

Anti-Inflammatory Activity and Clinical Efficacy of a 3-Month Levocetirizine Therapy in Mite-Allergic Children

Francesco Marcucci¹, Laura Giovanna Sensi¹, Piera Abate¹, Giusiana Allocca¹, Eleonora Ugolini¹, Giuseppe Di Cara¹ and Cristoforo Incorvaia^{*,2}

¹Institute of Pediatrics, Department of Medical and Surgical Specialty and Public Health, Perugia, Italy

²Allergy/Pulmonary Rehabilitation, ICP Hospital, Milan, Italy

Abstract: The non-sedating third generation antihistamine levocetirizine has ample evidence of efficacy in allergic rhinitis. *In vitro* studies suggested that levocetirizine has anti-inflammatory properties not simply related to the antihistamine activity but also to regulation of eosinophils. We performed a double-blind placebo-controlled study in 40 children allergic to house dust mites with persistent rhinitis with the primary aim to evaluate the anti-inflammatory efficacy of levocetirizine measuring eosinophil-related parameters and exhaled nitric oxide (eNO). After one month of treatment, a significant improvement in nasal symptom-medication scores was observed in actively but not in placebo-treated patients. After 3 months of treatment, a significant effect was detected on eosinophilic cationic protein (ECP) in nasal mucosa and on nasal eNO in active treated patients. This suggests that during treatment of mite-allergic children with levocetirizine the early improvement in nasal symptoms is due to the antihistamine activity, while more time is needed to achieve an effect on allergic inflammation.

Keywords: Antihistamines, levocetirizine, allergic rhinitis, inflammation, exhaled nitric oxide, eosinophilic cationic protein.

INTRODUCTION

Anti-inflammatory properties have been reported for several H1-antihistamines, including inhibition of nasal congestion, that is related to inflammation [1-4], down-regulation of cell adhesion molecule expression, and reduction of pro-inflammatory cytokine production [5]. These anti-inflammatory effects are argued to explain the high efficacy of these drugs in the treatment of allergic diseases [6]. In *in vitro* studies, some H1-antihistamines were shown to modulate immunological mechanisms involved in allergic inflammation, with a reduction of proinflammatory mediators, cytokines and chemokines, and of consequent inflammatory cell recruitment [7]. However, most of the antiinflammatory effects observed *in vitro* are evidenced at concentrations that are much higher than therapeutic levels, this suggesting a difference in their *in vitro* and *in vivo* anti-inflammatory potencies [6].

Levocetirizine dihydrochloride, the active enantiomer of cetirizine, is a third-generation oral histamine H1-receptor antagonist with an affinity for the human H1-receptor 2-fold higher than cetirizine [7-9], that is capable of inhibiting histamine and allergen-induced weal and flare reactions [10, 11] and showed high efficacy in long-term clinical studies [12, 13] including paediatric populations [14, 15]. Levocetirizine shows a higher dissociation half-life of 142 minutes, compared with 6 minutes for dextro-cetirizine [8]. This peculiarity explains its higher affinity, making the binding of this drug with the H1-receptor nearly irreversible and scarcely replaced by histamine in *in vitro* studies. Levocetirizine affects several major pro-inflammatory

processes. Several recent studies suggested an antiinflammatory activity of this drug, both directly, *via* its anti-H1 activity, and indirectly. These *in vitro* [16-19] and *in vivo* [20-21] studies showed that levocetirizine may inhibit chemotaxis and transendothelial migration of eosinophils due to eotaxin and adhesion molecules such as VCAM [18]. In addition, this drug reduces airway hyperreactivity to adenosine monophosphate (AMP) [22, 23]. Another study suggested that levocetirizine modulates the release of inflammatory mediators, such as cytokines, growth factors, proteinases, and antiproteinases produced by eosinophils *in vitro* [24]. However, the effect at therapeutic levels *in vivo* is still under investigation. A study in adolescents and adults has reported a significant effect on improving nasal congestion [25, 26]. Significant effects on nasal congestion have also been reported in adults suffering from persistent allergic rhinitis, as defined by the Allergic Rhinitis and its Impact on Asthma (ARIA) document [27].

Based on these data, and in order to confirm *in vivo* the anti-inflammatory activity of levocetirizine, we performed a study in children allergic to house dust mites with persistent rhinitis with or without intermittent asthma, in a double-blind placebo-controlled (DBPC) fashion, lasting more than the commonly suggested treatment period. Along with several secondary outcomes, the primary outcome was to evaluate anti-inflammatory efficacy of levocetirizine measuring nasal and bronchial exhaled nitric oxide (eNO) at baseline and after allergen specific nasal challenge (ASNC) with house dust mites.

MATERIAL AND METHODS

Patients

Sixty children (24 males, 36 females, mean age 9.73 +/- 2.11 years, range 6-12 years) allergic to house dust mites

*Address correspondence to this author at the Allergy/Pulmonary rehabilitation, ICP Hospital, Via Bignami 1, 20137 Milan, Italy; Tel: +390257993289; Fax: +39025799; E-mail: cristoforo.incorvaia@gmail.com

with persistent allergic rhinitis (AR) with/without intermittent asthma were enrolled in this study; 35 children were enrolled between September and October 2006, scheduled to end trial in March 2007; 25 children were enrolled in September and October 2007, scheduled to end trial in March 2008.

Diagnosis of rhinitis and asthma was defined according to clinical history, clinical evaluation during acute symptoms and FEV1 increase after inhaled salbutamol. Rhinitis severity was classified according to the ARIA international guidelines. Asthma severity was classified according to the Global Initiative on Asthma (GINA) international guidelines.

The study was performed according to a DBPC scheme. Randomization was done during visit 2, with inclusion of patients either in group 1 (active) or group 2 (placebo), according to an homogeneous distribution of recorded clinical scores during the wash-out period and to inflammation severity evaluated during visit 1.

Hospital's pharmacy provided both active drug and placebo, according to an internal scheme, with identical drug package. Active and placebo were administered at late evening. The trial lasted 4 months for each patient, and included four visits:

- Visit 1 (September-October): enrolment of patients, wash-out period of at least 2 weeks during which patients recorded in a diary both symptoms and rescue medication intake; measurement of nasal and bronchial eNO; measurement of nasal and bronchial eosinophil cationic protein (ECP).
- Visit 2: after the wash-out period patients who fulfilled all inclusion and exclusion criteria were randomly allocated in group 1 or 2. The visit included: delivery of the record diary cards, measurement of the asthma and rhinitis quality of life by the Asthma Quality of Life Questionnaire (AQLQ) and Rhinitis Quality of Life Questionnaire (RQLQ) by E. Juniper [28], and delivery of the first month of treatment; evaluation of skin prick tests; blood sampling for serum eosinophils, serum specific IgE and IL-8, nasal specific IgE and IL-8, nasal and bronchial ECP, nasal nuclear factor kappa B (NFkB); nasal and bronchial eNO (baseline and after ASNC), nasal tryptase (baseline and after ASNC); spirometry, nasal expiratory peak flow meter.
- Visit 3 (after the first month of therapy): return of the first month diary cards and of the unused drugs; delivery of a second record diary card and drugs for two months of treatment; nasal and bronchial eNO (baseline and after ASNC), nasal tryptase (baseline and after ASNC); spirometry, nasal expiratory peak flow meter.
- Visit 4 (after 3 months of therapy): return of the following two months diary cards and of the unused drugs; evaluation of skin prick tests; blood sampling for serum eosinophils, serum specific IgE and IL-8, nasal specific IgE and IL-8, nasal and bronchial ECP, nasal NFkB; nasal and bronchial eNO (baseline and after ASNC), nasal tryptase (baseline and after

ASNC); spirometry, nasal expiratory peak flow meter; AQLQ and RQLQ questionnaires.

The periods considered in the evaluation of data and in the statistical analysis were those between visit 1 and 2 (period V2), between visit 2 and 3 (period V3), and between visit 3 and 4 (period V4).

Inclusion Criteria

- Presence of mild-moderate persistent rhinitis (Total symptoms score, TSS $\geq$ 5) during the latest exposure period according to ARIA guidelines
- Presence of level 1 asthma according to GINA guidelines during the latest exposure period (symptoms less than once a week or nocturnal symptoms less than twice a month)
- Age range: 6-12 years
- Positive SPT for house dust mites (wheal diameter of at least 5 mm)
- Specific serum IgE for house dust mites of at least 3,5 KU/L

Exclusion Criteria

- Specific immunotherapy in the last 3 years
- Normal values of exhaled bronchial (< 20 ppm) and nasal (> 300 ppm) NO at baseline
- Low nasal (< 40 mcg/ml) or bronchial (< 30 mcg/ml) ECP values at baseline
- Positive SPT for moulds, Parietaria or animal epithelia
- Current systemic therapy with corticosteroids, antihistamines, cromones, antileukotrienes and calcineurin inhibitors. If present, a wash-out period of at least 4 weeks was performed
- Nasal polyposis or other mechanical obstructive diseases
- Severe systemic diseases
- Persistent asthma according to GINA guidelines

Skin Prick Tests

Skin prick tests (SPT) were performed with commercial glycerinated extracts (Stallergenes, Antony, France). The standard panel of allergens included Phleum pratense, Parietaria judaica, Juniperus ashei, Olea europaea, Dermatophagoides pteronyssinus, Alternaria tenuis, cat and dog epithelium, plus a positive (histamine 10 mg/mL) and a negative (normal saline) control. Wheals were contoured with a soft pen and transferred onto cello tape. A skin reaction of 5 mm was considered the threshold for positivity.

Serum IgE

Specific serum IgE was measured with UNICAP SYSTEM IGE FEIA method (Phadia, Uppsala, Sweden) for the same allergens tested in SPT.

Nasal-Specific IgE

Allergen-specific nasal IgE was measured using a previously described method [29], modified as follows. Briefly, seven allergen covalently coated paper disks plus a negative control disk with chicken albumin (Sigma Aldrich, Milan, Italy) were fixed on two sides of a plastic applicator (6 cm length and 0.6 cm width). The allergens were the same used for the prick test. The applicator carrying the disks was covered with a permeable polyamide monofilament membrane (03-64/45-SEFAR Heiden, Switzerland). The prepared device was inserted into a nostril of the subject and left inside for 20 min. The applicator was then removed from the nostril, disks were put into a tube containing a buffer solution (NaCl 0.9%, Tween 0.1% and NaN₃ 0.05%) and stored at 4 °C. All assays were performed at the same time. After 10 min washing, the allergen-coated disks were incubated for 30 min with a biotinate goat IgG anti-human IgE (VECTOR Laboratories, Peterborough, England) then washed and incubated for 30 min with streptavidine-beta-galattosidase (Boehringer Ingelheim, Milan, Italia), washed again and incubated for 10 min with the fluorimetric development kit (CAP System FEIA Phadia) and determined, as compared to a standard reference curve (CAP System FEIA IgE Phadia) performed with goat IgG antihuman IgE-coated paper disks (VECTOR Laboratories).

Nasal ECP and Tryptase

Tryptase and ECP levels were determined by ELISA (UniCAP Tryptase System FEIA and UniCAP ECP System FEIA, Phadia) adapted for mucosal sampling. For ASNC the measurement of tryptase was made 30 min after and that of ECP 24 hours after. Briefly, the sponges of the CAP System, coated with anti-tryptase or anti-ECP antibodies, were washed with physiologic solution and then mounted on an appropriate plastic stick and fixed by a permeable envelope. The plastic stick carrying the sponges was then inserted into a nostril and incubated in situ for 10 min fixed with a tape externally on the nose. The sponges were then removed and frozen at -20 °C in buffered (NaCl plus NaN₃ 0.1%) vials. At the end of the trial, all sponges were washed 3 times for 10 min with NaCl 0.9% and Tween 0.1%, reinserted into CAPs and run at the same time with Unicap System FEIA Phadia. The concentration of tryptase and ECP was expressed in mg/L according to a calibration curve.

Sputum ECP and Tryptase

Sputum was induced by having the subject inhaled hypertonic saline solution 4%. Briefly, prior to sputum induction, all subjects inhaled a beta2-agonist to avoid hypertonic saline solution-induced bronchoconstriction. The subjects were asked to rinse their mouth and throat every 5 min and then to try to cough sputum into a sterile plastic container. The nebulization was continued for at least 10 min and stopped after 20 min or earlier if a 2 mL sputum sample of good quality was obtained. The collected sample was weighted, mixed with an equal volume of a solution containing dithiothreitol (DTT) 0.1%, and then centrifuged

at 2000 g for 20 min. The supernatant was aspirated and ECP and tryptase were measured in duplicate using the UniCAP Tryptase System FEIA and UniCAP ECP System FEIA (Phadia).

Serum IL-8

Serum IL-8 was detected with ELISA method according to datasheet of Endogen (TEMA). Nasal IL-8 was detected with direct incubation at mucosal level with Whatman paper disks activated with BrCN, covalently coupled with anti IL-8 antibody.

NFkB

NFkB, obtained with nasal scraping (Markos Mephar S.p.a.), was detected in the collected cells according to Western Blot method using MAB2697 antibody.

Spirometry

Spirometry was performed with a Microloop Micro Medical spirometer (Micro Medical, Rochester, England). The subjects were asked to perform three times a maximum expiratory effort immediately following a maximum inspiration, with a rapid start, and the best forced expiratory volume in 1 second (FEV₁) was registered.

Exhaled NO

eNO was measured using a chemoluminescence NO analyzer (Eco Physics CLD 77 AM, Eco Physics, Switzerland) with a detection limit of 0.02 parts per million (ppm). The zero signal was calibrated with an air filter (Sievers AFL 01410-01) and the measurement scale was calibrated with a gas containing 100 ppm of NO in nitrogen (MESSER, Turin, Italy). Subjects at rest, sitting down and wearing a mouthpiece, performed a single exhalation from total lung capacity without breath-holding and were encouraged to maintain a constant nasal or mouth expiration throughout the manoeuvre.

ASNC, Nasal and Bronchial Clinical Scores

ASNC was performed at least 12 hours after the last drug intake, as levocetirizine and placebo were administered at late evening. ASNC was performed by spraying the vehicle into a nostril followed by increasing concentrations of house dust mite extract: 2, 4, and 8 BU/mL (Alk-Abello, Horsholm, Denmark). These concentrations correspond respectively to about 0.016, 0.032, and 0.064 mg per puff of the major allergen Der p 1. Nasal symptoms were evaluated attributing a score to itching, sneezing, rhinorrhea and blockage as follows: 0 = absent, 1 = mild, 2 = moderate, 3 = severe. Asthma symptoms considered were: coughing (asthma related), wheezing and breathing difficulty. Severity of asthma symptoms was evaluated attributing a score to each symptom as follows: 0 = absent, 1 = mild, 2 = moderate, 3 = severe. A total score increase of at least 3, between basal and after ASNC values, was used to define the threshold dose for the positive challenge.

Clinical Symptoms, Rescue Medication Intake and Quality of Life Evaluation

During the evaluation period, patients used daily diary cards to record symptoms, rescue medication use and side-effects. Symptoms of rhinitis (sneezing, rhinorrhoea, itching and nasal blockage), conjunctivitis (itching, redness and tearing) and asthma (cough, dyspnoea and wheezing) were assessed by the patients using a grading from 0 to 3 (0 = absent, 1 = mild, 2 = moderate and 3 = severe). The maximum symptom score was therefore 30.

Rescue medication use was scored as follows: 2 puffs of inhalant β -agonist = 1 point; 2 drops of corticosteroid eye drops = 2 points; 2 puffs of corticosteroid nasal spray = 2 points; 2 drops of antihistamine eye drops = 1 point; 2 puffs of antihistamine nasal spray = 1 point; 2 puffs of cromoglycate nasal spray = 1 point; 2 drops of cromoglycate eye drops = 1 point.

The combined score was calculated by adding the daily sum scores for medication intake to symptom scores.

Quality of life was measured using the specific questionnaires RQLQ and AQLQ.

Statistical Analysis

The Wilcoxon signed rank test for paired data was used to detect differences between basal and after-ASNC values of nasal and sputum tryptase, nasal and sputum ECP and eNO. Mann-Whitney test was used for intergroup analysis of the same values and for clinical scores evaluation. A p-value lower than 0.05 was considered significant.

The determination of sample size needed for this study was calculated, given that a difference of 20% between the two treatment arms for the sum score was considered to be clinically relevant. A two group t-test with a 0.05 two-sided significance level had 80% power to detect an effect size of 0.500 when the sample sizes in the two groups were 17 for nasal exhaled nitric oxide evaluation and 22 for bronchial exhaled nitric oxide. Considering a sample size of 30 patients, with a minimal significant difference of 13 ± 13 ppb for bronchial eNO and of 100 ± 100 ppb for nasal eNO the minimal potency ($1-\beta$) of such an evaluation was 0.968.

RESULTS

Of the 60 children enrolled in the study, 9 did not complete it, 3 because their families moved to another region and 6 because they did not present at follow-up visits. Of the 51 patients who completed the study 11 were not considered for analysis due to non-compliance with their rescue medication intake, as they had a prolonged intake of other drugs interfering with eNO and ECP production (5 patients oral antihistamines, 6 oral corticosteroids). Forty patients (18 males, 22 females, mean age 9.88 ± 1.96 years, range 6-12 years) were considered for statistical analysis of data, 21 from the active group, and 19 from the placebo group.

Bronchial eNO did not show any significant difference in ASNC values vs baseline, between the two groups (Table 1).

Nasal eNO after ASNC, showed significant reduction ($p < 0.01$) in the active group at the beginning of treatment (V2) and after 1 month of therapy (V3), but not after 3 months of treatment (V4). The placebo group showed a significant reduction after ASNC at each follow-up visit (Table 1).

Nasal tryptase showed significant increase ($p < 0.01$) after ASNC both in the active and in the placebo group, at each follow-up visit (data not reported).

Nasal ECP showed a significant ($p < 0.009$) reduction in the active group after 3 months of therapy, whereas it remained unchanged in the placebo group (Table 2).

There was a non-significant trend for bronchial ECP to increase in both groups between V2 and V4 (Table 2).

Spirometry and peak flow meter data did not show any differences between the two groups (data not reported). Serum eosinophils, serum and nasal specific IgE, nasal NFkB, and serum and nasal IL-8 did not show any significant difference after 3 months of treatment (data not reported).

Concerning the evaluation of clinical and rescue medication intake scores after one (V3), and 3 months of therapy (V4), both nasal and bronchial scores were highly affected by rescue medication.

Table 1. Nasal and Bronchial Nitric Oxide (Basal and After ASNC)

	Levocetirizine			Placebo		
	V2	V3	V4	V2	V3	V4
Basal eNO	32,2 (11,3-45,8)	23,1 (9,4-32,8)	26,2 (9,1-31,5)	31,7 (10,3-49,1)	21,4 (8,6-33,2)	18,8 (10,9-28,5)
after ASNC	28,3 (10,1-35,1)	21,4 (12,3-29,3)	26,2 (15,8-31,9)	24,2 (13,3-35,2)	22,8 (12,4-33,3)	16,5 (7,9-26,5)
	p=NS	p=NS	p=NS	p=NS	p=NS	p=NS
Basal nNO	848,09 (611,3-1024,9)	802,18 (587,3-993,2)	618,09 (484,4-864,3)	914,5 (634,5-1211,9)	920,85 (725,4-1098,7)	809,4 (699,3-954,2)
after ASNC	519 (344,3-712,9)	625,76 (493,5-792,1)	536,71 (398,5-722,1)	596,95 (415,4-696,3)	665,6 (512,2-765,7)	457,35 (354,4-597,6)
	p<0.01	p<0.01	p=NS	p<0.01	p<0.01	p<0.01

Table 2. Nasal and Bronchial ECP

	Levocetirizine			
	V2	V3	V4	p (V2-V4)
Nasal ECP	145,97 (74,5-200)	132,1 (61,2-200)	94,43 (45,9-174)	p<0.009
Bronchial ECP	35,36 (19,1-73,0)	79,68 (27,5-89,3)	64,81 (21,3-87,3)	p=NS
	PLACEBO			
	V2	V3	V4	p (V2-V4)
Nasal ECP	136,24 (67,2-200)	153,52 (77,1-200)	149,71 (68,1-200)	p=NS
Bronchial ECP	55,62 (31,9-77,8)	42,85 (28,9-73,2)	62,97 (37,7-93,2)	p=NS

In fact, nasal symptoms showed no significant differences, but nasal global (symptoms + drugs) scores showed a significant ($p<0.001$) increase (Table 3) which is due both to the significantly higher nasal rescue medication intake in the placebo group ($p<0.001$) and the significantly ($p<0.01$) lower rescue medication intake in the active group (Table 3).

RQLQ (Table 3) showed a significant ($p<0.01$) reduction in the active group between V3 and V4, which is not evident in the placebo group.

Bronchial clinical symptoms showed no significant differences between active and placebo after one (V3) and three months (V4) of treatment, while bronchial global (symptoms + drugs) scores showed a significant ($p<0.01$) increase in the placebo group if compared to active group due to the significant ($p<0.01$) higher rescue medication intake in the placebo group, evident since the first month of therapy (V3) (Table 4).

AQLQ showed no significant differences between active and placebo groups after 3 months of treatment (data not reported).

Comorbidity symptoms were not different in the two groups, 2 children in the active group had a single episode of sinusitis while 3 children in the placebo group had a single episode of sinusitis (2 patients) and otitis (1 patient).

After 3 months of treatment both groups reported no serious adverse events. Headache was reported in 3 patients of the active group and 2 patients of the placebo group, while one patient in the active group reported abdominal pain.

DISCUSSION

The primary endpoint of this trial was to evaluate the anti-inflammatory activity of a 3-months treatment with levocetirizine compared to placebo in children sensitized to house dust mites, suffering from persistent rhinitis with or without intermittent asthma, by measuring nasal eNO before and after allergen specific nasal challenge at baseline, after 1 month and after 3 months of treatment. The normal reduction of nasal eNO after specific nasal challenge was evident in both groups at baseline and after 1 month of treatment but not in the active group after 3 months of therapy with levocetirizine, suggesting an anti-inflammatory activity of this drug when used on a long term basis.

eNO is a validated noninvasive marker of airway inflammation in asthma, as its levels reflect airway inflammation in asthmatic subjects [30]. This was further confirmed by increased expression of one of the enzymes responsible for NO production - iNOS - in epithelial cells in bronchial biopsies taken from asthmatic patients compared to those of normal subjects [31, 32]. In patients with AR increased levels of basal nasal NO have also been measured, together with a reduction of its levels after specific nasal challenge [33], that suggests the use of this non invasive marker in the follow-up of allergic patients [34]. In previous studies, the reduction of nasal eNO after specific nasal challenge was so evident that the authors suggested to consider it as a marker of inflammation to be measured in the follow-up of patients with AR [35, 36].

The anti-inflammatory activity of levocetirizine indicated by eNO reduction was confirmed also by nasal ECP, which was significantly reduced in the active group after 3 months of treatment.

These data are in agreement with a recently published study in which levocetirizine effect on inflammation was evaluated measuring peak nasal inspiration flow rate after allergen specific and adenosine 5'-monophosphate nasal challenge [36]. On the contrary, bronchial eNO did not show any significant difference between treated and untreated patients. This data suggests the activity of levocetirizine only in upper airways inflammation but not in lung inflammation.

Table 3. Nasal Scores and Rhinitis Quality of Life

	Levocetirizine			Placebo		
	V3	V4		V3	V4	
Nasal Symptoms Score	74,28 (32-98)	76,88 (28-102)	p=NS	101,04 (53-136)	97,03 (79-129)	p=NS
Nasal Drug Score	35 (12-58)	6 (2-11)	p<0.01	32 (11-63)	221 (98-295)	p<0.001
Nasal Global Score	109,28	82,88	p=NS	133,04	318,03	p<0.001
Rhinitis Quality of Life (RQLQ)	29,92	15	p<0.01	26,77	26,15	p=NS

Table 4. Bronchial Scores

	Bronchial Symptom Score	
	V3	V4
Levocetirizine	14,36 (9-21)	14,64 (11-23)
Placebo	18,7 (11-24)	15,04 (9-24)
	p=NS	p=NS
	Bronchial Drug Score	
	V3	V4
Levocetirizine	79 (51-98)	50 (32-76)
Placebo	262 (194-306)	212 (134-295)
	p<0.01	p<0.01
	Bronchial Global Score	
	V3	V4
Levocetirizine	93,36	64,64
Placebo	280,7	227,04
	p<0.01	p<0.01

The initial increase of bronchial ECP detected in the active group during the first month of treatment, and not evident in the placebo group, is probably due to the high bronchial rescue medication intake of the placebo group if compared to active group, which may have reduced the ECP levels. The lack of transmission of inflammation from upper to lower airways after specific ASNC contrasts with statements in the ARIA document and with two studies by Braunstahl performed in adult patients [37, 38]. This difference might be explained by a previous study from our group conducted on children with AR and intermittent asthma, which indicated that children may present a lower bronchial inflammation both in terms of extent and persistence when compared to adult patients [39].

Possibly, this data reflects the absence of the peculiar immunological pattern at epithelial level which is responsible of the transmission of inflammation from upper to lower airways. To confirm the difference between children and adults, in another recent study 50 adults with persistent rhinitis with/without asthma treated with levocetirizine and placebo were evaluated concerning symptoms, quality of life, inflammatory cell count and mediators measurement, obtained by nasal scrapings and lavages. Both rhinitis and asthma symptoms and quality of life were significantly improved by active treatment compared with placebo, and eosinophils and neutrophils in nasal scraping were significantly decreased in the levocetirizine group vs baseline [40].

In the present study we assessed a number of inflammation parameters, including cells, mediators, cytokines, eNO, and NFkB, which acts as an important

transcription factor in inflammatory response by increasing the rates of transcription of the inflammatory genes [41]. The only significant differences in favour of levocetirizine were detected in nasal eNO and nasal ECP after 3 months of treatment. The fact that a significant effect on nasal symptoms/medication scores was found already after the first month of treatment clearly suggests that nose symptoms have an important histamine-mediated component which is effectively treated by the antihistamine activity of levocetirizine, while more time is needed to achieve an effect on allergic inflammation.

In conclusion, the results of this trial suggest that levocetirizine during long term treatment regimens may reduce the nasal, but not bronchial, inflammation that is present in children with mite-induced persistent allergic rhinitis. This anti-inflammatory effect is likely to be not due to the classic H1-receptor antagonist mechanism, but rather due to a direct influence on eosinophils, as previously suggested by other authors [18-19].

REFERENCES

- [1] Bachert, C.; Brostoff, J.; Scadding, G.K.; Tasman, J.; Stalla-Bourdillon, A.; Murrieta, M. Mizolastine therapy also has an effect on nasal blockade in perennial allergic rhinoconjunctivitis. *Allergy*, **1998**, *53*, 969-975.
- [2] Wilson, A.M.; Haggart, K.; Sims, E.J.; Lipworth, B.J. Effects of fexofenadine and desloratadine on subjective and objective measures of nasal congestion in seasonal allergic rhinitis. *Clin. Exp. Allergy*, **2002**, *32*, 1504-1509.
- [3] Horak, F.; Stubner, U.P.; Ziegelmayer, R.; Harris, A.G. Effect of desloratadine vs placebo on nasal airflow and subjective measures of nasal obstruction in subjects with grass pollen-induced allergic rhinitis in an allergen-exposure unit. *J. Allergy Clin. Immunol.*, **2002**, *109*, 956-961.
- [4] Ciprandi, G.; Cosentino, C.; Milanese, M.; Mondino, C.; Canonica, G.W. Fexofenadine treatment reduces nasal congestion in perennial allergic rhinitis. *Allergy*, **2001**, *56*, 1068-1070.
- [5] Agrawal, D.K. Anti-inflammatory properties of desloratadine. *Clin. Exp. Allergy*, **2004**, *34*, 1342-1348.
- [6] Walsh, G.M. Anti-inflammatory properties of antihistamines: an update. *Clin. Exp. Allergy Rev.*, **2005**, *5*, 21-25.
- [7] Albanesi, C.; Pastore, S.; Fanal-Belasio, E.; Girolomoni, G. Cetirizine and hydrocortisone differentially regulate ICAM-1 expression and chemokine release in cultured human keratinocytes. *Clin. Exp. Allergy*, **1998**, *28*, 101-109.
- [8] Gillard, M.; Van Der Perren, C.; Moguilevsky, N.; Massingham, R.; Chatelain, P. Binding characteristics of cetirizine and levocetirizine to human H(1) histamine receptors: contribution of Lys(191) and Thr(194). *Mol. Pharmacol.*, **2002**, *61*, 391-399.
- [9] Bloebaum, R.M.; Grant, J.A. Levocetirizine: the allergist's arsenal grows larger. *Expert Opin. Pharmacother.*, **2004**, *5*(7), 1581-1588.
- [10] Devalia, J.L.; De Vos, C.; Hanotte, F.; Baltes, E. A randomized double blind crossover comparison among cetirizine, levocetirizine and UCB 28557 on histamine-induced cutaneous responses in healthy adult volunteers. *Allergy*, **2001**, *56*, 50-57.
- [11] Frossard, N.; Strolin-Benedetti, M.; Purohit, A.; Pauli, G. Inhibition of allergen-induced wheal and flare reactions by levocetirizine and desloratadine. *Br. J. Clin. Pharmacol.*, **2008**, *65*, 172-179.
- [12] Bachert, C.; Bousquet, J.; Canonica, G.W.; Durham, S.R.; Klimek, L.; Mullol, J.; Van Cauwenberge, P.B. Levocetirizine improves quality of life and reduces costs in long-term management of persistent allergic rhinitis. *J. Allergy Clin. Immunol.*, **2004**, *114*, 838-844.
- [13] Canonica, G.W.; Fumagalli, F.; Guerra, L.; Baiardini, I.; Compalati, E.; Rogkakou, A.; Massacane, P.; Gamalero, C.; Riccio, A.M.; Scordamaglia, A.; Passalacqua, G. Levocetirizine in persistent allergic rhinitis: continuous or on-demand use? A pilot study. *Curr. Med. Res. Opin.*, **2008**, *24*, 2829-2839.
- [14] de Blic, J.; Wahn, U.; Billard, E.; Alt, R.; Pujazon, M.C. Levocetirizine in children: evidenced efficacy and safety in a 6-

- week randomized seasonal allergic rhinitis trial. *Pediatr. Allergy Immunol.*, **2005**, *16*, 267-275.
- [15] Potter, P.C.; Paediatric Levocetirizine Study Group. Efficacy and safety of levocetirizine on symptoms and health-related quality of life of children with perennial allergic rhinitis: a double-blind, placebo-controlled randomized clinical trial. *Ann. Allergy Asthma Immunol.*, **2005**, *95*, 175-180.
- [16] Wang, D.Y.; Hanotte, F.; De Vos, C.; Clement, P. Effect of cetirizine, levocetirizine and dextrocetirizine on histamine-induced nasal response in healthy adult volunteers. *Allergy*, **2001**, *56*, 339-343.
- [17] Jazrawi, E.; Stirling, R.; Chung, K. F. Effects of levocetirizine on eotaxin-induced eosinophil chemotaxis with or without interleukin-5 priming *in vitro*. *Allergy*, **2001**, *56*(Suppl 68), 138.
- [18] Thomson, L.; Blylock, M. G.; Sexton, D.W.; Campbell, A.; Walsh, G.M. Cetirizine and levocetirizine inhibit eotaxin-induced eosinophil transendothelial migration through human dermal or lung microvascular endothelial cells. *Clin. Exp. Allergy*, **2002**, *32*, 1187-1192.
- [19] Arnold, R.; Konig, B.; Campbell, A.; Freytag, C.C.; Konig, W. Levocetirizine modulates inflammatory responses of human neutrophils in the presence of the respiratory syncytial virus. *Allergy*, **2002**, *57*(Suppl 73), 146.
- [20] Ciprandi, G.; Cirillo, I.G.; Vizzaccaro, A.; Tosca, M.A. Levocetirizine improves nasal symptoms and air flow in patients with persistent allergic rhinitis: a pilot study. *Eur. Ann. Allergy Clin. Immunol.*, **2005**, *37*, 25-29.
- [21] Ciprandi, G.; Cirillo, I.G.; Vizzaccaro, A.; Tosca, M.A. Levocetirizine improves nasal obstruction and modulates cytokine pattern in patients with seasonal allergic rhinitis: a pilot study. *Clin. Exp. Allergy*, **2004**, *34*, 958-964.
- [22] Lee, D.K.; Bates, C.E.; Currie, G.P.; Lipworth, B. J. Comparative *in vivo* bioactivity of modern H1-antihistamines on AMP challenge in atopic asthma. *J. Allergy Clin. Immunol.*, **2003**, *111*, 337-341.
- [23] Lee, D.K.; Gardiner, M.; Haggart, K.; Fujihara, S.; Lipworth, B. J. Comparative effects of desloratadine, fexofenadine, and levocetirizine on nasal adenosine monophosphate challenge in patients with perennial allergic rhinitis. *Clin. Exp. Allergy*, **2004**, *34*, 650-653.
- [24] Hasala, H.; Janka-Junttila, M.; Moilanen, E.; Kankaanranta, H. Levocetirizine and cytokine production and apoptosis of human eosinophils. *Allergy Asthma Proc.*, **2007**, *28*, 582-589.
- [25] Potter PC; Paediatric Levocetirizine Study Group. Levocetirizine is effective for symptom relief including nasal congestion in adolescent and adult (PAR) sensitized to house dust mites. *Allergy*, **2003**, *58*, 893-899.
- [26] Patou, J.; De Smedt, H.; van Cauwenberge, P.; Bachert, C. Pathophysiology of nasal obstruction and meta-analysis of early and late effects of levocetirizine. *Clin. Exp. Allergy*, **2006**, *36*, 972-981.
- [27] Bousquet, J.; van Cauwenberge, P.; Khaltaev, N.; Eds. Allergic rhinitis and its impact on asthma. ARIA. In: Collaboration with the World Health Organization. *J. Allergy Clin. Immunol.*, **2001**, *108*, S1-S315.
- [28] Juniper, E.F.; Guyatt, G.H.; Dolovich, J. Assessment of quality of life in adolescents with allergic rhinoconjunctivitis: development and testing of a questionnaire for clinical trials. *J. Allergy Clin. Immunol.*, **1994**, *93*, 413-423.
- [29] Marcucci, F.; Sensi, L. A new method for IgE detection in nasal mucosa. *Clin. Exp. Allergy*, **1989**, *19*, 157-162.
- [30] Lopuhaa, C.E.; Out, T.A.; Jansen, H.M.; Aalberse, R.C.; Van der Zee, J.S. Allergen-induced bronchial inflammation in house dust mite allergic patients with or without asthma. *Clin. Exp. Allergy*, **2002**, *32*, 1720-1727.
- [31] Hamid, Q.; Springall, D.R.; Riveros-Moreno, V.; Chanez, P.; Howarth, P.; Redington, A.; Bousquet, J.; Godard, P.; Holgate, S.; Polak, J.M. Induction of nitric oxide synthase in asthma. *Lancet*, **1993**, *342*, 510-514.
- [32] Belvisi, M.; Barnes, P.J.; Larkin, S.; Yacoub, M.; Tadjkarimi, S.; Williams, T.J.; Mitchell, J.A. Nitric oxide synthase activity is elevated in inflammatory lung disease in humans. *Eur. J. Pharm.*, **1995**, *283*, 255-258.
- [33] Kharitonov, S.A.; Rajakulasingam, K.; O'Connor, B.; Durham, S.R.; Barnes, P.J. Nasal nitric oxide is increased in patients with asthma and allergic rhinitis and may be modulated by nasal glucocorticoids. *J. Allergy Clin. Immunol.*, **1997**, *99*, 58-64.
- [34] Boot, J.D.; de Kam, M.L.; Mascelli, M.A.; Miller, B.; van Wijk, R.G.; de Groot, H.; Cohen, A.F.; Diamant, Z. Nasal nitric oxide: longitudinal reproducibility and the effects of a nasal allergen challenge in patients with allergic rhinitis. *Allergy*, **2007**, *62*, 378-384.
- [35] Struben, V.M.; Wieringa, M.H.; Feenstra, L.; de Jonste, J.C. Nasal nitric oxide and nasal allergy. *Allergy*, **2006**, *61*, 665-670.
- [36] Vaidyanathan, S.; Nair, A.; Barnes, M.L.; Meldrum, K.; Lipworth, B.J. Effect of levocetirizine on nasal provocation testing with adenosine monophosphate compared with allergen challenge in allergic rhinitis. *Clin. Exp. Allergy*, **2009**, *39*, 409-416.
- [37] Braunstahl, G.J.; KleinJan, A.; Overbeek, S.E.; Prins, J.B.; Hoogsteden, H.C.; Fokkens, W.J. Segmental bronchial provocation induces nasal inflammation in allergic rhinitis patients. *Am. J. Respir. Crit. Care Med.*, **2000**, *161*, 2051-2057.
- [38] Braunstahl, G.J.; Overbeek, S.E.; KleinJan, A.; Prins, J.B.; Hoogsteden, H.C.; Fokkens, W.J. Nasal allergen provocation induces adhesion molecule expression and tissue eosinophilia in upper and lower airways. *J. Allergy Clin. Immunol.*, **2001**, *107*, 469-476.
- [39] Marcucci, F.; Passalacqua, G.; Canonica, G.W.; Frati, F.; Salvatori, S.; Di Cara, G.; Petrini, I.; Bernini, M.; Novembre, E.; Bernardini, R.; Incorvaia, C.; Sensi, L.G. Lower airway inflammation before and after house dust mite nasal challenge: an age and allergen exposure-related phenomenon. *Respir. Med.*, **2007**, *101*, 1600-1608.
- [40] Pasquali, M.; Baiardini, I.; Rogkakou, A.; Riccio, A.M.; Gamalero, C.; Descalzi, D.; Folli, C.; Passalacqua, G.; Canonica, G.W. Levocetirizine in persistent allergic rhinitis and asthma: effects on symptoms, quality of life and inflammatory parameters. *Clin. Exp. Allergy*, **2006**, *36*, 1161-1167.
- [41] Barnes, P.J. Nuclear factor-kappa B. *Int. J. Biochem. Cell. Biol.*, **1997**, *29*, 867-870.