# ANTIHISTAMINES ADDED TO AN ANTILEUKOTRIENE IN TREATING SEASONAL ALLERGIC RHINITIS : HISTAMINE AND LEUKOTRIENE ANTAGONISM

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**Summary**

Cysteinyl leukotrienes (CysLTs) are a key element of allergic inflammation. Thus, the antagonism of CysLTs appears a potential target of allergic rhinitis management. Antileukotrienes and antihistamines combined showed a synergistic effect in treating seasonal allergic rhinitis (SAR).

This double blind, parallel group, randomized study aimed at evaluating the clinical and anti-inflammatory effects exerted by two combinations of antihistamine plus antileukotriene in patients with SAR and mild intermittent asthma.

Thirty patients were treated with montelukast added to cetirizine (M-C group) or desloratadine (M-D group) for 2 weeks. The visits, including spirometry, nasal scraping and lavage, were performed in all subjects before and directly after the treatment. Evaluation parameters were: i) nasal symptoms, ii) number of eosinophils and neutrophils, and iii) IL5 and IL8 levels.

Both combinations significantly reduced: nasal symptoms ($p<0.001$), eosinophils and neutrophils ($p<0.001$), IL5 ($p<0.001$ for M-C groupand $p<0.01$ for M-D group), and IL8 levels ($p<0.001$ for M-C group and $p<0.05$ for M-D group). M-C combination reduced more significantly than M-D combination: nasal symptoms ($p<0.01$), inflammatory cells ($p<0.01$), and cytokines ($p<0.01$).

The present study demonstrates that these antileukotriene-antihistamine combinations are effective in relieving nasal symptoms in SAR. Moreover, these combinations exert additional anti-inflammatory activity as provided by reduction of inflammatory infiltrate and cytokine levels. Finally, cetirizine might exert more beneficial activity than desloratadine when added to montelukast.

**Key-words:** Seasonal allergic rhinitis; Montelukast; Cetirizine; Desloratadine; Cytokines; Inflammatory cells.

## INTRODUCTION

Allergic rhinitis is basically characterized by an inflammatory process that induces recruitment, accumulation, and activation of inflammatory cells (1).

The activation of inflammatory cells, including mast cells, basophils, eosinophils, antigen presenting cells, and neutrophils, leads to the release of arachidonic acid from the phospholipid layer of the cells' membranes. In turn, arachidonic acid is metabolized by the 5-lipoxygenase pathway to synthesize leukotrienes. Cysteinyl leukotrienes (CysLTs), including LTC4, LTD4, and LTE4, have been recognized as important proinflammatory mediators in allergic rhinitis (2).

Significant amounts of the CysLTs have been recovered from samples of nasal lavage of patients with allergic rhinitis (3). CysLTs induce physiologic responses in allergic rhinitis: increased vascular permeability, tissue edema, airway mucus secretion, impaired ciliary clearance, and eosinophil accumulation (4, 5). In addition, the inhalation of LTD4 causes significant nasal congestion (6). Taken all together these notions, the antagonism of CysLTs appears a potential target of allergic rhinitis management.

Montelukast is a selective antagonist of the CysLT1 receptor and is approved for use in the European Community and in the United States (7). There is increasing evidence suggesting a role of montelukast in relieving symptoms and improving quality of life in patients with allergic rhinitis (8).

Montelukast exerts additional anti-inflammatory activities, including the reduction of the number of peripheral blood eosinophils (9) and the serum concentrations of ECP, soluble ICAM-1, soluble IL2 receptor, IL4, and IL10 in asthmatic children (10, 11).

On the other hand, as histamine constitutes an important mediator in allergic rhinitis, antihistamines are widely prescribed (1).

Moreover, several trials showed that the combination of antileukotriene and antihistamine provided greater

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improvement in the treatment of SAR as recently reported by a review (8).

The present study aimed at evaluating the clinical and anti-inflammatory effects exerted by montelukast added to cetirizine or desloratadine in patients with SAR and mild intermittent asthma.

## METHODS

■ **Patients:** Thirty allergic subjects were evaluated: 18 males and 12 females, 18 to 35 years of age (mean 22.9). A detailed clinical history and a complete physical examination were carried out for each patient.

The diagnosis of SAR was made, according to guidelines (1), on the basis of history and skin prick test positivity for seasonal allergens (i.e. grasses and/or *Parietaria officinalis*), as previously described (12).

The asthma diagnosis and severity were established, according to GINA Criteria (13) and performing spirometry in all patients. All patients had mild intermittent asthma. Thus, short-acting b2-agonists as needed was the only treatment allowed during the study.

■ **Study design:** The study was conducted as double-blind, parallel groups, and randomized.

To evaluate the effects on clinical and inflammatory parameters in a group of allergic patients with SAR, we excluded all the subjects who had: perennial allergic rhinitis, acute and chronic upper respiratory infections, anatomic disorders, nasal polyps, and assumed antibiotics, nasal or oral corticosteroids, and antihistamines within the previous 4 weeks.

Fifteen patients were treated with montelukast (oral tablet 5 mg/daily) plus cetirizine (oral tablet 10 mg/daily) for 2 weeks: the M-C group.

Fifteen patients were treated with montelukast (oral tablet 5 mg/daily) plus desloratadine (oral tablet 5 mg/daily) for 2 weeks: the M-D group.

Visits included history, physical examination, spirometry, nasal scraping and lavage, and were performed at baseline and directly after treatment.

The study was approved by the Review Board and an informed oral consent was obtained from patients.

■ **Nasal symptoms:** The following symptoms were assessed by both the patient and the investigator before and after treatment: nasal obstruction, sneezing, rhinorrhea, and itchy nose. Each symptom was evaluated on the following scale: 0 = absent, 1 = mild (symptom was present but was not annoying or troublesome), 2 = moderate (symptom was frequently troublesome but did not interfere with either normal daily activity or sleep), and 3= severe (symptom was sufficiently troublesome to have interfered with normal daily activity or sleep). A total symptom score (TSS) as the sum of each symptom was considered.

■ **Spirometry:** It was performed by using a computer-assisted spirometer (COSMED, Eurasem, Milan, Italy), with optoelectronic whirlflow meter. Spirometry wa performed as stated by ERS (14), using the European Community for Steel and Coal (ECSC) reference equations.

■ **Nasal cytology:** Nasal cytologic specimens were obtained by scraping the head of the inferior turbinate with a cotton swab, as described elsewhere (12). 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 220g 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 (Sigma, Milan, Italy) and were analysed by optic microscope (Olympus U-SPT, Olympus Italy, Rome). The number of eosinophils and neutrophils 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 (15). This 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° (15).

■ **Cytokine assessment:** IL5 and IL8 were measured with ELISA method (R&D Systems, Denver, Colorado). This assay employed the quantitative sandwich enzyme immunoassay tecnique. The minimum detectable dose was less than 1 pg/mL for IL5 and less than 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 wasbound by the immobilized antibody. After washing away any unbound sub-

stances, 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 addedto the wells and color developped 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:** Parametric data were analyzed by two-way analysis of variance (ANOVA) with Duncan post-hoc and presented as means + SD. Non-parametric data were analyzed by Wilcoxon Signe Rank and Kruskal-Wallis tests to detect differences within and between groups respectively, and presented as medians (upper-lower quartiles). Values of $p<0.05$ were considered statistically significant.

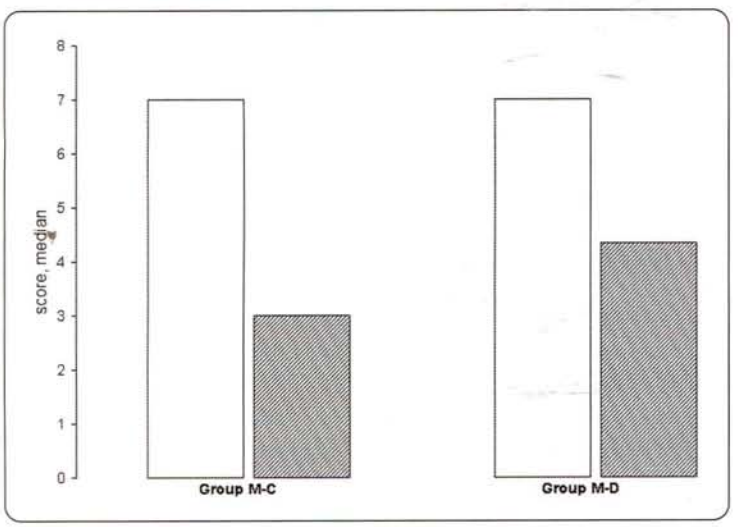



**Fig 1 :** Total Symptom Score expressed as median. Group M-C (Montelukast plus Cetirizine) and Group M-D (Montelukast plus Desloratadine). White bars before treatment; Striped bars after treatment.

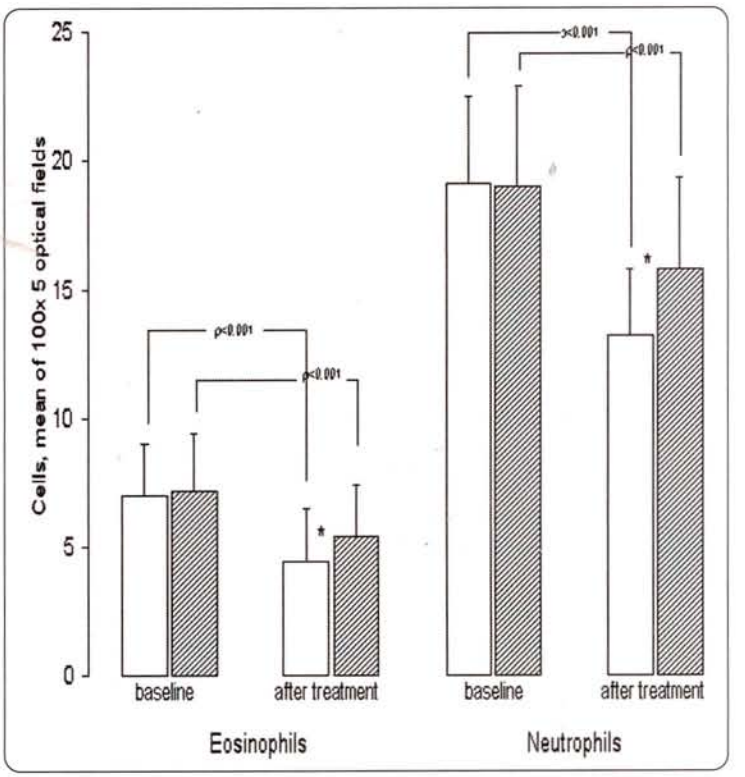



**Fig 2 :** Inflammatory cells at baseline and after treatment. White bars: Goup M-C, striped bars: Group M-D. * $p<0.01$.

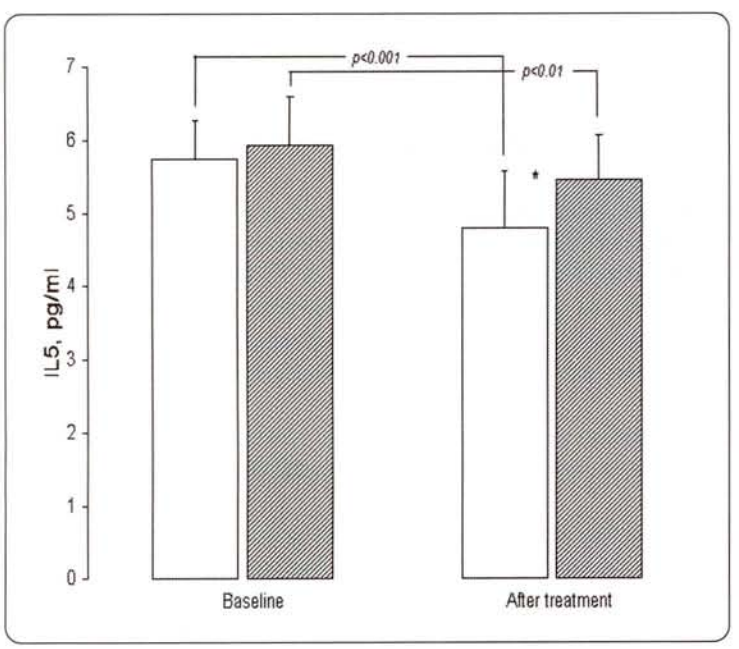



**Fig 3 :** L5 levels at baseline and after treatment. White bars: Goup M-C, striped bars: Group M-D. * $p<0.001$.

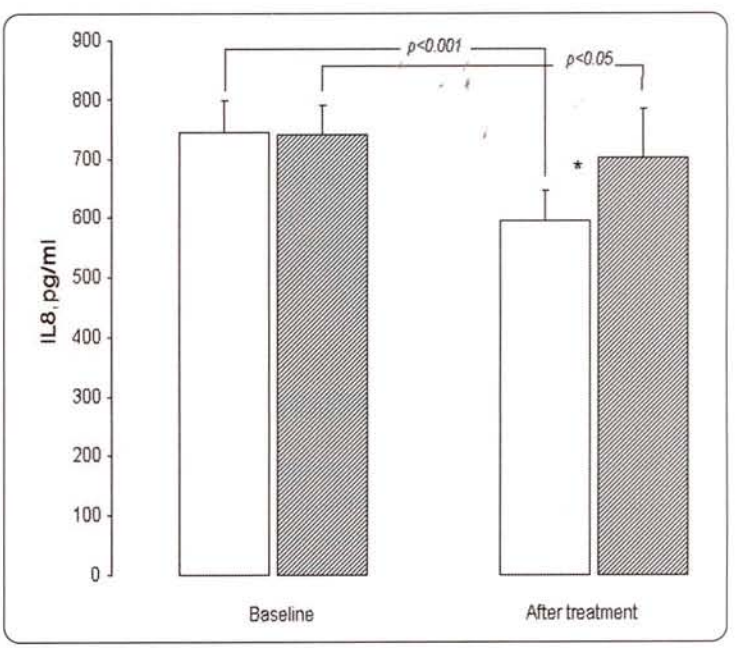



**Fig 4 :** IL5 levels at baseline and after treatment. White bars: Goup M-C, striped bars: Group M-D. * $p<0.001$.

## RESULTS

■ **Subjects:** Thirty allergic patients with SAR were examined. Ten patients were sensitised to *Parietaria officinalis,* eight patients were sensitized to grasses, and 12 subjects were polysensitised to both pollens.

All subjects assumed the prescribed therapy without significant side effects.

■ **Nasal symptoms:** At baseline total nasal symptom scores were not different between the two groups as reported in figure 1.

After treatment, TSS significantly decreased in both groups ($p<0.001$), but the reduction was significantly greater in M-C group than in M-D group ($p<0.01$).

■ **Spirometry:** FEV1 values were not significantly different in both groups before and after treatment: Group M-C 93 + 5 % of predicted at baseline and 93 + 3 after treatment; Group M-D 92 + 6 at baseline and 92 + 3 after treatment.

■ **Nasal cytology:** Figure 2 shows eosinophils and neutrophils before andafter treatment. At baseline, eosinophils and neutrophils were not different between the two groups. After treatment, both combinations significantly reduced eosinophils and neutrophils ($p<0.001$). M-C group showed a significantly greater reduction of both eosinophils and neutrophils in comparison with M-D group ($p<0.01$).

■ **Cytokine levels:** After treatment, IL5 levels were significantly reduced in both M-C group ($p<0.001$) and M-D group ($p<0.01$) as reported in Figure 3. M-C group showed a greater reduction than M-D group ($p<0.001$).

IL8 levels were significantly reduced in both M-C group ($p<0.001$) and M-D group ($p<0.05$) as reported in Figure 4. M-C group showed a greater reduction than M-D group ($p<0.001$).

## DISCUSSION

Allergic rhinitis is characterized by typical symptoms associated with inflammatory infiltrate. Nasal symptoms occur in sensitized subjects immediately after allergen exposure. They are elicited by the release of mediators from activated mast cells: two of the most abundant being histamine and cysteinyl leukotrienes. Histamine has been demonstrated to be mainly involved in the induction of snnezing, rhinorrhea, and nasal itching (the so called "irritative" symptoms), but it exertsan obstructive effect only at relatively high concentrations (16). On the contrary, CysLTs induce primarily nasal congestion with reduced effects on the other nasal symptoms (17). Thus, the combination of these two mediator antagonists could provide additional benefits in comparison with the blockage of a single mediator only. In this regard, several studies showed a beneficial effect in relieving nasal symptoms provided by the combination of antileukotriene with antihistamine in patients with SAR (8).

Moreover, an inflammatory infiltrate is always detectable in patients with allergic rhinitis when exposed to the causal allergen (18). Hallmark of allergic inflammation is the eosinophil infiltration, as its presence in the nasal smear is suggestive of allergic reaction. Eosinophil accumulation and activation are mainly regulated by the Th2-related cytokine IL5 (19).

Neutrophils are early recruited after allergen challenge following chemotactic signals exerted by IL-8, which is released by activated epithelium and mast cells and in turn activates neutrophils (20).

The present study showed that two combinations of antihistamine plus antileukotriene determined significant reduction of nasal symptoms and control of allergic inflammation in patients with SAR and mild intermittent asthma.

These findings partially confirm previous studies concerning both the clinical evaluation and the assessment of inflammatory parameters. Both cetirizine and loratadine, the precursor of desloratadine, have been showed to be able ofreducing neutrophil and eosinophil infiltrate in patients with SAR (12). This anti-inflammatory effect may be partially related to the modulation of proinflammatory cytokines such as IL5 and IL8.

Cetirizine modulates cytokine synthesis as provided by some experimental studies. Firstly, cetirizine has been demonstrated to reduce the release of IL8 from human epithelial cell line A549 (21). In addition, cetirizine decreased IL4, IL5 and IFNg gene expressions in nasal-associated lymphoid tissue of sensitised mice (22).

Desloratadine *in vitro* modulates the synthesis of some cytokines, including IL3, IL6, IL8, TNFa, and GM-CSF (23).

On the other hand, CysLTs promote eosinophil accumulation in the airways by specific mechanisms, including direct chemotaxis, enhanced production of some cytokines, upregulation of adhesion molecules both on eosinophils and endothelial cells, stimulation of bone marrow to produce eosinophils and to release eosino-

phil precursors, and increase of eosinophil activation and survival (4,5).

Montelukast has been demonstrated to be able of modulating some of these inflammatory events. In a murine model of allergen-induced asthma, montelukast treatment resulted in a decreased lung levels of IL4 and IL5 (24). Clinical studies demonstrated that montelukast reduced the blood eosinophil count (9). In addition, a reduced serum ECP concentration has been reported (10,25).

More recently, it has been demonstrated that montelukast reduced the serum concentrations of IL4 and IL10 in asthmatic children (10, 11).

At our knowledge, no study investigated the role of combined antihistamine plus antileukotriene therapy in seasonal allergic rhinitis on cytokine pattern. Thus, our study constitutes the first evidence concerning this issue.

Cytokine modulation by combined treatment might be due to a synergistic effect by these two pharmacologic classes.

Moreover, it seems that cetirizine should exert a greater immunomodulatory effect than desloratadine as provided by present data.

In conclusion, our findings provide a direct evidence of anti-inflammatory effects exerted by these combinations on upper airways. These data are consistent with the clinical activity and appear to be clinically relevant as provided by the symptomatic relief.

Anyway, we are aware that this experience concerned very few subjects. Thus, further studies should be addressed to investigate this issue in more numerous cohorts of allergic subjects.

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