

Comparison of Effect of Leukotriene Biosynthesis Blockers and Inhibitors of Phosphodiesterase Enzyme in Patients with Bronchial Hyperreactivity

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Abstract

Citation: Morina N, Haliti A, Iljazi A, Islami D, Bexheti S, Bozalija A, Islami H. Comparison of Effect of Leukotriene Biosynthesis Blockers and Inhibitors of Phosphodiesterase Enzyme in Patients with Bronchial Hyperreactivity. Open Access Maced J Med Sci. 2018 May 20; 6(5):777-781. <https://doi.org/10.3889/oamjms.2018.187>

Keywords: Bronchial asthma; Zileuton; Diprophylline and tiotropium bromide

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Received: 04-Mar-2018; **Revised:** 28-Mar-2018; **Accepted:** 30-Mar-2018; **Online first:** 23-Apr-2018

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

AIM: Blocking effect of leukotriene biosynthesis–zileuton and blocking the effect of phosphodiesterase enzyme–diprophylline in the treatment of patients with bronchial asthma and bronchial increased reactivity, and tiotropium bromide as an antagonist of the muscarinic receptor studied in this work.

METHODS: Parameters of the lung function are determined with Body plethysmography. The resistance of the airways (Raw) was registered and measured was intrathoracic gas volume (ITGV), and specific resistance (SRaw) was also calculated. For the research, administered was zileuton (tabl. 600 mg) and diprophylline (tabl. 150 mg).

RESULTS: Two days after in-house administration of leukotriene biosynthesis blocker–zileuton (4 x 600 mg orally), on the day 3 initial values of patients measured and afterwards administered 1 tablet of zileuton, and again measured was Raw and ITGV, after 60, 90 and 120 min. and calculated was SRaw; ($p < 0.01$). Diprophylline administered 7 days at home in a dose of (2 x 150 mg orally), on the day 8 to same patients administered 1 tablet of diprophylline, and performed measurements of Raw, ITGV, after 60, 90 and 120 min, and calculated the SRaw ($p < 0.05$). Treatment of the control group with tiotropium bromide - antagonist of the muscarinic receptor (2 inh. x 0.18 mcg), is effective in removal of the increased bronchomotor tonus, by also causing the significant decrease of the resistance (Raw), respectively of the specific resistance (SRaw), ($p < 0.05$).

CONCLUSION: Effect of zileuton in blocking of leukotriene biosynthesis is not immediate after oral administration, but the effect seen on the third day of cys-LTs' inhibition, and leukotriene B₄ (LTB₄) and A₄ (LTA₄) in patients with bronchial reactivity and bronchial asthma, which is expressed with a high significance, ($p < 0.01$). Blockage of phosphodiesterase enzyme–diprophylline decreases the bronchial reactivity, which is expressed with a moderate significance, ($p < 0.05$).

Introduction

Hyperactivity of the airways is manifested with acute bronchoconstriction. Recent studies indicate that blocking the effect of leukotriene biosynthesis is efficient in the treatment of hyperactivity and bronchial asthma. Effect of this medication is about the treatment of slight and moderate forms of bronchial asthma [1]. Effects of cys-LTs' appears not only because of the activation of cys-LT₁ receptor. But, also through cys-LTs' which trigger the vascular smooth muscle contraction [2] and stimulate activity of

the P-selectin produced by endothelial cells through receptor LT₂ [3].

Inhibitors of 5-lipoxygenase (5LO) and of the protein which activate 5LO (5-lipoxygenase activating protein–FLAP) such as GSK-2190915 (FLAP-inhibitor) acts not only in cysteinyl-leukotrienes but also inhibits the creation of LTB₄, which can be useful at neutrophile asthma [4]. It also inhibits the early and late asthmatic reaction during provoking test inhalator allergen. It also decreases the number of LTB₄ eosinophils in sputum.

Most of the authors agree that medicines which block the biosynthesis of leukotriene are the first line of asthma therapy, as an efficient alternative for reduction of inhaling doses of corticosteroids [5].

In the latest medical literature, highest importance is attributed to the caffeine, namely its capability to block the adenosine receptor. Adenosine receptor act through G-protein and that is why the possibility of new synthesis and their introduction to the specific blocker therapy is intensively studied, more powerful to these receptors. Adenosine causes contraction of airways smooth muscles and increases the release of histamine by mastocyte [6].

In the isolated preparation of frenicus-diaphragm, caffeine and theophylline increase the contractility of the diaphragm during the direct and indirect stimulation. In the isolated preparation of frenicus-diaphragm, methylxanthines cause the fatigue to be removed. Also, caffeine and theophylline remove the tiredness of the diaphragm during chronic obstructive pulmonary diseases (COPD). Deemed that this effect causes the removal of dyspnoea at the severe airways obstruction [7].

Inhibitors of phosphodiesterase such as diprophylline block the synthesis and secretion of inflammatory mediators from many types of cells including mastocytes and basophils. This effect of diprophylline may be caused as consequence of PDE inhibition and can be imitated in largest part with medicines that selectively inhibit the isoenzyme PDE4 [8]. In therapeutic concentration, therapeutic effects of diprophylline are related more with its anti-inflammatory effect rather than with bronchodilation effect. However, this remains to be proved.

Administration of these medicines to patient leads to the improvement of symptoms, lung function parameters, reduce of medicines usage, less aggravated breathing through the night, respectively improvement in all parameters necessary in the disease control process. They are also administered concomitantly with other antiasthmatics, such: agonists beta₂adrenergic receptor, corticosteroids, and antagonists of the muscarinic receptor, to which they have synergist and additive effect [9] [10].

Inhibitors of phosphodiesterase 4 (PDE4) have an immune modulatory effect. In the treatment of severe asthma, necessary are many doses which cannot be applied due to side effects to the gastrointestinal tract [11].

Inhalation forms of the medicines can improve these deficiencies and their therapeutic index [12].

Blocking effect of leukotriene biosynthesis – zileuton, orally administered and blocking the effect of phosphodiesterase enzyme-diprophylline administered in the treatment of patients with bronchial asthma and bronchial increased reactivity, and tiotropium bromide as an antagonist of the muscarinic receptor studied in this work.

Material and Methods

Examination performed in 14 patients with bronchial asthma and increased bronchial reactivity. At least 48 hours before the research of bronchial reactivity response, patients have not administered any of the bronchodilator substances. Examined were informed regarding the method of the functional pulmonary tests. Patients had asthma, with or without associated bronchitis.

Pulmonary function, composed of measurement of vital capacity (VC), forced expiratory volume in the first second (FEV₁), resistance in the airways (Raw) and intrathoracic gas volume (ITGV), was defined at the rest. The overall quantity of the intrathoracic gas volume (ITGV) was measured with the plethysmography method, including non-ventilated closed gas. If the residual functional capacity is taken from the ITGV, obtained by the plethysmography method, we will gain information regarding the quantity of closed gas due to a severe obstruction, cystic lungs, or pneumothorax. In healthy subjects with a normal pulmonary function, the volume of the intrathoracic gas is equal to the residual functional capacity. From the beta and alpha angles, assisted by tables, values of the airways resistance and volume of the intrathoracic gas are calculated. From gained values, the specific resistance was calculated:

$$SRaw = Raw \times ITGV$$

Raw and the SRaw were taken for analyses. Research of the bronchial response to different substances was done with the measurement of Raw and the SRaw as very sensitive indicators.

Basic and pulmonary function features researched provided in Table 1.

Table 1: Basic and pulmonary function features

| n | Age (yrs) | Height (cm) | Weight (kg) | VC (%) | FEV ₁ (%) | Raw (kPa L/s) | ITGV (L) |
|----|-----------|--------------|-------------|----------|----------------------|---------------|-----------|
| 14 | 45 ± 1.60 | 177.13 ± 1.5 | 71.77±0.5 | 3.41±3.5 | 3.02±1.26 | 0.19.5±0.1 | 3.05±3.05 |

Zileuton, as a blocker of leukotriene receptor (600 mg, tablet) administered per os 2 days in a row at home (4 x 600 mg) and on the 3rd day reported to the ambulance and measured initial values. One tablet administered orally at the ambulance, and measured Raw and ITGV after 60, 90 and 120 min. In the end, as control, administered was tiotropium bromide in the form of an aerosol and a dose of (2 inh. x 18 mcg), and Raw and ITGV values were measured again, and SRaw was calculated.

Diprophylline, as a blocker of leukotriene receptor, administered in-house for 7 days (2 x 150 mg) and on the 8th to the same patient administered 1 tablet of diprophylline, measured Raw and ITGV after 60, 90 and 120 min., with SRaw that was calculated afterwards. As above, the control group was treated

with tiotropium bromide-aerosol (antagonist of the muscarinic receptor), and after 5 minutes of administration, Raw and ITGV values were measured, and SRaw was calculated.

Used was the hypothesis that changes in the respiratory system are not important, not related to the development of bronchial asthma or other obstructive diseases, and not related to allergic manifestation.

Gained results grouped and analysed. Statistic data processing included determination of the average values (\bar{X}), standard deviation (SD), standard mistake (SEM).

Gained results tested with a t-test to ascertain significant changes in between examined groups. To compare groups, utilised was statistic computer software GraphPadInStat III.

Results

Results of this research, in patients with bronchial asthma, indicate that patients with increased bronchial reactivity 2 days after in-house administration of zileuton, a blocker of leukotriene biosynthesis, at a dose of (2 x 600 mg) reported to the ambulance and measured initial values.

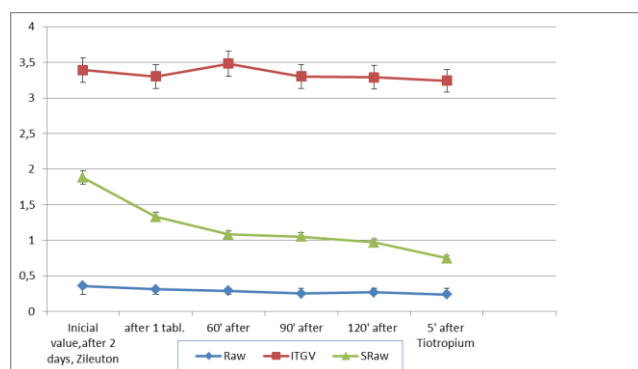


Figure 1: Effect of zileuton ($p < 0.01$), (2 x 600 mg, tablet), and tiotropium bromide ($p < 0.05$), (2 inh. x 18 mcg); in Raw, ITGV and SRaw. 2 days after in-house administration of zileuton (4 x 600 mg); ($n = 7$; $\bar{X} \pm SEM$)

One tablet administered orally and measured the Raw and ITGV again after 60, 90 and 120 min., and because of significant leukotriene biosynthesis inhibition ($p < 0.01$) reduced was the increased bronchomotor tonus. Diprophylline, as blocker of phosphodiesterase enzyme after in-house administration for 7 days (2 x 150 mg) and on the 8th administered 1 tablet of diprophylline, measured Raw and ITGV after 60, 90 and 120 min., with SRaw that was also calculated; as result of phosphodiesterase enzyme blockage (diprophylline), significantly was reduced the increased bronchomotor tonus ($p < 0.05$); also, as treatment of the control group with tiotropium bromide (2 inh. X 18 mcg), antagonist of the

muscarinic receptor, which is effective in removal of the increased bronchomotor tonus, by causing significant decrease of the resistance (Raw), respectively of the airways specific resistance (SRaw), ($p < 0.05$).

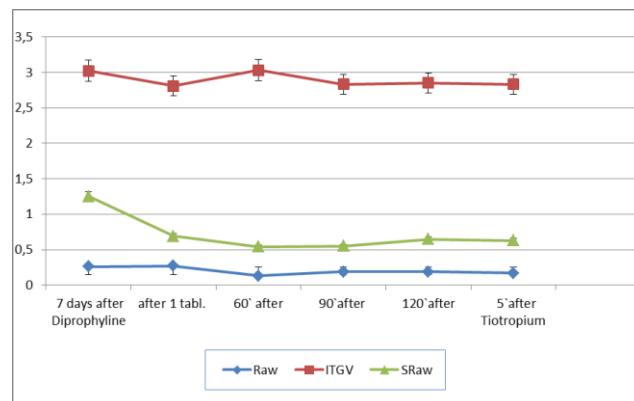


Figure 2: Effect of diprophylline ($p < 0.05$), (2 x 150 mg, tablet), and tiotropium bromide ($p < 0.05$), (2 inh. x 18 mcg); in Raw, ITGV and SRaw. 2 days after in-house administration of diprophylline ($n = 7$; $\bar{X} \pm SEM$)

Discussion

Clinical trials with blocking medicines of leukotriene biosynthesis were quite heterogeneous in response to the therapy, with patients that can be classified in two groups, those "responding" on the treatment and those "not responding" to it. For patients responding to the treatment with blockers of leukotriene receptor, pulmonology institution has recognised these medicines as an alternative to inhaled steroids, in small doses, to maintain slight chronic asthma under control.

Zileuton, as a blocker of the leukotriene biosynthesis, is a powerful selective inhibitor of the activity of 5-lipoxygenase and as such inhibits the biosynthesis of its products. Consequently, besides inhibition of cys-LTs', zileuton also inhibits the biosynthesis of leukotriene B₄ (LTB₄), which is a powerful chemotactic and another eicosanoid too, which depend on the synthesis of leukotriene A₄ (LTA₄). Theoretically, therapeutic effects of 5-lipoxygenase should include all those seen at the antagonist cys-LT₁, but also other effects which include inhibition of the LTB₄ and other products of 5-lipoxygenase. It is deemed that LTB₄ acts through receptor LT₁ and LT₂, by causing accumulation of neutrophils, but their role remains yet unclear [13] [14].

Some clinical trials indicated that blockers of the leukotriene biosynthesis have an affinity in the reduction of the dose of inhaled steroids necessary to control asthma exacerbation [15] [16]. If so, this can

be quite important, especially in children suffering from a more severe asthma.

Blocking effect of leukotriene biosynthesis-zileuton, in the treatment of patients with bronchial asthma and increased bronchial reactivity in comparison to the control group treated with tiotropium bromide applied via inhalation (as an antagonist of the muscarinic receptor) studied in this work. Two days after in-house administration of zileuton, and because of significant blockage of leukotriene biosynthesis ($p < 0.01$) decreased was the increased bronchomotor tonus in patients with emphasized reactivity; effect of tiotropium bromide is efficient in removal of increased bronchomotor tonus, causing significant decrease of the resistance (R_{aw}), namely of the specific resistance (SR_{aw}), ($p < 0.05$).

Blockers of the leukotriene biosynthesis at doses applied 2 days after in-house administration of zileuton to the same patient, cause lowering of systolic and diastolic blood pressure (BP), but not significantly ($p > 0.1$) [17].

Blockers of adenosine receptors and inhibitors of PDE can play a role in lung bronchodilation effect. Adenosine does not contract the isolated smooth muscle of human bronchi directly, but when inhaled it acts as powerful bronchoconstrictor at asthmatics [18]. Thus, inhibition of the adenosine function can contribute to the bronchodilation triggered by diprophylline at some asthmatics. Inhibition of PDE4 and PDE5 effectively relaxes the isolated smooth muscle of human bronchi. Thus, seems that inhibition of PDE may contribute to the bronchodilation effect of theophylline. Studies conducted with methylxanthine enprofylline (3-propylxanthine), which is studied a lot for asthma treatment in Europe, supports the role of PDE inhibition in bronchodilation effects of theophylline. Regarding bronchodilation, Enprofylline is more powerful than theophylline but is less powerful at inhibition of largest part of adenosine receptor types. The latter is to be carefully interpreted. Activation of subtype A_{2B} of adenosine receptor causes some pro-inflammatory effects, and both theophylline and enprofylline are powerful competitive antagonists of the A_{2B} adenosine receptor [19] [20].

Selective inhibitors of PDE4 are assessed in various clinical trials in asthma treatment and chronic obstructive pulmonary disease (COPD). In a study, cilomilast (Ariflo 15 mg two times a day for 10 weeks) significantly reduced infiltration of inflammatory cells, which is seen in the bronchial biopsies of patients with COPD. Further studies are necessary to determine the role of PDE4 inhibitors in asthma and COPD, but these medicines are promissory regarding new approaches to asthma therapy [21].

Our research indicates that due to result of inhibition of phosphodiesterase (doxofylline) reduced was the significantly increased bronchomotor tonus ($p < 0.05$); also, as treatment of the control group with

tiotropium bromide (antagonist of the muscarinic receptor), which is effective in removal of the increased bronchomotor tonus, by causing significant decrease of the resistance (R_{aw}), respectively of the airways specific resistance (SR_{aw}), ($p < 0.05$).

Further studies are necessary to determine the role of these medicines in moderated and severe asthma. Some clinical trials have shown that blockers of the leukotriene biosynthesis possess an affinity to reduce doses of inhaled steroids necessary to control asthma exacerbations [22]. Even though leukotriene inhibitors are efficient in the prophylactic treatment of slight asthma; their role in the asthma therapy is not defined. Most of the clinical trials with these medicines studied at the patients with slight asthma, who do not administer glucocorticoids. In general, studies show a modest, but important improvement to the pulmonary function and a decrease of symptoms and asthma exacerbation.

Based on results gained, it can be concluded as follows:

In patients with bronchial asthma and emphasized bronchial reactivity, an orally administered blocker of leukotriene biosynthesis-zileuton, 2 times a day (4 x 600 mg), causes emphasized and a significant decrease of the specific resistance (SR_{aw}) of airways, ($p < 0.01$).

Blocker of phosphodiesterase enzyme-diprophylline, orally administered for 7 days in a row at doses of 2 x 150 mg, also causes a significant decrease of the specific resistance (SR_{aw}) of airways, ($p < 0.05$).

Treatment of the control group with tiotropium bromide-antagonist of the muscarinic receptor (2 inh. x 0.18 mcg), is effective in removal of the increased bronchomotor tonus, by causing the significant decrease of the resistance (R_{aw}), respectively of the specific resistance (SR_{aw}), ($p < 0.05$).

Effect of a blocker of leukotriene biosynthesis – zileuton, applied to patients with reversible bronchial reactivity, is powerful only after two days of the intake, causing cys-LTs' inhibition, and blocking of leukotriene B₄ (LTB₄) and A₄ (LTA₄) biosynthesis, ($p < 0.01$).

Results, also indicate that blocker of phosphodiesterase enzyme significantly blocks connection to the adenosine receptor ($p < 0.05$), but their effect is weaker in the respiratory system rather that of the leukotriene biosynthesis blockers.

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