Contents lists available at ScienceDirect

Journal of Acute Disease

journal homepage: www.jadweb.org

Document heading doi: 10.1016/S2221-6189(13)60066-9

The effects of salbutamol in an experimental model with acute respiratory distress syndrome

Sema Yilmaz[1], Dinçer Yildizdas[2], Kenan Daglioglu[3], Arbil Acikalin[4], Can Acipayam[5], Ibrahim Bayram[5], Derya Gumurdulu[4], Atila Tanyeli[5]

[1]*Ondokuzmayıs University, Faculty of Medicine, Department of Pediatric, Hematology/Oncology, Samsun, Turkey*
[2]*Cukurova University Faculty of Medicine, Department of Pediatric Intensive Care, Adana, Turkey*
[3]*Cukurova University Faculty of Medicine, Department of Experimental Research and Practice Center of Medical, Adana, Turkey*
[4]*Cukurova University Faculty of Medicine, Department of Pathology, Adana, Turkey*
[5]*Cukurova University Faculty of Medicine, Department of Pediatric Hematology/Oncology, Adana, Turkey*

ARTICLE INFO

Article history:
Received 10 June 2012
Received in revised form 15 August 2012
Accepted 15 September 2012
Available online 20 November 2012

Keywords:
Acute respiratory distress syndrome
Salbutamol
Rat
Hyaline membrane
Edema
Critically ill
Hemorrhage

ABSTRACT

Objective: To investigate salbutamol effects on histopathologic features of acute respiratory distress syndrome (ARDS). **Methods:** ARDS was designed in Wistar albino male rats, 250-300 g in weight, by intratracheal instillation of physiological saline solution. Anesthezied and tracheotomized rats with ARDS were pressure-controlled ventilated. At the end of the 210 minutes, two hours past and nebulized salbutamol inhalation was tried. All rats were assigned to two groups: Group 1 (n=10) control group, given no treatment, group 2 (n=10) received salbutamol. Nebulized salbutamol inhalation was given in the dosage of 0, 15 mg/kg/dose. Rats were continued to be on ventilator through the experiment. After the last inhalation, two hours past and their both lungs were excised for histopathological examination. **Results:** Rat-model ARDS had similar histopathological appearance occuring during the acute phase of the acute respiratory distress syndrome in humans. A statistical difference was seen between control and salbutamol group (P=0.002) for HM. The margination of leukocytes was decreased in salbutamol group. The difference was significant (P<0.042). Hemorrhage and interstitial/intraalveolar edema were much lower in 0.15 mg/dose nebulized salbutamol group than that of control group. There was a significant difference statistically between two groups (P<0.001). **Conclusions:** Inhaled salbutamol therapy for ARDS is may be associated with the improvement of inflamation. Besides known effects of salbutamol, the reducing of infiltration of polymorphonuclear neutrophil leukocytes, interstitial/intraalveolar edema, perivascular and/or intraalveolar hemorrhage and hyaline membrane formation should be emphasized.

1. Introduction

The acute respiratory distress syndrome (ARDS) is characterized by diffuse inflammation of the lung's alveolar-capillary membrane in response to various pulmonary and extrapulmonary insults[1]. Pulmonary injury is occured by directly gastric aspiration, pneumonia, inhalational injury, pulmonary contusion or indirectly sepsis, trauma, pancreatitis, multiple transfusions of blood products mechanisms[2].

The pathologic features, margination of polymorphonuclear neutrophil leukocytes (PMNL) into the lung alveoli, interstitial/intraalveolar edema, perivascular and/or intraalveolar hemorrhage and hyaline membrane (HM) formation are consistent with the effects of the complex interaction of inflammatory mediators on alveolar epithelial and capillary endothelial cells[3]. ARDS are classified at different time as acute, subacute and chronic phases. In the acute phase (the first 1-6 d), there is evidence of interstitial and alveolar edema with accumulation of

*Corresponding author: Sema Yilmaz, MD, Ondokuzmayıs University, Faculty of Medicine, Pediatric Hematology/Oncology, Samsun/Turkey.
Tel: 0362 457 6070
Fax: 0362 457 6041
This work was supported by the Cukurova University Research (TF2011BAP20) Fund.

neutrophils, macrophages, and red blood cells in the alveoli. Prominent hyaline membranes is occured in the alveoli as well. In the subacute phase (the next 7-14 d), some of the edema has usually been reabsorbed, In the chronic phase (after 14 d), acute neutrophilic infiltration resolve with more mononuclear cells and alveolar macrophages in the alveoli[4].

The current treatment modalities and new therapeutic approaches primarily focused on the resolution of pathologic features in ARDS. These treatments include glucocorticoids, surfactants, inhaled nitric oxide, antioxidants, protease inhibitors, and a variety of other antiinflammatory treatments. Unfortunately, to date none of these pharmacologic treatments has proven to be effective[5]. The first step toward improving of ARDS is to remove the alveolar edema fluid to the lung interstitium, where net clearance can occur through lung lymphatics and the pulmonary microcirculation. The resolution of edema was reported in an animal models with ALI[6,7]. Perkins et al demonstrated that intravenous salbutamol, a selective β_2 adrenergic agonists, may reduce alveolar-capillary permeability, accelerate resolution of pulmonary edema and improve outcome in patients with ARDS[8]. Alveolar fluid clearance and arterial oxygen tension due to hydrostatic pulmonary edema were increased by aerosolized salmeterol[9,10].

The migration of large numbers of neutrophils can result in epithelial injury. β_2 adrenergic agonists reduce *in vitro* neutrophil adhesion to human alveolar epithelial cells[11] and endothelial cells[12-14]. Masclans JR *et al* reported that pulmonary sequestration of radio-labelled neutrophils was prevented by the treatment of 300 μg inhaled salbutamol in normal human volunteers[15]. Also salbutamol is a potent upregulator for repairing of human alveolar endothelial and epithelial cells[16]. In this article, we aimed to evaluate the effect of salbutamol inhalation on lung pathology in an experimental ARDS model.

2. Methods

2.1. Animals

Wistar albino male rats, weighing 250-300 g, were obtained from the Experimental Animal Research Center, Cukurova University Medical Faculty. The animals were kept in a temperature (21 ± 2) ℃ and humidity (60% ± 5%) controlled room in which a 12-12 h light-dark cycle was maintained. Animals were fed a standard rat chow diet, had access to water ad libitum, and were synchronized by the maintenance of controlled environmental conditions (light, temperature, feeding time, etc.) The experiments were performed in accordance with the guidelines for Animal Research from the National Institute of Health and were approved by the Committee on Animal Research at Cukurova University, Turkey.

2.2. Experimental design

Rats were anesthezied with 1.25 mg/kg body weight xylazine (Rompum, Bayer, Brazil, 2% solution) and 80 mg/kg body weight ketamine (Ketalar, Pfizer, USA, 50 mg/mL) intraperitoneally and instrumented in a manner previously described by German and Häfner et al[17,18] A catheter was placed into one carotid artery for blood gas parameters, partial arterial oxygen pressure (PaO_2) and partial arterial carbon dioxide pressure ($PaCO_2$). A tracheostomy was performed, and the trachea was canullated with polyethylene tubing, ID 1, 5 mm. They were pressure-controlled ventilated (Evita 4 Neoflow, Dräger, Germany) with 100% oxygen at a respiratory rate of 30 breaths/min, inspiration-expiration ratio of 1:2, peak inspiratory pressure of 15 cmH_2O at positive end-expiratory pressure (PEEP) of 2 cmH_2O. At the starting of the experiment, PaO_2 and $PaCO_2$ were evaluated under the described ventilatory settings. Before lavage, the peak inspiration pressure (PIP) was raised to 28 cmH_2O and PEEP to 8 cmH_2O. Rat lung parenchyma lavage was applied with 5 mL×6 mL of physiological saline solution per animal every half an hour through 210 min. The ventilation setting was not changed during the whole experimental period.

All rats with ARDS were assigned to two groups: Group 1 (n=10) control group, given no treatment, group 2 (n=10) received inhalation of 0.15 mg/kg/dose salbutamol (Ventolin®, Glaxo Smithkline, Boronia, Au, 2.5 mg/2.5 mL solution for nebulization) diluted in 4 mL of saline (0.9% NaCl). At two hours after the last lavage, control group rats were sacrified. Salbutamol was given four times during a 1 h period with a vibrating mesh nebulizer (Aeroneb Solo, Aerogen) placed on the ventilator. Physiological saline was added so that the inhalation time was 5 min. After the last inhalation, two hours past and rats were sacrified. Both lungs of all rats were excised for histopathological examination.

2.3. Light microscopic examination

Lung tissues were fixed in 10% neutral buffered formalin and was embedded in paraffin. Sections of tissue were cut at 5 μm, mounted on slides, stained with hematoxylin-eosin (H-E). The sections were examined by Olympus conventional CX21 light microscope. Grading was performed with respect to the severity of the pathological features [the margination and infiltration of polymorphonuclear neutrophil leukocytes (PMNL), interstitial/intraalveolar edema, perivascular and/

or intraalveolar hemorrhage and hyaline membrane (HM) formation] as grade 0 (clear lung paranchyma), grade 1 (25% of lung paranchyma), grade 2 (50% of lung paranchyma), grade 3 (75% of lung paranchyma) and grade 4 (100% of lung paranchyma)[17] (Table 2). Slides were coded and evaluated without any knowledge of the sacrifice time.

2.4. Electron microscopic examination

The tissues of 1 mm^3 thickness were immediately placed in 5% glutaraldehyde buffered at pH 7.4 with Millonig phosphate buffer for four hours. Samples were subsequently fixed in 1% osmic acid for two hours. After dehydration in acetone, they were embedded in araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and were examined in a Jeol JEM 1400 electron microscope.

2.5. Statistical analysis

A computer program (SPSS 11.0) was used for statistical analysis. Histopathological grading were expressed as means ± standard deviation (SD). While differences among the groups were detected, group means were compared with the using of the Mann-Whitney *U*-test. A *P*-value <0.05 was considered significant.

3. Results

At the starting of the experiment, the mean blood PaO_2 and $PaCO_2$ values were (350.34±75.69) and (52.16±5.89) mmHg before lavage, respectively. Directly after lavage (210 min after lavage), PaO_2 decreased to (57.55±12.10) mmHg and $PaCO_2$ increased to (73.27±5.60) mmHg (Table 1). The light microscopic examination showed that the histopathological variables of control group like hyaline membrane formation, margination of polymorphonuclear neutrophil leukocytes, interstitial/intraalveolar edema and perivascular and/or intraalveolar hemorrhage reached values similar to those of humans with the acute phase of ARDS (Figure 1 A,B,C,D). The grading of histopathological variables, median and means ± standard deviation (SD) values were presented for control and salbutamol group in Table 2.

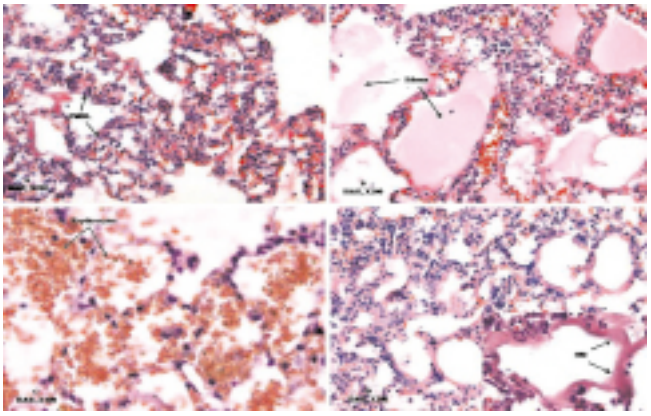

Figure 1. Light misroscopic examination of control group: A. Infiltration of polymorphonuclear neutrophil leukocytes into the lung alveoli (H&E×200). B. Interstitial and intraalveolar edema (H&E×400). C. Perivascular and/or intraalveolar hemorrhage (H&E×400). D. Hyaline membrane formation (H&E×200).

Table 1
Arterial blood gas parameters of control group.

	Before starting of the experiment (PaO_2 / $PaCO_2$)	210 min after lavage (PaO_2 / $PaCO_2$)
Minimum	255.40 / 40.80	35.40 / 62.70
Maximum	515.80 / 59.60	71.20 / 78.90
Mean±Std.Deviation	350.34±75.69 / 52.16±5.89	57.55±12.10 / 73.27±5.60

Table 2
Median, Mean±SD values and scores of Hyaline membrane (HM) formation, margination and infiltration of polymorphonuclear neutrophil leukocytes (PMNL) into the lung alveoli, interstitial/intraalveolar edema and perivascular and/or intraalveolar hemorrhage: Grade 0 (clear lung paranchyma), grade 1 (25% of lung paranchyma), grade 2 (50% of lung paranchyma), grade 3 (75% of lung paranchyma) and grade 4 (100% of lung paranchyma) in both lung.

Groups	Grading					*n*	Median (range)	Mean±SD	*P* value
	0(right/left)	1(right/left)	2(right/left)	3(right/left)	4(right/left)				
Group 1									
HM	–/–	–/–	2/3	5/3	3/4	10/10	3.0	3.1±0.7	
PMNL	–/–	–/–	2/1	6/7	2/2	10/10	3.0	3.0±0.6	
Edema	–/–	–/–	0/1	8/4	2/5	10/10	3.0	3.3±0.5	
Hemorrhage	–/–	–/–	–/–	4/5	6/5	10/10	4.0	3.5±0.5	
Group 2									
HM	1/1	4/3	2/2	2/3	1/1	10/10	2.0	1.9±1.2	0.002
PMNL	–/–	1/1	4/3	5/5	0/1	10/10	3.0	2.5±0.8	0.042
Edema	2/3	6/4	0/3	1/0	1/0	10/10	1.0	1.1±1.0	<0.001
Hemorrhage	–/–	8/7	2/3	0/0	0/0	10/10	1.0	1.2±0.4	<0.001

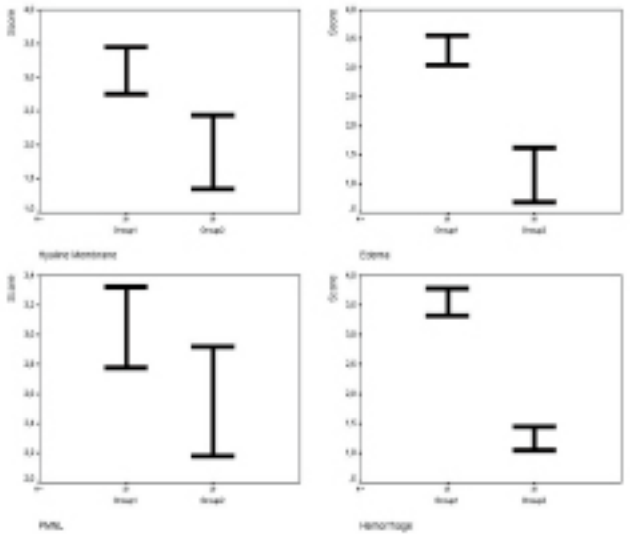

Figure 2. The comparision with control and salbutamol group for hyaline membrane formation, interstitial/intraalveolar edema, margination of polymorphonuclear neutrophil leukocytes (PMNL) into the lung alveoli and perivascular and/or intraalveolar hemorrhage.

The mean severity of margination of PMNL, interstitial/intraalveolar edema and perivascular and/or intraalveolar hemorrhage were (2.5 ± 0.8), (1.1 ± 1.0) and (1.2 ± 0.4) in group 2, respectively. The margination of PMNL was decreased in salbutamol group. The difference was significant (P=0.042). There was an apperant amelioration for interstitial/intraalveolar edema and perivascular and/or intraalveolar hemorrhage in nebulized salbutamol group. Hemorrhage and interstitial/intraalveolar edema was much lower in nebulized salbutamol group than that of control group (Figure 2). The difference was statistically significant (P<0.001). The mean severity of hyaline membrane formation was decreased from 3.1 ± 0.7 to 1.9 ± 1.2 in 0.15 mg/kg in salbutamol group. We found a statistical difference between control and salbutamol group (P=0.002) for HM. The light and electron microscopic examination of salbutamol and control group were shown in Figure 3 and 4, respectively.

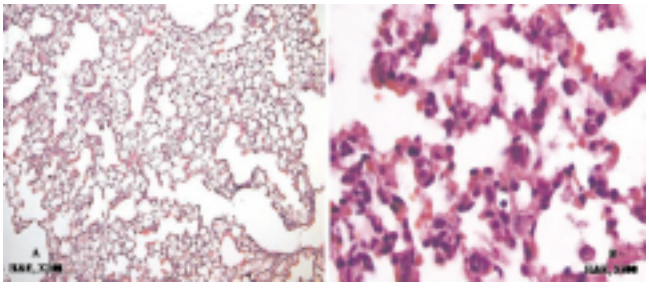

Figure 3. Light misroscopic examination of salbutamol group: A (H&E×100) and B (H&E×400). The decreasing of hyaline membrane formation, interstitial and intraalveolar edema, infiltration of polymorphonuclear neutrophil leukocytes into the lung alveoli and perivascular and/or intraalveolar hemorrhage were depicted.

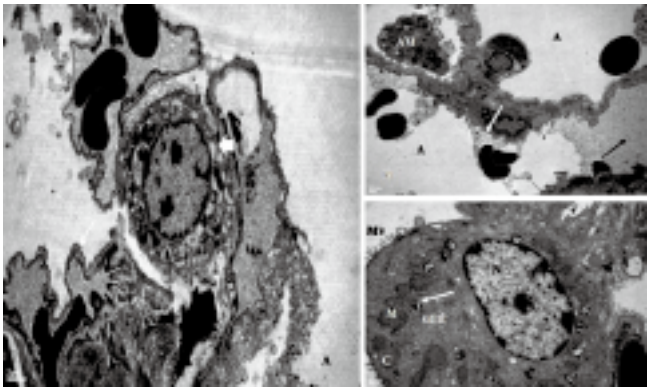

Figure 4. EM examination. A. Control group: Granular dens stractures (black arrow). Thick basal lamina on blood-air barrier area (white arrow) and increased intracytoplasmic vacualization of type II pneumocyte (thick white arrow). Salbutamol group: B. Destroyed alveolar wall integrity (white arrow), erythrocytes in alveolar space (A), dense granules (black arrow), alveolar macrophage (AM) next to the alveolar wall. C. A normal Type II pneumocyte. Microvilli (Mv), secretory vesicules (white arrow), mitocondrium (M), granular endoplasmic reticulum (GER) and nucleus were seen.

4. Discussion

The potential benefits of β-adrenergic stimulation in acute lung injury include epithelial protection, decreased neutrophil chemotaxis and activation, lower proinflammatory cytokine production, increased surfactant secretion, improved respiratory mechanics, and increased alveolar fluid clearance[19-21]. The present study sought to identify whether salbutamol was having an effect on the lung pathology of ARDS included infiltration of polymorphonuclear neutrophil leukocytes into the lung alveoli, interstitial/intraalveolar edema, perivascular and/or intraalveolar hemorrhage and hyaline membrane formation.

It is well documented that increased neutrophil accumulation and inflammation caused to alveolar-capillary permeability in the alveolar space. Data from previous clinical studies by using of salbutamol was attributable to prevention of leukocyte infiltration and chemotaxis[15,20]. Although the infiltration of polymorphonuclear neutrophil leukocytes into the lung alveoli was slightly decreassed after using of salbutamol in our study, the difference was significant between the salbutamol and control group. This result was compatible with previous literatures. Nebulized salbutamol inhalation in ARDS that had possible anti-inflammatory effects was addressed in former studies. Roca et al pointed that salbutamol inhalation might play a role in preventing the lipid peroxidation that occurs in ALI and ARDS patients[24]. Also different author clarified that salbutamol was a potent upregulator for repairing of human alveolar endothelial and epithelial cells[16]. In the present study, we could not examined the anti-inflammatory effect of salbutamol by proinflammatory cytokines investigation

because of our laboratory limitation.

Previous studies stated that injured epithelial alveolar barrier in ARDS causes to intraalveolar edema. Besides known effects of salbutamol, β_2 agonists have a important role in alveolar fluid clearance[4]. Decreased interstitial and intraalveolar edema were seen in our study. There was distinctive reduction of edema in the intralveolar area compare to the group given any treatment. Nebulized salbutamol could improve alveolar fluid clearance without adversely impacting systemic hemodynamics[19,23]. In Perkin's study, reduced alveolar-capillary permeability was shown after using of intravenous salbutamol in patients with ARDS[8]. Our histopathological findings were complying with recent literatures.

Intraalveolar hemorrhage may have a positive impact on inflammation in ARDS and may have clinical implications[8]. We found that perivascular and/or intraalveolar hemorrhage after administration of 0.15 mg/kg/dose of nebulized salbutamol were considerably lower than those control group. Therefore the optimal treatment effect of 0.15 mg/kg/dose nebulized salbutamol inhalation in four cycle with 15 min breaks could be helpful to decrease the hemorrhage of lung paranchyma during the management of ARDS in the critical care medicine. Even though the exact mechanism of decreased perivascular and/or intraalveolar hemorrhage could not be explain clearly in the present study, it might be attributable to reduced of alveolar-capillary permeability occured by salbutamol. In this setting, these data support the need for controlled clinical trials of the efficacy of inhaled nebulized salbutamol therapy in critically ill patients with ARDS.

Decreased surfactant production followed by diffuse epithelial damaged of alveoli resulted in hyaline membrane formation. We found that rats inhaled with salbutamol had less hyaline membrane formation than that of control group. Surfactant, a complex of lipids and proteins, reduces alveolar surface tension, has antibacterial properties, and prevents pulmonary edema formation. In one study, it was shown that the beta agonists, terbutaline and salmeterol, increased surfactant secretion by adult and fetal type II cells[22]. The mechanism of salbutamol on surfactant secretion can be attributed to its anti-inflammatory properties on the alveolar epithelium.

It is well known that nebulized salbutamol treatment is standard for asthma exacerbation in hospital emergency wards, and a dosage regimen of 0.15 mg/kg is widely used and recommended[25]. Nebulized salbutamol dose with 0.15 mg/body weight (kg) was decided based on describing in accordance with the Expert Panel Report 3[26]. We used a standard dose of 0.15 mg/kg/dose of nebulized salbutamol in every 15 min through one hour. Di Berardino et al presented that the duration of salbutamol inhalation as 30 min which is widely accepted for nebulization was found more useful for the management of patients with asthma[27]. Although no previous studies stated the duration of salbutamol inhalation in ARDS, we demonstrated that the histopathological findings were getting better with the duration of salbutamol inhalation mentioned above.

Our study had some limitations. First, respiratory mechanics could not be studied. Despite this, the aim of our study was primarily to assess the salbutamol effect with histopathologic examination in rats with ARDS. Second, as mention above, anti-inflammatory cytokin study could not be performed because of our insufficient laboratory facilities.

Besides known effects of salbutamol, it should be emphasized that nebulized salbutamol inhalation can be effective in the reducing of polymorphonuclear neutrophil leukocytes infiltration into the lung alveoli, interstitial and intraalveolar edema, perivascular and/or intraalveolar hemorrhage and hyaline membrane formation in ARDS. The present study supported recent suggestions that inhaled salbutamol therapy could be associated with a more rapid improvement of inflamation in ARDS. Therefore salbutamol could potentially increase the quality of life in terms of decreasing the duration of mechanical ventilation and hospitalization in critically ill patients with ARDS.

Conflict of interest statement

The authors have no conflicts of interest.

Acknowledgements

This work was supported by the Cukurova University Research (TF2011BAP20) Fund. Authors thank Kezban Bostaner and Recep Mutlu for their technical assistance.

Authors' contributions

SY coordinated and carried out all the experiments design, participated in all of the experiments and prepared the manuscript. DY provided the assistance in the design of the study. AA and DG examined and scored all pathological slides. CA and KD provided the assistance for all experiments. IB and AT participated in manuscript preparation. All authors have read and approved the content of the manuscript.

References

[1] Ware LB, Matthay MA. The acute respiratory distress syndrome. *N Engl J Med* 2000; **342**(18): 1334-1349.

[2] Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, et al. Report of the American-European consensus conference on ARDS: definitions, mechanisms, relevant outcomes and clinical trial coordination. The Consensus Committee. *Intense Care Med* 1994; **20**(3): 225-232.

[3] Tomashefski JF Jr. Pulmonary pathology of acute respiratory distress syndrome. *Clin Chest Med* 2000; **21**(3): 435-466.

[4] Matthay MA, Zemans RL. The acute respiratory distress syndrome: pathogenesis and treatment. *Annu Rev Pathol* 2011; **6**: 147-163.

[5] Cepkova M, Matthay MA. Pharmacotherapy of acute lung injury and the acute respiratory distress syndrome. *J Intensive Care Med* 2006; **21**(3): 119-143.

[6] Berthiaume Y, Folkesson HG, Matthay MA. Lung edema clearance: 20 years of progress: invited review: alveolar edema fluid clearance in the injured lung. *J Appl Physiol* 2002; **93**(6): 2207-2213.

[7] Matthay MA, Clerici C, Saumon G. Invited review: active fluid clearance from the distal air spaces of the lung. *J Appl Physiol* 2002; **93**(4): 1533-1541.

[8] Perkins GD, Gao F, Thickett DR. *In vivo* and *in vitro* effects of salbutamol on alveolar epithelial repair in acute lung injury. *Thorax* 2008; **63**(3): 215-220.

[9] Frank JA, Wang Y, Osorio O, Matthay MA. Beta-adrenergic agonist therapy accelerates the resolution of hydrostatic pulmonary edema in sheep and rats. *J Appl Physiol* 2000; **89**(4): 1255-1265.

[10] Sartori C, Allemann Y, Duplain H, Lepori M, Egli M, Lipp E. Salmeterol for the prevention of high-altitude pulmonary edema. *N Engl J Med* 2002; **346**(21): 1631-1636.

[11] Bloemen PG, van den Tweel MC, Henricks PA, Engels F, Kester MH, van de Loo PG. Increased cAMP levels in stimulated neutrophils inhibit their adhesion to human bronchial epithelial cells. *Am J Physiol* 1997; **272**(4): 580-587.

[12] Blease K, Burke-Gaffney A, Hellewell PG. Modulation of cell adhesion molecule expression and function on human lung microvascular endothelial cells by inhibition of phosphodiesterases 3 and 4. *Br J Pharmacol* 1998; **124**(1): 229-237.

[13] Derian CK, Santulli RJ, Rao PE, Solomom HF, Barret JA. Inhibition of chemotactic peptide-induced neutrophil adhesion to vascular endothelium by cAMP modulators. *J Immunol* 1995; **154**(1): 308-317.

[14] Perkins GD, Nathani N, McAuley DF, Gao F, Thickett DR. *In vitro* and *in vivo* effects of salbutamol on neutrophil function in acute lung injury. *Thorax* 2007; **62**(1): 36-42.

[15] Masclans JR, Barbera JA, MacNee W, Pavia J, Piara C, Lomena F. Salbutamol reduces pulmonary neutrophil sequestration of platelet-activating factor in humans. *Am J Respir Crit Care Med* 1996; **154**(2): 529-532.

[16] Spurzem JR, Gupta J, Veys T, Kneifl KR, Rennard SI, Wyatt TA. Activation of protein kinase A accelerates bovine bronchial epithelial cell migration. *Am J Physiol Lung Cell Mol Physiol* 2002; **282**(5): 1108-1116.

[17] Germann PG, Häfner D. A rat model of acute respiratory distress syndrome (ARDS): Part 1. Time dependency of histological and pathological changes. *J Pharmacol Toxicol Methods* 1998; **40**(2): 101-107.

[18] Häfner D, Germann PG: A rat model of acute respiratory distress syndrome (ARDS) Part 2, influence of lavage volume, lavage repetition, and therapeutic treatment with rSP-C surfactant. *J Pharmacol Toxicol Methods* 1999; **41**(2-3): 97-106.

[19] Groshaus HE, Manocha S, Walley KR, Russell JA: Mechanisms of beta-receptor stimulation-induced improvement of acute lung injury and pulmonary edema. *Crit Care* 2004; **8**(4): 234-242.

[20] Perkins GD, McAuley DF, Richter A, Thickett DR, Gao F. Benchto- bedside review: β_2-agonists and the acute respiratory distress syndrome. *Crit Care* 2004; **8**(1): 25-32.

[21] Perkins GD, McAuley DF, Thickett DR, Gao F. The β-Agonist Lung Injury Trial (BALTI): a randomized placebo-controlled clinical trial. *Am J Respir Crit Care Med* 2006; **173**(3): 281-287.

[22] Kumar VH, Christian C, Kresch MJ. Effects of salmeterol on secretion of phosphatidylcholine by alveolar type II cells. *Life Sci* 2000; **66**(17): 1639-1646.

[23] Atabai K, Ware LB, Snider ME, Koch P, Daniel B, Nuckton TJ, et al. Aerosolized β(2)-adrenergic agonists achieve therapeutic levels in the pulmonary edema fluid of ventilated patients with acute respiratory failure. *Intensive Care Med* 2002; **28**(6): 705-711.

[24] Roca O, Gómez-Ollés S, Cruz MJ, Muñoz X, Griffiths MJ, Masclans JR. Effects of salbutamol on exhaled breath condensate biomarkers in acute lung injury: prospective analysis. *Crit Care* 2008; **12**(3): R72.

[25] The Nebulizer Project Group of the British Thoracic Society Standards of Care Committee: Current best practice for nebulizer treatment. *Thorax* 1997; **52**(9): 838.

[26] National Heart, Lung, and Blood Institute. National Asthma Education and Prevention Program. Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma. *NIH Publication* 2007; **07**: 4051.

[27] Di Berardino F, Forti S, Piatti G, Fasano V. A comparative study of two different metered-dose inhaler-valved holding chambers in the administration of salbutamol. *Chest* 2010; **137**(2): 502-503.