Inhaled Fluticasone Propionate Downregulates Peripheral Blood T Lymphocyte Activation and Naturel Killer Cells in Asthma

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Abstract

Background: Inhaled corticosteroids (ICS) are widely used in the treatment of asthma although the exact mechanism of their action is not known. We investigated the effect of a 16-week therapy with inhaled fluticasone propionate (FP; 500 g/day as a dry powdered inhaler) on clinical indices as expressed by FEV_1, diurnal variation in peak expiratory flow rate ($PEFR_{var}$), bronchial hyperreactivity as expressed by histamine challenge test and T lymphocytes, naturel killer (NK) cells and T lymphocyte activation in peripheral blood in a group of symptomatic individuals with asthma.

Methods: Twelve symptomatic asthmatics (mean age was 32.8±1.9) were recruited in the study. Patients were given 500g/d FP for 16 weeks after a two-week run-in period. The results of peripheral blood T lymphocytes and NK cells of six nonasthmatic-nonatopic control subjects were compared with the asthmatics.

Results: A 16-week treatment with FP had a marked effect of improving clinical parameters including FEV_1 (p=0.02), $PEFR_{var}$ (p=0.002) and PC_{20} (p=0.004). Peripheral blood T lymphocyte activation marker CD25 which was higher than the controls at the beginning, was significantly reduced (p=0.02). The number of CD16/CD56 lymphocytes (Naturel killer=NK cells) were also reduced (p=0.002), although they were not significantly different from the controls.

Conclusion: This data suggested that T lymphocyte activation and the number of NK cells in peripheral blood of symptomatic asthmatics can be reduced by a 16-week inhaled fluticasone propionate treatment with the improvement in clinical indices as measured by FEV_1, PEFR and PC_{20} values.

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Key words: *Asthma, Fluticasone propionate, Inhaled corticosteroids, Therapy, T-lymphocytes, NK cells.*

Introduction

Anti-inflammatory therapy is considered as the first line therapy in asthma and, inhaled corticosteroids (ICS) are widely used (1). ICS are reported to be very effective in the treatment of asthma (2). The mechanism of their action is not exactly known. ICS have been shown to reduce proinflammatory cytokine expression, subepitelial collagen deposition, total and activated eosinophils and mast cells in biopsy (3), neutrophil chemotaxis and motility (4). Lymphocytes are prominent cells in the asthmatic airways (5,6). Activated T lymphocytes have been shown to be increased in the peripheral blood of the patients with clinical deterioration, and the percentage of activated cells decreased after the therapy with clinical improvement (7). A six-week therapy with inhaled beclomethasone dipropionate (BDP) has produced a significant reduction in the percentage of activated T lymphocytes both in bron-

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choalveolar lavage (BAL) and in peripheral blood of atopic asthmatics with clinical improvement (8). Fluticasone propionate (FP) is a relatively new ICS with a higher topical anti-inflammatory effect and it has been shown to improve clinical indices in the asthmatic patients (9). In this study, we have examined the effects of a 16-week treatment with FP 500 mg/day via dry powdered inhaler on lymphocyte subpopulations and their activation markers in peripheral blood from twelve nonatopic asthmatic patients and on clinical indices as expressed by forced expiratory volume in 1 second (FEV_1), diurnal variation in peak expiratory flow rate ($PEFR_{var}$) and bronchial hyperreactivity as expressed by PC_{20}.

Material and Methods

Subjects

Twelve patients with symptomatic asthma (mean age was 32.8±1.9, range 20-42) which required inhaled corticosteroid (ICS) for disease control were enrolled in the study from the outpatient clinic. The characteristics of the patients are shown in Table I. Seven of the 12 patients admitted to our outpatient clinic did not have a previous diagnosis of asthma. The rest of the patients were previously diagnosed as asthma and receiving inhaled beta -agonists only. All patients were considered severe enough to require ICS for disease control. They were diagnosed as asthma according to the standards of the American Thoracic Society (10). Seven patients were atopic as defined by the presence of a positive (a wheal 2 mm or more a diameter at 20 min) skin prick test to at least one of the common aeroallergens using standardized solutions (Laboratoire Des Stallergenes, France). Six nonasthmatic nonatopic control subjects with a mean age of 37.6±3.2 (range, 30-48) were included in the study. None of the patients were taking inhaled or systemic corticosteroids at least 6 months before the study and 5 had been taking only short acting inhaled β_2 -agonists. The patients had no upper or lower respiratory tract infection within 6 weeks prior to the study.

Table 1. Baseline characteristics of the patients.

Subject	Age/sex	Atopy	FEV_1*(%)	FEV_1#(%)
1	36 / F	Yes	65	63
2	32 / F	Yes	85	86
3	30 / M	No	64	64
4	42 / F	No	63	61
5	32 / F	Yes	100	98
6	22 / F	Yes	82	83
7	36 / F	No	72	70
8	34 / F	Yes	55	55
9	38 / F	No	100	95
10	40 / F	Yes	65	63
11	20 / F	No	91	90
12	32 / F	Yes	83	85

*= FEV_1 value at the first examination;
#= FEV_1 value at the end of the run-in period

Study Design

After a two-week run-in period, during which the patients recorded the morning and evening PEFR as the best of the three measurements using a mini Wright peak flow-meter and received only inhaled beta-agonists as required; blood was taken for lymphocyte subset analyses and patients received FP 500 g daily as a dry powdered inhaler for 16 weeks. Before completion of the study, the patients once again recorded PEFR measurements twice daily for a 2-week period. Diurnal variation of PEFR was calculated as the amplitude percentage of the mean (the difference between morning and evening values divided by the mean). On completion of the 16-week treatment period, a further blood sample was taken for analysis. FEV_1 values were determined with a dry bellows spirometry (Model S, Vitalograph Co., Buckingham, UK.) before and after the run-in period and at the end of the 16 weeks.

Nonspecific bronchial challenge test was done between 8:30–10:30 AM with a pari nebulizator as described before (11). Short acting β_2-agonists were stopped 6 hours before the histamine challenge test. None had an asthma attack or upper respiratory tract infection within 8 weeks before the challenge. After FEV_1 was determined, histamine diphospate solutions were inhaled in doubling concentrations from 0.03 mg/ml to 8 mg/ml. Histamine concentration causing more than 20% fall in baseline FEV_1 were expressed as PC_{20} which was obtained from the log dose-response curve.

Blood sample for lymphocyte analyses was drawn from six healty nonatopic-nonasthmatic controls. The control group was sampled once only. All the patients and the controls gave informed consent.

The study was done out of pollen season to reduce the environmental causes of exacerbation on allergic asthmatics.

Analysis of Lymphocytes

Peripheral blood lymphocytes and eosinophils numbers were counted using Coulter Counter STKS.

Flow cytometric analysis was performed on an Facs Calibur instrument (Becton Dickinson). Lymphocyte

phenotyping for T, naturel killer (NK) and active T lymphocytes antigens was performed by two color direct immunofluoresence using monoclonal antibodies directed against CD3, CD4, CD8, CD16/CD56, CD19, CD25 and HLA-DR. CD3 (T cells), CD3/CD4 (T helper), CD3/CD8 (T supressor), CD16/CD56 (naturel killer=NK cells), CD19 (pan B cells), CD5/CD25 and CD5/HLA-DR cells were performed in the blood from the patients and the controls.

Statistics

The values are expressed as the median (range). Data were analyzed by performing Wilcoxon signed rank test, Spearman Correlation matrix and Mann - Whitney U test when appropriate. P values less than 0.05 were considered significant. A personal computer program SPSS.WIN was used for all calculations.

Results

Table 2 shows the baseline median (range) numbers of eosinophils and the median (range) percentages of lymphocyte subtypes in peripheral blood (PB) of asthmatic patients and controls. There were no differences in CD3+ lymphocytes, CD3/CD4, CD3/CD8, CD16/CD56 (NK cells), CD19 and CD5/HLA-DR+ lymphocytes between asthmatics and controls (p>0.05). The numbers of eosinophils (p=0.009) and the percentages of CD25+ T lymphocytes (p=0.05) in the blood of asthmatics were higher than those in the controls (Table 2).

Table 2. Baseline numbers of eosinophils and percentages of T lymphocyte subtypes in the asthmatics and the control subjects†.

	Asthmatics	Controls	p
Eosinophils/mm³	470 (80-1600)	120 (65-252)	0.009
CD3+,%	71.1 (56.1-85)	66.7 (55.5-75.0)	NS
CD3/CD4,%	40.3 (27.9-50.7)	43.5 (37.6-54)	NS
CD3/CD8,%	25.4 (16.1-44.6)	23.5 (12.3-36.3)	NS
CD16/CD56,%	13.0 (7.0-28.3)	12.7 (8.6-21.3)	NS
CD19,%	9.1 (5.4-13.5)	10.8 (6.1-14.3)	NS
CD5/CD25,	5.6 (1.7-10.20)	3.0 (2.0-6.6)	0.05
CD5/HLA-DR,%	13.0 (3.9-27.8)	11.9 (2.2-23.4)	NS

†: Values are median (range); NS: nonsignificant.

Sixteen weeks of inhaled FP produced a significant improvement in diurnal variation of PEFR [from 13.3 (3.2-28.3)% to 9.5 (3.0-23.1)%; p=0.002), FEV_1 [from 77 (63-100)% to 85 (65-100)%; p=0.02] and PC_{20} values [from 0.14 (0.02-0.66) mg/ml to 0.18 (0.03-0.85) mg/ml, p=0.004). Absolute eosinophil numbers in PB of asthmatics showed significant reductions (from 470 (80-1600)/mm³ to 300 (95-600)/mm³, p=0.04) after the treatment (Figure 1).

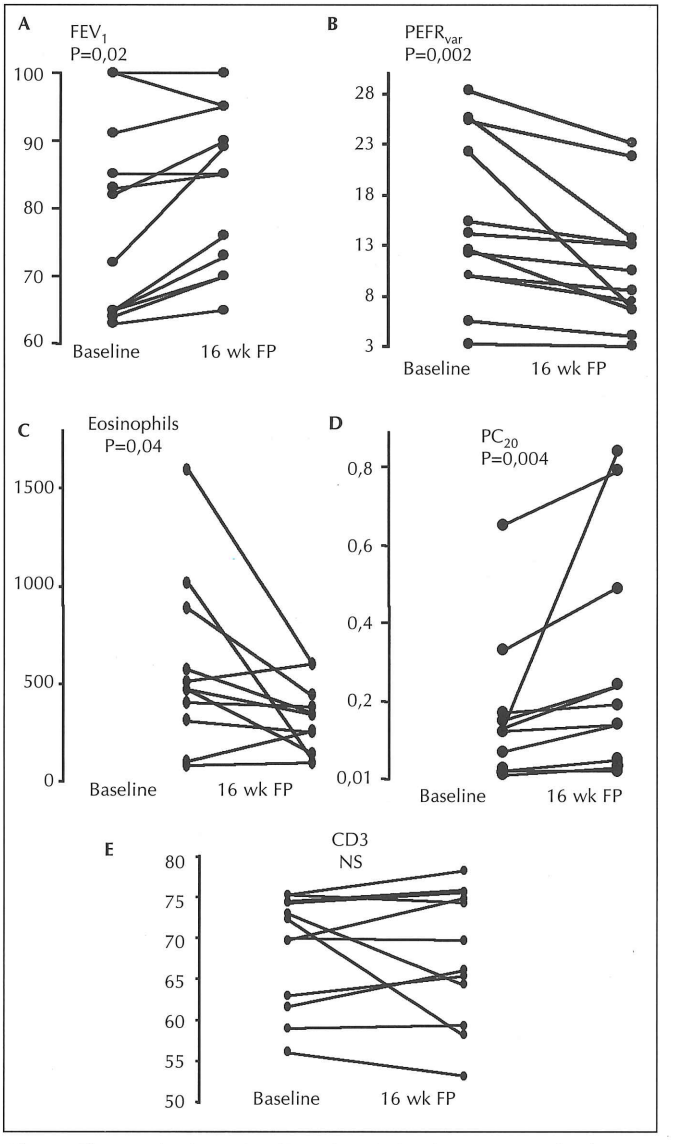

Fig. 1. Changes in FEV_1 (A), diurnal variation in PEFR (B), absolute numbers of eosinophils in peripheral blood (C), PC_{20} (D) and CD3 + T cells (E) from baseline to 16 weeks after treatment.

As shown in figure 2, phenotypes of PB cells before and after FP showed no significant differences in the percentages of CD3+ [71.1 (56.1-85.0)% versus 67.9 (53.2-78.2)%; p>0.05], CD3/CD4 [40.3 (27.9-50.7)% versus 45.1 (37.4-50.7)%; p>0.05], CD3/CD8 [25.4 (16.1-44.6)% versus 24.8 (3.7-35.8)%; p>0.05], CD19 [9.1 (5.4-13.5)% versus 12.0 (6.5-17)%; p>0.05] and CD5/HLA-DR [13.0 (3.9-27.8)% versus 10.4 (5.0-27.2)%; p>0.05) lymphocytes in the asthmatic patients. However there were significant reductions in the percentages of CD5/CD25 lymphocytes [5.6 (1.7-10.2)% versus 3.1 (0.1-5.6)%; p=0.02] and NK cells [13.0 (7-28.3)% versus 4.1 (0.8-8.3)%; p=0.002) after the treatment.

At the end of the study, T lymphocyte subtypes and their activation markers were not different as compared with the normal subjects (p>0.05), eosinophil numbers

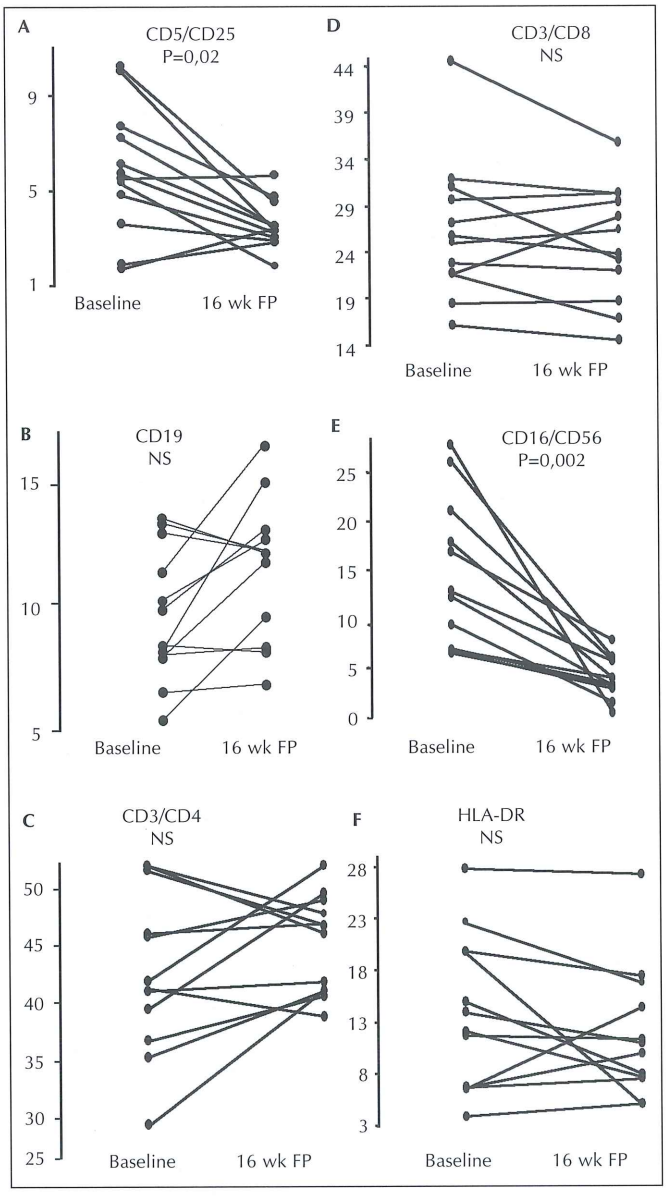

Fig. 2. *Percentages of peripheral blood CD5/CD25+ (A), CD19 (B), CD3/CD4+ (C), CD3/CD8+ (D), CD16/CD56+ (E), and CD5/HLA-DR+ (F) lymphocytes after the treatment with FP.*

in the asthmatics were found to be higher than the controls (p=0.02).

Discussion

In the present study, we demonstrated that a 16-week administration of FP 500 µg/d as a dry powdered inhaler improved clinical indices as expressed by diurnal variation of PEFR, FEV_1 and bronchial hyperreactivity with a significant downregulation of T lymphocyte activation and naturel killer cells in peripheral blood of asthmatics. All patients were symptomatic asthmatics and required anti-inflammatory therapy. Due to the practical and ethical limitations, we couldn't compare the results with a placebo drug; since delayed onset of the treatment with inhaled glucocorticoids has been shown to be associated with a reduced improvement of lung function and may lead to the development of chronic airway limitation, causing irreversible remodeling of the airways (11,12).

The class II major histocompatibility complex antigen HLA-DR and the α-chain of the IL-2 receptor (CD25) are the markers of T cell activation. FP has been considered to be a clinically potent drug in asthma and showed to improve clinical parameters as determined by FEV_1, PEFR and PC_{20} values (2,13). It has been shown that T cell activation is a feature of asthma in bronchoalveolar lavage (BAL) (14,15) and peripheral blood (16,17). Topical steroids have been shown to inhibit T lymphocyte activation and proliferation *in vitro* (18,19). In clinical studies, T cell activation reduced after treatment with steroids in peripheral blood (16,20) and BAL (8) of asthmatics. Wilson et al. also showed that HLA-DR expression in the peripheral blood responded to treatment with beclomethasone dipropionate 2000 µg/d for 6 weeks and suggested that the recirculation of supressed T cells from the airways into the circulation and a direct effect of systemically absorbed drug (8). Bootsma et al. recently demonstrated that FP 750 µg/day as metered dose inhaler caused significant decrease in the number of CD3/HLA-DR+ lymphocytes with a decrease in eosinophil numbers and serum ECP levels (20). In our study, we showed significant decrease in CD25+ T lymphocytes and eosinophil numbers in PB of symptomatic asthmatics. After the treatment with FP CD5/CD25+ lymphocytes decreased to nonsignificant levels, whereas eosinophils remained significantly elevated compared with the controls. Blood and tissue eosinophilia is a characteristic abnormality in asthma and eosinophil derived proteins cause epithelial damage and bronchial hyperressponsiveness in asthma (21). Corticosteroids decrease eosinophils in the peripheral blood by recruitment of eosinophils from the circulation, decrease in survival time, and suppression of bone marrow (22). Inhibition of eosinophils by corticosteroids through the cytokines such as GM-CSF and IL-5 which promote the survival of eosinophils was also shown previously (23, 24).

We did not find any significant correlation between the improvements of clinical parameters, decrease in eosinophil numbers and T cell activation markers (data not shown) in PB of the patients similar to the previous studies suggesting that decrease in cell numbers are not directly related with the clinical improvement (8,20).

We also determined the downregulation of NK cells after the treatment with FP. NK cells at baseline and after the therapy were not different in the asthmatics than controls. We think that the result is of interest. NK cells are large granulated lymphocytes having immunoregu-

latory functions in response to viral, bacterial and parasitic infections. These cells constitute 5-10% of the peripheral blood lymphocytes in humans and were shown to play an important role in host defense against infections (25). Recent studies have demonstrated that NK cells contribute to the development of eosinophilic inflammation by producing cytokines such as IL-4 and IL-5 in animal models (26, 27). Walker et al. (26) showed that depletion of NK cells not only reduced the levels of IL-5, but also inhibited the infiltration of eosinophils following allergen challenge. Warren et al. showed IL-5 production by human NK cells *in vitro* (28,29). These findings have been supported in a clinical study carried out by Krejsek J et al. (30) who showed increased numbers of NK cells in severe asthmatics, suggesting a relation between NK cells and clinical severity of asthma. The present study also demonstrated that the patients with a higher NK cells at the beginning showed less decrease in eosinophil number in peripheral blood, confirming that NK cells may contribute to increase of eosinophils in the asthmatics. Glucocorticoids have been shown to decrease the number of NK cells in peripheral blood (31). Our study may also confirm that ICS exert their effects by reducing NK cell dependent inflammation in asthma probably by the effect of systemically absorbed drug. This result requires to be confirmed by further clinical studies.

In summary, this study has shown that CD5/CD25+ T lymphocytes can be found in the peripheral blood of symptomatic asthmatics and inhaled FP 500 µg/d as a dry powdered inhaler improved clinical parameters expressed by FEV_1, diurnal variation of PEFR and bronchial hyperreactivity in association with a reduction of activated T lymphocytes and NK cells in peripheral blood of asthmatics.

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