

# Maximum Time of the Effect of Antileukotriene- Zileuton in Treatment of Patients with Bronchial Asthma

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## ABSTRACT

**Objective:** Maximum time of the effect of antileukotriene substances - Zileuton in the treatment of patients with bronchial asthma and increased bronchial reactivity and of the salbutamol as agonist of the beta<sub>2</sub> adrenergic receptor studied in this work. **Methods:** Parameters of the lung function are determined with Body plethysmography. Raw and ITGV were registered and specific resistance (SRaw) was calculated. Zileuton (Zyflo, tbl.. 600 mg), producer Cornerstone Therapeutics, USA was used in the research. **Results:** Results of this research, in patients with bronchial asthma, indicate that antileukotriene substances-Zileuton administered in a dose of 600 mg first day (oral route of administration 4 x 1 tbl..) has not caused significant decrease of the specific resistance of the airways (SRaw) (p value 0.1 > Alpha 0.05), whereas Zileuton administered two days in a row, in a dose of 600 mg (4 x 1 tbl.. a day), has caused significant decrease of the specific resistance of the airways (SRaw) (P value 0.03 < Alpha 0.05). Effect of the control with salbutamol (beta<sub>2</sub>-adrenergic receptor agonist) is efficient in the removal of the increased bronchomotor tone, causing significant decrease of the resistance (Raw), respectively of the specific resistance (SRaw), (p value 0.05 = Alpha 0.05). **Conclusion:** Formation of leukotrienes depends on the lipoxygenation of the arachidonic acid by 5-lipoxygenase. Zileuton is an active and powerful inhibitor of the activity of 5- lipoxygenase and as such inhibits generation of its products. Consequently, besides inhibition of cys-LTs', zileuton also inhibits the formation of leukotriene B<sub>4</sub> (LTB<sub>4</sub>), which is a powerful chemotactic of other eicosanoids too, which depend on the synthesis of leukotriene A<sub>4</sub> (LTA<sub>4</sub>). This suggests that the effect of antileukotrienes (Zileuton) is not immediate after oral administration, but the powerful effect of the Zileuton seen only after two days of inhibition of cys-LTs', and inhibition of leukotriene B<sub>4</sub> (LTB<sub>4</sub>) and A<sub>4</sub> (LTA<sub>4</sub>).

**Key words:** Respiratory system, zileuton, salbutamol..

## 1. INTRODUCTION

Research of the leukotrienes originates from the classic pharmacologic studies in 1940 from Kellaway and Trethewie (1). While studying albumin, they discovered a slow reacting substance, which stimulated smooth muscle. They named it as a slow reacting substance - SRS and based on the pharmacologic activity they managed to conclude that it was a unique substance, found only in immunologically sensitized tissues by an antigen. Decades later, SRS was called anaphylaxis slow reacting substance (SRS-A).

Two important discoveries occurred prior to proving of the SRS-significance to the allergic reaction. Firstly, it was the discovery of 1973 by the scientists of the pharmaceutical company "Fisons", who discovered the antago-

nists of the SRS-A named FPL 55712 (2) and second time was the discovery of the structure of SRS-A from Samuelson and his colleagues, which is a 5-lipoxygenase generated by arachidonic acid, by naming it cysteinyl-leukotriene (3). Immediately following this, with plenty of efforts, they seek from the various pharmaceutical industries to discover inhibitors of leukotrienes as important potential agents in treatment of asthma. This would have been achieved either by reduction of the leukotrienes synthesis via inhibition of the enzyme of 5-lipoxygenase or by antagonizing effects of leukotrienes in their receptor. These efforts proved successful in 1990s' with gaining of three new medicines administered in asthma treatment, respectively antagonists of leukotriene receptor zafirlu-

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kast, montelukast (4) and inhibitors of the leukotriene synthesis, zileuton (5). Zileuton is absorbed immediately after oral administration and extensively metabolized by CYP and UDP glucuronosyltransferase. Even in this case, initial medicine is responsible for the therapeutic effect. Zileuton is a medicine with short effect and a half-life of approximately 2.5 hours and also very much bound to the proteins (93%). Pranlukast is another antagonist of the receptor cys-LT1 administered in some countries in treatment of asthma, but not approved for administration. Inhibition of cys-LTs', which induces the contact of smooth bronchial muscles, included in the therapeutic effects of administration of these agents for relief of asthma symptoms. Formation of leukotrienes depends on the lipoxygenation of the arachidonic acid by 5-lipoxygenase. Zileuton is an active and powerful inhibitor of the activity of 5-lipoxygenase and as such inhibits generation of its products. Consequently, besides inhibition of cys-LTs', zileuton also inhibits the formation of leukotriene B4 (LTB4), which is a powerful chemotactic of other eicosanoids 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.

Pharmacologic effects of cys-LTs' occur not only as a consequence of the activation of cys-LT1 receptor; for example, cys-LTs' which trigger the vascular smooth muscle contraction (6) and stimulate expression of the P-selectin generated by endothelial cells via receptor LT2 (7). This provides another advantage of zileuton against zafirlukast and montelukast because inhibitors of 5-lipoxygenase will inhibit effects of cys-LTs' regardless subtypes of the receptor. In despite of the theory advantages, practically various studies showed that zileuton has no higher efficacy rather than antagonists of the receptor cys-LT1 in asthma treatment.

Work studied the effect of antileukotriene - Zileuton (maximum time of the effect) in the treatment of patients with bronchial asthma and increased bronchial reactivity, comparing it with control group treated with salbutamol ( $\beta_2$  adrenergic receptor agonist) applied via inhalation.

## 2. MATERIAL AND METHODS

Total amount of 21 patients with bronchial asthma and increased bronchial reactivity were subject to examination. Study included 21 patients. At least 48 hours prior research of bronchial reactivity response, patients has not administered any of the bronchodilator substances. Examined were informed regarding method of the functional pulmonary tests. Patients were suffering from asthma, with or without associated bronchitis. Average of the disease period was  $8 \pm 6$  years (from 4-20 years). Average of their age was  $35 \pm 7$  years (from 29 - 45 years), whereas average of relative weight was  $78 \pm 7\%$  (from 65 - 72%). The aim of the examination was explained to each of the patients in advance. Pulmonary function, composed of measurement of vital capacity (VC), forced expiratory volume in the first second ( $FEV_1$ ), resistance in the airways (Raw) and intrathoracic gas volume (ITGV), was defined at the rest. Overall quantity of the intrathoracic gas volume (ITGV) was measured with the pleth-

| N  | Age (v)       | Height (cm)       | Weight (kg)      | VC (%)         | FEV <sub>1</sub> (%) | Raw (kPa L/s)   | ITGV (L)        |
|----|---------------|-------------------|------------------|----------------|----------------------|-----------------|-----------------|
| 21 | $35 \pm 1,30$ | $173.19 \pm 1.17$ | $78.81 \pm 0.78$ | $3.19 \pm 3.2$ | $2.55 \pm 3.46$      | $0.29 \pm 0.01$ | $3.66 \pm 0.14$ |

Table 1. Basic airways characteristics

ysmography method, including closed gas that do not ventilate. If the residual functional capacity is taken from the ITGV, obtained by the plethysmography method, we will gain information regarding quantity of closed gas due to a severe obstruction, cystic lungs, or pneumothorax. In healthy people with a normal pulmonary function, volume of the intrathoracic gas is equal to the residual functional capacity. From gained values, specific resistance was calculated:  $SR_{raw} = Raw \times ITGV$ . Raw and the  $SR_{raw}$  were taken for analyses. Research of the bronchial response to different substances was done with the measurement of Raw and the  $SR_{raw}$  as very sensitive indicators. Basic and pulmonary function features of researched are provided in Table 1. In persons with bronchial asthma and increased bronchial reactivity ( $n=21$ ), Zileuton applied in a dose of 600 mg first day (oral route administration,  $4 \times 1$  tbl.). After measurement of initial values, 1 capsule 600 mg of zileuton applied and measured Raw and ITGV after 60, 90 and 120 min. In the end, in terms of control, applied salbutamol ( $\beta_2$ -adrenergic agonist) in the form of aerosol and in a dose of (2 inh.  $\times$  0,2 mg); Raw and ITGV values were measured again and  $SR_{raw}$  was calculated. Zileuton, as antagonist of leukotriene receptor (600 mg, tablet) administered orally 2 days in row at home ( $4 \times 600$  mg) and after 1 day, respectively 2 days reported at the ambulance and measured initial values, tablet administered orally at the

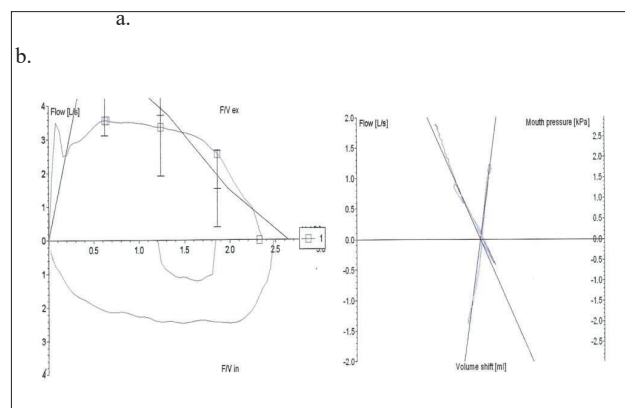


Figure 1. Measurement with Body plethysmography: a. Measurement of parameters of the gas volume in the sternum (ITGV); registration of flux-volume curve (inspiratory flux and expiratory flux-L/min); b. Resistance of airways (Raw-L/sec.) expressed in kPa.

ambulance, and measured Raw and ITGV after 60, 90 and 120 min. At the end, as control, administered salbutamol ( $\beta_2$ -adrenergic agonist) in the form of aerosol and in a dose of (2 inh.  $\times$  0,2 mg), and Raw and ITGV values were measured again and  $SR_{raw}$  was calculated. Gained results grouped and analyzed. Statistic data processing included determination of the average values (X), standard deviation (SD), standard mistake (SEM), and testing of significance of changes in between groups of patient treated with antileukotriene substances.

Gained results tested with a t-test in order to ascertain significant changes in between examined groups. In order to compare groups, utilized was statistic test ANOVA to prevent potential mistakes with t-test.

### 3. RESULTS

Results of this research, in patients with bronchial asthma, indicate that Zileuton administered in a dose of 600 mg first day (oral route administration, 4 x 1 tbl.) has not caused significant decrease of the specific resistance (SRaw) of airways (p value 0.1 > Alpha 0.05). Zileuton, administered 2 days in a row in a dose of 600 mg (4 x 1 tbl. per day), has caused significant decrease of the specific resistance (SRaw) of airways (p value 0.03 < Alpha 0.05). Treatment of the control group with salbutamol (agonist of beta<sub>2</sub>-adrenergic receptor) is also efficient in removal of the increased bronchomotor tone, causing significant decrease of the resistance (Raw), respectively of the specific resistance (SRaw), (p value 0.05 = Alpha 0.05) (Figure 2 and 3). Antagonists of leukotriene in doses administered 1 and 2 days after administration of Zileuton at home in the same patient, does not significantly cause decrease of the arterial systolic and diastolic pressure (AP) (p value 0.1 > Alpha 0.05) (Figure 4 and 5).

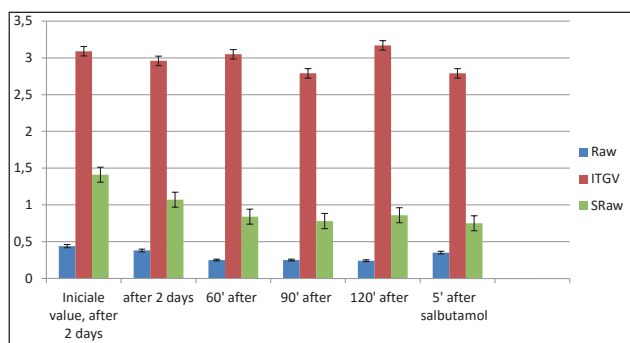


Figure 2. Effect of antileukotriene – Zileuton (600 mg; 4 x 1 tabl.), and salbutamol (2 inh. x 0.2 mg); in Raw, ITGV and SRaw; 2 days after administration of Zileuton at home in patients with emphasized reactivity; (n = 9; X ± SEM).

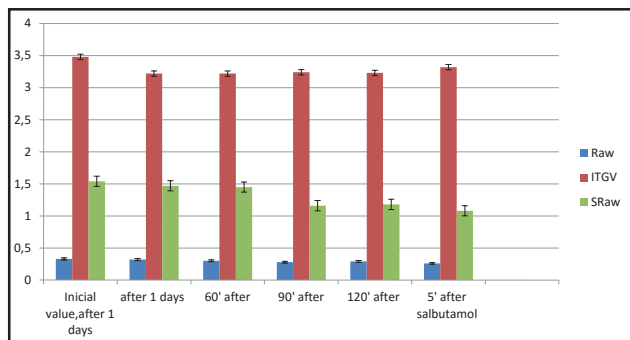


Figure 3. Effect of antileukotriene – Zileuton (600 mg; 4 x 1 tabl.), and salbutamol (2 inh. x 0.2 mg); in Raw, ITGV and SRaw; 1 day after administration of Zileuton in non-reacting patients; (n = 12; X ± SEM)

### 4. DISCUSSION

Antileukotrienes are newest form of anti-inflammatory medicine. Contact antigen-antibody results in degranulation of mastocyte and release of mediator substances: LTC-4, LTD-4 and LTE-4, which cause manifestation of the bronchoconstriction in asthma. From the power of their effect, major and clinical manifestation of asthma depends. There are two types of leukotrienes: antagonists of the receptor and inhibitors of the synthesis. Some of antileukotrienes called also modifiers of leukotriene. Antileukotriene blocks the effect of the component, which manifest contraction of smooth muscles, and block the accumulation of the inflammatory

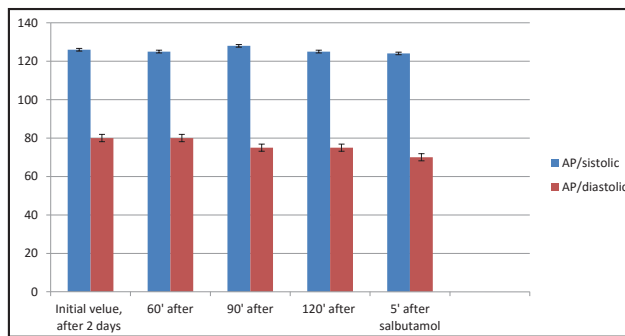


Figure 4. Effect of antileukotriene – Zileuton (4 x 600 mg), and salbutamol (2 inh. x 0.2 mg); to the arterial pressure (AP/ systolic/diastolic); 2 days after administration of Zileuton in patients with emphasized reactivity; (n = 9; X ± SEM).

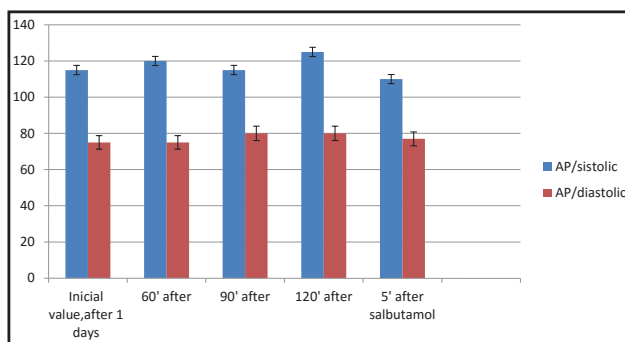


Figure 5. Effect of antileukotriene – Zileuton (4 x 600 mg), and salbutamol (2 inh. x 0.2 mg); to the arterial pressure (AP/ systolic/diastolic); 1 day after administration of Zileuton in non-reacting patients; (n = 12; X ± SEM).

cells, edema, and mucous secretion. They reduce the number of tissues and eosinophil cells. Latest research show that antileukotriene is effective in the therapy of asthma and easily administered orally. Therapeutic effect of these medicines lies in the treatment of slight and moderate forms of bronchial asthma, including other indication (e.g. asthma from the aspirin and reduction of the corticosteroids dosage) (8).

Results indicate that 2 days after administration of leukotriene antagonists - Zileuton at home, third day applied a capsule to the same patients. As a result of the blockage of leukotriene receptor (in a dose of 600 mg, oral route administration) significantly (p value 0.03 < Alpha 0.05) decreases the increased bronchomotor tone, same as the control effect with salbutamol (beta<sub>2</sub>-adrenergic agonist), which is also effective in removal of the increased bronchomotor tone, causing significant decrease of the resistance (Raw), respectively of the specific resistance (SRaw), (p value 0.05 = Alpha 0.05).

Antagonists of leukotriene in doses administered 2 days after administration of Zileuton, at the same patients, cause decrease of the arterial systolic and diastolic pressure (AP) but not significantly (p value 0.1 > Alpha 0.05). Leukotrienes are a product of the lipoxygenase metabolism process by arachidonic acid. Lipoxygenase is a cytosol and soluble enzyme, which can be found in lungs, thrombocyte, mastocyte, and leukocyte. Key enzyme of this process is 5-lipoxygenase - first enzyme in the leukotriene biosynthesis (“leuko” because it is found in blood white cells, whereas “triene” because it contains conjugated systems bound with double connection). During activation of cells, this enzyme places in the membrane surface and binds to the activated protein 5-lip-

oxygenase, necessary for the synthesis of leukotriene in intact cells. LTD<sub>4</sub> is approximately 1000 times more powerful than histamine in bronchoconstriction. Receptor responsible for the bronchoconstriction effect of leukotriene is *sys-LT<sub>1</sub>* receptor. Although each of *cys-LTs*' is agonist to this receptor, LTE<sub>4</sub> is less powerful than LTC<sub>4</sub> or LTD<sub>4</sub>. Zafirlukast and montelukast are selective competitive antagonists with high affinity for the receptor *cys-LT<sub>1</sub>* (9). Effects of *cys-LT*, born with the bronchial asthma, are not limited only in the contraction of smooth muscles. *Cys-LT* can increase the microvascular blood circulation, increase generation of mucous, and appearance of eosinophils and basophils in the airways (10). It is yet unknown how much this inhibition of leukotriene production contributes in the therapeutic effect of these medicines. Maybe, it is worth to mention that zafirlukast inhibits substantially also the appearance of basophils and lymphocytes in airways after experimental exposure of asthmatic people to an allergen (11, 12). Clinical trials with antileukotriene medicines 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" on it. For patients responding to the treatment with antileukotriene, heart, lungs, and blood treatment clinics have recognized these medicines as alternative to inhaled steroids, in small doses, in order to maintain slight chronic asthma under the control. More studies are needed to define the role of these medicines in moderate and severe asthma (13). Some clinical trials indicated that antagonists of leukotriene have an affinity in reduction of the dose of inhaled steroids necessary to control asthma exacerbations (14). If so, this can be quite important, especially in children suffering from a more severe asthma. Currently, it is impossible to foresee who would benefit more from a provided treatment. This forecast to response generally reflects our limited knowledge on the physiopathology of asthma. Moreover, some components of these changes are possible to explain by many pharmacogenetics factors (15).

Zafirlukast and montelukast, antagonists of leukotriene receptor, today are administered in treatment of bronchial asthma. First as addition to other antihistamines, whereas second in prevention of asthmatic attacks. Zileuton is in use, but seems not found yet a final place in the therapy. Iralkast is in the stage of preclinical research. Side effects of the patients administering zileuton are similar to those of patients administering placebo. In estimated 4 to 5% of the patients administering zileuton there is an increase of liver enzymes responsibly within 2 first months of treatment. Zileuton reduces the elimination of the theophylline, by increasing significantly plasma concentration. Zileuton also decreases the elimination of the warfarin. Due to many of the pharmacokinetic features, related to the safety, this medicine is not administered anymore in the USA. Hepatic enzymes should be monitored in patients that have just entered the treatment with zileuton, in order to be protected from a potential toxicity of the liver. Even though leukotriene inhibitors are efficient in the prophylactic treatment of slight asthma; their role in the asthma therapy is not clearly 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 improve-

ment to the pulmonary function and a decrease of symptoms and asthma exacerbations.

## 5. CONCLUSION

Results of this research indicate that Zileuton, administered in a dose of 600 mg (oral route administration, first day 4 x 1 tbl.) in patients with bronchial asthma has not caused significant decrease of the specific resistance (SR<sub>aw</sub>) of airways, (p value 0.1 > Alpha 0.05). Antileukotriene-Zileuton administered 2 days in a row, in a dose of 600 mg (4 x a day 1 tbl.), has caused significant decrease of the specific resistance (SR<sub>aw</sub>) of airways (p value 0.03 < Alpha 0.05). Treatment of the control group with salbutamol (agonist of beta<sub>2</sub>-adren-ergic receptor) is effective in removal of the increased bronchomotor tone, by causing significant decrease of the resistance (R<sub>aw</sub>), namely specific resistance (SR<sub>aw</sub>), (p value 0.05 = Alpha 0.05). This suggests that Zileuton is a powerful selective inhibitor of the activity of 5-lipoxygenase and as such inhibits generation of its products. Consequently, besides inhibition of *cys-LTs*' formation, Zileuton also inhibits the formation of leukotriene B<sub>4</sub> (LTB<sub>4</sub>), which is a powerful chemotactic of other eicosanoids too, which depend on the synthesis of leukotriene A<sub>4</sub> (LTA<sub>4</sub>). According to gained results, the effect of antileukotriene (Zileuton) is not immediate after oral administration, but the powerful effect of the Zileuton seen only after two days of inhibition of *cys-LTs*', and inhibition of leukotriene B<sub>4</sub> (LTB<sub>4</sub>) and A<sub>4</sub> (LTA<sub>4</sub>) based on the recordings made of the specific resistance of airways (SR<sub>aw</sub>).

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