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The role of nitric oxide and reactive nitrogen species in experimental ARDS

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1. ABSTRACT

The acute respiratory distress syndrome (ARDS) is associated with a mortality ranging between 40 – 80%. An effective drug therapy, however, is still missing. Many experimental models of ARDS exist enabling a better understanding of the underlying pathophysiology and allowing the establishment of novel therapeutic strategies for the treatment of patients suffering ARDS. Nitric oxide (NO) and reactive nitrogen species (RNS) are increasingly regarded as key substances within the development of ARDS. However, the definite pathomechanism is not completely understood. The purpose of this article is to review the latest results of experimental studies in reference to the role of NO and RNS in the pathogenesis of ARDS. In addition, current large animal models of ARDS are described and future therapeutic approaches are discussed.

2. INTRODUCTION

The acute respiratory distress syndrome (ARDS) is currently defined as a syndrome of inflammation and increased permeability, which is further characterized by a constellation of clinical, radiological, and physiological abnormalities that cannot be explained by left atrial or pulmonary capillary hypertension (1). Direct causes include pneumonia, toxic or smoke inhalation, pulmonary contusion, aspiration, and near-drowning. Among others sepsis, shock, severe extra thoracic trauma, burns, multiple transfusions, cardiopulmonary bypass surgery, or pancreatitis are indirect causes (2). With an incidence of 20-75 cases per 100,000 persons/year and a mortality rate between 30 and 75% ARDS and the less severe form acute lung injury (ALI) are major causes of morbidity and

mortality (3). The associated mortality of ARDS ranges between 40 - 80% (4-6).

Nitric oxide (NO) and reactive nitrogen species (RNS) are increasingly regarded as key substances within the development of ARDS. However, the definite pathogenesis is not completely understood yet. This may be one reason, why there is still no medical therapy with proven reduction of mortality in ARDS available. Experimental studies enable a better understanding of the underlying pathophysiology and allow the testing and establishment of novel therapeutic approaches for the treatment of ARDS patients. Therefore, they are of paramount clinical and socioeconomic importance.

The purpose of this article is to review the latest results of experimental studies in reference to the role of NO and RNS in the pathogenesis of ARDS. In addition, current large animal models of ARDS are described and future therapeutic approaches are discussed.

3. LARGE ANIMAL MODELS OF ALI AND ARDS

Several experimental models of ARDS have been described in the literature (7-14). However, large animal models mimic the pathophysiological response of humans to lung injury closer than cells or rodents. In addition, a greater number of hemodynamic data and histochemical analyses can be assessed and related to each other, facilitating the interpretation of the results. Our group developed different models of ALI and ARDS in sheep (8, 15-18).

The surgical preparation of the animals for our models has been described recently (19). In brief, the sheep are operatively prepared with an arterial and left atrial catheter as well as a Swan-Ganz catheter. Depending on the experiment, the lung and prefemoral lymphatic vessels are cannulated and continuously drained. After a recovery period of five to seven days, baseline data are collected. For single smoke inhalation injury, a tracheostomy is performed and the animals are subjected to 4 sets of 12 breaths of cold cotton smoke (a total of 48) under deep anesthesia (15, 20). The more severe model of combined burn and smoke inhalation injury includes an additional full thickness third degree burn of 40% of total body surface area (B+S) (8, 21-24). Following both injuries, the animals are mechanically ventilated for the observation period with a tidal volume of 15 mL/kg, a positive endexpiratory pressure of 5 cm H_2O and an inspired oxygen fraction (FiO_2) adjusted to keep the arterial partial oxygen pressure (PaO_2) between 80 and 120 mm Hg. Fluid resuscitation in B+S animals is performed according to the following protocol: mL/h = 4 x (body weight [kg] x burn area [% of total body surface area] / 2 / 8) for the first 8 hrs and mL/h = 4 x (body weight [kg] x burn area [% of total body surface area] / 2 / 16) for the rest of day one. Animals in the smoke inhalation injury only model receive 2 $mL \cdot kg^{-1} \cdot h^{-1}$.

Since sheep are quite as sensitive to endotoxin as humans, the first "sepsis" models were produced by bolus (0.75 $microg \cdot kg^{-1} \cdot 30min^{-1}$) (16) or continuous intravenous infusion (10 $nanog \cdot kg^{-1} \cdot min^{-1}$) of endotoxin (17). To increase the clinical relevance, endotoxin administration was replaced by a continuous intravenous infusion of live Pseudomonas aeruginosa (2.5 x 10^6 colony-forming units (cfu)/min) for 48 hrs (18, 25, 26). Although both models reflected the clinical conditions of sepsis well (26), the impairment of pulmonary function did not reach the ARDS defining criteria of a PaO_2/FiO_2 ratio below 200. Therefore, a combined smoke inhalation injury and sepsis model (SI+S) was developed. Following the smoke inhalation injury as described above, 5 x 10^{11} cfu of either Pseudomonas aeroginosa (7, 27) or methicillin-resistant Staphylococcus aureus (28) were placed by a bronchoscope in the right middle and lower lobe as well as in the left lower lobe of the lung. For the whole study period the animals were ventilated with a tidal volume of 15 mL/kg, a positive endexpiratory pressure of 5 cm H_2O and a FiO_2 of 1.0. Fluid resuscitation was adjusted to keep the hematocrit at baseline values ± 3%.

Despite certain differences in the extent of the individual parameters, both, the B+S and the SI+S injury, result in an ARDS as reflected by increased pulmonary fluid flux, pulmonary hypertension, pulmonary edema, consecutively increasing ventilatory pressures, and a reduction of oxygenation reflected by a drop of the PaO_2/FiO_2-ratio below 200.

4. ROLE OF NITRIC OXIDE IN THE PATHOGENESIS OF ARDS

Although NO is often described as toxic and a cause of many pathological processes such as vasodilation in sepsis, it is also an omnipresent messenger in all vertebrates, modulating neuronal activity and blood flow (29). The overproduction of NO, however, can lead to detrimental cell damage by reacting with reactive oxygen species (ROS), such as superoxide (O_2^-), and the resulting formation of RNS, such as peroxynitrite ($ONOO^-$). During the last years, the number of articles proposing a central role of these molecules in the pathogenesis of ARDS is increasing (30-33).

The majority of ROS are produced by polymorphonuclear cells (PMN) as part of the inflammatory response to the initial injury. In addition to ROS, NO is the second precursor of RNS. In the lung, NO derives from neutrophils, alveolar type II cells, activated macrophages, or endothelial cells (32). The NO synthase (NOS) catalyzes the formation of NO and L-citrulline from the amino acid L-arginine in a complex five-electron redox reaction. Three different isoforms of NOS have been identified in mammals: the neuronal (nNOS, NOS-1) and endothelial NOS (eNOS, NOS-3) are constitutive forms, while the inducible isoenzyme (iNOS, NOS-2) can be up-regulated by oxidative stress and systemic inflammation (34). Notably, in case of substrate (L-arginine) or cofactor limitation, like under pathophysiological conditions, NOS can also produce superoxide (35).

Superoxide is physiologically stabilized by the formation to hydrogen peroxide (H_2O_2) catalyzed by the

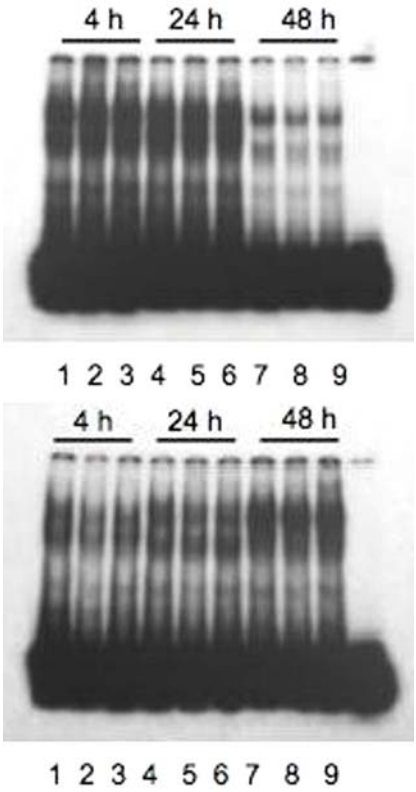

Figure 1. Electrophoretic mobility shift assay for nuclear factor kappa B in lung tissue at 4, 24, and 48 h post-injury (burn and smoke inhalation). Each 3 lanes are control, anti-p50, and anti-p65, respectively. Left picture: poly (adenosindiphosphate-ribose) polymerase (PARP) inhibitor (injury, PARP inhibitor), right picture: control (injury, no treatment).

superoxide dismutase. In case of increased NO plasma levels, however, the reaction with NO to form peroxynitrite is faster (36). Although this highly cytotoxic RNS represents the most cited example, all the ROS and RNS are able to interact with proteins, desoxynucleic acid (DNA) or lipids by oxidation or nitrosation causing inactivation of key enzymes like the glycolytic enzyme glyceraldehydes-3-phosphate dehydrogenase or DNA single strand breaks (33). DNA damage leads to the formation of poly (adenosindiphosphate (ADP) ribose) polymerase (PARP), a nuclear enzyme involved in DNA repair (37, 38). It has been shown that PARP activation can be induced by NO and its toxic products such as RNS (39). While the physiological function of PARP is beneficial, PARP overactivation, however, results in cellular adenosintriphosphate (ATP) depletion and consecutively cellular dysfunction and death (13, 37).

Both of our experimental ARDS models adequately reflect this pathophysiological pathway. Increased NO production is represented in a rise of its stable metabolites, nitrate and nitrite (NOx) in plasma and lung tissue (7, 23), the increased amount of iNOS messenger ribonucleic acid (mRNA) and protein in lung tissue proves its increased activity (35, 40). We also found increased levels of 3-nitrotyrosine (3-NT) in lung tissues (23, 41), a stable marker of the RNS peroxynitrite, as well as increased products of lipid peroxidation, an index of ROS activity (22, 41). The concentration of poly ADP ribose (PAR), the product of PARP, was also elevated in the lung (22, 40).

To investigate the pathomechanism of ARDS, several trials using selective iNOS inhibitors (mercaptoethyl-guanidine (MEG), aminoguanidine, or the most potent iNOS dimerization inhibitor BBS-2) have been performed (Table 1). However, they revealed contradictory results (for detailed results please see section 5). Notably, iNOS mRNA levels tended to be higher in animals given the iNOS inhibitor than in control animals (23), suggesting that the NO derived from iNOS might have a negative feed-back on iNOS formation. In addition, selective iNOS inhibition was sometimes even less effective as compared to non-selective NOS inhibition (40). These results suggested an additional source of NO in the pathway of ARDS.

The inhibition of PARP reversed the cardiopulmonary changes associated with ARDS in the same manner as the selective iNOS inhibitors (22, 41). Further analyses revealed the same attenuation of iNOS mRNA and protein levels in the lung as in animals treated with a selective iNOS inhibitor suggesting a relationship between PARP activation and iNOS activity (35). In addition to its role as a compound to repair injured DNA, PARP activates nuclear factor kappa B (NF-kappa B) (42, 43). NF-kappa B is known to up-regulate iNOS (44) and interleukin 8 (IL-8) (45). Our group was able to show that, beside increases in PARP and iNOS activity, NF-kappa B levels were elevated in lungs from ARDS sheep (22). Remarkably, these increases were reversed by PARP inhibition (Figure 1). The interpretation of these results reveals a problem. If PARP is responsible for the up-regulation of iNOS via NF-kappa B, then what NOS isoform is inducing RNS to damage DNA and the subsequent release of PARP?

The neuronal isoform nNOS is known to be present in multiple cells in the lung, e.g. epithelial, smooth muscle or glandular cells as well as in the parasympathic plexus and non-adrenergic as well as non-cholinergic nerve endings (46). Therefore, the effects of a selective nNOS inhibitor (7-nitroindazole, 7-NI)) in ARDS were investigated in both the B+S and the SI+S model (35, 40). 7-NI was most effective in the initial phase after the injury and attenuated the degree of pulmonary dysfunction in ovine ARDS similar to iNOS or PARP inhibitors (40). The most important result in respect to the determination of the ARDS pathomechanism, however, was that the concentration of iNOS mRNA in lung tissue in injured animals treated with the nNOS inhibitor was less than in injured animals treated with a selective iNOS inhibitor (Figure 2), suggesting that NO derived from nNOS up-regulates iNOS protein expression. In conclusion, the initial increase of NO in the development of ARDS seems to be caused by nNOS, while iNOS might be responsible for the perpetuation of the inflammatory process.

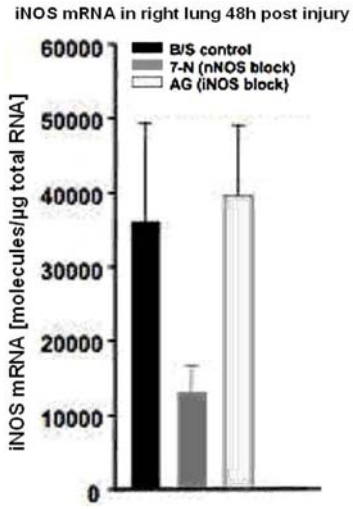

Figure 2. Levels of iNOS mRNA in the lung of sheep 48 h following the combination of burn and smoke inhalation injury (B+S). AG, aminoguanidine; 7-NI, 7-nitroindazole; values are expressed as means ± standard error.

Based on these experimental data, the following role of NO and RNS in the pathomechanism of experimental ARDS can be proposed (Figure 3): The primary injury induces the release of neuropeptides into the airway, which activate PMNs resulting in the production of ROS. Simultaneously, the activity of nNOS is up-regulated by neuropeptides causing the formation of relevant amounts of NO. NO reacts with ROS, e.g. superoxide, to form RNS such as peroxinitrite. These cytotocic products lead to nitration and oxidation of proteins, lipid peroxidation and DNA single strand breakage with subsequent activation of PARP. PARP overactivation results in depletion of ATP, finally causing cellular dysfunction or death. In addition, PARP up-regulates NF-kappa B, which increases the release of IL-8 resulting in attraction and activation of PMNs and consecutively in the production of ROS. NF-kappa B also stimulates iNOS formation thereby accelerating the production of NO. NO and ROS react to RNS leading to DNA damage, PARP activation and thus a positive feedback loop mechanism is established. The factors that are able to interrupt this vicious cycle may be of paramount importance for the treatment of ARDS.

5. THERAPEUTIC IMPLICATIONS BASED ON THE EXPERIMENTAL DATA

Because of the central role of NO, RNS and PARP within the pathogenesis of ARDS, the different NOS as well as PARP represent promising therapeutic targets. However, their physiological function has to be considered. Only the excessive stimulation causes morbidity and mortality. That is why an indiscriminate inhibition might be associated with detrimental consequences.

The first therapeutic approach was the non-selective NOS inhibition by L-nitro-arginine methyl ester (L-NAME). In edotoxemic and septic sheep L-NAME treatment prevented the drop in PaO_2/FiO_2 ratio, decreased pulmonary and transvascular fluid flux and markedly reduced fluid retention. In addition, hypoxic pulmonary vasoconstriction, a physiological reflex of the lung to distribute the blood flow to well ventilated areas in order to optimize pulmonary gas exchange, was partly preserved (47, 48). However, L-NAME treatment was also associated with several side effects: endotoxin related pulmonary hypertension as well as pulmonary edema were increased by L-NAME administration (17, 49-51). The total blockage of the endogenous vasodilator NO might be one reason for these side effects. A multicenter, randomized, double-blind, placebo-controlled trial in patients with septic shock revealed an increased mortality in patients treated with the non-selective NOS inhibitor 546C88 (52). In accordance to experimental trials pulmonary hypertension was one of the main side effects along with heart failure and systemic hypertension. However, the more rapid dose augmentation in this trial led to higher administrated doses as compared to a former multicenter, randomized, placebo-controlled trial in septic shock patients showing beneficial effects of iNOS inhibition with an acceptable safety profile (53).

To selectively inhibit the overexpression of NO, while preserving its physiologic function as an endogenous vasodilator, several selective iNOS inhibitors (MEG, BBS-2, aminoguanidine, ONO-1714, 1400 W) have been tested in different animal models of ARDS. The experimental trials and their main results are summarized in Table 1. The repetitive bolus administration of MEG and the continuous infusion of BBS-2 improved pulmonary gas exchange and reduced pulmonary edema in our ovine B+S model (8, 15, 23). In contrary, the pulmonary edema was not reduced by the administration of BBS-2 in septic sheep (7) or aminoguanidine in endotoxemic sheep (11). These differences between the B+S and the SI+S model emphasize the crucial role of the original injury and its specific pathophysiology for the treatment of ARDS patients. The impression that selective iNOS inhibition was most effective after 12 h post injury was confirmed by Okamoto and colleagues. In a mouse model of sepsis following peritonitis the authors revealed that the administration of ONO-1714 improved survival only when started 12 h post injury (9). Prophylactic treatment with 1400W was correlated with increased apoptosis rates in endotoxemic rats (12). The clinical impact of this result, however, is rather small because of the rodent model and the prophylactic approach. In endotoxemic pigs, the selective iNOS inhibition by continuous infusion of MEG did not show any beneficial effects on variables of oxidative stress (10). In conclusion, the results demonstrate possible differences in the role of iNOS in the pathogenesis of ARDS depending on the causal injury. However, no clinical trial has been performed using selective iNOS inhibitors so far.

There is growing evidence for the role of PARP activation in the pathogenesis of several human diseases (37, 38). The effects of PARP inhibition in experimental ARDS are promising (Table 2). In endotoxemic mice the repetitive bolus administration of the PARP-inhibitor PJ34

Table 1. Selectiv iNOS inhibition in experimental ARDS

Study design	Treatment	Main results
Smoke inhalation injury; sheep; 48 h (15)	MEG: 30 mg/kg i.v., every 8h, started after 1h; vs. placebo	Prevention of drop in PaO_2/FiO_2-ratio, decrease in airway blood flow, PVRI, Qs/Qt, pulmonary edema
Smoke inhalation and sepsis; sheep; 24 h (7)	BBS-2: 100 microg·kg^{-1}·h^{-1} cont. i.v., started after 1h vs. placebo	Prevention of drop in PaO_2/FiO_2-ratio, decrease in airway obstruction, ventilatory pressures, Qs/Qt, plasma NOx, pulmonary edema was not reduced
Burn and smoke inhalation; sheep; 48 h (8)	BBS-2: 100 microg·kg^{-1}·h^{-1}, started after 1h vs. placebo	Decrease in tracheal blood flow, plasma NOx-levels, airway obstruction
Burn and smoke inhalation; sheep; 48 h (23)	MEG: 30 mg/kg i.v. every 8h, started after 1h; vs. placebo	Decrease in lymph flow, NOx-plasma and -lymph levels and 3-nitrotyrosin
Endotoxin; pigs; 24 h (10)	MEG: 3 mg·kg^{-1}·h^{-1} cont. i.v., started after 12 h; vs. placebo	No beneficial effects on variables of oxidative stress
Endotoxin; sheep; 6 h (11)	Aminoguanidine: 10 mg/kg + 1 mg·kg^{-1}·h^{-1}, over 4h i.v., started after 2 h vs. placebo	No further increase in extravascular lung water, decrease in Qs/Qt Increase in PaO_2
Sepsis; mice; 48 h (9)	ONO-1714: 0.03 mg/kg s.c., every 12 h vs. placebo	No improvement when treatment started immediately or 6 h after injury; high iNOS mRNA in lung tissues in treatment groups decreased NOx-plasma levels Improved survival
Sepsis; rats; 0,6,12,18,24 h (72)	1400W: 1-10 mg/kg ONO-1714: 0.01-0.1 mg/kg Aminoguanidine: 5-30 mg/kg each i.p. after 1 h	Plasma NOx level decreased, useful indicator of lung NOS activity
Endotoxin; rats; 24 h (12)	1400 W: pre injury 20 mg/kg i.p. and every 8h vs. placebo	Increased apoptosis, caspase-3 and -7 activation; antiapoptotic effect of iNOS
Ischemia/reperfusion and oleic acid; rats; 6 h (73)	Aminoguanidine: 20 mg/kg 1 h pre injury vs. placebo	Decreased lung wet/dry-ratio, mRNA, iNOS, NO and TNF alpha

iNOS: inducible nitric oxide synthase; ARDS: acute respiratory distress syndrome; MEG: mercaptoethylguanadine; i.v.: intravenous; PaO_2/FiO_2 ratio: arterial partial pressure of oxygen-to-inspired oxygen fraction; cont: continuous; PVRI: pulmonary vascular resistance index; Qs/Qt: pulmonary shunt fraction; NOx: total amount of nitric oxide metabolites; i.p.: intraperitoneal; mRNA: messenger ribonucleic acid; NO: nitric oxide; TNF alpha: tumor necrosis factor alpha

Table 2. PARP inhibition in experimental ARDS

Study design	Treatment	Main results
Smoke inhalation and sepsis; sheep; 24 h (41)	INO-1001: 3 mg/kg bolus, 0.3 mg·kg^{-1}·h^{-1} cont. i.v. vs. placebo	Prevention of drop in PaO_2/FiO_2-ratio, decrease in ventilator pressure, Qs/Qt, lung wet-to-dry ratio and PARP level, no difference in iNOS and mRNA
Burn and smoke inhalation; sheep; 48 h (22)	INO-1001: 3 mg/kg bolus, 0.3 mg·kg^{-1}·h^{-1} cont. i.v. vs. placebo	Decrease in lung edema, airway blood flow and obstruction, ventilation pressures, vascular leakage, lipid peroxidation and plasma NOx-levels, prevention of drop in PaO_2/FiO_2
Sepsis; rabbits; 2, 4 and 6 h (13)	PJ34: 10 mg/kg bolus, 3 mg·kg^{-1}·h^{-1} cont. i.v.; vs. placebo	No difference in lung wet-to-dry-weight-ratio, positive blood cultures in 4/7 (PJ34) vs. 8/8 animals (placebo)
Endotoxin; mice; 24 h (54)	PJ34: 20 mg/kg i.p. after 1, 6 and 12 h vs. placebo	Decrease in TNF-alpha, myeloperoxidase activity, protein and NOx in BALF
Zymosan-activated plasma; mice; 24 h (74)	5-AIQ: bolus 3 mg/kg i.p. after 1 and 6 h vs. placebo	Decreased lung injury and expression of P-selectin and ICAM-1

PARP: poly (adenosindiphosphate ribose) polymerase; ARDS: acute respiratory distress syndrome; cont.: continuous i.v.: intravenous; PaO_2/FiO_2 ratio: arterial partial pressure of oxygen-to-inspired oxygen fraction; Qs/Qt: pulmonary shunt fraction; iNOS: inducible nitric oxide synthase; mRNA: messenger ribonucleic acid; NOx: total amount of nitric oxide metabolites; i.p.: intraperitoneal; TNF alpha: tumor necrosis factor alpha; NO: nitric oxide; BALF: bronchoalveolar lavage fluid; ICAM: intracellular adhesion molecule

led to decreased levels of inflammatory mediators in the brochoalveolar lavage fluid (54). Lobo *et al*. supposed that continuous infusion of PJ34 in septic rabbits might limit bacterial translocation. However, there was no difference in lung wet-to-dry ratios between treatment and placebo animals (13). Initial bolus administration followed by continuous infusion of the PARP inhibitor INO-1001 improved pulmonary gas exchange and decreased lung edema after B+S as well as after SI+S injury (22, 41). Beside these first promising reports, nothing is known yet about long-term consequences. Since PARP is involved in DNA repair, its inhibition might have carcinogenic effects (55).

Experimental trials using the selective nNOS inhibitor 7-NI for the treatment of ARDS revealed

Table 3. Selective nNOS inhibition in experimental ARDS

Study design	Treatment	Main results
Burn and smoke inhalation; sheep; 48 h (35)	7-nitroindazole: 1 mg·kg^{-1}·h^{-1} cont. i.v., started after 1h, vs. placebo	Prevention of drop in PaO_2/FiO_2-ratio and hypoxic pulmonary vasoconstriction, decrease in airway obstruction, ventilatory pressures, prevention of increases in NOx-plasma levels, iNOS mRNA and 3-nitrotyrosine
Smoke inhalation and sepsis; sheep; 24 h (40)	7-nitroindazole: 1 mg·kg^{-1}·h^{-1} cont. i.v., started after 1 h, vs. placebo, non-selective NOS and selective iNOS inhibition	Prevention of drop in PaO_2/FiO_2-ratio, decrease in airway obstruction, Qs/Qt, pulmonary edema and airway blood flow, prevention of increases in NOx-plasma levels
Smoke inhalation; rats; 24 h (14)	7-nitroindazole: 20 mg/kg i.p., vs. placebo	Increased PaO_2, decreased lung water, NO content, nNOS activity, super oxide dismutase and catalase concentration in the lung

nNOS: neuronal nitric oxide synthase; ARDS: acute respiratory distress syndrome; i.v.: intravenous; cont.: continuous; PaO_2/FiO_2 ratio: arterial partial pressure of oxygen-to-inspired oxygen fraction; NOx: total amount of nitric oxide metabolites; iNOS: inducible nitric oxide synthase; mRNA: messenger ribonucleic acid; i.p.: intraperitoneal; NO: nitric oxide

encouraging results (Table 3). The PaO_2/FiO_2- ratio was significantly higher in treated animals than in controls, while pulmonary edema and airway obstruction were decreased in both the B+S (35) as well as the SI+S model (40). In addition, the formation of RNS was blocked in nNOS treated animals as reflected by lung tissue concentrations of 3-nitrotyrosine that were at the level of sham animals (Figure 4). The nNOS inhibitor was similar effective as iNOS or PARP inhibition in attenuating the degree of pulmonary dysfunction in ovine ARDS. Remarkably, 7-NI was most effective in the initial phase post injury (40).

The combination of simultaneous administration of the selective iNOS inhibitor BBS-2 and the selective nNOS inhibitor 7-NI did not further improve pulmonary function as compared to single-drug infusion (unpublished data). Notably, some of these animals suffered from gut perforation. These results again emphasize the physiological role of NO for the immune defence and the paramount importance of specific treatment. Based on the notion that nNOS is primarily implicated in the early phase of ARDS (40) and iNOS in the later stage of the pathogenesis (8), our group recently investigated the effects of nNOS inhibition (7-NI) in the first 12 h after SI+S injury and selective iNOS inhibition (BBS-2) during the second 12 h of the experiment (56). Although this combination therapy reduced cardiopulmonary dysfunction, airway obstruction and nitrosative stress as compared to untreated animals, the effects were not superior to each of the mono-therapies of these inhibitors.

Against the presented evidence of beneficial effects of NO synthesis inhibition, the inhaled administration of NO for the treatment of ARDS patients seems to be even more questionable than before. Inhaled NO is thought to improve oxygenation and to reduce increased pulmonary vascular resistance by reversing the ventilation-perfusion mismatch in ARDS due to selective vasodilation in well ventilated lung areas. Based on limited data on efficacy, clinicians rapidly adopted this therapy; in 1997, 63% of European intensive care specialists reported using NO for ALI or ARDS (57). Several randomized, controlled trials as well as recent meta-analyses, however, revealed only a positive effect on the patient's oxygenation for 24 h (in some studies until 48 h) (58-60). No advantages in respect to mortality, ventilator-free days, duration of ventilation or pulmonary hypertension could be verified. Contrary, there even was a strong trend to an increased mortality in patients receiving NO as compared to placebo treatment (58).

Beside the possibly increased production of RNS due to NO administration, there are several other reasons for these findings. Long-term treatment with NO might lead to diffusion of NO into poorly ventilated areas abolishing the selective pulmonary vasoconstriction and consecutively increasing pulmonary shunt volume. In addition, surfactant inhibition, edematous changes, and continuing fibrosis may override any benefit of inhaled NO therapy. Having in mind that the main causes of death in ARDS patients are the underlying disease, multi organ failure and sepsis rather than hypoxemia (61), treatments aimed at modifying lung disease seem to be unlikely to beneficially influence the outcome. Although controversially discussed, unwanted side effects of inhaled NO like renal dysfunction and methemoglobinemia have been described (58). In contrast to the frequent use of inhaled NO in intensive care units, based on the current literature the standard treatment of ARDS patients with inhaled NO cannot be recommended. However, there are specific indications that still justify the use of inhaled NO. In contrast to children and adults, pulmonary artery resistance in newborns with ARDS is often higher than the systemic vascular resistance leading to right heart failure and subsequent death. Therefore, these newborns benefit from the vasodilating properties of inhaled NO (62, 63). In addition, short-term inhaled administration of NO might be considered as a rescue treatment to improve oxygenation for a short period in ARDS patient with acute, life threatening hypoxemia (60).

Summarizing the results of these studies, the following conclusions can be drawn:

- The interruption of the described vicious cycle consisting of ROS, NO and RNS syntheses and cell damage is necessary to attenuate the pulmonary dysfunction related to ARDS.
- The individual role of the different NOS isoforms and NO itself within the pathogenesis of ARDS may vary depending on the nature of the injury.
- Different NOS isoforms are activated at different time points of ARDS.

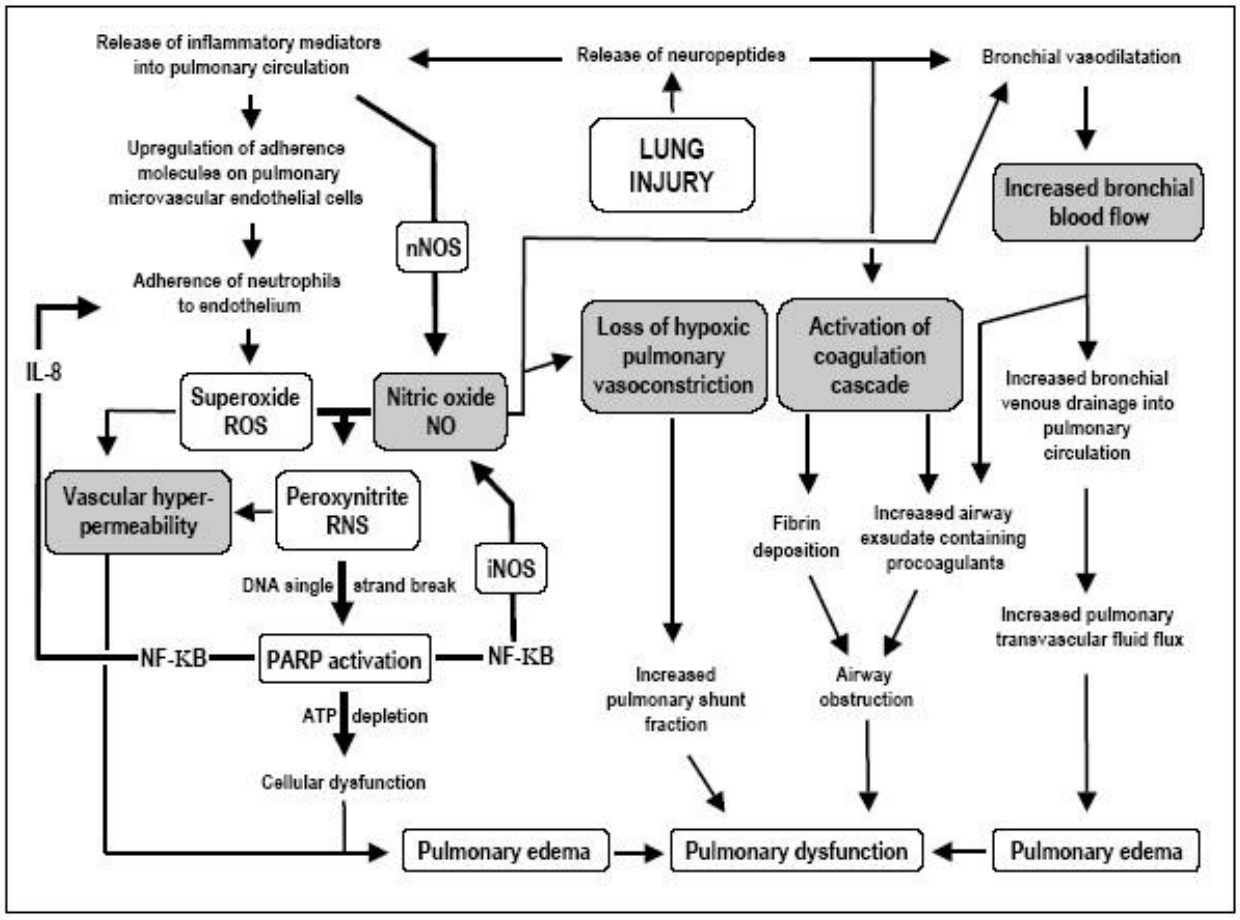

Figure 3. Proposed role of niric oxide and reactive nitrogen species in the current pathogenesis of acute respiratory distress syndrome. The neuronal nitric oxide synthase (nNOS) causes the initial overproduction of nitric oxide (NO) leading to formation of reactive nitrogen species (RNS). RNS cause poly (adenosindiphosphate-ribose) polymerase (PARP) activation, which up-regulates the inducible nitric oxide synthase (iNOS) resulting in a perpetuation of the vicious cycle. Depending on the causal injury, there may be differences from this scheme.

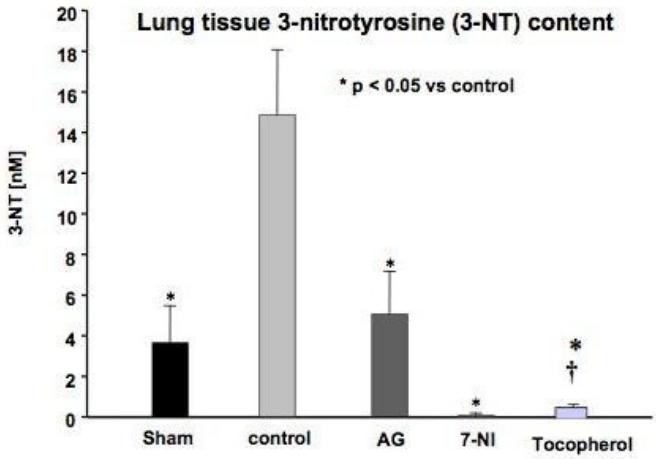

Figure 4. 3-nitrotyrosine (3-NT) levels 48 h after combined burn and smoke inhalation injury. Sham animals (no injury, no treatment) show a small amount of 3-NT suggesting some baseline activity. Control animals (injury, no treatment) show a large response that is markedly reduced by either treatment with aminoguanidine (AG), 7-nitroindazole (7-NI) or alpha-tocopherol. *, $p \leq 0.05$ vs. control; †, $p \leq 0.05$ vs. sham; values are expressed as means ± standard error.

- A single drug (e.g. NO formation inhibitor) is not sufficient to prevent the complex pathophysiological changes associated with ARDS.
- Based on the current literature, inhaled NO cannot be recommended for the standard care of ARDS patients.

The combination of different therapeutic strategies should be considered in the future. ROS and NO are produced physiologically by the organism. As long as the balance between oxidant and antioxidant effects is preserved no negative effects occur. Instead of solely trying to inhibit the formation of ROS and RNS by NOS or PARP inhibitors, it might, therefore, be reasonable to add antioxidant therapies. The nebulization of vitamin E, an oxidant scavenger, has been shown to improve pulmonary function after B+S injury (64-66). However, a combination therapy has never been investigated yet.

As demonstrated in Figure 3, there are additional or related mechanisms contributing to pulmonary dysfunction. One example is the activation of the coagulation cascade. Accordingly, different anticoagulants, like activated protein C (APC), heparin or antithrombin have been shown to ameliorate ARDS related pulmonary dysfunction in experimental and clinical trials (67). APC seems to be very promising in sepsis, because of the compensation of the sepsis-related protein C deficiency (68). Although very rare, the possible occurrence of severe bleeding events associated with APC treatment needs to be considered.

ARDS is further defined by increased permeability of pulmonary capillaries. Selective V1a receptor agonists might be able to reduce the degree of capillary leakage (69). In our SI+S model a selective V1a agonist reduced pulmonary lymph flow and fluid accumulation to the level of sham animals (70). In sepsis related ARDS the antibiotic therapy is of course of paramount importance for a successful treatment. The repetitive bolus administration of 3 g ceftazidime ameliorated the drop of the PaO_2/FiO_2 ratio, the airway obstruction and the pulmonary shunt fraction as compared to untreated animals. In addition, 3-NT concentrations in the lung remained at baseline levels (71).

Future experimental trials are warranted to evaluate the most beneficial time point and the most efficient way of administration for each of the compounds. The second step will consist in combining the different approaches according to the pathogenesis of the causal injury to finally reduce mortality in ARDS patients.

6. CONCLUSIONS

The current literature provides convincing evidence for the key role of NO and RNS in experimental ARDS. Within the inflammation process, neuropeptides activate nNOS to up-regulate its production of NO, which reacts with ROS to form cytotoxic products called RNS resulting in cellular dysfunction and death. RNS lead to activation of PARP and a subsequent increase in NF-kappa B, which induces the formation of iNOS accelerating the production of NO and perpenuating the vicious cycle. However, there is evidence that the different NOS isoforms are activated at different time points within the pathogenesis of ARDS. In addition, their role may vary depending on the causal injury. Last but not least, additional mechanisms like the activation of the coagulation cascade have to be considered. Future trials are warranted to develop more specific approaches, probably consisting of combined therapeutic strategies for the treatment of ARDS.

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Abbreviations: ARDS: acute respiratory distress syndrome; ALI; acute lung injury: NO; nitric oxide; RNS reactive nitrogen species; B+S: burn and smoke inhalation injury; FiO_2: inspired oxygen fraction; PaO_2: arterial partial oxygen pressure; SI+S: smoke inhalation injury and sepsis; ROS: reactive oxygen species; PMN: polymorphonuclear cells; NOS: nitric oxide synthase; nNOS: neuronal nitric oxide synthase; eNOS: endothelial nitric oxide synthase; iNOS: inducible nitric oxide synthase; DNA: desoxyribonucleic acid; PARP: poly (adenosindiphosphate-ribose) polymerase; ATP: adenosine triphosphate; NOx: total amount of nitric oxide metabolites; mRNA: messenger ribonucleic acid; 3-NT: 3-nitrotyrosine; PAR: poly adenosindiphosphate-ribose; MEG: mercaptoethyl-guanidine; NF-kappa B: nuclear factor kappa B; IL-8: interleukin-8; L-NAME: L-nitro-arginine-methylester; APC: activated protein C; 7-NI: 7-nitroindazole

Key Words: Acute Respiratory Distress Syndrome, Inhalation Injury, Nitric Oxide Reactive Nitrogen Species, Poly-(Adp-Ribose) Polymerase Inhibitor, Review

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