EXPERIMENTAL MODELS OF ACUTE LUNG INJURY: THEIR ADVANTAGES AND LIMITATIONS

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Abstract

Acute damage to the lung may originate from various direct and indirect reasons. Direct lung injury may be caused by pneumonia, near-drowning, aspiration, inhalation of toxic gases etc., while indirect lung injury is secondary, following any severe extra-pulmonary disease, e.g. sepsis, acute pancreatitis, or severe trauma. Due to a complex pathophysiology of the acute lung injury, the treatment is also extremely complicated and except for lung-protective ventilation there have been no specific treatment approaches recommended. An urgent need for a reliable and sufficiently effective treatment forces the researchers into testing novel therapeutic strategies. However, most of these determinations should be done in the laboratory conditions using animals. Complex methods of preparation of various experimental models of the acute lung injury has gradually developed within decades. Nowadays, there have been the models of direct, indirect, or mixed lung injury well established, as well as the models evoked by a combination of two triggering factors. Although the applicability of the results from animal experiments to patients might be limited by many factors, animal models are essential for understanding the pathophysiology of acute lung injury and provide an exceptional opportunity to search for novel therapeutical strategies.

Key words: acute lung injury, acute respiratory distress syndrome, direct lung injury, indirect lung injury, animal model

INTRODUCTION

Acute lung damage has been classified under the nosological unit termed *acute respiratory distress syndrome* (ARDS). ARDS may result from various reasons and occur in all age groups. Diffuse alveolar damage, lung edema, inflammation, and ventilation-perfusion mismatch finally lead to a decreased lung compliance, pulmonary hypertension, and severe hypoxemia (1).

The incidence of ARDS in adults varies between 30–80 per 100,000 population due to genetic variability in the geographical regions, the quality of health care, and due to differences in the diagnostic criteria. Although improvement in understanding the pathophysiology of ARDS and use of lung-protective ventilation have partially improved the prognosis, the incidence of
ARDS is still high with mortality around 40 % (2).

In this situation it is reasonable to use suitable animal models of ARDS which may bring new information on the pathophysiology of the acute respiratory distress and provide a possibility to evaluate various novel therapeutic approaches.

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ACUTE LUNG INJURY

Definitions of acute lung damage

The American-European Consensus Conference in 1994 recommended basic criteria for ARDS:

a) acute hypoxemia, defined as a ratio of arterial partial pressure of oxygen (PaO_2) and fraction of inspired oxygen (FiO_2); for ARDS: PaO_2/FiO_2 is <200 mmHg (26.7 kPa), for a milder form of ARDS called *acute lung injury* (ALI): PaO_2/FiO_2 200 mmHg (26.7 kPa) – 300 mmHg (40 kPa)

b) bilateral infiltrates on the chest X-ray

c) no increase in the pulmonary artery wedge pressure (3).

More recently the so-called Berlin Definition in 2012 considered 3 categories of ARDS according to severity of hypoxemia: mild (PaO_2/FiO_2 200-300 mmHg), moderate (PaO_2/FiO_2 100–200 mmHg), and severe (PaO_2/FiO_2 ≤ 100 mmHg) form of ARDS on the level of positive end-expiratory pressure (PEEP) ≥ 5 cmH_2O (0.5 kPa) (4).

For experimental studies where a lung injury is evoked artificially and other clinically relevant signs except of hypoxemia cannot be determined the term *acute lung injury* is used (5).

Triggering factors of ARDS

ARDS may develop from direct reasons, such as pneumonia, aspiration of the gastric content, or inhalation of toxic gases, or from indirect ones which generate as a consequence of severe systemic injury, e.g. in sepsis, severe trauma with shock, or acute pancreatitis (**Table 1**).

Table 1 Triggering factors of ARDS in adults (Adapted from Ref. 1)

Risk factors of ALI/ARDS	
Direct lung injury	**Indirect lung injury**
Often Pneumonia Aspiration of gastric content	*Often* Sepsis Severe trauma with shock Repetitive transfusions of blood products
Rare Lung contusion Fat embolism Near-drawning Inhalational injury (smoke, gases) Reperfusion edema after lung transplantation or lung embolectomy	*Rare* Acute pancreatitis Drug abuse Burns Cardiopulmonary by-pass Injury of head Disseminated intravascular coagulation

Pathophysiology and clinical picture of ARDS

Acute (exudative) phase of ARDS (<7 days from the insult) is characterized by a rapid onset of respiratory insufficiency, increased breathing rate, respiratory alkalosis, and diffuse alveolar infiltrates on the chest X-ray. A diffuse damage of alveolar epithelial and/or endothelial cells leads to massive formation of lung edema and ventilation-perfusion mismatch, pulmonary vasoconstriction, and finally to reduced lung compliance and severe hypoxemia (6). Activated neutrophils, alveolar macrophages, and fixed lung cells produce proinflammatory cytokines [interleukins (IL)-1β, -6, -8, TNFα etc.], proteolytic enzymes, reactive oxygen species (ROS), and other bioactive substances which further potentiate the tissue damage. In addition, lung injury is aggravated by dysfunction of pulmonary surfactant or its insufficient production by type II cells. Damage to the endothelial cells leads to increased microvascular permeability and edema

formation, vasomotor dysfunction, and increased production of microthrombi. Increasing afterload of the right ventricle results in pulmonary hypertension (7).

The extent of damage to microvascular endothelium and/or alveolar epithelium strongly depends on the triggering insult (8). In the direct (pulmonary) ARDS there dominates the injury to alveolar epithelial cells, with increased concentrations of cytokines (TNFα, IL-1β, IL-6 and IL-8) in the lung tissue or in the bronchoalveolar lavage fluid (BALF) and alveolar edema formation, demonstrating local lung inflammation. Damage to the type II cells can be proven by changes in specific surfactant proteins (SP), particularly in SP-D, and damage to the type I cells by increase in soluble receptor for advanced glycation end products (sRAGE) (9). In the extrapulmonary ARDS, systemic inflammation and injury of the capillary endothelium are dominant, with interstitial edema and increased plasma levels of cytokines (IL-6, IL-8). Damage to the endothelium is confirmed by increased levels of von Willebrand factor and angiopoietin-2 (9). Epithelial/endothelial damage is also accompanied by a release of components of extracellular matrix (e.g. matrix metalloproteinases, MMP) (10) and by an activation of coagulation, which might be proven by increased plasminogen activator inhibitor (PAI)-1 and decreased protein C (11). Complex effect of the above-mentioned factors influences the balance of pro- and apoptotic factors in the lung as well. In ARDS, apoptosis (programed cell death) of epithelial cells is increased and apoptosis of neutrophils is delayed, which leads to a longer persistence of neutrophils at a site of injury and a more serious injury to the tissue (12).

Within several days after the insult, the acute phase changes fluently to *fibroproliferative phase* with various degree of cell proliferation, formation of collagen deposits, and neovascularization, finally leading to healing, or to irreversible lung fibrosis (6).

MODELS OF ALI

Animal models represent an important bridge between the patients and the laboratory. Since human studies provide information about the onset and development of the pathological changes, some mechanisms which have been difficult to test in humans may be easily

Table 2 Features of ARDS in humans (Adapted from Ref. 5)

Clinical features	Acute onset Diffuse bilateral alveolar injury Acute exudative phase Repair with fibrosis
Physiological changes	Ventilation/perfusion mismatching Increase in intrapulmonary shunt fraction Severe hypoxemia Decreased lung compliance Impaired clearance of alveolar fluid
Biological changes	Increased endothelial and epithelial permeability Increase in extravascular lung water Increased proinflammatory cytokines in the lung Activation of proteolytic enzymes Abnormalities of blood coagulation
Pathological changes	Neutrophil infiltration of alveoli Intra-alveolar coagulation and deposition of fibrin (formation of hyaline membranes) Abnormalities of pulmonary surfactant Injury to alveolar epithelium with denudation of the basement membrane

tested in animals. Animal models can reproduce the mechanisms and consequences of ARDS in humans including the clinical, physiological, biological, and pathological changes (5) (**Table 2**).

However, while in humans the criteria for ARDS have been well defined, these criteria cannot be directly translated into animal models. Therefore, the Official American Thoracic Society Workshop suggested these main features of experimental ALI:

– histological evidence of the tissue injury
– alteration of the alveolar capillary membrane
– presence of the inflammatory response
– evidence of the physiological dysfunction (13) (**Table 3**).

Table 3 Features of ALI in animal models (Adapted from Ref. 13)

Histological evidence of tissue injury	Very relevant • Accumulation of neutrophils in the alveoli or interstitium • Formation of hyaline membranes • Proteinaceous debris in the alveoli (fibrin strands) • Thickening of the alveolar wall • Enhanced injury standardized by a histology score Somewhat relevant • Evidence of hemorrhage • Areas of atelectasis • Gross macroscopic changes as a discoloration of the lungs
Alteration of the alveolar capillary membrane	Very relevant • Increase in extravascular lung water content • Accumulation of an exogenous protein or tracer in the airspaces or the extra vascular compartment • Increase in total protein concentration in BALF • Increase in concentration of high molecular weight proteins in BALF (e.g., albumin, IgM) • Increase in the microvascular filtration coefficient Somewhat relevant • Increase in lung wet/dry weight ratio • Translocation of a protein from the airspaces into plasma • Increased lung lymph flow • High lymph protein concentration
Inflammatory response	Very relevant • Increase in the absolute number of neutrophils in BALF • Increase in lung MPO activity or protein concentration • Increase proinflammatory cytokines in lung tissue or BALF Somewhat relevant • Increase in procoagulatory activity • Increased expression of adhesion molecules • Conversion of the neutrophilic alveolitis into a mononuclear alveolitis with time • Increase in complement factors and MMP
Evidence of physiological dysfunction	Very relevant • Hypoxemia (PaO_2 <60 mmHg or SpO_2 < 90%) • Increased alveolar-arterial oxygen difference [(Aa)DO_2] Somewhat relevant • PaO_2/FiO_2 <200 mmHg • Increase in spontaneous minute ventilation • Increase in spontaneous respiratory rate

Abbreviations: BALF: bronchoalveolar lavage fluid, IgM: immunoglobulin M, MMP: matrix metalloproteinases, MPO: myeloperoxidase, PaO_2: arterial partial pressure of oxygen, FiO_2: fraction of inspired oxygen, SpO_2: saturation of hemoglobin by oxygen.

To determine whether ALI with required characteristics has occured in animals the Workshop advised that at least three of the four „main features“ of ALI should be present, with at least one of the „very relevant“ measurements, and preferably one or two additional measurements. However, not all of the measurements can be determined in each study (13) (**Table 3**).

When modeling any human lung injury the differences between the species should be considered, particularly the species differences in innate immune response (differences in Toll-like receptors, mononuclear phagocyte system, production of nitric oxide, chemokines and chemokine receptors) and differences in animal size which can limit the value of results received from the animal model (5).

According to the primary target three types of animal models of ALI have been used:
- models with primary injury to epithelium
- models with primary injury to endothelium
- models with injury of both epithelium and endothelium (5) (**Table 4**).

Table 4 Most frequently used models of ALI (Adapted from Ref. 5)

Model	*Model induction*
Models with primarily targeted capillary endothelium	
Oleic acid model	Oleic acid instilled into peripheral or central vein or artery
Endotoxin model	Intravenous administration of LPS
Models with primarily targeted alveolar epithelium	
Model of acid aspiration	Intratracheal instillation of HCl
Model of hyperoxia	Exposition to high concentrations of oxygen
Model of surfactant depletion	Repetitive lung lavage with saline
Model of ventilator-induced lung injury	Overventilation
Model of pulmonary fibrosis	Intratracheal instillation of bleomycin
Models targeting both the epithelium and endothelium	
Model of ischemia/reperfusion	Ischemia followed by reperfusion
Model of sepsis	Intravenous/intratracheal instillation of LPS

Abreviations: HCl: hydrochloric acid, LPS: lipopolysaccharide.

ANIMAL MODELS WITH A PRIMARY INJURY TO THE LUNG EPITHELIUM

Model of surfactant depletion induced by saline lavage

The model of surfactant depletion is usually evoked in anesthesized animals after a short period of stabilization during which the animal is oxygen-ventilated. Then, a lung injury is caused by an instillation of saline (volume 10–30 ml/kg) *via* an endotracheal tube and its immediate suctioning. This procedure is repeated until a target hypoxemia is reached in two following measurements and then the experiment continues with a ventilatory or pharmacological therapy (14–17).

A lung lavage with saline removes a significant portion of pulmonary surfactant which increases the alveolar surface tension. This leads to alveolar collapse, increase in alveolar-arterial oxygen gradient, and an early onset of hypoxemia, as well as the production of lung edema (5, 18). A partial removal of the surfactant enhances the transmigration of polymorphonuclears (PMN) into the alveoli and increases the synthesis of pro-inflammatory cytokines (14, 15). Histological evaluation showed areas with the alveolar collapse changing with overdistended regions and peribronchial edema, thicker alveolo-capillary membrane, desquamation of epithelium and endothelium, necrosis of type I cells, and damage to the basement membrane (19).

There are several disadvantages of this model as well. The animals require general anesthesia, insertion of tracheal cannula, and artificial ventilation. Except for neonates, the surfactant depletion just rarely occurs in the absence of severe alveolar damage, e.g. in near-drowning or as a consequence of a primary injury to lung epithelium and exudation of protein-rich edema fluid (5, 18). Therefore, the models of surfactant depletion are suitable for simulation of an insufficient surfactant production in the premature neonates. For this purpose young-to-adult rabbits are often used, while the body weight, airway diameter, and lung compliance are very close to those in the neonates which allows the use of comparable ventilatory parameters.

Model of lung injury induced by meconium

Other model suitable for modeling of neonatal diseases is a model of meconium aspiration syndrome (MAS) (20). MAS originates in the term and post-term neonates due to the aspiration of the first faeces (meconium) which can be prematurely evacuated from the intestine due to intrauterine stress, stimulation of *nervus vagus,* or fetal maturation. The inhaled meconium causes airway obstruction with subsequent alveolar atelectasis, increased right-to-left shunts and hypoxemia, hypercapnia, and acidosis. When meconium reaches the alveoli the dysfunction of surfactant triggers a collapse of alveoli and small airways, decrease in lung compliance, and development of lung edema, neutrophil-mediated inflammation, and cell apoptosis (21, 22).

Models of MAS are performed in anesthesized animals by intratracheal instillation of meconium suspension prepared from lyophilized neonatal meconium. According to the used animal species and the design of the experiment various combinations of meconium concentrations and dose volumes can be used (22, 23). For instance, meconium concentration of 25 mg/ml (representing 10 % of the original meconium) accentuates alveolar component with a predominant surfactant dysfunction and inflammation, while the instillation of higher meconium concentrations accentuates the airway obstruction. Before the administration the meconium can be filtered through a gauze or blotting paper to separate large particles. However, the instillation of non-filtered suspension better resembles the situation in the neonates (21, 24).

For the creation of MAS models, various animal species have been used. Advantageous is the use of neonatal animals immediately after the labor, which simulates better the aspiration of meconium on the background of postnatal changes of the lungs from liquid-filled to air-filled organ (25). However, due to technical and ethical difficulties associated with preparation of the model, several days up to several weeks-old animals, mainly piglets, young rabbits, or rats, are used instead of animal pups immediately after the labor. On the other hand, use of several weeks-old piglets and rabbits can be advantageous for testing of artificial ventilation or intratracheal delivery of treatments, as piglets and rabbits have similar airway size and body weight to those in the neonates (26-29).

Model of lung injury induced by hyperoxia

Models of hyperoxia-induced ALI are induced by overproduction and detrimental action of ROS due to inhalation of high oxygen concentrations (5). An abundant production of ROS leads to the oxidation of proteins and the peroxidation of membrane lipids and nucleic

acids, which results in a lung cell apoptosis (30) and increased production of pro-inflammatory cytokines (31).

To create a hyperoxia model the animals are housed in a sealed cage and inhale high oxygen concentrations. The exposure of animals to normobaric oxygen within 3-4 days caused a damage to type I cells, necrosis of endothelial cells, formation of interstitial and intraalveolar edema, increased platelet adhesion, and PMN accumulation and longer exposure (60–70 hours) resulted in death (32). The limitation of this model is a requirement for specialized equipment to ensure a delivery of appropriate oxygen concentrations for a longer time.

Model of lung injury induced by mechanical ventilation

Mechanical ventilation, particularly ventilation with high volumes or high pressures, can produce a lung injury and inflammation which is called *ventilator-induced lung injury* (VILI). Overstretching of alveolar walls results in early endothelial and epithelial breaks, edema, formation of hyaline membranes, and increased migration and activation of neutrophils (33, 34). The extent of lung injury depends on the used ventilatory volumes and pressures as well as on the level or absence of PEEP. For instance, large volume ventilation results in alveolar hemorrhage, production of hyaline membranes, neutrophilic infiltration, decrease in lung compliance, and worsened gas exchange (35), whereas ventilation with small volumes can reduce inflammation and histopathological damage of the tissue (15).

The limitation of this model is the requirement for general anesthesia, tracheotomy, and mechanical ventilation, whereas ventilatory and cardiovascular parameters should be monitored. In addition, there are also differences between iatrogenic VILI induced in animals and injury caused by excessive ventilation in humans where ARDS had originated from other reasons.

Model of acid aspiration

To create the model of aspiration of gastric contents the tratracheal instillation of hydrochloric acid (HCl) with a low pH (usually of 0.1 N HCl at a dose of 1–4 ml/kg) induces a biphasic lung injury. The first phase (<2 h after HCl instillation) is characterized by an increased vascular permeability probably due to physiochemical reactions to the acid. The second peak is reached within 3–4 h after HCl instillation with neutrophilic recruitment and acute inflammation (36). The situation could be accompanied by alveolar hemorrhage, intraalveolar and interstitial edema, decrease in lung compliance, and hypoxemia, as well as an increased pulmonary vascular resistance and pulmonary artery pressure and elevated shunt fraction (37, 38).

The disadvantage of this model is that except to HCl, gastric content includes other potentionally detrimental substances such as food particles, bacterial cell wall products, cytokines, etc. To elicit ALI model of gastric content aspiration closer to a clinical situation the whole gastric fluid containing particles can be used (39, 40).

Model of lung injury induced by intratracheal bleomycin

Although the bleomycin model is considered for a model of lung fibrosis, it has some features of ALI as well. Bleomycin creates a complex with oxygen and metals leading to the production of ROS, DNA breaks, and cell death. Bleomycin can be delivered intravenously, intratracheally, intraperitoneally, or subcutaneously, most often in mice or rats. While the intravenous administration primarily targeting the endothelium requires several weeks to exert required changes in the lung, the intratracheal administration primarily targeting epithelium can produce lung fibrosis already after a single dose of bleomycin (41). Early after the intratracheal bleomycin administration a patchy neutrophilic alveolitis and a fibrosis can be found as well as elevated counts of neutrophils in the BALF and high concentrations of pro-inflammatory cytokines (42). This model is relatively technically easy and reproducible but has little relevance to clinics.

ANIMAL MODELS WITH A PRIMARY INJURY TO THE CAPILLARY ENDOTHELIUM

Model induced by intravenous administration of lipopolysaccharide (LPS)

An intravenous delivery of LPS causes the changes resembling the sepsis. LPS is a membrane glycolipid of Gram-negative bacteria which binds to a specific LPS binding protein (LBP) and forms LPS:LBP complex. This complex activates CD14/TLR4 receptor structure on many cells including monocytes and macrophages and triggers an inflammation.

Early after an intravenous administration of LPS an apoptosis of capillary endothelial cells may precede other tissue damage and trigger a release of various mediators including cytokines, adhesion molecules, and tissue factors (43, 44). This initial phase is characterized by leukopenia, decreased cardiac output and decreased arterial pressure, and increased pressure in pulmonary artery. Within several hours hypoxemia and increased alveolar-arterial oxygen gradient can be observed. Contrary to intratracheal LPS delivery (see also in subchapter 3.3.1), PMN infiltration into the lung is relatively small (5). In animals the response to LPS can vary according to the presence or absence of pulmonary intravascular macrophages (PIMs). Animal species with PIMs (sheep, cattle, pigs, cats, goats, horses, etc.) can develop pulmonary inflammation after very small doses of LPS (in μg/kg range), while species without PIMs (humans, dogs, rats, mice, rabbits, etc.) require much higher doses (in mg/kg range) (5, 18).

The advantage of this model is its high reproducibility. LPS activates innate immune reactions through TLR4 pathways and has a low direct toxicity to cells *in vitro*. However, intravenous LPS administration does not provide such severe injury to endothelium and epithelium as observed in human ARDS induced by live bacteria (5).

Model of oleic-acid lung injury

The model of oleic-acid lung injury mimicking pulmonary lipid embolism in patients with long bone trauma can be induced by an intravenous delivery of oleic acid *via* a peripheral or central vein or directly into the right atrium or the pulmonary artery. A dose of oleic acid in the range of 0.06–0.15 ml/kg is mixed with saline and injected within 20–30 min, or partionated into 3–4 equal aliquots. Because of insolubility in water oleic acid can be dissolved in ethanol or emulsified in the blood prior to the administration (5, 18).

The oleic-acid induced lung injury can be caused by an unsaturation of oleic acid and its binding to biological membranes, or covalent binding to Na^+ channels and Na^+-K^+ ATPase in epithelial cells impairing sodium transport and leading to edema formation (45). The effects of oleic acid are detectable immediately, with maximum changes at 12 h. Oleic acid causes a dose-dependent damage to endothelial cells followed by an epithelial injury with swelling and a necrosis of type I cells and a lung edema. The mentioned changes impair the gas exchange due to the ventilation/perfusion mismatch, decreased lung compliance, and increased shunts (46, 47).

The advantages of this model include early and rapid elicitation of the lung injury and a high reproducibility in different animals. The disadvantage of the model is the fact that just few cases of ARDS are associated with a long bone trauma or a lipid injury (5, 18).

ANIMAL MODELS WITH INJURY TO BOTH EPITHELIUM AND ENDOTHELIUM

Models of lung injury due to sepsis

Sepsis can be induced by an administration of live bacteria, by a creation of an endogenous infection, e.g. by cecal ligation and puncture, or by an administration of bacterial products, e.g. endotoxin (see also in the subchapter 3.2.1).

The models using live bacteria can differentiate according to the route of administration, the size of bacterial inoculum, and bacteria and animal species (5). The intravenous administration of bacteria bolus is followed by hypotension and leukopenia which can progress

to septic shock, intravascular coagulation, and death. If the animal survives, the acute phase is followed by a hemodynamic stabilization with PMN lung sequestration, increased vascular permeability, increase in shunts and pulmonary artery pressure, and intravascular thrombosis (48).

The model of the lung injury secondary to peritonitis can be induced by a ligation and perforation of the cecum. This leads to the development of peritonitis, leukopenia, neutrophilic inflammation, interstitial and alveolar edema, hypoxemia, and pulmonary hypertension over several days while the onset is less abrupt (49, 50) and less severe intra-alveolar inflammation and hyaline membranes formation are found compared to a direct lung injury (5).

The administration of live bacteria into the lung *via* intratracheal or intranasal route leads to pneumonia and may exert a systemic manifestations of sepsis (51). For exposure to aerosol a special equipment including a whole body exposure chamber and a nebulizer should be used, however, multiple animals can be infected simultaneously, bacteria symmetrically reach both lungs, and anesthesia is not needed. An intratracheal administration of LPS or bacteria requires anesthesia and tracheotomy or puncture of the trachea, however, the delivered dose of LPS or bacteria is precisely known. Also the delivery *via* intranasal route requires the anesthesia and the homogenity of delivery of the material throughout the lungs is questionable, however, this method is technically simple and timesaving (52).

Models of ischemia/reperfusion

Ischemia followed by reperfusion either in the lung or in any distant vascular bed can result in the lung injury. For instance, after a lung transplantation the reimplantation response includes non-cardiogenic lung edema, inflammation, and hypoxia (5). In elicitation of the model the lungs are subjected to ischemia by clamping the pulmonary artery which preserves the bronchial circulation, or by clamping the hilum which stops the whole blood flow. Ischemia of inflated lung for 2 h followed by 2 h of reperfusion causes a structural damage of both alveolar endothelium and epithelium (53). The severity of the lung injury depends on the inflation state of the lung (deflated or inflated), the extent of ischemic bed (pulmonary, bronchial circulation, venous return), the duration of ischemia and reperfusion, experimental preparation (*in vivo* or isolated perfused lung), and animal species (5). Interestingly, ischemia/reperfusion injury of one lung lobe causes inflammatory changes and lung edema also in the contralateral lung (54).

The advantages and disadvantages of animal models of lung injury are listed in **Table 5**.

COMBINATION OF THE MODELS

To provide a model closer to the clinical ARDS it is reasonable to combine two or more insults. However, it is hard to determine the extent of lung injury caused by the individual insults (5).

For instance, if saline-lavaged animals are ventilated with high volumes and no PEEP, the lung injury similar to ARDS with an increased microvascular permability, PMN infiltration, and generation of hyaline membranes can be obtained (16, 19). Similarly, an instillation of LPS into the lung of surfactant-depleted animals can aggravate an intensity of the inflammation (55).

Changes related to mechanical ventilation can be different in normal and inflamed lungs. Nevertheless, they are additive to changes induced by other factor (e.g. LPS) and the resulting injury is dependent on the type and extent of lung inflammation (56). In several models of ALI the use of mechanical ventilation is required for elicitation of the model. Deleterious effects of ventilation can be reduced by ventilation with small volumes and low ventilatory pressures.

Table 5 Advantages and disadvantages of the most frequently used animal models of ARDS (Adapted from Ref. 5, 13, 18 and 36)

Model of ARDS	Advantages	Disadvantages
Model induced by saline lung lavage	Relatively stable Easy and fast preparation of the model Suitable as a model of immature lung	Surfactant depletion rarely develops in adults, except of near-drowning Requirement for anesthesia and artificial ventilation
Model induced by neonatal meconium	Very good stability Easy and fast preparation of the model Obvious inflammation and pulmonary vasoconstriction	Situation limited to neonatal MAS, not in adults Requirement for anesthesia and artificial ventilation
Model induced by hyperoxia	Stable model	Requirement for special equipment (cages, delivery system for oxygen) Longer time for preparing the model (several days)
Model induced by mechanical ventilation	Stable model	Requirement for anesthesia and mechanical ventilation Rarely develops in humans separately from primary insult causing lung injury requiring ventilation
Model induced by HCl	Very good stability Easy and fast preparation of the model Obvious inflammation and pulmonary vasoconstriction	Instillation of whole gastric juice including particles considered for more suitable and clinically closer insult
Model induced by intratracheal bleomycin	Easy and fast preparation of the model	Not stable model in mice Low clinical relevance
Model induced by intravenous LPS	Clinically relevant model Easy and fast preparation of the model	Inter-species differences in response to LPS
Model induced by oleic acid	Easy and fast preparation of the model Obvious inflammation and pulmonary vasoconstriction	Rare occurance of pulmonary lipid embolism
Model induced by intratracheal LPS	Clinically relevant model Easy and fast preparation of the model	Requirement for anesthesia and tracheotomy
Model of sepsis induced by ligation and perforation of the cecum	Clinically relevant model Fast development of the changes	Requirement for surgical intervention Preparation of the model technically difficult
Model induced by ischemia/reperfusion	Clinically relevant model Fast development of the changes	Requirement for surgical intervention Preparation of the model technically difficult

CONCLUSIONS

Several types of animal models can be used to simulate the changes in the acutely damaged lungs. However, the origin and development of ARDS in humans are complex and multifactorial. The patient´s lung can be affected by a primary illness (e.g. sepsis) and/or can be affected also by therapeutical approaches (e.g. mechanical ventilation). The course of ARDS is influenced by genetic or hereditary factors, susceptability to the infectious agents, concomittant diseases, etc. Therefore, no single animal model reproduces all the characteristics of human ARDS and most of the existing animal models are relevant for only limited aspects of ARDS in humans. Nevertheless, although the applicability of results from animal experiments to patients might be limited, animal models are essential for understanding the pathophysiology of ALI/ARDS and provide an excellent opportunity to search for novel therapeutical strategies.

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