

Comparison of the effects of fluticasone propionate, aqueous nasal spray and levocabastine on inflammatory cells in nasal lavage and clinical activity during the pollen season in seasonal rhinitis

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Summary

Background Treatment options for allergic rhinitis include antihistamines, decongestants, anticholinergics, cromolyn sodium and corticosteroids. As the nose is a small organ, comprising less than 1% of total body mass and surface area, it seems logical to confine treatment of rhinitis to the diseased organ.

Objective To evaluate the effects of therapy with intranasal fluticasone propionate (FP), both on subjective symptoms and pathophysiological mechanisms, in rhinitis patients during pollen season when the patients were symptomatic.

Methods We used a double-blind, placebo (PLA)-controlled, randomized, double dummy, parallel group study of the effect of 6 weeks treatment. The double-blind comparison was made between the following three treatments: FP aqueous nasal spray, 200 µg taken once daily, levocabastine (LEV) nasal spray, 200 µg taken twice daily and PLA nasal spray. Clinical evaluation and the levels of cells and mediators in nasal washing were performed before and after treatments. Twenty-four patients (11 men and 13 women, aged 17–50 years, mean age 30.1 ± 8.5) with strictly seasonal allergic rhinitis to *Parietaria* entered the study. Clinical evaluation and the levels of inflammatory cells (eosinophils and activated eosinophils, i.e. EG_2^+) and their mediators (tryptase, eosinophil cationic protein, eosinophil protein X and neutrophil myeloperoxidase) in nasal-lavage were performed before and after treatments.

Results Treatment with FP significantly increased, with respect to placebo, the percentage of days without sneezing ($P < 0.001$), nasal blockage ($P < 0.001$), rhinorrhea ($P < 0.001$), nasal itching ($P < 0.001$). Furthermore, treatment with FP showed additional benefits with respect to LEV. The percentage of days without nasal blockage was significantly higher in the FP group than in the placebo group ($P = 0.018$). The same applied to rhinorrhea ($P = 0.009$). The percentages of days without sneezing and itching were instead not significantly different between the two groups. As expected, no significant differences were observed in baseline medians of the rhinitis symptom scores as well as in mean values of all mediators and eosinophils in nasal lavages of the various groups under study. After treatment the mean of subjective symptoms as well as all values in nasal lavage level fell significantly only in the FP group, whereas no significant changes were observed either in LEV or PLA groups. Accordingly, significant differences were observed at the end of the treatments between the values of fluticasone group vs LEV and PLA group values.

Significant correlations between these values and symptom scores were found, according with literature data suggesting a pathogenetic role for these mediators and eosinophils in rhinitis.

Conclusion FP (200 µg once daily) affords a significant degree of improvement in rhinitis control during pollen season, as measured by subjective and objective parameters, compared with LEV (200 µg twice daily) and PLA. The therapeutic benefits of intranasal FP are reflected in, and may be caused by, the decrease in nasal inflammatory cells.

Keywords: EG₂⁺, eosinophil cationic protein, eosinophil protein X, eosinophils, fluticasone propionate, levocabastine, myeloperoxidase, placebo, seasonal rhinitis, tryptase

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Introduction

The allergic response is biphasic whether studied in the nose, lungs or skin. The early phase reaction occurs within 30 min after natural allergen exposure or challenge and may be followed by the late-phase reaction 3–11 h later. The pathophysiology of allergic rhinitis is characterized by ongoing tissue inflammation which follows the classic type I immediate hypersensitivity reaction [1–9]. The reaction in the nose is characterized by the classic symptoms of sneezing, itching, congestion, rhinorrhea and nasal blockage. Other symptoms, such as popping of the ears, throat clearing and coughing are less common [3].

Treatment options for allergic rhinitis include antihistamines, decongestants, anticholinergics, cromolyn sodium and corticosteroids [3,10]. H₁-antagonistic drugs are the most frequently used medication in the treatment of allergic rhinitis. In general, their onset of action is rapid, and they reduce sneezing, pruritus and rhinorrhea, but not nasal blockage. Histamine acts through three receptors, referred to as H₁, H₂ and H₃. Activation of H₁ receptors causes smooth-muscle contraction, increases vascular permeability, increases the production of mucus, and activates sensory nerves to induce pruritus and reflexes such as sneezing. Some antihistamines have local anaesthetic properties whose potency actually exceeds that of procaine. In high concentrations, antihistamines reduce the release of histamine from basophils and mast cells both *in vitro* and *in vivo*. Treatment with antihistamines has also been claimed to reduce the production of leucotrienes and kinins. These properties should offer an advantage in treatment, but the clinical benefits of these actions have not been documented [3]. Antihistamines applied topically to the nose are as effective as oral antihistamines. Although there is little difference with regard to speed of onset of clinical activity, unwanted effects are reduced and the preparations may be prescribed without risk of interaction with any concomitant medications [11–13].

Glucocorticoids are the most potent medications available for the treatment of allergic rhinitis [2]. The known side-effects of systemic administration have led to the development of topical preparations. The efficacy of these preparations is equal to or exceeds that of their oral counterparts, and they are far safer. These agents all act by regulating gene expression, interfering with multiple steps in the inflammatory process [14–19]. Treatment via the oral route has little effect on the immediate response to nasal provocation with antigen but markedly diminishes the subsequent hyperresponsiveness to antigen and late-phase reactions. It also reduces the influx of eosinophils after antigenic stimulation. The number of eosinophils, the presence of eosinophil cationic protein (ECP), and the number of mast cell progenitors are also reduced by topical steroids during seasonal exposure to allergens. In marked contrast with systemic steroids, topical steroids also affect the early response. Another difference is that topical steroids inhibit the influx of neutrophils as well as eosinophils [2,20].

The fluorinated, topically active glucocorticoid fluticasone propionate (FP) (Glaxo Laboratories Ltd, Greenford, UK), has been shown to be safe and effective in the treatment of both seasonal and perennial rhinitis [21–23]. When taken by the inhaled route, FP has shown significant therapeutic efficacy in the management of asthma. Results of clinical studies suggest a therapeutic index which should be higher than other currently available inhaled corticosteroids [24]. Furthermore, FP has been shown to be more effective than the second-generation antihistamine as terfenadine and loratidine [25–28].

However, as discussed above, the studies performed on nasal lavage fluids when topical intranasal corticosteroids are administered before allergen challenge shows inhibition of the release of the mediators of both early- and late-phase reactions. Recruitment and activation of eosinophils and neutrophils is also inhibited, and that might decrease some of the subsequent steps of the inflammatory process. However, these effects have been reported in studies in which the primary emphasis has been on efficacy. In addition, a few

data, if any, are available on the effect of the treatment on mediators from nasal fluids in seasonal allergic rhinitis [21–29]. In this context we present the effects of therapy with intranasal FP both on subjective symptoms and pathophysiological mechanisms, in rhinitis patients during pollen season. We used a double-blind, placebo (PLA)-controlled, randomized, double dummy, parallel group study of the effect of 6 weeks' treatment. The double-blind comparison was made between the following three treatments: FP aqueous nasal spray, 200 µg taken once daily, levocabastine (LEV) (Livostin, Janssen-Cilag B.V., Tilburg, The Netherlands) nasal spray, 200 µg taken twice daily and PLA nasal spray. Clinical evaluation and the levels of cells and mediators in nasal washing were performed before the start of the study and at the end of the treatments.

Methods

Subjects

Twenty-four patients (11 men; aged 17–50 years, mean age 30.1 ± 8.5) with strictly seasonal allergic rhinitis to Parietaria entered the study. The study was performed during the pollen season when the patients were symptomatic. Their allergy had been verified by their medical history, a skin prick test (SPT) and RAST. The patients had no symptoms of asthma, urticaria, or eczema, and their symptoms were present only during the pollen season for at least 4 years before the study. These patients had taken part to the multicentre Italian study FLNC02 [30]. Informed consent according to the rules of our department ethics committee was obtained from each patient.

Allergen

In the Mediterranean area there are some typical allergenic plants, such as *Parietaria* and *Olea europaea*. *Parietaria*, an Urticacea, is the most important hay fever-provoking plant in southern Italy. *Parietaria* grows especially on walls inside towns and has two very long cycles of flowering. The largest pollination period of *Parietaria* takes place from March to the end of June. A lower pollination period occurs from the end of August to October. Accordingly, the clinical manifestation of pollinosis caused by *Parietaria* are present for a long period, often multiseasonal, particularly in Spring and sometimes also in Autumn [31–36].

Parietaria pollen count per m³ of air were continuously taken with a volumetric pollen trap (Burkard Manufacturing, Rickmansworth, UK) placed on the roof of the hospital building at a height of approximately 20 m. Microscopic analysis was performed at magnification of 400×, and results calculated as mean counts per day. During the study treatment period, it was documented a concentration

of *Parietaria* over 50 grains/m³ (range 53–247). These data confirmed that the study period was largely coincident with the pollen season, when recruited patients were expected to be symptomatic [37].

SPTs were performed and evaluated in accordance with EAACI (European Academy of Allergy and Clinical Immunology) Subcommittee on Allergen Standardization and skin tests using a standard allergen panel (Neo Abellò, Milan, Italy) [38]. The panel included the following extracts: five pollens (grass, *Parietaria*, mugwort, birch and *Olea europaea*), two moulds (*Alternaria* and *Cladosporium*), two animal danders (cat and dog), two house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), a negative control (glycerinated saline), and a positive control (histamine, 10 mg/mL). A skin test response was regarded as positive if the weal diameter was 3 mm greater than that of the glycerinated saline control.

Study design

This was a randomized double-blind, double dummy, PLA-controlled parallel group study of the effect of 6 weeks' treatment. The double-blind comparison was made between the following three treatments: FP aqueous nasal spray, 200 µg taken once daily, LEV nasal spray, 200 µg taken twice daily and PLA nasal spray. The nasal lavages were performed before starting the study and at the end of the treatments. The treatment period began 1 week after starting of the pollen season without any run-in.

Assessment of symptoms

Patients were instructed to record their daily symptoms on diary card. Nasal symptoms included nasal blockage, nasal itching, sneezing and rhinorrhea. Nasal symptoms were scored as follows: 0, none; 1, mild (occasionally present); 2, moderate (rather frequent); 3, severe (persistent). Total symptom scores ranged from 0 to 12 and represented the sum of scores of nasal blockage, nasal itching, sneezing and rhinorrhea. Before and after the treatment period (week 0 and 6) the patients gave an overall assessment of their rhinitis symptoms. Rhinoscopic examination was performed to confirm nasal blockage and rhinorrhea. Furthermore, the assessment of efficacy was based on the analysis of the percentages of symptom-free days for symptom of: sneezing, rhinorrhea, nasal blockage and nasal itching.

Nasal fluid

Nasal lavage was performed using a disposable metered-dose nasal inhaler (Markos, Monza, Italy) filled with sterile, room-temperature normal saline solution. The device

consisted of a plastic cup with two rooms. The central compartment was filled with sterile saline solution with a syringe; the external compartment collected the liquid after washing. Total input of saline solution was approximately 8 mL (4 mL in each nostril for 5 min). To collect the nasal washings, the subjects were instructed to actively breathe during a Valsalva manoeuvre to harvest nasal fluid in the cup. Obtained samples were stored on ice and centrifuged at 400g for 10 min at 4°C and supernatant aliquots stored at -70°C. The individual variation in the recovered vs introduced volume was $86 \pm 8\%$ [39].

Laboratory tests

Tryptase, eosinophil protein X (EPX) and neutrophil myeloperoxidase (MPO) levels were determined using commercial radioimmunoassays (Kabi-Pharmacia AB Uppsala, Sweden). For tryptase, the detection limit was 0.5 U/L and the interassay variation was less than 6% [39]. For EPX, the detection limit was 3 ng/mL, and the interassay variation was less than 6.1%. For MPO, the detection limit was 8 ng/mL, and the interassay variation was less than 5.4%.

The assay of nasal fluid ECP was performed with a Fluorescent Enzyme Immunoassay (FEIA) using the Pharmacia CAPTM system (Pharmacia AB, Uppsala, Sweden). The detection limit was 2 ng/mL and the interassay variation was less than 5.9% [39].

Nasal cytology

Nasal eosinophil counts were performed on nasal lavage. One cytopsin slide for each sample (1×10^4 cells in 170 µL for slide) was centrifuged at 500g for 10 min in a Shandon cytocentrifuge (Shandon Southern Ltd, Runcorn, UK). Slides were immediately fixed in 95% ethyl alcohol and dipped in Wright–Giemsa stain. The slides were examined under oil immersion by light microscopy at magnification of 400×. Eosinophil counts were expressed as percentage of three hundred cells counted. All specimens were examined by the same microscopist. Cell-ruptures in pellet sometimes were observed, but they never exceeded 1–2% of cells and therefore not taken into account.

A flow cytometric technique, based on fixation with formaldehyde and permeabilization with octyl-glucopyranoside (FOG) of eosinophils from nasal washing was used according to Hallden *et al.* [40], with minor modifications. Briefly, cell suspensions (1×10^5 cells/mL) were washed in PBS-EDTA and subsequently fixed in 200 µL of 4% paraformaldehyde (PFA) for 10 min at 4°C to stabilize cell membranes, washed in PBS-EDTA and permeabilized by incubation in 0.74% *n*-octyl-glucopyranoside (OG, Sigma, St Louis, MO, USA) for 6 min at 20°C. The detection of intracellular ECP was performed on FOG-treated cell

suspension, which were incubated with 10 µL of anti-ECP (10 µg/mL) mAb (Clone EG₂; Kabi Pharmacia, Sweden) or irrelevant isotype matched mAb for 30 min at 4°C, washed in PBS-EDTA and further incubated with FITC-conjugated F(ab')₂ antimouse IgG. Unlabelled mouse IgG1 (10 µg/mL) (Coulter Inc, Hialeah, FL, USA) served as background control. The eosinophil preparations were resuspended in 500 µL PBS-EDTA and samples were run on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA, USA) equipped with an argon laser emitting at 488 nm. Forward and side scatter were collected as linear signals and all fluorescent emissions were collected on a four-decade logarithmic scale. FITC signals were measured at 530 nm and spectral overlap was minimized by electronic compensation with Calibrate Beads (Becton Dickinson) before each determination series. A minimum of 10 000 events was acquired in list mode using Cell Quest[®] software (Becton Dickinson). In single parameter fluorescence histograms, results were expressed as mean fluorescence intensity (MFI) ratio of positive cell populations [40,41].

Statistics

The assessment of efficacy was based on the analysis of the percentages of nasal symptom-free days. The comparison between the treatments was performed using Kruskal–Wallis test. Sum of symptom scores before and after the treatments were expressed as median (range) and were compared using a non-parametric test (Kruskal–Wallis). Mediators and eosinophils levels were expressed as mean values $\pm$ SEM and were analysed using ANOVA test (one-way) to compare values within the groups and by Student Newman–Keuls test to compare mean values between the treatments. Putative correlation between mediators and eosinophils levels in the lavage and sum of score symptoms, before and after the study, were analysed using Spearman's correlation coefficient analysis.

Results

Clinical data

All patients completed the study and no adverse effect was reported. Evidence from patient's diary cards showed that treatment with FP over a 6-week period during pollen season significantly increased, with respect to placebo, the percentage of days without sneezing (45 vs 24%; $P < 0.001$), nasal blockage (51 vs 25%; $P < 0.001$), rhinorrhea (70 vs 30%; $P < 0.001$), nasal itching (72 vs 46%; $P < 0.001$). Furthermore, treatment with FP showed additional benefits with respect to LEV. The percentage of days without nasal blockage was significantly higher in the FP group than in the placebo (51 vs 30; $P = 0.018$). The same applied to

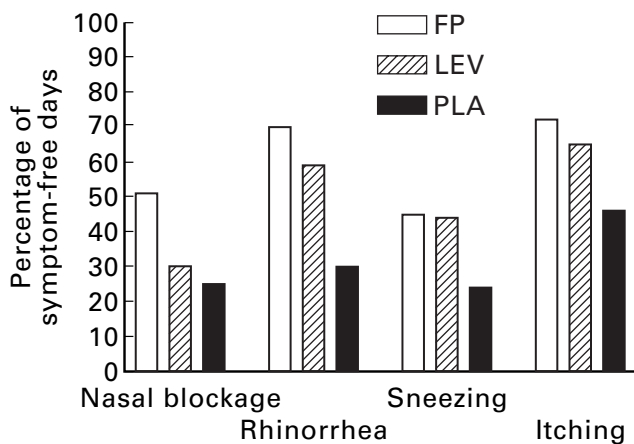


Fig. 1. Median number of symptom-free days during treatments with fluticasone propionate aqueous nasal spray 200 µg, once daily (FP), or levocabastine 200 µg, given twice daily (LEV) or placebo (PLA).

rhinorrhea (70 vs 59; $P=0.009$). The percentages of days without sneezing (45 vs 44) and itching (72 vs 65) were instead not significantly different between the two groups (Fig. 1). As expected, before the study the medians of the total rhinitis symptom scores were not significantly different between the various groups, i.e. 8 (range 6–12) in FP group, 8.5 (range 2–12) in the LEV group and 7.5 (range 5–12) in PLA group. At the end of the study the median of the rhinitis symptom scores were significantly lower in FP group than in LEV and PLA groups, respectively, 2.5 (range 0–4), 6.5 (range 1–12) and 7.5 (range 5–12) ($P<0.05$).

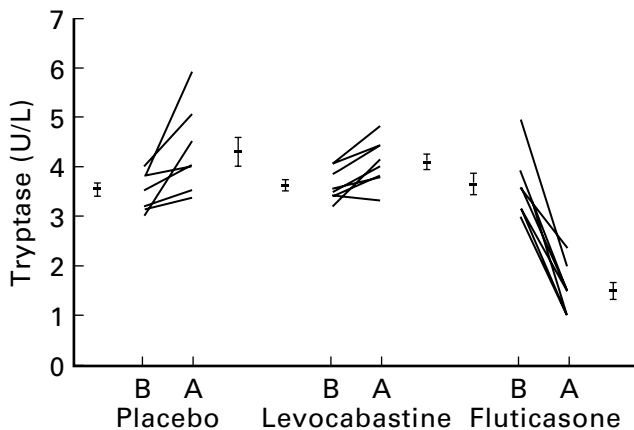


Fig. 2. Individual and mean $\pm$ SEM tryptase levels in nasal lavages (B) before and (A) after PLA, LEV or FP treatment. No significant differences were observed in baseline values (3.55 ± 0.13 , 3.63 ± 0.11 , 3.66 ± 0.22 U/L, respectively). After FP treatment, the mean of tryptase in nasal lavages level fell significantly in FP group to 1.5 ± 0.17 U/L ($P<0.01$), whereas no significant changes were observed either in the PLA (4.32 ± 0.29 U/L) or LEV (4.10 ± 0.16 U/L) groups.

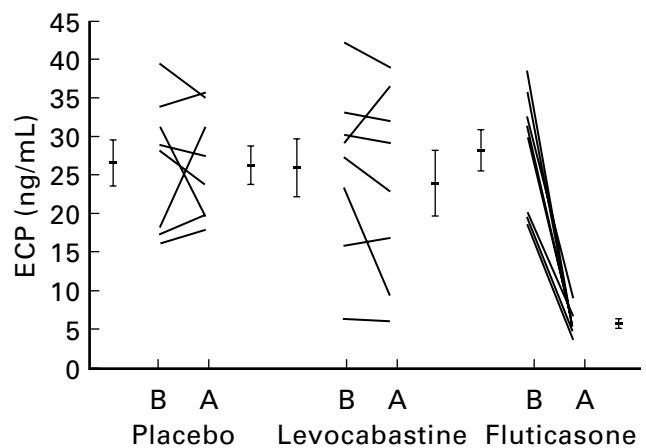


Fig. 3. Individual and mean $\pm$ SEM ECP levels in nasal lavages (B) before and (A) after PLA, LEV or FP treatment. No significant differences were observed in baseline values (26.65 ± 3.03 , 25.92 ± 3.86 , 28.28 ± 2.76 ng/mL). After FP treatment, the mean of ECP in nasal lavages level fell significantly in the FP group to 5.72 ± 0.60 ng/mL ($P<0.01$), whereas no significant changes were observed both in the PLA (26.32 ± 2.52 ng/mL) and in LEV (23.97 ± 4.34 ng/mL) groups.

Mediator levels in nasal fluid

No significant differences were observed in baseline mean values of all mediators in nasal lavages of the various groups under study (Figs 2–4). After treatment, the mean value of tryptase in nasal lavages had significantly fallen only in FP

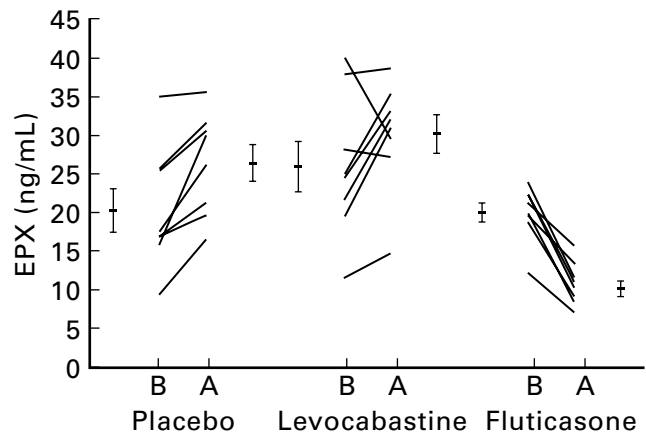


Fig. 4. Individual and mean $\pm$ SEM EPX levels in nasal lavages (B) before and (A) after PLA, LEV or FP treatment. No significant differences were observed in baseline values (20.23 ± 2.80 , 25.96 ± 3.30 , 19.93 ± 1.24 ng/mL). After FP treatment, the mean of EPX in nasal lavages level fell significantly in the FP group to 10.78 ± 0.1 ng/mL ($P<0.01$), whereas no significant changes were observed either in the PLA (26.31 ± 2.36 ng/mL) or LEV (30.07 ± 2.53 ng/mL) groups.

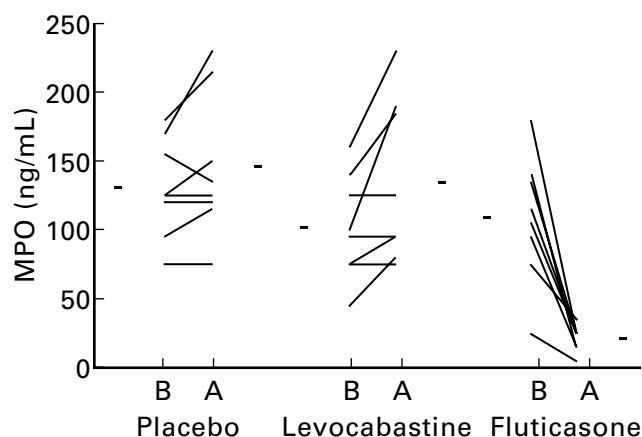


Fig. 5. Individual and mean MPO $\pm$ SEM levels in nasal lavages (B) before and (A) after PLA, LEV or FP treatment. No significant differences were observed in baseline values (130.62 ± 12.76 , 101.87 ± 13.42 , 108.75 ± 16.44 ng/mL). After FP treatment, the mean of MPO in nasal lavages level fell significantly in FP group to 21.25 ± 3.2 ng/mL ($P < 0.01$), whereas no significant changes were observed both in PLA (145.62 ± 18.45 U/L) and in LEV (134.37 ± 20.90 ng/mL) groups.

group ($P < 0.01$), whereas no significant changes were observed in either the LEV or PLA groups (Fig. 2). Accordingly, significant differences were observed at the end of the treatments, in mean value of tryptase in nasal lavages from FP group vs those both from LEV and from PLA ones ($P < 0.05$).

With regard to eosinophil mediators ECP (Fig. 3) and EPX (Fig. 4), both mean values in nasal lavages fell significantly in FP group ($P < 0.01$), whereas no significant changes were observed both in LEV and in PLA groups. Accordingly, significant differences were observed at the end of the treatments, in means of both ECP and EPX in nasal lavages level of FP group vs LEV and PLA ones ($P < 0.05$).

Exactly the same results were obtained by analysing in the different groups the levels of neutrophil mediator MPO (Fig. 5). After treatment the mean of MPO in the level of nasal lavages fell significantly only in the FP group ($P < 0.01$), whereas no significant changes were observed either in the LEV or PLA groups. Accordingly, significant differences were observed at the end of the treatments, in MPO mean values in nasal lavages from the FP group vs those from LEV and PLA ($P < 0.05$).

Eosinophil counts

Before the treatment, no significant differences in nasal samples were observed between the different groups, either for the percentage of eosinophils or activated eosinophils, i.e. EG_2^+ cells (analysed as MFI) (Figs 6 and 7). After the

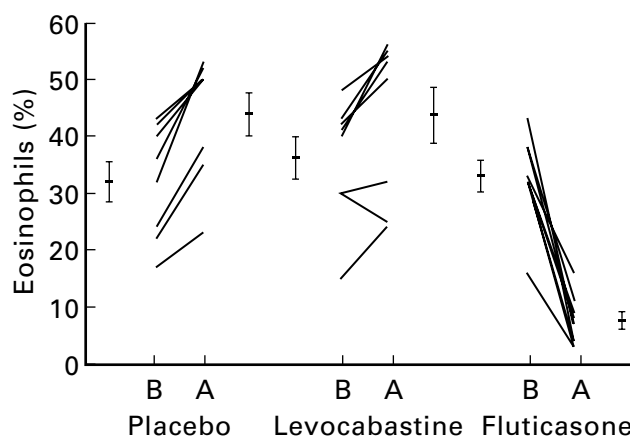


Fig. 6. Individual and mean $\pm$ SEM eosinophil percentages in nasal lavages (B) before and (A) after PLA, LEV or FP treatment. No significant differences were observed in baseline values (32.0 ± 3.51 , 36.12 ± 3.73 , 33.12 ± 2.8 %, respectively). After FP treatment, the mean of eosinophil percentage in nasal lavages level fell significantly in the FP group to 7.6 ± 1.58 % ($P < 0.05$), whereas no significant changes were observed either in the PLA (43.87 ± 3.80 %) or the LEV (43.62 ± 4.97 %) groups.

treatments, both values decreased only in the patients treated with FP ($P < 0.05$), whereas no significant differences were detected in the other groups under study. Accordingly, significant differences were observed between patients treated with FP and patients treated with LEV and with PLA ($P < 0.05$) (Figs 6 and 7).

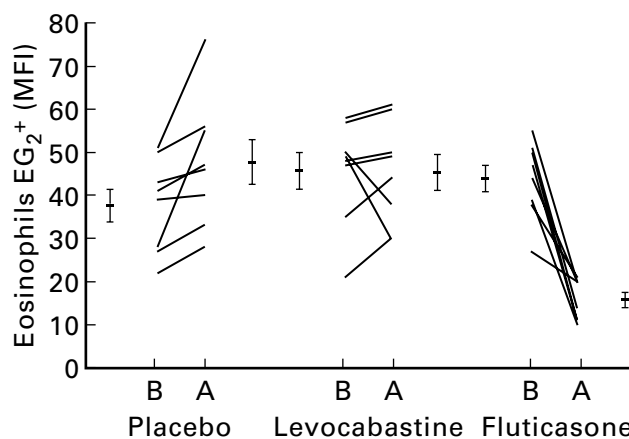


Fig. 7. Individual and mean $\pm$ SEM activated eosinophil (EG_2^+) values (expressed as mean fluorescence intensity) in nasal lavages before and after PLA, LEV or FP treatment. No significant differences were observed in baseline values (37.62 ± 3.83 , 45.62 ± 4.30 , 43.8 ± 3.17 MFI, respectively). After FP treatment, the mean of EG_2^+ in nasal lavages level fell significantly in FP group to 15.75 ± 1.76 MFI ($P < 0.05$), whereas no significant changes were observed either in the PLA (47.62 ± 5.32 MFI) or LEV (45.25 ± 4.27 MFI) groups.

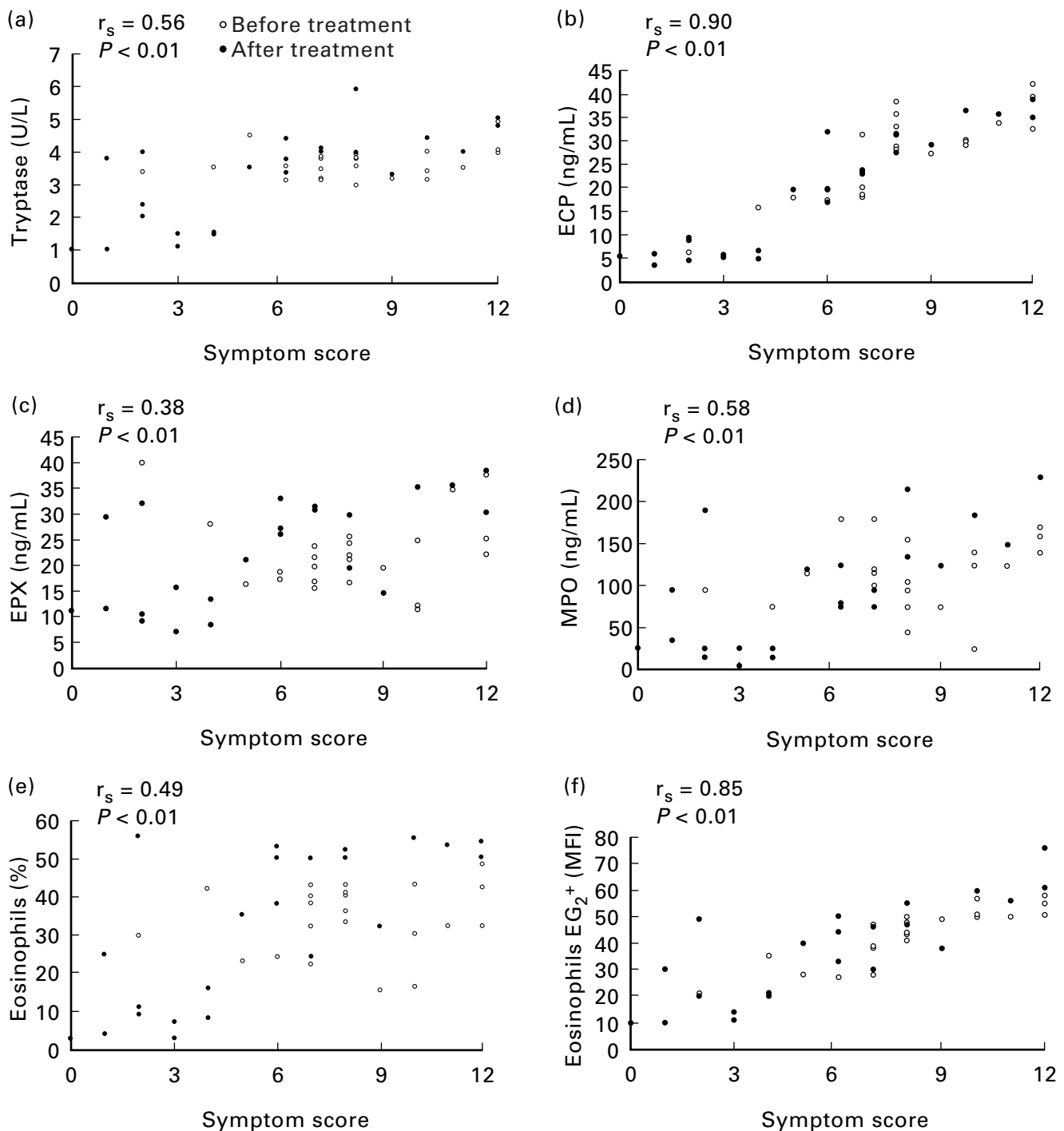


Fig. 8. Correlation between symptom score and levels of tryptase (a), ECP (b), EPX (c), MPO (d), eosinophil (e) and EG_2^+ (f). Individual values before and after treatment.

Correlations

We also analysed patient data before and after treatment, for correlation between symptom score and levels of tryptase (Fig. 8a), ECP (Fig. 8b), EPX (Fig. 8c), MPO (Fig. 8d),

eosinophil (Fig. 8e) and EG_2^+ (Fig. 8f). As shown, significant correlations between these values and symptom score were found, according to literature data that suggest a pathogenetic role for these mediators and eosinophils in rhinitis [2,8,9,37,39,41–44].

Discussion

The results of this comparative study demonstrate that fluticasone propionate administered once daily produces a significant reduction in the nasal lavage of tryptase, eosinophil mediators, ECP and EPX, MPO and the percentage of eosinophils and activated eosinophils, i.e. EG_2^+ cells (analysed as MFI) in patients with seasonal allergic rhinitis compared with levocabastine given twice daily. In addition FP treatment reduces nasal blockage, rhinorrhea, sneezing and itching, and its clinical efficacy with respect to the nasal blockage and rhinorrhea was greater than that produced by LEV. As expected, LEV only had a significant effect on sneezing and itching measured as a percentage of symptom-free days, whereas it failed to produce any change in pathophysiological mechanisms. In fact, as already suggested, LEV is an effective H_1 -receptor antagonist without anti-inflammatory properties [45].

Allergic rhinitis is associated with mucosal inflammation, characterized by the influx and activation of inflammatory cells. Activation of inflammatory cells may be inferred through elevated levels of their mediators in nasal lavage fluid. Histamine is not a reliable marker of mast cell and basophil degranulation in the nose, since nasal lavage fluid in normal subjects contains high levels of histamine [39]. Tryptase is a more reliable marker of mast cell activation. Under natural conditions, eosinophils are detected in allergic patients only during exposure to allergen. The accumulation of eosinophils and their products have been observed during allergen-induced late-phase reactions, and they are cytotoxic to the respiratory epithelium. A significant number of neutrophils have also been demonstrated in the nasal secretions in allergic disease. They are also recruited during late-phase reactions. The importance of neutrophils in allergic reactions in humans is still controversial. However, neutrophils have the potential for producing considerable airway damage by virtue of their capacity to generate potent lipid mediators and release the contents of their granules as MPO [2,4–9,46–48]. Over the last decade, a new model to explain allergic inflammation has evolved. In this model, Langerhans' cells and T cells play an important role. Langerhans' cells possess a high-affinity receptor for IgE. Allergen provocation results in stimulation of T cells by the IgE-positive Langerhans' cells. The T cells produce a number of cytokines which stimulate IgE production as well as the inflammatory reaction. These cytokines may be derived from a variety of sources besides T lymphocytes, like mast cells and eosinophils, and contribute to the underlying inflammatory process in rhinitis [45,49,50].

However, the inflammatory cell changes are evident in relation to clinical disease expression, and the symptoms of rhinitis, i.e. nasal pruritus, sneezing, rhinorrhoea, nasal obstruction and loss of sense of smell, may be explained

on the basis of mediator release from inflammatory cells [39,42–44,49–52].

As discussed in the introduction, treatment options for allergic rhinitis include antihistamines, decongestants, anticholinergics, cromolyn sodium and corticosteroids [2,10,53]. It is apparent from an understanding of the inflammatory process which underlies rhinitis that prevention of airway inflammation, with prophylactic therapy, is the optimal approach to control disease expression and that, while having some efficacy, inhibition of individual mediator release or end organ receptor antagonism will not give complete disease control. However, the nose is a small organ, comprising less than 1% of total body mass and surface area, and it is therefore logical to confine treatment of rhinitis to the diseased organ [2,10,48,53].

Topical corticosteroids have proven efficacy in the treatment of allergic rhinitis and have been shown, with regular administration, to inhibit both the immediate and late response to allergen provocation. Intranasal corticosteroids have been shown to have an effect on a number of different cells involved in the nasal allergic inflammatory reaction, which may explain their potent effect in clinical nasal allergy studies. Many clinical studies have shown equal efficacy of topical corticosteroids on all three major symptoms of allergic rhinitis, i.e. sneezing, watery rhinorrhea and nasal blockage, in contrast with antihistamine treatment, which has little effect on nasal blockage. In fact, topical steroids but not H_1 -antihistamines control the inflammatory cell influx that increases the potential for leucotriene generation responsible for nasal blockage and rhinorrhoea. The lack of effects on cell influx contributes to the lesser benefit of H_1 -antihistamines in allergic rhinitis as observed in this and other studies [2,14–20,54–56].

The aim of the present study was to evaluate the effects of therapy with intranasal FP both on subjective symptoms and pathophysiological mechanisms, in rhinitis patients during pollen season. FP is a topically active corticosteroid molecule based on the androstane nucleus. A key feature is the 17β -carbothioate ester, which is metabolized on first pass through the liver to yield the 17β -carboxylic acid, an inactive metabolite. Due to the first pass metabolism, the bioavailability of the absorbed fraction is negligible, resulting in minimal systemic adverse effects. The rate of association with the receptor is faster and the rate of dissociation slower than with standard corticosteroid. It is more potent *in vitro* than standard corticosteroids in inhibiting anti-CD3-induced human T-lymphocyte proliferation, cytokine generation, in attenuating tumour necrosis factor- α -induced endothelial cell adhesion molecule expression, and in increasing secretory leucocyte protease inhibitor levels in airway epithelial cells. It is also more potent and longer-acting than other corticosteroids in inhibiting oedema formation, interleukin-5 (IL-5)-induced blood eosinophilia,

and IL-5 or platelet activating factor-stimulated eosinophil accumulation in the lung. FP has demonstrated marked vasoconstrictor potency in normal subjects and inhibited antigen-induced mucosal platelet activating factor/eicosanoid production in rhinitis patients. FP therefore has increased intrinsic glucocorticoid potency and high topical anti-inflammatory activity. In clinical studies there were no significant adverse effects, including effects on plasma cortisol concentrations [21–28,53,57–60].

The anti-inflammatory activity of FP has previously been demonstrated in studies on subjects with both seasonal and perennial allergic rhinitis. Particularly, its efficacy has been demonstrated in the treatment of allergic rhinitis, both reducing clinical and inflammatory parameters [21–23,49,60]. In addition, a recent study has demonstrated that FP exerts a significant action on early- and late-phase clinical events following specific nasal challenge, also reducing the cellular influx during the late phase. This event is likely due to the modulation of ICAM-1 expression on epithelial cells [61].

As discussed in the introduction, in these studies the primary emphasis has been on efficacy, and spare data are available on the effect of the treatment on mediators from nasal fluids in seasonal allergic rhinitis. In the present study we show the effects of therapy with intranasal FP both on subjective symptoms and pathophysiological mechanisms, in rhinitis patients monosensitive to *Parietaria* during pollen season. After treatment the values of eosinophils and inflammatory mediators in nasal lavages as well as subjective symptoms had significantly fallen only in FP group, whereas no significant changes were observed either in the LEV or PLA groups. The inhibitory effect of FP on the activation of mast cells, eosinophils and neutrophils was clearly demonstrated by the reduced level of their mediators in nasal lavage fluid, i.e. tryptase, ECP and EPX, and MPO. Furthermore, the effect on eosinophil has been shown by the decreased influx of total and activated eosinophils, i.e. EG_2^+ [41]. Concerning neutrophils, it is worth noting that neutrophils appeared to have a short life span in tissue and that the true cellular load of neutrophils is very difficult to assess [48]. However, the presence of MPO in nasal lavage before the treatment and its reduction after FP shows that the FP also acts on the neutrophils [48]. Significant correlations between these values and symptom score have been found, according with literature data suggesting a pathogenetic role for these mediators and eosinophils in rhinitis [42,43]. However, the results of this study indicate that FP has the ability to influence both the cells of the early and the delayed phase of the allergic reaction. As discussed above, several effects of FP may well account for these results. In addition our preliminary observations suggest that *in vitro* FP inhibits IL-4 and IL-5 production by T cells and it is able to induce eosinophil apoptosis, according to Bradding *et al.* [62].

In conclusion, the results of the clinical, cytological and mediator components of the present study clearly show that the symptoms of seasonal allergic rhinitis to *Parietaria* are due to influx and/or activation of nasal inflammatory cells including mast cells, neutrophils and eosinophils, as demonstrated by the correlation between symptom score and levels of cells and mediators. Present and previous studies suggest that the therapeutic benefits of intranasal FP are reflected in, and may be caused by, the decrease in nasal inflammatory cells [14,29,42,49,63].

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