The Effect of Budesonide Delivered by High-Frequency Oscillatory Ventilation on Acute Inflammatory Response in Severe Lung Injury in Adult Rabbits

Nikolett NEMCOVA[1], Petra KOSUTOVA[2], Maros KOLOMAZNIK[2], Stanislav MATEFFY[3], Zuzana TURIANIKOVA[2], Andrea CALKOVSKA[1], Pavol MIKOLKA[1,2]

[1]Department of Physiology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovak Republic, [2]Biomedical Centre Martin, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovak Republic, [3]Diagnostic Center of Pathology in Presov, Unilabs Slovakia, s.r.o., Martin, Slovak Republic

Received March 30, 2023
Accepted September 15, 2023

Summary

The inflammation present in acute respiratory distress syndrome (ARDS) and thereby associated injury to the alveolar-capillary membrane and pulmonary surfactant can potentiate respiratory failure. Even considering the high mortality rate of severe ARDS, glucocorticoids appear to be a reasonable treatment option along with an appropriate route of delivery to the distal lung. This study aimed to investigate the effect of budesonide therapy delivered intratracheally by high-frequency oscillatory ventilation (HFOV) on lung function and inflammation in severe ARDS. Adult New Zealand rabbits with respiratory failure (P/F<13.3 kPa) induced by intratracheal instillation of hydrochloric acid (HCl, 3 ml/kg, pH 1.5) followed by high tidal ventilation (V_T 20 ml/kg) to mimic ventilator-induced lung injury (VILI) were treated with intratracheal bolus of budesonide (0.25 mg/kg, Pulmicort) delivered by HFOV (frequency 8 Hz, MAP 1 kPa, ΔP 0.9 kPa). Saline instead of HCl without VILI with HFOV delivered air bolus instead of therapy served as healthy control. All animals were subjected to lung-protective ventilation for 4 h, and respiratory parameters were monitored regularly. *Postmortem*, lung injury, wet-to-dry weight ratio, leukocyte shifts, and levels of cytokines in plasma and lung were evaluated. Budesonide therapy improved the lung function (P/F ratio, oxygenation index, and compliance), decreased the cytokine levels, reduced lung edema and neutrophils influx into the lung, and improved lung architecture in interstitial congestion, hyaline membrane, and atelectasis formation compared to untreated animals. This study indicates that HFOV delivered budesonide effectively ameliorated respiratory function, and attenuated acid-induced lung injury in a rabbit model of severe ARDS.

Key words

Budesonide • High-frequency oscillatory ventilation • ARDS model • Lung function • Inflammation

Corresponding author

P. Mikolka, Department of Physiology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Mala Hora 4C, 03601 Martin, Slovak Republic. E-mail: pavol.mikolka@uniba.sk

Introduction

Acute respiratory distress syndrome (ARDS) is a common clinical syndrome of acute respiratory failure because of diffuse lung inflammation and edema, characterized by the acute onset of bilateral alveolar opacities and hypoxemia. ARDS is associated with significant mortality, with an overall in-hospital death rate of approximately 40 percent [1]. ARDS can be triggered by a variety of causes including both infectious and non-infectious triggers (e.g. pneumonia, pancreatitis, aspiration of gastric contents, non-pulmonary sepsis, hemorrhage, and severe traumatic injuries with shock and multiple transfusions) [2,3]. These triggers can injure the lung directly due to local inflammation, or indirectly because of systemic inflammatory and injury mediators. Rapid onset, histological evidence of tissue injury, altered alveolar capillary barrier, inflammatory response, and signs of physiological dysfunction are the major characteristics of experimental ARDS in animals [4].

Management of ARDS involves addressing the

underlying causes of the lung injury and providing supportive care and measures to optimize oxygenation and gas exchange, including mechanical ventilation, fluid-restrictive strategies, and prone positioning, to prevent iatrogenic lung injury [5]. Due to the high mortality rate of severe ARDS, additional therapeutic strategies are required. Despite intensive research, drugs such as glucocorticoids (GCs), surfactants, inhaled nitric oxide, antioxidants, protease inhibitors, and other anti-inflammatory drugs have been investigated with conflicting results of their therapeutic effects [6]. In general, GCs are not currently the recommended treatment for ARDS. However, during over-inflammation in ARDS, the use of GC appears to provide a benefit by reducing excessive inflammation and the associated tissue injury. GCs counteract lung injury by improving epithelial permeability, reducing edema, inhibiting local, and systemic inflammation, and reducing apoptosis [7,8]. Budesonide is a non-halogenated GC with potent anti-inflammatory effects, induces vasoconstriction, inhibits mucosal edema, reduces cellular exudation, and remodels airway tissue [9,10], commonly used to treat asthma and many other lung diseases [11]. Furthermore, treatment with budesonide results in satisfactory pulmonary protective effects in the treatment of ARDS in clinical [12-14] and experimental studies [15-17].

The efficacy of a treatment mostly depends on the techniques by which the drug is delivered. Pulmonary delivery has become an attractive but equally challenging method of local drug delivery [18]. High-frequency oscillatory ventilation (HFOV) is a type of mechanical ventilation delivered small rapid breaths of gas to the lung at a very high frequency that is used to support breathing in critically ill patients with acute respiratory failure. Several studies have investigated the use of HFOV for drug delivery [19-21]. HFOV exhibits a unique characteristic of local particle deposition due to the rapid ventilation process and the strong influence of the endotracheal tube jet [22]. In addition, during HFOV, the complexity of aerosolized drug delivery is significantly increased because of high frequency and low tidal volumes [23,24].

The heterogeneous nature and uneven injury to the lung parts of ARDS requires a proper discussion not only about the suitability of dosing regimens, timing, and duration of GCs therapy, but also about the possibilities of drug delivery to the lung. Therefore, we hypothesized that the budesonide delivered intratracheally by HFOV improves lung function and architecture, and attenuates inflammation in an animal model of severe ARDS.

Material and Methods

Animal instrumentation

This study was approved by the National Veterinary Board of Slovakia and the local Ethics Committee of the Jessenius Faculty of Medicine, Comenius University. Adult New Zealand white male rabbits with body weight (b.w.) of 2.5 (0.3) kg were handled according to the Federation of European Laboratory Animal Science Associations (FELASA) standards and instrumented in accordance with our previous study [25]. Randomization was performed at the beginning of the experiment. After initial intramuscular sedation with tiletamine and zolazepam (15 mg/kg b.w.; Zoletil, Virbac, France) and xylazine (5 mg/kg b.w.; Xylariem, Riemser, Germany), the animals were placed on 37 °C controlled heating surgical table in a supine position prior to the surgical procedures. The left and right marginal ear veins and right femoral artery were cannulated for continuous intravenous (i.v.) infusion of tiletamine and zolazepam (10 mg/kg/h), Ringer's acetate solution (10 ml/kg/h), arterial blood sampling and arterial pressure monitoring. Tracheotomy was performed and an endotracheal tube was inserted. All animals were paralyzed with atracurium besylate i.v. (0.7 mg/kg/h; Tracrium, Aspen Pharma, Ireland) and were mechanically ventilated (Aura V, Chirana, Slovak Republic) with positive end-expiratory pressure (PEEP) of 0.5 kPa, tidal volume (VT) 6-8 ml/kg, inspiration expiration rate (I:E) 1:2, respiratory rate (RR) 40 breaths per minute (bpm) and inspiratory oxygen fraction FiO_2 of 1.0 throughout the experiment. Finally, 4 h after the treatment administration, the animals were euthanized under deep anesthesia by injection of a lethal dose of potassium chloride i.v. Electrocardiographic monitoring with subcutaneous electrodes and invasive arterial pressure monitoring were performed continuously using a PowerLab 8/30 multichannel recorder (AD Instruments, Germany). Gas exchange and acid-base balance parameters were measured from arterial blood samples using a blood gas analyzer (RapidLab TM348, Bayer Diagnostics, Germany). Ventilation parameters, e.g. plateau airway pressure (Paw), static lung-thorax compliance (C_{stat}), dynamic lung-thorax compliance (C_{dyn}), mean alveolar pressure (MAP), and positive end-expiratory pressure (PEEP), were measured by in-build sensors and Aura V ventilator software. The following

parameters were calculated: P/F = a ratio between arterial oxygen partial pressure (pO_2) and a fraction of inspired oxygen (FiO_2); oxygenation index (OI) = (mean airway pressure × FiO_2)/pO_2; alveolar-arterial oxygen gradient (AaG) = [FiO_2 (Patm − PH_2O) − pCO_2 / 0.8] − pO_2, where Patm is barometric pressure and PH_2O is the pressure of water vapor; and ventilation efficacy index (VEI) = 3800/(peak inspiratory pressure (PIP)-PEEP) × RR × pCO_2.

The experimental model of ARDS

A two-hit experimental model of severe ARDS was induced by a combination of acid aspiration followed by high-tidal volume injurious ventilation. First insult, bolus of hydrochloric acid (HCl, 3 ml/kg b.w., pH 1.5) was intratracheally instilled in the left and right lateral positions of the animal with stabilization period for 15 min. Subsequently, the lungs were ventilated with high tidal volumes (HVT) with target VT 20 ml/kg, zero PEEP, RR 20-30 bpm, I:E 1:2, and FiO_2 1.0 to mimic ventilator-induced lung injury (VILI). Hypocapnia was accepted without additional reduction of RR. Arterial blood gases were analyzed every 15 min during HVT ventilation until P/F ratio decreased to ≤13.3 kPa, equal to P/F ≤100 mm Hg, which corresponds to severe ARDS according to the Berlin definition [3]. The standard HVT ventilation time of 30 min was extended by 15 min in 3 animals to meet the defined criteria for severe ARDS. Saline (3 ml/kg b.w.) was instilled instead of HCl and no HVT ventilation was applied, in addition air bolus instead of budesonide was delivered by HFOV in the Saline group (n=9).

Treatment protocol

After fulfilling the lung injury criteria, the animals were assigned to the following groups (n=9 in each): (1) Model group, animals with severe ARDS without treatment; (2) Model+Bud group, ARDS animals with budesonide treatment. Glucocorticoid budesonide (Pulmicort susp inh, AstraZeneca, Great Britain) was administered intratracheally as a bolus (0.25 mg/kg) using a syringe connected to a catheter inserted into the trachea above the carine in the supine position during high-frequency oscillatory ventilation (HFOV). The therapeutic protocol consisted of a 1 min budesonide instillation and a 1 min stabilization period before and after the administration of the therapy. HFOV was set to a frequency 8 Hz, MAP 1 kPa, ΔP 0.9 kPa, and I:E 1:2. Afterwards, animals were subjected to lung-protective ventilation for an additional 4 h (VT 6-8 ml/kg, PEEP 0.5 kPa, RR 40 bpm, I:E 1:2, and FiO_2 1.0). In Model group for five animals, PEEP was increased up to maximum 1 kPa in cases where oxygen saturation (SaO_2) fell below 87 %. PEEP was gradually increased every 30 min in increments of 0.1 kPa to reach the minimum required level. For the rest of the animals, a fixed PEEP of 0.5 kPa was sufficient throughout the experiment. Post-treatment physiological data, such as blood gases and respiratory parameters, were recorded at 30, 60, 120, 180, and 240 min after the administration of the therapy. All 27 animals survived the entire protocol.

Post-mortem analyses

Blood samples from arteria were taken at the end of the experimental protocol. Plasma was obtained by centrifugation (3000 rpm for 15 min, 4 °C). The thorax was opened by a sternotomy and the lung were removed with the clumped trachea at the carina level. The left lung was lavaged twice with saline (10 ml/kg b.w.) to obtain the bronchoalveolar lavage fluid (BALF). In BALF, total and differential white blood cells (WBC) were estimated using the Sysmex XT-2000i veterinary hematology analyzer (Sysmex, Sweden).

Tissue samples from the right lung were used to assess the wet-to-dry (W/D) lung weight ratio. Lung strips from apical, medial, and caudal areas were weighed before and after drying in an oven at 60 °C for 48 h to calculate the W/D ratio, and the extent of lung edema.

A tissue sample from the caudal medial right lung was fixed in 10 % buffered formalin for subsequent histological analysis. Formalin-fixed lung samples were embedded in a paraffin, sectioned, and stained with hematoxylin and eosin. Histological analysis was performed blindly by a veterinary pathologist (SM) and a scoring system was used to determine the lung injury as described previously [4]. Neutrophil infiltration, interstitial congestion, and the hyaline membrane were scored according to: 1 – normal lung, 2 – moderate, 3 – intermediate, 4 – severe; perivascular edema, emphysema: 0 – absent, 1 – mild-moderate, 2 – moderate-severe, 3 – severe; hemorrhage, atelectasis: 0 – absent, 1 – present. The sum of scores was used to assess the total lung injury score.

Plasma and centrifuged BALF (1500 rpm for 15 min) were used for the determination of the concentration of cytokines. The levels in pg/ml of tumor necrosis factor-alpha (TNFα) and interleukin (IL)-1β, -6, -8, and -10 were quantified using rabbit-specific ELISA kits (Fine Biotech Co., China). ELISA assays were performed in duplicate according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using the statistic software Prism 9 (GraphPad, USA). The results are presented as mean and standard deviation (SD). The normality of the data was tested using the Shapiro-Wilk test. Two-way ANOVA with Tukey's *post hoc* test in parameters with dynamic changes and Kruskal-Wallis non-parametric test for group comparisons were performed. The chi-square test or Fisher's exact test for qualitative binary variables (absent/present) and Mann-Whitney non-parametric test for multiple variables (1-4 scale) were performed to analyze histological findings. A p value below 0.05 was considered statistically significant.

Results

Lung function parameters

Data from 27 male rabbits were used for the analysis. At the beginning of the experiments, there were no significant differences in the basal values (BV) of respiratory parameters between all groups (for all $p>0.05$). Induction of lung injury by hydrochloric acid aspiration followed by high-volume ventilation caused a significant deterioration in all observed lung function parameters in both Model groups. The deterioration was observed compared to BV (time sequence ARDS vs. BV; for all $p<0.001$), however with no significant differences in ARDS time point (Model+Bud group vs. Model group, for all $p>0.05$). The respiratory parameters, including the ratio of arterial oxygen partial pressure to fraction of inspired oxygen (P/F), oxygenation index (OI), alveolar-arterial oxygen gradient (AaG) and static compliance (C_{stat}) had been seriously altered after two insults compared to healthy animals (Model group vs. Saline group) and this alteration persisted until the end of the experiment (Fig. 1).

The high-frequency oscillatory (HFO) delivery of budesonide into the lung immediately significantly improved P/F ratio, AaG, OI in 30 min and C_{stat} in 60 min after drug instillation compared to untreated animals (Model+Bud group vs. Model group; for P/F, AaG, C_{stat} $p<0.01$, for OI $p<0.001$) and this positive effect persisted until the end of the experimental protocol (Fig. 1). Partial pressure of oxygen (pO_2) was significantly altered from 120 min ($p<0.01$), oxygen saturation (SaO_2) from 30 min ($p<0.01$) and dynamic lung-thorax compliance (C_{dyn}) from 60 min after budesonide therapy (Model+Bud group vs. Model group) (Table 1).

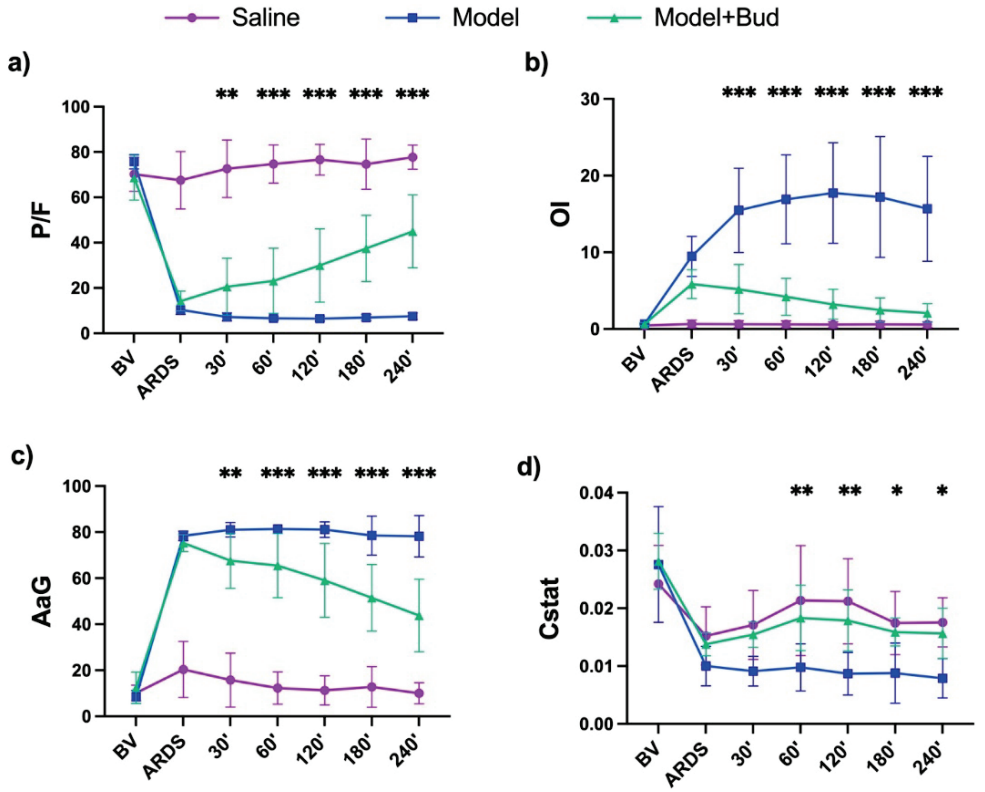

Fig. 1. Respiratory parameters: (**a**) the ratio of arterial oxygen partial pressure to fraction of inspired oxygen (P/F, kPa), (**b**) oxygenation index (OI), (**c**) alveolar-arterial oxygen gradient (AaG, kPa) and (**d**) static compliance (C_{stat}, ml/kPa) before (basal value, BV), in acute lung injury conditions (ARDS) and during 240 min after therapy in Saline, Model and Model+Bud groups. Data are presented as mean and SD. Statistical comparisons for Model+Bud vs. Model * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Table 1. Respiratory parameters: partial pressure of oxygen (pO_2), partial pressure of carbon dioxide (pCO_2), pH, oxygen saturation (SaO_2), mean airway pressure (MAP), dynamic lung-thorax compliance (C_{dyn}), and ventilation efficacy index (VEI) before (basal value, BV) and after induced ARDS and within 4 h after administration of the therapy (Th) in Saline group, Model group and Model+Bud group treated with budesonide. Data are presented as mean and SD. Statistical comparisons for Model+Bud vs. Model * p<0.05, ** p<0.01, *** p<0.001.

	BV	ARDS	30 min Th	60 min Th	120 min Th	180 min Th	240 min Th
pO_2 (kPa)							
Saline	45.02 (12.13)	63.52 (19.28)	68.3 (20.12)	65.62 (22.86)	66.55 (21.46)	65.07 (22.43)	67.57 (21.45)
Model	51.62 (7.24)	11.57 (8.99)	8.99 (3.21)	7.69 (1.70)	8.17 (3.35)	10.71 (8.09)	11.26 (8.82)
Model+Bud	48.05 (6.90)	14.24 (4.36)	20.57 (12.53)	23.13 (14.37)	29.96 (16.16)**	37.45 (14.61)***	44.96 (16.11)***
pCO_2 (kPa)							
Saline	4.03 (0.90)	3.83 (1.04)	3.72 (1.06)	3.57 (0.89)	3.62 (1.04)	3.64 (1.14)	3.83 (1.45)
Model	4.38 (0.49)	4.15 (0.51)	4.81 (0.71)	5.46 (1.77)	5.02 (0.75)	5.54 (1.16)	5.25 (0.85)
Model+Bud	4.85 (0.78)	4.44 (0.82)	5.5 (1.03)	5.16 (0.7)	4.82 (0.46)	4.89 (0.60)	5.05 (0.54)
pH							
Saline	7.589 (0.05)	7.55 (0.06)	7.51 (0.07)	7.45 (0.11)	7.44 (0.07)	7.39 (0.08)	7.35 (0.10)
Model	7.59 (0.04)	7.47 (0.14)	7.42 (0.08)	7.38 (0.09)	7.31 (0.11)	7.24 (0.12)	7.20 (0.12)
Model+Bud	7.57 (0,10)	7.48 (0.1)	7.34 (0.07)	7.34 (0.07)	7.33 (0.07)	7.31 (0.07)	7.29 (0.07)
SaO_2 (%)							
Saline	99.77 (0.20)	99.83 (0.13)	99.84 (0.10)	99.86 (0.10)	99.82 (0.17)	99.83 (0.14)	99.81 (0.20)
Model	99.89 (0.03)	96.80 (1.78)	90.16 (7.09)	85.70 (8.79)	83.84 (9.87)	85.30 (10.72)	87.24 (7.93)
Model+Bud	99.82 (0.08)	97.41 (1.88)	96.63 (3.38)**	97.66 (2.22)***	98.77 (1.20)***	99.22 (1.18)***	99.48 (0.75)***
MAP (kPa)							
Saline	0.44 (0.07)	2.28 (2.59)	2.50 (2.93)	2.42 (2.73)	2.40 (2.74)	2.31 (2.58)	2.32 (2.57)
Model	0.47 (0.04)	1.95 (3.02)	1.90 (2.66)	1.98 (3.01)	2.28 (3.64)	2.43 (3.97)	2.58 (4.28)
Model+Bud	0.44 (0.05)	0.76 (0.08)	0.74 (0.10)	0.68 (0.07)	0.70 (0.10)	0.75 (0.08)	0.78 (0.16)
C_{dyn} (ml/kPa)							
Saline	0.02 (0.005)	0.01 (0.005)	0.01 (0.005)	0.01 (0.007)	0.01 (0.006)	0.01 (0.005)	0.01 (0.004)
Model	0.02 (0.01)	0.009 (0.002)	0.008 (0.001)	0.009 (0.003)	0.007 (0.003)	0.008 (0.004)	0.007 (0.002)
Model+Bud	0.02 (0.004)	0.01 (0.001)	0.01 (0.004)	0.01 (0.004)*	0.01 (0.004)**	0.01 (0.002)	0.01 (0.003)*
VEI							
Saline	2.75 (0.63)	2.52 (1.15)	3.30 (1.39)	3.55 (1.61)	3.74 (1.35)	3.24 (1.01)	3.46 (1.54)
Model	3.02 (1.84)	1.39 (0.55)	1.13 (0.48)	1.20 (0.48)	0.91 (0.39)	1.10 (0.61)	0.96 (0.46)
Model+Bud	2.77 (0.63)	1.44 (0.45)	1.38 (0.54)	1.75 (0.65)	1.74 (0.59)	1.52 (0.41)	1.52 (0.53)

Inflammation in the BALF and plasma

The deterioration of lung function in the Model group was highlighted by the increase of all observed markers of inflammation in bronchoalveolar lavage fluid (BALF) at the end of the experiment compared to the Saline group; p<0.001 for interleukin (IL)-1β, -6, -8, -10 and tumor necrosis factor-alpha (TNFα) (Fig. 2). Budesonide significantly decreased levels of IL-8, -10 and TNFα (Model+Bud vs. Model; for all p<0.01) (Fig. 2b, d, e). In plasma at the end of the experiment, a significant increase of all markers of inflammation IL-1β, -6, -8, -10, and TNFα was observed in untreated animals compared to healthy control (Model group vs. Saline group; for all p<0.001). Budesonide therapy significantly decreased IL-1β, -8 (p<0.001) and IL-10, TNFα (p<0.01) compared to untreated Model animals (Model+Bud group vs. Model group).

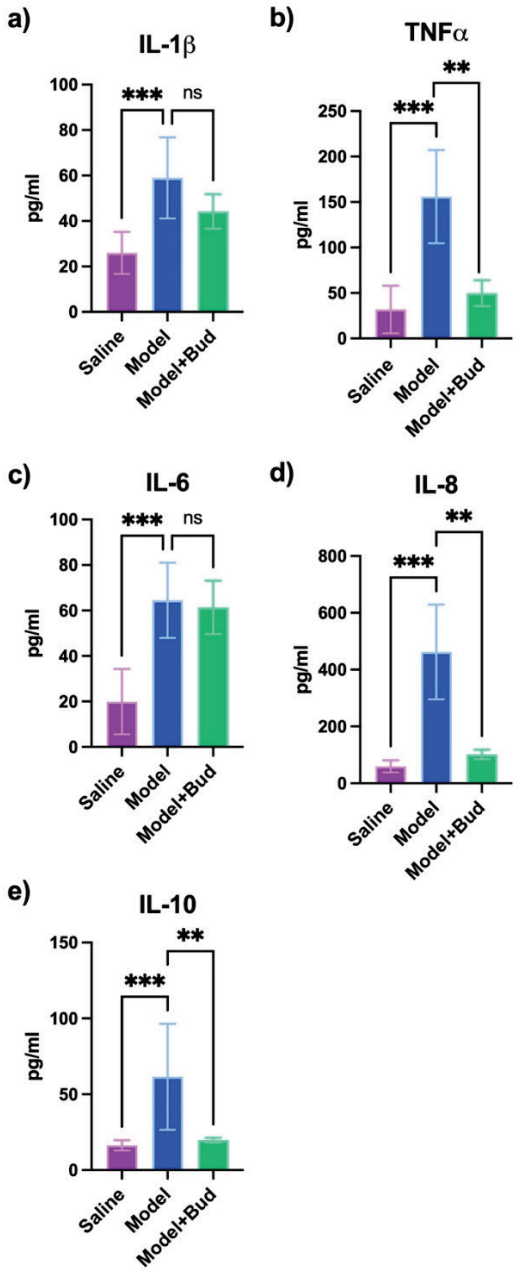

Fig. 2. Levels of cytokines (**a-e**) IL-1β, -6, -8, -10, and TNFα (all in pg/ml) in the BALF in Saline, Model and Model+Bud groups. Data are presented as mean and SD. Statistical comparisons for Model+Bud vs. Model vs. Saline ** p<0.01, *** p<0.001.

White blood cells in the BALF and plasma

In the BALF, a significant decrease was observed for total leukocyte count after budesonide therapy (Model+Bud vs. Model; p<0.05) (Fig. 3a). For differential leukocyte count, a significant decrease in the percentage of neutrophils (p<0.001) and significant increase in the percentage of lymphocytes (p<0.01) were observed after budesonide therapy compared to the untreated animals (Model+Bud group vs. Model group) (Fig. 3b). In the plasma, a significant change was observed after 3 h after budesonide therapy (Model+Bud vs. Model; p<0.001) (Fig. 3c). For differential leukocyte count in plasma, a significant increase in the percentage of neutrophils and significant decrease of lymphocytes were noticed compared to untreated animals, the opposite effect was observed in BALF (Model+Bud group vs. Model group; p<0.001) (Fig. 3d).

Edema formation and histological analyses of lung tissue

Formation of the lung edema expressed as a wet-to-dry lung weight ratio (W/D ratio) significantly increased in the Model group compared to the Saline (p<0.001) (Fig. 4a). Budesonide administration significantly decreased the total W/D ratio (p<0.05) and W/D of the apical medial, medial medial (p<0.01), caudal medial, caudal dorsal and caudal ventral (p<0.001) section of the lung compared to untreated animals (Model+Bud vs. Model group) (Fig. 4a, b).

Histological analysis showed marked deterioration in all observed histopathological features in the Model group compared to the Saline group (for all p<0.01). Budesonide therapy significantly attenuated neutrophil infiltration (p<0.05), interstitial congestion, hyaline membrane (p<0.001), and atelectasis (p<0.01) compared to untreated animals (Fig. 4d, e, f, g). Overall, the total lung injury score was significantly increased in the Model group compared to the Saline (p<0.001) and was reduced by budesonide administration (Model+Bud group vs. Model group, p<0.05) (Fig. 4c).

In Model, the lung tissue showed signs of acute lung injury compared to Saline group (Fig. 5). Deterioration of the architecture of the alveolar spaces with the presence of proteinaceous material and erythrocyte extravasates was observed. In the air spaces of the alveoli, there were numerous free polymorphonuclear cells, mainly neutrophils, sometimes even purulent, protein material, and eosinophilic macrophages. The peripheral parts of the alveolar spaces were filled with edematous fluid. Edematous excess and inflammatory cellularization were present in the perivascular tissue. Budesonide administration improved lung architecture. The lung parenchyma showed the presence of proteinaceous material, sporadic hyaline material, and erythrocyte extravasates. Rarely, free polymorphonuclear cells – neutrophils and eosinophilic macrophages were presented in the alveoli. The perivascular tissue was with signs of mild edematous excess (Fig. 5c).

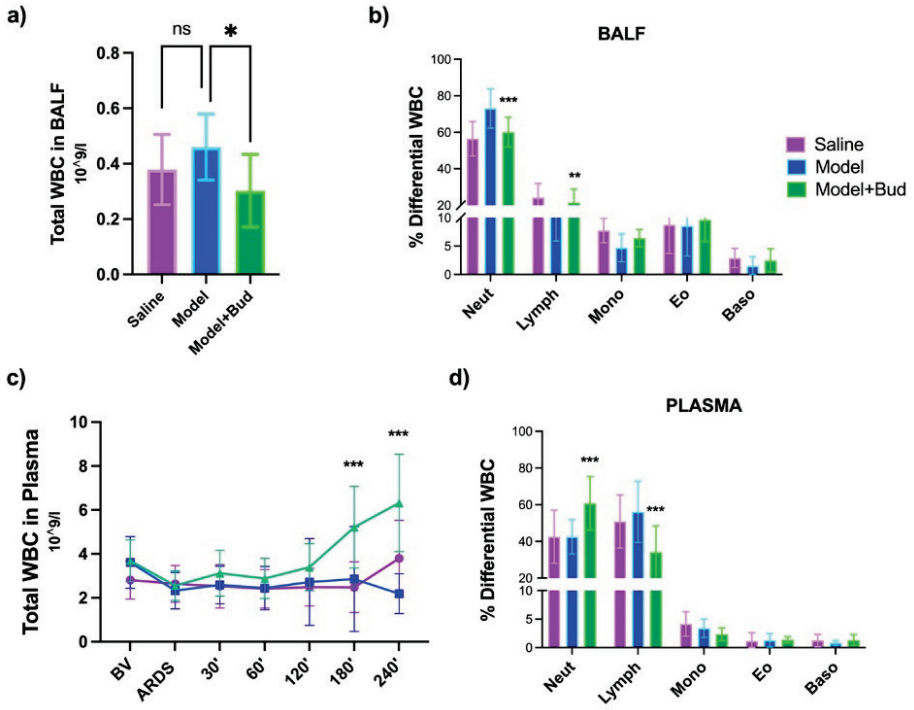

Fig. 3. White blood cells (WBC). (**a**) Total and (**b**) differential WBC count in bronchoalveolar lavage fluid (BALF), and (**c**) total and (**d**) differrential WBC count in the plasma in Saline, Model, and Model+Bud groups. Abbreviations: neutrophils (Neut), lymphocytes (Lymph), monocytes (Mono), eosinophils (Eo), and baso-phils (Baso). Data are presented as mean and SD. Statistical comparisons for Model+Bud vs. Model * p<0.05, ** p<0.01, *** p<0.001.

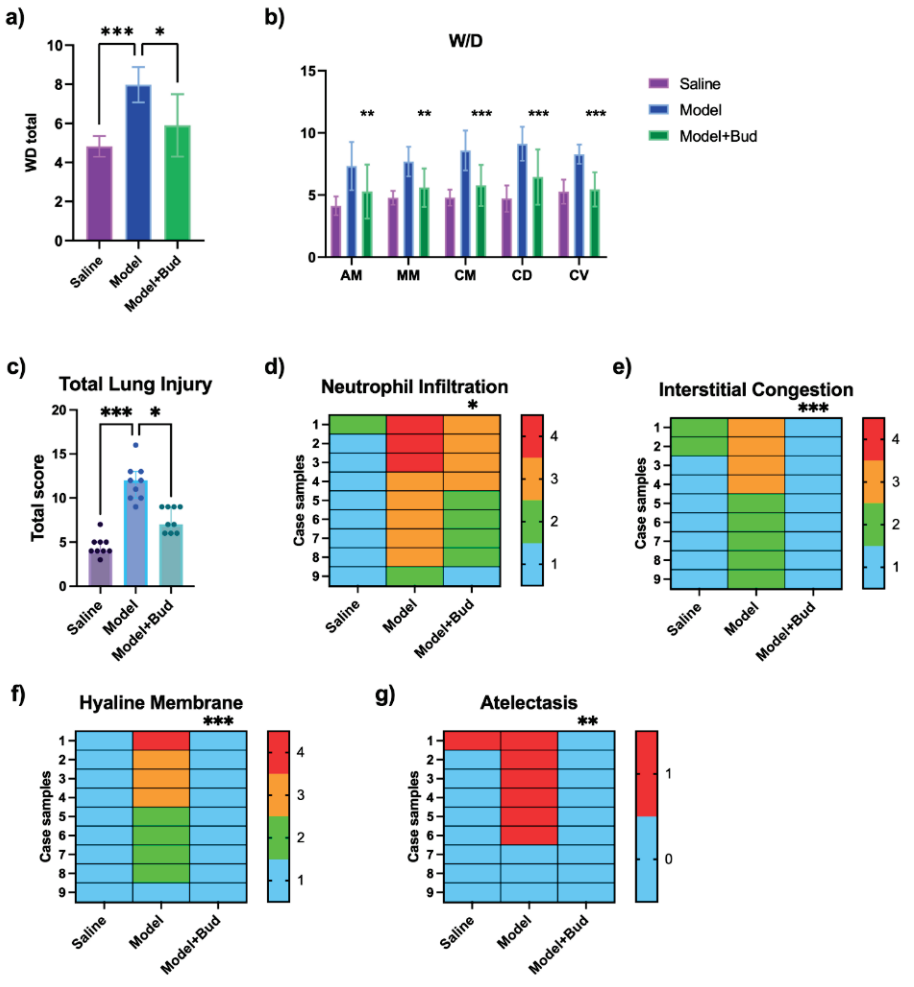

Fig. 4. (**a**) Total lung edema formation expressed as wet-to-dry (W/D) lung weight ratio, (**b**) W/D of apical, medial and caudal regions of the lungs; semiquantitative histo-logical evaluation of (**c**) total lung injury score, (**d**) neutrophil infiltration, (**e**) interstitial congestion, (**f**) hyaline membrane, and (**g**) atelectasis in Saline, Model and Model+Bud groups. Abbreviations: apical medial (AM), medial medial (MM), caudal medial (CM), caudal dorsal (CD), caudal ventral (CV). Data are presented as mean and SD. The color of each cell represents the semiquantitative scoring of the histopathological features of one animal according to scoring systems: 1 – normal lung, 2 – moderate, 3 – intermediate, 4 – severe deterioration or 0 – absent, 1 – present. Statistical comparisons for Model+Bud vs. Model * p<0.05, ** p<0.01, *** p<0.001.

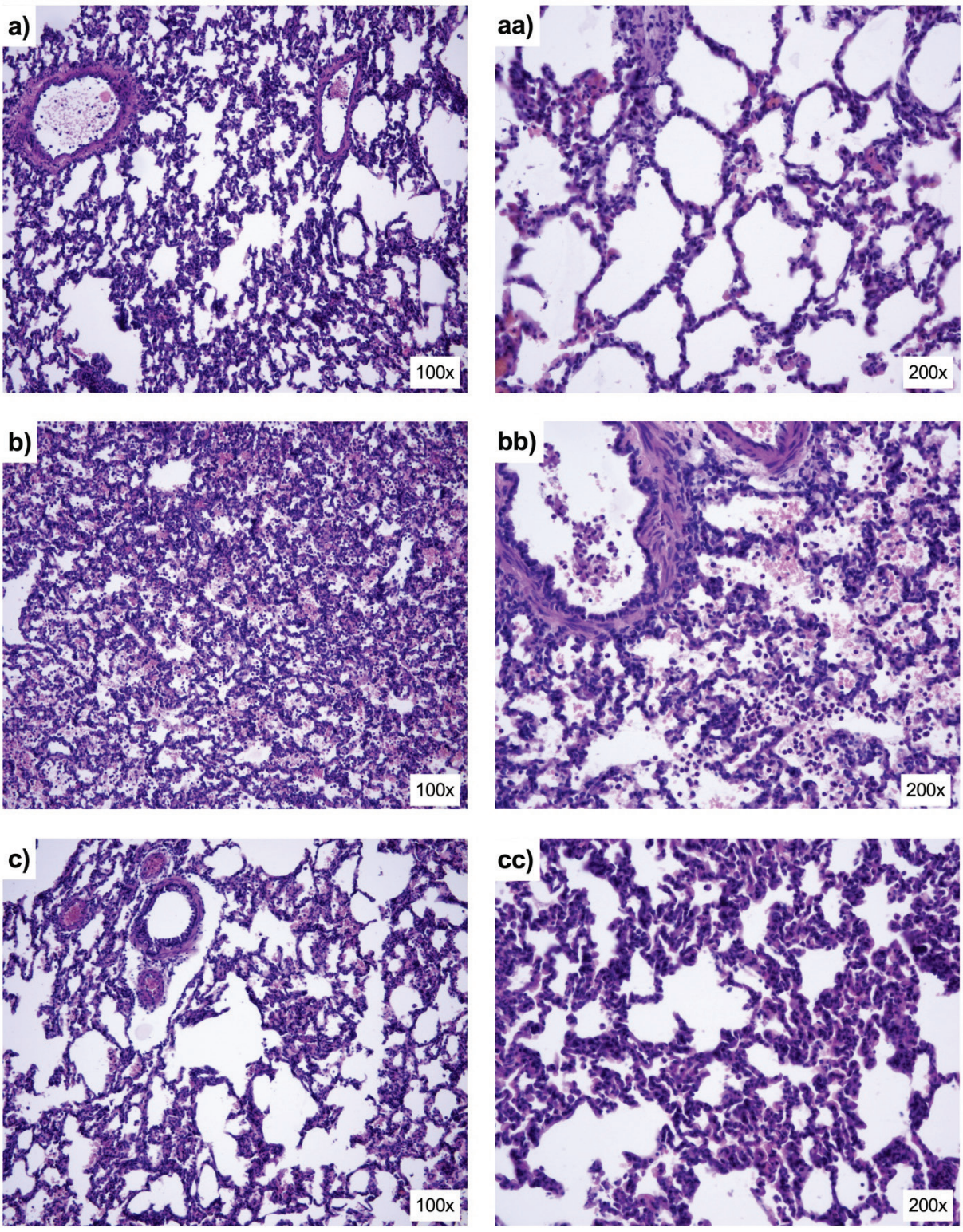

Fig. 5. Histological lung images of healthy (Saline group, **a, aa**), untreated ARDS (Model group, **b, bb**), and budesonide treated animals (Model+Bud, **c, cc**). In Saline group, the lung showed a normal appearance with aerated alveolar spaces, without pathological cellularization, however, with minor protein aggregates in the septa. Perivascular and peribronchial tissue was free of signs of edematous extension and inflammatory cellularization (a, aa). In Model group, the pulmonary parenchyma revealed changes in the alveolar spaces' architectural structure, and showed the presence of proteinaceous material, massive hyaline membranes, and diffuse miscellaneous inflammatory cell infiltrate (b, bb). In Model+Bud group, the lung parenchyma was without significant disturbance in the architecture of the alveolar spaces, rarely with the presence of proteinaceous and hyaline material, and polymorphonuclear cells (c, cc). The magnification 100× was used for a, b, c and 200× for aa, bb, cc.

Discussion

Acute respiratory distress syndrome (ARDS) is a severe and life-threatening form of respiratory failure that can occur in response to a variety of insults to the lungs, including infection, trauma, and aspiration injury. Inflammatory insults, either locally from the lung or systemically from extra-pulmonary sites, affect bronchial epithelium, alveolar macrophages, and vascular endothelium. Increased alveolar-capillary permeability leads to the development of alveolar edema and proteinosis. Alveolar edema reduces gas exchange leading to hypoxemia, reduces lung compliance, and increases the need for mechanical ventilation [26,27].

To date, there are currently no guidelines that cover all aspects of ARDS. However, several guidelines for ventilatory management such as lung protective ventilation with low tidal volume, high level PEEP, prone position, high-frequency oscillatory ventilation (HFOV), and/or adjunctive therapies such as fluid strategy, corticosteroids, extracorporeal membrane oxygenation (ECMO), extracorporeal carbon dioxide removal ($ECCO_2R$), omega-3 fatty acids enteral nutrition are available [28,29]. Based on this recommendation, in this study, we tried to combine the two approaches: glucocorticoid therapy and HFOV delivery. Budesonide is an inhaled glucocorticoid that inhibits a variety of inflammatory cells and reduces the production of inflammatory mediators with significant anti-inflammatory effects [10]. Several clinical and experimental studies have shown that budesonide also leads to satisfactory pulmonary protective effects in the treatment of ARDS [12-14,17]. The use of HFOV can significantly increase drug deposition in the lungs and we tried to use and evaluate this principle of drug delivery. HFOV works by delivering small volume of gas into the lungs at a very high frequency, usually ranging from 5 to 15 Hz with V_T 1-3 ml/kg [30]. These small, quick breaths help to maintain adequate gas exchange in the lung, improve oxygenation, prevent lung injury, and may reduce the risk of ventilator-induced lung injury (VILI) [19,20]. In addition, several studies have investigated the use of HFOV for drug delivery, due to the rapid process of ventilation and the strong influence of the flow of the endotracheal tube [19,21,22,31].

In this study, the hydrochloric acid (HCl) aspiration was used in an animal model to reproduce severe ARDS caused by gastric acid aspiration, as a realistic preclinical model for studying ARDS in a controlled and repeatable way [1,32]. Using additional high-volume ventilation, ARDS-like injury was created in adult rabbits to mimic a clinical scenario where two harmful variables are causing severe lung injury. Biophysical forces associated with mechanical ventilation and associated VILI can contribute to the deterioration of the alveolar-capillary membrane and increase its permeability and inflammation [33]. After induction of the severe ARDS condition, we observed deterioration in all lung function parameters (P/F, OI, AaG, C_{stat}) of the untreated group until the end of the experiment as well as our previous experiments [15,17,25,34]. After meeting the criteria for severe ARDS, budesonide was administered as a bolus intratracheally using HFOV, and animals were conventionally ventilated for additional 4 h. The single intratracheal dose 0.25 mg/kg of budesonide was set according to our previous results [17,35]. Treatment with budesonide improved respiratory parameters markedly 30 min after the instillation and this effect persisted till the end of the experiment, similar to previous studies [10,17,36].

Acute inflammation and neutrophil accumulation in the lungs are commonly observed in both patients and animal models of ARDS [37,38]. The ARDS is associated with influx of leukocytes into the alveolar spaces. We observed an increase of total leukocyte count and neutrophils in the BALF in Model group compared to Saline group in accordance with other studies [17,25,39]. Budesonide therapy significantly decreased total leukocyte and neutrophil counts and increased lymphocyte counts in BALF compared to untreated animals. Neutrophil activation is associated with the release of cytotoxic molecules, including granulated enzymes, reactive oxygen metabolites, bioactive lipids, and cytokines [40]. In BALF and plasma in Model group, there were increased levels of pro-inflammatory cytokines (TNFα, IL-1β, IL-6, IL-8, and IL-10), which is consistent with previous results [15,17,25]. Intra-pulmonary deposition of budesonide significantly reduced inflammatory cell infiltration and inflammation. In our study, this was demonstrated by the fact that the levels of inflammatory markers such as TNFα, IL-8, and IL-10 were significantly lower compared to the Model group. Consistent with previous results [14,17,41]. This neutrophil-dependent form of lung injury is characterized by injury to the alveolar epithelium, alveolar hemorrhage, and intra-alveolar and interstitial edema [27]. The inflammatory events lead to histological changes typical of an acute exudative phase [42]. Histological analysis

showed markedly deterioration in all observed histopathological features; neutrophil infiltration, interstitial congestion, hyaline membrane, and atelectasis in Model group compared to Saline. Budesonide therapy significantly attenuated these lung injury manifestations and contributed to lung architecture stability.

The deterioration of the alveolar-capillary membrane in ARDS is a key factor in the development of respiratory failure associated with the loss of aerated lung tissue and the systemic effects that can occur in this condition [2,38]. VILI-dependent alveolar wall overload results in endothelial and epithelial breaks contributing to interstitial edema. Restoration and severity of injury to the alveolar-capillary barrier and the degree of elevation of microvascular pressure contribute to edema formation [43]. In this study, degree of the lung edema formation expressed as lung wet-to-dry weight ratio (W/D) increased in Model group, while treatment with budesonide significantly reduced these changes, similar to another study [17].

The effect of HFOV as a drug transporter is still debatable. Most often, GCs are applied to the lungs using a nebulizer [11]. However, administration of an aerosol drug to a ventilated patient is difficult, and only a very small amount of aerosol successfully reaches the distal lung. In total, approximately 10 % of the nebulized drug reaches the lungs in adult patients, and even less than 1 % in newborns [44]. The use of HFOV depending on the frequency used had a similar effect on the distribution of particles in the lungs as exogenous surfactant as a drug transporter [45]. In addition, HFOV improved budesonide delivery to distal part of the lung [46]. Also, the effect of budesonide itself, which acts directly in the lungs, is not negligible [47-49].

In conclusion, the use of budesonide in the treatment of ARDS is still a topic of ongoing research, and its effectiveness will depend on a variety of factors, including the underlying cause and severity of the condition, as well as the patient's individual medical history and current condition. In our experimental setting, budesonide significantly prevents the migration of polymorphonuclear leukocytes into the lung and modulates their activation which could inhibit local inflammation and alleviate respiratory failure. Using HFOV for drug delivery can help distribute aerosolized drugs more evenly in the lungs, potentially improving drug efficacy because of rapid gas oscillations. In addition, HFOV delivers small volumes of gas, so this approach can deliver drugs in smaller doses and achieve similar therapeutic effects [21]. Promising results in this study may be explained by the following: (1) early budesonide administration may be more effective than late ARDS treatment; (2) local homogenous delivery using HFOV; and (3) both genomic and nongenomic GC mechanisms, because the effects of budesonide were observed as early as 30 min after delivery to the lung [50,51]. We are aware of the limitations of this study, e.g. interspecies differences, laboratory setting, and primary focus on early and acute ARDS without testing mortality or long-term effects. While the administration of budesonide with high-frequency oscillatory ventilation could potentially have synergistic effects on lung function, it is important to note that this would require further research to determine a safe and effective therapeutic protocol.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

The authors thank M. Petraskova, M. Hutko, D. Kuliskova and Z. Remisova for technical assistance. Funding for the study was provided by the Scientific Grant Agency of the Slovak Republic (VEGA 1/0004/21, VEGA 1/0097/23) and Grant of the Comenius University (UK/101/2023).

References

1. Bellani G, Laffey JG, Pham T, Fan E, Brochard L, Esteban A, Gattinoni L, ET AL. Epidemiology, Patterns of Care, and Mortality for Patients With Acute Respiratory Distress Syndrome in Intensive Care Units in 50 Countries. JAMA 2016;315:788-800. https://doi.org/10.1001/jama.2016.0291
2. Fanelli V, Vlachou A, Ghannadian S, Simonetti U, Slutsky AS, Zhang H. Acute respiratory distress syndrome: new definition, current and future therapeutic options. J Thorac Dis 2013;5:326-334.
3. Force ADT, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, ET AL. Acute respiratory distress syndrome: the Berlin Definition. JAMA 2012;307:2526-2533. https://doi.org/10.1001/jama.2012.5669

4. Matute-Bello G, Downey G, Moore BB, Groshong SD, Matthay MA, Slutsky AS, ET AL. An official American Thoracic Society workshop report: features and measurements of experimental acute lung injury in animals. Am J Respir Cell Mol Biol 2011;44:725-738. https://doi.org/10.1165/rcmb.2009-0210ST
5. Saguil A, Fargo MV. Acute Respiratory Distress Syndrome: Diagnosis and Management. Am Fam Physician 2020;101:730-738.
6. Boyle AJ, Mac Sweeney R, McAuley DF. Pharmacological treatments in ARDS; a state-of-the-art update. BMC Med 2013;11:166. https://doi.org/10.1186/1741-7015-11-166
7. Zhang L, Wang Z, Xu F, Ren Y, Wang H, Han D, Lyu J, Yin H. The Role of Glucocorticoids in the Treatment of ARDS: A Multicenter Retrospective Study Based on the eICU Collaborative Research Database. Front Med (Lausanne) 2021;8:678260. https://doi.org/10.3389/fmed.2021.678260
8. Zhao Q, Shi JX, Hu R, Li Q, Zhang CY, Li JS. Effect of glucocorticoids on mortality in patients with acute respiratory distress syndrome: A meta-analysis. Exp Ther Med 2019;18:4913-4920. https://doi.org/10.3892/etm.2019.8156
9. Donnelly R, Seale JP. Clinical pharmacokinetics of inhaled budesonide. Clin Pharmacokinet 2001;40:427-440. https://doi.org/10.2165/00003088-200140060-00004
10. Mohamed HS, Meguid MM. Effect of nebulized budesonide on respiratory mechanics and oxygenation in acute lung injury/acute respiratory distress syndrome: Randomized controlled study. Saudi J Anaesth 2017;11:9-14. https://doi.org/10.4103/1658-354X.197369
11. Murphy KR, Hong JG, Wandalsen G, Larenas-Linnemann D, El Beleidy A, Zaytseva OV, Pedersen SE. Nebulized Inhaled Corticosteroids in Asthma Treatment in Children 5 Years or Younger: A Systematic Review and Global Expert Analysis. J Allergy Clin Immunol Pract 2020;8:1815-1827. https://doi.org/10.1016/j.jaip.2020.01.042
12. Tripathi S, Saili A. The effect of steroids on the clinical course and outcome of neonates with meconium aspiration syndrome. J Trop Pediatr 2007;53:8-12. https://doi.org/10.1093/tropej/fml018
13. Festic E, Carr GE, Cartin-Ceba R, Hinds RF, Banner-Goodspeed V, Bansal V, Asuni AT, ET AL. Randomized Clinical Trial of a Combination of an Inhaled Corticosteroid and Beta Agonist in Patients at Risk of Developing the Acute Respiratory Distress Syndrome. Crit Care Med 2017;45:798-805. https://doi.org/10.1097/CCM.0000000000002284
14. Deliloglu B, Tuzun F, Cengiz MM, Ozkan H, Duman N. Endotracheal Surfactant Combined With Budesonide for Neonatal ARDS. Front Pediatr 2020;8:210. https://doi.org/10.3389/fped.2020.00210
15. Gao W, Ju N. Budesonide inhalation ameliorates endotoxin-induced lung injury in rabbits. Exp Biol Med (Maywood) 2015;240:1708-1716. https://doi.org/10.1177/1535370215584938
16. Mikolka P, Kopincova J, Kosutova P, Cierny D, Calkovska A, Mokra D. Lung inflammatory and oxidative alterations after exogenous surfactant therapy fortified with budesonide in rabbit model of meconium aspiration syndrome. Physiol Res 2016;65(Suppl 5):S653-S662. https://doi.org/10.33549/physiolres.933529
17. Mokra D, Kosutova P, Balentova S, Adamkov M, Mikolka P, Mokry J, Antosova M, Calkovska. Effects of budesonide on the lung functions, inflammation and apoptosis in a saline-lavage model of acute lung injury. J Physiol Pharmacol 2016;67:919-932.
18. Plaunt AJ, Nguyen TL, Corboz MR, Malinin VS, Cipolla DC. Strategies to Overcome Biological Barriers Associated with Pulmonary Drug Delivery. Pharmaceutics 2022;14:302. https://doi.org/10.3390/pharmaceutics14020302
19. Meyers M, Rodrigues N, Ari A. High-frequency oscillatory ventilation: A narrative review. Can J Respir Ther 2019;55:40-46. https://doi.org/10.29390/cjrt-2019-004
20. Ritacca FV, Stewart TE. Clinical review: high-frequency oscillatory ventilation in adults--a review of the literature and practical applications. Crit Care 2003;7:385-390. https://doi.org/10.1186/cc2182
21. DiBlasi RM, Crotwell DN, Shen S, Zheng J, Fink JB, Yung D. Iloprost drug delivery during infant conventional and high-frequency oscillatory ventilation. Pulm Circ 2016;6:63-69. https://doi.org/10.1086/685080
22. Alzahrany M, Banerjee A, Salzman G. Flow transport and gas mixing during invasive high frequency oscillatory ventilation. Med Eng Phys 2014;36:647-658. https://doi.org/10.1016/j.medengphy.2014.01.010

23. Guerin C, Fassier T, Bayle F, Lemasson S, Richard JC. Inhaled bronchodilator administration during mechanical ventilation: how to optimize it, and for which clinical benefit? J Aerosol Med Pulm Drug Deliv 2008;21:85-96. https://doi.org/10.1089/jamp.2007.0630
24. Ari A, Fink JB. Factors affecting bronchodilator delivery in mechanically ventilated adults. Nurs Crit Care 2010;15:192-203. https://doi.org/10.1111/j.1478-5153.2010.00395.x
25. Mikolka P, Kosutova P, Kolomaznik M, Mateffy S, Nemcova N, Mokra D, Calkovska A. Efficacy of surfactant therapy of ARDS induced by hydrochloric acid aspiration followed by ventilator-induced lung injury - an animal study. Physiol Res 2022;71(Suppl 2):S237-S249. https://doi.org/10.33549/physiolres.935003
26. Aggarwal NR, King LS, D'Alessio FR. Diverse macrophage populations mediate acute lung inflammation and resolution. Am J Physiol Lung Cell Mol Physiol 2014;306:L709-L725. https://doi.org/10.1152/ajplung.00341.2013
27. Matthay MA, Zemans RL, Zimmerman GA, Arabi YM, Beitler JR, Mercat A, Herridge M, ET AL. Acute respiratory distress syndrome. Nat Rev Dis Primers 2019;5:18. https://doi.org/10.1038/s41572-019-0069-0
28. Griffiths MJD, McAuley DF, Perkins GD, Barrett N, Blackwood B, Boyle A, Chee N, ET AL. Guidelines on the management of acute respiratory distress syndrome. BMJ Open Respir Res 2019;6:e000420. https://doi.org/10.1136/bmjresp-2019-000420
29. Fujishima S. Guideline-based management of acute respiratory failure and acute respiratory distress syndrome. J Intensive Care 2023;11:10. https://doi.org/10.1186/s40560-023-00658-3
30. Rettig JS, Smallwood CD, Walsh BK, Rimensberger PC, Bachman TE, Bollen CW, Duval EL, ET AL. High-Frequency Oscillatory Ventilation in Pediatric Acute Lung Injury: A Multicenter International Experience. Crit Care Med 2015;43:2660-2667. https://doi.org/10.1097/CCM.0000000000001278
31. Garner SS, Wiest DB, Bradley JW. Albuterol delivery by metered-dose inhaler in a pediatric high-frequency oscillatory ventilation model. Crit Care Med 2000;28:2086-2089. https://doi.org/10.1097/00003246-200006000-00070
32. Chimenti L, Morales-Quinteros L, Puig F, Camprubi-Rimblas M, Guillamat-Prats R, Gomez MN, Tijero J, ET AL. Comparison of direct and indirect models of early induced acute lung injury. Intensive Care Med Exp 2020;8(Suppl 1):62. https://doi.org/10.1186/s40635-020-00350-y
33. Kallet RH. Mechanical Ventilation in ARDS: Quo Vadis? Respir Care 2022;67:730-749. https://doi.org/10.4187/respcare.09832
34. Kosutova P, Mikolka P, Mokra D, Calkovska A. Anti-inflammatory activity of non-selective PDE inhibitor aminophylline on the lung tissue and respiratory parameters in animal model of ARDS. J Inflamm (Lond) 2023;20:10. https://doi.org/10.1186/s12950-023-00337-y
35. Mikolka P, Kopincova J, Tomcikova Mikusiakova L, Kosutova P, Antosova M, Calkovska A, Mokra D. Effects of surfactant/budesonide therapy on oxidative modifications in the lung in experimental meconium-induced lung injury. J Physiol Pharmacol 2016;67:57-65.
36. Liu H, Wang Z, Ren L, Zhang G, Zhao D, Guo Y, Wang L, Feng H. Clinical Efficacy of Budesonide/Glycopyrronium Bromide/Formoterol in the Treatment of Patients with Acute Respiratory Distress Syndrome and Its Effect on Inflammatory Factors. Evid Based Complement Alternat Med 2022;2022:8150444. https://doi.org/10.1155/2022/8150444
37. Pierrakos C, Karanikolas M, Scolletta S, Karamouzos V, Velissaris D. Acute respiratory distress syndrome: pathophysiology and therapeutic options. J Clin Med Res 2012;4:7-16. https://doi.org/10.4021/jocmr761w
38. Bos LDJ, Ware LB. Acute respiratory distress syndrome: causes, pathophysiology, and phenotypes. Lancet 2022;400:1145-1156. https://doi.org/10.1016/S0140-6736(22)01485-4
39. Hong S, Jian C, Wang H, Wang X, Xing L, Qiao L. Effects of different doses of methylprednisolone therapy on acute respiratory distress syndrome: results from animal and clinical studies. BMC Pulm Med 2022;22:348. https://doi.org/10.1186/s12890-022-02148-y
40. Narasaraju T, Yang E, Samy RP, Ng HH, Poh WP, Liew A-A, Phoon MC, ET AL. Excessive neutrophils and neutrophil extracellular traps contribute to acute lung injury of influenza pneumonitis. Am J Pathol 2011;179:199-210. https://doi.org/10.1016/j.ajpath.2011.03.013

41. Mokra D, Mokry J, Drgova A, Petraskova M, Bulikova J, Calkovska A. Intratracheally administered corticosteroids improve lung function in meconium-instilled rabbits. J Physiol Pharmacol 2007;58 Suppl 5(Pt 1):389-398.
42. Castro CY. ARDS and diffuse alveolar damage: a pathologist's perspective. Semin Thorac Cardiovasc Surg 2006;18:13-19. https://doi.org/10.1053/j.semtcvs.2006.02.001
43. Sibbald WJ, Short AK, Warshawski FJ, Cunningham DG, Cheung H. Thermal dye measurements of extravascular lung water in critically ill patients. Intravascular Starling forces and extravascular lung water in the adult respiratory distress syndrome. Chest 1985;87:585-592. https://doi.org/10.1378/chest.87.5.585
44. Watterberg KL, Clark AR, Kelly HW, Murphy S. Delivery of aerosolized medication to intubated babies. Pediatr Pulmonol 1991;10:136-141. https://doi.org/10.1002/ppul.1950100217
45. Alzahrany M, Banerjee A. Aerosolized drug delivery in patient-specific lung model during invasive high frequency oscillatory ventilation. J Aerosol Sci 2015;81:1-20. https://doi.org/10.1016/j.jaerosci.2014.11.005
46. Esmaeilizand R, Rocha T, Harrison A, Gray S, Fusch G, Dolovich M, Mukerji A. Efficiency of budesonide delivery via a mesh nebulizer in an in-vitro neonatal ventilator model. Pediatr Pulmonol 2020;55:2283-2288. https://doi.org/10.1002/ppul.24897
47. Lin CH, Jeng MJ, Yang YC, Hsiao YH, Kou YR. Comparison of different dosing strategies of intratracheally instilled budesonide on meconium injured piglet lungs. Pediatr Pulmonol 2017;52:891-899. https://doi.org/10.1002/ppul.23697
48. Yu H, Lv M, Zhang S, Zou K, Qian Y, Lv S. Combination therapy with budesonide and acetylcysteine alleviates LPS-induced acute lung injury via the miR-381/NLRP3 molecular axis. PLoS One 2023;18:e0289818. https://doi.org/10.1371/journal.pone.0289818
49. Dong L, Zhu Y-H, Liu D-X, Li J, Zhao P-C, Zhong Y-P, Chen Y-Q, ET AL. Intranasal Application of Budesonide Attenuates Lipopolysaccharide-Induced Acute Lung Injury by Suppressing Nucleotide-Binding Oligomerization Domain-Like Receptor Family, Pyrin Domain-Containing 3 Inflammasome Activation in Mice. J Immunol Res 2019;2019:7264383. https://doi.org/10.1155/2019/7264383
50. Chang X, Li S, Fu Y, Dang H, Liu C. Safety and efficacy of corticosteroids in ARDS patients: a systematic review and meta-analysis of RCT data. Respir Res 2022;23:301. https://doi.org/10.1186/s12931-022-02186-4
51. Mokra D, Mikolka P, Kosutova P, Mokry J. Corticosteroids in Acute Lung Injury: The Dilemma Continues. Int J Mol Sci 2019;20:4765. https://doi.org/10.3390/ijms20194765