SELECTIVE INHIBITION OF PHOSPHODIESTERASE 7 (PDE7) BY BRL50481 IN HEALTHY AND OVALBUMIN-SENSITIZED GUINEA PIGS

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Abstract

Phosphodiesterase (PDE) inhibitors may have significant clinical benefit in respiratory diseases associated with inflammation. The aim was to evaluate effects of selective PDE7 inhibitor (BRL50481) on citric acid induced cough, *in vivo* and *in vitro* airway smooth muscle reactivity in both healthy and ovalbumin sensitized guinea pigs, as well as its effectiveness in changes of blood cells count.

Tested drugs were administered intraperitoneally to male guinea pigs once daily for 7 days either vehicle 10% DMSO (dimethyl sulfoxide) 3 ml/kg (as control) or BRL50481 1 mg/kg. Double chamber whole body plethysmograph was used for evaluation of citric acid (0.6 M) induced cough and specific airway resistance. Organ bath method was used for measurement of tracheal and lung tissue strips contractions evoked by cumulative doses (10^{-8} – 10^{-3} mol/L) of acetylcholine (ACH) and histamine (HIS).

In healthy guinea pigs we did not observe significant effect of tested drug BRL50481 on *in vitro* contractions (similarly to *in vivo* conditions). The effect on cough was in healthy animals negligible. In ovalbumin-sensitized animals, more pronounced *in vitro* relaxing effect of BRL50481 in HIS induced contractions was observed with similar results *in vivo* and no significant change in number of cough efforts.

Our results suggest that PDE7 inhibitors have stronger anti-inflammatory effect compared to its direct effect on smooth muscle and cough.

Keywords: BRL50481, cough, ovalbumin, PDE7, airway hyperresponsiveness

INTRODUCTION

Two decades ago, the first evidence on the presence of phosphodiesterase (PDE) 7 in T cells was confirmed (1). Later on, its inhibition by different selective PDE7 inhibitors was shown to be an effective tool in experimental influence of T cells activation and proliferation (2,3,4).

Chronic airways diseases associated with bronchoconstriction and inflammation like bronchial asthma, and chronic obstructive pulmonary disease (COPD) are historically treated by several agents from a group of xanthine derivatives (5). Their mechanism of action includes a non-selective inhibition of PDE. However, in therapeutically relevant plasma concentrations there are several other mechanisms involved in their effects (6). In previous studies, the antitussive effects of xanthine derivatives were confirmed, with no particular discussion about underlying mechanism (5,7,8).

Adverse effects associated with their administration are based on their relatively mixed and low specific mechanism of action, interactions with other drugs, and a narrow therapeutic range (9,10). Thus, the use of selective (PDE3, PDE4, PDE7) or dual (PDE3/4, PDE4/7) PDE inhibitors in the therapy of these diseases and influencing cough could be beneficial (11,12).

Selective inhibitors of PDE have attracted increasing attention in the therapy of respiratory diseases (13,14). PDE isoenzymes play an important role in the regulation of airways diameter and smooth muscle functions. As major PDE isoforms in the airways, PDE3 and

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PDE4 were confirmed (both hydrolyzing cAMP). However, airway smooth muscle and especially some inflammatory cells present in respiratory system contain other PDE isoenzymes responsible for hydrolysis of cAMP, e.g. PDE 7.

It is widely known that PDEs are represented as 11 superfamilies of metalophosphohydrolases, hydrolyzing cAMP and cGMP to their inactive metabolites (15). Selective inhibitors of PDE4 are considered as the most important therapeutic tools. Rolipram as the mostly used first generation inhibitor of PDE4 was not yet introduced into clinical practice due to its adverse effects at higher doses (nausea, vomiting). Second generation of PDE4 inhibitors (roflumilast, cilomilast) have better perspective, as they maintained anti-inflammatory and immunomodulating effects with lower incidence of adverse effects (16). Nowadays there are no relevant data about their antitussive effects, but roflumilast was recently approved for clinical use in adult patients with severe COPD (17,18).

Furthermore, an inhibition of PDE3 seems to be the most suitable target in affecting the airway reactivity and cough (19). However, PDE3 is expressed in different tissues like airway smooth muscle, myocardium, vessels, and gastrointestinal tract, leading to potential adverse effect from non-respiratory organs.

BRL50481, a selective PDE7 inhibitor, was found to be effective in suppression of some inflammatory cells in *in vitro* conditions, like human monocytes, lung macrophages, and CD8+ T-lymphocytes (20). Furthermore, some other roles were recently found, i.e. protection of dopaminergic neurons in animal models of Parkinson disease (21). Thus, their therapeutic potential in airway inflammatory disease should be evaluated, in order to get more relevant data about the effects of PDE7 in respiratory system. To elucidate the participation of PDE7 izoenzymes in cough and bronchoconstriction, effects of selective PDE7 inhibitor BRL50481 on cough, *in vivo* and *in vitro* airway reactivity as well as blood cells count were assessed in healthy and ovalbumin-sensitized guinea pigs.

MATERIAL AND METHODS

The study protocol was approved by local Ethics Committee at Jessenius Faculty of Medicine, Comenius University in Martin, Slovakia. 32 healthy, male guinea pigs (250-350 g) were used for the study. They were kept in an animal house and had food and water *ad libitum*. In two groups of animals (n=8 in each), airway hyperresponsiveness was induced with antigen (ovalbumin) and the other two groups were used as naïve controls without sensitization (n=8 in each). From the two groups, the first one was left without treatment – only solvent, 10% dimethylsulfoxide (DMSO) at the dose of 3.0 mL/kg was used. All animals in the second group were treated with PDE7 inhibitor BRL 50481 (Sigma Aldrich, Germany) at the dose of 1.0 mg/kg b.w., dissolved in 10 % DMSO (3.0 mL/kg b.w.). The same dosing schema was applied in healthy as well as in ovalbumin sensitized guinea pigs.

Antigen-induced airway hyperresponsiveness

Sensitization of animals by antigen ovalbumin, which causes airway reactivity changes on immunological basis, was performed during 14 days (5,22). The allergen (1% ovalbumin) was administered on the 1st day of sensitization intraperitoneally (0.5 mL) and subcutaneously (0.5 mL), on the 3rd day intraperitoneally (1 mL) and on the 14th day only by inhalation (30 seconds). The airway reactivity to mediators of bronchospasm was followed *in vivo* immediately after the ovalbumin inhalation and *in vitro* after sacrificing the animal. In treated groups, BRL50481 or DMSO were administered 30 minutes before the nebulization (19).

Cough reflex assessment

The method of chemically-induced cough was used for assessing the cough reflex. The following two methods for detection of cough were used to distinguish the cough effort from sneezing and movements: a) the changes of the expiratory airflow interrupting the basic

respiratory pattern during cough were measured by pneumotachograph connected to the head chamber of bodyplethysmograph; b) the typical cough reflex movement and sound recognized by two trained observers (23). The number of coughs evaluated on the basis of sudden enhancement of expiratory flow accompanied by a typical cough movement and sound during 2 minutes nebulization of citric acid aerosol in concentration of 0.6 M in saline and during following 2 min was counted.

Evaluation of in vivo airway reactivity

In vivo airway reactivity was evaluated using a double chamber whole body plethysmograph immediately after administration of bronchoconstrictors (19). Specific airway resistance and its changes after a short-term inhalation (2 min) of bronchoconstricting agent (histamine) at a concentration of 10^{-6} mol/L in saline) were considered as an indicator of the *in vivo* reactivity changes. For comparison, reactivity after nebulization of saline was used. Between two exposures there was an interval of minimum 5 min. During intervals, fresh air was insufflated into the nasal chamber.

Evaluation of in vitro airway reactivity

After sacrificing the animals, trachea and lungs were immediately excised. Tracheal strips (approximately 15 mm) were cut on the opposite side of a smooth muscle. Lung tissue strips (2 x 2 x 15 mm) were cut from the margin of upper lobe of right and left lungs. The strips were mounted between two hooks and placed into the 30 mL organ chambers containing Krebs-Henseleit`s buffer (NaCl 110.00 mmol/L, KCl 4.80 mmol/L, $CaCl_2$ 2.35 mmol/L, $MgSO_4$ 1.20 mmol/L, $KHPO_4$ 1.20 mmol/L, $NaHCO_3$ 25.00 mmol/L, and glucose 10.00 mmol/L in glass-distilled water). The chambers were maintained at 36.5 ± 0.5 °C and aerated continuously with a mixture of 95 % O_2 and 5 % CO_2 to maintain pH 7.5 ± 0.1. One of the hooks was connected to a force transducer (TENSIL10, RES Martin, Slovakia) and an amplifier (TEMES 1052, RES Martin, Slovakia), and tension changes were recorded online using special computer software (TEMES 1, RES Martin, Slovakia). The tissue strips were initially set to 4 grams of tension for 30 min (loading phase). Then, in each strip the tension was readjusted to a baseline value of 2 grams for another 30 min (adaptation phase). During both periods, the tissue strips were washed at 10 min intervals. Cumulative doses of histamine (10^{-8} to 10^{-3} mol/L, Sigma-Aldrich, Germany) were added after the adaptation phase had been finished and a continuous recording of contractions was made (5,24). Data of the tracheal tissue reactivity are shown in grams (g) of the smooth muscle tension.

Hematological assay

Samples of blood were taken immediately after sacrificing the animals and total white blood cells (WBC) count was determined by hematology analyzer BC-5500 (Mindray, China) as well as in Bürker's chamber after staining by Türck. Differential leukocytes count in blood was evaluated by hematology analyzer BC-5500 (Mindray, China) as well as microscopically after panchromatic staining by Pappenheim and a relative count of neutrophils, monocytes, basophils, eosinophils, and lymphocytes was determined (in %) (25).

Statistical analysis

Data are shown as median and interquartile range (cough), and means ± SE (in vivo and in vitro reactivity). For statistical analysis Student's *t-test* was used. A $p<0.05$ was considered statistically significant.

RESULTS

Intraperitoneal administration of PDE7 inhibitor BRL50481 did not lead to significant decrease in number of cough efforts in healthy and ovalbumin sensitized guinea pigs (Fig. 1).

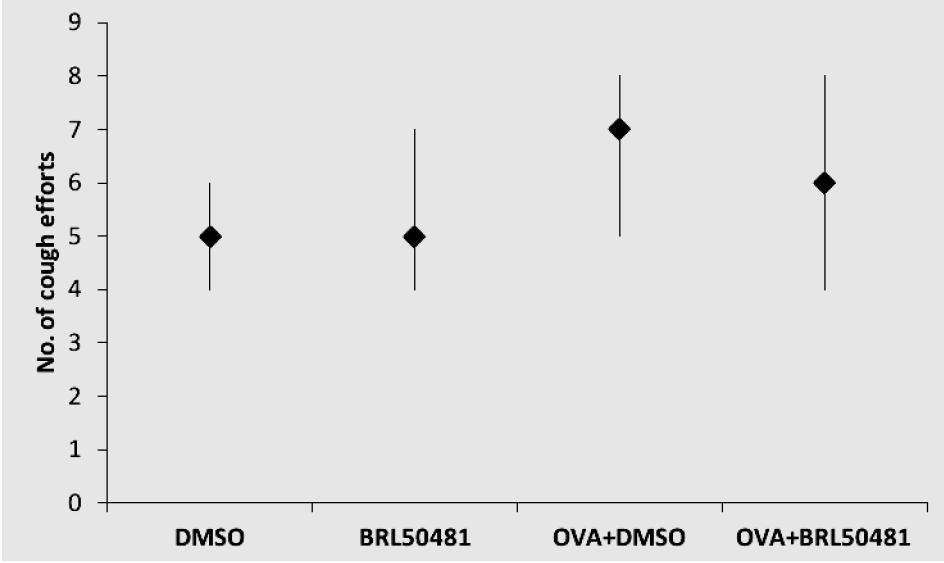

Fig. 1. Number of cough efforts during (2 min) and after (2 min) inhalation of citric acid aerosol in healthy (DMSO, BRL50481) and ovalbumin-sensitized guinea pigs (OVA+DMSO, OVA+BRL50481) after 7-days pre-treatment with vehicle (10 % DMSO, 3.0 ml/kg b.w.), and BRL50481 (1 mg/kg b.w.). Data are shown as median and interquartile range.

Furthermore, the ability of selective PDE7 inhibitor to affect the airway responsiveness was evaluated. Specific airway resistance measured in the whole body double chamber plethysmograph was used as a marker of *in vivo* airway responsiveness. Administration of BRL50481 at the dose of 1.0 mg/kg caused a significant decrease of specific airway resistance after histamine nebulization only in ovalbumin-sensitized guinea pigs (Fig. 2).

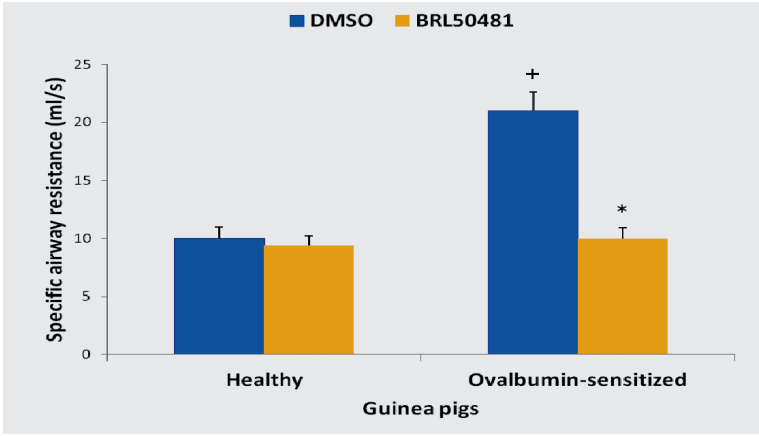

Fig. 2. Specific airway resistance after inhalation of histamine (10^{-6} mol/L) in healthy and ovalbumin-sensitized guinea pigs after 7-days pre-treatment with vehicle (10 % DMSO, 3.0 ml/kg b.w.), and BRL50481 (1 mg/kg b.w.). (+ $p<0.05$ vs. healthy, * $p<0.05$ vs. DMSO)

In vitro testing of tracheal smooth muscle tissue strips contractility of healthy guinea pigs to cumulative doses of histamine demonstrated no significant suppression of strips reactivity only after BRL50481 pretreatment. In ovalbumin-sensitized guinea pigs, contractions evoked by histamine were significantly decreased in tracheal strips by pretreatment with BRL50481 (Fig. 3).

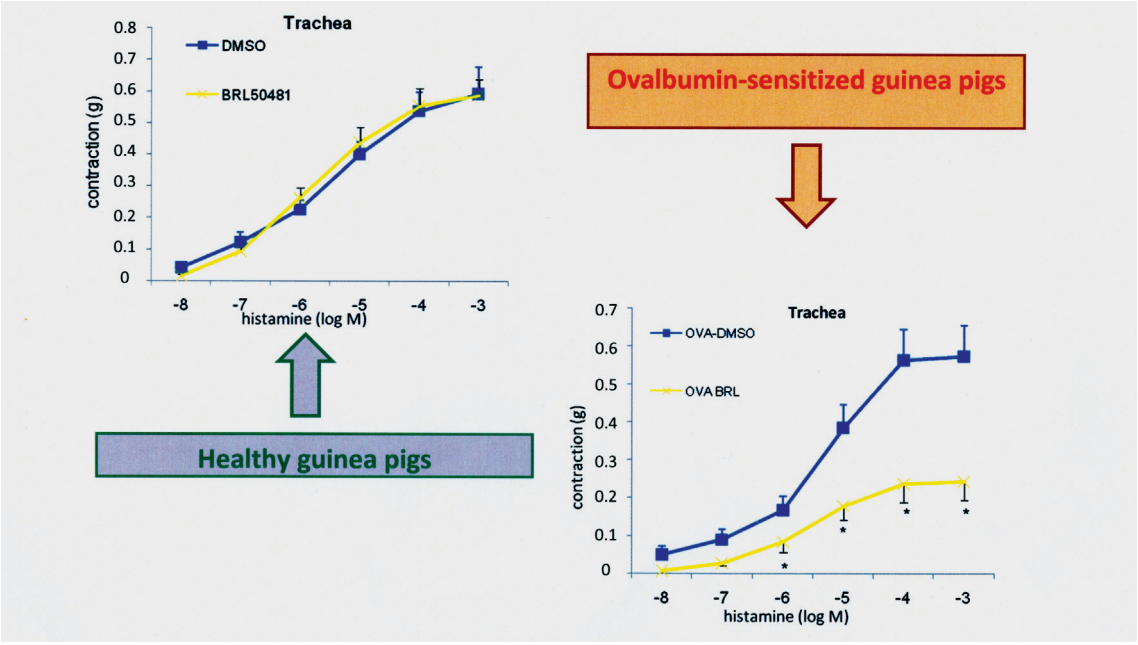

Fig. 3. In vitro airway reactivity to histamine in healthy (left) and ovalbumin-sensitized guinea pigs (right) after 7-days pre-treatment with vehicle (10 % DMSO, 3.0 ml/kg b.w.), and BRL50481 (1 mg/kg b.w.). (* $p<0.05$ vs. DMSO or OVA DMSO)

Hematological examination was focused on changes in total WBC count and in their differential count. We found that sensitization with ovalbumin led to a significant increase in circulating WBC with increasing representation of eosinophils at the expense of lymphocytes. Administration of BRL50481 to healthy guinea pigs did not cause any significant changes in WBC count (only tendency to decrease the relative count of eosinophils and monocytes was observed). In ovalbumin-sensitized guinea pigs, significant decrease in number of WBC as well as in relative count of eosinophils compared to non-treated animals was observed (Table 1).

Type of cells	Healthy	BRL50481	Ovalbumin-sensitized	OVA + BRL50481
No. of Leu ($x10^9$/l)	**2.38**	**2.1**	**4.8+**	**2.71***
Neu (%)	20.3	18.3	19.0	22.8
Ly (%)	**76.7**	**80.2**	**78.4**	**73.5**
Mo (%)	2.0	1.1	1.6	1.2
Eo (%)	**0.62**	**0.3**	**2.3+**	**1.1***
Ba (%)	0.26	0.2	0.1	1.2

Table 1. Blood cells count (absolute and relative) in peripheral blood of healthy and ovalbumin-sensitized guinea pigs after 7-days pre-treatment with vehicle (10 % DMSO, 3.0 ml/kg b.w.), and BRL50481 (1 mg/kg b.w). (+ $p<0.05$ vs. healthy, * $p<0.05$ vs. DMSO or OVA DMSO)

DISCUSSION

Selective inhibitors of PDE (especially PDE3 and PDE4) have been extensively studied for their anti-inflammatory action and their inhibition was previously confirmed as suitable target for influencing the airway inflammation as well as contractility of airway smooth muscle (13). Several clinical studies testing second generation of PDE4 inhibitors (piclamilast, cilomilast, roflumilast) confirmed their bronchodilating, anti-inflammatory and antitussive effects previously demonstrated in experimental conditions. Furthermore, it emphasizes the necessity of testing other PDE isoforms (26,27,28).

PDE7 enzymes are cAMP specific but insensitive to rolipram. They are encoded by two genes PDE7A and PDE7B with significant expression of PDE7A1 protein in T-cell lines, peripheral T-lymphocytes, epithelial cell lines, airway and vascular smooth muscle cells, lung fibroblasts, and eosinophils (29). Thus, the inhibition of this isoenzyme could be an effective tool in suppressing the airway hyper-responsiveness and inflammation.

There are several selective PDE7 inhibitors, like e.g. VP1.15 and S14 synthesized in the Instituto de Quimica Medica (30), pyrimidine inhibitors (31), purine inhibitors (32), ASB16165 synthesized in Asubio Pharma Co Ltd in Japan (4), thiadiazol derivative PF0332040 synthesized by Pfizer (3). Another selective PDE4 inhibitor has some activity against PDE7 isoenzyme – T-2585 synthesized by the Discovery Research Laboratory in Japan (2).

In our experiments, BRL50481 as the only commercially available selective PDE7 inhibitor (20) was used and led to significant decrease of *in vivo* airway reactivity as well as in *in vitro* conditions. The most significant drop of contractile responses to histamine was observed in ovalbumin-sensitized animals. This suggests its potential use in clinical states associated with allergic inflammation, which was mimicked by ovalbumin sensitization. Furthermore, the patients may benefit from its synergistic effect when administered with a PDE4 inhibitor (based on own unpublished data as well as data published by other authors, 33). This concept was described in literature and new dual PDE inhibitors are studied (e.g. PDE3/4 and PDE4/7) for their anti-inflammatory action (34).

As there were so far no relevant data about antitussive effects of PDE7 inhibitors, our results indicate their alternative efficiency in influencing the cough. However, in our experiments we did not reach the significance level. Nevertheless, the model we used is considered as model of asthma, with predominance of eosinophils (19). The blood cells count changes done in our experiment confirmed the increase in eosinophil relative count in peripheral blood, accompanied by increase in absolute white blood cells count after 14 days ovalbumin sensitization of guinea pigs. A significant decline in absolute number of WBC as well as relative number of eosinophils after 7 days lasting administration of BRL50481 indicates anti-inflammatory effects of PDE7 inhibition in the selected model of airway hyperresponsiveness. Similarly to previous studies (2), pre-treatment with PDE7 inhibitor BRL 50481 decreased the relative count of lymphocytes. However, this decrease was in our experiments not statistically significant, probably due to the fact we did not distinguish between B- and T-line of lymphocytes. Furthermore, no suppressive effect on neutrophils count, typical for selective PDE4 inhibitors, was observed.

Nevertheless, other models, like COPD models with prevailing neutrophils, should be tested, in order to exclude the efficacy of these groups of drugs in influencing the cough (33).

In conclusion, we did not observe any antitussive effect of tested PDE7 inhibitor BRL50481. Nevertheless, the significant suppression of airway hyper-responsiveness both in *in vivo* and *in vitro* conditions by this PDE7 inhibitor as well as positive changes in absolute and relative white blood cells counts indicate stronger anti-inflammatory effects compared to its direct effect on smooth muscle and cough.

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Acknowledgement
Authors thank to M. Gruchalakova, M. Kocmalova, and M. Repcakova for technical assistance. This study was supported by VEGA grant 1/0030/11 and by the Centre of Experimental and Clinical Respirology II. – "Project co-financed from EU sources".

Received: Oct, 26, 2011
Accepted: Dec, 2, 2011