacturer's instructions. RNA samples were reversed-transcribed to produce cDNA using a High Capacity cDNA Reverse Transcription

Kit (Applied Biosystems). TaqMan primers and probes were designed using Primer Express primer design software (Applied Biosystems). The sequences of the H1R primers were : sense primer, 5'-CAGAGGATCAGATGTTAGGTGATAGC-3' ; antisense primer, 5'-AGCGGAGCCTCTTCCAAGTAA-3'. The sequence of the probe was as follows : FAM-CTTCTCTCGAACGGACTCAGATACCACC-TAMRA. The PUM1 primer and probe kit (Hs 00206469-m1, Applied Biosystems) was used as an internal standard.

HeLa cells were cultured at 37℃ under a humidified 5% CO_2 atmosphere in Minimal Essential Medium-α (α-MEM) containing 8% fetal calf serum and 1% antibiotic-antimycotic (Invitrogen). HeLa cells were cultured to 70% confluence in 6-well dishes then serum-starved for 24 h and treated with dexamethasone at doses of 0.1, 1, and 10 μM for 1 h. Cells were then treated with 100 μM histamine for 3 h then cells harvested with 700 μl of RNAiso Plus (Takara Bio Inc. Kyoto Japan) and total RNA and cDNA were prepared by a previously reported method (9). For real-time quantitative polymerase chain reaction (RT-qPCR), GAPDH levels were used to standardize the amount of starting material (Applied Biosystems), and data were expressed as the ratio of H1R mRNA to GAPDH mRNA. Transcripts were subjected to a 40-cycle, 3-step PCR program using the GeneAmp 7300 Sequence Detection System (Applied Biosystems). The size and reaction specificity of the amplicon were confirmed by agarose gel electrophoresis. Identification of PCR products was carried out using a genetic analysis system (SEQ8000 ; Beckman Coulter, Inc., Fullerton, CA, USA).

Immunoblot analysis

After HeLa cells were treated with 10 uM dexamethasone for 1 h before stimulation with 100 uM histamine, whole cell extracts were prepared at various time points as described previously (9). Ten μg of total protein per sample was separated on a 10% SDS-PAGE gel then transferred to a nitrocellulose membrane (Bio-Rad). The membrane was briefly rinsed in Tris-buffered saline with 0.1% Tween 20 (TBS-T) then incubated for 1 h at room temperature in TBS-T with 3% BSA (Sigma). The membrane was then incubated overnight at 4℃ with one of the following primary antibodies : PKCδ (C-20), sc-937, 1:1000 ; ERK (K-23), sc-94, 1:1000 ; phospho-ERK (E-4), sc-7383, 1:1000, (Santa Cruz Biotechnology) ; phospho-PKCδ (Tyr311), #2055S, 1:500 (Cell Signaling). The membrane was then incubated in the appropriate secondary antibody (goat anti-rabbit IgG (H ± L)-HRP conjugate (#170-6515, 1:10,000, Bio-Rad) or Immun-Star goat anti-mouse-HRP conjugate (#170-5047, 1:10,000, Bio-Rad) and proteins visualized using Immobilon Western Chemiluminescent HRP substrate (Millipore).

Statistical analysis

Data are presented as means ± SEM. Statistical analyses were performed with unpaired *t* tests or one-way ANOVA using Dunnett's test by the GraphPad Prismsoftware (GraphPad-SoftwareInc., LaJolla, CA). P values of < 0.05 were considered statistically significant.

RESULTS

Effect of INCS administration on H1R mRNA levels in the nasal mucosa of healthy participants.

Levels of H1R mRNA in the nasal mucosa of healthy participants receiving INCS once a day for a week were significantly decreased, compared to those before INCS administration (Fig. 1). No localized adverse events, such as nasal burning or epistaxis were observed.

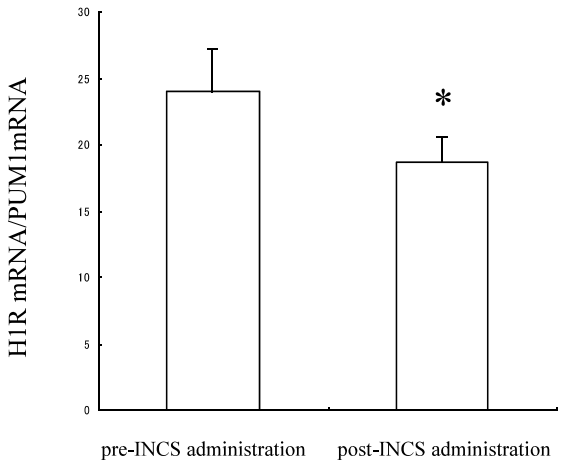

Figure 1. Effect of intranasal corticosteroid (INCS) administration on histamine H_1 receptor (H1R) mRNA levels in the nasal mucosa of healthy participants.
The expression of H1R mRNA in the nasal mucosa of 16 healthy participants receiving INCS once a day for one week was significantly lower than that before administration. Data are presented as means ± SEM. *$p < 0.05$ versus pre-INCS administration.

Effects of dexamethasone on basal and histamine-induced H1R mRNA levels, and histamine-induced phosphorylation of PKCδ and ERK in HeLa cells.

HeLa cells expresses H1R endogenously (10). Treatment with dexamethasone at a dose of 1 μM significantly decreased basal levels of H1R mRNA in HeLa cells (Fig. 2A). Histamine significantly increased levels of H1R mRNA in HeLa cells and dexamethasone at doses from 0.1-10 μM significantly suppressed histamine-induced up-regulation of H1R mRNA (Fig. 2B).

Stimulation with histamine also increased phosphorylation of PKCδ and ERK in HeLa cells (11). Immunoblot analysis showed that dexamethasone did not suppress histamine-induced PKCδ phosphorylation (Fig. 3). On the other hand, dexamethasone suppressed histamine-induced ERK phosphorylation in HeLa cells (Fig. 3).

DISCUSSION

In the present study, we showed that INCS administration for a week decreased levels of H1R mRNA in the nasal mucosa of healthy participants with no history of allergic rhinitis. The finding suggests that INCS down-regulates H1R gene expression in the nasal mucosa in vivo. We also showed that dexamethasone decreased basal levels of H1R mRNA in HeLa cells, suggesting that corticosteroid suppressed basal transcription of H1R. Corticosteroids are effective drug for treating allergic diseases, and the different mechanisms by which steroids exert their anti-allergic properties have been described (12). Recently, INCS was used for a prophylactic treatment of pollinosis (13) and a double-blinded, randomized, and placebo-controlled study showed that prophylactic administration of INCS before pollen dispersion was more effective than post-onset treatment or a placebo (14). Taken together, it is suggested that pre-seasonal INCS administration prevents the forthcoming type-1 allergy symptoms due to its suppression of basal transcription of H1R in the nasal mucosa. Prophylactic INCS administration has also been suggested to suppress priming effect, minimal persistent

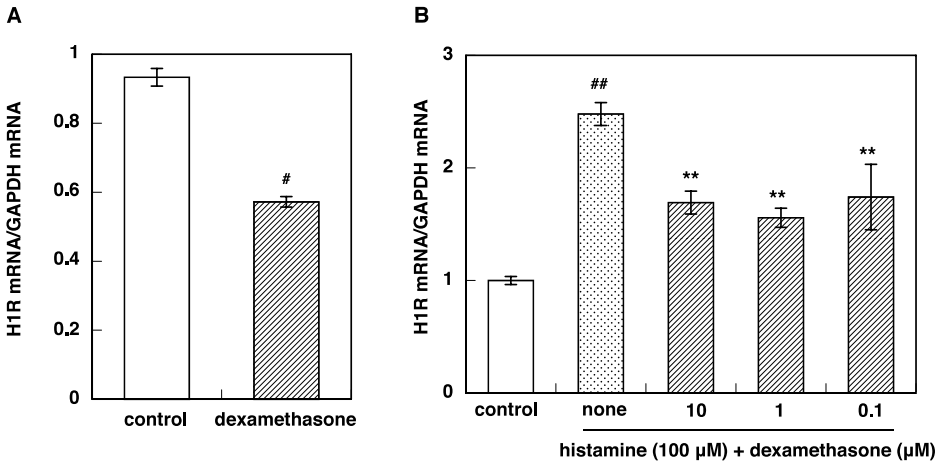

Figure 2. Effects of dexamethasone on basal (A) and histamine-induced (B) H1R mRNA levels in HeLa cells. (A) HeLa cells were treated with dexamethasone (1 μM) for 1 h. (B) HeLa cells were treated with dexamethasone (0.1-10 μM) for 1 h before 100 μM histamine treatment for 3 h. Then, the cells were harvested and total RNA was isolated. Amount of H1R mRNA was determined by quantitative RT-PCR. Data are presented as means ± SEM (n = 3-9). ★★, $p < 0.01$ versus histamine ; ##, $p < 0.01$, #, $p < 0.05$ versus control.

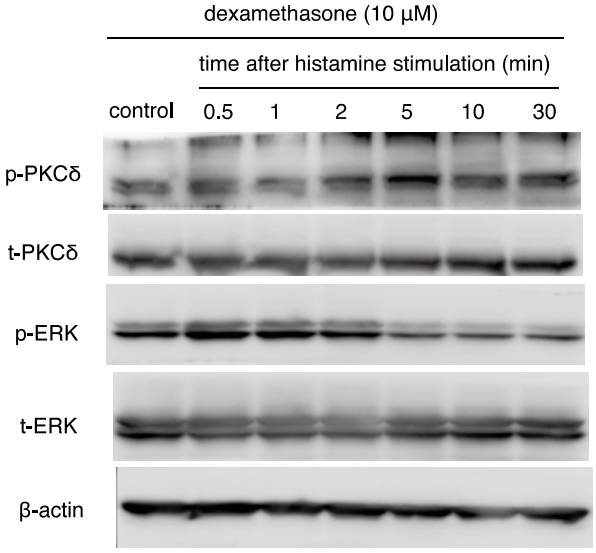

Figure 3. Effects of dexamethasone on histamine-induced phosphorylation of PKCδ and ERK in HeLa cells.
HeLa cells were serum-starved for 24 h. The cells were treated with 10 μM dexamethasone for 1 h before stimulation with 100 μM histamine and phosphorylation levels of PKCδ and ERK at various timepoints were determined using immunoblot analysis. Data show that in the presence of dexamethasone, p-ERK density decreased as time goes by, while p-PKC increased after treatment with histamine. p-PKCδ : phospho PKCδ ; t-PKCδ : total PKCδ ; p-ERK : phospho ERK ; t-ERK : total ERK.

inflammation and eosinophil migration in the nasal mucosa of patients with allergic rhinitis (15).

In the present study, we showed that dexamethasone also suppressed histamine-induced up-regulated H1R gene expression in HeLa cells, as reported previously (10). We also showed that immunoblot analysis showed that dexamethasone suppressed histamine-induced ERK phosphorylation, but not PKCδ phosphorylation in HeLa cells. The PKCδ/ERK/poly (ADP-ribose) polymerase-1 (PARP-1) signaling pathway was involved in histamine-induced up-regulation of H1R gene expression in HeLa cells after PKCδ and ERK activation by phosphorylation (11). Therefore, it is suggested that ERK is a target molecule of dexamethasone to suppress transcriptional activation of H1R.

Because we previously showed that corticosteroids suppressed histamine-induced transcriptional activation of H1R in the nasal mucosa of a rat model of allergic rhinitis (16), it is assumed that INCS would suppress histamine-induced up-regulation of H1R in the nasal mucosa of patients with pollinosis. Although the hypothesis should be proved in the further study, the findings in the present study suggest that prophylactic administration of INCS before pollen dispersion suppresses transcriptional activation of H1R, as well as its basal transcription in the nasal mucosa, resulting in the prevention of worsening of nasal symptom during peak pollen season in patients with pollinosis.

In conclusion, we showed INCS down-regulated H1R gene expression in the nasal mucosa of healthy participants with no history of allergic rhinitis. We also showed that dexamethasone, a corticosteroid inhibited basal transcription and transcriptional activation of H1R in HeLa cells through its suppression of ERK phosphorylation in the PKCδ/ERK/PARP-1 signaling involved in H1R gene transcription. These data suggest that pre-seasonal prophylactic administration of INCS suppresses both basal and pollen-induced up-regulation of H1R gene expression in the nasal mucosa of the patients with pollinosis, leading to prevention of the exacerbation of nasal symptoms during peak pollen season.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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AUTHORS CONTRIBUTION

YK and HM designed the study and wrote the manuscript. SK and TF carried out experimental work. KN, EK, KM, TA, and GS contributed to data collection and interpretation of results. HF and NT supervised the research and wrote the manuscript. All authors read and approved the final manuscript.

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