

## Effect of montelukast compared with inhaled fluticasone on airway inflammation

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### Summary

**Background** Inhaled corticosteroids are currently regarded as the gold standard in anti-inflammatory therapy, however, leukotriene receptor antagonists have been ascribed anti-inflammatory properties.

**Objective** We directly compared the anti-inflammatory effects of inhaled fluticasone propionate (FP, 100 µg Diskus, twice daily) and oral montelukast (MON 10 mg, nocte) in bronchial biopsies of patients with asthma in a double-blind, double-dummy, parallel-group design.

**Methods** Bronchial biopsies, serum and urine samples were collected from 36 atopic asthmatics before and after 8 weeks of treatment. Activated T cells (CD25<sup>+</sup>), eosinophils (MBP<sup>+</sup>) and mast cells (tryptase<sup>+</sup>) were analysed by immunohistochemistry. Serum eosinophil cationic protein (ECP) and IL-5 were analysed by radio and enzyme immunoassay (EIA), respectively. Urinary 9α-11β-PGF<sub>2</sub> and leukotriene E<sub>4</sub> (LTE<sub>4</sub>) were measured by EIA.

**Results** A comparison of changes from baseline [FP/MON ratio (95% confidence interval)] of activated T cells was not different when subjects were treated with FP compared to treatment with MON [1.00 (0.18–4.86); *P* = 0.924]. Following treatment, mast cells in the FP group were significantly lower than in the group treated with MON [0.39 (0.16–0.97); *P* = 0.041]. There was no difference in the number of eosinophils in the lamina propria following either treatment [0.54 (0.05–2.57); *P* = 0.263]. However, treatment with FP resulted in a significantly greater decrease in serum ECP, compared to treatment with MON [0.37 (0.25–0.71); *P* = 0.002].

**Conclusions** FP appears to be superior to MON as an anti-inflammatory therapy in mild asthmatics.

**Keywords** airway inflammation, asthma, fluticasone propionate, montelukast

Submitted 18 September 2003; revised 23 March 2004; accepted 7 May 2004

### Introduction

Asthma is a chronic disease associated with variable levels of airflow obstruction. Considerable evidence has been obtained to show that airway inflammation is a major factor in the pathogenesis of asthma and associated bronchial hyper-responsiveness (BHR). The inflammatory pattern in asthma is multicellular in nature, with mainly mast cells, eosinophils, and T lymphocytes participating in the response. Furthermore, it is known that mediators of inflammation such as cytokines and chemokines contribute to the orchestration of the inflammatory process [1]. As airway inflammation appears to be a critical etiologic feature of asthma, it has become the target of therapy, with inhaled corticosteroids (ICS) being currently regarded as the gold standard in anti-inflammatory therapy [2]. Treatment with ICS leads to consistent reductions in the number and activation of mast

cells, macrophages, T lymphocytes and eosinophils in the bronchial epithelium and submucosa [3, 4].

Leukotriene receptor antagonists (LTRAs) have been shown to improve pulmonary function, symptom control and to reduce asthma exacerbations [5]. Effects of LTRAs on inflammatory markers are not well defined. Treatment with LTRA montelukast (MON) has resulted in a significant decrease in serum eosinophil cationic protein (ECP) in both sputum [6] and peripheral blood eosinophils [7]. Calhoun et al. [8] have reported reductions in histamine and TNF-α concentrations in bronchoalveolar lavage (BAL) fluid after segmental allergen challenge following treatment with zafirlukast. However, there is a paucity of biopsy data relating to possible anti-inflammatory effects of LTRAs in the asthmatic airway. Nakamura et al. [9] analysed bronchial biopsies before and after 4 weeks of treatment with pranlukast (225 mg b.i.d.). There was a reduction in activated eosinophils, T cells and mast cells compared with the placebo treated group.

The positioning of LTRAs in asthma management guidelines continues to be controversial. In studies of mild and moderate asthmatics, directly comparing low-dose ICS and LTRAs, ICS have been shown to have a modest superiority

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with respect to lung function and BHR [10–13]. However, studies of oral and inhaled asthma treatments show that patient acceptance and compliance are significantly greater for oral than for inhaled preparations [14]. In addition, there is a general consensus that ICS do not affect LT biosynthesis *in vivo* [15]. These data have led to the suggestion that LTRAs could replace ICS as first line therapy in mild persistent asthmatics [16]. The primary obstacle to such an approach is the lack of information regarding the anti-inflammatory effects of LTRAs in the asthmatic lung.

The aim of the current study is to assess if MON has anti-inflammatory effects in the asthmatic lung and if these effects are comparable with those elicited by the gold standard treatment, ICS. Such information should prove valuable in placing LTRAs in treatment guidelines.

## Methods

### Subjects

Thirty-six (20 M/16 F, mean age  $27.1 \pm 1.7$ , range 19–57 years) non-smoking, atopic, mild asthmatics receiving  $\leq 800 \mu\text{g}$  of beclomethasone dipropionate or equivalent, were entered into the study. Atopy was defined as a positive skin prick test to house dust mite or two other commonly inhaled allergens. Subjects were required to have an forced expiratory volume in 1 s ( $\text{FEV}_1$ ) of  $\geq 60\%$  of the predicted value, a  $\Delta\text{FEV}_1 \geq 12\%$  to salbutamol and a provocative concentration of methacholine (MCh) to cause a 20% drop in  $\text{FEV}_1$  ( $\text{PC}_{20}\text{MCh}$ ) of  $\leq 4 \text{ mg/mL}$ . Patients with a history of respiratory infection or exacerbation of asthma in the month preceding the run-in period were excluded from the study. None of the study subjects had used oral or systemic corticosteroids 1 month prior to the run-in period. All participants gave informed written consent to the study, which was approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, the Netherlands.

**Study design** The study was a randomized, double-blind, double-dummy, parallel-group design. After a run-in period of 8 weeks subjects entered an 8 weeks' treatment period. At the beginning of the run-in period, subjects discontinued use of their inhaled steroid. Subjects were provided with a salbutamol Diskus (200  $\mu\text{g}$ /puff) rescue inhaler for use on an as-needed basis (up to 4 puffs daily) during the run-in and treatment period for symptomatic relief. Treatment with salbutamol was allowed up to 8 h prior to study measurements. On the last day of week seven ( $\pm 2$  days) of the run-in period, baseline spirometry was performed and  $\text{PC}_{20}\text{MCh}$  determined. On the same occasion, blood and urine samples were collected for measurement of inflammatory mediators.

At the end of the run-in period in week 8 ( $\pm 2$  days), subjects underwent bronchoscopy, and on the same day were randomly assigned to receive fluticasone propionate (FP) (100  $\mu\text{g}$  b.i.d) by Diskus inhaler and a placebo MON capsule or placebo FP and a MON capsule (10 mg nocte). After 7 weeks on treatment, pulmonary function and MCh provocations were performed. Subjects had a second bronchoscopy 1 week later, following 8 weeks of treatment.

**Pulmonary function** Pulmonary function tests were performed at the same time in the morning, using a heated pneumotachometer system (Jaeger, Würzburg, Germany). MCh provocation was performed using the standardized 2 min tidal-breathing technique [17].

### Bronchial biopsies

All bronchial biopsies were taken by the same pulmonary physician (S.E.O.). After intra-muscular pre-medication with atropine dinitrate (0.5 mg), oropharyngeal anaesthesia was accomplished with topical xylocaine spray 1%. The vocal cords, trachea and bronchial tree were anaesthetized with oxybuprocaine. The fibre-optic bronchoscope (BF, type P20 D; Olympus Tokyo, Japan) was introduced into the airway via the oral route and mucosal biopsies were taken from the segmental carinae of the left and right lung. At least three and up to six biopsies were taken from each patient.

Bronchial biopsies were embedded in Tissue-Tek II optimal cutting temperature (OCT) compound (Sakura Finetek USA Inc., Torrance, CA, USA), snap frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$ .

**Immunohistochemistry** Once all biopsy specimens were collected, at least four to six sections 6  $\mu\text{m}$  in thickness were serially cut from the specimens onto poly-L-lysine coated glass slides (Sigma Diagnostics, St Louis, MO, USA). Sections were then air-dried for 30 min and stored at  $-80^\circ\text{C}$  until use.

Immunostaining was carried out with:  $\alpha\text{CD}25$  (ACT-1) (Becton Dickinson, San Jose, CA, USA),  $\alpha$ -Major basic protein (MBP; clone BMK13, Sanbio BV, Uden, the Netherlands) and  $\alpha$ -tryptase (Chemicon Brunswick Chemie, Temecula, CA, USA).

Binding of the antibodies was detected by the immuno-alkaline phosphatase anti-alkaline phosphatase (APAAP) method. Sections were fixed in acetone for 10 min at room temperature (RT), rinsed in phosphate buffered saline (PBS) pH 7.2 and placed in a semi-automatic stainer (Shandon, Pittsburgh, PA, USA). Slides were sequentially incubated with bovine serum albumin (BSA) 2% in PBS for 10 min, incubated with 10% normal rabbit serum in PBS (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, the Netherlands) for 10 min. Slides were then incubated with the appropriate monoclonal antibodies for 60 min at RT. Subsequently, sections were rinsed twice in PBS for 5 min and incubated with a rabbit anti-mouse (1:50) immunoglobulin serum for 30 min. Slides were then rinsed twice in PBS, incubated with APAAP (1:50) (Dako, Glostrup, Denmark) for 30 min at  $20^\circ\text{C}$ , rinsed in PBS and Tris buffer (pH 8.0), and incubated for a further 30 min with New Fuchsin substrate (Chroma, Stuttgart, Germany) as chromogen. Negative controls were included for each section by omitting primary layer reagents, and positive controls were performed on sections of human palatine tonsil.

Positively stained areas in the lamina propria (depth 100  $\mu\text{m}$  below the reticular basement membrane) were quantified using an image analysis system (Quantimed, Leica, Rijswijk, the Netherlands). Images were taken at  $10 \times 10$  magnification. A minimum of 100 000  $\mu\text{m}^2$  per immunostaining was analysed for a given subject at a given time point. Thresholds for hue, saturation and intensity of colour were

set for each immunohistochemical stain and these were kept constant during the analysis of the entire sample set. This approach allows for a densitometric analysis. The ratio of positive-stained area divided by the total area analysed was taken as measure for each immunohistochemical staining.

**Analysis of cellular and soluble markers** Subjects provided a 3 h urinary collection on the morning of the 2 study days. Urine was collected in a 24 h urinary container without any addition of preservatives. Urinary and serum samples were stored at  $-20$  and  $-80^{\circ}\text{C}$ , respectively, until analysis.

The total number of blood eosinophils was determined by haemocytometric differential cell count (Sysmex NE 8000, Sysmex GmbH Norderstedt, Germany).

Blood for ECP determination was allowed to clot for 60 min at RT and was centrifuged at  $1200 \times g$  for 10 min. Serum ECP was quantified with a double antibody radioimmunoassay (Pharmacia & UpJohn, Uppsala, Sweden). The lower limit of detection for ECP was  $3 \mu\text{g/L}$ .

Serum levels of IL-5 were quantified by a sandwich ELISA (Pharmingen, San Diego, CA, USA). The lower limit of detection for IL-5 was  $2 \text{ pg/mL}$ .

Enzyme immunoassay (EIA) analysis of urinary  $9\alpha,11\beta$ -PGF<sub>2</sub> and LTE<sub>4</sub> were performed with polyclonal antisera and acetylcholinesterase-linked tracers (Cayman Chemical Company, Ann Arbor, MI, USA). A peptidoleukotriene immunoaffinity resin (Cayman Chemical) was utilized for urinary cysteinyl-leukotrienes (LTC<sub>4</sub>, LTD<sub>4</sub>, LTE<sub>4</sub>) purification prior to EIA. The detection limit for both assays was  $7.8 \text{ pg/mL}$ . Creatinine was measured in all urine samples by a colorimetric assay, using an alkaline picrate method. Hence, urinary data are expressed as nanogram of compound per millimole filtered creatinine, in order to correct for possible variations in diuresis.

**Data analysis** Calculations of geometric mean PC<sub>20</sub> values were performed on log-transformed data. Treatment effects in outcome variables with a Gaussian-shaped residual distribution were estimated as baseline-adjusted mean differences (95% confidence interval(CI)) between the treatment groups (FP minus MON) and tested using ANCOVA, using baseline as a covariate. Mean changes within either treatment group were tested using unpaired *t*-tests. Data are summarized as means (95% CI).

For variables with a non-Gaussian-shaped (positively skewed) distribution, changes from baseline (post minus pre) after logarithmic transformation were compared between the two treatment groups using the Mann–Whitney test and expressed as a ratio (FP to MON) and 95% CI. Changes within either treatment group were tested using paired the Wilcoxon tests after logarithmic transformation. Data are presented as medians (with ranges). A *P*-value of  $<0.05$  was considered significant.

## Results

All of the 36 subjects enrolled completed the study. No adverse events were reported in either of the treatment groups.

## Pulmonary function

For baseline characteristics see Table 1. Over the 8-week period no significant change from baseline in FEV<sub>1</sub> (% pred) was recorded following treatment with FP or MON. However treatment with FP resulted in a significantly greater increase in FEV<sub>1</sub>, compared to treatment with MON [ $-3.88$  ( $-7.71$  to  $-0.04$ );  $P = 0.048$ ] (Table 2). No significant increase from baseline in PC<sub>20</sub> (mg/mL) was recorded following treatment with either FP or MON (Table 2). Improvements in the FP group did not differ from those observed in the MON group [ $-0.14$  ( $-1.02$  to  $0.74$ );  $P = 0.743$ ] (Table 2).

**Bronchial biopsies** At baseline, there were no statistically significant differences in CD25 (activated T cells), tryptase (mast cells) or MBP (eosinophils) between the two treatment groups.

Activated T cells [median (range)] were not significantly reduced from baseline following 8 weeks of treatment with FP [ $0.00$  ( $0.00$ – $84.1$ ) vs.  $0.00$  ( $0.00$ – $52.5$ );  $P = 0.925$ ], or MON [ $0.00$  ( $0.00$ – $52.5$ ) vs.  $0.00$  ( $0.00$ – $84.1$ );  $P = 1.000$ ] (Fig. 1a). A comparison of changes from baseline [FP/MON ratio (95% CI)] of activated T cells was not different when subjects were treated with FP compared to treatment with MON [ $1.00$  ( $0.18$ – $4.86$ );  $P = 0.924$ ].

Mast cell numbers were significantly decreased from baseline in the group treated with FP [ $0.58$  ( $0.11$ – $4.27$ ) vs.  $0.22$  ( $0.02$ – $0.95$ );  $P = 0.007$ ] (Fig. 1b). A non-significant reduction in mast cells in the group treated with MON was observed [ $0.43$  ( $0.07$ – $3.25$ ) vs.  $0.33$  ( $0.02$ – $1.55$ );  $P = 0.700$ ]. Following treatment, mast cells in the FP group were significantly lower than in the group treated with MON [ $0.39$  ( $0.16$ – $0.97$ );  $P = 0.041$ ].

There was a non-significant decrease in the number of eosinophils in the lamina propria following treatment with both FP [ $3.37$  ( $0.18$ – $11.4$ ) vs.  $0.60$  ( $0.00$ – $8.59$ );  $P = 0.203$ ] and

**Table 1.** Baseline characteristics

|                            | FP               | MON              |
|----------------------------|------------------|------------------|
| Patients, <i>n</i>         | 17               | 19               |
| Age, years*                | 31.6 (19–57)     | 24.5 (19–49)     |
| FEV <sub>1</sub> (L)†      | 3.08 ± 0.69      | 3.14 ± 0.75      |
| FEV <sub>1</sub> (% pred)† | 84.9 ± 9.7       | 88.3 ± 13.6      |
| PC <sub>20</sub> (mg/mL)‡  | 0.44 (0.09–2.15) | 1.00 (0.05–2.81) |

\*Mean (range). †Mean ± SE. ‡Geometric mean (range).

FP, fluticasone propionate; MON, montelukast; FEV<sub>1</sub>, forced expiratory volume in 1 s.

**Table 2.** Pulmonary function

|                           | FP                         | MON                          | <i>P</i> -value* |
|---------------------------|----------------------------|------------------------------|------------------|
| FEV <sub>1</sub> (L)      | 0.06 ( $-0.02$ to $0.14$ ) | $-0.13$ ( $-0.26$ to $0.0$ ) | 0.043            |
| FEV <sub>1</sub> (% pred) | 1.4 ( $-1.0$ to $3.7$ )    | $-3.2$ ( $-6.6$ to $0.2$ )   | 0.048            |
| PF (L/min)                |                            |                              |                  |
| PC <sub>20</sub> (mg/mL)† | 0.69 ( $-0.05$ to $1.44$ ) | 0.48 ( $-0.09$ to $1.04$ )   | 0.743            |

Mean change (CI) from baseline shown for all values.

†denotes geometric mean. \* $P < 0.005$ : FP vs. MON

CI, confidence interval; FP, fluticasone propionate; MON, montelukast; FEV<sub>1</sub>, forced expiratory volume in 1 s.

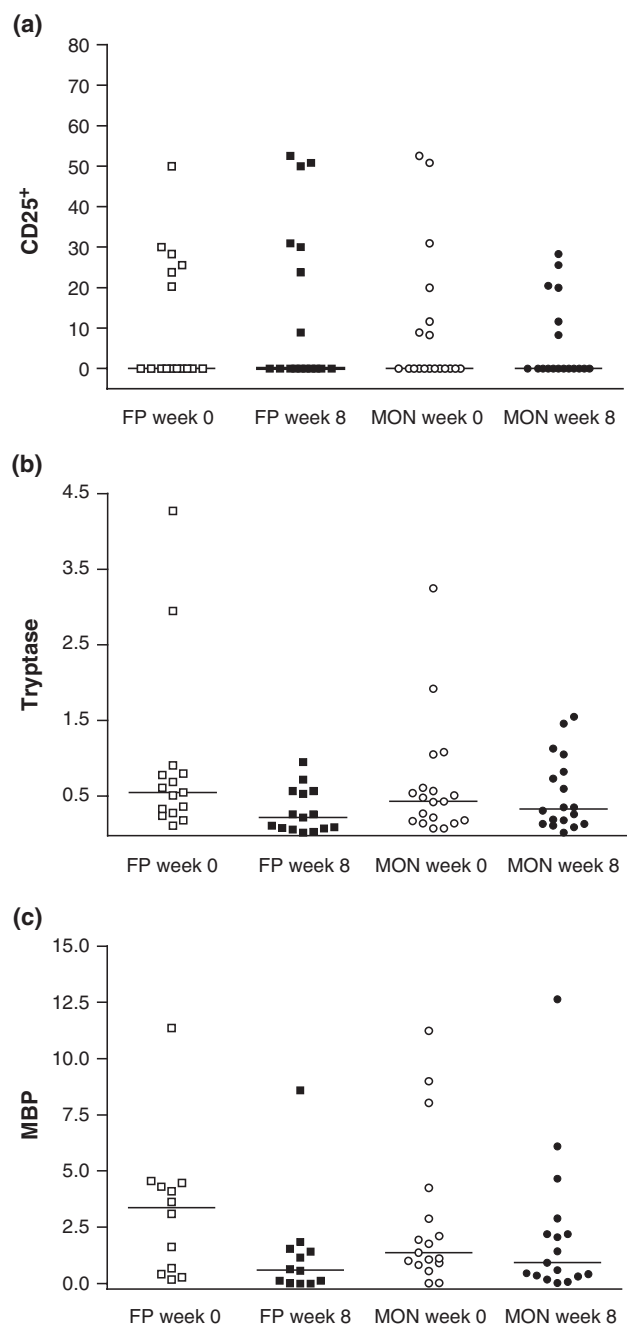


**Fig. 1.** Percentage of positively stained (a) CD25<sup>+</sup>, (b) tryptase and (c) Major basic protein (MBP) cells in the lamina propria before (open symbols) and after eight weeks of treatment (closed symbols) with fluticasone propionate (FP) (squares) and montelukast (MON) (circles). The line represents the median.

MON [1.37 (0.01–11.2) vs. 0.93 (0.02–12.6);  $P = 0.847$ ] (Fig. 1c). There was no difference between the treatments [0.54 (0.05–2.57);  $P = 0.263$ ].

**Cellular and soluble markers** Blood eosinophils were significantly reduced from baseline following 8 weeks of treatment with FP [0.212 (0.04–0.89) vs. 0.189 (0.00–0.66);  $P = 0.015$ ], but not after treatment with MON [0.214 (0.05–0.48) vs. 0.201 (0.00–0.55);  $P = 0.441$ ]. However, the

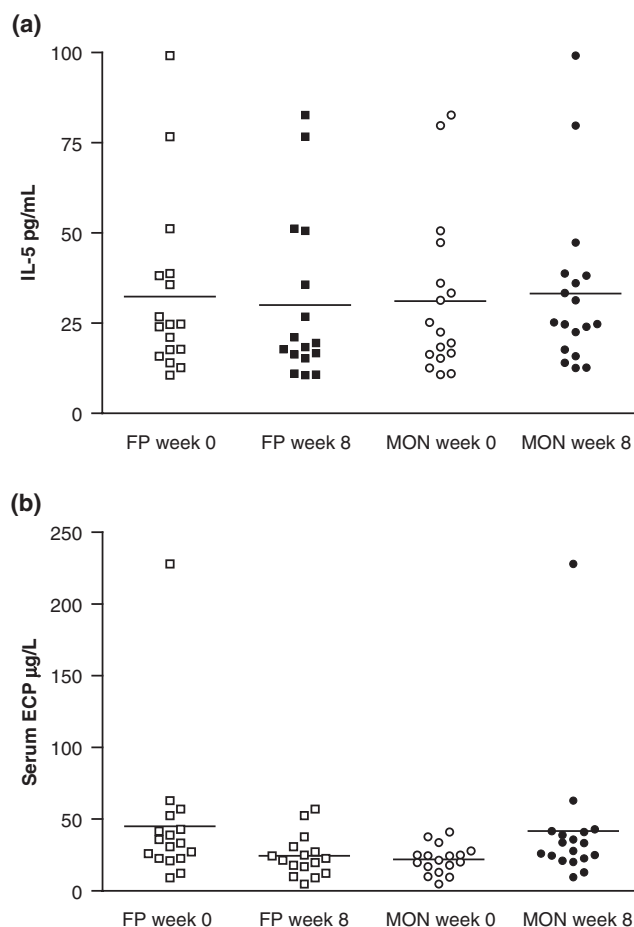


**Fig. 2.** Levels of serum (a) IL-5 (pg/mL) and (b) eosinophil cationic protein (ECP, µg/L) before (open symbols) and after 8 weeks of treatment (closed symbols) with fluticasone propionate (FP) (squares) and montelukast (MON) (circles). The line represents the median.

reduction in the number of eosinophils was not different between the two groups [0.87 (0.50–1.13);  $P = 0.121$ ].

Similarly, there was a significant decrease from baseline in serum ECP in the FP-treated group [33.5 (9.2–228) vs. 22.2 (4.80–57.1);  $P = 0.011$ ], not seen in the MON-treated group [21.5 (4.8–41.1) vs. 30.8 (9.6–228);  $P = 0.105$ ] (Fig. 2a). Treatment with FP resulted in a significantly greater decrease in serum ECP, compared to treatment with MON [0.37 (0.25–0.71);  $P = 0.002$ ].

Serum IL-5 levels were not significantly affected by treatment with FP [24.7 (10.6–99.2) vs. 19.0 (10.6–82.7);  $P = 0.706$ ], or MON [22.5 (10.8–82.7) vs. 25.0 (12.6–99.2);  $P = 0.744$ ] (Fig. 2b). No significant differences between the two treatment groups were observed [0.73 (0.44–1.71);  $P = 0.669$ ].

There was a modest decrease from baseline in the urinary levels of  $9\alpha,11\beta$ -PGF<sub>2</sub> [74.3 (24.9–147) vs. 69.2 (27.6–239);  $P = 0.804$ ] (Fig. 3a), and LTE<sub>4</sub> [17.8 (6.10–150) vs. 13.7 (7.00–40.3);  $P = 0.890$ ] (Fig. 3b), following treatment with FP, while a non-significant increase was documented in both urinary metabolites in the MON-treated group. Following 8 weeks of therapy, there was no difference between the treatment groups in urinary levels of  $9\alpha,11\beta$ -PGF<sub>2</sub> [1.11 (0.59–1.93);  $P = 0.706$ ] and LTE<sub>4</sub> [1.19 (0.38–1.74);  $P = 0.891$ ].

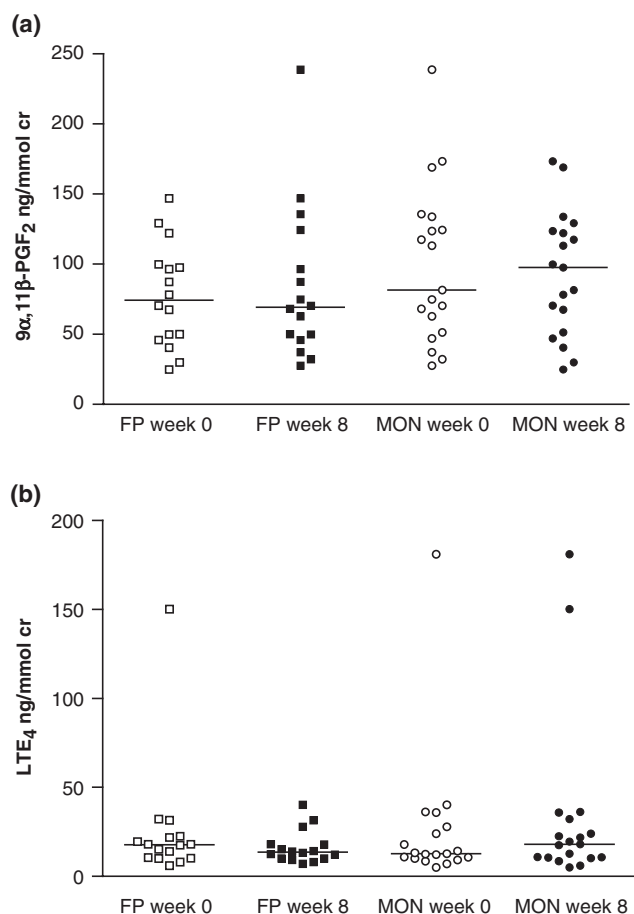


**Fig. 3.** Levels (ng/mmol creatinine (cr)) of urinary (a) 9 $\alpha$ ,11 $\beta$ -PGF<sub>2</sub> and (b) leukotriene E<sub>4</sub> (LTE<sub>4</sub>) before (open symbols) and after 8 weeks of treatment (closed symbols) with fluticasone propionate (FP) (squares) and montelukast (MON) (circles). The line represents the median.

## Discussion

Airway inflammation appears to be a critical aetiological feature of asthma, thus, it has become the prime target of therapy. ICS are currently regarded as the gold standard in anti-inflammatory therapy. LTRAs have also been proposed as anti-inflammatory agents. To the best of our knowledge, this is the first study to compare the anti-inflammatory properties of an LTRAs with those of an ICS on markers of inflammation within the bronchial tissue.

Treatment with FP led to a significantly greater reduction of tryptase<sup>+</sup> cells compared to the group treated with MON. The reduction in mast cells in the bronchial mucosa of mild-to-moderate asthmatics following treatment with inhaled corticosteroids is well documented [18, 19]. Nakamura et al. [9] have reported a reduction in the number of mast cells in the bronchial mucosa of 17 mild-to-moderate asthmatics, following 4 weeks of treatment with the LTRA pranlukast in a double-blind, placebo-controlled study. The lack of a significant decrease in mast cell numbers following treatment with MON in the current study is therefore, unlikely to be because of insufficient power to detect changes. Rather, the subjects in the Nakamura study were moderate asthmatics, and the authors used a different monoclonal antibody to

detect mast cells (AA1). In support of the current findings, the change in tryptase levels in sputum supernatants obtained from 40 moderate asthmatics treated for 4 weeks in a double-blind, randomized, cross-over study, decreased significantly after FP treatment, while there was a non-significant increase in tryptase levels following treatment with MON [20].

Levels of the mast cell marker 9 $\alpha$ ,11 $\beta$ -PGF<sub>2</sub> decreased after FP treatment but increased in the group treated with MON, despite a decrease in mast cell numbers in both groups. A similar observation in bronchial biopsies has been made by O'Sullivan et al. [21]. The authors speculated that MON might indirectly affect cyclooxygenase or 11-ketoreductase resulting in increased quantities of PGD<sub>2</sub>, the parent compound, or 9 $\alpha$ ,11 $\beta$ -PGF<sub>2</sub>, respectively.

One of the most prominent features of bronchial inflammation in asthma is the pronounced eosinophilia [22]. In the current study, there was a non-significant decrease in MBP<sup>+</sup> cells in both groups. Although the percentage decrease of MBP<sup>+</sup> cells was greater in the FP-treated group (82%), compared with MON-treated group (32%), there was no significant difference between the two groups. O'Sullivan et al. have previously reported a non-significant decrease in eosinophil numbers in the bronchial submucosa in mild asthmatics following 2 weeks of treatment with low-dose FP (100  $\mu$ g) [23]. In contrast to the current findings, 4 weeks of treatment with the LTRA pranlukast (450 mg) resulted in a significant reduction from baseline in the number of EG2<sup>+</sup>, but not EG1<sup>+</sup> cells in bronchial biopsies from mild-to-moderate asthmatics [9]. MBP is a pan eosinophil marker and as such, will detect both activated and non-activated eosinophils. This could potentially explain the inconsistency between the data. If LTRAs do, in fact, stabilize activated eosinophils, as suggested by the authors of the aforementioned study, it is not surprising that we could not document any significant decrease in serum IL-5 levels in the MON-treated group.

A number of studies have documented a decrease in sputum eosinophils following treatment with LTRAs [6, 7, 23]. Kanniess et al. [20] have failed to document a significant decrease in the percentage of sputum eosinophils following 4 weeks of treatment with MON, and Diamant et al. [24] demonstrated that pre-treatment with MON did not attenuate the increase in sputum eosinophils following allergen challenge. It is worth noting that the relationship between the cellular content in sputum and airway tissue has not been yet been fully clarified. Grootendorst et al. has compared inflammatory cell counts in induced sputum, BAL and bronchial biopsies. They found no significant correlation between the number of eosinophils/mL sputum and the number of EG2<sup>+</sup> eosinophils/mm<sup>2</sup> lamina propria in bronchial biopsies in 18 clinically stable mild-to-moderate asthmatics [25].

There was a significant reduction in the number of peripheral blood eosinophils and serum ECP in the FP-treated group, in contrast to the group treated with MON. Dempsey et al. [12] have reported that 4-weeks treatment with inhaled hydrofluoroalkane triamcinolone acetate 450  $\mu$ g/day ex-actuator dose in patients with mild persistent asthma resulted in significant reduction of blood eosinophils and serum ECP, whereas no effect on these indices was observed with MON (10 mg once daily). In contrast, in a study with a

similar number of subjects to that reported here, Pizzichini et al. [7] have previously documented that 4 weeks of treatment with MON resulted in a decrease in peripheral blood eosinophils. Yoshida et al. [6] have also observed a significant decrease in peripheral blood eosinophils numbers following 4 weeks of treatment with pranlukast (450 mg). It should be noted, however, that baseline levels of blood eosinophils were 40–53% higher in these studies than baseline levels documented in the current study, potentially allowing more room for improvement.

No change was observed in the number of CD25<sup>+</sup> cells following treatment with either FP or MON. Subjects were in stable phase of their asthma, which is illustrated by the low basal level of CD25 expression. This may explain why no effect of either treatment could be demonstrated.

Pulmonary function parameters, FEV<sub>1</sub> and PC<sub>20</sub> MCh, were not significantly different from baseline following treatment with FP or MON. We have previously shown that 100 µg b.i.d of FP has no significant effect on FEV<sub>1</sub> or PC<sub>20</sub> in mild asthmatics with similar baseline pulmonary function values as the subjects in the current study [26]. It could be argued that the subjects did not have active disease and this is why there was no significant improvement in FEV<sub>1</sub> or PC<sub>20</sub> when subjects were treated with FP or MON. However, FEV<sub>1</sub> decreased by 120 and 140 mL in the FP and MON groups, respectively, over the 8 week run-in period (data not shown). Treatment with FP proved superior to MON with respect to FEV<sub>1</sub>. Busse et al. [11] have previously shown that treatment with low-dose FP (88 µg b.i.d) over a period of 24 weeks was more effective than MON (10 mg) at increasing morning FEV<sub>1</sub> in a group of moderate asthmatics. A 12-week multicentre study conducted in 451 asthmatics randomized to receive either FP (88 µg b.i.d) or zafirlukast (20 mg b.i.d) demonstrated that the inhaled corticosteroid was more effective than the LTRA in increasing morning FEV<sub>1</sub> [27]. We could not demonstrate any difference in improvements observed in PC<sub>20</sub> following 8 weeks of treatment with either drug. In patients using ICS, alone and in combination with long-acting β<sub>2</sub>-agonists, MON has been shown to significantly attenuate BHR to adenosine monophosphate [28]. Adenosine monophosphate has been found to be more sensitive in detecting changes in underlying airway inflammation compared with PC<sub>20</sub> MCh [29]. This may explain why improvements in BHR were not observed in the current study. There is accumulating evidence that airway remodelling most likely occurs secondary to the inflammatory process [30]. Because of this link between airway inflammation and remodelling, a decrease in inflammatory cell numbers, even in the absence of an effect on BHR or FEV<sub>1</sub> we believe is clinically significant. Furthermore, Ward et al. [31] have recently reported that changes in airway physiology, inflammation and remodelling following long-term treatment with FP (750 µg for 12 months) were not temporally concordant.

In this study we show that MON has limited anti-inflammatory effects, while 8 weeks of treatment with FP significantly decreased the number of mast cells in the bronchial submucosa of mild asthmatics. In addition, the number of peripheral blood eosinophils and serum ECP was significantly decreased following treatment with FP. In conclusion, the results support current asthma treatment

guidelines [2] that recognize inhaled corticosteroids as the most effective anti-inflammatory treatment in mild persistent asthma. Future prospective studies should investigate the effectiveness of LTRAs in modulating airway inflammation in moderate-to-severe asthma.

## Acknowledgements

The authors thank the staff of the Lung Function Laboratory, in particular Carolien Vons and Yvonne de Feijter for their excellent technical assistance, Martijn Akveld for his advice and study coordination and to the patients who participated in this study. This study was supported by GlaxoSmithKline R&D, UK (study: FMS40006).

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