

EFFECTS OF INHALED GLUCOCORTICOSTEROIDS TREATMENT ON CLINICAL AND EOSINOPHILIC INFLAMMATORY PARAMETERS IN PATIENTS WITH BRONCHIAL ASTHMA

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Inhaled glucocorticosteroids are the most efficient anti-inflammatory drugs used in asthma treatment that can bring the improvement of clinical symptoms as well as lung function. Eosinophils (Eo) are the key effector cells in asthmatic inflammation, and determination of their number and concentration of mediators which can bring about eosinophilic activation-interleukin-5 (IL-5) would contribute to the evaluation of anti-inflammatory treatment effects in asthma patients.

The aim of this study was to compare clinical parameters and eosinophilic inflammation parameters in patients with asthma, after 4-week treatment with fluticasone-propionate (FP) in a daily dose of 500 µg.

The study involved 39 patients with bronchial asthma as well as 17 healthy subjects (controls). Asthma symptom scores, FEV₁, FEV₁/FVC, total number of Eo in peripheral blood and IL-5 concentration in serum were measured in all subjects, before and after FP 500 treatment.

There was a significant decrease in asthma symptom scores ($p < 0.001$) and improvement of FEV₁ and FEV₁/FVC ($p < 0.05$) after FP 500 treatment. There was also a statistically significant negative correlation between asthma symptom score and FEV₁ before and after the treatment ($r = -0.415$, $p < 0.01$; $r = -0.346$, $p < 0.05$). The concentration of eosinophilic inflammatory parameters (Eo, IL-5) was significantly reduced after the treatment ($p < 0.05$) in groups of patients with larger number of eosinophiles prior to the therapy.

Besides lung function normalisation and improvement of disease symptoms after the treatment, there were higher concentrations of eosinophilic inflammatory parameters that point to persistent inflammation of airways during well-controlled asthma. It is necessary to constantly compare the symptoms of disease, lung function, severity of disease and level of inflammation parameters in order to assess the treatment effects of inhaled glucocorticosteroids. *Acta Medica Medianae* 2010;49(1):43-47.

Key words: bronchial asthma, glucocorticosteroids, inflammation, fluticasone-propionate

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Introduction

It is now well established that inflammation of the airways plays a central role in the pathophysiology of asthma and leads to bronchospasm, mucus hypersecretion, and airway narrowing with remodeling (1). Asthmatic inflammation develops as a result of complex interactions between inflammatory cells (eosinophils, T-lymphocytes, mast cells, macrophages and epithelial cells) and mediators. Eosinophils (Eo) are the key effector cells in asthmatic inflammation, and determination of their number and concentration of mediators which can bring about eosinophilic activation such as interleukin-5 (IL-5) would contribute to the evaluation of anti-inflammatory treatment effects in asthma patients (2-4).

Inhaled glucocorticosteroids (IGCS) are the most efficient anti-inflammatory drugs used in asthma treatment which can lead to the improvement of clinical symptoms as well as lung function. Inhaled glucocorticosteroids have significantly changed the treatment of asthma and they represent the main source in the treatment of persistent asthma. Researches, so far, have conferred knowledge that IGCS improve the symptoms of the disease and lung function and that they reduce inflammation after several weeks of treatment (5-7). Efficiency of IGCS in the treatment of bronchial asthma is explained by increasing of beta receptors expression on the cell surface, mediators' synthesis inhibition, reduction of cellular income and stabilization of the cell wall (8). Although eosinophils contain glucocorticosteroid receptors (GR), they are relatively insensitive to glucocorticosteroid effects, and the effect is rather indirect by the inhibition of stimulating factors, like IL-5, granulocyte macrophage colony stimulating factor (GM-CSF) that affect surviving of eosinophils (9). That way, IGCS indirectly cause the reduction of eosinophils

in the airways and blood circulation due to the induction of eosinophilic apoptosis and programmed death of eosinophils (10). There is lots of evidence that IGCS inhibit the increase in the number of circulating eosinophils and reduce the markers of inflammation concentration in serum (11-13). Although there is evidence of the association between the airway inflammation, asthma and anti-inflammatory treatment, it is also evident that the concept „asthma is inflammation“ is too simple, and therefore it is necessary to additionally determine clinical parameters, besides parameters of inflammation, in observing the treatment effects of IGCS in bronchial asthma.

The aim of this study was to compare clinical parameters (symptoms of disease and parameters of lung function) and eosinophilic inflammation parameters (total number of eosinophils in blood and serum concentration of IL-5) between healthy individuals and patients with bronchial asthma, before and after a 4-week treatment with fluticasone propionate (FP) in doses of 500 µg per day.

Patients and methods

Subjects

The study included 39 patients with asthma (20 females and 19 males) who were examined at the Institute for Pulmonary Diseases, aged from 15 to 39 years, mean age 28, 95 years. The control group consisted of 18 subjects, aged from 21 to 35 years, mean age 27,56 years, with no history of asthma and atopic disease. The diagnosis of asthma and classification according to severity of the disease was based on the criteria from the Global initiative of asthma guidelines (14). Newly diagnosed asthma patients were classified based on clinical presentation, immunologic status (IgG, IgA, IgM, IgE, immune complexes) and allergic testing into two groups: extrinsic bronchial asthma (EBA) (N=24) and intrinsic bronchial asthma (InBA) (N=15).

Symptom score

Patients with bronchial asthma completed a questionnaire about the most frequent symptoms (daytime cough, daytime wheeze, daytime breathlessness and nighttime asthma) and degree of these symptoms (1 - no symptoms, 2 - mild, 3 - moderate and 4 - severe) during the past 4 weeks.

Lung function

Lung function parameters (FVC, FEV1, FEV1/FVC) were measured by using American Thoracic Society/ European Respiratory Society (ATC/ERS) standards on spirometer Masterscope PC Jaeger Viasys, Germany.

Eosinophilic inflammation parameters

Total eosinophil count in peripheral blood was measured using the May-Grünvald-Giemse

method. Interleukin-5 serum concentration measurement was performed according to ELISA method (commercial test by R&D systems, Minneapolis, USA). Minimal detectable concentration for IL-5 was 3,0 pg/mL.

Statistical analysis

Comparison of average values of numerical signs (x) between two groups of patients was performed by Student t-test, comparison of frequency of atributive signs by Mantel- Henszel chi-square test; the correlation between the different parameters was done by calculating the Spearman`s rank correlation coefficient. A p value of 0,05 or less was considered statistically significant. Statistical analysis was performed on PC using programs Microsoft office , Excel 2007 and SPSS programme, version 10.0.

Results

There was no statistically significant difference between asthma patients group and healthy individuals group regarding the gender (H=0,008, p=0,928) and age of patients (t=-0,89, p=0,373). Thus, the influence of gender and age on the obtained results was excluded.

After the 4-week treatment with FP500, statistically significant decrease of the symptom score occurred (p<0,001) (Table 1). There were found statistically significantly lower values in asthma patients group than in control group by analyzing the parameters of lung function (FEV1, FEV1/FVC) (p<0,001). Statistical significance of values of FEV1 and FEV1/FVC in patients subgroups occurred after corticosteroid treatment (p<0,05) (Tables 2 and 3).

Table 1. Average values of symptom score in patients with BA

Group	n	Before treatment	After treatment
BA	39	9,82±1,47	5,36±1,48*
EBA	24	9,50±1,44	5,00±1,10*
InBA	15	10,33±1,39	5,93±1,83*

Values given as mean ±SD.

* p< 0,001 compared to the same group before treatment

Table 2. Average values of lung function parameters Forced expiratory volume in 1 second

Group	n	FEV1(%)	
		Before treatment	After treatment
Control	18	107,64±13,93	
BA	39	86,64±18,33*	100,21±12,14#
EBA	24	87,21±18,78*	103,71±11,70#
InBA	15	85,73±18,19*	94,60±10,96 **, ##

Values given as mean ±SD

* p<0,001 compared to K

** p<0,05 compared to K

p<0,001 compared to the same group before treatment

p<0,05 compared to the same group before treatment

There was a significant correlation between the lung function parameters among each other and with symptom score. There was a statistically significant negative correlation between FEV1 and symptom score before and after the treatment ($r = -0,415$, $p < 0,01$; $r = -0,346$, $p < 0,05$).

This study included the evaluation of eosinophilic inflammation in asthma patients by determining of the eosinophil blood count and serum IL-5 concentration. There is a statistically significant difference between average values of total eosinophil count of asthma patients group and control group ($p < 0,05$). There was not a significant difference between subgroups EBA and InBA, but the EBA group had considerably higher values than the control one ($p < 0,05$). The number of circulating eosinophils decreased after the treatment, but there was a statistically significant decrease also in BA group and EBA subgroup ($p < 0,05$) (Table 4).

Table 3. Average values of FEV1/FVC (% predicted)

Group	n	FEV1/FVC(%)	FEV1/FVC(%)
		Before treatment	After treatment
Control	18	84,20±6,84	
BA	39	73,42±9,64*	81,67±10,77#
EBA	24	71,99±9,04*	81,56±9,66#
InBA	15	75,70±10,43**	81,86±12,70##

Values given as mean ±SD

* $p < 0,001$ compared to K

** $p < 0,05$ compared to K

$p < 0,001$ compared to the same group before treatment

$p < 0,05$ compared to the same group before treatment

Table 4. Average values of total eosinophils count in patients with BA

Group	n	Eo	Eo
		Before treatment (10 ⁶ /L)	After treatment (10 ⁶ /L)
Control	18	216,67±98,51	
BA	39	323±167,74*	282,05±150,21#
EBA	24	354,17±184,10*	300,00±169,39*#
InBA	15	273,33±127,98	253,33±112,54

Values given as mean ±SD

* $p < 0,05$ compared to K

$p < 0,05$ compared to the same group before treatment

Table 5. Average values of interleukine- 5 concentration

Group	n	IL-5	IL-5
		Before treatment (pg/mL)	After treatment (pg/mL)
Control	18	7,51±7,85	
BA	39	51,55±35,40*	45,75±31,81*
EBA	24	53,47±38,26*	47,56±33,76*
InBA	15	48,46±31,31*	42,86±29,32*

Values given as mean ±SD

* $p < 0,001$ compared to K

Between the control group and BA group, there was a statistically significant difference

between average IL-5 concentrations as well as between all subgroups of asthma patients and control group. After the treatment, IL-5 values decrease, but without statistical significance. Statistically significant difference in values of IL-5 between all subgroups of asthma patients and control group remained after the treatment (Table 5). The correlation between the eosinophil blood count and concentration of serum IL-5 before the treatment was on the limit of statistical significance ($r = 0,308$, $p = 0,057$), but with statistical correlation after the treatment ($r = 0,406$, $p < 0,05$).

Discussion

Controlling of asthma symptoms is very important for treatment of this disease. Although they represent rough index of airway obstruction, the symptoms point to patient perception of disease activity and therefore the need for medication. That is the reason for determining the symptom score in every asthma patient according to daily and night symptoms and average severity of attacks. There was statistically significant decrease of symptom score after the treatment in all observed groups of patients. Literature data also show that a 4-week treatment of patients with moderately severe asthma, with high doses of FP brings about a significant improvement of symptom score and FEV1 (12, 15). By comparing the effects of FP 2000 and FP 500 after a 2-week treatment it was determined that the symptom score significantly improves, more distinctly with FP 2000 (16).

The analysis of lung function (FEV1, FEV1/FVC) shows statistically significant decrease in the group of patients with bronchial asthma compared to the control group of healthy individuals. After the treatment with inhaled glucocorticosteroids, there is a significant increase of the lung function parameters. The obtained data are in keeping with literature data that point to significant improvement of lung function parameters after a 2-weeks treatment with FP 500 (16). There are data that show significant improvement of FEV1 and symptom score in patients with moderate-severe asthma after a 4-weeks treatment with high doses of FP (from 1000 to 1500 µg per day) (12, 15).

Determination of total number of eosinophils and mediators that lead to eosinophil activation such as IL-5 can be useful in observing the treatment effect of inhaled corticosteroids (17).

Results of this study show that the number of eosinophils was significantly higher in the group of patients with BA in relation to the group of healthy individuals. After the treatment with FP500, the number of eosinophils was significantly reduced in a group of patients with bronchial asthma (BA), and especially in a group of patients with atopic asthma (EBA) that had significantly larger number of eosinophils even before the treatment. There are literature data that are in accordance with the obtained results that patients with the larger number of eosinophils

had better reaction to the treatment with FP (13). There is a significant improvement of FEV1 and the symptoms of the disease after the 4-week treatment with high doses of FP (1000mcg per day) in patients with moderate-severe asthma and larger number of eosinophils in sputum and blood (12). If treatment effects of FP 2000 and FP 500 are compared, with significant improvement of symptom score and FEV1 after a 2-weeks treatment, while the number of eosinophils in blood serum is significantly lower after FP 2000 but not after FP 500. FP 2000 is acting against systemic inflammatory parameters both directly and indirectly. With its direct acting, FP reduces the airway inflammation, thus influencing the decrease in levels of chemokines, cytokines and reduced attraction of blood eosinophils into the lung. Indirectly, after systemic absorption from the lung tissue, there is the suppression of eosinophil progenitors in peripheral blood and bone marrow, which results in reduced number of blood eosinophils (16). Comparing the treatment effect of high doses of inhaled FP and oral corticosteroids, it has been established that they equally influenced the improvement of lung function and symptoms of the disease, and that FP rapidly reduced the number of eosinophils in sputum with distinct local effect in the lungs, while oral corticosteroids more efficiently influence the decrease in the number of eosinophils in blood (18).

The results of this theses show that after the treatment with inhaled corticosteroids the reduction of IL-5 value occurs, but without statistical significance. Statistically significant difference in the values of IL-5 between all groups of patients with bronchial asthma and the group of healthy individuals is kept after the treatment as well. The obtained data are in keeping with literature data that show the decrease of serum IL-5 after the treatment with inhaled corticosteroids, without statistical significance (19). Comparing the concentration of IL-5 in

sputum of patients with bronchial asthma in relation to healthy individuals, significantly higher values were obtained. Significant decrease of values of IL-5 occurs after a 4-weeks treatment with fluticasone propionate (3). Higher concentrations of IL-5 were recorded in bronchoalveolare lavate of patients with bronchial asthma that were not treated with IGKS compared to patients treated with low doses of inhaled corticosteroids (20). After a short, intensive treatment with oral corticosteroids, a significant decrease in the number of eosinophils and serum IL-5 occurs, but the values are still higher than in healthy individuals (11, 21, 22).

After the treatment with inhaled corticosteroids in the group of patients with bronchial asthma and all subgroups, statistically significant decrease of symptom score and improvement of lung function occur (23). Determination of eosinophilic inflammation parameters (blood eosinophil count and concentration of serum IL-5) shows that the airways inflammation persists during well-controlled asthma (22-25).

Conclusions

After the treatment with inhaled corticosteroids, a statistically significant decrease of symptom score and improvement of lung function occur. Parameters of eosinophilic inflammation are decreased after corticosteroid treatment, with statistical significance in asthma patients groups with increased eosinophil count before treatment.

Besides the lung function normalisation and improvement of disease symptoms after the treatment, there are higher concentrations of eosinophilic inflammatory parameters that point to persistent inflammation of airways during well-controlled asthma. It is necessary to constantly compare the symptoms of disease, lung function, severity of disease and level of inflammation parameters in order to assess the treatment effects of inhaled glyocorticosteroids.

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EFEKAT TERAPIJE INHALACIONIM GLUKOKORTIKOSTEROIDIMA NA KLINIČKE PARAMETRE I PARAMETRE EOZINOFILNE INFLAMACIJE KOD BOLESNIKA SA BRONHIJALNOM ASTMOM

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Inhalacioni glukokortikosteroidi (IGKS) su najefikasniji antiinflamatorni lekovi koji se koriste u terapiji astme, dovodeći do popravljavanja kliničkih simptoma i poboljšanja plućne funkcije. Eozinofili su ključne efektorne ćelije u astmatičnoj inflamaciji. Određivanje njihovog broja i koncentracija medijatora, koji dovode do aktivacije eozinofila, kao što je interleukin-5 (IL-5), doprinelo bi praćenju efekata antiinflamatorne terapije u bronhijalnoj astmi.

Cilj ove studije bio je upoređivanje kliničkih parametara i parametara eozinofilne inflamacije kod bolesnika sa bronhijalnom astmom pre i posle četiri nedelje terapije flutikazon propionatom (FP) u dozi od 500 µg dnevno.

Studijom je obuhvaćeno 39 bolesnika obolelih od bronhijalne astme i 17 zdravih ispitanika. Kod svih bolesnika je određivan simptom skor, FEV₁, FEV₁/FVC, ukupan broj Eo u krvi i koncentracija IL-5 u serumu, pre i posle terapije FP od 500 µg dnevno (FP 500).

Nakon terapije FP 500, dolazi do statistički značajnog smanjenja simptoma skora ($p < 0,001$) i povećanja FEV₁ i FEV₁/FVC ($p < 0,05$). Statistički značajna negativna korelacija je postojala između simptoma skora i FEV₁, pre i posle terapije ($r = -0,415$, $p < 0,01$; $r = -0,346$, $p < 0,05$). Koncentracija parametara eozinofilne inflamacije (Eo i IL-5) se smanjuje nakon terapije, uz statistički značajno smanjenje broja eozinofila ($p < 0,05$) u grupama bolesnika koji su i pre terapije imali značajno veći broj eozinofila.

I pored normalizovanja plućne funkcije i popravljavanja simptoma bolesti, povišene koncentracije parametara eozinofilne inflamacije i nakon terapije ukazuju da inflamacija disajnih puteva perzistira i tokom dobro kontrolisane astme. U cilju procene terapijskog efekta inhalacionih glukokortikosteroida neophodno je stalno vršiti poređenje simptoma bolesti, plućne funkcije, stepena težine bolesti i nivoa parametara inflamacije. *Acta Medica Medianae* 2010; 49(1): 43-47.

Ključne reči: bronhijalna astma, glukokortikosteroidi, inflamacija, flutikazon propionat