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A Novel Experimental Model of Acute Respiratory Distress Syndrome in Pig

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Summary

Acute respiratory distress syndrome (ARDS) is severe medical condition occurring in critically ill patients and with mortality of 33-52 % is one of the leading causes of death in critically ill patients. To better understand pathophysiology of ARDS and to verify novel therapeutical approaches a reliable animal model is needed. Therefore we have developed modified lavage model of ARDS in the pig. After premedication (ketamine and midazolam) 35 healthy pigs were anesthetized (propofol, midazolam, morphin, pipecuronium) and orotracheally intubated and ventilated. Primary ARDS was induced by repeated cycles of lung lavage with a detergent Triton X100 diluted in saline (0.03 %) heated to 37 °C preceded by pre-oxygenation with 100 % O_2. Single cycle included two subsequent lavages followed by detergent suction. Each cycle was followed by hemodynamic and ventilation stabilization for approx. 15 min, with eventual administration of vasopressors according to an arterial blood pressure. The lavage procedure was repeated until the paO_2/FiO_2 index after stabilization remained below 100 at PEEP 5 cm H_2O. In 33 pigs we have achieved the desired degree of severe ARDS (PaO_2/FiO_2<100). Typical number of lavages was 2-3 (min. 1, max. 5). Hemodynamic tolerance and the need for vasopressors were strongly individual. In remaining two animals an unmanageable hypotension developed. For other subjects the experimental ARDS stability was good and allowed reliable measurement for more than 10 h. The present model of the ARDS is clinically relevant and thus it is suitable for further research of the pathophysiology and management of this serious medical condition.

Key words

Acute respiratory distress syndrome • Animal model • Pig • Lavage

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Introduction

Acute respiratory distress syndrome (ARDS) is severe medical condition occurring in critically ill patients triggered by an injury to the alveolocapillary membrane from any of a variety of causes, resulting in fluid accumulation and an acute respiratory failure with severe hypoxemia, which requires arteficial ventilation support. In contrast to substantial progress in understanding of its pathophysiology, ARDS still has high mortality of 33-52 % which is one of the leading causes of death in critically ill patients (Ranieri 2012).

In 1967, Ashbaugh described firstly ARDS in 12 patients with severe acute respiratory failure which had severe hypoxemia that was refractory to supplementation of oxygen, but which in some cases was responsive to the application of positive endexpiratory pressure (PEEP) (Ashbaugh 1967). Pulmonary inflammation, edema, and hyaline membranes were observed on autopsy of these patients. In 1994, the American-European Consensus Conference (AECC),

published a definition that defined ARDS as a respiratory failure with acute onset of hypoxemia (the ratio of partial pressure of arterial oxygen measured in mm Hg to fraction of inspired oxygen, PaO_2/FiO_2<200 mm Hg) with bilateral infiltrates on X-ray, and in the absence of left atrial hypertension. They also defined a new entity termed acute lung injury (ALI), which used the same definition but with a less stringent criterion for hypoxemia (PaO_2/FiO_2<300 mm Hg) (Bernard 1994). Since 2011 a new "Berlin" definition is used that divides the severity of the condition into three degrees, mild ARDS: 300 mm Hg>PaO_2/FIO_2>200 mm Hg, moderate ARDS: 200 mm Hg>PaO_2/FIO_2>100 mm Hg, severe ARDS: PaO_2/FIO_2≤100 mm Hg (Ranieri 2012).

Primary and secondary ARDS differs from which side of the alveolocapillary membrane the triggering insult in the lungs emerges (Pelosi 2003). Primary (pulmonary, direct) damage to the lungs can be: aspiration, diffuse pulmonary infections (bacterial, viral, etc.), drowning, toxic inhalation or lung contusion. Secondary (extrapulmonary, indirect) damage to the lungs can occurre in: shock, sepsis, systemic inflammatory response syndrome (SIRS), polytrauma without a primary chest injuries, massive transfusion (transfusion-related acute lung injury – TRALI), burn trauma, pancreatitis, cardiopulmonary bypass, systemic intoxication, disseminated intravascular coagulation (DIC), malignancy or eclampsia.

ARDS developes in three pathophysiological phases: i) first acute-exudative, with increased lung permeability, ii) second subacute, where there is a capillary and alveolar obstruction due to inflammation and tissue damage due to the disbalance between coagulation and fibrinolysis, and there is a reduced production of surfactant with possibility of damage to the lung parenchyma by mechanical ventilation, and iii) late post reparation stage when we can find a remodeling of the pulmonary parenchyma and fibroproliferative changes.

Animal models provide a bridge between clinical and the laboratory research. Hypotheses based on clinical observations can be directly tested in animal models. To reliably mimic human ARDS an animal model should reproduce the acute injury to the epithelial and endothelial barriers in the lungs together with the acute inflammatory response. Ideally, the injury should evolve over the time if the animals are supported for prolonged periods. One of the most difficult aspects of modeling ARDS is that the lungs of humans can be affected by the mechanisms involved in the primary illness (e.g. sepsis) and/or they can be affected by therapeutic modalities used for supportive care (e.g. mechanical ventilation). This increases the complexity of the ideal animal model of lung injury, as it requires the incorporation of specific treatment modalities into experimental protocols, adding additional variables to the experimental design. No single animal model reproduces complete characteristics of ARDS and most of the existing experimental models are relevant for limited aspects of known clinical situations (Matute-Bello 2008).

In the course of evaluating novel treatment strategies to ARDS three approaches in experimental conditions to model ARDS are widely used: the surfactant washout lavage model (LAV model), intravenous injection of oleic acid (OAI model) or endotoxin, lipopolysaccharide (LPS model).

LAV model

Saline lavage model was developed by Lachmann *et al.* (1980), based on the observation that ARDS is associated with depletion of surfactant from the air spaces and reduced concentrations of surfactant-associated proteins in bronchoalveolar lavage (BAL) fluid.

Mechanism of lung injury by repeated lavage with saline reduces the surfactant lipid concentration in alveolar lining fluids, alters alveolar surface tension, facilitates alveolar collapse and increases the likelihood of mechanical injury to the alveolar walls during repeated cycles of alveolar opening/closure during mechanical ventilation. Surfactant depletion leads to the injury of the alveolar epithelium and to the exudation of protein-rich edema fluid into the alveolar spaces. It is characterized by increased protein permeability, infiltration of polymorphonuclear neutrophils (PMN) into the air spaces and interstitium, accumulation of PMNs and dysregulation of their activation, increased cytokine production (TNF), and in the later stages by hyaline membrane formation (Matute-Bello 2008, Wang 2008). The acute morphologic changes show some similarities with human ARDS as perivascular edema, large areas of atelectasis which decrease lung compliance and impair gas exchange. The alveolar-capillary membrane is also thickened and severely deformed. Alveolar type I. cells appear necrotic while alveolar type II. cells are largely unaffected. Unlike the lung morphology of ARDS patients, there is no severe epithelial damage reported with this animal model. However, repeated lavage combined with an injurious ventilation strategy does produce significant epithelial damage (Ballard-Croft 2012).

Surfactant washout protocols considerably vary between induced lung injury studies. Lung lavage is commonly performed in adult pigs, dogs and sheep (20-40 kg) with heated normal saline (37-39 °C) alone or with low concentrations of detergent, such as 0.2 or 0.5 % of Tween 80 to further inactivate the surfactant (Kobayashi 1996, Musch 2004). With the animal in the supine position, the bilateral lungs are lavaged repeatedly with an aliquot of 10-30 ml/kg of fluid per lavage cycle until the targeted lung injury level is achieved (Wang 2008, Lethvall 2008, Kirmse 1998, Fuchs 2005, Zick 2006, Bellardine Black 2007). In order to acquire a uniform degree of injury in the whole lung, some researchers (Musch 2004, Bellardine Black 2007) shift the animals from the supine to the prone position or vice versa between the lavages. The criteria for the target lung injury are typically set as the PaO_2/FIO_2<100 (Wang 2008). The major advantage of this model is that it provides an ideal way to test the effects of different ventilatory strategies on the development of tissue injury because the tissue injury results simultaneously from the ventilatory strategy than and from the saline lavage and surfactant depletion. The main technical disadvantage of this model is that the animals require intubation, mechanical ventilation, and general anesthesia. Another reported disadvantage of this model is rapid reversibility (Ballard-Croft 2012, Kloot 2000, Wang 2008) making this model unsuitable for studies investigating the inflammatory pathophysiology of ARDS (Ballard-Croft 2012).

OAI model

The oleic acid model was first developed to mimic ALI/ARDS that is caused by fat embolism because oleic acid is the most prevalent fatty acid in pulmonary emboli (Schuster 1994, Ballard-Croft 2012). The OAI model is usually performed by continuous infusion of OA into the central vein through a central venous catheter, widely used dose of OA is 0.06-0.15 ml/kg, which is mixed thoroughly with 15-20 ml of normal saline and injected slowly by a syringe pump within 20-30 min to achieve PaO_2/FIO_2 ratio of 80-120 mm Hg. Sometimes a smaller dose 0.01 ml/kg is used to produce a very slight lung injury or a dose as high as 0.3 ml/kg (Kloot 2000, Ballard-Croft 2012) is applied to achieve a quite severe lung injury. Apart from the normal saline, pure or high concentration of ethanol or freshly drawn arterial blood has been used as the dilution liquid for oleic acid. Membrane damage caused by the direct binding of oleic acid to biological membranes and direct toxicity to endothelial cells play a role in oleic acid-mediated lung injury. Similar to clinical ARDS, oleic acid produces a lung injury that is morphologically heterogeneous with areas of minimal lung damage alongside areas of severe injury. Morphological changes in the early phase of oleic acid-induced lung injury occur rapidly within minutes, with endothelial cell necrosis followed by alveolar I. epithelial cell necrosis with both cell types detaching from their basement membranes, at 2 to 3 h after oleic acid exposure, severe capillary congestion and interstitial/ intraalveolar edema are present (Ballard-Croft 2012).

The hemodynamic response to infusion of oleic acid is rapid, with severe hypoxemia occurring within hours. Pulmonary hypertension with increased pulmonary artery pressure and pulmonary vascular resistance and the right-side heart failure with decreased mean arterial pressure and cardiac output may lead to sudden death as a complication of this model.

LPS model

Sepsis is the most common cause of secondary human ARDS. One of the key mediator of sepsis-induced ARDS is endotoxin / lipopolysaccaride (LPS), a proinflammatory molecule from the outer membrane of gramnegative bacteria. LPS used to induce ARDS in pigs, dogs and sheep is usually extracted from *Escherichia coli*. In pigs and sheep, a dosage of 1-100 ug/kg of LPS is diluted in normal saline and infused intravenously over a half hour to several hours (Wang 2008, Ballard-Croft 2012). In dogs, extremely high doses of endotoxin (1.1 mg/kg) are routinely needed to induce the ARDS symptomatology due to their tolerance to endotoxin.

The most prominent alteration was the sequestration of PMNs in the alveolar septa, spaces and interstitium accompanied by congestion, hemorrhage and interstitial edema which caused a thickening of alveolar walls, another alteration was the dramatically increased number of alveolar macrophages. This model is associated with cardiac instability, with an initial increase of cardiac output and mean arterial pressure followed by a significant drop down, due to changes in systemic vascular resistance. Pulmonary hypertension due to pulmonary vasoconstriction can also accompany this ALI/ARDS model. LPS model has some significant disadvantages, preparations vary in purity and can be contaminated with bacterial lipoproteins and other bacterial materials. LPS model does not cause the severe endothelial and epithelial injury that occurs in humans with ARDS (Matute-Bello 2008). The development of

a stable lung injury by these dosages of LPS infusion usually takes several hours (Matute-Bello 2008, Wang 2008).

Comparison between human ADRS and three commonly used ADRS models is shown in Table 1.

Table 1. Comparison of various ARDS models.

Pathological characteristics	Human ALI/ARDS	Animal LAV model	Animal OAI model	Animal LPS model
ARDS category	pulmonary/ extrapulmonary	pulmonary	extrapulmonary	extrapulmonary
Oxygenation	↓↓↓	↓↓↓	↓↓↓	↓↓↓
Compliance	↓↓↓	↓↓↓	↓↓↓	↓↓↓
Atelectasis	+++	+++	++	++
Neutrophil infiltrate	+++	+	+++	+++
Macrophage infiltrate	++	+/-	++	++
Alveolar proteinaceous edema	+++	+/-	+++	+++
Perivascular/peribronchial edema	+++	+++	+++	+++
Endothelial cell necrosis	+++	+	+++	+++
Epithelial cell necrosis	+++	+	+++	+++
Congestion	++	+	+++	+++
Alveolar hemorrhage	+	+/-	+++	+++
Intravascular coagulation	+	?	+	+
Extravascular coagulation	+	?	++	?
Reversibility	slow	rapid	several hours	several hours
Time to induce	hours	minutes to hours	several (>2) hours	several (2-4) hours
Stability of model		tend to improve	improve after several hours, unpredictable, may cause sudden death	several hours

The major disadvantages of used LAV model is rapid reversibility, major problem of oleic acid model is unpredictable hemodynamic instability and LPS model usually achieves clinical relevance after long time.

The aim of this study was to establish clinically relevant experimental model of acute respiratory distress syndrome in pigs with rapid onset and stable in time thus suitable for preclinical research of this live threatening condition.

Methods

All animal experiments were performed in an accredited animal laboratory at the Institute of Physiology, First Medical Faculty, Charles University, Prague, in accordance with Act No. 246/1992 Coll., on the protection of animals against cruelty. 35 crossbred Landrace female pigs (*Sus scrofa domestica*) with an average body weight of 48 kg (35-60 kg) underwent ARDS protocol over four years. The animals were premedicated with midazolam 15 mg i.m. followed by ketamim (Narketan 10 %) 20 mg/kg = 10 ml i.m. Animals were placed in supine position on a heated pad, temperature was kept in the normal range for pig (38-39 °C). After cannulation of ear vein the induction of anesthesia was performed by propofol 2 % 100 mg and then animals were intubated with a cuffed endotracheal tube (I.D. 7.5 mm) and connected to a conventional ventilator Hamilton G5 (Hamilton Medical, Bonaduz, Switzerland). Anesthesia was maintained as TIVA (total intravenous anesthesia) by continuous infusion of propofol 2 % (8-10 mg/kg/h IV) combined with morphine

1 % (0.1-0.2 mg/kg/h IV) and midazolam (5 mg/h IV). Myorelaxation was maintained by pipecuronium (4 mg boluses every 45 min). Initial rapid infusion of 1 000 ml of normal saline was given intravenously, followed by a continuous infusion of 100-250 ml/h to reach and maintain CVP (central venous pressure) of 6 to 7 mm Hg. Continuous infusion of heparin (500 IU/kg/h IV) was used to prevent clotting in the catheters. Mean arterial pressure (MAP) >50 mm Hg was maintained by continuous infusion by Norepinephrine (0-0.6 ug/kg/min IV). Venous and arterial cannulas for monitoring of central venous pressure (CVP, femoral vein), pulmonary artery pressure (PAP, jugular vein) and arterial blood pressure (ABP, a. femoral artery) were inserted. Vital sign monitoring (ECG, ABP, SpO_2, PAP, $etCO_2$) was performed by monitor BSM - LIFE SCOPE (Nikoh Kohden, Japan). Arterial blood gases, i.e. partial pressure of oxygen (PaO_2), carbon dioxide ($PaCO_2$) and pH, were continuously measured by CDI 500 (Terumo, Tokyo, Japan*)*. All respiratory parameters including static compliance (Cstat), positive end-expiratory pressure (PEEP), mean airway pressure (Pmean) were obtained from Hamilton G5 (Hamilton Medical, Bonaduz, Switzerland). All the signals were recorded synchronously using a LabChart system (ADInstruments, Oxford, UK).

To induce primary ARDS repeated cycles of lung lavage with nonionic detergent Triton X100 (T8787 Sigma Aldrich) diluted in normal saline (0.03 %, Triton 0.45 ml in 1500 ml 0.9 % NaCl, 25-42 ml/kg) were performed. Each cycle included two subsequent lavages followed by airway suction. The maneuver lasted up to 120 s, and was typically performed after pre-oxygenation with FiO_2=1.0 for 5 min. Retrieved lavage solution was opaque and after 2-3 cycles it had typical light pink color. Each cycle was followed by hemodynamic and ventilation stabilization for approx. 15 min, with eventual administration vasopressors according to MAP. Ventilator settings was Adaptive Support Ventilation (ASV), minute ventilation 180 % of body weight-predicted value PEEP 5, FiO_2 1.0 or 0.5 to keep SpO_2>90 %. The lavage procedure was repeated until the paO_2/FiO_2 index after stabilization remained below 100 at PEEP 5 cm H_2O.

Statistic

From a total of 35 animals that underwent the ARDS protocol 15 were included in statistical analysis of paO_2/FiO_2 (p/F) and compliance (C). There were no obvious differences between included and excluded animals, but complete, high quality data both during induction and follow-up were obtained only in 15 animals.

For statistical analysis of paO_2/FiO_2 (p/F) and compliance (C) we used non-parametric Friedman test for repeated measurements and Dunn's tests for between pairs defined conditions.

Results

33 out of 35 animals survived the protocol. The desired degree of severe ARDS (i.e. PaO_2/FiO_2<100) was achieved in all survivors. Typical number of lavages needed was 2-3 (min. 1, max. 5). Figure 1 shows a typical course of the experiment-induction of ARDS and following recruitment maneuvers. After the second lavage (LAV2) the paO_2/FiO_2 index was consistently below 100, followed by improvement after recruitment maneuver(s). As demonstrated in Figures 2 and 3 there was a significant decrease of paO_2/FiO_2 (p/F) index after lavage 2 (base vs. LAV2 p=0.001), and decrease of compliance (C, ml/cm H_2O) after lavage 1 and lavage 2, (base vs. LAV1 p=0.01, base vs. LAV2 p=0.01). There were no significant differences between lavage 1 and 2 and 3 compared with the final status (LAV1 vs. final NS, LAV2 vs. final NS, LAV3 vs. final NS), which indicates clinically proven stability of this model.

Hemodynamic tolerance and need for vasopressor was strongly individual (Norepinephrine 0-0.5 mcg/kg/min). In two animals an unmanageable hypotension developed and despite an early start of cardiopulmonary resuscitation (CPR) these two pigs died before completion of the experiments (mortality 5.7 %). For the others the experimental ARDS stability was good and allowed for experimental measurement for more than 10 h. The average duration of the protocol (LAV1 – FINAL) was 7:18 (SD 1:17, h:min).

Histopathological findings were similar to human ARDS. Figure 4 demonstrates a typical perivascular edema and the lymphatics which appear dilated (arrows on panel A), panel B shows perivascular and peribronchial edema. The lungs exhibit typical overdistended and atelectatic changes (see panel C). The majority of alveoli contain no cells. The alveolar-capillary membrane is thickened and we can find alveolar epithelium with denudation of the basement membrane (panel D).

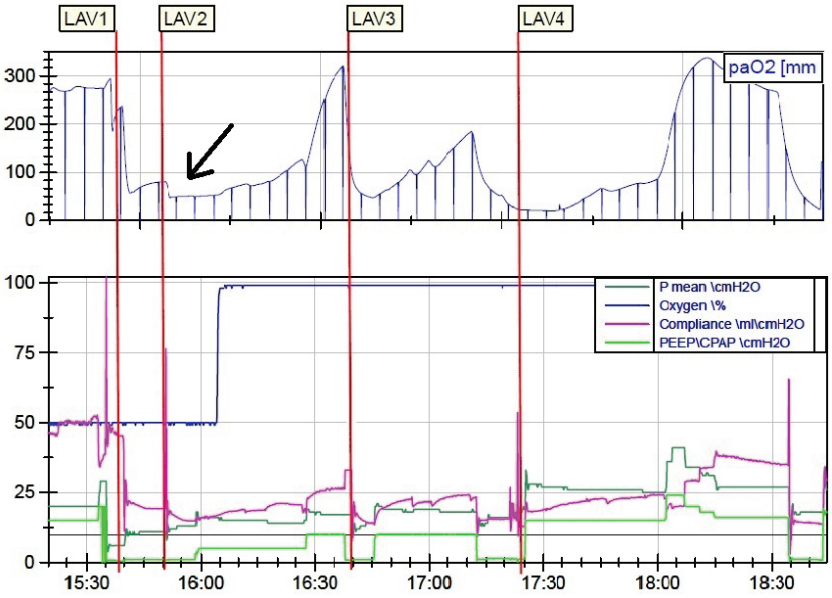

Fig. 1. A typical course of the experiment – induction of ARDS and following recruitment maneuvers, after the second lavage (LAV2) is an index paO_2/FiO_2 stable below 100 (arrow), followed by improvement after recruitment maneuver.

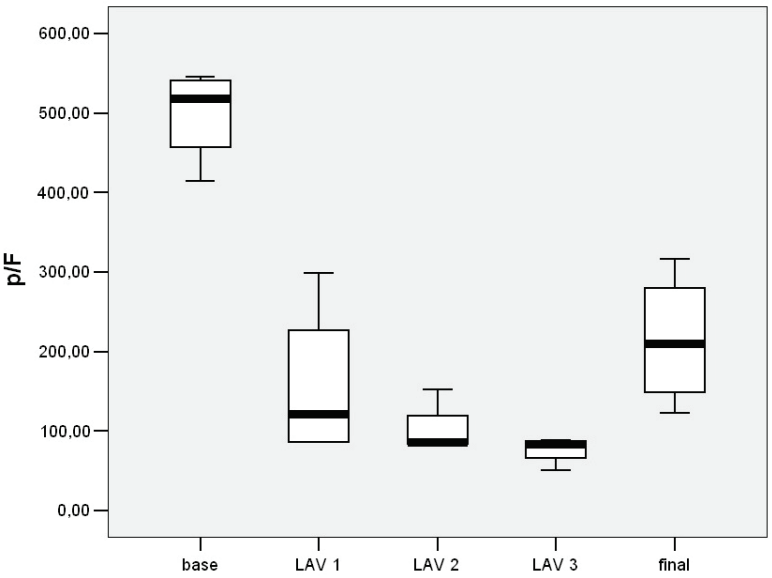

Fig. 2. Changes of paO_2/FiO_2 (p/F) index after each lavage, base vs. LAV1 NS, base vs. LAV2 p=0.001, LAV1 vs. final NS, LAV2 vs. final NS, LAV3 vs. final NS. LAV – lavage, base – baseline reading, final – final reading at protocol termination.

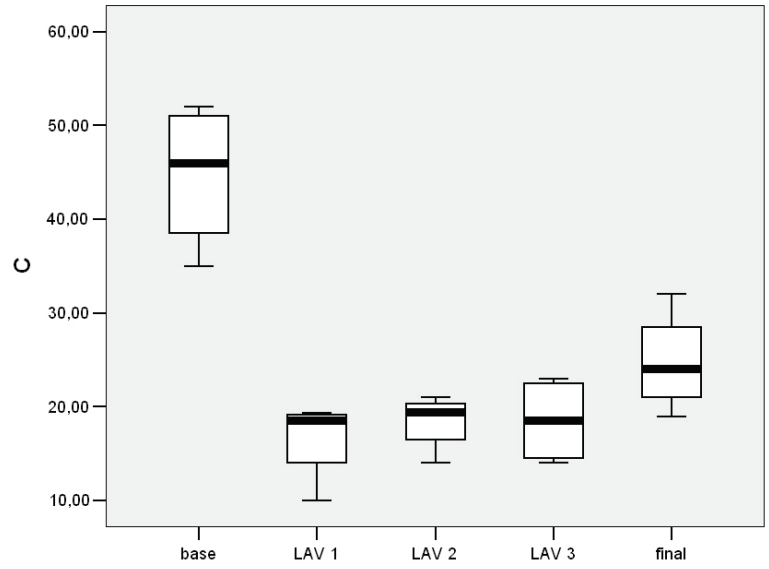

Fig. 3. Changes of compliance (C, ml/cm H_2O) after each lavage. Base vs. LAV1 p=0.01, base vs. LAV2p=0.01, LAV1 vs. final NS, LAV2 vs. final NS, LAV3 vs. final NS. LAV – lavage, base – baseline reading, final – final reading at protocol termination.

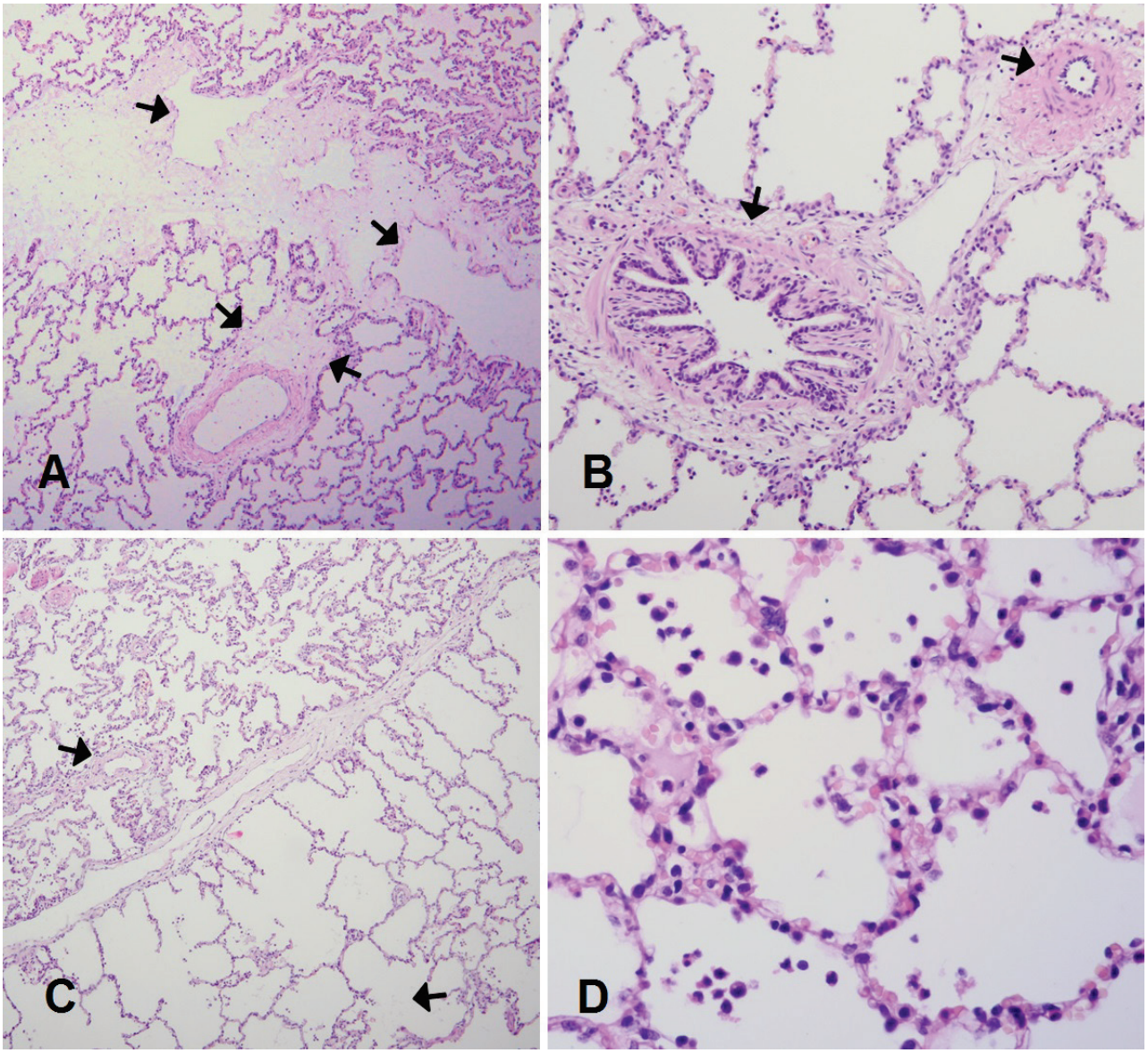

Fig. 4. Histopathology of the lungs. **A.** Perivascular edema and the lymphatics appear dilated (arrows) (hematoxylin-eosin staining (H&E), x100). **B.** Perivascular and peribronchial edema (arrows) (H&E, x400). **C.** The lungs showed overdistended and atelectatic changes (arrows). The majority of the alveoli contain no cells (H&E, x100). **D.** The alveolar-capillary membrane is thickened. Alveolar epithelium with denudation of the basement membrane (H&E, x600).

Discussion

Our major aim was to achieve a rapid induction, yet durable ARDS model for preclinical research purposes and for clinical training of ventilation strategies during severe ARDS. Therefore we have developed our Triton X100 LAV model. Both conventional and later also nonconventional modes of mechanical ventilation were successfully used with the ARDS model (data not shown). Based on ventilatory, biochemical and histopathology data we consider the model very relevant and reproducible.

Pulmonary functions and histopathology

Already after the first lavage, the lung compliance (Cstat) dropped rapidly to very low values (<20 vs. >40 ml/cm H_2O at baseline). Also index paO_2/FiO_2 declined sharply to nearly the target value of 100. However, at this stage the model is easily reversible by increased oxygen fraction in the inspired air and/or increased PEEP. After the second lavage the model met the criteria for severe ARDS but in some subjects it could still be partially reverted to less severe stages (ALI) requiring further lavage/s. However, too many lavages resulted in hemodynamic failure and/or ARDS that could not be managed by neither conventional

nor non-conventional ventilation. Nonetheless, we consider the inducibility rapid since 2-3 lavages were typically sufficient compared to 8 described by others (Ballard-Croft 2012). We attribute this to using Triton X100 detergent in our lavage. Triton could also be responsible for greater lung injury than just the washout of surfactant with the normal saline. As seen in the histological findings, the typical histopathology signs of human ARDS were found in presented model.

Hemodynamic stability

In all experimental ARDS models hemodynamics is challenged. In our model the hemodynamic response to lavages varied greatly (from no change to cardiac arrest) but almost always it could be rather easily managed by timely and careful administration of vasopressors. Probably even more important safety factor was the preoxygenation and strict limiting the maneuver duration to below 120 s. Generally, we consider the presented model very safe in terms of unexpected hemodynamic failure.

Model stability

The ARDS naturally tends to develop in time since over the time more pathophysiological mechanisms are involved such as ventilator/lung interaction, immune response, activation of coagulation and others. The effect of lavage tends to weaken over the time while other mechanisms (ventilator injury) contribute to ARDS. Optimally, the balance is established between the two trends. Our study was not aimed at long-term stability and the ventilation protocol (i.e. regimen, airway pressure, use of recruitment maneuvers) was not identical in all subjects. Despite this variability the compliance and oxygenation index did not significantly vary from LAV1 or LAV2 values thus indicating reasonable stability over about 7 h.

Limitations

This modified LAV model shows some typical drawbacks of a model of primary ARDS. The initial lavage does not resemble any typical clinical insult leading to ARDS. Even very prompt clinical ARDS would evolve over many hours before a patient is treated by mechanical ventilation, so systemic inflammatory response would already be underway. Detergent that seems to help greatly in maintaining the ARDS condition is quite rarely an ethiological agent in clinical setup. Since most of the subjects were used for clinical training of severe ARDS management, the ventilation protocol was not unified among individual animals thus long term data include rather large variability.

In conclusion, the present novel experimental ARDS model in pigs has significant advantages compared to existing ones. These include rapid onset (hours), good reproducibility, low mortality and improved stability over the time (10 h). Since histopathology indicates good clinical relevance, we consider that the model well suited for preclinical research and testing of novel approaches in the management of a life threatening condition such as ARDS.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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Abbreviations

ABP – arterial blood pressure, ALI – acute lung injury, ARDS – acute respiratory distress syndrome, ASV – adaptive support ventilation, Cstat – lung compliance, static, $etCO_2$ – end-tidal CO_2 concentration, FiO_2 – fraction of inspired oxygen, H&E – hematoxylin-eosin, IV – intravenous, LAV – lavage, LPS – lipopolysaccharide, MAP – mean artery pressure, NS – not significant, paO_2 – O_2 partial pressure, PAP – pulmonary artery pressure, PEEP – positive end-expiratory pressure, Pmean – mean airway pressure, PMN – polymorfonuclear/s, SIRS – systemic inflammatory response, SpO_2 – saturation of hemoglobin with oxygen in peripheral arterial blood, TIVA – total intravenous anesthesia, TNF – tumor necrosis factor.

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