Effects of Roflumilast, a Phosphodiesterase-4 Inhibitor, on the Lung Functions in a Saline Lavage-Induced Model of Acute Lung Injury

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Summary

Acute lung injury (ALI) is associated with deterioration of alveolar-capillary lining and transmigration and activation of inflammatory cells. Whereas a selective phosphodiesterase-4 (PDE4) inhibitor roflumilast has exerted potent anti-inflammatory properties, this study evaluated if its intravenous delivery can influence inflammation, edema formation, and respiratory parameters in rabbits with a lavage-induced model of ALI. ALI was induced by repetitive saline lung lavage (30 ml/kg). Animals were divided into 3 groups: ALI without therapy (ALI), ALI treated with roflumilast i.v. (1 mg/kg; ALI+Rofl), and healthy ventilated controls (Control), and were ventilated for following 4 h. Respiratory parameters (blood gases, ventilatory pressures, lung compliance, oxygenation indexes etc.) were measured and calculated regularly. At the end of experiment, animals were overdosed by anesthetics. Total and differential counts of cells in bronchoalveolar lavage fluid (BAL) were estimated microscopically. Lung edema was expressed as wet/dry lung weight ratio. Treatment with roflumilast reduced leak of cells ($P<0.01$), particularly of neutrophils ($P<0.001$), into the lung, decreased lung edema formation ($P<0.01$), and improved respiratory parameters. Concluding, the results indicate a future potential of PDE4 inhibitors also in the therapy of ALI.

Key words

Roflumilast • Lung injury • Inflammation • Lung functions • Lung edema

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Introduction

The acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are clinical syndromes characterized by decreased lung compliance, bilateral pulmonary infiltrates, and hypoxemia which have direct or indirect lung etiology (Ware 2006, Matthay *et al.* 2012). An acute exudative phase of the syndromes involves an alveolar-capillary leakage in conjunction with a leukocyte extravasation. This is followed by a fibroproliferative phase which includes hyaline membrane formation, persistent inflammation, and proliferation of alveolar epithelial cells and mesenchymal cells. In the lung inflammation, recruitment of circulating polymorphonuclears (PMN) is essential for a host defense and initiates a specific immune response; whereas a pathological hallmark of ALI/ARDS is an uncontrolled transmigration of neutrophils into the lung interstitium and alveolar space and their recruitment (Grommes and Soehnlein 2011, Matthay *et al.* 2012).

There is a variety of inflammatory reactions activated in ALI/ARDS, with complex interactions between the cells and mediators where some of them have not been completely elucidated yet. However, therapeutic modification of these reactions by anti-inflammatory agents can suppress the inflammatory changes and finally improve the patient's status. For instance, 3',5'-cyclic adenosine monophosphate (cAMP) is an intracellular signaling molecule which regulates a broad range of cell processes. cAMP is formed from ATP by action of enzyme adenylate cyclase. Signaling

initiated by cAMP formation is partially terminated *via* degradation of the compound by phosphodiesterases (PDEs) which hydrolyse cAMP to inactive 5′-AMP (Maurice *et al.* 2014). From the PDEs family of eleven known classes, eight are able to hydrolyse cAMP. Among them, PDE4 is proposed to play an exceptional role in ALI as it is expressed in inflammatory and immunomodulatory cells including neutrophils and macrophages, and in the lung airway epithelial cells, endothelial cells, smooth muscle cells etc. (Houslay *et al.* 2005). Thanks to modulation of PDE4 in these cells, PDE4 inhibitors have become valuable therapies for chronic lung diseases such as chronic obstructive pulmonary disease (COPD) and bronchial asthma (Parikh and Chakraborti 2016). Nevertheless, there is a lack of information on potential benefit of PDE4 inhibitors in acute lung injury. Therefore, the purpose of this study was to investigate whether a selective PDE4 inhibitor roflumilast can positively influence the respiratory parameters, migration of PMN and formation of lung edema in animals with ALI induced by repetitive saline lung lavage.

Methods

General design of experiments

Experimental protocols were performed in accordance with the ethical guidelines and were authorized by the local Ethics Committee of Jessenius Faculty of Medicine and by National Veterinary Board.

In the study, young New Zealand white rabbits of both genders and a mean body weight (b.w.) of 2.5±0.3 kg were used. Animals were anesthetized with intramuscular tiletamine + zolazepam (15 mg/kg b.w.; Zoletil, Virbac, France) and xylazine (5 mg/kg b.w.; Xylariem, Riemser, Germany), followed by an infusion of tiletamine + zolazepam (10 mg/kg/h i.v.). A tracheotomy was performed and an endotracheal tube was inserted. Catheters into the femoral artery and right atrium for sampling the arterial and mixed venous blood and into the femoral vein to administer anesthetics were inserted. Animals were paralyzed with pipecuronium bromide (0.3 mg/kg b.w./30 min; Arduan, Gedeon Richter, Hungary) and subjected to ventilator Aura V (Chirana, Slovakia) and were ventilated conventionally with following settings: frequency (f) of 40/min, fraction of inspired oxygen (FiO_2) of 1.0, time of inspiration (Ti) 50 %, positive end-expiratory pressure (PEEP) 0.5 kPa, and peak inspiratory pressure (PIP) to achieve a tidal volume (V_T)<6 ml/kg b.w. After 15 min of stabilization, respiratory parameters were recorded and blood samples for analysis of blood gases (RapidLab 348, Siemens, Germany) were taken. One group of animals served as healthy ventilated and non-treated controls (Control group, n=6). In other animals, the lung injury was induced by repetitive lung lavage with saline (0.9 % NaCl, 30 ml/kg b.w., 37 °C) which was instilled into the endotracheal cannula in the semi-upright right and left lateral positions of the animal and was immediately suctioned by a suction device. Lavage was performed 6-12 times, until PaO_2 decreased to <26.7 kPa in 2 measurements at 5 and 15 min after the lavage. When the criteria of the ALI model were full-filled, respiratory parameters were recorded and blood samples taken again. Then, animals were treated with roflumilast (1 mg/kg b.w.; ALI+Rofl group, n=8) which was given intravenously during 2 min, or were left without therapy (ALI group, n=8). All animals were oxygen-ventilated (FiO_2 1.0, frequency 40/min, PEEP 0.5 kPa, V_T <6 ml/kg b.w.) for an additional 4 h after administration of the treatment. Blood gases and respiratory parameters were measured at 0.5, 1, 2, 3, and 4 h of the treatment. At the end of experiment, animals were sacrificed by an overdose of anesthetics.

Measurement of respiratory parameters

During experiment, animals were ventilated with ventilator Aura V (Chirana, Slovakia). Ventilatory parameters, such as V_T, FiO_2, minute ventilation, Ti, f, ventilatory pressures [mean airway pressure (MAP), PIP and PEEP], static (Cst) and dynamic (Cdyn) lung compliance, and airway resistance (Raw) were automatically measured by in-build sensors and software and were displayed on the screen of the ventilator. Partial pressures of oxygen and carbon dioxide (PaO_2 and $PaCO_2$), arterial pH, arterial oxygen saturation (SaO_2), and parameters of acid-base balance were measured with blood gas analyzer (Rapidlab 348, Siemens, Germany). Ventilation efficiency index was calculated as:

$VEI = 3800/[(PIP-PEEP) \times frequency \times PaCO_2]$.

The oxygenation index (OI) was calculated as:

$OI = [MAP\ (kPa) \times FiO_2\ (\%)]/PaO_2\ (kPa)$.

The PaO_2/FiO_2 ratio, the PF for short, was calculated as:

$PF\ ratio = [PaO_2\ (mmHg) \times 100]/FiO_2\ (\%)$.

Alveolar partial pressure of O_2 (P_AO_2 mmHg) was expressed as:

$P_AO_2 = \{[FiO_2\ (\%)/100] \times (Patm - PH_2O)\} - [PaCO_2\ (mmHg)/RQ]$,

where atmospheric pressure (Patm) at a sea level is 760 mmHg, PH_2O is 47 mmHg, and RQ (respiratory quotient) is 0.8. Alveolar-arterial (A-a) gradient was calculated as:

A-a gradient = P_AO_2 (mmHg) - PaO_2 (mmHg). Right-to-left pulmonary shunts (Qs/Qt) were calculated as described elsewhere (Vodoz *et al.* 2009).

Counting of cells in the BAL fluid

After sacrificing the animal, lungs and trachea were excised. The left lung was lavaged 3-times by saline (0.9 % NaCl, 37 °C, dose of 10 ml/kg b.w.). Total number of cells in the BAL fluid was determined by an automated cell analyzer (Countess, Invitrogen, USA). Bronchoalveolar lavage (BAL) fluid was then centrifuged at 1500 rpm for 15 min. Differential count of cells in the BAL fluid sediment was evaluated microscopically after staining by May-Grünwald/Giemsa-Romanowski.

Lung edema formation (wet/dry lung weight ratio)

Strips of the right lung tissue were weighed, dried at 60 °C for 24 h and the wet/dry weight ratio was determined.

Statistics

GraphPad Prism (Version 6.01) software was used for statistical analyses. Statistical differences between the groups were determined by analysis of variance (ANOVA) with Bonferroni *post hoc* test or Kruskal-Wallis test. Results are presented as average with error bars indicating standard error of the mean (means ± SEM).

Results

Body weights of animals and initial values of the parameters before induction of the ALI model were comparable between the groups (all $P>0.05$).

Respiratory parameters

Lung lavage with saline induced an acute respiratory insufficiency characterized with decrease in lung compliance, increase in right-to-left pulmonary shunts with subsequent hypoxemia, hypercapnia and acidemia compared to non-lavaged controls (all $P<0.001$, Tables 1 and 2). These changes have been reflected also by worsening in clinically relevant indexes of oxygenation (all $P<0.001$, Table 2). As a result of insufficient gas exchange, ventilatory pressures (MAP and PIP) had to be elevated to higher values than in controls (Table 1). Roflumilast administration significantly improved PaO_2 from 1 h ($P<0.01$, Table 2) and $PaCO_2$ from 30 min ($P<0.05$, Table 1), but had no positive effect on pH compared to ALI group ($P>0.05$, Table 1). Roflumilast enhanced also other respiratory parameters compared to non-treated ALI group, i.e. MAP ($P<0.001$), PF ratio ($P<0.05$), and P_AO_2 ($P<0.01$) from 30 min of the therapy, AAG ($P<0.05$), VEI ($P<0.05$), and Cdyn ($P<0.01$) from 1 h of the therapy and OI ($P<0.05$) from 2 h of the therapy (Tables 1 and 2).

Cells in the BAL fluid

Repetitive lung lavage significantly elevated a total number of cells in the BAL fluid compared to controls ($P<0.001$ for ALI vs Control), while roflumilast inhibited the infiltration of cells into the lungs ($P<0.01$ for ALI+Rofl vs ALI) (Fig. 1). As shown in Figure 2, induction of ALI model significantly increased the percentage of neutrophils ($P<0.01$ for ALI vs Control) and decreased number of monocytes-macrophages ($P<0.01$ for ALI vs Control). Roflumilast therapy lowered number of neutrophils ($P<0.001$ for ALI+Rofl vs ALI) and elevated relative count of monocytes and macrophages ($P<0.001$ for ALI+Rofl vs ALI).

Lung edema formation

Compared with controls, wet/dry weight ratio significantly increased in the ALI group ($P<0.01$). Roflumilast reduced lung wet/dry weight ratio vs ALI group ($P<0.01$) (Fig. 3).

Discussion

The neutrophil-mediated inflammation and lung edema formation are fundamental signs of ALI. We have assumed that administration of PDE4 inhibitor can alleviate these changes what can finally improve the lung functions of animals with model of ALI. Results of our study showed that intravenous delivery of 1 mg/kg b.w. of roflumilast reduced transmigration of cells, particularly of neutrophils into the lung, decreased generation of lung edema and enhanced gas exchange and other respiratory parameters in comparison with non-treated animals with ALI.

PDE4 inhibitors have previously demonstrated a number of anti-inflammatory actions including the inhibition of cell trafficking and microvascular leakage, release of cytokines and chemokines from inflammatory

Table 1. Respiratory parameters before and after induction of ALI model (Before/After ALI) and after administration of the therapy (Th) in healthy ventilated and non-treated animals (Control group), in non-treated animals with ALI (ALI group) and in animals with ALI treated with roflumilast (ALI+Rofl group).

	Before ALI	After ALI	0.5 h Th	1 h Th	2 h Th	3 h Th	4 h Th
***MAP** (kPa)*							
Control	0.78±0.02	0.79±0.01	0.77±0.02	0.76±0.02	0.71±0.01	0.74±0.02	0.74±0.02
ALI	0.80±0.02	1.04±0.02[a]	1.03±0.04[a]	1.04±0.05[a]	1.08±0.06[a]	1.04±0.08[a]	1.09±0.06[a]
ALI+Rofl	0.80±0.02	0.99±0.04	0.90±0.04[e]	0.90±0.05[e]	0.90±0.04[e]	0.85±0.02[e]	0.91±0.06[e]
***PaCO$_2$** (kPa)*							
Control	4.22±0.17	4.37±0.15	4.30±0.14	4.21±0.10	3.89±0.29	3.69±0.29	3.85±0.08
ALI	3.98±0.21	6.18±0.14[a]	6.43±0.23[a]	6.65±0.34[a]	6.57±0.26[a]	6.19±0.22[a]	6.01±0.38[a]
ALI+Rofl	3.52±0.22	6.04±0.26	5.52±0.36[c]	5.16±0.35[b]	4.94±0.29[a]	4.73±0.32[b]	4.53±0.29[b]
pH							
Control	7.55±0.01	7.52±0.04	7.43±0.02	7.37±0.02	7.32±0.02	7.23±0.04	7.22±0.03
ALI	7.53±0.02	7.18±0.02[a]	7.19±0.03[a]	7.19±0.03[a]	7.13±0.04[a]	7.06±0.04[b]	7.02±0.05[a]
ALI+Rofl	7.58±0.03	7.23±0.03	7.23±0.03	7.18±0.03	7.08±0.03	7.03±0.02	7.03±0.03
VEI							
Control	19.0±0.9	18.2±0.6	18.5±0.6	18.9±0.4	20.6±0.9	22.6±2.6	20.6±0.4
ALI	20.5±1.1	12.9±0.3[b]	12.4±0.5[b]	12.1±0.5[a]	12.2±0.4[a]	12.9±0.5[a]	13.5±0.8[a]
ALI+Rofl	23.3±1.5	13.3±0.6	14.9±1.1	15.8±1.2[f]	16.4±1.0[e]	17.3±1.3[e]	18.0±1.3[e]
***Cdyn** (ml/kPa)*							
Control	14.5±0.5	14.9±0.5	15.1±0.5	15.6±0.4	15.3±0.4	14.7±0.4	15.3±0.5
ALI	13.0±0.8	7.2±0.9[a]	7.7±0.4[a]	6.2±0.9[a]	6.4±0.9[a]	4.4±1.2[a]	5.1±1.0[a]
ALI+Rofl	14.0±0.8	7.8±0.6	9.3±1.2	9.7±1.2[e]	10.0±1.3[e]	10.0±1.2[d]	9.3±1.2[f]
***Qs/Qt** (%)*							
Control	3.8±0.6	5.1±1.8	3.7±0.8	6.1±1.6	5.8±0.4	4.4±0.8	1.3±0.5
ALI	5.6±1.2	23.8±0.5[a]	24.2±0.6[a]	25.0±0.3[a]	25.0±0.3[a]	25.0±0.2[a]	24.8±0.3[a]
ALI+Rofl	4.0±0.8	23.0±1.1	18.2±2.8	16.6±3.5[e]	15.8±3.4[e]	16.6±4.0[e]	13.4±3.6[d]

Abbreviations: MAP: mean airway pressure, $PaCO_2$: arterial partial pressure of carbon dioxide, pH: arterial pH, VEI: ventilation efficiency index, Cdyn: dynamic lung compliance, Qs/Qt: intrapulmonary shunts. Statistical comparisons: for ALI vs Control: [a] $P<0.001$, [b] $P<0.01$, [c] $P<0.05$; for ALI+Rofl vs ALI: [d] $P<0.001$, [e] $P<0.01$, [f] $P<0.05$. Data are expressed as means ± SEM.

cells, production of reactive oxygen species (ROS), expression of cell adhesion molecules, attenuated bronchoconstriction etc. (Rabe 2010). These effects are attributed to maintained cAMP levels and enhanced protein kinase activity (PKA) (Bender and Beavo 2006). Roflumilast is a representant of the second generation of PDE4 inhibitors which was in 2010 approved to manage COPD (Field 2011). Roflumilast has shown to reduce a neutrophil infiltration, to decrease a release of inflammatory mediators, to inhibit a fibrotic remodeling, and to reduce an oxidative stress (Rabe 2010). In addition, although roflumilast is not known as a potent bronchodilator, modest improvements in FEV_1 have been documented in clinical trials (Rabe *et al.* 2005, Garnock-Jones 2015). Nevertheless, majority of the information on roflumilast action is related to its oral administration. In our study, roflumilast was administered intravenously as this route of delivery is more suitable for anesthetized, tracheotomized and ventilated rabbits with model of ALI as well as for patients with ALI/ARDS.

In the present study, ALI model was induced by repetitive lung lavage with saline what removed a significant portion of pulmonary surfactant. This model of surfactant depletion is one of standardly used animal models of ALI which is usually elicited in rabbits (Matute-Bello *et al.* 2008, Ronchi *et al.* 2012). Although this model does not provide such massive migration of cells and so convincing extent of inflammation as it was demonstrated for lipopolysaccharide (LPS)-induced models of ALI, disturbance of an alveolar-capillary lining early after the

Table 2. Parameters expressing oxygenation before and after induction of ALI model (Before/After ALI) and after administration of the therapy (Th) in healthy ventilated and non-treated animals (Control group), in non-treated animals with ALI (ALI group) and in animals with ALI treated with roflumilast (ALI+Rofl group).

	Before ALI	After ALI	0.5 h Th	1 h Th	2 h Th	3 h Th	4 h Th
PaO_2							
Control	81.04±1.29	78.69±3.56	81.52±1.59	76.94±4.22	77.24±4.05	81.9±1.72	87.83±1.54
ALI	78.21±3.18	16.71±1.88[a]	21.09±4.33[a]	17.34±5.19[a]	12.32±1.52[a]	12.88±0.99[a]	18.62±5.08[a]
ALI+Rofl	79.74±2.05	20.34±4.09	39.29±10.12	45.37±12.00[e]	48.44±11.62[d]	51.79±13.62[d]	56.76±11.03[d]
OI							
Control	1.00±0.02	0.99±0.03	0.93±0.03	0.95±0.03	0.89±0.02	0.92±0.02	0.85±0.03
ALI	1.05±0.07	7.57±1.04[a]	7.98±1.22[a]	9.02±2.14[a]	9.09±2.03[a]	9.36±2.4[a]	7.57±1.43[a]
ALI+Rofl	1.06±0.04	7.71±0.93	5.99±1.76	5.73±1.77	4.56±1.38[f]	3.82±1.61[f]	2.27±0.73[e]
PF ratio							
Control	602.3±9.0	590.2±26.7	611.5±11.9	577.0±31.7	579.3±30.4	614.2±12.9	659.7±11.7
ALI	591.1±25.9	104.8±11.3[a]	102.4±10.8[a]	84.2±8.2[a]	92.0±13.2[a]	98.3±8.3[a]	105.6±11.7[a]
ALI+Rofl	595.1±16.5	140.2±20.3	269.0±82.4[f]	340.9±90.1[e]	364.0±87.3[d]	389.5±102.7[d]	426.6±83.2[d]
P_AO_2							
Control	673.4±1.6	672.0±1.4	672.7±1.3	673.6±0.9	676.6±1.5	678.4±2.7	676.9±0.8
ALI	675.7±1.9	652.2±2.1[a]	648.5±3.9[a]	650.7±3.2[a]	651.4±2.4[a]	655.0±2.1[a]	656.6±3.6[a]
ALI+Rofl	680.0±2.0	655.7±2.7	661.3±3.4[e]	663.3±3.1[e]	664.9±3.0[e]	667.9±2.7[e]	666.6±4.9[f]
AAG							
Control	612.0±11.7	593.9±33.6	606.6±14.7	561.9±41.1	563.3±38.0	601.9±13.5	663.2±14.6
ALI	586.6±23.3	110.8±11.6[a]	150.9±38.7[a]	136.6±45.3[a]	147.5±56.1[a]	157.6±62.3[a]	164.4±62.4[a]
ALI+Rofl	597.6±16.8	138.8±17.0	269.9±71.4	306.4±85.0[f]	328.6±83.1[f]	347.9±95.4[f]	385.5±82.2[e]

Abbreviations: PaO_2: arterial partial pressure of oxygen, OI: oxygenation index, PF ratio: PaO_2/FiO_2, P_AO_2: alveolar partial pressure of oxygen, AAG: alveolar-arterial gradient of oxygen. Statistical comparisons: for ALI vs Control: [a] $P<0.001$, [b] $P<0.01$, [c] $P<0.05$; for ALI+Rofl vs ALI: [d] $P<0.001$, [e] $P<0.01$, [f] $P<0.05$. Data are expressed as means ± SEM.

lung lavages caused a significant recruitment of inflammatory cells, particularly of neutrophils, into the lung. Activated neutrophils and alveolar macrophages induce an injury of epithelial and endothelial cells, enhance an apoptosis of epithelial cells, cause a degradation of surfactant proteins, stimulate a generation of ROS in the lung what results in a destruction of the basement membrane, alteration of alveolar-capillary permeability and final deterioration of oxygenation (Ware 2006, Grommes and Soehnlein 2011). In addition, migrating groups of neutrophils mechanically damage the alveoli and worsen an accumulation of fluid in the alveolar space (Kobr *et al.* 2010). In our study, roflumilast treatment reduced a severity of the lavage-induced lung injury, in part by diminishing the infiltration of neutrophils into the lung. Inhibited accumulation of neutrophils in the lung after treatment with PDE4 inhibitors were presented also in a model of LPS-induced lung injury (Kubo *et al.* 2012), in the airway disease models (Bundschuh *et al.* 2001) as well as in rats with a saline-lavage induced model of ALI (Häfner and Germann 2000).

Repetitive lavage by saline used for induction of ALI model in this study triggered a generation of lung edema. Pulmonary edema is one of typical pathophysiological features of ALI/ARDS which can be clearly distinguished from cardiogenic pulmonary edema (Zemans and Matthay 2004, Schmickl *et al.* 2015). The pulmonary edema in ALI originates from altered permeability of the alveolar epithelial and/or capillary barriers. Although epithelial barriers are tighter than endothelial, these two have functional interactions (Matthay *et al.* 2012). The endothelial barrier is sealed by tight and adherens junctions, both of which are targeted during acute inflammation what results in the formation of intercellular gaps with consecutive extravasation of the proteinaceous fluid (Vandenbroucke *et al.* 2008, Spindler *et al.* 2010). Treatment with intravenous roflumilast in our study effectively attenuated the formation of lung

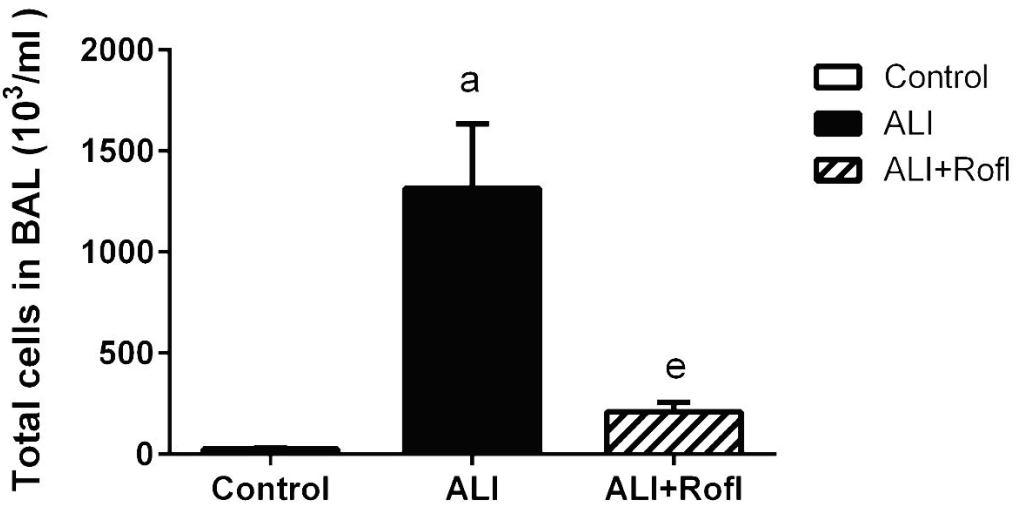

Fig. 1. Total count of cells in the BAL fluid at the end of experiment after systemic therapy of roflumilast (ALI+Rofl) in rabbit model. Statistical comparisons: for ALI vs Control: [a] P<0.001; for ALI+Rofl vs ALI: [e] P<0.01.

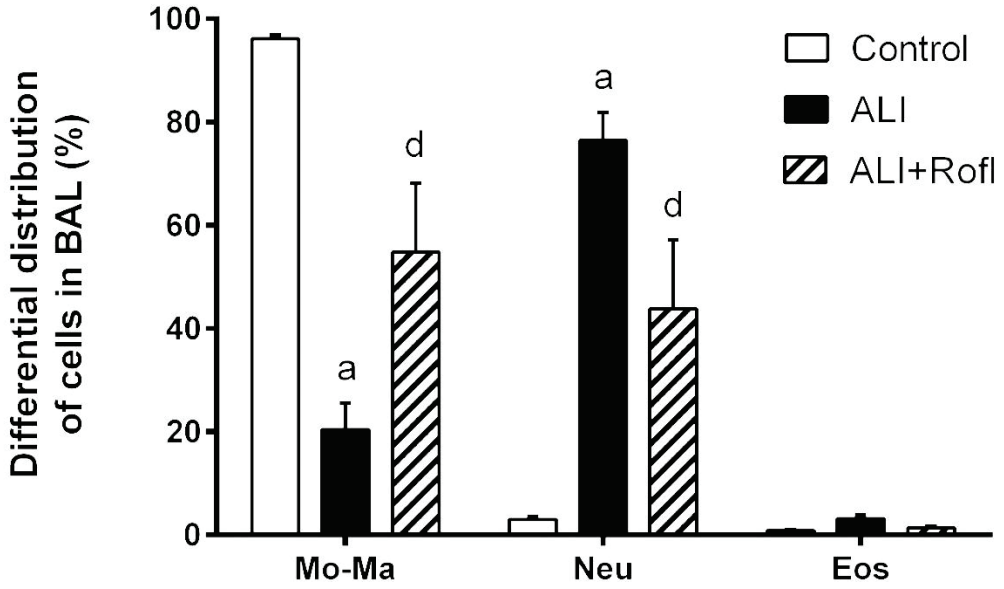

Fig. 2. Differential count (percentage) of cells in the BAL fluid at the end of experiment. Abbreviations: Mo-Ma: monocytes-macrophages, Neu: neutrophils, Eos: eosinophils. Statistical comparisons: for ALI vs Control: [a] P<0.001; for ALI+Rofl vs ALI: [d] P<0.001.

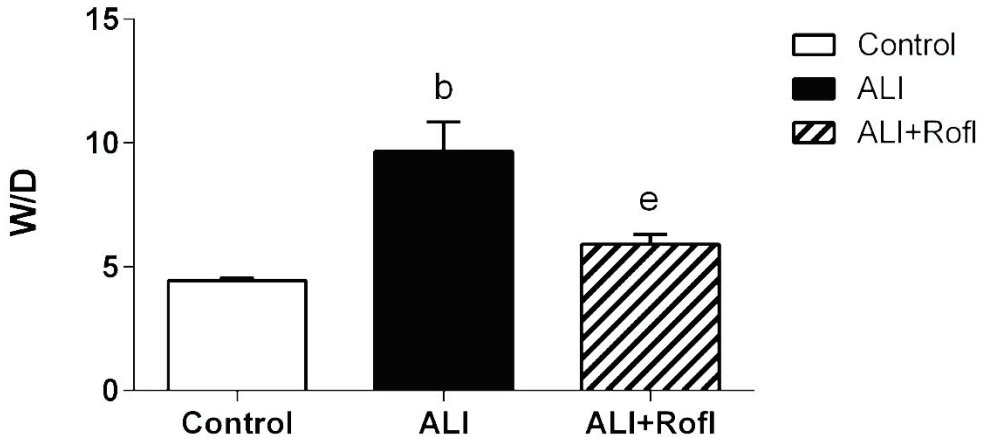

Fig. 3. Wet/dry weight ratio of the rabbits. The lobe of the right lung was excised after systemic therapy of roflumilast in rabbit model. Statistical comparisons: for ALI vs Control: [b] P<0.01; for ALI+Rofl vs ALI: [e] P<0.01.

edema expressed by wet-dry lung weight ratio. We can presume that roflumilast alleviated a lavage-induced breakdown of the endothelial barrier what was associated with reduced fluid loss and increased microcirculatory flow. Roflumilast is known to inhibit microvascular leakage and edema formation (Bundschuh *et al.* 2001), likely *via* cAMP regulation of the stability of intercellular junctions by PKA- or EPAC/Rap1-dependent activation of small GTPase Rac1 (Adamson *et al.* 1998, Birukova *et al.* 2008, Spindler *et al.* 2010). Increased cAMP levels effectively stabilize the endothelial barriers under resting conditions and in acute inflammation (Birukova *et al.* 2008, Spindler *et al.* 2010). Consistently, increased cAMP levels in the endothelial cells prevented increased endothelial permeability by inflammatory mediators *in vitro* and in single postcapillary venules *in vivo* (Adamson *et al.* 2003, Schlegel and Waschke 2009, Beckers *et al.* 2010). PDE4 inhibitors reduced the vascular leakage and deposition of alveolar fibrin or production of hyaline membranes resulting from plasma protein leakage also in a rat model of neonatal lung injury (de Visser *et al.* 2008) and in rats with saline lavage-induced model of ALI (Häfner and Germann 2000). The use of agents increasing cAMP decreased the formation of pulmonary edema in the isolated lungs of models with lung injury (Schmidt *et al.* 2008, Witzenrath *et al.* 2009), as well.

The above mentioned transmigration and activation of inflammatory cells and lung edema formation in our study resulted in a worsening of the respiratory parameters. Early after the lung lavages, the oxygenation and efficacy of ventilation decreased and these changes were relatively stable during the whole experiment in the ALI model group. Similar findings were previously presented by other researchers (Ronchi *et al*. 2012, Kamiyama *et al*. 2014). Administration of roflumilast led to significant improvement of blood gases as well as of measured respiratory parameters and calculated indexes compared to non-treated animals with ALI, whereas the changes become significant within 2 h after roflumilast delivery. We presume that the rapid improvement in oxygenation is related to a decrease in the right-to-left pulmonary shunts. Our results are in accordance with findings that PDE4 is present also in the pulmonary artery smooth muscle cells and that exposure to hypoxia increases expression of several PDE4 isoforms (Millen *et al.* 2006). From this reason, PDE4 inhibitors have been considered for perspective treatments of neonatal pulmonary hypertension (Farrow and Steinhorn 2011).

Concluding, intravenous administration of roflumilast reduced PMN migration into the lung, decreased lung edema formation and improved respiratory parameters in rabbits with repetitive saline lung lavage-induced ALI. These results indicate that PDE4 inhibitors could be of benefit also in the treatment of ALI/ARDS in patients. However, further extensive testing in the laboratory and clinical conditions is needed before this treatment can be recommended.

Conflict of Interest

There is no conflict of interest.

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