Short Communication

Combination of Leukotriene Receptor Antagonist With Antihistamine Has an Additive Suppressive Effect on the Up-regulation of H1-Receptor mRNA in the Nasal Mucosa of Toluene 2,4-Diisocyanate-Sensitized Rat

Wakana Kuroda1, Yoshiaki Kitamura1*, Hiroyuki Mizuguchi2, Yuko Miyamoto2, Bukasa Kalubi1, Hiroyuki Fukui2, and Noriaki Takeda1

1Department of Otolaryngology, 2Department of Molecular Pharmacology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima 770-8503, Japan

Received October 31, 2012; Accepted March 11, 2013

Abstract. An attempt was made to clarify the additive suppressive effects of pranlukast, a cysteinyl leukotriene–receptor (LTR) antagonist, in combination with chlorpheniramine, an antihistamine, on the up-regulation of histamine H1–receptor (H1R) mRNA in toluene 2,4-diisocyanate (TDI)-sensitized rats. Although pre-treatment with pranlukast partially, but significantly, suppressed TDI-induced up-regulation of H1R mRNA and nasal symptoms, pre-treatment with the combination of pranlukast and chlorpheniramine significantly suppressed them in a manner greater than either drug alone. These findings suggest that the additive therapeutic effect of the combination of LTR antagonist and antihistamine is due to their additive suppression of H1R up-regulation.

Keywords: antihistamine, cysteinyl leukotriene receptor antagonist, histamine H1–receptor

Histamine is a major chemical mediator in the development of allergic rhinitis, causing sneezing and rhinorrhea. Cysteinyl leukotrienes (LTs) are also important mediators, as intranasal administration of LT in patients with allergic rhinitis causes nasal congestion, but not the sneezing or rhinorrhea seen in challenges with histamines (1). Thus, histamine and LT have different roles in the pathogenesis of allergic rhinitis.

We previously demonstrated that antihistamines are effective in reducing nasal symptoms by suppressing the up-regulation of histamine H1–receptor (H1R) gene expression in the nasal mucosa of toluene 2,4-diisocyanate (TDI)-sensitized rats, in addition to their histamine-blocking effect at H1R (2 – 4). In addition to antihistamines, leukotriene-receptor (LTR) antagonists were demonstrated to be effective in the treatment of allergic rhinitis (5). Recently, it was reported that the combination therapy with antihistamine and LTR antagonist was more effective than either treatment alone (6, 7). The additive therapeutic effects of LTR antagonist in combination with antihistamine on sneezing or rhinorrhea were also reported (6). Furthermore, it was reported that LTs increase the number of H1R in mouse and human cells (8). These findings lead to our hypothesis that LTR antagonists inhibit histamine signaling in the development of allergic rhinitis and LTR antagonist in combination with antihistamine additively suppresses the up-regulation of H1R in the development of allergic rhinitis, resulting in the higher efficacy of the combination therapy.

In the present study, to clarify this hypothesis, we examined the effects of pranlukast, an LTR antagonist and chlorpheniramine, an antihistamine, on TDI-induced nasal symptoms and up-regulation of H1R mRNA in the nasal mucosa of TDI-sensitized rats. The additive suppressive effects of pranlukast in combination with chlorpheniramine were then examined.

Six-week-old male Brown Norway Rats (Japan SLC, Hamamatsu) were used in the present study. Rats were kept in a room maintained at a constant temperature (22°C ± 1°C), 50% humidity, and a 12-h light/dark cycle. A 10-μl aliquot of 10% solution of TDI (Wako Chemical Co., Tokyo) in ethyl acetate was painted bilaterally on the nasal vestibules once a day for five consecutive days.

*Corresponding author. ykitamura@clin.med.tokushima-u.ac.jp
Published online in J-STAGE on April 25, 2013
doi: 10.1254/jphs.12250SC
This sensitization procedure was repeated at 2-day interval. Nine days after the second sensitization, 10% TDI solution was re-applied to the nasal vestibules to provoke sneezing in previously sensitized rats. Control rats were treated only with the vehicle (ethyl acetate) by the same procedure. The number of sneezes was scored during a 10-min period after TDI provocation. The study was approved by the Ethical Committee for Animal Studies of Institute of Health Biosciences, the University of Tokushima Graduate School.

Pranlukast (Ono Pharmaceutical Co., Osaka) in 0.5% carboxymethyl cellulose sodium salt was orally administered 1 h before TDI provocation at doses of 30, 200, and 1000 mg/kg, while d-chlorpheniramine (Wako Chemical Co.) in saline was intraperitoneally administered 15 min before TDI provocation at a dose of 30 mg/kg. In the vehicle group, saline and/or 0.5% carboxymethyl cellulose sodium salt was given instead of a drug.

Nasal mucosa was removed from the nasal septum, collected in RNAlater (Takara Biochemicals, Tokyo), and stored at −80°C until assayed. After that, the nasal mucosa was homogenized using a Polytron (Model PT-K; Kinematica AG, Littau/Luzern, Switzerland). Total RNA was then isolated using TRIzol reagent (Invitrogen Corp., Carlsbad, CA, USA) in accordance with the manufacturer’s instructions.

RNA samples were reverse-transcribed to cDNA using Superscript II reverse transcriptase (Invitrogen). TaqMan primers and probe were designed using the Primer Express primer design software (Applied Biosystems, Foster City, CA, USA). The sequences of the H1R primers were as follows: sense primer, 5’-TATGTGT TCCGGGCTGCACT-3’; antisense primer, 5’-CGCCAT GATAAAACCCAACTG-3’, while the sequence of the probe was as follows: FAM-CCGAGAGCGGAGCCA-TAMRA. Rodent glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primer and probe reagents (Applied Biosystems) were used as an internal standard. Real time polymerase chain reaction (PCR) was conducted using a GeneAmp 7300 Sequence Detection System (Applied Biosystems). Amplicon size and reaction specificity were confirmed by agarose gel electrophoresis. Identification of the PCR products was verified by sequencing using a genetic analysis system (Beckman CEQ 8000; Beckman Coulter, Fullerton, CA, USA). To determine whether the amplification products were derived exclusively from the RNA, a reverse transcriptase (RT)-negative reaction was run in which the enzyme was replaced by RNase-free water in each sample.

Statistical analysis was performed by Fisher’s paired least-significant-difference tests. P-values of < 0.05 were considered statistically significant.

Intranasal application of TDI induced repeated sneezes in TDI-sensitized rats. The mean number of sneezes (17.0/10 min) was significantly reduced by pre-treatment with either chlorpheniramine (30 mg/kg, i.p.) or pranlukast (200 mg/kg, p.o.) to 7.5/10 min and 13.3/10 min, respectively. Moreover, pre-treatment with both chlorpheniramine and pranlukast significantly and completely inhibited TDI-induced sneezes (Table 1).

Conversely, the expression of H1R mRNA in the nasal mucosa was increased after TDI provocation in sensitized rats. However, pre-treatment with pranlukast (200 and 1000 mg/kg) significantly suppressed the up-regulation of H1R mRNA in the nasal mucosa of TDI-sensitized rats 4 h after TDI provocation in a dose-dependent manner (Fig. 1). Although pre-treatment with either

### Table 1. Additive suppressive effects of combination of pranlukast with chlorpheniramine on the number of sneezes induced by TDI provocation in TDI-sensitized rats

<table>
<thead>
<tr>
<th></th>
<th>vehicle (30 mg/kg)</th>
<th>chlorpheniramine (30 mg/kg)</th>
<th>pranlukast (200 mg/kg)</th>
<th>chlorpheniramine (30 mg/kg) plus pranlukast (200 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17.0 ± 1.0</td>
<td>7.5 ± 3.5*</td>
<td>13.3 ± 0.6*</td>
<td>0.1 ± 0.2**</td>
</tr>
</tbody>
</table>

*P < 0.05 vs. vehicle; **P < 0.01 vs. chlorpheniramine or pranlukast (n = 4).

Fig. 1. Effect of pranlukast on the expression of H1R mRNA in the nasal mucosa of TDI-sensitized rats 4 h after TDI provocation. Columns and vertical bars represent means ± S.E.M. as the ratio against the expression of H1R mRNA of the control group. *P < 0.05 vs. vehicle (n = 4).
chlorpheniramine or pranlukast significantly suppressed the up-regulation of H1R mRNA in the nasal mucosa of TDI-sensitized rats, this suppressive effect was greater with the combination of both drugs, compared to that of either drug alone (Fig. 2).

In the present study, intranasal application of TDI induced repeated sneezes and pre-treatment with chlorpheniramine significantly reduced TDI-induced sneezes in TDI-sensitized rats. These findings are in line with our previous findings (2, 3) and indicate that the release of histamine in the nasal mucosa of sensitized animals is induced by TDI provocation through neurogenic inflammation (2) and that antihistamine plays an inhibitory role on TDI-induced nasal hypersensitivity symptoms by blocking H1R. However, the suppression of the up-regulation of H1R by chlorpheniramine was found to be partial (Fig. 2) and this evidence suggests that the up-regulation of H1R in the nasal mucosa comprises a histamine-mediated mechanism and a non-histamine-mediated mechanism. In addition, we demonstrated that histamine increased H1R expression at both mRNA and protein levels in HeLa cells and that this up-regulation was inhibited by antihistamines, but not histamine H2-receptor antagonist (9), indicating that the up-regulation of H1R in the nasal mucosa of TDI-sensitized rats is partially induced by histamine released after TDI provocation. Pre-treatment with pranlukast significantly and dose-dependently suppressed TDI-induced up-regulation of H1R mRNA expression in nasal mucosa of TDI-sensitized rats (Fig. 1). Therefore, it is suggested that LT is in part a mediator of the up-regulation of H1R mRNA in nasal mucosa. This hypothesis was supported by the findings that there is an increase in the number of H1R by LTs in mouse and human cells (8).

The mechanism of LT-mediated up-regulation of H1R is unknown, although histamine up-regulates the expression of H1R mRNA in HeLa cells through protein kinase Cδ (PKCδ)-mediated H1R gene transcription (10). However, it was reported that treatment of OVA-sensitized mice with PKCδ inhibitors (GF109203X and rottlerin) before provocation diminished not only the CysLT production but also the effects of LT such as eosinophil infiltration and AHR (11), suggesting the involvement of PKCδ in the LT signaling pathway. Furthermore, it was reported that LTs up-regulated H1R expression (8) and the activation of CysLT1 receptor by LTD4 induced stress-fiber formation through the PKCδ pathway (12). From these evidences, one can imagine that PKCδ is one of the signal molecules involved in LT signaling and activation of PKCδ by LT may affect the expression level of H1R.

We further showed that pre-treatment with pranlukast in combination with chlorpheniramine suppressed TDI-induced up-regulation of H1R mRNA in the nasal mucosa of TDI-sensitized rats in a manner greater than that with either drug alone. The additive suppressive effect of pranlukast in combination with chlorpheniramine was also observed on TDI-induced sneezes in TDI-sensitized rats. These observations corroborate previous clinical findings that showed the additive therapeutic effects of LTR antagonist in combination with antihistamine (6, 7). We previously demonstrated that anti-allergic compounds that suppress the up-regulation of H1R gene expression alleviated allergy-like symptoms in TDI-sensitized rats (13). We also demonstrated that intranasal application of histamine to rats increased the H1R and IL-4 mRNA expression (13, 14) and the expression level of H1R was strongly correlated with the expression level of IL-5 (15), suggesting the crosstalk with H1R signaling and Th2 cytokine signaling in patients with pollinosis and suppression of H1R signaling could inhibit Th2 cytokine signaling. Therefore, suppression of H1R signaling through the inhibition of the PKCδ signaling pathway by LTR antagonists might be one of the mechanisms for the additive therapeutic effects from the combined treatment.

In conclusion, the present study demonstrated that compared to the effect of individual drugs, the combination therapy with LTR antagonist and antihistamine is more effective for the treatment of nasal hypersensitivity symptoms in allergic rhinitis model rats. Our data suggest that LTR antagonists suppress the H1R signaling and this might be one of the mechanisms for the additive therapeutic effects from the combined treatment.
Acknowledgment

This work was financially supported by a Grant-in-Aid from Ministry of Health, Labour and Welfare of Japan (Suppression of Histamine H1 Receptor Gene Expression Using Natural Sources: a New Therapeutic Strategy for Allergic Disease).

References