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Montelukast effects on inflammation in allergic rhinitis: a double blind placebo controlled pilot study

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Key words

Allergic rhinitis, montelukast, eosinophils

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Summary

It has been demonstrated that Leukotriene modifiers reduce rhinitis symptoms, but montelukast preventive effect on inflammatory cells pattern in intranasal challenge studies has not been already assessed. This pilot study has been designed to explore the montelukast effects in preventing early/late inflammatory cells response to specific allergen challenge in persistent rhinitis. After a 4 week wash-out period, patients were randomised to receive montelukast/placebo for 4 weeks. Pre-post treatment nasal washing and scraping before and after specific nasal challenge were performed. No difference in baseline inflammatory cells count before and after treatment was shown between groups. Despite at a basal level a decrease of inflammatory cells in active group after treatment was observed, the statistical significance was not reached. The generalised mixed model showed that, after therapeutic interventions, the inflammatory cells increased 30' and 6 hour after challenge but, only in the active group the cells amounting was less for eosinophils (-34%), macrophages (-56%), lymphocytes (-45%) and neutrophils (-46%; p=0.001). The longitudinal generalised linear model with just one time variable showed a decrease of all inflammatory cellular types although a significant relevance was reached only for macrophages (p= 0.038) and neutrophils (p=0.001). The modulatory effect on neutrophils and macrophages could lead to montelukast still unexplored effects. Specific trials, sized according to the results of this pilot exploratory study, could add relevant evidences concerning the leukotrienes receptors antagonist treatment of specific rhinitis and asthma phenotypes.

Background

Allergic rhinitis represents the most common immune-mediated disease and its prevalence is progressively increasing (1). Nowadays, the allergic reaction is considered not only an immediate short-lived phenomenon, but a dynamic process. Allergen exposure induces mast cells activation and release of mediators and cytokines which induce inflammatory cell recruitment and activation at the target organ level (2). Eosinophils play a crucial role in this process through the release of cystenyl leukotrienes (3-6). Leukotrienes increase after allergen challenge (7) and this is related to a symptoms increase (8).

Clinical trials (7-12) have shown that leukotriene modifiers can reduce rhinitis symptoms in, but montelukast preventive effect on inflammatory cells pattern in intranasal challenge studies has not been assessed yet.

This pilot study has been designed to explore montelukast effects in preventing early and late inflammatory cells response to specific allergen challenge in persistent rhinitis.

The primary objective of the study is to evaluate the effects of 4 weeks montelukast treatment versus placebo on

lymphocytes, macrophages, neutrophils and eosinophils during early and late allergic response to nasal specific challenge.

Materials and methods

Study design

A predefined sample of 20 adult patients suffering from persistent allergic rhinitis according to A.R.I.A. guidelines (13) were enrolled from May 2007 to February 2008 in this double-blind, 8 weeks, two arm, parallel group study. After a 4 weeks run-in period, patients were randomly assigned to receive montelukast or placebo for 4 weeks. At visit 0, after the acquisition of the informed consent, a complete medical history of the patient was collected, physical examination and inhalant-allergens skin prick test were performed, and therapeutic wash out period was started.
Both at visit 1 (4 weeks after visit 0) and at visit 2 (4 weeks after visit 1) haematochemical tests (haemachrome, liver and kidney function, erythrocyte sedimentation rate pregnancy test in females), basal spirometry, rhinitis symptoms score (Total 4 Symptom Score - T4SS), nasal washing and scraping before and after specific nasal challenge, delivery/withdrawal of the drug of the study were performed.
Montelukast was supplied as sodium salt dispensed as a film-coated tablet. The daily dose was 10 mg administered per os as one tablet at bedtime, with or without food, for 4 weeks. The placebo tablet had a matching image. After 15 days (±1) from the treatment period end, a follow-up visit was performed.
The following inclusion criteria were adopted: positive skin prick test (≥++) for indoor allergen (house dust mite) in the last 6 months, availability to participate in the study (informed consent), and a T4SS for rhinitis ≥6 out of 12 during the week before the enrolment. Patients aged less than 18 or requiring asthma treatment other than β-2 short acting inhaled agonists on demand or affected by any other inflammatory (e.g. infectious rhinosinusitis), neoplastic and anatomical nasal abnormalities were excluded.
Before starting the treatment, the following medications were banned: decongestionants within 3 days, ketotifen and nedocromil or chromoglycate within 14 days, antihistamines within 15 days, oral, intravenous, intramuscular, corticosteroids and topical corticosteroids within 30 days and astemizole within 90 days.
The study protocol was approved by the Ethical Committee.

Study procedures

Specific nasal challenge
Nasal challenge was performed with a validated methodology according to the guidelines of the European Academy of Allergy and Clinical Immunology (14).
Increasing doses of house dust mite extract (*Dermatophagoides farinae* 50%, *Dermatophagoides pteronyssinus* 50%) (Allerkin®, Lofarma, Milan) were administered at standard time intervals (15 minutes) until a clinically relevant nasal hypersensitivity reaction was elicited. Allergen extract was prepared as freeze-dried micronized powder with lactose as the vehicle and is stored in ready to use capsules containing 20 to 40 allergenic units (A.U.). Allergen was nebulised into the nose by a powder insufflator delivering one puff/volume (90+/-2 ml) in one nostril, while the patient was holding his breath in full inspiration to avoid bronchial delivery.
Clinical evaluations were performed at baseline and after challenge by T4SS, which assesses the presence and grade of four symptoms (nasal itching, sneezing, rhinorrea, nasal blockage) on a four point Likert scale (0= no symptoms, 1= mild, 2= moderate, 3= severe) with a 0-12 range. Allergen doses were increased gradually (20, 40, 60, 80 A.U) until the threshold dose was reached. This was defined as the allergen dose that elicited an increase of>1 in the total score. The subjective allergen dose elicited symptoms before and after treatment was registered (14, 15).

Nasal lavage and scraping
Early and late response to specific challenge were assessed by nasal lavage fluid and then by nasal scraping, performed at baseline, 30 minutes after the final nasal provocation (when total symptoms score, TSS≥7 was reached) and 6 hours after the end of specific nasal challenge. Nasal lavage was performed as it follows: the subjects were seated with their necks extended approximately 30° from the vertical while holding breath; 5 millilitres of lactated Ringer's solution, pre-warmed to 37°C, were instilled into each nostril. Approximately 10 s after instillation, the fluid was expelled into a plastic tray and transferred to a 10 ml propylpropylene tube. Then the nasal fluid was centrifuged at 1350 x g for 10 minutes; the supernatants were harvested and centrifuged again, then stored at –20°.
Nasal scraping was taken from the anterior part of the inferior turbinates where they jut into the nasal cavity, with the aid of a torch and nasal speculum using a rhinoprobe device.

After the nasal scraping, the nasal probe was dipped in a plastic tray with phosphate-buffer saline (PBS) and transferred to a 10 ml polypropylene tube. The recovered fluids were centrifuged at 220 g for 10 minutes, and each pellet was resuspended in PBS (2 ml). Eosinophilic Cationic Protein (ECP) in nasal fluid and differential cell count (number of eosinophils, neutrophils, lymphocytes and macrophages) in scraping samples were assessed. The concentration of ECP in the samples was quantified by fluoroenzyme immunoassay according to the manufacturer's protocol (UniCAP; Phadia).
Cell suspension was filtered to reduce mucus quantity, and cytospin slides were prepared by using standard techniques. Both technician slide preparation and investigator slide lecture were performed in a blind fashion.
Smears were stained with Diff Quick stain differentiate between eosinophils, neutrophils, lymphocytes, macrophages and were analysed by optic microscope. The number of inflammatory cells was expressed as a percentage of cells. Samples were examined in a blinded fashion.

Statistical analysis
Intra and inter-group cells' values at basal level, before and after treatment were assessed using t-test.
Due to the longitudinal clustered nature of this clinical investigation, the predictive role of the treatment defined as the linear time trend across inflammatory cells categories was assessed through a longitudinal generalized linear mixed model for repeated measures. This model takes into consideration the within-subject correlation and allows estimating a relative risk (RR) index as a measure of association between each predictor and the study outcomes. The values of the parameters and their 95% confidence intervals are obtained by maximum likelihood estimation (16, 17).

Results

Twenty patients (10 males and 10 females) were enrolled and randomly assigned to active treatment with montelukast or placebo; mean age was respectively 39.2 ± 12.61 and 31 ± 10.19 years. At baseline mean FEV1 value was 91.2% (SD 5.3) predicted and mean total symptom score was 7.55 (SD 1.14).
All enrolled patients showed allergic sensitisation to dermatophagoides farinae; moreover, 5 patients also showed sensitisation to grass, 4 to parietaria officinalis, 4 to hazel, 6 to dog's epithelium, 3 to cat's epithelium, 2 to cypress, 2 to olive tree, 1 to alternaria, 3 to birch. 7 patients were mono-sensitive to mite.

As reported in the CONSORT flow diagram (Fig. 1), all patients completed visit 1; 3 drops-out (one in the active group, 2 in the placebo) due to non-health-related reason were registered at visit 2. Moreover, a patient refused to undergo haematochemical tests.
No difference in baseline inflammatory cells count before and after treatment between groups was reported (Tab. 1).
Despite a decrease of inflammatory cells in active group after treatment was observed at a basal level, it did not reach the statistical significance.
Inflammatory cells response after specific challenge in both groups is reported in Table 2.
The generalised linear mixed model showed that, after therapeutic interventions, the inflammatory cells increased 30' and 6 hours after the specific challenge in both treatments groups, but, compared to placebo, the cells amount was smaller for eosinophils (-34%; ns), macrophages (-56%; ns), lymphocytes (-45%; ns) and neutrophils (-46%; p=0.001) in the active group. The increase for time unit variance from basal to the first measurement (30') and from the first to the second measurement (6 hour) using the longitudinal generalised linear model with just one time variable showed a decrease of all inflammatory cellular types, although a significant relevance, in active versus placebo, was reached for macrophages (p= 0.038) and neutrophils (p=0.001) (Tab. 3, Fig. 2).
No statistical significant difference between groups concerning emathological safety parameters and ECP level in nasal fluid was found.

Figure 1 - CONSORT Flow Diagram

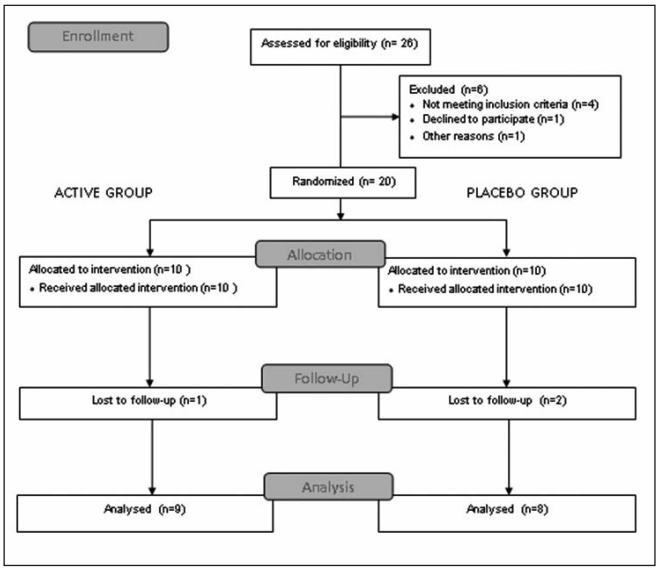


Table 1 - Cells' values (%) at basal level, before and after treatment: comparison between active and placebo group

	Active Treatment group	Placebo group	T test	p-value
	Mean ± SD	Mean ± SD		
Visit 1				
Eosinophils	0.89 ± 1.27	1.1 ± 1.52	-0.326	0.748
Macrophages	0.89 ± 1.05	2.0 ± 2.40	-1.060	0.303
Neutrophils	3.44 ± 4.30	3.1 ± 2.08	0.3450	0.734
Lymphocytes	0.44 ± 0.88	0.6 ± 0.84	-0.530	0.602
Visit 2				
Eosinophils	0.56 ± 0.73	1.00 ± 1.06	-1.013	0.327
Macrophages	0.56 ± 0.73	1.50 ± 1.77	-1.470	0.162
Neutrophils	2.22 ± 4.47	2.25 ± 2.25	-0.016	0.988
Lymphocytes	0.11 ± 0.33	0.13 ± 0.35	-0.083	0.935

Table 2 - Specific challenge inflammatory cells response (%) in active and placebo groups before and after treatment

		Active group				Placebo			
		Mean ± SD		Paired t test	p value	Mean ± SD		Paired t test	p value
		Visit 1	Visit 2			Visit 1	Visit 2		
Eosinophils	Basal	0.89 ± 1.27	0.56 ± 0.73	1.41	0.195	1.25 ± 1.67	1.00 ± 1.06	0.45	0.668
	30 min	0.89 ± 1.05	0.78 ± 0.44	0.24	0.813	0.88 ± 1.13	1.25 ± 1.49	-0.53	0.612
	6 h	1.86 ± 0.90	1.71 ± 1.60	0.24	0.818	2.13 ±2.59	2.5 ± 2.83	-0.89	0.402
Macrophages	Basal	0.89 ± 1.05	0.56 ± 0.73	0.756	0.471	1.63 ± 1.77	1.50 ± 1.77	0.22	0.836
	30 min	1.33 ± 1.12	0.78 ± 0.83	2.29	0.051	1.13 ± 1.55	1.25 ± 1.28	-0.55	0.598
	6 h	1.13 ± 1.46	0.13 ± 0.35	1.87	0.104	1.63 ± 1.85	0.75 ± 0.71	1.51	0.176
Neutrophils	Basal	3.44 ± 4.30	2.22 ± 4.47	1.47	0.179	3.13 ± 2.36	2.25 ± 2.25	0.64	0.543
	30 min	1.89 ± 3.14	1.67 ± 2.12	0.18	0.863	3.38 ± 6.00	2.63 ± 1.92	0.32	0.755
	6 h	10.5 ± 12.72	1.88 ± 1.64	1.93	0.095	7.25 ± 10.36	6.00 ± 4.96	0.37	0.723
Lymphocyites	Basal	0.44 ± 0.88	0.11 ± 0.33	1.41	0.195	0.75 ± 0.89	0.13 ± 0.35	1.67	0.140
	30 min	0.33 ± 0.71	0.22 ± 0.44	0.36	0.729	0.25 ± 0.46	0.63 ± 0.74	-1.16	0.285
	6 h	0.25 ± 0.46	0.25 ± 0.46	0.00	1.00	0.38 ± 0.74	0.25 ± 0.46	0.55	0.598

Discussion

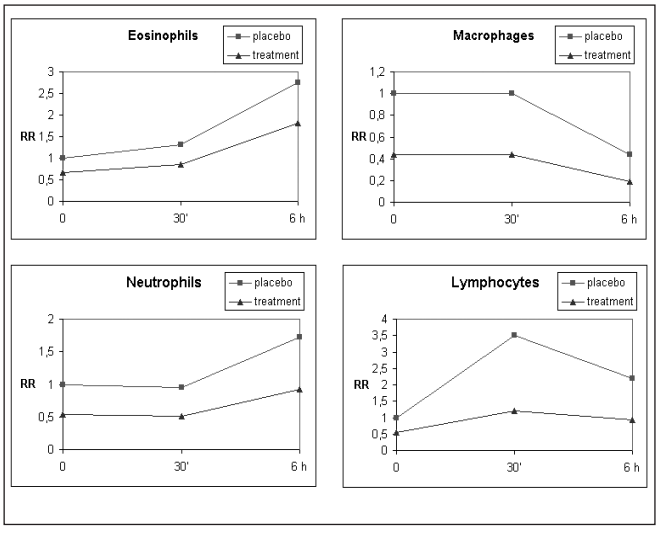
Montelukast induces its therapeutic effects mainly through antagonism of Cysteinil Leukotrienes mediated bronchoconstriction, recruitment and activation of inflammatory cells, enhancement of vascular permeability, bronchial hyper-reactivity and airways remodelling. Recent evidence underlined that montelukast has secondary anti-inflammatory activities, maybe unrelated to CyLT Rs antagonism. It has been reported that montelukast inhibits 5-lypoxygenase in both activated neutrophils and monocytes/macrophages (18, 21, 22). The inhibition of CyslTS synthesis could represent an additional therapeutic activity that may contribute to the control of corticosteroid insensitive neutrophil-mediated inflammation (23, 24). Other authors report that antileukotrienes drugs are able to inhibit the transcription nuclear factor kB in allergen activated human monocytes or lypopoliysaccharide or tumor necrosis factor stimulate monocyte/macrophage cells life (25-28) resulting in a reduction of cytokine such as IL-8. Moreover, Tintinger et al. have recently reported that montelukast cause dose-related inhibition of the chemoattractent-activated proinflammatory activities of isolated human neutrophils (18) reducing LTB4 production also through an increase of intracellular cAMP levels.
Although the above mentioned montelukast secondary ef-

Table 3 - Time unit variance from basal to first measurement and form first to second measurement

Longitudinal Generalised Linear Model					
Response visit 2	Covariates	Comparison	RR	95% Confidence Interval	p-value
Eosinophils	Time	linear trend	1.72	1.26 - 2.34	0.001
	Treatment	placebo	1.00	-	-
		active vs placebo	0.65	0.35 - 1.19	0.161
Macrophages	Time	linear trend	0.71	0.50 - 1.02	0.062
	Treatment	placebo	1.00	-	-
		active vs placebo	0.40	0.16 - 0.95	0.038**
Lymphoid cells	Time	linear trend	1.32	0.68 - 2.56	0.413
	Treatment	placebo	1.00	-	-
		active vs placebo	0.69	0.18 - 2.70	0.595
Neutrophils	Time	linear trend	1.35	1.10 - 1.67	0.005
	Treatment	placebo	1.00	-	-
		active vs placebo	0.54	0.38 - 0.76	0.001**

fect could be particularly significant in other diseases such as COPD, cystic fibrosis, viral bronchiolitis, idiopathic pulmonary fibrosis, their potential effects in allergic rhinitis need to be explored. This pilot exploratory study has been performed in other to evaluate the potential montelukast effects in preventing early and late inflammatory cells response to specific allergen challenge in persistent rhinitis. Since previous studies on the topic were not available, the population sample was defined a priori in order to expose the minimal number of patients to allergen specific challenge. On these bases the population sample resulted too small to achieve a definitive result in active versus placebo analysis. As a matter of fact, considering these preliminary results, power analysis was performed to calculate the minimum sample size required to detect a difference between active and placebo groups. Sixty-nine subjects per group are necessary to notice a significant difference at the 95% level and with a power of 80% on eosinophils at visit 2 (mean values ± SD in both groups 0.56±0.73 and 1±1.06). The underpower of this pilot study explains the lack of results when modulatory effects of montelukast on eosinophils were assessed. Therefore, the results of this pilot study were sufficient to show an intra-active group effect of montelukast in modulating neutrophyls recruitment. The results of the study provide the background to further studies. Nevertheless, some suggestions can be drawn. These results should be taken into consideration on the above mentioned evidences as regards cyclic AMP-dependent inhibition of neutrophil pro-inflammatory activity (18) and the sCD14 LTD(4) mediated decrease induced by montelukast treatment (19, 20).
Until now the clinical research on leukotrienes inhibitors has been mainly focused on their effects on eosinophils activities. The modulatory effect on neutrophils and macrophages could lead to montelukast still unexplored effects. The specific immunological pattern of rhinitis patients (i.e. eosinpohilic infilatate in Th2 response) can

Figure 2 - Relative risk of post intervention cells increasing at 30' and 6 hrs post challenge from baseline in active treatment and placebo group.



change during the time or can be modulated by the presence of intercurrent phenomena, such as infections. Future research on patient populations with different cellular immunological response to concomitant noxoius agents (i.e. allergic rhintis with comorbid infectious rsinusitis) could lead to define new phenotypes of disease that can benefit from antileukotrienes approach. Specific trials, sized according to the results of this pilot exploratory study, could add relevant evidences concerning the rhinitis treatment with multicellular involvement.

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