

CETIRIZINE REDUCES CYTOKINES AND INFLAMMATORY CELLS IN CHILDREN WITH PERENNIAL ALLERGIC RHINITIS

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SUMMARY: Cetirizine has been demonstrated able of reducing nasal inflammatory infiltration in children with allergic rhinitis and cytokine production in in vitro studies.

The aim of this double-blind, placebo-controlled, and randomized study was to evaluate cytokine pattern and inflammatory cells in children with perennial allergic rhinitis, before and after treatment with cetirizine or placebo.

Twenty children with perennial allergic rhinitis were evaluated, 13 males and 7 females (mean age 13.4 years). Inflammatory cells and cytokines were evaluated by scraping and nasal lavage, before and after 2-weeks administration of cetirizine or placebo.

IL4 and IL8 were measured by immunoassay and inflammatory cells were counted by conventional staining.

Cetirizine treatment induced a significant decrease of IL4 ($p<0.01$) and IL8 levels ($p=0.01$). A significant reduction of the inflammatory cells was detected in actively-treated children, both concerning neutrophils and eosinophils ($p<0.01$). Moreover, cetirizine significantly reduced nasal obstruction score ($p=0.007$).

This study shows the cetirizine effectiveness in exerting anti-inflammatory activity by modulating cytokine pattern and by reducing inflammatory infiltration in children with perennial allergic rhinitis.

Key-words: Children - Perennial allergic rhinitis - Inflammatory cells - Cytokines - Cetirizine.

INTRODUCTION

Allergic rhinitis is the most frequent immune-mediated disease and its prevalence is progressively increasing. Allergic rhinitis is sustained by an inflammatory response (1). Allergen exposure induces mast cell activation with consequent release of mediators and cytokines (such as IL-4, IL-5, IL-8, TNF- α) that induce inflammatory cell recruitment and activation at the target organ level (2).

Eosinophil presence on nasal smear can be considered suggestive for an allergic reaction. Also neutrophils are early recruited after allergen challenge as a consequence of IL8 chemotactic activity.

Cetirizine, the carboxylated metabolite of hydroxyzine, is a highly selective antihistamine, with additional antiallergic activities since it prevents changes consequent to allergen specific challenge (3) and reduces

eosinophil and neutrophil infiltration at the site of the allergic reaction (4).

Furthermore, cetirizine reduces the minimal persistent inflammation (i.e. neutrophil and eosinophil infiltration and ICAM-1 expression on epithelial cells) at nasal level in asymptomatic children with asthma due to mite allergy (5).

Anti-inflammatory properties of antihistamines are still under investigation (6). Therefore, the present study was designed to assess the possible anti-inflammatory activity of cetirizine in children with perennial allergic rhinitis.

MATERIALS AND METHODS

■ **Subjects:** Twenty allergic children were prospectively evaluated, 13 boys and 7 girls, mean age 13.4 years (range 12-16 years). A detailed clinical history and a complete physical examination, including allergy evaluation, were performed.

The diagnosis of perennial allergic rhinitis was made on the basis of skin prick test for a panel of common allergens described elsewhere (3, 4, 5) and according to international guideline (7).

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■ **Study design:** To evaluate the cetirizine effect on cytokine and cellular pattern in a group of children with perennial allergic rhinitis, we excluded all the children who met the following exclusion criteria: acute upper respiratory infection and use of antibiotics, nasal or oral corticosteroids, and antihistamines within the previous 4 weeks.

The study was conducted as double-blind, placebo-controlled, and randomised during the winter to exclude the interference of pollen exposure.

Ten children were treated in a double blind manner with cetirizine (10 mg/daily in the morning), and ten children with placebo for 2 weeks.

Nasal lavage and nasal cytology were performed in all subjects before and thereafter the treatment.

The study was conducted according the Helsinki declaration and an informed oral consent was obtained from children's parents.

■ **Nasal symptoms:** As nasal obstruction represents an annoying symptom in allergic rhinitis, mainly in the perennial form, we focussed our attention on this symptom. We evaluated this issue at the two visits. An arbitrary score was used according to previous studies (4, 8). A 5-point scale was employed with 0 indicating free nasal patency and four indicating complete block to nasal airflow passage.

■ **Nasal cytology:** Nasal cytologic specimens were obtained by scraping the head of the inferior turbinate with a cotton swab, as described in previous reports (4, 5, 8). Briefly, after the nasal scraping, the cotton tip of the swab was immersed in a plastic tray with phosphate-buffered saline (PBS) and transferred to a 10 mL polypropylene tube. The recovered fluids were centrifuged at 220 g per minute for 10 minutes, and each pellet was re-suspended in PBS (2 mL). Cell suspensions were filtered to reduce the quantity of mucus, and cytospin slides were prepared by using standard techniques.

Smears were stained with Diff Quik stain and were analysed by optic microscope (Olympus U-SPT). The number of inflammatory cells was expressed as a mean of 10 optical fields at 100x magnification. Samples were examined in a blinded fashion.

■ **Nasal lavage:** A lavage of the nasal cavity was done using 5 mL of physiologic saline, according to standard methods described elsewhere (8). This procedure was performed with the patient's head bent backwards during closure of the soft palate. The recovered fluid was comparable in all groups, it was collected to dose cytokine levels and stored at -20° (8).

■ **Cytokine measurement:** The cytokines were measured with the ELISA method (R&D Systems, U.S.A.).

This assay used the quantitative sandwich enzyme immunoassay technique. The minimum detectable level was less than 1 pg/mL for IL4 and 10 pg/ml for IL8. A monoclonal antibody specific for each cytokine was pre-coated onto a microplate. Standards and samples were pipetted into the wells and each cytokine present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for cytokine was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of cytokine bound in the initial step. The color development was stopped and the intensity of the color measured.

■ **Statistical analysis:** Changes on inflammatory cells and cytokines at baseline and after treatment were assessed by two factor repeated-measures ANOVA with Duncan post hoc test. Differences in nasal obstruction score at baseline and after treatment were assessed within groups by Wilcoxon Signed Rank test, and between groups by Mann-Whitney U test. Correlation between symptoms score and inflammatory parameter were evaluated by Spearman test. P values <0.05 were considered statistically significant. Data were presented as means $\pm$ SD or median (upper-lower quartiles).

RESULTS

■ **Subjects:** Twenty allergic children with persistent rhinitis were examined. Twelve children were sensitised to *Dermatophagoides farinae* and *pteronysinus*, whereas 8 were polysensitised to mites and pollens. All children assumed the prescribed therapy without significant side effects.

■ **Nasal symptoms:** At baseline, nasal obstruction scores were not different between the two groups. After treatment, nasal obstruction significantly decreased in cetirizine group only ($p=0.007$), and was significantly different in comparison with placebo-group ($p=0.02$) as reported in table 1.

■ **Nasal cytology:** Figure 1 summarized symptoms and eosinophils reduction after cetirizine administration. At baseline, eosinophils tended to be lower in placebo group than in cetirizine group, but without significant difference ($p>0.05$), while neutrophils were not different between the two groups. After treatment with cetirizine, both inflammatory cells decreased significantly ($p<0.01$, figure 2). However, after treatment only neutrophils were significantly different from placebo ($p<0.01$).

■ **Cytokine levels:** After treatment, IL4 levels were significantly decreased in cetirizine group only ($p=0.01$) with a significant difference with placebo group ($p<0.01$) (figure3). Equally, IL8 values significantly decreased in cetirizine group only ($p=0.03$) with a significant difference with placebo group ($p=0.01$) (figure 4).

■ **Correlations:** At baseline, in patients treated with cetirizine, there was a significant correlation between eosinophils and nasal obstruction score ($r=0.65$, $p<0.05$), which tended to be significant also after treatment ($r=0.60$, $p=0.06$). In addition, there was a significant correlation between neutrophils and nasal obstruction score after cetirizine treatment ($r=0.68$, $p=0.03$).

	Cetirizine group	Placebo group	
Pre	3 (2-3)	2.5 (2-3)	$P=0.66$
Post	1 (1-2)*	2 (2-3)	$P=0.02$

Table 1: Nasal obstruction score.

Data are expressed as median (lower-upper quartiles); *, $p=0.007$.

DISCUSSION

There is consent concerning the role played by cytokines and leukocytes as pathogenetic factors in the maintenance and amplification of inflammatory events in perennial allergic rhinitis (9, 10, 11).

However, very few data are available about the possible *in vivo* anti-inflammatory activity exerted by antihistaminic treatment in this issue.

Cetirizine has been demonstrated to reduce the levels of expression of IL4 in the nasal-associated lymphoid tissue of ovalbumin-sensitised mice (12). In addition, cetirizine reduced the eosinophil count in the nasal septal mucosa (12).

Arnold and coll. demonstrated that cetirizine diminished the release of IL8 from human epithelial cell line A549 (15). A549 cells were stimulated with PMA and $TNF\alpha$, respectively, and cetirizine lowered IL8 gene expression on those cells (15). Furthermore, these authors showed that cetirizine decreased the amount of accessible DNA-binding site of the nuclear transcription factor kappa B (NF-kB).

NF-kB plays a crucial role in the pathway of allergic inflammation. Actually, the 5'-flanking regions of both genes coding for ICAM-1 and IL8 contain putative binding sites for the transcription factor NF-kB (14).

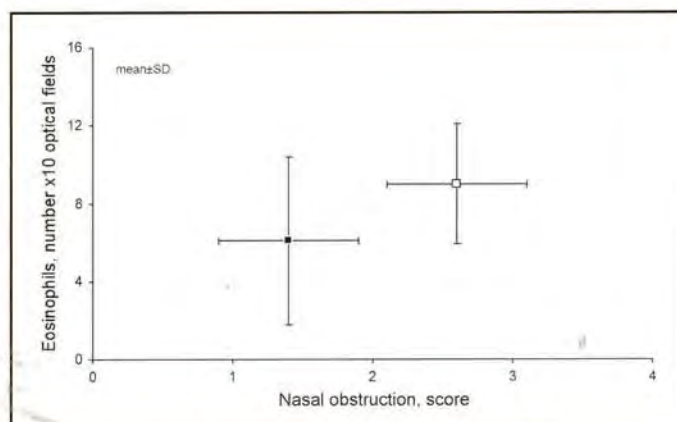


Figure 1: Nasal obstruction scores and eosinophil counts in allergic children after treatment. Black square refers to cetirizine group, white square refers to placebo group. Data are expressed as means + SD.

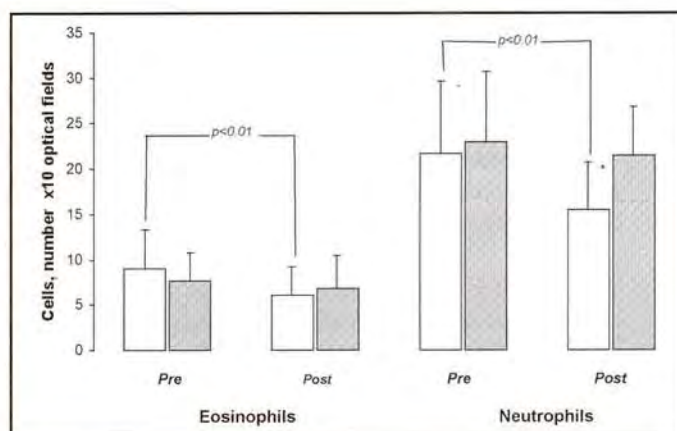


Figure 2: Eosinophil and neutrophil counts in allergic children before and after treatment. Open bars refer to cetirizine group, darkened bars to placebo group. Data are expressed as means + SD. * = $p<0.001$.

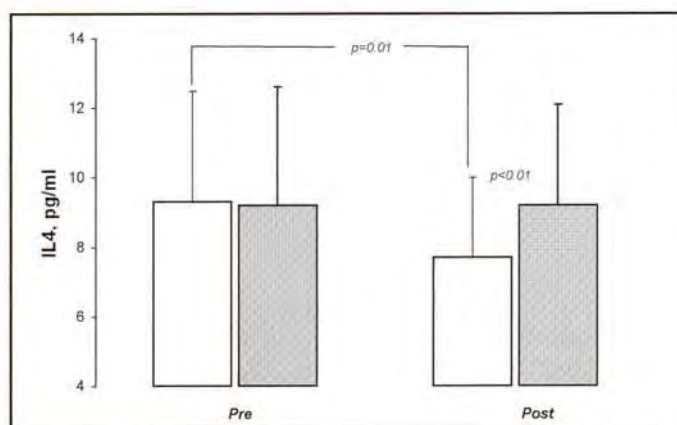


Figure 3: IL4 levels in allergic children before and after treatment. Open bars refer to cetirizine group, darkened bars to placebo group. Data are expressed as means + SD.

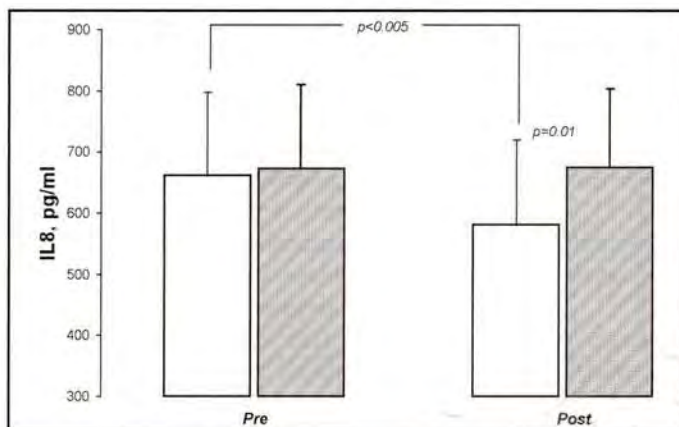


Figure 4: IL8 levels in allergic children before and after treatment. Open bars refer to cetirizine group, darkened bars to placebo group. Data are expressed as means + SD.

These authors hypothesized that cetirizine might exert its inhibitory activity on the signal transduction pathway of $\text{TNF}\alpha$ and PMA upstream of NF- κB (13).

Therefore, the present study was aimed at investigating the potential anti-inflammatory activity of cetirizine in allergic children with persistent allergic rhinitis and concomitant chronic rhinosinusitis.

We showed that cetirizine treatment significantly reduced IL4 and IL8 levels in the nasal lavage fluid. Our findings are consistent with the above-mentioned experimental data concerning the cetirizine capacity of reducing cytokine production in experimental models (12, 13).

Moreover, this study confirms previous findings concerning the cetirizine effects on inflammatory cells, mainly regarding the property of reducing eosinophil and neutrophil infiltration in children with seasonal allergic rhinitis (4).

The clinical relevance of our results is supported by the significant reduction of nasal obstruction score

and the relationship between symptoms and cellular infiltration.

In conclusion, this study provides evidence of immuno-modulatory activity exerted by cetirizine by reducing *in vivo* cytokine levels. Further studies should be addressed to correlate clinical and functional data with immunopathological parameters in allergic subjects treated with antihistamines.

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