

Effects of desloratadine citrate disodium injection on rat models of ovalbumin-induced allergic rhinitis: involvement of T-cell responses modulation

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Background: Allergic rhinitis (AR) is a very common worldwide problem; patients display a number of symptoms, such as sneezing, nasal itching, and rhinorrhea, and their lifestyle is affected. Desloratadine is a novel, long-acting inhibitor of histamine. However, very little is known about the effect of desloratadine citrate disodium injection (DLC injection) on AR, and the underlying mechanisms are yet unexplored. Herein, we sought to explore the effects and mechanisms of actions of DLC injection in ovalbumin (OVA)-induced immune responses in a rat model of AR.

Methods: Sixty rats were subjected to immunization with OVA (intraperitoneal [IP]), followed by a nasal challenge with OVA. Drugs or saline were given daily for treatment. Nasal symptoms and histology of the nasal mucosa were examined. Cytokines such as interleukin (IL)-4, IL-12, interferon (IFN)- γ , adhesion molecules such as soluble vascular cell adhesion molecule 1 (sVCAM-1), and inducible nitric oxide synthase (iNOS) expression were assessed by enzyme-linked immunosorbent assay (ELISA) kit. Nitric oxide (NO) concentration was also measured by NO assay kit.

Results: DLC treatment (intravenous [IV]) significantly decreased the frequency of sneezing and nasal scratching and

alleviated nasal inflammation by increasing the serum levels of IFN- γ and IL-12, while lowering the expression of IL-4. Thus, DLC (IV) treatment led to a marked elevation in T-helper 1/T-helper 2 (Th1/Th2) ratio when administered in the AR rats. The expression of sVCAM-1, iNOS, and NO were also reversed.

Conclusion: DLC (IV), given after an allergen challenge, improved Th1 cytokines level and restrained Th2 responses alleviating the symptoms of AR. Our results indicate that DLC injection may exhibit such effects through the modulation of T-cell responses. © 2015 ARS-AAOA, LLC.

Key Words:

allergic rhinitis; desloratadine citrate disodium injection; nasal symptoms; T-cell balance; iNOS

How to Cite this Article:

Yuan X, Ghosh A, Jie Q, He G, Wu Y. Effects of desloratadine citrate disodium injection on rat models of ovalbumin-induced allergic rhinitis: involvement of T-cell responses modulation. *Int Forum Allergy Rhinol.* 2015;XX:1-7.

Allergic rhinitis (AR), asthma, and atopic eczema frequently coexist and represent worldwide health concerns with increasing prevalence in all age groups.¹⁻³ AR

results in the synthesis and release of immunoglobulin E (IgE) exhibiting a number of symptoms such as sneezing, nasal itching, and rhinorrhea. In AR, histamine is primarily released along with Th2 cytokines (interleukin [IL]-4, IL-5, and IL-13), which activate the effector cells, such as eosinophils, basophils, and mast cells, with consequent release of the inflammatory mediators.^{4,5}

Histamine institutes several immediate hypersensitive reactions, which are mainly responsible for triggering the allergic responses.^{6,7} Extensive reports have evidenced the promising therapeutic effects of histamine H1-receptor antagonists on various aspects of allergic inflammatory responses.^{7,8} Desloratadine citrate disodium (DLC), a new, long-acting histamine H1-receptor antagonist that has an improved solubility compared to that of desloratadine, has higher potential for reducing sneezing, nasal itching, and rhinorrhea associated with AR.^{6,9} Moreover, like

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Funding sources for the study: Hefei Industrial Pharmaceutical Institute Co., Ltd., Hefei, China; China Pharmaceutical University, Nanjing, China. Potential conflict of interest: All the authors declare that, the results of this study belong to the company, there is no potential conflict of interest.

Received: 27 January 2015; Revised: 2 June 2015; Accepted: 10 June 2015

DOI: 10.1002/alr.21594

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desloratadine, DLC does not cause central nervous system sedation⁶ and is considered as a suitable therapeutic treatment in patients of any age. The DLC injection has been developed with special focus on the patients who have difficulty in swallowing. Besides, it can be useful for acute therapy because of its rapid onset and long duration of action.

CD4⁺ T-cells can be divided into 2 distinct subsets of effector cells: Th1 cells associated with inflammation and Th2 cells that can secrete cytokines highly associated with allergic reactions.^{10–12} Recent evidence indicates that H1-receptor antagonists have the potential effect of altering T-cell imbalance, ranging from elevation of Th1 cytokines to reduction of Th2 cytokines released in AR.¹³ However, only a few studies have examined their direct effect on T-cell balance maintenance.¹⁴ In this study, we focused on evaluating the effects of DLC (IV) administration on T-cell responses in an AR model of rats. Interferon (IFN)- γ and IL-4, representing the Th1 and Th2 cell phenotypes, respectively, both play crucial roles in the inflammatory cascade and have been found in serum samples of AR rats.^{15,16} Herein, we detected the expression of IFN- γ and IL-4 to find whether DLC (IV) administration could alter the ovalbumin (OVA)-stimulated T-cell responses and attenuate allergic nasal inflammation.

AR is a complex disease that is likely to be involved with dysregulated CD4⁺ T-cell differentiation. As desloratadine citrate disodium has been gaining popularity in China with its increasing use as an antiallergic agent, we developed its injection and further evaluated its antihistaminic efficacy. In this study, we used OVA-sensitized AR rats to gain an insight into whether DLC (IV) administration could cure the pathological symptoms by normalizing the T-cell imbalance.

Materials and methods

Animals

All the experimental procedures on animals were approved by the Committee for Ethical Use and Care of Animals, China Pharmaceutical University. Male Sprague-Dawley rats (160 to 200 g) were obtained from Shanghai Experimental Animal Center (Shanghai, China). Animals were housed in an air-conditioned room at an ambient temperature of $24 \pm 1^\circ\text{C}$, $55\% \pm 5\%$ relative humidity, and 12-hour controlled light-dark cycle (lights on at 7:00 AM) and under pathogen-free conditions. Food and water were available *ad libitum* throughout the study except before the commencement of the study when they were deprived of food for 12 hours before the experiment.

Materials

OVA (grade V) was purchased from Sigma (St. Louis, MO, USA). Aluminum hydroxide ($\text{Al}(\text{OH})_3$) gel was purchased from Thermo (Rockford, IL, USA). Anti-rat IL-4/IL-12/IFN- γ /soluble vascular cell adhesion molecule

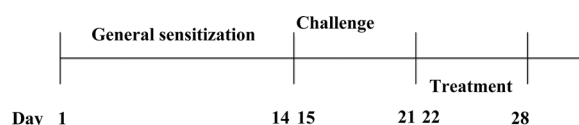


FIGURE 1. Schematic diagram for sensitization of AR model rats. AR = allergic rhinitis.

TABLE 1. Summary for sensitization of AR model rats

Group	General sensitization	Challenge	Treatment
Normal	Saline	Saline	Saline
AR	OVA-sensitized	OVA-challenged	Saline
Positive	OVA-sensitized	OVA-challenged	Chlorpheniramine
DLC (0.3 mg/kg)	OVA-sensitized	OVA-challenged	DLC
DLC (0.6 mg/kg)	OVA-sensitized	OVA-challenged	DLC
DLC (1.2 mg/kg)	OVA-sensitized	OVA-challenged	DLC

AR = allergic rhinitis; DLC = desloratadine citrate disodium; OVA = ovalbumin.

1 (sVCAM-1)/inducible nitric oxide synthase (iNOS) enzyme-linked immunosorbent assay (ELISA) kits were purchased from Yifei Biotech (Shanghai, China). Nitric oxide (NO) assay kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Drug treatment in animals

A total of 60 Sprague-Dawley rats were randomly divided into the following 6 groups ($n = 10$): normal; AR (allergic rhinitis); positive (chlorpheniramine maleate injection); and desloratadine citrate disodium injection (DLC injection: 0.3 mg/kg, 0.6 mg/kg, and 1.2 mg/kg) groups.

AR in rats was induced by a previously described method¹⁷ with minor modifications (Fig. 1).

Briefly, rats were sensitized on every other day for 2 weeks starting from the first day (ie, days 1, 3, 5, 7, 9, 11, and 13) with 1 mL physiological saline containing 0.3 mg OVA and 10 mg $\text{Al}(\text{OH})_3$ through an intraperitoneal (IP) route. The normal group was given 1 mL saline without OVA. The $\text{Al}(\text{OH})_3$ gel was used as an adjuvant. Then, the animals were challenged by a nasal administration of OVA every day from day 15 to day 21 by dripping the OVA (2% solution in physiological saline) into the bilateral nasal cavities (50 μL each nostril) using a micropipette. The normal group was given saline only.

From day 22 to day 28, rats were treated with 50 μL 2% OVA solution in saline in the form of intranasal drops on every other day (ie, days 22, 24, 26, and 28) for a persistent effect on the nasal sensitization. The normal group was treated with saline drops only. Drugs were administered intravenously 30 minutes before nasal antigen challenge from day 22 to day 28. Saline was replaced in the AR and normal groups (Table 1).

Evaluation of the nasal allergic symptoms

The nasal allergic symptoms were observed and recorded from day 22 to day 28. During this period, the animals received drugs every day from day 22 to day 28 while they underwent antigen challenge every other day. DLC injection and Chlorpheniramine maleate injection was administered intravenously by tail vein at a volume of 2 mL/kg 30 minutes before the nasal antigen challenge. After the antigen challenge, nasal allergic symptoms were evaluated by the frequencies of sneezing and nasal scratching for 30 minutes.

Histological examination

On day 28, the rats were euthanized and parts of the nasal septum mucosa were collected. Then the septum mucosa specimens were immediately fixed with 10% formaldehyde and embedded in paraffin. Samples were sliced into 4-mm-thick sections, and then each section was stained by hematoxylin and eosin (H&E).¹⁸ Histopathological changes in the septal mucosa sections were observed under the microscope.

Assay of the serum cytokine levels by ELISA and the level of NO by NO kit

Blood samples were drawn from the abdominal aorta immediately after the evaluation of nasal allergic symptoms mentioned above. The blood samples were centrifuged at 799 g for 10 minutes and the serum content was collected and samples were prepared accordingly. Cytokines such as IL-4, IL-12, and IFN- γ , adhesion molecules such as sVCAM-1 production, and iNOS expression were assessed by the respective Rat ELISA Kits according to the manufacturers' instructions. The absorbance profiles were read at 450 nm. NO was also assessed by an NO assay kit following the manufacturer's instructions and the absorbance was read at 550 nm.

Statistical analysis

All data were analyzed using SPSS 17.0 software (IBM, Armonk, NY) and expressed as mean $\pm$ SD. A value of $p < 0.05$ was considered significant. One-way analysis of variance (ANOVA) test was used to assess the numeric variables among different groups for IL-4, IL-12, IFN- γ , sVCAM-1, iNOS, NO levels, sneezing and nasal scratching counts, and Th1/Th2 ratio.

Results

DLC injection alleviated the nasal allergic symptoms induced by OVA

Notably, the nasal allergic symptoms in the AR group were significantly higher exhibiting more frequent sneezing and nasal scratching than that in the normal group ($p < 0.01$) (Fig. 2). The positive control group as well as the DLC (IV)-treated groups (0.3, 0.6, 1.2 mg/kg) showed significantly

lower incidents of sneezing and nasal scratching ($p < 0.01$ for all the DLC (IV)-treated groups) (Fig. 2). And the DLC (IV)-treated groups (0.6, 1.2 mg/kg) showed similar effects with the positive drug-treated group.

Histological examination revealed the inhibitory effect of DLC injection on OVA-induced eosinophil infiltration

The histological findings in the nasal mucosa sections are shown in Figure 3. The normal group exhibited no sign of eosinophil infiltration, whereas a large number of eosinophil infiltration was observed in the AR group compared with those in the normal group. Parenteral administration of DLC and chlorpheniramine maleate after OVA challenge potentially inhibited the eosinophil infiltration (Fig. 3C-F). The nasal inflammation was significantly less in the DLC-treated (0.6, 1.2 mg/kg) groups (Fig. 3E, F) and the positive group (Fig. 3C) compared to that of the AR group.

DLC injection reversed the cytokine release in the serum as measured by ELISA

To investigate the effects of DLC (IV) treatment on the T-cell phenotype, we examined the production of cytokines (IL-4 representing Th2 cytokines, IFN- γ representing the Th1 phenotype; IL-12 representing any alteration in the polarity of the evolving T-cell response to Th1 phenotype).

As shown in Figure 4A, IL-12 production was significantly lowered in the AR group compared to that of the normal group ($p < 0.01$). After treatment, IL-12 production significantly increased in the animals injected with DLC (0.6, 1.2 mg/kg) and chlorpheniramine compared to that of the AR group (DLC 0.6 mg/kg, $p < 0.05$; others, $p < 0.01$).

As shown in Figure 4B, the IFN- γ level, a Th1 cell phenotype, was significantly lowered in the AR groups compared to that in the normal group ($p < 0.01$). But the IFN- γ level in the DLC (IV)-treated groups was significantly higher than that in the AR group (DLC 0.3 mg/kg, $p < 0.05$; $p < 0.01$ for the other doses), which was correlated with the increased Th1 level. The group administered 0.6 mg/kg DLC showed a similar profile to the positive group.

On the other hand, the expression of IL-4, a topical representative of the Th2 cells, was higher in the AR groups compared with the normal group ($p < 0.01$). The expression of IL-4 was lowered in the DLC (IV)-treated groups (0.6 mg/kg and 1.2 mg/kg) compared with the AR group ($p < 0.01$), as shown in Figure 4C.

The Th1/Th2 ratio is an important factor that can help explaining some of the mechanisms underlying the AR inflammatory conditions. As shown in Figure 4D, the Th1/Th2 ratio was decreased in the AR group compared to that of the normal group ($p < 0.01$). Whereas the Th1/Th2 ratio was increased in the DLC (IV) (0.6 mg/kg and 1.2 mg/kg)-treated groups and positive group compared to that of the AR group ($p < 0.01$). This revealed that DLC

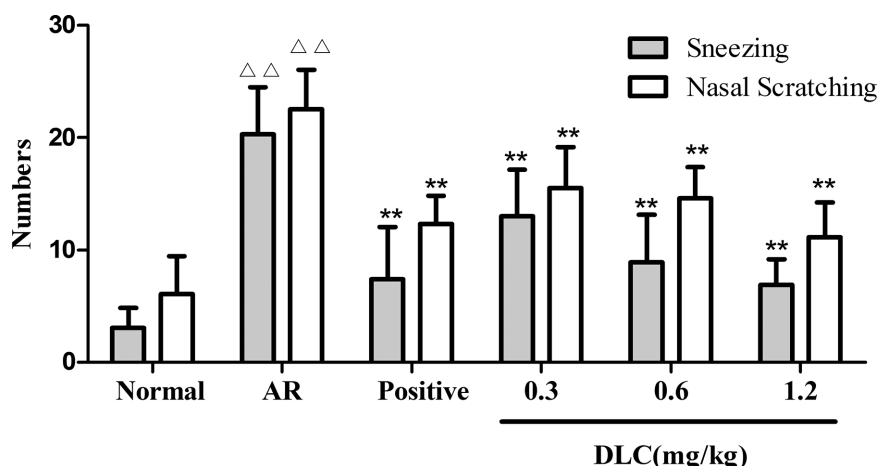


FIGURE 2. Treatment with DLC and chlorpheniramine relieved the numbers of sneezing and nasal scratching in OVA-sensitized rats ($n = 10$ in each group). We assessed the nasal symptoms by counting the numbers of sneezing and nasal scratching within 30 minutes after the last administration on day 28, 10 rats in each group, all values represent mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$ compared with AR group; $\Delta p < 0.05$, $\Delta\Delta p < 0.01$ compared with normal group. AR = allergic rhinitis; DLC = desloratadine citrate disodium; OVA = ovalbumin; positive = chlorpheniramine maleate; SD = standard deviation.

may modulate the polarization of T-cell responses and inhibit the production of Th2 cytokines in AR. The DLC (1.2 mg/kg)-treated group showed efficacy similar to that of the positive group (0.6 mg/kg).

To explore the inhibitory effect of DLC (IV) on adhesion molecules, we detected the serum expression of sVCAM-1 (Fig. 5A), which is correlated with VCAM-1 expression in the nasal mucosa. The AR group showed a higher level of sVCAM-1 compared to that of the normal group ($p < 0.01$). But the DLC-treated groups showed a lowered expression of sVCAM-1 compared to that of the AR group ($p < 0.01$ compared with AR). The effects at the 1.2-mg/kg and 0.6-mg/kg doses of DLC were similar to that in the positive group.

AR sensitization induced a significant rise in the serum iNOS level ($p < 0.01$ compared with normal), which was modulated by DLC (IV) (0.6 mg/kg and 1.2 mg/kg) treatment ($p < 0.01$ for both compared to that of the AR group) (Fig. 5B). The 1.2 mg/kg DLC-treated group and the positive group exhibited a similar effect. Consequently, and most certainly, the NO level also took a prominent elevation in the AR group ($48.54 \pm 7.78 \mu\text{mol/L}$, $p < 0.01$ compared with normal), which was alleviated by DLC (IV) treatment. DLC 0.6 mg/kg lowered the NO level to $37.33 \pm 4.11 \mu\text{mol/L}$ ($p < 0.05$ compared with AR). DLC 1.2 mg/kg and the positive drug were effective to a significant extent ($p < 0.01$ compared with AR). These observations indicate that iNOS to NO generation can be weakened by DLC injection.

Discussion

AR occurs in a 2-phase reaction pattern, termed the “early-phase” and the “late-phase” reaction. In the early-phase response, IgE activates mast cells and basophils, leading to the release of inflammatory mediators such as histamine and cytokines.^{17,19} In the late-phase response, lipid

mediators such as the leukotrienes or platelet activating factor are synthesized by activated mast cells that cause the local inflammatory response, which is characterized by assemblies of eosinophils, neutrophils, macrophages, and basophils. Infiltration of the inflammatory cells release a number of inflammatory factors and several enzymes, which lead to persistent inflammation and may cause tissue injury.¹⁸ AR is characterized by sneezing, rhinorrhea, and itching, which are caused by an elevation in the local inflammatory cytokines.^{20,21} Therapeutic strategies against AR have often been concentrated on blocking the effects of histamine because of its major contribution to nasal allergic reactions.²² In our studies, DLC, a non-sedative, novel histamine H1-receptor antagonist, when administered IV, alleviated nasal inflammatory symptoms as exhibited by reduced nasal itching and sneezing. Additionally, infiltration of inflammatory cells in the nasal mucosa in AR rats was also normalized by DLC (IV) treatment, which indicates that DLC injection can effectively alleviate the nasal allergic symptoms.

Very few studies have focused on the effect of DLC on the T-cell regulatory loop. To explore these unknown mechanisms, we studied the effect of DLC (IV) treatment on the expression of both Th2-related and Th1-related cytokines in the serum of the OVA-induced AR rats. In this study, we confirmed that DLC injection could improve the levels of IFN- γ and IL-12 while reducing IL-4 production in the serum of the AR rats (Fig. 4).

IFN- γ and IL-12 play a key role in the Th1-cytokine-related inflammatory and autoimmune reactions and in altering the inflammatory responses.¹⁵ It has been suggested that a histamine inhibitor might limit the Th2 responses by modulating IL-12 expression.²³ Previous studies have revealed that IL-12 promoted IFN- γ production, which had significant regulatory roles in diminishing Th2 inflammation.^{23–25} On the other hand, the pivotal role of IL-4 is already well known for inducing and maintaining

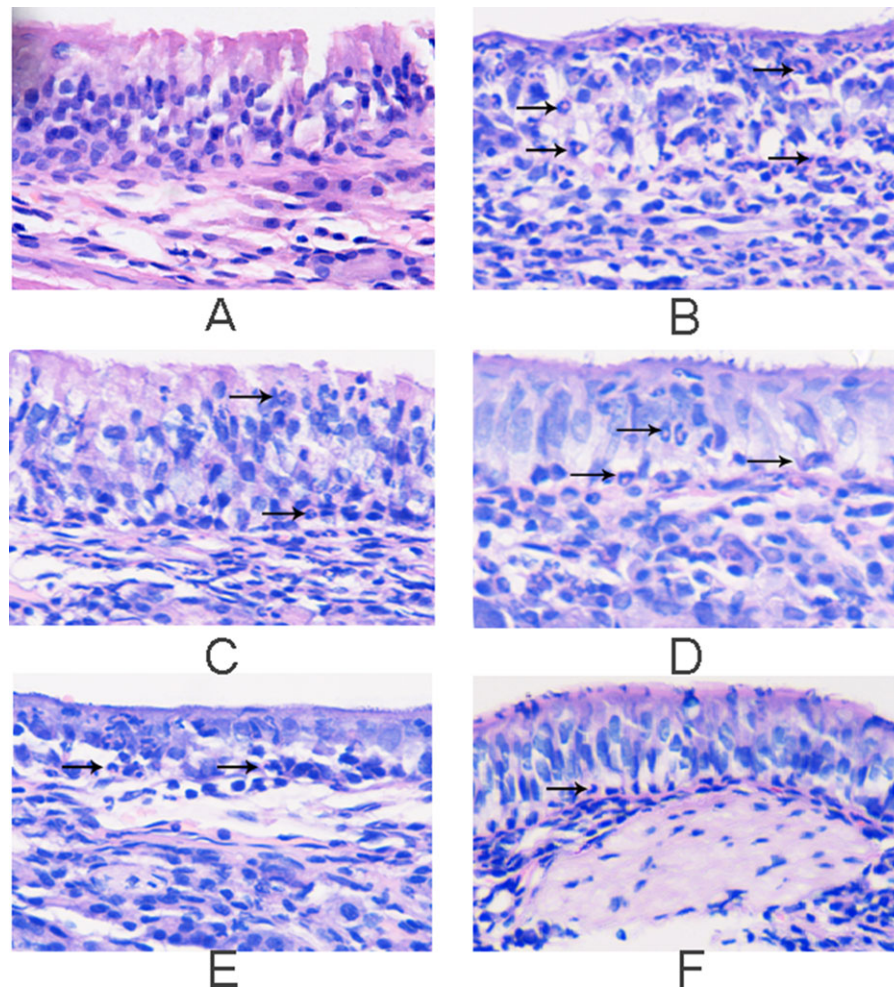


FIGURE 3. Light photomicrographs of H&E stained sections of nasal mucosa from the normal or sensitized rats after intranasal OVA challenge. H&E staining rat septum mucosa. (A) The normal group showed a normal state. (B) In the OVA-sensitized group, the mucosa was significant with infiltration of the inflammatory cells such as eosinophils. (C) In the positive group, infiltration of only a few inflammatory cells was seen, particularly eosinophils. (D) In the DLC (0.3 mg/kg) group, infiltration of inflammatory cells was still observed. (E) In the DLC (0.6 mg/kg) group, part of infiltration of inflammatory cells was restrained. (F) In the DLC (1.2 mg/kg) group, little infiltration of eosinophils can be seen. Infiltration was markedly minimized in treatment group (F) compared with the AR group (B). Eosinophil cells are marked by an arrow. (A-F) Original magnification $\times 400$. AR = allergic rhinitis; DLC = desloratadine citrate disodium; H&E = hematoxylin and eosin; OVA = ovalbumin; positive = chlorpheniramine maleate.

Th2 polarization in allergic responses.^{13,26} IL-4 can promote IgE synthesis, upregulate adhesion molecules such as the VCAM-1, and cause elevated mucus production.²⁷ Thus, it can be concluded that DLC might diminish AR partly through lowering the IL-4 cytokine, which is a key player in induction, maintenance, and amplification of the inflammatory responses in AR.

These findings support the idea that DLC (IV) treatment attenuates AR by balancing the T-cell responses. This was consistent with the development of the systemic Th1 responses and the inhibition of the local Th2 responses, resulting in an improved Th1/Th2 ratio.^{24,28} This suggests that DLC might act by modulating the Th1/Th2 ratio.

After the antigenic intrusion, an enhanced expression of iNOS in OVA-challenged rats suggested its key role in the pathology of AR.^{29,30} An enhanced production of NO strongly correlates with an increase in the iNOS activity.³¹

A marked increase in NO production, an important factor contributing to Th1/Th2 immunity, aggravates the allergic context.^{32,33} The expression of VCAM is marked by sVCAM-1 in the serum, and its increasing vascular permeability in the inflamed areas.³⁴ The increasing levels of sVCAM-1 or iNOS deteriorates such conditions to a greater extent. Levels of sVCAM-1, iNOS, and NO decreased after DLC (IV) administration in our investigation, suggesting a protective role of DLC (IV) in AR through the modulation of the iNOS pathway.

Recent studies have shown that nuclear factor κ B (NF- κ B) mediates the gene transcriptions for IL-12, IFN- γ , and IL-4.¹⁵ A noticeable characteristic of NF- κ B is that it can promote the expression of cytokines that are responsible for T-cell recruitment and activation.^{35,36} It is reasonable to believe that persistent NF- κ B activation in severe AR can result in a strong inflammatory environment by lowering the Th1/Th2 ratio and vice versa.³⁷ Simultaneously with

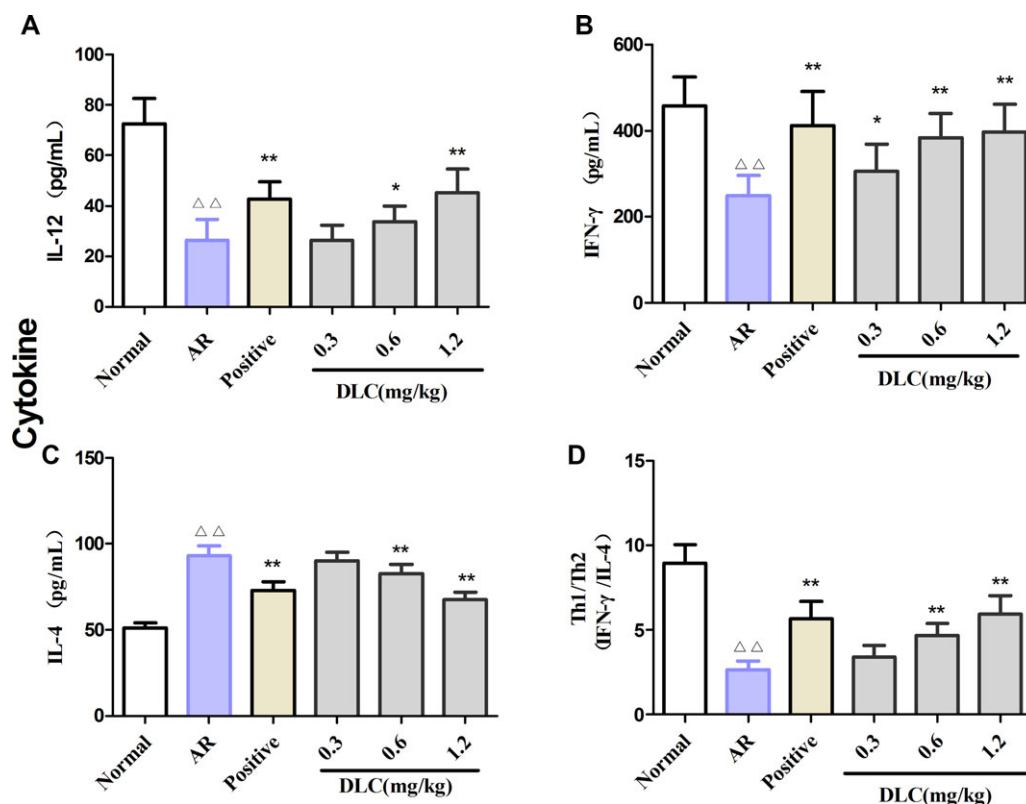


FIGURE 4. Th1 and Th2 cytokines expression and Th1/Th2 ratio in serum of AR models of rats. ELISA assessment showed concentrations of IL-12 (A), IFN-γ (B), IL-4 (C), and Th1/Th2 (D) (n = 10 in each group). All values represent mean ± SD. **p* < 0.05, ***p* < 0.01 compared with AR group; [△]*p* < 0.05, ^{△△}*p* < 0.01 compared with normal group. AR = allergic rhinitis; DLC = desloratadine citrate disodium; ELISA = enzyme-linked immunosorbent assay; IFN = interferon; IL = interleukin; positive = chlorpheniramine maleate; SD = standard deviation; Th = T helper.

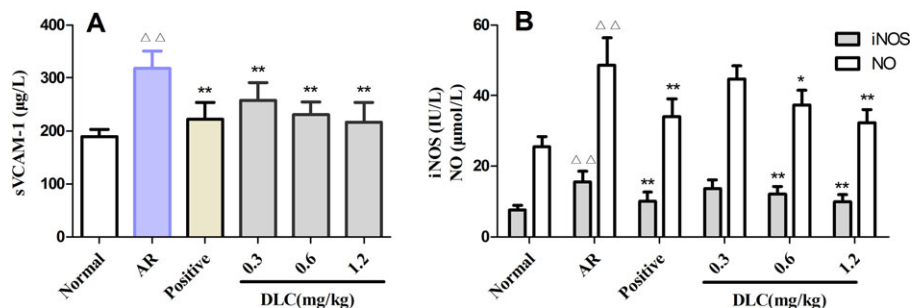


FIGURE 5. sVCAM-1, iNOS, and NO expression in serum of AR models of rats. ELISA assessment showed concentration of sVCAM-1 (A) and iNOS and NO (B) (n = 10 in each group). All results were representative by mean ± SD. **p* < 0.05, ***p* < 0.01 compared with the AR group; [△]*p* < 0.05, ^{△△}*p* < 0.01 compared with the normal group. AR = allergic rhinitis; ELISA = enzyme-linked immunosorbent assay; iNOS = inducible nitric oxide synthase; NO = nitric oxide; positive = chlorpheniramine maleate; SD = standard deviation; sVCAM-1 = soluble vascular cell adhesion molecule 1.

T-cell activation, NF-κB also can enhance the activity of several enzymes and adhesion molecules, such as iNOS, intercellular adhesion molecule 1 (ICAM-1), and VCAM-1. Based on the current perspectives, we further suggest that DLC (IV) administration might alleviate the symptoms of AR by modulating the nuclear localization of the NF-κBp65 subunits.

In summary, we have shown that the H1-R antagonist DLC, when administered intravenously, alleviated the symptoms of AR in a rat model of OVA-sensitized AR. Our studies further suggest that DLC (IV) administration might act through the inhibition of the production

of Th2 cytokines. However, the clinical efficacy of DLC injection in relieving AR and consequent nasal inflammatory symptoms needs further confirmation through human trials. Understanding of the AR pathophysiology is a complex regime and may be controlled by several not-yet-understood mechanisms. Herein, we have suggested some of those possible mechanisms by which DLC may alleviate the symptoms of AR, and further insights are required to explore more of the unknown mechanisms. The anti-histaminic drugs are generally prescribed as a combination therapy with glucocorticosteroids because of their limited potential in clinical use. Whether monotherapy with DLC

through parenteral administration can have a prominent advantage over the other antihistaminic drugs needs further investigation. However, in this study, we have shown the pharmacological efficacy of DLC (IV), a novel antihistaminic class of drug, which should be studied more extensively in order to establish stronger basis for its wider therapeutic use. We have also suggested several mechanisms by which this drug may alleviate the symptoms of AR that can serve for a better understanding of the AR pathophysiology and help in finding novel therapeutic targets for this disease.

Conclusion

In this study, we confirmed the pharmacological efficacy of DLC injection and reported the mechanisms of action through which it modulates the AR symptoms. It restrained

the Th2 responses that triggered the inflammatory cascade in AR and improved the level of Th1 cytokines. VCAM-1, iNOS, and NO production were also inhibited following DLC (IV) administration. Therefore, DLC injection, which effectively relieved the nasal inflammatory symptoms and altered the T-cell responses in our experimental paradigm, should be investigated further for its clinical application. ④

Acknowledgments

We acknowledge the grant and drug support from Hefei Industrial Pharmaceutical Institute Co., Ltd., Hefei, China; and China Pharmaceutical University (CPU), Nanjing, China. The education of Arijit Ghosh at CPU is supported by the China Govt. Scholarship-Chinese University Program for the international students borne by the China Scholarship Council (Grant# 2013GXZB28).

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