Pathophysiology of acute lung injury in combined burn and smoke inhalation injury

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ABSTRACT
In the U.S.A., more than 1 million burn injuries occur every year. Although the survival from burn injury has increased in recent years with the development of effective fluid resuscitation management and early surgical excision of burned tissue, the mortality of burn injury is still high. In these fire victims, progressive pulmonary failure and cardiovascular dysfunction are important determinants of morbidity and mortality. The morbidity and mortality increases when burn injury is associated with smoke inhalation. In the present review, we will describe the pathophysiological aspects of acute lung injury induced by combined burn and smoke inhalation and examine various therapeutic approaches.

ACUTE LUNG INJURY AND BRONCHIAL CIRCULATION
ARDS (acute respiratory distress syndrome) is one of the major complications of thermal injury. There are several factors that affect the pulmonary function. In patients with extensive cutaneous burns in which the burned area exceeds 30% of the TBSA (total body surface area), capillary hyperpermeability occurs not only at the injured site, but also in regions distant from the injury [1,2]. Vascular hyperpermeability leads to a large amount of fluid flux from the circulating plasma to the interstitial spaces. This lung oedema formation is even more severe when the thermal injury is associated with smoke inhalation [3]. The investigators in our laboratory have shown previously that both smoke inhalation alone [4,5] and combined burn and smoke inhalation injury [3] causes pulmonary microvascular hyperpermeability to both fluid and protein. Pulmonary oedema formation could be affected by changes in both systemic and pulmonary blood supply. Bronchial blood flow enters into the pulmonary vasculature through the various bronchopulmonary anastomoses. Airway (trachea) blood flow increases approx. 20-fold in sheep with combined burn and smoke inhalation injury [3,6]. Abdi et al. [7] reported that bronchial blood flow was increased approx. 8-fold after smoke inhalation injury in sheep. The bronchial artery occlusion either by ligation or ethanol injection after smoke inhalation injury improved pulmonary function markedly. Reduced bronchial blood flow reversed the fall in pulmonary gas exchange and an increase in lung lymph flow and lung water content seen in sheep with smoke inhalation injury [8]. We have described previously [3,6] the pathophysiological responses to the combined burn and smoke inhalation injury in sheep. Acute lung injury in this model is characterized by an

Key words: acute lung injury, burn and smoke inhalation, cytokine, nitric oxide (NO), pathophysiology; poly(ADP-ribose) polymerase (PARP).
Abbreviations: ARDS, acute respiratory distress syndrome; BALF, bronchoalveolar lavage fluid; COX, cyclo-oxygenase; HPV, hypoxic vasoconstriction; IL-1, interleukin-1; IL-8, interleukin-8; LPS, lipopolysaccharide; NO, nitric oxide; NOx, nitrite and nitrate; NOS, NO synthase; eNOS, endothelial NOS; iNOS, inducible NOS; MPO, myeloperoxidase; nNOS, neuronal NOS; NF-κB, nuclear factor κB; O2−, superoxide; ONOO−, peroxynitrite; PaO2/FiO2, arterial partial pressure of O2/inspired fraction of O2; PARP, poly(ADP-ribose) polymerase; RNS, reactive nitrogen species; TNF-α, tumour necrosis factor-α; TPA, tissue plasminogen activator.
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increase in transpulmonary fluid flux (lung lymph flow), lung water content and a fall in the $\text{PaO}_2/\text{FiO}_2$ (arterial partial pressure of $\text{O}_2$/inspired fraction of $\text{O}_2$) ratio with increased pulmonary shunt fraction. Tissue injury is also shown by histological changes. These injured animals showed approx. 10-fold increase in lung lymph flow and the $\text{PaO}_2/\text{FiO}_2$ ratio was below 200 48 h after the injury. All abnormal changes were associated with marked airway obstruction with subsequent increase in ventilatory pressures (peak and pause airway pressures). Although the exact mechanisms of these pathological changes are not completely clear, several mechanistic aspects have been investigated. One of the possible candidates is NO (nitric oxide).

**ROLE OF NO IN ACUTE LUNG INJURY**

It is well known that NO plays an important role in the pathogenesis of conditions that are frequently complicated by ARDS, such as sepsis and multiple trauma. We have reported previously [9,10] an up-regulation of NO in burn and smoke inhalation injury. Plasma NOx (nitrite and nitrate), a stable metabolite of NO, was increased approx. 2- to 2.5-fold.

NO is formed from arginine by the enzyme NOS (NO synthase). There are three isofoms of NOS: nNOS (neuronal NOS), eNOS (endothelial NOS) and iNOS (inducible NOS). nNOS and eNOS are referred to as cNOS (constitutive NOS). The other isoform is induced by cytokines and bacterial products [11,12]. These cytokines and bacteria are present in multiple traumas, including the combination of burn and smoke inhalation. With combined thermal and inhalation injuries, cytokines, such as IL-1 (interleukin-1), are up-regulated in lung tissue [13]. In addition, translocation of endotoxin and bacteria from the intestine into the systemic circulation has been reported to occur after burn plus smoke inhalation injury [14,15]. Both IL-1 and endotoxin activate NF-$\kappa$B (nuclear factor-$\kappa$B), which induces the synthesis of iNOS. iNOS catalyses the production of large amounts of NO and, under conditions of substrate or cofactor limitation, may also synthesize $\text{O}_2^-$(superoxide) [16]. The formation of NO in the lung results in the loss of HPV (hypoxic vasoconstriction), the physiological process that diverts blood flow from alveoli that are not being ventilated and perfuses the alveoli that are being ventilated [17,18]. When the loss of HPV is present, vasodilation occurs in the low or non-ventilated areas of the lung, leading to ventilation/perfusion mismatching, which, in turn, results in poor oxygenation [19]. At high concentrations, NO becomes a potential pro-inflammatory and cytotoxic factor by reacting with $\text{O}_2^-$ to form the toxic product ONOO$^-$(peroxynitrite) [20], which can oxidize/nitrate other molecules or decay and produce even more damaging species, such as the hydroxyl radical [21]. ONOO$^-$ may damage the alveolar capillary membrane [22], resulting in additional pulmonary oedema (Figure 1). We have reported previously [9] the presence of ONOO$^-$ in the airway and parenchyma of the lung of sheep subjected to burn and smoke inhalation injury. We have also reported [23] that the plasma concentration of L-arginine is markedly depleted after burn and smoke inhalation injury. In conditions where arginine is depleted, NOS produces $\text{O}_2^-$, which also can cause tissue injury. Exogenous arginine treatment was shown [23] to ameliorate the pulmonary function in sheep subjected to combined burn and smoke inhalation injury. Recently, our laboratory [9] has demonstrated the involvement of iNOS in the pathogenesis of acute lung injury in sheep with combined burn and smoke inhalation injury. This evidence was initially obtained with L-NAME (N$^\text{N}$-nitro-L-arginine methyl ester), a non-selective NOS inhibitor, and subsequently with MEG (mercaptoethyl guanidine), a specific iNOS inhibitor. A key role of iNOS-derived NO in the pathogenesis of acute lung injury in combined burn and smoke inhalation injury model was described in our recent study [6] using the truly selective and potent iNOS dimerization inhibitor BBS-2, which would selectively inhibit de novo synthesized iNOS. The selectivity of this compound for iNOS is 1500 and 620 times higher than for eNOS and nNOS respectively [24]. The iNOS inhibitor significantly improved pulmonary status [i.e. pulmonary gas exchange and pulmonary vascular permeability (Figure 2), and histological changes]. Thus the role of iNOS-derived NO in the pathogenesis of acute lung injury induced by burn and smoke inhalation is significant.

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Currently, there is a significant debate over whether the inhibition of NO is beneficial or harmful and, if the excessive amount of NO should be suppressed, what isoform should be targeted. Recently, it was reported [25] that the non-selective NOS inhibitor 546C88 increased mortality in patients with septic shock. Non-specific NOS inhibitors inhibit all three isoforms of NOS, including eNOS, which adversely affects cardiovascular function. We have hypothesized that certain NOS isoforms could be expressed at certain time points depending on the type of injury. More targeted and selective inhibition of NOS isoforms at their maximal activity could be beneficial in the management of multiple organ failure in this model. Our recent studies suggest that iNOS may dominate the pathophysiology of the acute lung injury in a combined burn and smoke inhalation model. There are studies that have indicated that nNOS may be induced in pathological conditions [26]. We have also reported [27] a possible role of nNOS-derived NO in the pathogenesis of acute lung injury in sheep with sepsis. A specific nNOS inhibitor, 7-nitroindazole, was shown to reverse the pathological changes in sheep with smoke inhalation and pneumonia [27]. If eNOS-derived NO is harmful, it may present a therapeutic target similar to iNOS. To test this hypothesis, further studies should investigate the effects of a truly selective nNOS inhibitor on a model of combined burn and smoke inhalation injury. Recently, we have reported [27a] that a non-steroidal anti-inflammatory agent (ketorolac) improved pulmonary function in sheep subjected to burn and smoke inhalation by inhibiting an excessive NO. There are a number of studies showing that NOS and COX (cyclo-oxygenase) are co-localized in a variety of inflammation disorders [27b]. The interplay between excessive NO and COX should be investigated in the future studies.

**ROLE OF PARP [POLY (ADP-RIBOSE)-POLYMERASE] IN ACUTE LUNG INJURY**

It has been proposed that RNS (reactive nitrogen species)-mediated injury is related to DNA damage and the consequential activation of the nuclear enzyme PARP [28]. PARP is a chromatin-bound enzyme constitutively expressed in most cell types [29] and is involved in DNA repair [30]. The overactivation of PARP in response to oxidant-mediated DNA single-strand breaks leads to a fall in ATP and NAD$,+$, resulting in cellular dysfunction and ultimately to necrotic cell death.

PARP has been shown recently [31] to be involved in the regulation of inflammatory processes, being functionally associated with important transcription factors, most notably NF-$\kappa$B. Liaudet et al. [32] reported that the activation of PARP-1 is a central mechanism of LPS (lipopolysaccharide)-induced acute lung inflammation and that PARP-1 suppression resulted in the reduction of proinflammatory cytokines, such as TNF-$\alpha$ (tumour necrosis factor-$\alpha$), and chemokines, such as MIP-1 (macrophage inflammatory protein-1). The authors [32] also reported that PARP-1 inhibition resulted in suppressed MPO (myeloperoxidase) activity in lung tissue of mice. Pulido et al. [33] have shown that inhibition of PARP prevented the decrease in lung ATP levels and attenuated pulmonary dysfunction induced by LPS. It was shown that ONOO$^{-}$-dependent activation of PARP caused energy depletion and increased permeability in human pulmonary epithelial cells in vitro (Figure 3). PARP inhibition has also been shown [34,35] to result in...
the reduction of oedema formation in septic and non-septic models of lung inflammation. We have shown [34] that PARP inhibition results in a significant improvement in pulmonary function, as evidenced by improved oxygenation, reduced pulmonary vascular permeability and reduced lung water content in sheep with combined burn and smoke inhalation injury. Post-treatment with a PARP inhibitor was also shown [35] to improve cardiopulmonary dysfunction in sheep with smoke inhalation and pneumonia-induced sepsis. The pathological changes seen in injured (burn/smoke) non-treated animals were associated with a marked increase in PARP activation in lung tissue. PARP inhibition in these animals resulted in a marked decrease in plasma NOx levels. Liaudet et al. [32] reported that LPS-induced increase in BALF (bronchoalveolar lavage fluid) NOx was significantly reduced by PARP inhibition. The authors also showed that PARP-deficient mice have significantly less NOx compared with wild-type mice [32].

Activation of NF-κB causes an up-regulation of iNOS, thus accelerating the formation of NO and RNS. As mentioned above, RNS are potent stimulants of the PARP activation because of their high potential to damage DNA. Thus activation of iNOS and PARP may be linked. To test this hypothesis, further studies are needed to determine PARP activation in sheep subjected to burn and smoke inhalation injury and treated with iNOS inhibitor.

It is particularly interesting that the accumulation of neutrophils in the lung tissue in sheep with burn and smoke inhalation injury, evaluated by measuring MPO activity, was attenuated by a PARP inhibitor [34]. Histological examination revealed extravasated neutrophils in lung tissue of these sheep. A large amount of neutrophils were found in the lung lymph and airways (in the form of an obstructive cast). It is well known that oxygen radicals and elastase released from activated neutrophils, if sufficiently extensive, can cause tissue injury [36,37]. Thus the inhibition of excessive PARP activation could improve pulmonary function through (i) the inhibition of excessive NO, and (ii) the activation of neutrophils by interacting with transcriptional factors, such as NF-κB (Figure 3).

**ROLE OF AIRWAY OBSTRUCTION IN ACUTE LUNG INJURY**

One of the major causes of progressively worsening pulmonary gas exchange is airway obstruction. In a previous study, we have shown [38] that there was significant airway obstruction with a mean reduction in cross-sectional area of approx. 29% in bronchi, 11% in bronchioles and 1.2% in respiratory bronchioles in sheep 48 h after being subjected to combined burn and smoke inhalation injury. Obstructive cast material occludes the lumen of the airway, resulting in hypoventilation or focal loss of ventilation. The blood vessels in the under-ventilated areas fail to constrict normally, causing a perfusion/ventilation mismatch. This transfer of blood from a ventilated area to a non-ventilated part results in poor oxygenation of arterial blood, which leads to hypoxaemia changes in organs. In addition, obstruction of part of the bronchial tree results in the hyperventilation of the non-occluded parts, thus increasing airway pressure when volume-controlled mechanical ventilation is given [39]. The over-stretching of the alveoli by high pressure results in mechanical trauma or volutrauma of the non-obstructed alveoli, thus worsening oxygenation [40]. This over-stretching of the alveoli also induces synthesis and secretion of pro-inflammatory chemokines, such as IL-8 (interleukin-8) [41], which is a major chemo-tactic factor for neutrophils. IL-8 has been shown [42] to mediate smoke inhalation-induced lung injury. It was also reported that inflammatory mediators, such as TNFα and IL-1, are induced by mechanical ventilation [43]. Previously, we have shown [38] that airway obstructing material is composed of fibrin, neutrophils, mucus and epithelial cell debris, leading us to hypothesize that pathogenic therapy targeting those factors could represent a more effective form of airway management. In our laboratory, we tested the hypothesis that a reduction of a fibrin clot in the airway could reduce the degree of airway obstruction. For our experiments, we studied the effects of two different types of anticoagulants: those that prevented fibrin formation and those that lysed already formed fibrin clots. We reported that the prevention of fibrin clot formations in the airways using anticoagulants (delivered via nebulization), such as heparin [44] and antithrombin [45], was beneficial in treating sheep subjected to smoke inhalation and pneumonia. Interestingly, these anticoagulants failed to ameliorate the lung injury in sheep with combined burn and smoke inhalation injury. Although the exact mechanism of this discrepancy is not completely understood, Murakami et al. [46] reported that antithrombin concentration in BALF in sheep with smoke inhalation and pneumonia was much higher than in sheep with burn and smoke inhalation injury. Their findings may explain why heparin is effective in one case and not in the other. The administration of antithrombin improved pulmonary function in sheep with burn and smoke inhalation injury only when combined with heparin. It is a well-known fact that antithrombin exerts a much more potent anticoagulant effect as an antithrombin-heparin complex. The exact mechanism by which antithrombin nebulization alone ameliorates acute lung injury in a smoke inhalation and pneumonia model, but not in burn and smoke inhalation model, remains unclear. At the present time, we can only speculate that the anti-inflammatory property of
Acute lung injury in combined burn and smoke inhalation injury

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**Figure 4 Airway obstruction-related pulmonary dysfunction**

Occluded part of the alveola is hypo- or non-ventilated, resulting in pulmonary shunt fraction, which leads to ventilation/perfusion mismatch. Open alveolae are overstretched when mechanical ventilation is present, leading to barotraumas. Overstretching of the alveolar wall induces cytokine release, such as IL-8, a leucocyte chemoattractant.

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the antithrombin may be dominated in sheep with sepsis induced by smoke inhalation and pneumonia. The anticoagulants we used were nebulized starting 2 h after the injury; however, fire victims are often admitted to specialized burn units well after 2 h after sustaining their injuries. In those patients, it is possible that fibrin could already have begun to form clots in the airway in which case the use of fibrinolytic agents is the only choice available to physicians. Considering this fact, we tested the effect of TPA (tissue plasminogen activator, which converts plasminogen into plasmin, leading to break of both fibrinogen and fibrin) on burn and smoke inhalation-induced pathologies in sheep. Nebulization of TPA starting at 4 h after the injury markedly reduced the degree of the airway obstruction, resulting in improved oxygenation and microvascular permeability (Figure 1). Following pulmonary epithelial injury, plasma exudes into the distal airway. Extravascular plasma rapidly clots because of the procoagulant properties of the pulmonary epithelium and alveolar macrophages. Fibrinolytic activity of the lavage fluid in patients with ARDS is increased in contrast with increased PAI-1 (plasminogen activator inhibitor-I). In the alveolar space, fibrin or fibrinogen can impair surfactant function [47], thereby contributing to atelectasis. Both fibrin and fibrinogen provide adhesion sites for inflammatory cells that are recruited to the site of tissue damage [48]. Thus direct delivery of anticoagulants including fibrinolytic agents into the airways might be an effective treatment strategy.

As mentioned previously, airway blood flow increases dramatically in sheep with combined burn and smoke inhalation injury. We have reported that iNOS inhibitors or PARP inhibitor significantly reduced the increased airway blood flow. The decrease in airway blood flow in these treated animals was associated with significantly lower airway obstruction scores. Consequently, ventilatory pressures were also lower in these sheep. Thus it is possible that effective airway management based on this treatment strategy could reduce the degree of acute lung injury in cases of thermal injury associated with smoke inhalation (Figure 4).

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**CONCLUSION**

In this review, we have attempted to summarize some of the pathophysiological aspects of the acute lung injury induced by thermal injury associated with smoke inhalation. Although the exact mechanism of acute lung injury in patients with thermal injury remains unclear, we have described the important roles played by excessive NO, PARP activation and airway obstruction in this process.

We believe that future studies should seek to investigate in detail the interplay between PARP activation and iNOS. Because the link between iNOS and COX has been well described in previous studies, the effect of specific COX inhibitors should be tested. We also suggest that the inhibition of excessive NO, especially iNOS-derived NO, and PARP activation with effective airway management using various aerosolized anticoagulants might be an option of the effective strategy for treating patients with combined burn and smoke inhalation injury.

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