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Role of nitric oxide in shock: the large animal perspective

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1. ABSTRACT

Excessive nitric oxide (NO) formation plays important roles in the pathogenesis of shock and multiple organ failure in sepsis and acute lung injury (ALI). Evidence from studies in large animal models of shock provide further insight into the role of NO and the varying nitric oxide synthase (NOS) isoforms. Nonselective NOS inhibition in sepsis models reversed sepsis-induced derangements in hemodynamic status, but was associated with side effects such as pulmonary vasoconstriction and decreases in global oxygen delivery. Results from studies on specific inhibition of inducible NOS (iNOS, NOS-2) and neuronal NOS (nNOS, NOS-1) in sepsis models remain inconclusive, but suggest that both isoenzymes are involved in the pathophysiological processes. While the long-term effects of NOS inhibition in models of burn and inhalation injury remain unknown, specific iNOS inhibition attenuated ALI without worsening injury-related pulmonary hypertension. Further investigation in large animal models is warranted to clarify the time course of increased expression and/or activity of different NOS isoenzymes and the effects of specific inhibition of the NOS isoforms at different time points.

2. INTRODUCTION

Nitric oxide (NO) is an endogenous vasodilator generated from L-arginine through catalysis by a family of enzymes called NO synthases (NOS). Three different genetic isoforms of NOS have been identified in mammals (1); in contrast to the constitutively synthesized isoenzymes endothelial NOS (eNOS, NOS-3) and neuronal NOS (nNOS, NOS-1), the inducible NOS (iNOS, NOS-2) is up-regulated by diverse stress stimuli such as oxidative burst and systemic inflammation. Constitutively produced NO is involved in various physiologic processes, including neurotransmission and the regulation of vascular tone and blood flow (2). Under pathophysiological conditions, however, endotoxin or inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), interferon gamma (IFN-gamma) and tumor necrosis factor alpha (TNF-alpha) may lead to an increased expression of iNOS. The resulting overproduction of NO is thought to be an important factor in the pathogenesis of shock and multiple organ failure resulting from sepsis and acute lung injury (2-4).

Importantly, a significant amount of research on the role of NO in shock states of various etiologies has

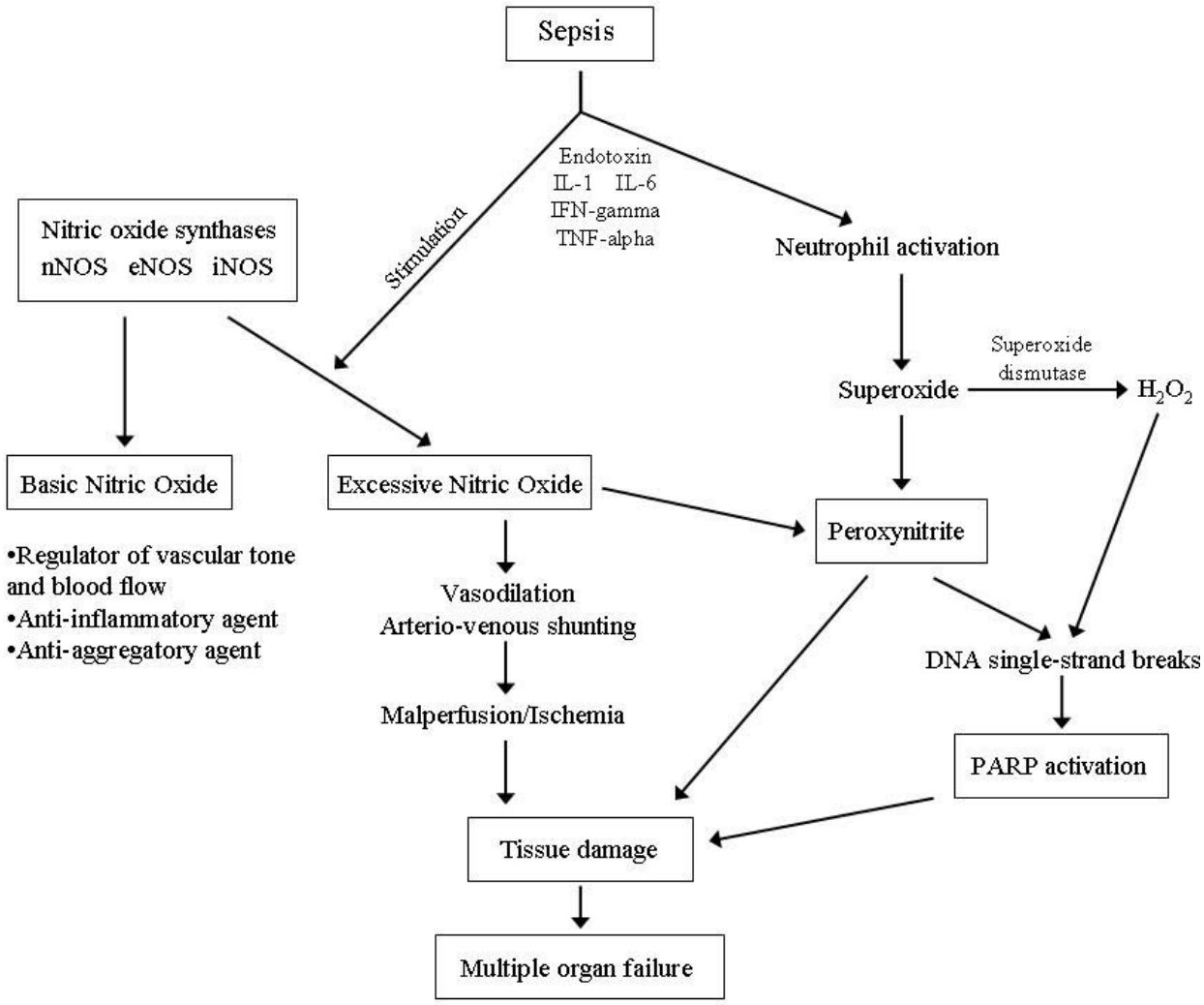

Figure 1. Possible role of nitric oxide in the pathophysiology of sepsis. Excessive nitric oxide production leads to vasodilation and arterio-venous shunting as well as peroxynitrite formation and poly(ADP-ribose) polymerase (PARP) activation, both contributing to tissue damage and multiple organ failure. The roles of the different nitric oxide synthase (NOS) isoenzymes at different time points of sepsis are not sufficiently identified.

been conducted in large animal models. This review examines the role of NO production and its pharmacological inhibition in large animal models of septic shock and/or acute lung injury.

3. ROLE OF NITRIC OXIDE IN ENDOTOXEMIA AND SEPSIS

Sepsis is a state of sustained infection, resulting in a severe systemic inflammatory response and, ultimately, shock. Despite significant improvements in critical care medicine during the last few decades, the mortality in septic shock remains high (5). The pathophysiological changes in patients with sepsis are typically characterized by systemic vasodilation to metabolically inactive tissues (6). The resultant systemic arterial hypotension reduces blood flow to organs that are metabolically active. The ensuing misdistribution of systemic and microvascular blood flow leads to an impairment of tissue oxygenation, finally resulting in multiple organ failure (7, 8). Excessive formation of NO may be critically involved in these vascular changes (Figure 1). Via its secondary messenger, cyclic guanosine monophosphate, NO activates the myosin phosphatase and, by dephosphorylating myosin, causes vasodilation (8). Moreover, excessive NO formation may activate potassium channels in vascular smooth muscles, thereby causing vaso-relaxation (9, 10). The amount of NO production within the vascular system may vary at different anatomical sites, resulting in different degrees of vasodilation. Consequently, an underperfusion of metabolically active tissue and an overperfusion of metabolically inactive tissues may occur, possibly contributing to the deficient oxygen extraction, tissue hypoxia and lactic acidosis often observed in patients with septic shock (2).

Increased plasma and urine levels of the stable NO byproducts nitrate and nitrite in septic patients, combined with the identification of the endothelium-derived relaxing factor as NO, led to the assumption that NO may be involved in the pathogenesis of cardiovascular changes in septic shock (4, 11). Since then, a vast number of studies have been performed, investigating the role of NO in the pathogenesis of septic shock. Most research in that field has been conducted in animal models. Large animal models are most suitable to study the pathophysiology of septic shock and the effects of various treatment strategies because they exert circulatory alterations that closely mimic the hemodynamic changes in patients with sepsis (12-14), whereas rodents produce NO at a much greater rate.

In response to continuous infusion of endotoxin or live bacteria, sheep typically develop significant decreases in systemic vascular resistance and blood pressure, while cardiac output and pulmonary pressure markedly increase. Furthermore, endotoxin infusion in sheep is associated with impairments of global oxygen transport and significantly increased regional blood flows (15-22). Notably, these endotoxin-related changes could be largely reversed by administration of the nonselective NOS inhibitor L-nitro-arginine-methylester (L-NAME), indicating that increased NO synthesis has a major role in the cardiovascular alterations in ovine endotoxemia. Infusion of L-NAME in endotoxemic ewes restored systemic vascular resistance and mean arterial pressure, while heart rate and cardiac index decreased. In addition, nonselective NOS inhibition in sheep reversed the endotoxin-induced elevation in cardiac output, oxygen delivery and regional blood flows (19-21).

Like sheep, pigs exert a hyperdynamic circulation and a substantial decrease in systemic vascular resistance in response to continuous infusion of endotoxin (23-25). In an animal model of endotoxic shock in swine, Santak *et al.* (25) confirmed the significant role of increased NO formation in endotoxin-induced cardiovascular changes. Infusion of the nonselective NOS inhibitor N-monomethyl-L-arginine (L-NMMA) reversed the hyperdynamic circulation close to pre-endotoxin levels. The authors also reported an attenuation of the endotoxin-related increase in NO_3^- production by L-NMMA. However, despite hemodynamic stabilization, L-NMMA administration in pigs failed to beneficially influence the endotoxin-induced disturbances of both intestinal and liver energy balance (26, 27).

The role of NO in the pathogenesis of cardiovascular changes in response to endotoxin or tumor necrosis factor (TNF) has been investigated in a dog model (28-31). In anesthetized dogs, TNF, a cytotoxin produced by macrophages in reaction to bacterial endotoxin, induced a significant fall in mean arterial pressure, which was completely reversed by bolus infusion of L-NAME (28). Similarly, the decreases in systemic vascular resistance and mean arterial pressure following endotoxin infusion in dogs were inverted by bolus administration of L-NMMA (29). In the same animal model, Zhang *et al.* (30) tested the effects of methylene blue, an inhibitor of soluble guanylate cyclase, on cardiopulmonary hemodynamics in endotoxic shock. Methylene blue increased systemic vascular resistance and arterial pressure in a dose-dependent manner, while organ blood flows decreased.

Taken together, these findings in dogs are in agreement with those made in sheep and pigs, suggesting that NO is critically involved in the pathophysiology of cardiovascular alterations due to endotoxemia in large animals. However, nonselective NOS inhibition in large animals was also associated with several unfavorable side effects. Inhibition of NOS by L-NAME and L-NMMA apparently reversed the endotoxin-related hyperdynamic circulation, as indicated by decreases in cardiac output, oxygen delivery and regional blood flows, including hepatic, portal, mesenteric and renal blood flow (15, 19-21, 30, 31). Nonetheless, it should be noted that NO is not only involved in pathophysiological processes (e.g., iNOS), but constitutively produced NO is an important physiological regulator of vascular tone and blood flow (e.g., nNOS, eNOS). Especially in sepsis, a condition of increased oxygen demand, it appears deleterious to inhibit all NOS isoforms to the same extent, because this process may lead to a further dysregulation of local vascular tone and regional perfusion, thereby possibly fostering tissue hypoxia and organ failure.

Furthermore, administration of L-NAME in sheep, dogs and pigs aggravated endotoxin-related pulmonary hypertension (15, 17, 20, 21, 25, 28-33) and pulmonary edema (17). This phenomenon may be explained by the blunt of vasodilatory effects of constitutively produced NO due to nonselective NOS inhibition. In this regard, it could be demonstrated that concomitant inhalation of NO decreased pulmonary hypertension (17, 32, 33) and ameliorated pulmonary edema (17) in experimental endotoxemia.

In interpreting these findings, it appears desirable to selectively inhibit iNOS without affecting the beneficial effects of constitutively expressed NOS. Several studies on selective iNOS inhibition using different compounds have been conducted. However, the results of these investigations remain largely inconclusive.

Booke *et al.* (34) investigated the effects of S-ethylisothiourea (S-EITU), a selective iNOS inhibitor *in vitro*, in healthy sheep and sheep exposed to continuous infusion of live bacteria. Since the overproduction of iNOS is believed to be responsible for septic vasodilation, S-EITU was expected to cause a more intense vasoconstriction under septic conditions. However, the effects of S-EITU on hemodynamics and regional blood flows were comparable in both septic and healthy sheep, suggesting either that S-EITU does not selectively inhibit iNOS or that other mediators besides NO play a significant role in septic vasodilation in sheep. Employing different selective iNOS inhibitors in an animal model of porcine endotoxemia also yielded controversial results. Administration of mercaptoethylguanadine (MEG) decreased the amount of expired NO and prevented the fall

in systemic blood pressure without affecting cardiac output, but failed to improve the disturbances in hepatosplanchnic metabolism (24).

In contrast, the application of N- [3-(aminomethyl) benzyl] acetamidine hydrochloride (1400W) reversed the endotoxin-associated disturbances in the systemic circulation and attenuated the impairment of intestinal and hepatocellular oxygenation and energy state (23, 35). Matejovic *et al.* (36) investigated the selective iNOS inhibitor L-N6-(1-iminoethyl)-lysine (L-NIL) in a model of continuous *Pseudomonas aeruginosa* infusion in pigs. Besides an inhibition of plasma nitrate/nitrite (NOx) levels and a stabilization of systemic hemodynamics, the authors observed several beneficial effects of L-NIL on hepatosplanchnic metabolism, including a mitigation of the sepsis-associated impairment of hepatosplanchnic redox state and liver lactate clearance, as well as an attenuation of mesenteric and hepatic venous acidosis.

Recently, an ovine model has been developed that induces sepsis by instillation of live *Pseudomonas aeruginosa* bacteria into the airways following acute lung injury by smoke inhalation (37, 38). This large animal model resembles the pathophysiological conditions of hyperdynamic sepsis in humans more closely than models of continuous intravenous endotoxin or bacteria infusion and may, therefore, provide further insight into the role of increased NO formation and the value of selective pharmacological NOS inhibition. However, while the administration of aminoguanidine, a specific inhibitor of iNOS, significantly inhibited the increase in plasma NOx concentrations in this model, it failed to prevent the drop in mean arterial pressure and pulmonary gas exchange and pulmonary shunt fraction. Likewise, the increases in lung wet-to-dry weightratio and bronchial blood flow were not inhibited by aminoguanidine (38). It remains unclear why aminoguanidine was effective in reversing the endotoxin-induced changes in sheep, as reported by Evgenov *et al.* (39), but this may be related to the differences in the two animal models. Endotoxin infusion in sheep produces an acute and severe response, although the animals usually recover spontaneously after discontinuation of infusion. Instillation of live bacteria into the lungs produces a subacute response, likely with a different pathophysiology. In the same ovine model of sepsis following acute lung injury, Enkhbaatar *et al.* (40) investigated the effects of BBS-2, a newer and more potent selective iNOS inhibitor. Although BBS-2 significantly improved the pulmonary gas exchange and partially attenuated airway obstruction and increased ventilatory pressures, lung water content (lung wet-to-dry weight ratio) was not affected and septic vasodilation could not be reversed.

These results indicate that increased iNOS expression is only partially responsible for the pathophysiological alterations in sepsis induced by smoke inhalation and bacterial instillation in the airway in sheep. On the other hand, nonselective NOS inhibition improved those changes (38), suggesting that constitutively-produced NOS may be more critically involved in the septic process. Neuronal NOS is a constitutively expressed isoform of NOS and its activation is regulated by the intracellular concentration of calcium. It is present in both the central and peripheral nervous system (41). The presence of nNOS in the airway epithelium, airway smooth muscle, submucosal glands, blood vessels, non-adrenergic non-cholinergic nerve endings, and in the airway intrinsic parasympathetic plexus has been described (42). We have also identified nNOS in the airway and goblet cells of the bronchi (43). This is another finding that differentiates large animals including humans from mice and rats since these small animals lack the large mucous secreting cells. The results of these studies led to the hypothesis that nNOS-derived NO in the lung could possibly participate in the pathogenesis of lung injury associated with sepsis. To test this hypothesis, Enkhbaatar *et al.* (44, 45) investigated the effects of 7-nitroindazole (7-NI), a specific inhibitor of nNOS in septic sheep. The administration of 7-NI significantly inhibited the increased plasma NOx levels, suggesting that the up-regulation of NO was, at least in part, due to the nNOS isoenzyme. In contrast to the specific iNOS inhibitor BBS-2, 7-NI significantly attenuated the drop in mean arterial pressure in sheep. Furthermore, nNOS blockade with 7-NI significantly improved the pulmonary gas exchange as well as reductions in lung water content, histological airway obstruction, and airway pressures. The fact that the specific nNOS inhibitor 7-NI reduced all these pathophysiological indices indicates that nNOS-derived NO could be an essential pathogenetic factor. Importantly, 7-NI inhibited the plasma NOx levels especially during the initial 12 hours after induction of sepsis, while the reduction was weaker during the second 12 hour interval after injury. These results suggest that early formation of NO was mainly derived from nNOS; and nNOS may be important in the up-regulation of iNOS during the later course of sepsis. In support of this relationship we found that iNOS mRNA is increased in a model of ARDS and that this increase was attenuated in animals that had been treated with a nNOS inhibitor (unpublished data). This hypothesis agrees with the finding that inhibition of plasma NOx levels by the selective iNOS inhibitor BBS-2 was greater at later time points (40).

Large amounts of NO exert potential cytotoxic and pro-inflammatory effects by reacting with superoxide radicals, yielding reactive nitrogen species such as peroxynitrite. Peroxynitrite exerts a deleterious influence by oxidizing/nitrating/nitrosating various other molecules or decaying and producing even more damaging species such as hydroxyl radicals (3, 46). Nitric oxide-mediated tissue injury may be related to DNA damage and subsequent activation of the nuclear enzyme poly (ADP-ribose) polymerase (PARP) (47, 48). After activation by DNA single-strand breaks, PARP catalyzes ADP-ribose subunits to nuclear proteins. This process depletes intracellular NAD^+ and reduces the rate of glycolysis, electron transfer and ATP formation. Excessive PARP activation in response to immense oxidant-induced DNA strand breakage causes cell necrosis (47-49). It has been demonstrated that PARP activation can be induced by NO or its toxic products such as peroxynitrite (50) (Figure 1).

There is good evidence of increased PARP activation and its detrimental effects in various large animal models of sepsis. Administration of the potent PARP inhibitor PJ34 in septic pigs following fecal peritonitis abolished injury-related poly (ADP-ribose) accumulation and formation of nitrotyrosine, a marker of oxidative/nitrative stress. In addition, inhibition of PARP synthesis by PJ34 significantly improved survival and attenuated both sepsis-induced hemodynamic changes as well as cytokine response (51). Murakami *et al.* (52) reported that administration of INO-1001, another PARP inhibitor, improved the acute lung injury induced by smoke inhalation and pneumonia in sheep. INO-1001 treatment attenuated the sepsis-induced worsening of pulmonary gas exchange and pulmonary shunt fraction. Moreover, INO-1001 reduced pulmonary histological injury and attenuated poly (ADP-ribose) formation in the lung.

4. ROLE OF NTRIC OXIDE IN BURN AND INHALATION INJURY

Despite the fact that care of burn victims has significantly improved with the use of broad-spectrum antibiotics, effective fluid resuscitation and early surgical removal of burned tissue, the mortality of burn victims with inhalation injury remains high (53, 54). In these patients, progressive pulmonary dysfunction and cardiovascular failure frequently occur, culminating in multiple organ failure and death.

Pulmonary edema formation after inhalation injury may be caused by critical changes in pulmonary blood flow and alterations in capillary permeability. Especially in patients with concomitant extensive cutaneous burns, vascular hyperpermeability occurs not only at the injured site, but also in regions distant from the injury (55, 56), leading to a fluid shift from the intravascular to the interstitial space. The loss of fluid from the circulation results in hypovolemic shock unless adequate fluid resuscitation is performed (57). The combination of capillary hyperpermeability and fluid resuscitation may lead to an excessive accumulation of fluid in the interstitial space of the lung. The ensuing pulmonary edema formation represents a major source of morbidity and mortality in burn patients (58).

In sheep, bronchial blood flow increases approximately 8-fold after smoke inhalation injury alone (59, 60), and tracheal blood flow increases approximately 20-fold after combined burn and inhalation injury, resulting in an impairment of pulmonary gas exchange and an increase in lung fluid content (61, 62). It has been demonstrated that these changes were all markedly improved by bronchial artery occlusion either by ligation or ethanol injection (63-65), suggesting that bronchial circulation also plays a crucial role in the pathophysiology of lung edema formation that occurs after smoke inhalation injury.

The pathophysiological response to combined smoke and inhalation injury in sheep has been described previously (43, 61, 62, 64-71). Acute lung injury in this large animal model is characterized by significant increases in transpulmonary fluid flux and lung water content (wet-to-dry weight ratio), as well as significant decreases in PaO_2/FiO_2 (partial arterial O_2 pressure/inspired O_2 fraction) ratio. These changes are associated with the occurrence of marked airway obstruction and increases in ventilatory pressures.

As an essential regulator of vasotonus and microcirculatory blood flow, including vascular permeability (72), NO is thought to be critically involved in the regulation of bronchial blood flow (73). In acute lung injury, however, disturbances of NO synthesis in the lung tissue may, at least in part, account for the observed pathophysiological alterations (Figure 2). It has been demonstrated that human lung epithelium cells express iNOS (74), and that iNOS is up-regulated after burn and smoke inhalation injury in sheep (71).

Plasma NOx levels are known to be significantly increased in sheep exposed to burn and inhalation injury, as compared to uninjured control animals (61, 69). This increase in NOx levels can be eliminated by NOS inhibition (61, 71). Because inhalation injury mainly affects the lung, arginine metabolism in lung tissue has been measured by using the stable isotope (^{15}N) arginine as a tracer in this ovine model. 24 hours after injury, lung arginine metabolism was markedly increased and could be significantly attenuated by administration of the nonselective NOS inhibitor L-NAME, suggesting that excessive NO may be responsible for the increased arginine metabolism (75). It has also been reported that the NOS enzymes can become uncoupled when arginine levels are reduced resulting in the formation of superoxide and peroxynitrite (76). We reported that the levels of arginine are markedly reduced following burn and inhalation injury but that the restoration of arginine levels reduced the pathophysiology seen with inhalation injury, suggesting that reactive oxygen and nitrogen species may be generated by NOS in this situation (77). To address these issues experiments were carried out in sheep with combined burn and smoke inhalation injury that were treated with vitamin E which scavenges reactive oxygen species. This treatment with tocopherols prevented increases in 3-nitrotyrosine, a peroxynitrite marker, as well as much of the pathophysiology that was noted in untreated subjects with burn and smoke inhalation injury (67, 68).

The effects of different specific iNOS inhibitors on pulmonary function and vascular permeability have been investigated in combined burn and smoke inhalation injury in sheep (61, 71, 78). Inhibition of iNOS in these studies congruently resulted in a significant amelioration of pathologically altered variables, providing evidence that iNOS is a key mediator of pulmonary pathology in this model. Administration of iNOS reversed the impairment of pulmonary gas exchange in addition to reducing pulmonary shunt fraction, tracheal blood flow, lung water content, and lung lymph flow. Furthermore, signs of histologically determined airway obstruction as well as increased ventilatory pressures were significantly attenuated. However, the exact mechanism by which NO formation contributes to the development of acute lung injury has not yet been clarified.

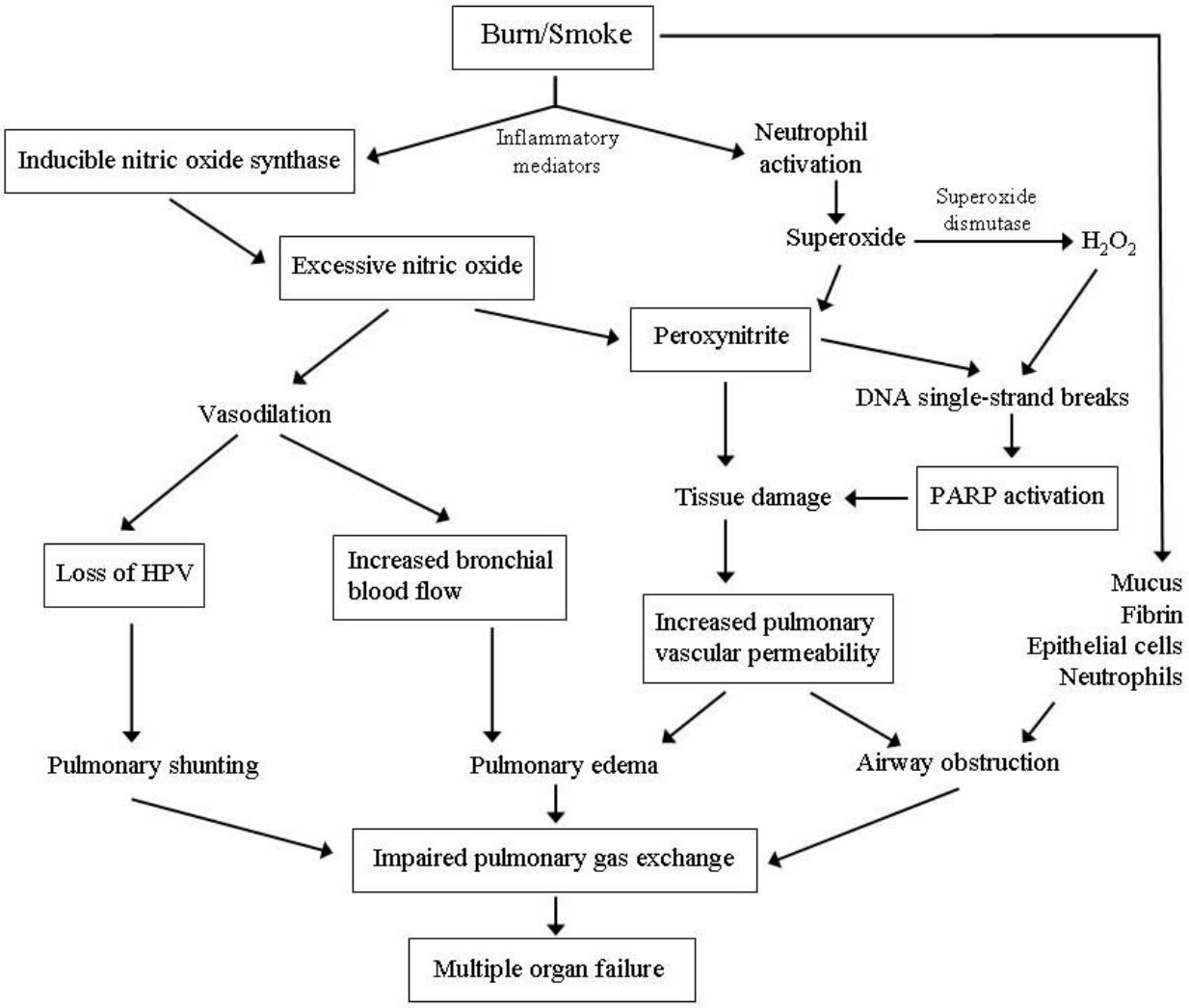

Figure 2. Possible role of nitric oxide in the pathophysiology of burn and inhalation injury. Excessive nitric oxide production by the inducible nitric oxide synthase causes pulmonary vasodilation, leading to the loss of hypoxic pulmonary vasoconstriction (HPV) and increased bronchial blood flow which, in turn, result in pulmonary shunting and pulmonary edema. Both nitric oxide-induced peroxynitrite formation and poly(ADP-ribose) polymerase (PARP) activation cause increased pulmonary vascular permeability. In combination with mucus secretion, fibrin clotting, as well as congregation of neutrophils and epithelial cell debris, airway obstruction occurs. Pulmonary shunting, pulmonary edema and airway obstruction result in impaired pulmonary gas exchange and ultimately multiple organ failure.

Hypoxic pulmonary vasoconstriction (HPV) is a physiologic reflex that matches lung perfusion to ventilation in order to optimize pulmonary gas exchange. Vasoconstriction occurs in under-ventilated, hypoxic areas of the lung, resulting in a diversion of blood flow from the unventilated to ventilated alveoli (79). Combined burn and smoke inhalation injury has recently been demonstrated to impair HPV in sheep (80). Excessive formation of NO may lead to a critical disturbance in pulmonary vasoregulation with a subsequent loss of HPV. This pathomechanism may play an important role in the pulmonary changes following inhalation injury (Figure 2).

As described above, large amounts of NO exert potential pro-inflammatory and cytotoxic effects by reacting with superoxide radicals to form reactive nitrogen species such as peroxynitrite (81-83). Peroxynitrite may damage the alveolar capillary membrane, resulting in increased pulmonary vascular permeability and edema formation (83). Nitrotyrosine, a marker of peroxynitrite production, is markedly increased in lung tissue of sheep exposed to burn and smoke inhalation injury (80). This increase could be significantly attenuated by selective iNOS inhibition.

Excessive NO production and peroxynitrite formation cause DNA single-strand breakage with subsequent activation of PARP (50). To elucidate the role of PARP activation in burn and inhalation injury, Shimoda *et al.* (69) administered the selective PARP inhibitor INO-

1001 in an ovine model. INO-1001 attenuated the observed deterioration in pulmonary gas exchange, lung edema formation, increases in airway blood flow and airway pressure, as well as histological lung injury. These findings suggest that PARP is involved in the lung damage caused by combined burn and inhalation injury in sheep (Figure 2).

Additional studies have been performed to asses the effects of selective iNOS inhibition on extrapulmonary co-morbidities in burn and smoke inhalation injury in sheep (66, 70). The injury induced systemic vascular leakage as evidenced by hemoconcentration and increased prefemoral lymph flow, an effect that could be reversed by iNOS inhibition. Moreover, iNOS inhibition attenuated both injury-associated myocardial depression and impaired renal function. These findings establish an important role of iNOS in the pathogenesis of systemic morbidity and multiple organ failure in combined burn and inhalation injury.

5. CONCLUSIONS

Results from large animal models prove the significant role of NO, subsequent peroxynitrite formation and PARP activation in the pathophysiology of shock and organ failure, resulting from both sepsis and combined burn and inhalation injury. Nonselective NOS inhibition has been reported to improve sepsis-related derangements in hemodynamic status, while simultaneously inducing significant adverse effects such as decreases in global oxygen delivery and organ blood flow, as well as increases in pulmonary vascular resistance. These results from large animal models are consistent with data available from clinical studies. Although administration of the nonselective NOS inhibitor 546C88 has been demonstrated to promote the resolution of shock in septic patients (84), the drug actually increased mortality in a recent phase III trial (85). Notably, the protocol of the latter study allowed a more rapid dose escalation of 546C88, resulting in the application of higher doses. These findings suggest that lower doses of a nonselective NOS inhibitor may be more beneficial in human septic shock, possibly even improving survival (85). However, since the only available phase III study in this field demonstrated increased mortality in patients treated with nonselective NOS inhibitors, further clinical trials will be problematic. Future large animal studies may provide valuable information in determining whether lower doses of nonspecific NOS inhibitors improve sepsis-related cardiopulmonary dysfunction with reduced side effects.

Furthermore, it may be crucial to know which NOS isoforms are involved in the pathophysiology of sepsis, as well as their respective time points, when considering possible treatment strategies. It has been generally believed that NO derived from constitutive NOS exerts physiological, regulatory effects, while NO from iNOS is detrimental. This assumption needs to be revised, as recent experimental studies indicate that constitutive NOS isoforms are also involved in the pathophysiology of sepsis (86, 87). However, different isoforms may be increasingly expressed at different time points. Investigations on specific iNOS and nNOS inhibitors suggest that the early pathophysiological changes in ovine sepsis were induced by NO derived from nNOS, while NO from iNOS expression may account for the derangements in the later course of sepsis. Results from a rat model of sepsis following peritonitis indicate that the administration of a selective iNOS inhibitor improves survival only when given 12 hours after injury (88). The initiation of iNOS inhibition at an earlier time point in the same animal model even increased mortality. Future studies in large animal models are needed to shed light on the time course of different NOS isoenzyme expression in sepsis and the effects of their selective inhibition at different time points. Knowledge from these studies may allow for the development of more differentiated treatment strategies.

Existing evidence about the role of NO in burn and inhalation injury is more conclusive. Specific inhibition of iNOS attenuated acute lung injury without worsening injury-related pulmonary hypertension. However, the long-term effects of iNOS inhibition in burn and smoke inhalation injury have not yet been evaluated. In this regard, it is necessary to keep in mind that inhibition of iNOS may blunt the physiological, bactericidal properties of NO, possibly resulting in suppression of the host defense system with subsequent superinfection of burn tissue. Furthermore, NO physiologically exerts anti-aggregatory effects on different cell types, including platelets. Blunting these properties may increase the risk of blood clotting and embolism. Therefore, further studies should investigate the long-term effects of iNOS inhibition after burn and smoke inhalation injury in large animals.

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Abbreviations: NO: nitric oxide; NOS: nitric oxide synthase; eNOS: endothelial NOS; nNOS: neuronal NOS; iNOS: inducible NOS; IL-1: interleukin 1; IL-2: interleukin 2; IFN: interferon gamma; TNF: tumor necrosis factor alpha; L-NAME: L-nitro-arginine-methylester; L-NMMA: N-monomethyl-L-arginine; S-EITU: S-ethylisothiourea; MEG: mercaptoethylguanadine; L-NIL: L-N6-(1-iminoethyl)-lysine; NOx: nitrate/nitrite; 7-NI: 7-nitroindazole; DNA: desoxyribo-nucleic acid; PARP: poly(ADP-ribose) polymerase; NAD: nicotinamide adenine dinucleotide; ATP: adenosine triphosphate; HPV: hypoxic pulmonary vasoconstriction

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