Lymphocyte expression of CD4+CD25^{hi} and adhesion molecules in children with Atopic dermatitis: the effect of Levocetirizine treatment

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There is considerable evidence that several novel H1 antihistamines possess anti-allergic/ anti-inflammatory properties, through inhibition of leukocyte activation. Levocetirizin and other H1 antihistamines are considered central to the treatment of atopic dermatitis (AD) associated pruritis; however there is a lack of studies of possible anti-inflammatory effect of these drugs in children with AD. In this study, we investigated the lymphocyte sub-population profile in the peripheral blood of 15 children with AD at baseline and following two weeks of levocetirizine treatment. The clinical symptoms and flow cytometric analysis of the percentage expression of CD4+CD25+ subsets on T cells, as well as expression of the adhesion molecules; CD4+CD54+ (ICAM-I) and CD4+CD62+ (L-Selectin) on T cells were evaluated. The children exhibited a reduction in the percentages of the eosinophil count ($p<0.05$) as well as major clinical symptoms, itching/ scratching ($p<0.05$) and the subsequent bleeding of lesions ($p<0.05$); however the total symptom score was not significantly changed. A significant increase was observed in CD4+CD25^{hi} Treg cells while CD4+CD54+ (ICAM-I) cells were significantly decreased, and no significant change was observed in other populations. Reduction of CD4+CD54+ may be associated with suppression of IgE production and hence reduced mast cell recruitment into the inflammatory sites, on the other hand; expansion of CD4+CD25^{hi} indicates that Treg-mediated host immune defenses are augmented. Our study suggests the potential of the anti-inflammatory effects of levocetirizine in allergic inflammation.

Key words: Dermatitis, Treg cells, Adhesion molecules, Levocetirizine.

Introduction

Atopic dermatitis (AD) is a common chronic inflammatory skin disorder with intense pruritis and typical cutaneous symptoms, and is frequently seen in patients with a family history of atopy (1). Patients with AD are a heterogeneous group of whom about 80%

show immediate type skin reactions and elevated serum IgE levels (2). In most cases AD appears in early childhood, current lifetime prevalence is estimated to be between 10-20% in children and 1-3% in adults (3). For diagnosis, at least three of both the major and minor criteria (pruritis, typical morphology and distribution, chronic or relapsing skin lesions, personal or family history of atopy etc) should be present (4). The pathogenesis of AD involves a complex inflammatory process which is not yet fully understood and is constantly undergoing revision as more data become available (5, 6). Recent studies indicate that the marked elevation of IgE is the result of T-cell dysregulation in AD patients (7). Over the past few years it has become increasingly clear that T-cells contribute to the abnormal regulation of the immune response in atopic diseases. Th2-type CD4+ T cells appear to be crucial but still little is known about the contribution of other subsets of T cells (8).

In recent years, a specific subset of regulatory T cells bearing a CD4+CD25+ T-cell phenotype has been the focus of extensive investigation (9). These T cells, endowed with distinct immuno-modulatory properties, are important components of the homeostasis of the immune system, as impaired CD4+CD25+ T-cell activity can cause both autoimmune and allergic diseases (10, 11). There is evidence of the role of $CD4^{+}CD25^{+}$ regulatory T cells in suppressing T-cell responses to allergens (12, 13). Expression of the transcription factor, Foxp3, is critical to the development of $CD4^{+}CD25^{hi}$regulatory T cells with suppressor function. It was recently reported that human $CD4+CD25^{hi}$ T cells associated with inflammatory diseases such as AD may be a mixture of activated effector T cells and regulatory T cells, the two subtypes were identified on the basis of differential expression of the chemokine receptor CCR6 (14). Furthermore this study found that activated $CD25^{hi}$ T cells that lack expression of CCR6 promote TH2 responses.

Recent studies have demonstrated that several adhesion molecules play a critical role in the recruitment and migration of leucocytes to sites of inflammation in various diseases (15, 16). Important adhesion molecules expressed on leucocytes or endothelial cells include intercellular adhesion molecule-1 (ICAM-1) and L-selectin. The levels of adhesion molecules have been reported to increase in patients with allergic diseases (17-20). Higher levels of adhesion molecules in serum samples from atopic individuals may reflect the up-regulation of cell-surface ICAM-1 expression in allergic inflammation.

There is now considerable evidence from both in vitro and in vivo studies that several novel H1 antihistamines possess anti-allergic/ anti-inflammatory properties, through inhibition of leukocyte activation and reduction of ICAM1 expression on epithelial cells (21, 22, 23). Levocetirizine, as a R-entantiomer of Cetirizine dichloride having high bioviability and rapid onset of action, is effective for treatment of allergic rhinitis and chronic urticaria, showing several anti-inflammatory effects that are observed at clinically relevant concentrations that may enhance its therapeutic benefit. (21, 24). Levocetirizin and other H1 antihistamines are considered central to the treatment of AD associated pruritis and are widely used despite a lack of double blind randomized clinical trials (25). Also, there is lack of studies of the possible anti-inflammatory effect of H1 antihistamines in children with AD.

The aim of this study was to investigate the effect of levocetirizine on lymphocyte expression of $CD4+CD25^{hi}$ T cells and the adhesion molecules ICAM-I and L-selectin in children having a moderate – form of atopic dermatitis from early childhood.

Patients and Methods

Patients

The study included 15 atopic children; 9 females and 6 males with an age range of 7-14 years old (mean age 12.36 ± 0.9) diagnosed with moderate to severe atopic dermatitis. All patients were diagnosed from early childhood (before 5 years of age). The diagnosis of atopic dermatitis was based on a constellation of typical clinical features, such as extended eczematous lesions with pruritis and scratching of affected areas. Chronic or relapsing dermatitis was frequently associated with personal or family history of atopic disease. Atopy was confirmed by the increased level of specific IgE to one or more inhalant and /or food allergens (food allergy was implicated in approximately one third of our patients). Severity of the disease was assessed by a physician on the basis of skin condition experienced over the past 6 weeks, expressed as a total clinical symptoms score (TCSS) (1-12) which included the following: a) skin thickening: 1= mild, 2 = moderate, 3 = severe; b) skin itching/scratching: 1= mild, without significant changes in daily activities and without night sleep disturbance, 2 = moderate with occasional night sleep awakening, 3 = severe itching with frequent sleep disturbance; c) location of the conditions:1 = mild: flexuous side of arms and/or legs, 2 = moderate: +lesions on the neck and face, 3 = severe: +lesions on other part of the body with excessive dryness / scaling or blisters; d) number of times/ year that symptoms flare up: 1 = mild: 1-2 times , 2 = moderate: 3-5 times, 3 = severe: more than 6 times. Only children with moderate to severe dermatitis (clinical score ranged 8-12), were enrolled in the study. Patients on antihistamines and topical corticosteroids within the previous week were excluded. Fourteen healthy children with no history or sign of atopic diseases; 8 females and 6 males with an age range of 8- 15 years old (mean age 13.5 ± 0.6) served as a control group. Blood samples were collected from children with AD at baseline and following two weeks of treatment with levocetirizine (5 mg/ day); one blood sample was collected from each healthy child. All samples were collected in the early morning. Informed consent was obtained from the parents of the patients and the controls.

Measurement of Lymphocyte subpopulations

Five ml of peripheral venous blood were collected from each subject in EDTA tubes and analyzed within 4-6 hours. Fifty µl of blood were incubated for 30 min at room temperature with 5 µl of flourescein-isothiocyanate (FITC), phycoerythrin (RD1) or PerCP (peridin chlorophyll protein) conjugated monoclonal antibodies (mAb), to surface markers of interest. The cells were then treated with Q-prep (Coulter Corporation, Hialeah, FL, USA) for hemolysis, stabilization and amplification of the antigen-antibody reaction and fixation with paraformaldehyde. Two and three color fluorescence analysis using an automated flow cytometer (Coulter Epics Altra) was performed. Positive analysis regions for cells expressing specific surface antigens were compared with isotypic controls and the specific binding of fluorophore-conjugated monoclonal antibodies was analyzed according to standard methods recommended by the manufacturer. Monoclonal antibodies specific for human CD4+CD54+ (ICAM1+ T cells), CD4+CD62+ (L-Selectin+ T cells) and CD4+ CD25+ (activated T cells – some with a regulatory phenotype) were used. All fluorophores were purchased from Immunotech, Beckman Coulter Corporation, Hialeah, FL, USA. Typical histogram data are depicted in Fig. 1, showing CD4+ and CD25+ subpopulations. The total population of CD4+ cells are mostly contained in areas B1 +M1 + M2, with CD4 + $CD25+_{low}$ cells represented in area M1;

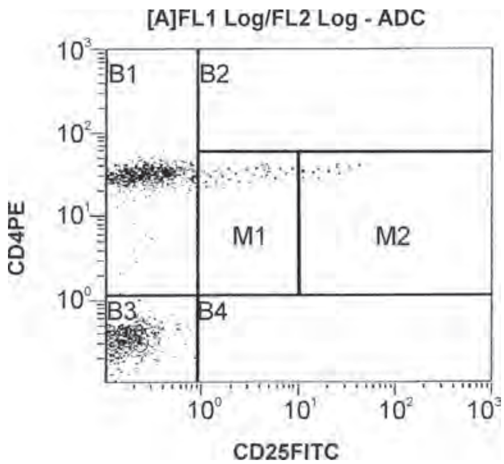

Figure 1 Expression of CD4+CD25+ subpopulations in one participant in the study. CD4+CD25+^{low} cells are represented in area M1; and CD4+CD25+^{hi} in area M2.

CD4 + CD25+hi in area M2 and CD4+ cells in area B1 considered to express negligible levels of CD25 (i.e. these are non-activated T cells). The frequency of CD4 + CD25+low was calculated as the frequency ratio for M1/B1 +M1 +M2 and the frequency of CD4 + CD25+hi as M2/B1 +M1 + M2. This analysis was used for cells taken from each participant in the study.

Statistical analysis

Data are presented as box plots displaying medians and interquartile ranges (IR) for the variables that exhibited statistically significant differences when compared between the study groups. As the variables evaluated were not distributed normally, the mean comparisons were done by non-parametric analysis (Kruskall–Wallis and, if significant, Mann–Whitney *U* test). All reported *p*-values represented two-tailed tests and $p \leq 0.05$ was considered statistically significant. Non-parametric Spearman correlations were performed to measure the association between variables. Statistical analyses were performed using the SPSS for Windows Program Version 14 (Norusis/SPSS Inc.).

Results

Levocetirizine treatment of AD patients had improved quality of life expressed as fewer disturbances of night sleep. The analysis of the clinical symptom score showed that levocetirizine had reduced the itching/scratching circle (p=0.011) as well as the bleeding of lesions (p=0.006) (Fig.2); how-

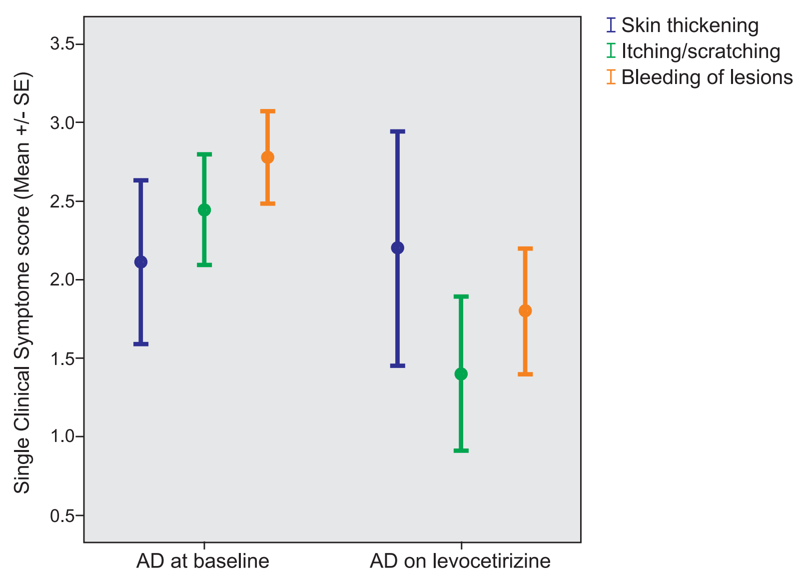

Figure 2 Box plot representation of the single symptoms score in AD patients. Comparisons were made between the groups studied: AD group at baseline (n = 15), AD following levocetirizine treatment and the control group (n =7). Statistical differences (*) were considered significant at p < 0.05.

Table 1 Median and IR (interquartil ranges) of eosinophils and T lymphocyte subpopulations in atopic dermatitis at baseline, following levocetirizine treatment and in controls

	Atopic Dermatitis at Baseline		Atopic Dermatitis on Levocetirizine		Control	
	Median	IR (75-25)	Median	IR (75-25)	Median	IR (75-25)
Eosinophils	7.85**	14.35-3.68	2.5ψ	6.2-1.93	2.2	3.2-1.2
CD4+CD25+ cells	2.97	4.45-2.0	1.93	2.47-1.3	2.4	2.3-0.5
CD4+CD25^{hi}	0.33*	0.56-0.3	0.99ψ	1.85-0.61	1.2	1.5-0.21
CD4+CD25^{low}	1.65	2.65-1.4	1.58	2.1-1.0	1.0	2.0-0.34
CD4+CD54+ cells	8.0**	9.2-6.1	2.5ψψ	2.9-1.99	2.3	5.5-1.7
CD4+CD62+ cells	8.1	17.8-6.5	10.95	15.5-10.9	3.9	19.3-2.7

Comparisons were made between eosinophil count and T lymphocyte expression of surface antigens in AD and control, statistical differences were considered significant at p<0.05.
**p<0.05, ** p<0.01 versus healthy control, ψ P<0.05, ψψ p<0.01 versus baseline*

ever the total symptom score was not significantly changed. As shown in Table 1, levocetirizine treatment significantly reduced the percentages of eosinophils (p= 0.027). Lymphocyte expression of CD4+CD25+ T cells with two subsets: CD4+CD25^{hi} cells and CD4+CD25^{low}cells are shown. Following levocetirizine treatment, no significant change was observed in the percentage of CD4+CD25+ cells (median 1.93; IR: 2.47-1.3) versus baseline (median 2.97; IR: 4.45-2.0; p= 0.132), the percentage of CD4+CD25^{hi} was significantly increased (median 0.99; IR: 1.85-0.61) versus baseline (median 0.33; IR: 0.56-0.3; p= 0.048), while the CD4+CD25^{low} subset was not significantly changed (median 1.58; IR: 2.1-1.0) versus baseline (median 1.65; IR: 2.65-1.4; p= 0.295). CD4+CD54+ T cell subset (ICAM-I) was significantly reduced (median 2.5; IR 2.9-1.99) versus baseline (median 8.0; IR 9.2-6.1; p= 0.024) (Table 1 and Fig. 4), on the other hand CD4+CD62+ T cell subset (L-selectine) was not significantly changed (median 10.95; IR 15.5-10.9) versus baseline (median 8.1; IR 17.8-6.5; p= 0. 241).

Discussion

The management of AD is difficult due to the fact that its pathogenesis is still obscure. A major therapeutic challenge is to reduce the itching/ scratching circle, which could be achieved by controlling chronic allergic inflammation. H1-antihistamines are widely used in AD patients for the control of pruritis, despite the lack of double blind randomized clinical trials (25, 26). Antihistamine action in the treatment of allergic disease is the competitive antagonism of histamine binding to cellular receptors. Recently, many studies have shown that H1-antihistamines, beside their antihistaminic effects, have additional anti-inflammatory properties (5, 21, 22, 23). They are capable of inhibiting inflammatory cell migration and activation, and adhesion molecule expression in tissues affected by allergic inflammation (24, 27). Such effects are already known in the treatment of seasonal allergic rhinitis (28) and chronic urticaria (29) both in adults and children. However few studies have addressed the anti-inflammatory activities of H1- histamine antagonists in AD patients (30, 31). Levocetirizine, as an active enantiomer of cetirizine, is one of the most recent antihistamines and is indicated for symptomatic relief in different allergic diseases, with clear evidence of possessing anti-inflammatory activities (32), which could be useful in the treatment of AD patients.

We evaluated the efficacy of levocetirizine in fifteen AD patients to determine whether two weeks of treatment (5 mg once daily) would induce clinical improvement shown through CSS and changes in inflammatory parameters. As expected, our results were similar to the findings of other studies which showed significant improvement, expressed as reduction of CSS, particularly itching/ scratching (Fig. 2) and subsequent improvement in quality of life expressed as less disturbed night sleep.

Our understanding of the complex inflammatory process in AD is constantly undergoing revision as more data become available (26). It is already known that interaction among susceptible genes and environmental factors activate different immune cells and their products, leading to clinical manifestation. The phenotype of AD depends on factors which have an important impact on the severity of the disease. T cell-driven inflammation appears to be crucial in the pathogenesis, characterised by skin infiltration with migrating T lymphocytes. Although CD4+ T cells appear to be crucial in AD pathophysiology (8), little is known about the role of a specific subset of T cells bearing a CD4+CD25+ phenotype in AD patients (32, 33). As shown in our results, we have evaluated that a particular T cell subset in peripheral blood is a parameter of allergic inflammation. We found no significant difference in the percentages of these cells following levocetirizine treatment (Table 1). A particularly interesting outcome of treatment with this drug is the apparent induction of an expanded CD4+CD25+hi subpopulation ($p<0.05$) (Fig. 3) which suggests that it might augment Treg-mediated host immune defence. It has been shown that histamine stimulation of H4 receptors suppresses pathogenic processes and promotes expansion of peripheral blood Treg subpopulations (34). These findings raise the possibility that levocetirizine-H1 interaction may converge or reinforce histamine stimulation of H4 receptors.

Eosinophil participation in allergic inflammation depends on maintenance of cell viability and function. Eosinophils are recruited and activated at the site of inflammation, releasing a wide variety of mediators (33). Similar to the results shown by Segwik (27) that cetirizine is capable to affect eosinophil survival in patients with AD, our results showed a significant reduction in eosinophil count (Table 1).

Additionally, similar to other studies (6, 32, 35), our results confirmed the possible immune modulating role of levocetirizine, through reduction of adhesion molecule expression, especially ICAM-I (Fig. 4). Possibly, levocetirizine is capable of regulating the release of cytokines and chemokines and consequently reduces recruitment of the inflammatory cells (28). In contrast to ICAM-I molecules, we could not find significant reduction in L- selectin (Table 1). Such results could be a consequence of the fact that the selectin family mediates tethering and rolling of leukocytes while the Ig superfamily, including ICAM -1, is critical for the firm adhesion (6). It is also possible that ICAM –1 has closer cooperation with L-selectin, to mediate optimal leukocyte rolling (36), which we did not observe in this study. Reduction of expression of ICAM –1 and the selectin family may be responsible for suppression of IgE production, as explained by Shimada et al (6), which could reduce rapid mast cell recruitment into the inflammatory sites. Our results confirm previous analyses (28, 36) of the anti-inflammatory effects of levocetirizine in allergic inflammation. CONCLUSION: This study demonstrates that levocetirizine induces *in vivo* suppression of eosinophils as well as ICAM I expression on CD4+ T cell of AD patients, on the other hand, expansion of CD4+CD25+hi Treg cells was observed. These findings may indicate the important immunomodulatory effects of this drug and suggest future investigation of the cellular and molecular mechanisms underlying the role of antihistamine in immunoregulation.

References

1. Allam, JP, Novak N. The pathophysiology of atopic eczema Clin Exp Dermatol. 2006;31(1):89-93.
2. Jenerowicz D, Czarnecka-Operacz M, Silny W. Selected eosinophyl proteins as a marker of inflammation in atopic dermatitis patients. Acta Dermatol Croat. 2006;14(2):73-80.
3. Leung DYM, Bieber T. Atopic dermatitis. The Lancet. 2003;361:151-60.
4. Hanifin JM, Rajka G. Diagnostic feature of atopic dernmatitis. Acta Dermatovenerologica. 1980;114 (Suppl 114):87-92.
5. Jenerowic D, Czarnecka –Operatcz Msilnv W. Selected eosinophil protein asa marker of inflammation in atopic deramatitis patients. Acta Dermattovenerol Croat. 2006;14:(2):73-80.
6. Shimada Y, Hasegava M, Kaburagi Y, Hamagushi Y, at al. L selectin or ICAM1 deficiency reduce an immediate type hypersensitivity response by preventing mast cells recruitment in repeated elicitation of contact hypersensitivity. J Immunol. 2003; 15;170(8):4325-34.
7. Lurzius-Spencer M, Halone M, Lohman IC, Martinez FD, Wright AL. Prenatal factors associated with the development of eczema in the first year of life.. Ped Allergy and Immunol. 2005;16:19-26.
8. Henino A, Vocanson M,Toussaint Y, Rodet K et al. Skin infiltrating CD8+T cells initiate Atopic Dermatitis lesion. J Immunol. 2007;178(9):5571-7.
9. Shi H-Z Qin X-J. CD4+CD25+regulatory T lymfocites in allergy and asthma. Allergy. 2005;60:986-95.
10. Shwarz RH. Natural regulatory T cels and self tolerance. Nat Immunol. 2005;6:327-30.
11. Umetsu DT, Akbary O, De Kruy. RH. Regulatory T cells control development of allergic diseases and asthma. JACI. 2003;112:480-7.
12. Ling EM, Smith T, Nguyen XD, Pridgeon C, Dallman M, Arbery J, et al. Relation of CD4CD25 regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. Lancet. 2004;363:608-15.
13. Walker MR, Kasprowicz DJ, Gersuk VH, Van Landeghen M, Buckner JH, Ziegler SF. Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4CD25- T cells. J Clin Invest. 2003;112:1437-43.
14. Reefer AJ, Satinover SM, Solga MD, Lannigan JA, Nguyen JT, Wilson BB, Woodfolk JA. Analysis of CD25hiCD4+ "regulatory" T-cell subtypes in atopic dermatitis reveals a novel T(H)2-like population. J Allergy Clin Immunol. 2008;121(2):415-22.
15. Springer TA. Adhesion receptors of the immune system. Nature. 1990;346:425-34.
16. Wegner CD, Gundel RH, Reilly P, Haynes N, Letts LG, Rothlein R. Intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. Science. 1990;247: 456-9.
17. Kojima T, Ohno A, Aoki T, Hayashi N, Kobayashi Y. Circulation ICAM-1 levels in children with atopic dermatitis. Ann Allergy. 1994;73:351–5.
18. Hashimoto S, Imai K, Kobayashi T, et al. Elevated levels of soluble ICAM-1 in sera from patients with bronchial asthma. Allergy. 1993;48:370–2.
19. Chihara J, Yamamoto T, Kurachi D, Nakajima S. Soluble ICAM-1 in sputum of patients with bronchial asthma. Lancet. 1994;343:1108.
20. Ohashi Y, Nakai Y, Tanaka A, Kakinoki Y, Washio Y. Soluble adhesion molecules in middle ear effusions from patients with chronic otitis media with effusion. Clin Otolaryngol Allied Sci. 1998;23(3):231-4.
21. Caproni M, Volpi W, Giomi B, Torchia D, Del Bianco E, Fabbri P. Cellular adhesion molecules in chronic urticaria: modulation of serum levels occurs during levocetirizine treatment. Br J Dermatol. 2006;155:1270-4.
22. Traddi-Hoffmann C, Munster I, Ring J, Behrendt H. Impact of desloratadine in and loratadine on the crosstalk between human keratinocytes and leucocites: Implication for anti-inflammatory activities of antihistamines. Int Arch Allergy Immunol. 2006;4:315-20.
23. Leurs R, Church MK, Taglialatela M. H1-antihistamines: inverse agonism, anti-inflammatory actions and cardiac effects. Clin Exp Allergy. 2002;32:489–98.
24. Walsh GM. Levocetirizine: an update. Curr Med Chem. 2006;13(22):2711-5.
25. Dieogen T. Long term treatment with cetirizin of infants with atopic dermatitis: a multi country, double blind randomized, placebo-controled trial (the ETAC trial) over 18 months. Pediatr Allergy Immunol. 2002;13:278-86.
26. Leung DYM, Nicklas RA, Li JT, Bernstein L, Blessing-Moore J, Boguniewicz M, et al. Disease management of atopic dermatitis: an updated practice parameter. Annals of Allergy, Asthma & Immunology. 2004;93(3); S1-S21.
27. Sedgwick JB, BusseWW. Inhibitory effect of Cetirizine on cytokine-enhanced in vitro eosino-

phyl durvival. Ann Allergy Asthma Immunol. 1997;78:581-5.

28. Lokey RF, Widlitz MD, Mitchell DQ et al. Comparative study of cetirizine and terfenadine versus placebo in the symptomatic management of s seasonal allergic rhinitis.Ann Allergy Asthma Immunol 1996;76:448-54.
29. Breneman D, Bronsky EA, Bruce S, Kalivas JT, Klein GL, Roth HL, Tharp MD, Treger C, Soter N. Cetirizine and astemizole therapy for chronic idiopathic urticaria: a double-blind, placebo-controlled, comparative trial. J Am Acad Dermatol. 1995;33(2 Pt 1):192-8.
30. Gonda A, Gal I, Szanto S, Sarraj B, Hunyadi J, Mikecz K, Glant TT. CD44 but not I selectin is critically involved in leucocite migration int skin in a murine model of allergic dermatitis. Exp Dermatol. 2005;14:700-8.
31. Albanezi C, Pastore E, Fanales-Belasio E, Girolomoni G. Cetirizine and hydrocortisone differentially regulate ICAM 1 expression and chmokine release in cultured human keratinocytes. Clin Exper Allergy.1998;28:101-9.
32. Wu P, Mitchel S, Walsh GM. A new antihistamine levocetirizine inhibits eosinohil adhesion to vascular cell adhesion molecule-1 under flow conditions. Clin Exper Allergy. 2005;35:1073-9.
33. Kroegel C, Virchow JC Jr, Luttmann W, Walker C, Warner JA. Pulmonary immune cells in health and disease: the eosinophil leucocyte (Part I). Eur Respir J. 1994;7(3):519-43.
34. Morgan RK, McAllister B, Cross L, Green DS, Kornfeld H, Center DM, Cruikshank WW. Histamine 4 receptor activation induces recruitment of FoxP3+ T cells and inhibits allergic asthma in a murine model. J Immunol. 2007;178:8081-9.
35. Fumagalli F, Baiardini I, Pasquali M, Compalati E, Guera L, Massacane P, Canonica GW. Anihistamines: do they work? Further well-controlled trials involving larger samples are needed. Allergy. 2004;59 (Suppl. 78):74-7.
36. Steeber DA, Tang ML, Green NE, Zhang XQ, Sloane JE, Tedder TF. Leukocyte entry into sites of inflammation requires overlapping interactions between the L-selectin and ICAM-1 pathways. J Immunol. 1999;163(4):2176-86.