Efficacy of Surfactant Therapy of ARDS Induced by Hydrochloric Acid Aspiration Followed by Ventilator-Induced Lung Injury – an Animal Study

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Summary

The development of acute respiratory distress syndrome (ARDS) is known to be independently attributable to aspiration-induced lung injury. Mechanical ventilation as a high pressure/volume support to maintain sufficient oxygenation of a patient could initiate ventilator-induced lung injury (VILI) and thus contribute to lung damage. Although these phenomena are rare in the clinic, they could serve as the severe experimental model of alveolar-capillary membrane deterioration. Lung collapse, diffuse inflammation, alveolar epithelial and endothelial damage, leakage of fluid into the alveoli, and subsequent inactivation of pulmonary surfactant, leading to respiratory failure. Therefore, exogenous surfactant could be considered as a therapy to restore lung function in experimental ARDS. This study aimed to investigate the effect of modified porcine surfactant in animal model of severe ARDS (P/F ratio ≤13.3 kPa) induced by intratracheal instillation of hydrochloric acid (HCl, 3 ml/kg, pH 1.25) followed by VILI (V_T 20 ml/kg). Adult rabbits were divided into three groups: untreated ARDS, model treated with a bolus of poractant alfa (Curosurf®, 2.5 ml/kg, 80 mg phospholipids/ml), and healthy ventilated animals (saline), which were oxygen-ventilated for an additional 4 h. The lung function parameters, histological appearance, degree of lung edema and levels of inflammatory and oxidative markers in plasma were evaluated. Whereas surfactant therapy with poractant alfa improved lung function, attenuated inflammation and lung edema, and partially regenerated significant changes in lung architecture compared to untreated controls. This study indicates a potential of exogenous surfactant preparation in the treatment of experimental ARDS.

Key words

ARDS • Surfactant replacement therapy • Poractant alfa • Lung function • Inflammation • Two-hit model

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Introduction

Aspiration-induced lung injury is recognized as an independent risk factor for the subsequent development of acute respiratory distress syndrome (ARDS) [1,2]. In addition, patients with ARDS receive supportive care and mechanical ventilation. However, the biophysical forces associated with ventilation might contribute to both increased inflammation and endothelial-epithelial permeability, a phenomenon known as ventilator-induced lung injury (VILI) [3,4]. Although preventive strategies exist in the clinic to protect patients from these phenomena, the combination of acid aspiration and VILI may represent a relevant experimental model of surfactant inactivation, moreover, it partially captures the multifactorial nature of ARDS [5].

In animal models, the acid component of gastric aspirates is often modeled by intratracheal instillation of

hydrochloric acid (HCl). The presence of HCl in the lung leads to the loss of pulmonary microvascular integrity and extravasation of fluid and protein into the airways and alveoli [2]. In addition to inhibiting oxygen diffusion, edema fluid contains plasma proteins and other substances that can directly interfere with the function of the alveolar surfactant and inactivate it. The deterioration of the surfactant, together with the formation of edema, the mismatch between ventilation and perfusion, and inflammation, leads to a reduction in lung compliance and hypoxemia that further deteriorate the lung function [6,7]. The participation of surfactant dysfunction in acid aspiration pneumonitis was confirmed by the finding that exogenous surfactant treatment improves pulmonary function only after inhibitory plasma proteins are removed by the lavage [8]. Aspiration-induced lung injury is characterized by an acute neutrophilic inflammatory response, with elevated levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), macrophage inflammatory protein 2 (MIP-2), cytokine 1-induced neutrophil chemoattractant (CINC-1) and monocyte chemotactic protein 1 (MCP-1) [2], as well as increased levels of oxidants and proteases and complement activation [9].

The current treatment of aspiration syndromes is primarily supportive with an emphasis on the management of hypoxemia. In particular, invasive mechanical ventilation is the mainstay for most of ARDS patients [10]. On the other hand, surfactant therapy in ARDS has led to controversial results, which may be related to variations in surfactant composition, biophysical activity, susceptibility to inactivation, and dose. Among the currently available exogenous surfactant preparations, the animal-derived ones are preferred [11,12]. For instance, poractant alfa is a naturally derived surfactant from minced porcine lungs containing 1 % SP-B and SP-C, which has greatly improved outcomes in neonatal RDS patients and is well tolerated [13]. Exogenous surfactant use was demonstrated in pre-clinical animal studies to improve lung function and reduce pulmonary edema [14,15]. The use of pulmonary surfactant in adult ARDS improved oxygenation during the first 24 h after treatment and appeared to reduce the duration of ventilation [12,16,17]. In contrast, other authors do not support the clinical efficacy of exogenous surfactant in inflammatory lung disease to improve mortality and oxygenation in adult patients with ARDS [11]. A recent retrospective study investigated the feasibility, efficacy, and safety of poractant alfa for COVID-19 induced ARDS in adults. There were no acute effects, decompensation, as well as no impact on the duration of mechanical ventilation and mortality [18]. In such a undecided situation, another perspective can be beneficial.

We hypothesized that poractant alfa, because of its properties and high resistance to inactivation, would be effective in the treatment of severe ARDS condition. To recognize the therapeutic potential of poractant alfa in experimental ARDS condition with a multifactorial nature, a two-hit model of ARDS was established in adult rabbits and the effects of the exogenous surfactant on lung function and inflammation were evaluated.

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, Martin. Adult New Zealand white male rabbits with body weight (b.w.) of 2.5±0.3 kg were instrumented in accordance with the previous study [19-21]. The animals were mechanically ventilated (Aura V, Chirana, Slovakia) with a positive end-expiratory pressure (PEEP) of 0.2 kPa, tidal volume (V_T) 6 ml/kg, inspiration expiration rate (I:E) 1:2, respiratory rate (RR) of 40 breaths per minute (bpm), and inspiratory oxygen fraction FiO_2 of 1.0 throughout the experiment. The animal was sacrificed by a lethal dose of KCl and the lungs were removed and processed *post-mortem*. 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 TM^{348}, Bayer Diagnostics, Germany). Ventilation parameters, e.g. plateau airway pressure (Paw), static compliance (C_{stat}) and positive end-expiratory pressure (PEEP), were measured by in-build sensors and Aura V ventilator software. The following parameters were calculated: P/F = calculated as a ratio between arterial oxygen partial pressure (PaO_2) and a fraction of inspired oxygen (FiO_2); oxygenation index (OI) = (mean airway pressure × FiO_2) /PaO_2; and alveolar-arterial gradient (AaG) = [FiO_2 (P_{atm} – PH_2O) – $PaCO_2$ /0.8] – PaO_2, where P_{atm} is barometric pressure and PH_2O is the pressure of water vapor.

The experimental model of ARDS

The two-hit lung injury was performed in two phases. First, hydrochloric acid – HCl (3 ml/kg b.w., pH 1.25) was intratracheally instilled in the right and left lateral positions of the animal with conventional ventilatory stabilization for 15 min. Subsequently, the lungs were ventilated with high tidal volumes (HTV) to mimic ventilator-induced lung injury (VILI) with target V_T 20 ml/kg, zero PEEP, RR 20-30 bpm, I:E 1:2 and FiO_2 1.0. Arterial blood gases were analyzed every 15' during HTV ventilation until P/F ratio decreased to ≤13.3 kPa, equal to P/F ≤100 mm Hg and is graded as severe condition according to the Berlin definition of ARDS [22]. In two animals, the standard HTV ventilation time of 30' was extended by 15' to meet the defined criteria for ARDS. Saline (3 ml/kg b.w.) was instilled instead of HCl and no high tidal ventilation was applied in the saline group.

Treatment protocol

After fulfilling the lung injury criteria, the animals were randomly assigned to the following two groups (n=8 for each group): (i) ARDS group, model of acute lung injury without treatment; (ii) Poractant alfa group, model with surfactant treatment (2.5 ml/kg, 200 mg phospholipids/kg) which was given as a bolus instillation into the trachea above the carina with the animal placed in semi-upright right and in the left lateral position (50 % of the dose was administered in each position). The administered dose is recommended in the guidelines for the treatment of neonatal RDS [23] and simultaneously used in previous experimental studies of ARDS [14,15]. The third group of healthy animals (iii) received saline. After the treatment procedure, all animals were ventilated for 4 h in a volume controlled mode (V_T 6 ml/kg, PEEP 0.5 kPa, RR 40 bpm, I:E 1:2, and FiO_2 1.0). PEEP was increased up to 1 kPa in cases where SaO_2 fell below 87 %. PEEP was increased gradually to reach the minimum required level. Post-treatment physiological data, such as blood gases and respiratory parameters, were recorded at 15, 30, 60, 120, 180, and 240 min after therapy administration.

White blood cells in the blood

Artery blood samples for total leukocyte counting were taken regularly during the experiment. At the end, the percentage of differential white blood cells (WBC) was estimated. Total and differential leukocyte counts values were evaluated using the veterinary hematology analyzer Sysmex XT-2000i (Sysmex, Sweden).

Post-mortem analyzes

Samples of arterial blood were harvested at the end of the experiment. Plasma was obtained by centrifugation (3000 rpm for 15 min, 4 °C) and was subsequently used for determination of concentrations of cytokines and oxidation markers. The levels in pg/ml of tumor necrosis factor-alpha (TNFα) and interleukin (IL)-1β, -6, -8 were quantified using rabbit-specific ELISA kits (Cloud-Clone Corp., USA). The OxiSelect™ Nitrotyrosine ELISA Kit was used for evaluation of oxidation of proteins expressed at a 3-nitrotyrosine nanomolar concentration (nM 3NT) and the OxiSelect™ TBARS Assay Kit was used to detect of oxidation of lipids expressed as malondialdehyde at a micromolar concentration (μM MDA) (both Cell Biolabs Inc., USA).
After sacrificing the animal, lungs and trachea with endotracheal tube were excised. The right lung was ligated. The left lung was lavaged twice with saline at a dose 10 ml/kg b.w. using 50 ml syringe connected to endotracheal tube for fluid administration and withdrawal. Tissue samples from the right lung were immersed in 10 % buffered formalin for two weeks for tissue fixation or 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, the extent of lung edema. Bronchoalveolar lavage fluid (BALF) was centrifuged at 1500 rpm for 15 min and total protein content was determined in BALF supernatant by the Bradford colorimetric method. The BALF recovery was approximately 84 % with no significant differences between the groups (mean ± SD for Saline 84.9±1.3, ARDS 84.7±4.4, Poractant alfa 83.7±3.9).

Formalin-fixed lung samples were embedded in 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 total lung injury score as described previously [15,24].

Statistical analysis

The number of animals in the groups was determined by calculating the statistical power of the test using G*Power software. Given the test, n=7 represents the edge of statistical power, but without losing the significance of differences that may exist in populations. Therefore, we set the number of animals in the groups to eight to comply with the 3R principle. Data analysis was performed using statistical software Graph Pad Prism 6.01 (USA). Results are presented as

mean ± standard deviation (*SD*). The normality of the data was tested using the Shapiro-Wilk test. Two-way analysis of variance (ANOVA) with Dunnett's multiple comparison test for dynamic change parameters and Kruskal-Wallis non-parametric test for group's comparison were used. A $p<0.05$ was considered statistically significant.

Results

Data from 24 male rabbits were used for the analysis. All animals survived the entire protocol. There were no significant differences in the initial values of respiratory parameters between all groups (for all $p>0.05$) at the starting phase of the experiments (basal value, BV). The induction of the experimental ARDS condition affected the lung function parameters compared to basal values (BV), for P/F, OI and AaG; for all $p<0.001$, however, there were no significant differences in these parameters between the acute lung injury groups at the observed time point for the Model (ARDS group vs. Poractant alfa; for all $p>0.05$).

Lung function parameters

Induction of lung injury by aspiration of hydrochloric acid followed by injurious high-volume ventilation caused a significant deterioration in the lung function parameters. All observed respiratory parameters, including the ratio of arterial oxygen partial pressure to fraction of inspired oxygen (P/F), oxygenation index (OI), alveolar-arterial gradient (AaG), static compliance (C_{stat}), plateau airway pressure (Paw) and positive end-expiratory pressure (PEEP) had been severely altered after the two insults compared to the salines (for all $p<0.001$) and this trend persisted till the end of the experiment (Fig. 1).

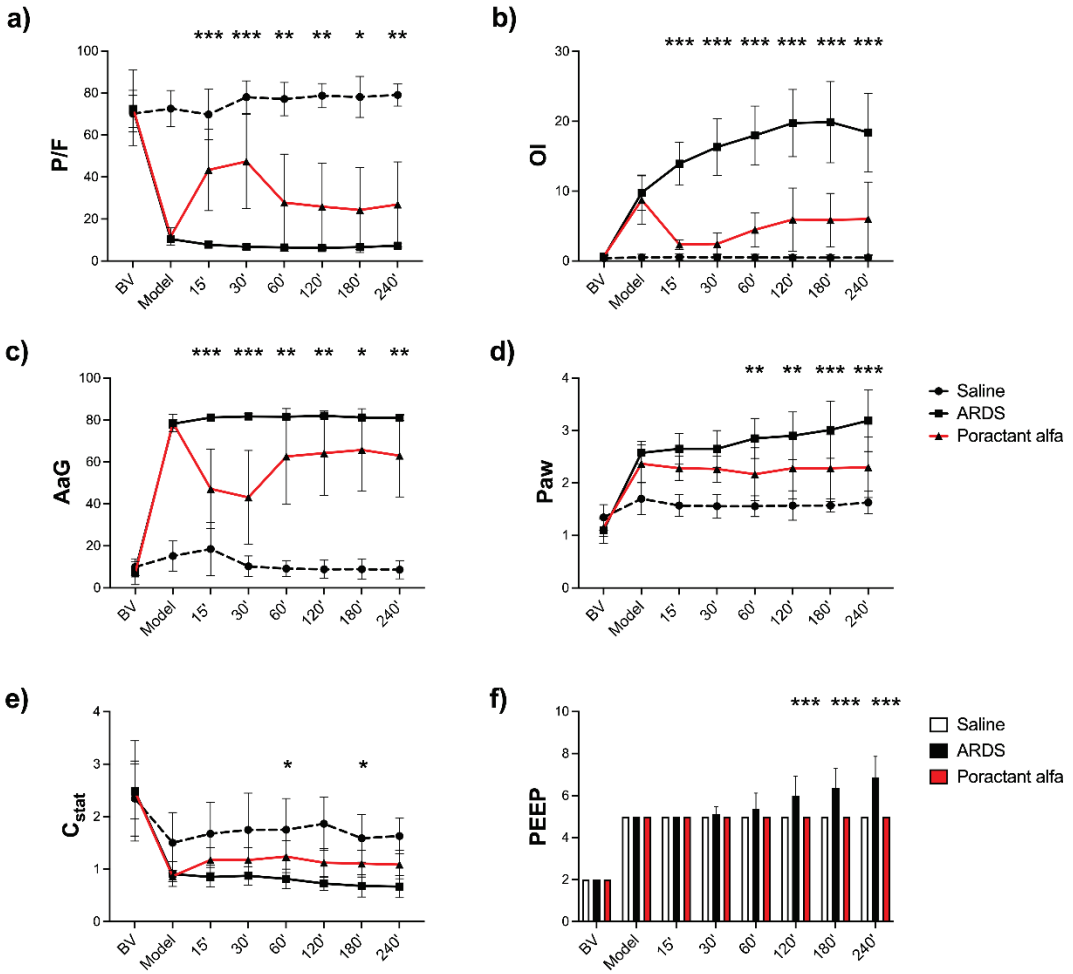

Fig. 1. Respiratory parameters: (**a**) the ratio of arterial oxygen partial pressure to fraction of inspired oxygen (P/F, kPa), (**b**) oxygenation index (OI), (**c**) alveolar-arterial gradient (AaG, kPa), (**d**) Plateau airway pressure (Paw, kPa), (**e**) Static compliance (C_{stat}, ml/cm H_2O) and (**f**) Positive end-expiratory pressure (PEEP, kPa) before (basal value, BV) and in acute lung injury condition (Model) and during 240 min after therapy in Saline, ARDS and Poractant alfa groups. Data are presented as means ± SD. Statistical comparisons for Poractant alfa vs. ARDS group * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

The poractant alfa significantly improved P/F ratio, OI, AaG, Paw and PEEP compared to ARDS animals, whereas the effect for P/F, OI and AaG had a rapid onset observed already at 15 and 30 min (p<0.001) and persisted until the end of the experiment (at 240 min Th p<0.01) (Fig. 1). Paw was significantly altered from 60 and PEEP from 120 min after administration of surfactant therapy. Poractant alfa significantly improved Cstat at 60 and 180 min versus untreated ARDS animals (p<0.05) (Fig. 1e).

Inflammation and oxidation in the plasma

The lung deterioration after two-hits in the ARDS group was highlighted by the increase of all observed markers of inflammation and oxidation compared to saline in plasma at the end of the experiment; for tumor necrosis factor-alpha (TNFα) and interleukin (IL)-1β, -6, -8 and the marker of lipid oxidation thiobarbituric acid-reactive substances (TBARS) the p value represents <0.05, and for the marker of protein oxidation 3-nitrotyrosine (3NT) p<0.01 (Fig. 2). Poractant alfa significantly decreased cytokine and oxidation marker levels compared to ARDS animals, for all p<0.05, except for no significant difference for IL-8.

White blood cells in the blood

In the blood, the total leukocyte count in both injured groups (ARDS and Poractant alfa groups) decreased immediately after the two hits compared to the saline group (p<0.05), significantly only at two time points of observation 120 and 240 min, with a more obvious difference for the ARDS group vs. Saline (p<0.001) at the end of the experiment (Fig. 3a). Lung deterioration in the ARDS group led to significant shift in the percentage of neutrophils (p<0.05) and lymphocytes (p<0.05) compared to Saline at the end of the experiment (Fig. 3b). No differences in total and differential leukocyte count were observed after poractant alfa therapy compared to untreated ARDS animals.

Lung edema formation and protein content in BALF

Lung edema expressed as a wet-dry lung weight ratio (W/D ratio) increased in ARDS group vs. salines (p<0.001), and similarly the total protein content (p<0.001) in bronchoalveolar lavage fluid (BALF) also increased. Surfactant therapy decreased the total W/D ratio (p<0.001) and the protein content (p<0.01) compared to ARDS animals (Fig. 4a, b). In each section of the lung, surfactant therapy significantly decreased

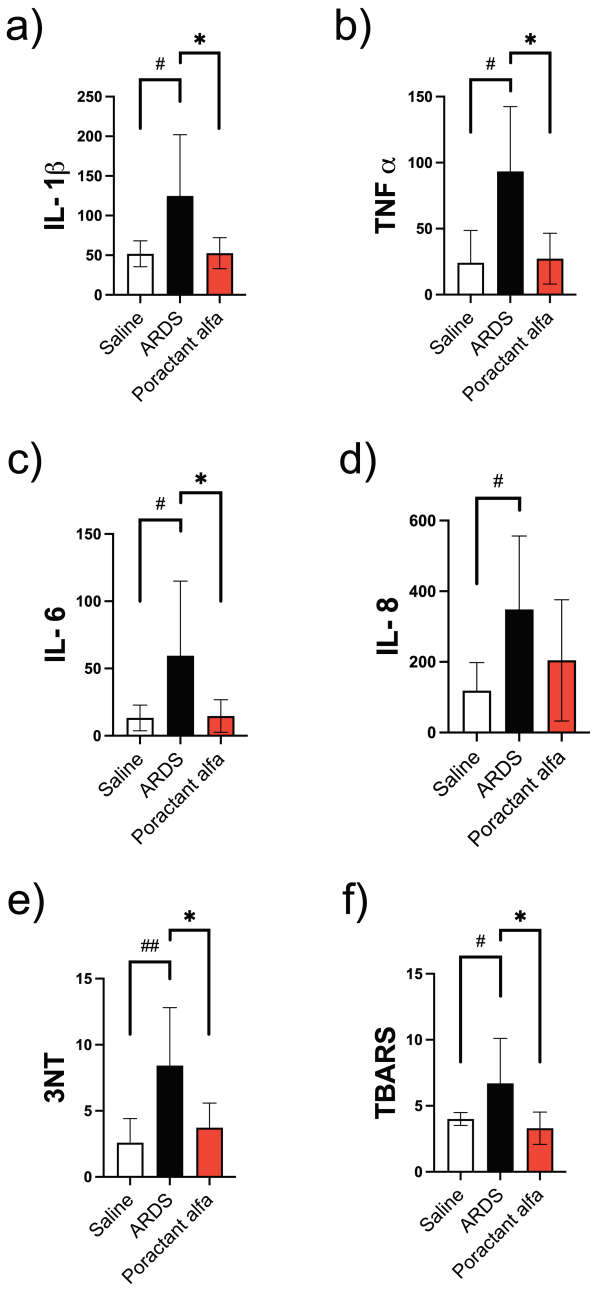

Fig. 2. Levels of cytokines (**a-d**) IL-1β, TNFα, IL-6 and IL-8 (all in pg/ml) and oxidation markers (**e**) 3-nitrotyrosine (3NT, in nM), (**f**) thiobarbituric acid-reactive substances (TBARS, in μM MDA) in plasma in Saline, ARDS and Poractant alfa groups. Data are presented as means ± SD. Statistical comparisons: $^{\#}p$<0.05, $^{\#\#}p$<0.01, * p<0.05.

W/D compared to ARDS group (for all lung parts p<0.001 vs. Saline) (Fig. 4b).

Histological analysis

In ARDS group with HCl instillation and ventilator-induced lung injury, the pulmonary parenchyma displayed a diffuse miscellaneous inflammatory cell infiltrate in the alveoli and partially disrupted tissue architecture with collapsed alveoli.

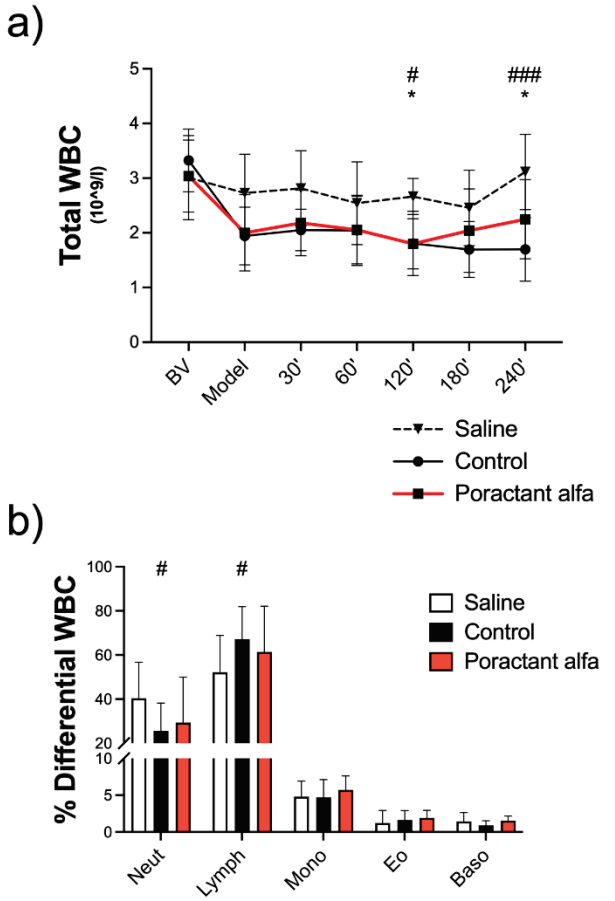

Fig. 3. (**a**) Total and (**b**) percentage of differential white blood cells (WBC) count in the blood in Saline, ARDS and Poractant alfa groups. Abbreviations: neutrophils (Neut), lymphocytes (Lymph), monocytes (Mono), eosinophils (Eo), and basophils (Baso). Data are presented as means ± SD. Statistical comparisons for Poractant alfa vs. Saline * $p<0.05$; for ARDS vs. Saline $^{\#}$ $p<0.05$, $^{\#\#\#}$ $p<0.001$.

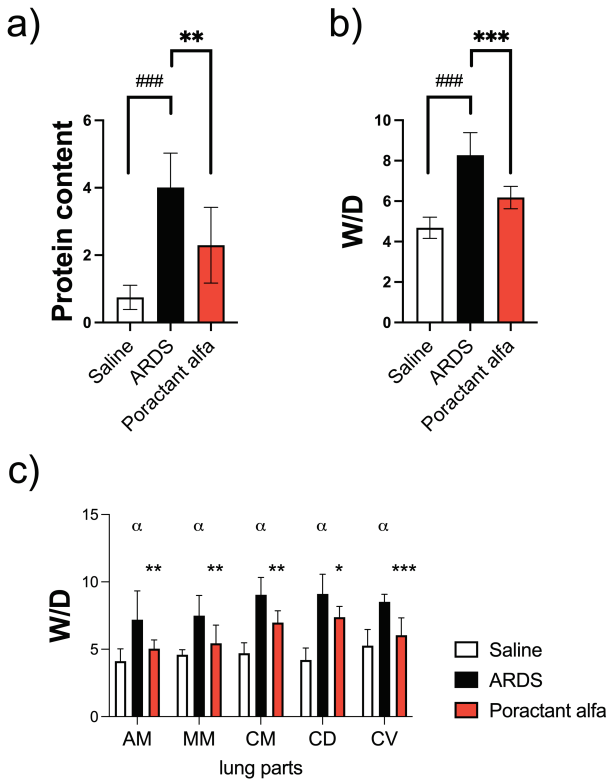

Fig. 4. (**a**) Protein content in bronchoalveolar lavage fluid (mg/ml); (**b**) Total lung edema formation expressed as wet-dry (W/D) lung weight ratio; (**c**) W/D of apical, medial and caudal regions of the lungs in Saline, ARDS and Poractant alfa groups. Abbreviations: apical medial (AM), medial medial (MM), caudal medial (CM), caudal dorsal (CD), caudal ventral (CV). Data are presented as means ± SD. Statistical comparisons: for Saline vs. ARDS $^{\alpha}$ $p<0.001$; for Poractant alfa vs. ARDS * $p<0.05$, ** $p<0.01$, *** $p<0.001$; $^{\#\#\#}$ $p<0.001$.

Arterioles with a perivascular inflammatory cell infiltrate were observed. The alveoli displayed an acute cell reaction with a predominant accumulation of neutrophils and plasmocytes, activated pneumocytes, and numerous erythrocytes. In some places, massive hyaline membranes and protein debris were present on the alveolar surface (Fig. 5a). Surfactant therapy improved lung architecture and alleviated the acute inflammatory processes characteristic for ARDS condition. In the poractant alfa group, the lung showed a normal appearance, but slightly thickened alveolar septa displayed rare inflammatory cell infiltrate with neutrophils, plasmocytes, and a few erythrocytes. The alveolar spaces were airy with inconspicuous protein debris. The peribronchial space around the terminal bronchi displayed increased lymphocyte aggregates (Fig. 5b). Poractant alfa therapy significantly attenuated total lung injury ($p<0.05$) compared to untreated ARDS animals (Fig. 5d).

Discussion

Since gastric content aspiration represents an important cause of ARDS [25], the acid aspiration model using hydrochloric acid (HCl) can be used as a realistic clinical model to study this condition in a controlled and repeatable manner [26]. Following the aspiration of dilute HCl, lung injury is characterized by a biphasic response. The early phase injury is mediated by capsaicin-sensitive neurons, as well as the direct caustic effects of low pH on the airway epithelium, and is followed by an immediate neutrophilic inflammatory response within 4-6 h [27]. The combination of these mechanisms leads to loss of microvascular integrity, extravasation of fluid, and protein into the airways and alveoli. The presence of edema fluid can affect airway compliance, and plasma proteins in edema fluid can directly interfere with alveolar surfactant function. A neutrophilic airway influx occurs when stomach

contents of low pH are aspirated [28]. Therefore, intratracheal instillation of HCl could represent a suitable *in vivo* model of ARDS [2,29,30]. However, in clinical practice, ARDS typically present with an initializing event, followed by the need for mechanical ventilation, while inadequate ventilation acts as an additional noxious factor that could deteriorate the lung function.

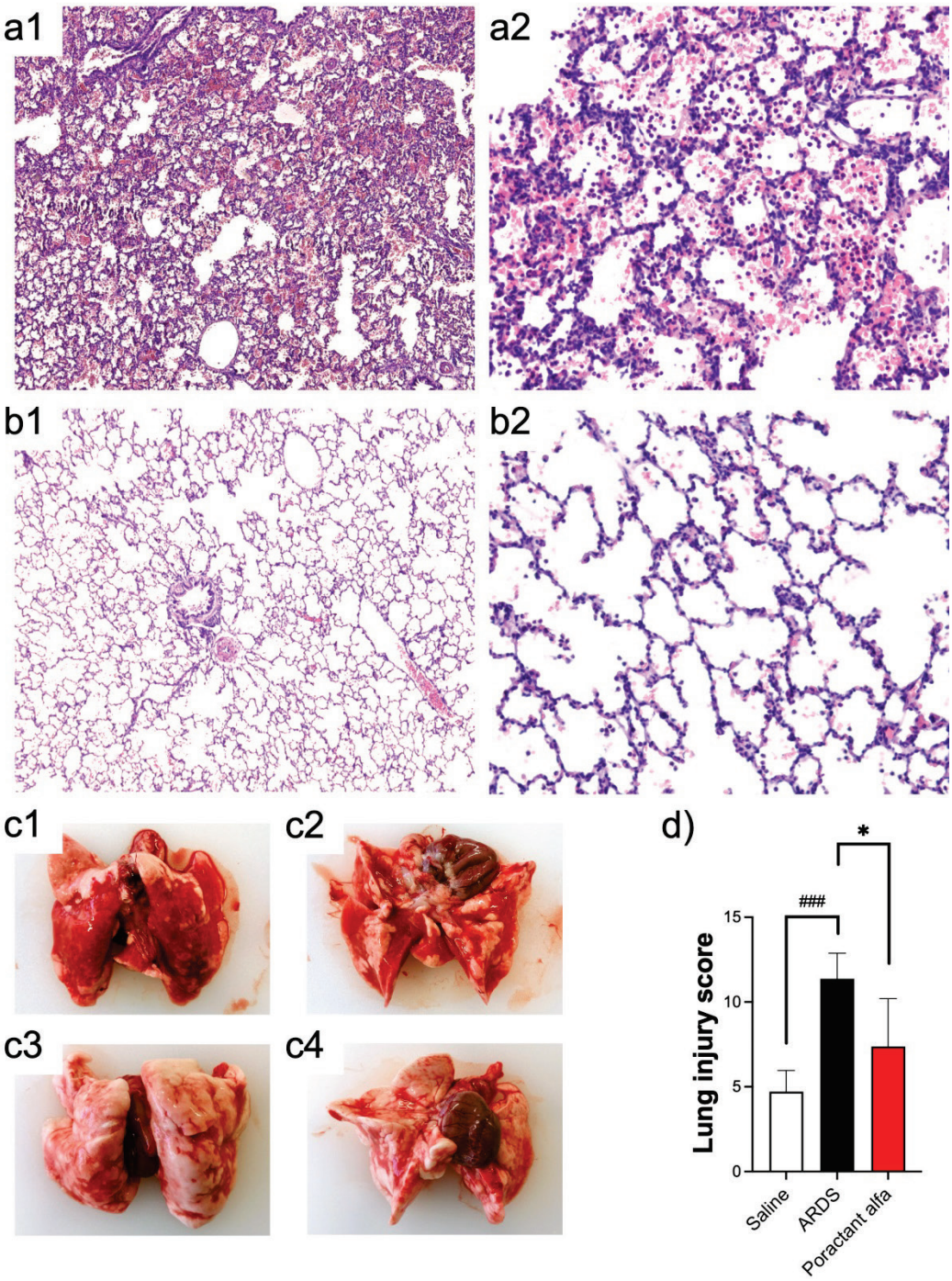

Fig. 5. Histological analysis. Lung sections of untreated ARDS animals (**a1, a2**) and Poractant alfa treated animals (**b1, b2**); the macroscopic appearance of the ARDS lungs (**c1 ventral, c2 dorsal part**) and poractant alfa (**c3, 4**); total lung injury score (**d**) after the experiments. In ARDS group, the pulmonary parenchyma showed a diffuse miscellaneous inflammatory cell infiltrate, predominantly neutrophils and plasmocytes with activated pneumocytes, and erythrocytes, collapsed alveoli, massive hyaline membranes and protein debris (**a1, 2**). In Poractant alfa group, the lung showed a normal appearance with aerated alveolar spaces, but the slightly thickened alveolar septa displayed rare inflammatory cell infiltrate (**b1, 2**). Macroscopically extensive atelectasis was observed on the lung surface in ARDS group (**c1, 2**) compared to poractant alfa (**c3, 4**). The magnification 50× was used for a1, 2 and 200× for b1, 2. Data are presented as means ± SD. Statistical comparisons: $^{###}$ $p<0.001$, * $p<0.05$.

In an effort to resemble a clinical situation with two deleterious factors inducing lung injury, a combination of intratracheal instillation of HCl and high-volume ventilation was used to induce ARDS-like damage in adult rabbits. The biophysical forces associated with mechanical ventilation might contribute to ventilator-induced lung injury (VILI) characterized by increased inflammation and permeability of the alveolar-capillary membrane [31]. Animals ventilated with the high-volume strategy had markedly more severe lung injury compared to animals ventilated with the high-pressure low-volume strategy [32-34]. Ventilation with high lung volumes leads to alveolar rupture, air leakage, and regional lung overdistension [4]. This model may be particularly relevant for capturing the clinically relevant multifactorial etiology of ARDS and for studying direct ARDS that develops after acid aspiration followed by harmful mechanical ventilation.

After induction of the lung injury model, the lung function parameters (P/F, OI, AaG) of the control group remained worsened until the end of the experiment, which indicates that our two-hit model of ARDS is stable. The two-hit intervention caused the parameters P/F, OI, AaG, Paw and C_{stat} to deteriorate within minutes, which is consistent with earlier findings [15,35,36]. Once the criteria for ARDS were met, surfactant therapy was administered as a bolus intratracheally, and animals were ventilated for an additional four hours. Treatment with poractant alfa improved P/F, OI, AaG parameters markedly 15 min after the instillation of bolus and this effect persisted until the end of the experiment. Improvement in Paw and C_{stat} after surfactant therapy appeared slightly later. Similar results were observed in previous studies, in which surfactant therapy was administered in various animal models of ARDS [14,15,37,38]. The 'open lung effect' induced by surfactant delivery was also seen in the macroscopic appearance, with less atelectasis regions on the lung surface after poractant alfa therapy compared to untreated injured lungs.

In response to lung damage, there is a significant influx of leukocytes, particularly neutrophils, from the circulation to the interstitium and alveolar gaps occurs [39]. In our study, the alveoli displayed an acute cell reaction with predominant neutrophils and plasmocytes and activated pneumocytes in the histological lung section at four hours after ARDS induction. The activation of neutrophils and macrophages is associated with the production of pro-inflammatory cytokines [35,40,41]. Furthermore, activated neutrophils and linked oxidative bursts can cause oxidative damage of proteins and lipids [42]. In our two-hit ARDS model, increased levels of pro-inflammatory cytokines (TNFα, IL-1β, IL-6, and IL-8) and markers of protein nitrosylation (3-nitrotyrosine, 3NT) and lipid peroxidation (TBARS) were observed in plasma. These observations are consistent with previous findings [21,43-45].

The critical point determining the success of ARDS therapy is the reduction of the inflammatory response in the early phase of ARDS. In this study, treatment with poractant alfa significantly reduced inflammation and oxidative modification and reduced lung edema compared to the untreated ARDS group. The surfactant has immunomodulatory characteristics that regulate innate and acquired pulmonary immunity and inflammatory processes [46]. Surfactant is known to decrease the release of pro-inflammatory cytokines and chemokines and reduce the activation of lung inflammatory cells such as neutrophils, and macrophages [46,47]. Therefore, the therapeutic efficacy of the surfactant is related not only to its biophysical characteristics but also to its anti-inflammatory features. In this study, the anti-inflammatory benefit of surfactant was demonstrated by the levels of inflammatory and oxidative markers (TNFα, IL-1β, IL-6, 3NT and TBARS) being significantly lower than those observed in the control group. The potent anti-inflammatory and linked antioxidative properties of natural surfactants have been described previously [48].

Inflammatory mediators and bioactive substances cause endothelial and epithelial cells to be damaged, resulting in increased permeability across the alveolar-capillary membrane and the formation of pulmonary edema [42,49]. Lung edema is one of the manifestations of ARDS that might advance to hypoxemia and impaired carbon dioxide excretion [50]. Lung wet/dry (W/D) weight ratio has been shown to reflect the integrity of the alveolar-capillary barrier and the degree of pulmonary edema [51,52]. In the current work, the ARDS group showed increased lung edema formation and protein content in BALF, while treatment with poractant alfa significantly reduced these changes. It may be related to reducing the surface tension of the alveoli by surfactant and therefore reducing collapsibility of the alveoli and subsequent prevention of fluid transduction [53].

The respiratory failure induced by two-hit could mimic the primary alveolar permeability problem in

ARDS, due to deterioration to alveolo-capillary membrane. Experimental setting in this study leads to flooding of the alveolar spaces with proteinaceous edema, which may contain potent surfactant inhibitors, such as albumin, fibrinogen [54]. Serum proteins may compete with surfactant aggregates, reducing surfactant adsorption (e.g. albumin). In addition, the inflammatory processes and associated oxidation of protein contribute to surfactant inactivation [55-57]. Upon pathological mechanisms, the presence of proteins which are not normally found in the alveolar space may also affect other biological functions by interacting with surfactant-proteins. The inflammation processes and secretory phospholipase A2 (sPLA2) produced by alveolar macrophages, facilitating surfactant phospholipid hydrolysis and production of inflammatory mediators, including the release of free fatty acids, which may in turn change surfactant fluidity and structure, significantly impacting on surfactant biophysical function [57,58]. This explains the pathobiological complexity of ARDS in context of surfactant. The syndrome, although appears with a common clinical trait, may have different underlying mechanisms of injury. In bronchoalveolar lavages, edema fluid, or endotracheal aspirates from patients with ARDS or other disorders involving lung injury, there have been observations of impairments in lung surfactant activity and deficits in the content or composition of active large surfactant aggregates [59]. Therefore, the lung environment of ARDS is destructive not only for endogenous but also for exogenous surfactant and their activity. Exogenous surfactant therapy seems not effective when airspaces are flooded with inhibitory serum proteins [60]. There are several strategies to protect surfactant against inactivation; e.g. more surface-active or catabolism-resistant surfactants production, surfactant protection using direct inhibitors or reduction of inflammation and oxidation, and higher or repeated surfactant doses [57]. Last mentioned strategy could improve oxidation and thus prevent high tidal ventilation and associated VILI of patient with severe ARDS, which can be considered a benefit of this treatment. Exogenous surfactant can replace inactivated surfactant, expanding the supply of intraalveolar surfactant components and boosting the formation of endogenous surfactant, thereby increasing the ratio of surfactant to inhibitors in alveoli [61]. Additionally, the use of surfactant therapy in ARDS suggests greater effectiveness in cases of direct pulmonary injury as opposed to indirect forms [62-64].

This work has a number of limitations, including the fact that our experimental technique determined V_T based on body weight rather than inspiratory capacity. Even though most published research utilized an approach similar to ours, there may be more variation in the degree of lung damage as a result. We only analyzed the effects of poractant alfa (inflammatory process and gas exchange) after 4 h, thus we can't comment on how lung damage would progress. The early and acute stage of ARDS was the study's main focus. Long-term consequences or mortality were beyond the scope of this investigation. For the purpose of reducing the impact of any possibly confounding factors, all rabbits were of the same gender. To simulate the human clinical condition, we did not begin the trial when the animals were infected with ARDS, but rather when all animals had met the criteria for ARDS. Furthermore, we use acid with a pH of 1.25, which is lower than the pH usually used in these models, because most ICU patients have gastric juice pH ranging from 3.0 to 4.0, and pH 4.0 has been linked to lung harm, suggesting that this model is clinically relevant. Human aspiration of gastric fluid is not merely inhalation of HCl, but of more complex gastric contents such as particulate debris, bacterial products, and cytokine suspensions. Food particles, which combine synergistically with acid to cause lung injury, are not included in the solutions we provide. This omission may limit our model's physiologic validity. We work with initially normal lungs with normal endogenous surfactant levels, although surfactant levels can be altered in ARDS.

In conclusion, the objective of this study was to determine the effect of the natural modified exogenous surfactant, the poractant alfa, on lung function parameters, inflammation, and lung edema in an established two-hit model of ARDS in rabbits. Our results demonstrated that poractant alfa decreased the levels of pro-inflammatory cytokines and markers of oxidative stress and reduced the formation of lung edema. Mitigation of surfactant dysfunction, a primary effect of surfactant in the alveoli, and inhibition of inflammation alleviated respiratory insufficiency, as shown by an improvement in the parameters of lung function. Therefore, exogenous surfactant appears to be a valuable option to maintain oxygenation in severe ARDS, but further research in this field is necessary.

Conflict of Interest

There is no conflict of interest.

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