Asano K, et al., J Clin Immunol Immunother 2014, 1: 002
DOI: 10.24966/CIIT-8844/100002

HSOA Journal of Clinical Immunology and Immunotherapy

Research Article

Enhancement of CC10 Production from Human Nasal Epithelial Cells by Histamine H_1 Receptor Antagonists, Desloratadine and Levocetirizine *in Vitro* and *in Vivo*

Takahiro Suzuki[1], Kazuhito Asano[2]*, Atsuko Furuta[1], Ken-ichi Kanai[1], Yasuyuki Hinohira[1] and Hitome Kobayashi[1]

[1]*Department of Otolaryngology, School of Medicine, Showa University, Tokyo*

[2]*Division of Physiology, School of NRS, Showa University, Yokohama, Japan*

Abstract

Background and Objective: CC10 is well known to be an immuno-suppressive protein secreted from airway epithelial cells after inflammatory stimulation and plays important roles in the development of allergic disorders. Although the third generation histamine H_1 receptor antagonists, Levocetirizine (LCT) and Desloratadine (DLT) are developed and used for the treatment of allergic disorders with remarkable success, the influence of these agents on CC10 production is not fully understood. In the present study, we examined the influence of a histamine H_1 receptor antagonist, Cetirizine (CT), LCT, Loratadine (LT) and DLT on CC10 production *in vitro* and *in vivo*.

Methods: Nasal epithelial cells (5×10^6 cells/ml) were stimulated with 20 ng/ml TNF-α in the presence of various concentrations of the agents for 24 h. CC10 levels in culture supernatants were examined by ELISA. We also examined the influence of the agents on CC10 mRNA expression and mRNA translation by RT-PCR and wheat germ cell-free protein synthesis technique, respectively. In the second set of experiments, pollinosis patients against Japanese cedar pollen were treated orally with 5 mg LCT once a day for 4 weeks during Japanese pollen season (January 2014 to April 2014). CC10 levels in nasal secretions were also examined by ELISA.

Results: The addition of LCT and DLT into epithelial cell cultures caused increase in the ability of cells to produce CC10 in response to TNF-α stimulation as well as their mother drugs, CT and LT. The minimum concentrations that caused significant increase were 0.05 μM for LCT and 0.01 μM for DTL, which are lower levels than that induced by their mother drugs, CT and LT. Although the treatment of cells with LCT and DLT caused inhibition of CC10 mRNA expression, which was increased by TNF-α stimulation, these agents increased the translation of CC10 mRNA to produce specific proteins. Oral administration of LCT also increased CC10 levels in nasal secretions from pollinosis patients along with attenuation of clinical symptoms.

Conclusion: The ability of histamine H_1 receptor antagonists, LCT and DLT to enhance CC10 production may account, at least in part, for the clinical efficacy of the agent on allergic disorders, including allergic rhinitis.

Keywords: CC10; Allergic rhinitis; Nasal epithelial cells; Production; Levocetirizine

***Corresponding author:** Kazuhito Asano, Division of Physiology, School of Nursing and Rehabilitation Sciences, Showa University, 1865 Touka-Ichiba, Midori-ku, Yokohama 226-8555, Japan, Tel: +81-45-985-6538; Fax: +81-45-985-7583; E-mail: asanok@med.showa-u.ac.jp

Citation: Suzuki T, Asano K, Furuta A, Kanai K, Hinohira Y et al. (2014) Enhancement of CC10 Production from Human Nasal Epithelial Cells by Histamine H_1 Receptor Antagonists, Desloratadine and Levocetirizine *in Vitro* and *in Vivo*. J Clin Immunol Immunother 1: 002.

Received: June 19, 2014; **Accepted:** August 04, 2014; **Published:** August 18, 2014

Introduction

Allergic rhinitis is well known to be allergic inflammatory responses in the nasal mucosa. It occurs when an allergen (e.g. pollen, dust and animal dander, etc.) is inhaled by an individual with a sensitized immune system. In such individuals, allergen triggers the production of IgE antibodies for specific allergen(s), which binds to receptors on the surface of mast cells and basophils [1,2]. On re-exposure to the relevant allergen(s), cross-linking of adjacent IgE molecules occurs, and mast cell degranulation takes place, releasing a variety of chemical mediators, such as histamine, leukotriene and prostaglandins, among others. These chemical mediators are responsible for the development of symptoms of allergic rhinitis including sneezing, swelling and inflammation of the nasal passages, and hyper-secretion of mucus [2]. From these established concept, histamine H_1 receptor antagonists, so-called antihistamines are recommended as the first choice drugs for the treatment and prevention of allergic rhinitis.

It is well known that the human body is an exquisite machine and fends off many challenges from environments to its maintenance of balance. This ability of the body is called homeostasis and is nothing but its capability to regulate and control the inner environment physiologically, so that the body functions under a constant stability when exposed to certain fluctuating conditions in the external environment. The endocrine system and endogenous peptides secreted after several stimuli are well accepted to major part in maintaining homeostasis, as well as the sympathetic nervous system. From the point of view, we examined the influence of endogenous peptides on the development of allergic rhinitis and reported that nasal secretions from patients with allergic rhinitis contained much lower levels of Thioredoxin (TRX), which show the action of both anti-inflammatory and immunomodulation, as compared with that from normal subjects [3]. We also observed that oral administration of epinastine hydrochloride and fexofenadine hydrochloride, the second generation histamine H_1 receptor antagonists, into allergic rhinitis patients for two weeks increases the contents of TRX in

nasal secretions along with attenuation of clinical symptoms [3]. These reports may suggest that the activity of histamine H_1 receptor antagonists on the production (or secretion) of endogenous peptides may contribute to the clinical efficacy of the agents on allergic rhinitis.

Clara cell 10-kDa protein (CC10) is a member of secretoglobin family and the major constituent of secretory granules of Clara cells in the bronchi and nasal epithelial cells. CC10 constitutively expressed by the epithelial lining of airways and isolated from mammals, including rats, rabbit and human [4]. It is reported that pro-inflammatory cytokines such as IFN-γ and TNF-α modulate CC10 mRNA expression in Clara cells and results in increase in protein secretion from cells [5]. CC10 is also reported to exert anti-inflammatory effects through the suppression of the activity of both phospholipase A2 and transglutaminase, which are responsible for the development of allergic inflammation [4,6]. Furthermore, it is recognized that CC10 can inhibit inflammatory cell chemotaxis, and down-regulate Th2 T cell differentiation, including cytokine production [7-10,6]. In human cases, low levels of CC10 were detected in nasal inflammatory diseases, such as chronic rhinosinusitis and allergic rhinitis [11-13], suggesting that CC10 may play essential roles in the development of nasal inflammatory diseases.

Recently, third generation histamine H_1 receptor antagonists, Desloratadine (DLT) and Levocetirizine (LCT), are developed and used for the treatment of allergic diseases such as allergic rhinitis and atopic dermatitis with remarkable success [14-16]. Although the attenuating effects of these agents on clinical condition of allergic patients has been recognized to depend, in part, on antagonistic action on the histamine H_1 receptor [14,16], these drugs are also well known to exert inhibitory action on the synthesis and release of chemical mediators from mast cells and eosinophils in response to immunological and non-immunological stimulation [14]. It is reported that DLT can inhibit the production of cytokines and chemokines from mast cells and epithelial cells as well as the expression of adhesion molecules, which are involved in the development of allergic inflammation [14]. However, the influence of these agents on CC10 production is not well understood. The present study, therefore, was undertaken to examine the influence of the third generation histamine H_1 receptor antagonists, DLT and LCT, on CC10 production from nasal epithelial cells in response to inflammatory stimulation *in vitro*. We also examined the influence of third generation histamine H_1 receptor antagonists on the appearance of CC10 in nasal secretions through the choice of pollinosis patients against Japanese cedar pollen who were treated with LCT.

Materials and Methods

Reagents

Cetilizine (CT), LCT, loratadine (LT) and DLT were purchased from Toronto Research Chem., Inc. (North York, ON, Canada) as preservative free pure powders. These agents were dissolved in SABM medium (Lonza Co., Ltd., Walkersville, MD, USA) at appropriate concentrations just before use. Human recombinant TNF-α was purchased from R and D Systems, Inc. (Minneapolis, MA, USA). This was also dissolved in SABM medium at 40 ng/ml.

Subjects and treatment

The subjects were 10 male patients with Japanese cedar pollen-sensitized rhinitis, who were recruited from the Otolaryngology Outpatient Clinic of the SASAKI Hospital (Yokohama, Japan) under written informed consent, which was approved the Ethics Committee of Showa University. Pollinosis was diagnosed by otorhinolaryngologists in accordance with the established criteria on the basis of patient history and rhinoscopic examination. To confirm the diagnosis and demonstrate allergen-caused pollinosis, skin prick testing (mean wheal diameter at least 4 mm greater than negative control) and a nasal provocation test were performed with commercial crude extracts used for *in vitro* and *in vivo* (Torii Pharmaceutical Co., Ltd., Tokyo, Japan). The number of eosinophils in nasal secretions obtained after the provocation test was also examined using smears stained with Giemsa solution. We also recruited 4 male healthy subjects from the member of the SASAKI Hospital (Yokohama, Japan) under written informed consent, which was also approved the Ethics Committee of Showa University. The characteristics of the subjects used in this study are shown in Table 1. Pollinosis patients were orally treated with 5 mg LCT (Glaxo SmithKline, Tokyo, Japan) once a day for 4 weeks during Japanese cedar pollen season (January 2014 to April 2014).

	Controls	**Patients**
Age, years (range)	44-61	36-39
Number of subjects	4	10
Sex	Male	Male
Disease	Nonallergic	Mild
Medication	None	None
Serum IgE (U/ml)	41.6 ± 8.9	145.6 ± 12.7
IgE RAST score		
Cj	0	50.8 ± 11.2
Aa	0	**0**
Ap	0	**0**
Dg	0	**0**
Df	0	**0**
Af	0	**0**
Cd	0	**0**
Dd	0	**0**
Blood Eosinophil Count, %	3.4 ± 0.5	**15.5 ± 0.8**
Skin prick test	-	**+++**[a]
Nasal provocation test		
Symptoms	-	**+++**[b]
Smear cytology	<1% eosinophils	>30% eosinophils

Table 1: Characteristic of Subject used for treatment.

Cj: *Cryptomeria japonica*; Aa: *Ambrosia artemisilifolia*; Ap: *Artemisia princeps*; Dg: *Dactylls glomerata*; Df: *Dermatophagoides farinae*; Af: *Aspergillus fumigates*; Cd: *Cat dander*; Dd: *Dog dander*.

[a]Wheal reaction>13 mm and flare reaction>30 mm against *C. japonica* alone.

[b]Positive for sneezing/itch, watery rhinorrhea and nasal blockage against *C. japonica* alone.

Recovery of nasal secretions

Nasal secretions were obtained as previously described [3,17]. Briefly, filter papers (Whatman No. 42) were cut into 7 × 30 mm. A filter strip was placed on the anterior portion of the inferior turbinates of the right and left nose and left for 5 min. They were then cut into small pieces and suspended in PBS and rocking 12 h at 4°C to prepare the extract of nasal secretions. After measuring IgA concentration in the extract with ELISA (Bethyl Laboratories, Inc., Montogomery, TX,

USA) according to the manufacturer's recommendations, they were stored at –80°C until used.

Nasal symptom scores

Nasal discharge and congestion were scored from 0 to 3 (0=no, 1=mild, 2=moderate, 3=severe symptoms). The number of sneezes during one h were counted and transformed into a score (0=0 sneezes, 1=1-4 sneezes, 2=5-9 sneezes, and 3=10 or more sneezes). A total symptom score was calculated by adding these three scores.

Nasal epithelial cell preparation and culture

Nasal polyp tissues were washed 5 times with phosphate buffered saline that contained 500 mg/ml streptomycin, 500 U penicillin and 5.0 μg/ml amphotericin B. These tissues were then treated with 0.1% protease type XIV for 12 h at 4°C. Epithelial cell layers were then obtained and vigorously mixed with pipets to obtain single cell suspension. The cells were suspended in SABM medium at a concentration of 5×10^5 cells/ml. The cell suspension was introduced into 24-well tissue culture plates in triplicate that were coated with human Type I collagen (Becton Dickinson Co., Ltd., Bedford, MA, USA) and cultured for 72 h when number of cells reached approximately 5×10^6 cells/well. The cells were then stimulated with 20 ng/ml TNF-α [18,19] in the presence of various concentrations of agents. After 24 h, culture supernatants were collected and stored at –40°C until used. In the case of examining mRNA expression, cells were cultured in a similar manner for 12 h. In all experiments, agents were added to cell cultures 2 h before TNF-α stimulation.

Assay for CC10 mRNA expression

Poly A^+ mRNA was separated from cultured cells with oligo(dT)-coated magnetic micro beads (Milteny Biotec, Bergisch Gladbach, Germany). The first-strand cDNA was synthesized from 1.0 mg of Poly A^+ mRNA using a Superscript cDNA synthesis kit (Invitrogen Corp., Carlsbad, CA, USA) according to the manufacturer's instructions. Polymerase Chain Reaction (PCR) was then carried out using a Gene Amp 5700 Sequence Detection System (Applied Biosystems, Forster City, CA, USA). The PCR mixture consisted of 2.0 μl of sample cDNA solution (100 ng/μl), 25.0 μl of SYBR-Green Mastermix (Applied Biosystems), 0.3 μl of both sense and antisense primers, and distilled water to give a final volume of 50.0 μl. The reaction was conducted as follows: 4 min at 94°C, followed by 40 cycles of 15 s at 95°C and 60 s at 60°C. β-actin was amplified as an internal control. mRNA levels were calculated by using the comparative parameter threshold cycle and normalized to GAPDH. The nucleotide sequences of the primers were as follows: for CC10, 5'-ATGAAACTCGCTGTCACCCT-3' (sense) and 5'-ATTACACAGTGAGCTTTGGGCTAT-3' (antisense) [20], and for GAPDH, 5'ATCTGGCACCACACCTTCTACAATGAGCTGCG-3' (sense) and 5'-CGTCATACTCCTGCTTGCTGATCCACATCT-GC-3' (antisense) [21].

Preparation of TRX specific mRNA

To prepare TRX specific mRNA for cell-free protein synthesis, the first-strand cDNA synthesized as above was amplificated with a Takara PCR Amplification kit using specific primers for CC10 in a final volume of 30 μl. The PCR conditions were as follows: 5 min at 94°C, followed by 30 cycles of 20 s at 94°C, 30 s at 58°C, and 30 s at 72°C. After measuring mRNA contents, samples were stored at –80°C until used.

Cell-free protein synthesis

Cell-free protein synthesis was performed using wheat germ cell-free protein synthesis core kits (Cell-Free Sciences Co., Ltd., Yokohama, Japan). The reaction mixture consisted of 2.0 μl of reaction buffer, 1.7 μl of creatine kinase (10 μg/ml), 1.0 μl of ribonuclease inhibitor (40 U/ml), 10.0 μl of wheat germ extract, 33.5 μl of specific mRNA (0.4 μg/μl) and distilled water contained various concentrations of the agents to give a final volume of 50.0 μl. The reaction mixture (50 μl) was then introduced into each well of 96 well plates that contained 250 μl of reaction buffer. The plates were incubated at 26°C for 24 h and the solutions were stored at –40°C until used.

Assay for CC10

CC10 levels in supernatants obtained from both cell culture and cell-free protein synthesis system were examined by the commercially available CC10 (clara cell protein^R) ELISA test kits (BioVendor Lab. Med. Inc., Brno, Czech Republic) according to the manufacturer's recommendations. Since our previous report clearly showed that individual specificity was observed in the ability of nasal epithelial cells to produce CC10 after TNF-α stimulation [17], the CC10 levels in culture supernatants were expressed as the % of non-stimulated control. CC10 levels in nasal secretions were also measured by the commercially available CC10 (clara cell protein^R) ELISA test kits (BioVendor Lab. Med. Inc.) in a similar manner and the results were expressed as the mean pg/ng IgA ±SE. The minimum detection levels of CC10 ELISA test kit was 46 pg/ml.

Statistical analysis

Statistical significance between control and experimental groups was examined by ANOVA followed by Dunette's multiple comparison test. Paired t-test was used to examine the statistical significance between before and after treatment with LCT. Data analysis were performed by using ANOVA for Mac (SPSS Inc., Chicago, IL, USA). The level of significance was considered at a P value of less than 0.05.

Results

Increased CC10 production induced by LCT and DLT in nasal epithelial cells *in vitro*

The first experiments were undertaken to examine the influence of LCT and DLT on CC10 production from nasal epithelial cells in response to TNF-α stimulation. To do this, nasal epithelial cells were stimulated with 20 ng/ml TNF-α in the presence of various concentrations of either LCT or DLT for 24 h, and CC10 levels were assayed by ELISA. As shown in Figure 1A, LCT caused increase in CC10 levels in culture supernatants when the cells were treated with LCT at more than 0.05 μM. The data in Figure 1B also show that the treatment of cells by DLT at more than 0.01 μM increased the ability of nasal epithelial cells to produce CC10 in response to TNF-α stimulation. The second set of experiments was designed to examine whether CT and LT, which are mother drugs for LCT and DLT, respectively, could also increase CC10 production from cells stimulated with TNF-α. As shown in Figure 2A and 2B, addition of CT and LT into cell cultures caused increase in CC10 levels in culture supernatants obtained from cells after TNF-α stimulation. The minimum concentration of these agents that caused significant increase were 0.1 μM and 0.03 μM, respectively.

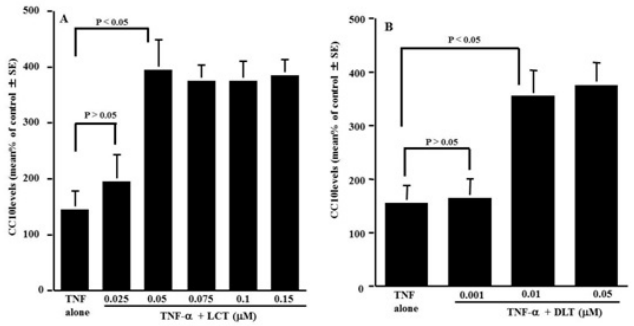

Figure1: Influence of levocetirizine (LCT) and desloratadine (DLT) on CC10 production from nasal epithelial cells after TNF-α stimulation *in vitro*. Nasal epithelial cells at approximately 5 × 10^6 cells were stimulated with 20 ng/ml TNF-α in the presence of various concentrations of the agents. After 24h, the culture supernatants were obtained and assayed for CC10 levels by ELISA.

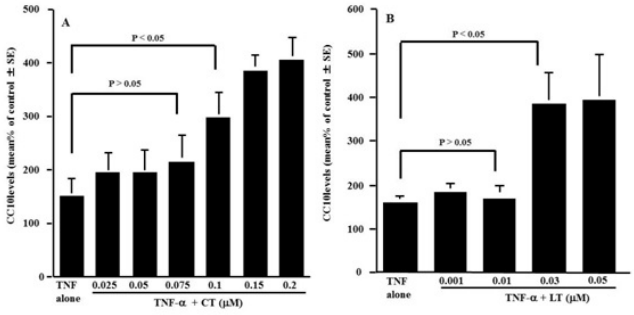

Figure 2: Influence of Cetrizine (CT) and Loratadine (LT) on CC10 production from nasal epithelial cells after TNF-α stimulation *in vitro*. Nasal epithelial cells were stimulated with 20 ng/ml TNF-α in the presence of various concentrations of the agents. After 24 h the culture supernatants were collected and assayed for CC10 levels by ELISA. The data are expressed as % of control (non-stimulated) ±SE of five different subjects.

Influence of LCT and DLT on CC10 mRNA expression in nasal epithelial cells *in vitro*

The third experiments were undertaken to examine the influence of LCT and DLT on CC10 mRNA expression in nasal epithelial cells *in vitro*. As shown in Figure 3A and 3B, stimulation of cells with TNF-α caused significant increase in CC10 mRNA levels as compared with non-stimulated control. Although the addition of LCT at 0.05 μM into cell cultures scarcely affected CC10 mRNA expression induced by TNF-α stimulation, LCT at more than 0.075 μM caused significant suppression of CC10 mRNA expression (Figure 3A). Treatment of nasal epithelial cells with DLT at 0.001 μM did not suppress CC10 mRNA expression, which was increased by TNF-α stimulation (Figure 3B). However, DLT at more than 0.01μM caused significant suppression of CC10 mRNA expression (Figure 3B).

Influence of LCT and DLT on cell-free protein synthesis

The fourth experiments were carried out to examine the influence of LCT and DLT on protein synthesis using cell-free protein synthesis system. As shown in Figure 4A, although the addition of LCT at 0.025 μM into cell-free protein systems did not increase CC10 synthesis, LCT at more than 0.05 μM caused increase in CC10 levels in the solutions as compared with that of control. Furthermore, the data in Figure 4B also clearly showed that DLT at more than 0.01 μM significantly increased the ability of wheat germ extract to produce CC10 as compared with the control (0 μM).

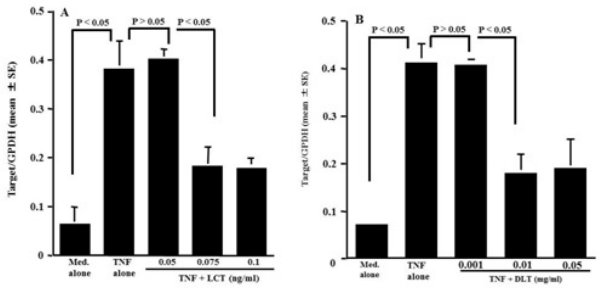

Figure 3: Influence of Levocetrizine and Desloratadine (DLT) on CC10 mRNA expression in nasal epithelial cells after TNF-α stimulation *in vitro*. Nasal epithelial cells were stimulated with 20ng/ml TNF-α in the presence of various concentrations of the agents. After 12h, Poly A+ mRNA was obtained from the cultured cells and CC10 mRNA expression was examined by real-time RT-PCR. The data are expressed as a mean ratio calculated as Target gene/GPDH.

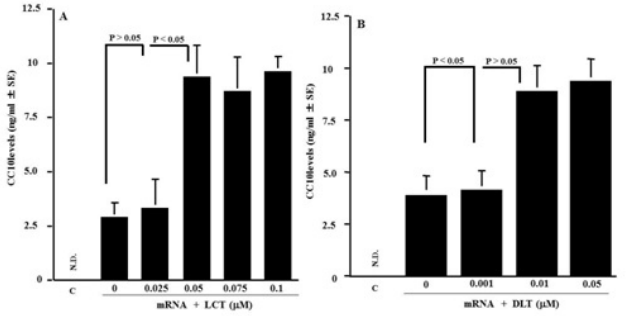

Figure 4: Influence of Levocetirizine (LCT) and Desloratadine (DLT) on CC10 production in cell-free protein synthesis system. CC10 levels in samples were examined by ELISA. The data expressed as the mean SE of five. ND: Not Detected (lower than 46 pg/ml) C: control.

Influence of LCT treatment on CC10 appearance in nasal secretions from patients with pollinosis

The fifth set of experiments was designed to examine whether LCT could also increase CC10 levels *in vivo*. To do this, patients with Japanese cedar pollinosis were orally administered 5 mg LCT once a day for 28 days during Japanese cedar pollen season, and CC10 levels in nasal secretions were examined by ELISA. As shown in Figure 5, oral administration of LCT into patients caused significant increase in CC10 levels in nasal secretions as compared with before treatment.

Influence of LCT treatment on clinical symptoms in patients with pollinosis

The final experiments were performed to examine whether oral administration of LCT could favorably modify the clinical conditions of patients. As shown in Table 2, the clinical symptom scores decreased after treatment.

Discussion

The present results clearly show that the third generation histamine H_1 receptor antagonists, LCT and DLT, enhance the ability of nasal epithelial cells to produce CC10 induced by TNF-α stimulation as well as their mother drugs, LT and CT *in vitro*. The minimum concentrations that cause significant increase in CC10 levels are 0.05 μM for LCT and 0.01 μM for DLT, which are lower levels that observed in their mother drugs and therapeutic blood levels [14,16].

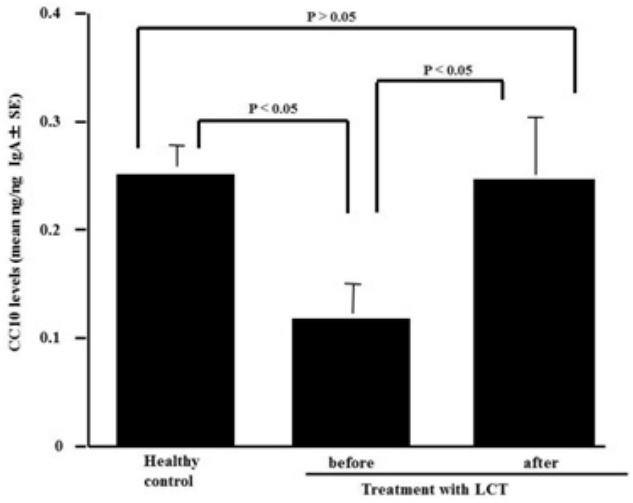

Figure 5: CC10 levels in nasal secretions from pollinosis patients before and after treatment with Levocetilizine (LCT) for 4 weeks.

	Treatment	
Symptoms	**Before**	**After***
Sneezing	2.4 ± 0.5	0.5 ± 0.4
Nasal discharge	2.5 ± 1.2	1.6 ± 1.0
Congestion	2.2 ± 1.1	0.8 ± 0.4

Table 2: Changes in clinical symptom scores observed in pollinosis patients treated with levocetirizine for 4 weeks.

**P*<00.5 as compared with before treatments

CC10 is a secretory protein composed of two identical subunits of 70 amino acids joined by two disulfide bonds [22,19]. Although CC10 was initially discovered in the rabbit uterus and believed to be a marker of the action of progesterone in a mammalian species [23], it is now revealed that CC10 possesses anti-inflammatory and immunomodulatory effects [7-10,6]. The role of CC10 on the development of allergic inflammation was extensively studied using experimental murine model. CC10 knock-out mice represent exaggerated allergic inflammation with intense infiltration of eosinophils in both lung [7,8] and nasal cavity [13]. In human cases, recent studies indicate that CC10 expression in nasal mucosa is down-regulated in patients with allergic rhinitis and chronic rhinosinusitis with nasal polyps [11,12]. Furthermore, it is also reported that CC10 levels in nasal secretions and nasal lavage fluids obtained from patients with allergic rhinitis was much lower than those from healthy control [11,24,25], indicating that CC10 may contribute to the induction and development of airway inflammatory diseases, especially allergic rhinitis. Together with these reports, the present results may suggest that enhancement of the ability nasal epithelial cells to produce CC10 by histamine H_1 receptor antagonists underlie the therapeutic mode of action of the agent on allergic rhinitis. To further confirm this speculation, we then examine the influence of the agents on the production of CC10 *in vivo* using pollinosis patients treated with LCT. The present results clearly show that nasal secretions obtained from patients before treatment contained much lower levels of CC10 as compared with those from healthy control subjects, and that oral administration of LCT into pollnosis patients caused an increase in CC10 levels in nasal secretions along with favorable modification of clinical symptoms. Our previous observation [17] showing that oral administration of fexofenadine hydrochloride, a second generation histamine H_1 receptor antagonist, into pollinosis patients for 2 weeks caused an increase in CC10 levels in nasal secretions and results in attenuation of clinical conditions in patients also supports our speculation.

Our previous works clearly showed that histamine H_1 receptor antagonists inhibit the production of inflammatory mediators, such as cytokines and chemokines from both epithelial cells and fibroblasts after inflammatory stimulations through the suppression of mRNA expression [1,26,27]. On the other hand, the present results clearly show that treatment of nasal epithelial cells with DLT and LCT caused an increase in CC10 protein production in spite of the suppression of its mRNA expression induced by TNF-α stimulation. The reasons for this discrepancy are not clear at present. The process of protein synthesis in cells requires two different steps: in the first step, transcription, mRNA is synthesized from DNA in the nucleus. mRNA formed then comes out through nuclear membrane into cytoplasma where it binds to mRNA-binding site on ribosome and starts protein synthesis, which is call translocation. The final experiments, therefore, were undertaken to examine whether LCT and DLT could increase the translocation activity of CC10 mRNA. Cell free protein assay revealed that the addition of LCT and DLT at concentrations showing suppressive effects on CC10 mRNA expression could increase CC10 protein synthesis. These results strongly suggest that there is the possibility that LCT and DLT increase the translation of CC10 mRNA, resulting in production and secretion of large amount of CC10 from TNF-α stimulated epithelial cells. Although glucocorticoids, dexamethasone and cortisol, are well known to exert their immunosuppressive effects through the suppression of inflammatory protein mRNA expression, they are reported to enhance the ability of airway cells to produce CC10 after inflammatory stimulation, and this is due, in part, to an increase in translatable activity of CC10 mRNA by glucocorticoids [28-30]. These reports may also support our speculation that the translation of CC10 mRNA in nasal epithelial cells is enhanced by LCT and DLT and results in increase in the ability of cells to produce CC10 after TNF-α stimulation.

In conclusion, the present results may suggest that some of therapeutic mode of action of histamine H_1 receptor antagonists, especially LCT and DLT, in allergic diseases such as allergic rhinitis and atopic allergy depend on their ability to increase the production of CC10 from epithelial cells in response to inflammatory stimulation through the enhancement of the translatable activity of CC10 mRNA.

Conflict of Interest

The authors report no conflict of interest in this work.

References

1. Asano K, Kanai KI, Suzaki H (2004) Suppressive activity of fexofenadine hydrochloride on metalloproteinase production from nasal fibroblasts in vitro. Clin Exp Allergy 34: 1890-1898.
2. Howarth PH (1998) Pathogenic mechanisms: a rational basis for treatment. British Med J. 316:757-761.
3. Furuta A, AsanoK, Kanai K, Hirano K, Sanbe T, et al. (2010) Enhancement of thioredoxin production by epinastine hydrochloride in vitro and in vivo. Jpn Pharmacol Ther. 38: 965-70.
4. Johansson S, Keen C, Ståhl A, Wennergren G, Benson M (2005) Low levels of CC16 in nasal fluid of children with birch pollen-induced rhinitis. Allergy 60: 638-642.
5. Elia J, Aoki A, Maldonado CA (2003) Regulation of uteroglobin/Clara cell protein expression after acute lung exposure to an organophosphoreted insecticide. Histochem Cell Biol 120: 33-39.

6. Sohn J, Kim TI, Yoon YH, Kim JY, Kim SY (2003) Novel transglutaminase inhibitors reverse the inflammation of allergic conjunctivitis. J Clin Invest 111: 121-128.

7. Chen LC, Zhang Z, Myers AC, Huang SK (2001) Cutting edge: altered pulmonary eosinophilic inflammation in mice deficient for Clara cell secretory 10-kDa protein. J Immunol 167: 3025-3028.

8. Hung CH, Chen LC, Zhang Z, Chowdhury B, Lee WL, et al. (2004) Regulation of TH2 responses by the pulmonary Clara cell secretory 10-kd protein. J Allergy Clin Immunol 114: 664-670.

9. Johansson S, Wennergren G, Aberg N, Rudin A (2007) Clara cell 16-kd protein downregulates T(H)2 differentiation of human naive neonatal T cells. J Allergy Clin Immunol 120: 308-314.

10. Shiyu S, Zhiyu L, Mao Y, Lin B, Lijia W et al. (2011) Polydatin up-regulates clara cell secretory protein to suppress phospholipase A2 of lung induced by LPS in vivo and in vitro. BMC Cell Biol. 12: 31-44.

11. Benson M, Fransson M, Martinsson T, Naluai AT, Uddman R et al. (2007) Inverse relation between nasal fluid Clara Cell Protein 16 levels and symptoms and signs of rhinitis in allergen-challenged patients with intermittent allergic rhinitis. Allergy 62: 178-183.

12. Liu Z, Lu X, Zhang XH, Bochner BS, Long XB et al. (2009) Clara cell 10-kDa protein expression in chronic rhinosinusitis and its cytokine-driven regulation in sinonasal mucosa. Allergy 64: 149-157.

13. Wang H, Long XB, Cao PP, Wang N, Liu Y, et al. (2010) Clara cell 10-kD protein suppresses chitinase 3-like 1 expression associated with eosinophilic chronic rhinosinusitis. Am J Respir Crit Care Med 181: 908-916.

14. Henz BM (2001) The pharmacologic profile of desloratadine: a review. Allergy 65: 7-13.

15. Sadowska-Woda I, Bieszczad-Bedrejczuk E, Rachel M (2010) Influence of desloratadine on selected oxidative stress markers in patients between 3 and 10 years of age with allergic perennial rhinitis. Eur J Pharmacol 640: 197-201.

16. Schoepke N, Church MK, Maurer M (2013) The inhibition by levocetirizine and fexofenadine of the histamine-induced wheal and flare response in healthy Caucasian and Japanese volunteers. Acta Derm Venereol 93: 286-293.

17. Nogaki T, Asano K, Furuta A, Kanai K, Suzaki I, et al. (2012) Enhancement of clara cell 10-kD protein (CC10) production from nasal epithelial cells by fexofenadine hydrochloride. Asian Pac J Allergy Immunol 30: 139-145.

18. Cowan MJ, Huang X, Yao XL, Shelhamer JH (2000) Tumor necrosis factor alpha stimulation of human Clara cell secretory protein production by human airway epithelial cells. Ann N Y Acad Sci 923: 193-201.

19. Yao XL, Levine SJ, Cowan MJ, Logun C, Shelhamer JH (1998) Tumor necrosis factor-alpha stimulates human Clara cell secretory protein production by human airway epithelial cells. Am J Respir Cell Mol Biol 19: 629-635.

20. Yamamoto O, Takahashi H, Hirasawa M, Chiba H, Shiratori M, et al. (2005) Surfactant protein gene expressions for detection of lung carcinoma cells in peripheral blood. Respir Med 99: 1164-1174.

21. Suzaki I, Asano K, Kanei A, Suzaki H (2013) Enhancement of thioredoxin production from nasal epithelial cells by the macrolide antibiotic, clarithromycin in vitro. In Vivo 27: 351-356.

22. Bally R, Delettré J (1989) Structure and refinement of the oxidized P21 form of uteroglobin at 1.64 A resolution. J Mol Biol 206: 153-170.

23. Lombardero M, Nieto A (1981) Glucocorticoid and developmental regulation of uteroglobin synthesis in rabbit lung. Biochem J 200: 487-494.

24. Broeckaert F, Bernard A (2000) Clara cell secretory protein (CC16): characteristics and perspectives as lung peripheral biomarker. Clin Exp Allergy 30: 469-475.

25. Yang KD, Ou CY, Chang JC, Chen RF, Liu CA et al. (2007) Infant frequent wheezing correlated to Clara cell protein 10 (CC10) polymorphism and concentration, but not allergy sensitization, in a perinatal cohort study. J Allergy Clin Immunol. 120: 842-848.

26. Kanai K, Asano K, Watanabe S, Kyo Y, Suzaki H (2006) Epinastine hydrochloride antagonism against interleukin-4-mediated T cell cytokine imbalance in vitro. Int Arch Allergy Immunol 140: 43-52.

27. Shoji N, Asano K, Furuta A, Hirano K, Suzaki H (2011) Effect of histamine H1 receptor antagonists on TARC/CCL17 and MDC/CCL22 production from CD14+ cells induced by antigenic stimulation in vitro. Int Arch Allergy Immunol 155: 38-51.

28. Cui YH, Wang YY, Liu Z (2011) Transdifferentiation of Clara cell 10-kDa protein secreting cells in experimental allergic rhinitis. Am J Rhinol Allergy 25: 145-151.

29. Mukherjee AB, Zhang Z, Chilton BS (2007) Uteroglobin: a steroid-inducible immunomodulatory protein that founded the Secretoglobin superfamily. Endocr Rev 28: 707-725.

30. Roth FD, Quintar AA, Uribe Echevarría EM, Torres AI, Aoki A, et al. (2007) Budesonide effects on Clara cell under normal and allergic inflammatory condition. Histochem Cell Biol 127: 55-68.