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Comparison of methylxanthines (Doxofylline and Diprophylline) effect in patients with bronchial hyperreactivity and bronchial asthma.

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ABSTRACT

Comparison effect of methylxanthines substances – Doxofylline and Diprophylline in treatment of patients with bronchial asthma and bronchial increased reactivity, and tiotropium bromide as antagonist of the muscarinic receptor studied in this work. Parameters of the lung function are determined with Body plethysmography. Raw and ITGV were registered and specific resistance (SRaw) was also calculated. Doxofylline (tabl. 400 mg), and Diprophylline (tabl. 150 mg) administered in the research. Results of this research indicate that Methylxanthines (Doxofylline and Diprophylline) applied 7 days at home in a dose of (2 x 400 mg and 2 x 150 mg), on the day 8 to same patients applied 1 tablet of doxofylline, respectively diprophylline, and performed measurements with Body plethysmography (Raw, ITGV and calculated SRaw); as result of blockage of adenosine receptors (doxofylline), and as a result of inhibition of phosphodiesterase enzyme (diprophylline) 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 (Raw), respectively of the specific resistance (SRaw), ($p < 0.05$). Methylxanthines at the doses applied 7 days after administration of doxofylline and diprophylline at home to the same patients, have not caused meaningful change in systolic and diastolic blood pressure (BP) ($p > 0.1$). This suggests that methylxanthines act as competition antagonists of adenosine receptors (doxofylline) and as inhibitor of phosphodiesterase enzyme (diprophylline). Results indicate that methylxanthines significantly inhibit connection to the receptor adenosine receptor ($p < 0.05$), identically as inhibitors of phosphodiesterase enzyme ($p < 0.05$). Therefore, they can be successfully used as medicines in treatment of bronchial asthma.

Keywords: Bronchial asthma, Doxofylline, Diprophylline and Tiotropium bromide.

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INTRODUCTION

Bronchial hyperreactivity is main cause and critical factor of bronchial obstruction development. Mechanism of its development is not entirely clear. Certainly, it must do with effect of many factors including activity disorder of autonomous nerve system, inflammatory reaction in the bronchus wall, immunologic mechanism with genetic predispose, and all these as reaction to specific and non-specific stimulus. Sufficient recognition of these mechanisms helps enough not only to understand the cause of bronchial obstruction but also in treatment of diseased from bronchial asthma and chronic obstructive pulmonary disease [1].

Pathophysiologic mechanism and development of inflammation to asthma is very complex, and it is logical to request multiple therapeutic approaches for proper control of the disease particularly in those related to the long-term treatment and prevention of medicine effect.

Untreated bronchial hyperreactivity ends up in a disease of bronchial asthma [1]. In clear majority of patients, inflammation appears because of lymphocyte Th2 activation, whereas cell and molecular mechanisms "non Th2", which associate the inflammation, are not fully defined. Typical example is Th2 that induces the inflammatory processes and hypersensitive reactivity of type I. This reaction is related to genetic atopic predispose, and capability of antigen to penetrate the epithelial barrier and create contacts with cells of congenital and acquired immunity.

Mostly, it is about aeroallergen of which majority is hydro soluble protein (grass, harmful seeds and tree pollen) whilst others (dermatophagoides) have proteolytic action, which facilitates penetration to the previously intact mucous.

In addition, latest research indicates that epithelia of bronchi mucous in patients with asthma from the morphologic aspect is changed intrinsically because of tight junctions. This insufficient physical barrier facilitates supplementary the penetration not only of allergen but also of viruses, pollutant, and other cell particles [2].

Nowadays, deemed that epithelial cells have key role in cause of sustainable chronic inflammation, because their activation and damage cause release of substantial number of mediator most important of which are as follows:

- Chemotactic factor, especially Rantes and eotaxin;
- Cytokinin such IL-23, IL-33, and
- Thymic stromal lymph protein [3].

Released mediator cause activation of dendrite cells (DC), which takes allergen and present them to the lymphocyte T of class II molecule (MHC) [4].

Th2 lymphocyte represent a very important source of cytokinin Th2 such: IL-4, IL-5 and IL-13, and without their presence there is no further development of allergic inflammatory process. Lately, deemed that IL-13 is synthesized only in Th2 cells acquired from the immunity. Almost never found cells named nuocytes from the acquired immunity because such cells are infiltrate for stimulation of IL-33 (largely also of IL-25) [5,6].

Due to synergic effect IL-4 and IL-13 in B lymphocyte, appears the change of isotype of IgM chain and generation of specific IgE anticorps, which bind to the surface of mastocyte and basophil through receptor with high affinity (FcεRI), and surface of mastocyte, eosinophil, DC, Langerhans cells, and thrombocyte through receptor FcεRII (CD23).

Viral or bacterial infection contribute to the development of asthma with the help of acquired immunity cells activation such macrophage or "natural killer" (NK) cells, and activation of lymphocyte Th1 and Th17. IL-10 and IL-17 activate the generation of Th-17, and have powerful chemotactic effect on neutrophils by causing their accumulation in submucous, which correlates to severe forms of asthma and resistance to corticosteroids [7,8].

Development of allergic reaction is possible only in cases of existence of successful suppression of protective mediator. Group of T-regulator cells (Treg) plays a significant role in adjustment of immunity reaction.

Airways chronic inflammation in bronchial asthma causes damage of epithelia of restoring protective mucous, increase of secretion of growth factors and airways remodelling. Remodelling includes the whole bronchial tree and involves all parts of bronchi walls causing smooth muscle hypertrophy, hyperplasia of cup cells, thickening of subepithelial wall of basal membrane and angiogenesis. Because of these changes caused thickening of the wall and decrease of bronchi elasticity, damage of pulmonary function and development of bronchoconstrictive irreversible changes [9].

Comparison effect of methylxanthines substances – Doxofylline and Diprophylline in treatment of patients with bronchial asthma and bronchial increased reactivity, and tiotropium bromide as antagonist of the muscarinic receptor studied in this work.

MATERIAL AND METHODS

Examination conducted in patients with bronchial asthma and increased bronchial reactivity. Selection of patients for this study was done based on anamnestic data, clinical–laboratory findings, and functional examination of respiratory system.

Study included 20 ambulatory patients and 13 cases taken for analysis.

Average of disease duration was 11 ± 6 years (from 4-20 years).

Average of their age was 44 ± 7 years (from 29 – 45 years), whilst average weight was $70 \pm 7\%$ (from 62 – 72%). Purpose of the examination was clarified in advance to each of the patient. 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.

At least 48 hours prior research of bronchial reactivity response, patients were not administered bronchodilation substances. Examined were informed regarding method of the functional pulmonary tests. Patients were suffering from asthma, with or without associated bronchitis.

Besides measurement of these parameters of lungs ventilator function, defined was the curve of maximum expiratory flow volume (MEF). Curve (MEF) was registered in a sitting position with same breathing action as forced vital capacity.

Person has breathed through the mouth (closed nose) via opening of pneumotachograph tube. Air flow was measured with the help of pneumotachograph, whilst volume through volume-integrator. MEF curve was registered in the X-Y writer (Hewlett-Packard).

In the ordinate was marked the flow, and volume marked in the abscissa. Several parameters were calculated, whereas for analysis taken maximum expiratory flow with expiration 25, 50 and 75% at VC (MEF₂₅, MEF₅₀, MEF₇₅ - l/s.).

These parameters taken to analysis because they found in the part of the curve, which at first depends from pulmonary mechanical features and not by the expiratory force and because that they are more susceptible than FEV₁ in measurement of bronchial reactivity. Prior provoking of bronchoconstriction, defined were at least two reproduction curves of MEF and measured blood pressure and pulse.

General resistance of airflow in airways (Raw) and intrathoracic gas volume (ITGV) researched at the patients. Patient has been placed in the cabin of hermetically closed plethysmograph and connected through air mask to the pneumotachograph through which breathed the air. During inspire with expansion of the sternum, air in cabin is compressed whilst in lungs decompressed, namely it comes to the decrease of intrathoracic pressure with proportional increase of air pressure in the cabin.

During expire appears the contrary situation: increase of intra-alveolar pressure and respective decrease of the pressure in cabin. At the end of silent expire, when there is no flow, very soon appears the balance of pressure in alveoli, bronchi with pressure in the mouth.

Therefore, measurement of the pressure with mouth provides accurate information for alveolar pressure. Change of the pressure in mouth and cabin is checked by two susceptible manometers.

Overall quantity of the intrathoracic gas volume (ITGV) was measured with the plethysmography 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 the beta and alpha angles, assisted by tables, values of the airways resistance and volume of the intrathoracic gas are calculated. From gained values, specific resistance was calculated ($SRaw = Raw \times ITGV$).

Raw and the SRaw were taken for analyses. Research of the bronchial reaction to different substances was done with the measurement of Raw and the SRaw as very sensitive indicators than parameters calculated from the MEF curve, thus, they are very significant in the research of bronchoconstriction and bronchodilation. Values realized of MEF₂₅, MEF₅₀, indicate that calculated parameters from the flow-volume curve during volumes in fine pieces of lungs are more sensitive than classic indicators of the measured obstruction with spirometry (FEV₁, 100 x FEV₁/FVK). Comparison of direct gained variables of Raw, and SRaw and indirect indicators of airways obstruction (FEV₁, 100 x FEV₁/FVK, RME₂₅, MEF₅₀) is very important at patients with bronchial asthma and pulmonary obstructive disease. Plethysmography was utilized to measure the overall quantity of intrathoracic gas volume (ITGV), including closed gas that does 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 the beta and alpha angles, assisted by tables, values of the airways resistance and volume of the intrathoracic gas are calculated. From gained values, 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.

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 patients treated with antileukotrienes.

Gained results tested with a t-test. Statistic computer software GraphPadInStat III used to process the data. Used was the hypothesis that changes in the respiratory system are not relevant, and not related to the development of bronchial asthma or other obstructive diseases, and not related to allergic manifestation.

RESULTS

Methylxanthines (Doxofylline and Diprophylline) applied 7 days at home in a dose of (2 x 400 mg and 2 x 150 mg), on the day 7 to same patients applied 1 tablet doxofylline, respectively diprophylline, and performed measurements with Body plethysmography (Raw, ITGV and calculated SRaw); significantly increased bronchomotor tonus ($p < 0.05$) was reduced as a result of blockage of adenosine receptor (doxofylline), and as a result of inhibition of phosphodiesterase enzyme (diprophylline); 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 (Raw), respectively of the specific resistance (SRaw), ($p < 0.05$). See fig. 2 and 3.

Methylxanthines (Doxofylline and Diprophylline) at the doses applied 7 days after administration at home to the same patients, have not caused meaningful change in systolic and diastolic blood pressure (BP) ($p > 0.1$). See fig. 4 and 5.

Table 1. Basic airways characteristics

N	Age (yrs.)	Height (cm)	Weight (kg)	VC (%)	FEV1 (%)	Raw (kPa L/s)	ITGV (L)
13	40.13 ± 1,110	178.19 ± 1.11	72.81 ± 0.78	108.11±2.2	100.33±2.56	0.12±0.01	3.08±0.12

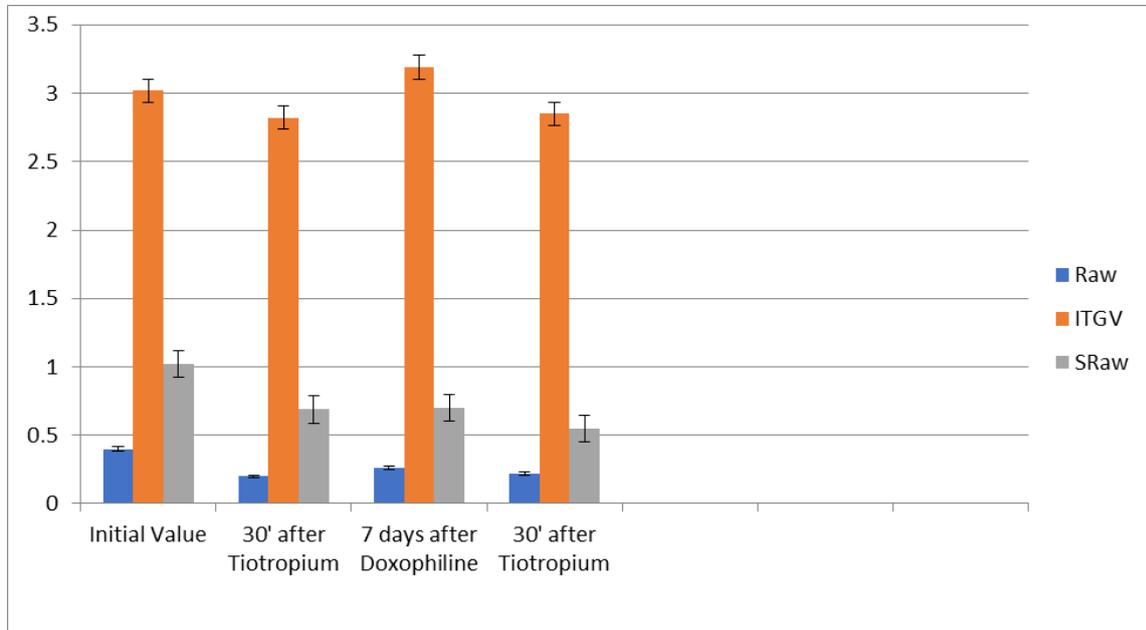


Fig. 2. Effect of Doxofylline (tablet 2 x 400 mg), and Tiotropium bromide (2 inh. x 18 mcg); in Raw, ITGV and SRaw. (n = 7; X ± SEM).

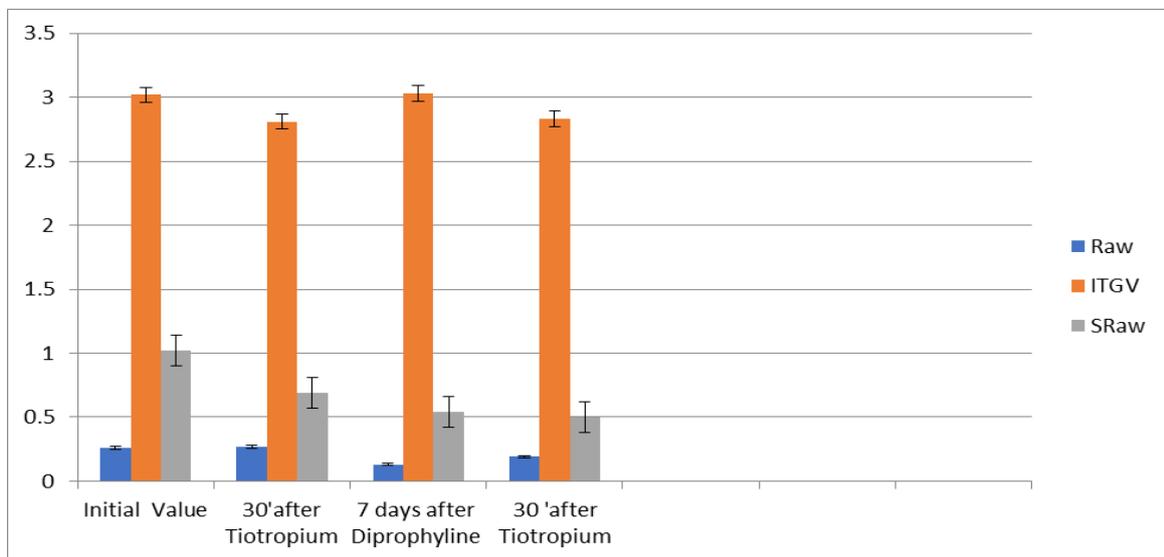


Fig. 3. Effect of Diprophyllyne (tablet 2 x 150 mg), and Tiotropium bromide (2 inh. x 18 mcg); in Raw, ITGV and SRaw (n = 6; X ± SEM).

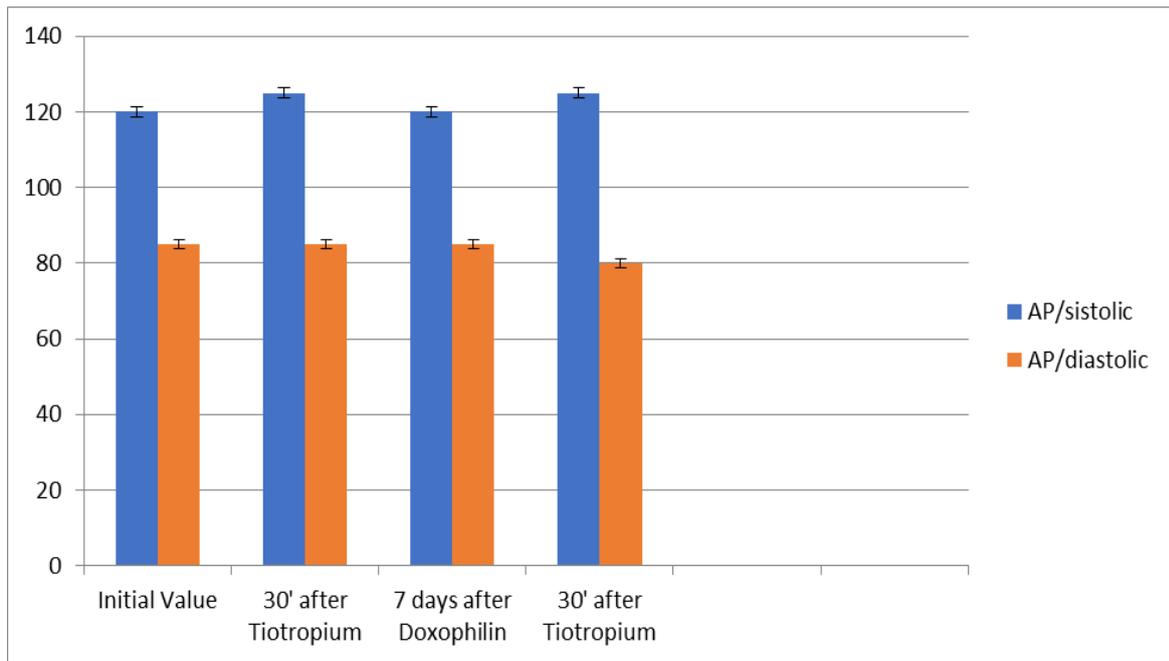


Fig.4. Effect of Doxofylline (tablet 2 x 400 mg), and Tiotropium bromide (2 inh. x 18 mcg); to systolic and diastolic blood pressure. (n = 7; X ± SEM).

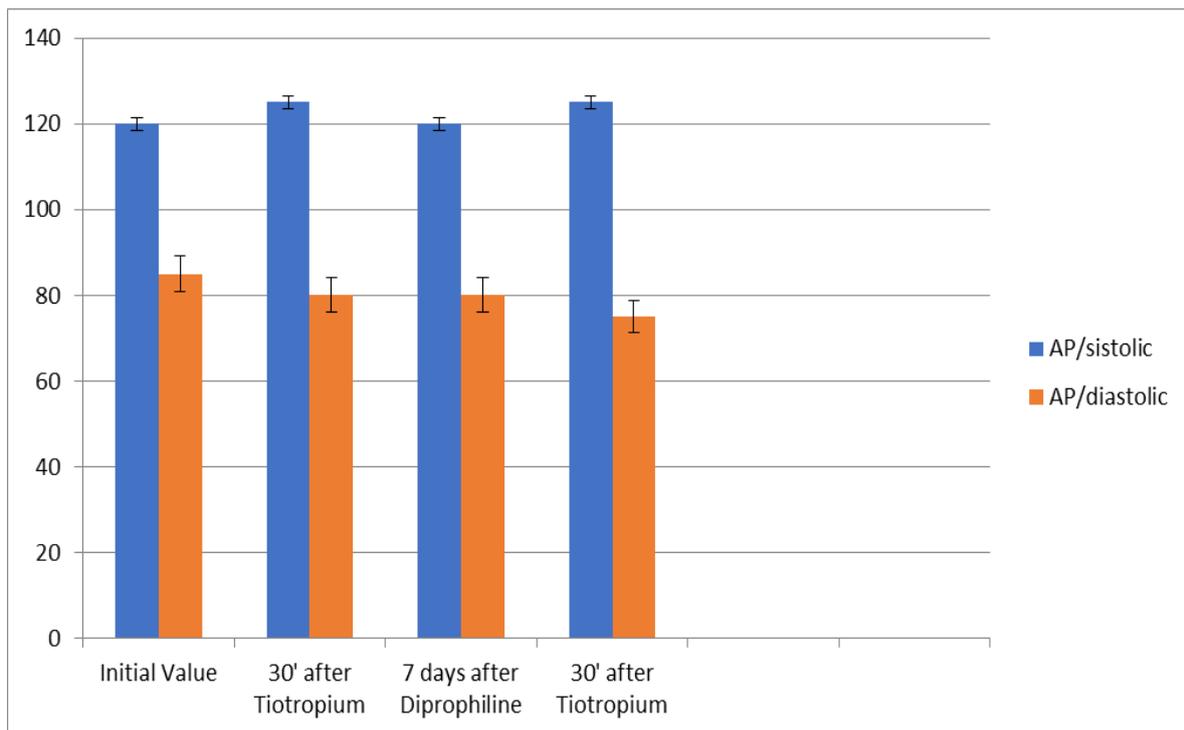


Fig.5. Effect of Diprophyliline (tablet 2 x 150 mg), and Tiotropium bromide (2 inh. x 18 mcg); to systolic and diastolic blood pressure (n = 6; X ± SEM).

DISCUSSION

Chronic diseases therapy requires complex and inclusive approach for treatment of patients that will control not only acute manifestation but also chronic consequences of disease itself. In line with this, global initiatives for treatment and prevention of asthma (GAN) aims reduction of exacerbation and hospitalization figures to achieve better life quality. These aims can be achieved with multifactorial attitude, which means

elimination of external irritant and allergic factors, applying of adequate therapeutic regime, monitoring of changes in the status of disease, medication of comorbidity, education of patients and establishment of partnership relation in between the patient and physician.

Having in mind that generally asthma cannot be cured, it is necessary to achieve an adequate balance between therapeutic aim, selection of therapy and eventually, treatment of side effects because of the selection of not proper medication or medication for other purposes.

As per latest information, xanthenes improve the contraction of diaphragm, which is relevant for their role in respiratory system. Over the years was thought that derivatives of methylxanthines cause bronchodilation through inhibition of phosphodiesterase, by preventing enzymatic detachment of 3', 5'-AMP-c: afterwards seen that these action appear only in the presence of high doses of the medication. Certain number of new mechanisms was proposed:

1. Antagonism of prostaglandins
2. Inhibition of calcium ion flux to the smooth musculature
3. Stimulation of endogen catecholamines
4. Inhibition of release of mediators from mastocyte and leukocyte
5. Antagonism of the adenosine receptor

Theophylline is one of most effective medicines used often in asthma treatment. Nonetheless, its negative effect in central nerve system and cardiovascular system is known and limits its administration.

Bronchospasmolytic activity of theophylline can be attributed to inhibition of phosphodiesterase and mobilizing calcium and antagonist activity in A_1 -adenosine receptors. It is well known that adenosine has significant role in the control of central nerve, cardiovascular, and endocrine system. Adenosine receptors were divided into subtypes A_1 and A_2 , from which A_1 intermediates inhibition of creation of adenylate cyclase and it is responsible for bronchoconstriction, whilst A_2 intermediates stimulation of adenylate cyclase and thus, it is responsible for bronchodilation.

Third subclass of adenosine receptor, A_3 found in heart and lungs of sheep's and an interaction with these receptors leads to inhibition of adenylate cyclase.

Xanthines are effective in asthma treatment, but mechanism of action remains yet unclear. Theophylline is a competition antagonist in adenosine receptors [10,11]. Adenosine can act as autacoid and transmitter with numerous biologic effects. Observation representing special importance to asthma are those where adenosine can cause bronchoconstriction at asthmatics and empower the release from human mastocyte of immunologically triggered mediators [10]. Therefore, inhibition of adenosine effect is to be considered when attempting to explain the mechanism of theophylline effect [12].

Anti-inflammatory effect of theophylline may appear also as consequence of its capability to activate deacetylase in the nucleus. Theoretically, deacetylation of histone can reduce the transcription of some pro-inflammatory genes and emphasize the effect of corticosteroids.

Theophylline relaxes effectively airways smooth muscles, and this bronchodilation can contribute to its acute therapeutic effective ness in asthma.

Both antagonism of adenosine receptors and inhibition of PDE can play a significant role in its bronchodilation effect. **Adenosine** does not contract directly the isolated smooth muscle of human bronchi, but when inhaled it acts as powerful bronchoconstrictor at asthmatics [12]. So, inhibition of the adenosine function can contribute to the bronchodilation triggered by theophylline at some asthmatics. Inhibition of PDE4 and PDE5 effectively relaxes 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. Speaking of 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

proinflammatory effects and both theophylline and enprofylline are powerful competitive antagonists of the A_{2B} adenosine receptor [10,12,13].

Our results indicate that the significantly increased bronchomotor tonus was reduced ($p < 0.05$) because of blockage of adenosine receptor (doxofylline); 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 specific resistance (SR_{aw}), ($p < 0.05$).

Theophylline inhibits synthesis and secretion of inflammatory mediators from many types of cells including mastocytes and basophils. This effect of theophylline may appear as consequence of PDE inhibition and can be imitated in largest part with medicines that inhibit selectively isoenzyme PDE4 [14]. In therapeutic concentration, therapeutic effects of theophylline are related more with its anti-inflammatory effect rather than with bronchodilation effect, however, this remains to be proved.

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 candidate in innovative approaches at asthma therapy.

Our research indicates that due to result of inhibition of phosphodiesterase (diprophylline) 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 specific resistance (SR_{aw}), ($p < 0.05$).

Moderated doses of caffeine can provoke deep feelings of anxiety, fear or panic to some individuals. Even patients with a history of low or moderated doses of coffee experience tension, anxiety, dysphoria following administration of 400 mg or more of this medicine. In new-borns treated with theophylline for apnoea because of premature birth can provoke disorders of cycle sleep-wake [14,15], but effects coming during a long-term use in behaviour and cognitive growth are yet to be defined. Concern that treatment of asthmatic children with theophylline may cause depression, hyperactivity or other worries about behaviour has been increased.

This type of dependence towards exposure on theophylline can contribute in the difficulty to define relation between severity of clinical symptoms with plasma concentration of the medicine, being more careful in treatment of intoxicated patients, which has undergone regular therapy with theophylline [14].

At patients who yet has asthma symptoms, administered was the therapy with inhaled glucocorticoids and to steroid regimen may be added agonists of β_2 -adrenergic receptor in long terms and with satisfactory results. Once used widely, today methylxanthines are used less because of the modest effects and a small therapeutic window. Selective inhibitors of PDE4, which may have the same efficacy but with less side effects are being assessed in clinical studies. New agents are aiming to act over the specific mechanisms, which are significant at the commencement and progression of asthma. These include antagonists of adenosine receptors of leukotriene and the therapy with anti-IgE, omalizumab.

CONCLUSION

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

- ❖ Methylxanthines– Doxofylline administered for 7 consecutive days at a dose of 400 mg orally, causes significant decrease of specific resistance (SR_{aw}) of airways ($p < 0.05$).
- ❖ Methylxanthines– Diprophylline administered for 7 consecutive days at a dose of 150 mg orally, also causes significant decrease of specific resistance (SR_{aw}) of airways ($p < 0.05$).

- ❖ Treatment of the control group with Tiotropium bromide (antagonist of muscarinic receptors) is effective in removal of increased bronchomotor tonus, by causing significant decrease of resistance (R_{aw}), respectively of specific resistance (SR_{aw}), ($p < 0.05$).
- ❖ This suggests that methylxanthines act in the same scale as competition antagonists of adenosine receptors (doxofylline) and as inhibitors of phosphodiesterase (diprophylline).
- ❖ Our results indicate that methylxanthines significantly inhibit connection to the adenosine receptors ($p < 0.05$), identically as inhibitors of phosphodiesterase ($p < 0.05$). Therefore, they can be used successfully as medicines in medication of bronchial asthma.

Abbreviations:

Th1, Th2, and Th17 – lymphocytes
IL-4, IL-5, IL-13, IL-17, IL-25, IL-33 – cytokinin
FcεRI, FcεRII - thrombocyte receptors
NK - natural killer
T – group of regulatory cells (Treg)
VC – vital capacity
FEV₁ – forced expiratory volume in the first second
Raw – airways resistance
ITGV – intrathoracic gas volume
LRMEV - maximum expiratory flow volume curve
FEV₁, 100 x FEV₁/FVK - Tiffeneau-Pinelli index
MEF₂₅, MEF₅₀, MEF₇₅ - l/s. - maximum expiratory flow when expire 25, 50 and 75% at VC
X - average value
SD – standard deviation
SEM – standard error of the mean
GAN – global initiatives for treatment and prevention of asthma
A₁, A₂, and A₃ – adenosine receptors
PDE, PDE4, PDE5 - cyclic nucleotide phosphodiesterase
COPD - Chronic obstructive pulmonary disease
MHC – class II molecules
IgE – specific antibodies
IgM - specific antibodies
DC – dendrite cells

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