

MIZOLASTINE AND FEXOFENADINE MODULATE CYTOKINE PATTERN AFTER NASAL ALLERGEN CHALLENGE

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Summary

Allergen specific nasal challenge (ASNC) is an optimal model to study the pathophysiological mechanisms sustaining allergic inflammation, particularly the cytokine pattern. Antihistamines have been accepted as a highly effective therapy for allergic rhinitis.

The aim of this double blind, randomised, placebo controlled study was the evaluation of symptoms and cytokines, during the early phase, after a single dose of mizolastine (10 mg), fexofenadine (120 mg) or placebo, using the model of ASNC.

A total of 30 patients with allergic rhinitis underwent nasal challenge 6 hours after treatment. The following parameters were evaluated 30 minutes after ASNC (i.e. early phase): nasal symptoms (rhinorrhea, itching, sneezing, obstruction), and cytokine pattern, including IL1, IL6, and TNF α .

Mizolastine was associated with early phase reduction of: i) clinical symptoms ($p < 0.03$), ii) cytokine levels of IL1 ($p = 0.003$), IL6 ($p < 0.007$), and TNF α ($p < 0.003$) in comparison with placebo group.

Fexofenadine significantly inhibited IL6 ($p < 0.004$) and TNF α ($p < 0.004$) levels in comparison with placebo.

The present findings demonstrate that mizolastine exerts a significant effect on early phase events, reducing symptoms and pro-inflammatory cytokines. Fexofenadine reduces TNF α and IL6 levels only. These effects appear to be clinically relevant for mizolastine.

Key-words: Nasal challenge - Cytokines - Mizolastine - Fexofenadine - Allergic inflammation - Early phase.

INTRODUCTION

Allergic rhinitis is characterised by sneezing, rhinorrhea, nasal itching and obstruction. All these symptoms are caused by mediators released from inflammatory cells. In fact, the nasal mucosa of allergic rhinitis is constantly infiltrated by inflammatory cells (including eosinophils, T cells, and mast cells) during allergic reaction (1, 2). Inflammatory cells are also activated, thus they can release mediators and cytokines, which maintain and amplify the allergic inflammation.

The nasal challenge is an optimal model to study the pathophysiological mechanisms leading to the allergic inflammation, since it is a safe, reproducible, and reliable test (3, 4).

Nasal challenge induces an immediate clinical response (the so called early phase reaction) in sensitised

subjects, identical to that occurring after natural exposure to allergen. It has been recently demonstrated that this early phase is characterised by a contextual mucosal infiltration by activated inflammatory cells (3).

Inflammatory cell recruitment is a very complex phenomenon, involving several mechanisms and occurring through different steps (5). A main pathogenic mechanism is the involvement of adhesion molecule system: particularly, the interaction between molecules expressed on the surface of inflammatory cells and endothelial cells (6). Early adhesion molecule expression is upregulated by proinflammatory cytokines, including IL1, IL6, and TNF α . The main source of these cytokines derives from activated mast cells (7). Cytokines produced by mast cells include molecules initiating and boosting Th2 type immunological reactions as well as a broad spectrum of proinflammatory, growth promoting and chemotactic responses for several cell types. Therefore, inhibition of the production and secretion of these cytokines may represent a crucial tool for treating allergic inflammation.

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Recent evidence has demonstrated that some antihistamines can also exert anti-inflammatory activity via this pathway (8). Particularly, recent *in vitro* studies have previously demonstrated that mizolastine (9) and fexofenadine (10) modulate cytokine pattern.

The aim of the present study was to evaluate the effect of mizolastine and fexofenadine pre-treatment on clinical response and inflammatory phenomena (i.e., cytokine pattern) during the early phase, following ASNC. IL1, IL6, and TNF α were chosen because of their documented role in the initiation of allergic inflammation cascade and acute phase reaction.

METHODS

STUDY DESIGN

The study was designed as a parallel group, double blind, placebo-controlled and randomised trial. Thirty adult patients sensitised to *Parietaria officinalis* pollen and suffering from allergic rhinoconjunctivitis, were enrolled outside the pollen season when symptom-free. They were randomised into 3 groups receiving one single dose of mizolastine (10 mg) or fexofenadine (120 mg) or matched placebo, assumed 6 hours before ASNC. The effects of this pre-treatment were evaluated by means of ASNC. The following parameters were evaluated: clinical symptoms, and cytokine levels recovered from nasal lavage fluids during early phase (i.e. 30 min after ASNC).

The study was performed according to the Declaration of Helsinki and was approved by the Review Board; an informed consent was obtained from each patient.

SUBJECTS

We studied 30 subjects, aged among 18 and 27 years (mean 23).

All subjects suffered from seasonal allergic rhinoconjunctivitis caused solely by *Parietaria officinalis* pollen. All of them had a positive history for at least the two previous years and a positive skin prick test ($> +++$, histamine= $+++$) only for the specific allergen. All subjects were asymptomatic.

Local nasal examination did not reveal any abnormality in all subjects. On admission, no subject suffered from any upper or lower airway respiratory disease for 6 weeks before the study. Subjects were not treated with topical or systemic drugs in the preceding 4 weeks and during the study, and they did not previously receive specific immunotherapy.

ALLERGEN

The allergen extract (the same employed for the skin prick test, kindly provided by Stallergenes, Saronno, Italy), was stored as a lyophilised powder at 4°C, and reconstituted in the diluent (saline human albumin, 0.03%) within 24 h before the test.

The concentration was expressed as AUR/mL; a single dilution was employed: 100 AUR/mL.

ALLERGEN SPECIFIC NASAL CHALLENGE (ASNC)

Nasal challenge was performed following a methodology previously validated (3). Briefly, two 10 mL lavages with lactated Ringer's solution at 37° were performed to establish a stable baseline. Challenge with the vehicle used for the allergen was performed to rule out any non-specific hyperreactivity to the delivery system.

Allergen was applied to the nose using a metered pump spray delivering 90 $\pm$ 2 microliters in one nostril, with patients holding their breath in full inspiration to avoid bronchial delivery. After spraying, patients were allowed to wipe their nose but not to blow it.

CLINICAL EVALUATION

The clinical reaction was assessed as total symptom score according to an arbitrary four-point rating scale, from 0 to 3 (0= absent, 1= mild, 2= moderate, 3= severe) (3).

Total symptom score comprised nasal itching, sneezing, rhinorrhea, and obstruction.

CYTOKINES ASSESSMENT

The cytokines were measured with ELISA method (R&D Systems, U.S.A.). This assay employed the quantitative sandwich enzyme immunoassay technique. The minimum detectable dose was typically less than 1 pg/mL for all cytokines. 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

Since the parameters are non-normally distributed, the Wilcoxon and Mann Whitney tests were used for intragroup and intergroup, respectively, comparisons.

RESULTS

All patients completed the study and no adverse effect was reported.

All subjects were symptom free at baseline. Therefore, the 3 groups were considered homogeneous at baseline.

■ **Clinical data** (figure 1) : Mizolastine pretreatment significantly reduced the Total Symptom Score in comparison with placebo, evaluated 30 minutes after ASNC ($p < 0.03$). Fexofenadine did not exert a significant protective effect.

■ **Cytokines dosages** (figure 2) : Mizolastine group showed a significant reduction of $\text{TNF}\alpha$ ($p < 0.003$), IL1 ($p = 0.0003$) and IL6 ($p < 0.007$) in comparison with placebo group and of IL6 ($p < 0.001$) in comparison with fexofenadine group.

Fexofenadine pre-treatment significantly reduced $\text{TNF}\alpha$ ($p < 0.004$) and IL6 ($p < 0.004$) levels in comparison with placebo group.

DISCUSSION

It is now widely accepted that allergic rhinitis is sustained by an inflammatory response. After mast cell activation, mediator and cytokine release occurs, associated with mucosal inflammatory cell infiltration (including Th2 lymphocytes, eosinophils and mast cells).

Antihistamines constitute a first-line treatment for allergic rhinitis (11). Some of these molecules were shown to exert additive activities unrelated to the blockade of histamine at its receptor and defined as "antiallergic properties" (12).

Mizolastine is a potent and selective antihistamine indicated in allergic rhinitis and chronic idiopathic urticaria; the recommended daily dose is 10 mg. The pharmacological activity of mizolastine is due to a selective blockage of H1 histamine receptors (13). Moreover, mizolastine inhibits histamine release from mast cell and recruitment of inflammatory cells (neutrophils and eosinophils) (14). In

addition, mizolastine inhibits 5-lipoxygenase activity and reduces the release of leukotrienes in *in vitro* studies (15). Finally, mizolastine is able of modulating cytokine release as shown by three studies. Particularly, mizolastine inhibits $\text{TNF}\alpha$ release in *in vitro* animal study (16), from human nasal polyp cells (9), and from HaCaT keratinocytes (17). This last study demonstrated also the ability of reducing the release of both IL1 and IL6 (17).

Fexofenadine is a second generation antihistamine, which is the main active metabolite of terfenadine. Fexofenadine induced a reduced release of IL8 and GM-CSF from human nasal epithelial cells (18). In addition, fexofenadine significantly inhibited IL6 release from human lung macrophages (10). This elegant study evidenced that physiologically relevant concentrations of histamine induce the release of IL6 from human macrophages isolated from the lung parenchyma (10).

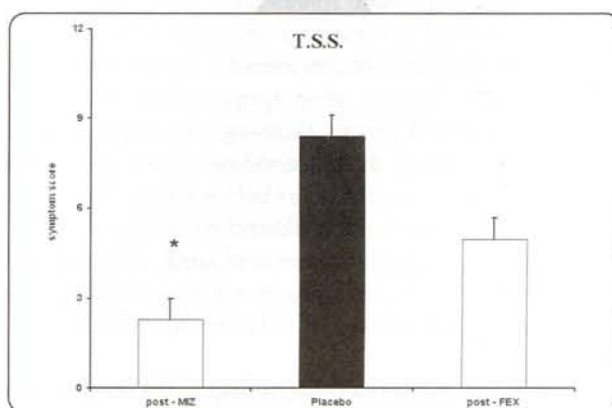


Figure 1 : Total symptom score evaluation after pre-treatment with mizolastine (white bar), placebo (black bar) or fexofenadine (grey bar) during the early phase phase. Data are expressed as mean + SD.

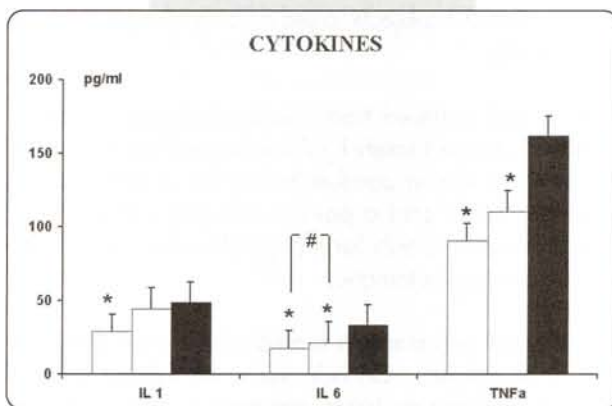


Figure 2 : Cytokine levels after pre-treatment with mizolastine (white bar), fexofenadine (grey bar) or placebo (black bar) during the early phase phase. Data are expressed as mean ± SD.

IL6 is a multifunctional cytokine involved in inflammatory and immune responses (19). IL6 participate to allergic inflammation in a number of ways. IL6 is a stem cell factor and a B cell proliferating and activating factor (19). IL6 is involved in mast cell proliferation and differentiation (20), it may promote Th2 responses and IgE synthesis (21), and stimulates airway epithelial cells to release IL8 (22). These data support the important role played by IL6 in modulating allergic inflammation. In this regard, it has been shown that fexofenadine inhibits *in vitro* histamine-induced exocytosis, IL6 production, and Ca⁺⁺ signalling (10).

On the other hand, IL1 and TNF α are pro-inflammatory cytokines that play a crucial role in allergic rhinitis, with a primary role concerning the early phase reaction. In fact, they up-regulate adhesion molecule expression on endothelial and epithelial cells, promoting inflammatory cell influx (7).

Our study represents the first evidence of *in vivo* modulation of these cytokines by these antihistamines.

Mizolastine exerted a significant protective effect on symptoms appearing after ASNC. This activity may be due in part to its antihistaminic effect and also to its anti-allergic activity as documented by the significant inhibition of IL1, IL6, and TNF α . Moreover, these effects on symptoms and inflammatory events during the early phase may explain the rapid symptom relief demonstrated in patients with perennial allergic rhinitis (23).

On the contrary, fexofenadine did not exert a significant protective effect on symptoms, but exerted an inhibitory effect on TNF α and IL6 release. Anyway, IL6 was significantly more influenced by mizolastine than by fexofenadine. This finding may, at least in part, explain the more relevant clinical activity exerted by mizolastine.

In addition, our findings partially confirm a very recent study that demonstrates the ability of modulating early phase events by fexofenadine (24). Nevertheless, fexofenadine did not inhibit the release of histamine and tryptase from mast cells (24). It has to mention that fexofenadine was administered in this study at higher dosage (i.e. 180 mg) which is not recommended for rhinitis. In another study, fexofenadine did not affect the cytokine release during the late phase after nasal challenge (25).

Moreover, as the leukotrienes and the pro-inflammatory cytokines play a crucial role in the allergic inflammation, with a primary emphasis concerning the nasal congestion (5), these inhibitory effects on the release of

these mediators could explain the efficacy of mizolastine in improving nasal obstruction in patients with perennial allergic rhinitis (26).

In conclusion, in addition to its antihistaminic activity that exerts a symptomatic relief, mizolastine also inhibits the release of pro-inflammatory cytokines, including IL1, IL6, and TNF α , in this *in vivo* study. This anti-inflammatory activity may be considered as clinically relevant as sustained by the rapid symptom relief.

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IMPACT OF ASTHMA MAJOR FOCUS FOR AAAAI IN 2004 AAAAI WILL EDUCATE PATIENTS, PHYSICIANS ON BEST TREATMENT OPTIONS

(SAN FRANCISCO, March 23, 2004) - More than 20 million Americans have asthma - a chronic disease that is becoming more prevalent, and more severe. Asthma leads to an estimated 14 million missed days of school each year and 1.8 million visits to emergency departments. About 12 million people have asthma attacks each year, and nearly 5,000 people die from asthma.

But with proper management and treatment, people with asthma can control their disease and their symptoms - a message the American Academy of Allergy, Asthma and Immunology (AAAAI) will stress in 2004.

"Asthma is an extremely serious disease, but it does not have to be a debilitating one", said Michael Schatz, MD, MS, who was installed as the AAAAI's 2004-2005 president during its Annual Meeting, March 19-23 in San Francisco.

"During the next year, the AAAAI will place a strong emphasis on educating patients, physicians and other health care providers about best treatment and management options for controlling asthma", Schatz said.

This will include developing asthma adherence training, assessing emergency room visits for asthma and creating a surveillance system for monitoring asthma and allergy medications in pregnant women.

"The AAAAI is committed to improving the lives of patients with asthma and other allergic diseases, and these projects will provide strong educational and research opportunities to do that", Schatz said.

An important aspect of all these efforts will be emphasizing the Guidelines for the diagnosis and management of asthma developed by the National Asthma Education and Prevention Program (NAEPP).

"These guidelines are the gold standard for asthma treatment, and all physicians who care for patients with asthma

should understand and follow their recommendations", said Gail Shapiro, MD, a past President of the AAAAI and the AAAAI's representative to the NAEPP Professional and Patient Education Subcommittee.

The NAEPP Guidelines provide detailed information on classifying asthma and what the appropriate treatment is based on that classification. They also recommend when patients should be referred to an asthma specialist.

"Any patient, no matter whether their asthma is mild or severe, who has had even one life-threatening asthma exacerbation should be working with a specialist to get their asthma symptoms under control", Shapiro said.

For most asthma patients, the guidelines recommend inhaled corticosteroids as the first line of treatment. However, studies have shown that only a small percentage of patients at risk for life-threatening asthma attacks are actually taking inhaled corticosteroids, said Richard Nicklas, MD, a fellow of the AAAAI and Chair of the AAAAI Asthma Mortality Committee.

"Physicians and patients need more education on how important inhaled corticosteroids are for managing their asthma", Nicklas said.

In particular, educating patients who rely on Emergency Departments for asthma care will be an important aspect of the AAAAI's 2004 efforts. Studies have shown that children with asthma account for up to 17% of all pediatric Emergency Department visits and 8% of all direct medical expenses are from Emergency Department visits.

"Many patients who come to the Emergency Department for asthma treatment could avoid these visits if their asthma was better controlled. We want to reach these patients, and their health care providers, to emphasize the importance of prevention", said Carlos Camargo, MD, a Fellow of the AAAAI and emergency physician at Massachusetts General Hospital.