Pathophysiological Basis of Smoke Inhalation Injury
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Smoke inhalation injury results in serious respiratory failure. When smoke inhalation injury is combined with burn injury or pneumonia, the physiological responses are different and more severe than those of smoke inhalation injury alone. Treatment strategies should be planned based on these pathophysiological aspects.

Smoke inhalation injury is a serious health threat to victims of house fires, explosions, and other disasters involving fire and smoke. The clinical symptoms and prognosis of smoke inhalation injury are often exacerbated by additional burn injury or bacterial infection (such as pneumonia). From our experience using an ovine model of inhalation injury, we have found that the acute lung injury (ALI) resulting from a combination of smoke inhalation and burn injury or pneumonia is more severe than that resulting from smoke inhalation injury alone. We have also observed that a combination of smoke inhalation and pneumonia results in a severe septic response in sheep.

We will review the pathophysiological aspects of smoke inhalation injury and note the various treatment strategies being currently investigated. Because of space limitations, this review will not discuss the pathophysiology of toxic gas inhalation or oropharyngeal and/or tracheobronchial thermal injury. These topics are important issues to be considered elsewhere.

Bronchial blood flow

The lungs have two separate blood supplies (the systemic and the pulmonary), each of which can contribute to lung edema. Under normal conditions, the pulmonary blood supply is equivalent to the cardiac output, whereas the bronchial blood flow is ~1% of the cardiac output. After inhalation injury, there is a marked increase in bronchial blood flow, which results in pulmonary edema. In an ovine smoke inhalation model, airway blood flow increases eightfold or more in the main stem bronchi after the injury, whereas cardiac output, and thus blood flow to the peripheral tissues, remains relatively unchanged (1). Bronchial blood flow enters into the pulmonary vasculature through various bronchopulmonary anastomoses. It has been suggested that the bronchial circulation plays a significant role in the spread of injury from the airway to the parenchyma. We have investigated the effect of bronchial artery ligation or ethanol injection after inhalation injury in sheep (11) and have found that the decrease in gas exchange [PaO$_2$/FiO$_2$ (P/F) ratio], the increase in lung lymph flow, and the lung wet/dry weight ratio were all improved by these bronchial artery occlusion techniques. Therefore, we have concluded that the bronchial circulation contributes to edema formation in the lung that occurs after ALI caused by smoke inhalation injury. This phenomenon has been confirmed by other investigators (4).

There are several mediators involved in the regulation of bronchial circulation, including nitric oxide (NO), a potent vasodilator. It has been reported that NO synthase (NOS) inhibitors reduce the increase in bronchial blood flow. The may be other factors, such as neurotransmitters, involved in this phenomenon, but they are still under investigation.

Role of NO

NO is produced from the reaction L-arginine (Arg) $\rightarrow$ NO + L-citrulline by the presence of NOS. There are three known NOS isoforms: NOS-1 (neuronal NOS or nNOS), NOS-2 (inducible NOS or iNOS), and NOS-3 (endothelial NOS or eNOS). nNOS and eNOS are calcium dependent and constitutively expressed. iNOS is calcium independent and induced in various cells, such as macrophages, by inflammatory cytokine stimulation. As we discussed above, NO is a vasodilator and regulates the microcirculation. In addition, NO is a potent inhibitor of platelet aggregation, neutrophil adhesion, and cytokine production. These effects are important for maintaining microcirculatory blood flow. However, once the production of NO gets extremely high, NO begins to function as a free radical and becomes involved in inflammation. When NO reacts as a free radical, it is highly reactive and interacts with various substances, such as oxygen free radicals. Under certain conditions, NO reacts with superoxide (O$_2^-$) to form peroxynitrite (ONOO$^-)$, an extremely potent oxidant that contributes to cellular injury, including lipid peroxidation, nitrosylation of different molecules, sodium channel interaction, and interaction with different transitional metals. Under normal conditions, NO is rapidly scavenged by the heme group of hemoglobin and metabolized to nitrate (NO$_3^-$) and nitrite (NO$_2^-$). However, under inflammatory conditions, such as those caused by inhalation injury, leukocytes are activated, which results in the expression of adhesion molecules on the surface of leukocytes and endothelial cells. When leukocytes adhere to the endothelial cells by way of adhesion molecules, hemoglobin is no longer accessible to the small gap between endothelial cells and neutrophils. Because activated neutrophils and monocytes produce O$_2^-$ radicals, NO easily reacts

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with \( O_2^- \) to form \( \text{ONOO}^- \) in this gap. This reaction may also happen in the extracellular space or in the alveolar space, where hemoglobin does not exist. As a result, \( \text{ONOO}^- \) formation causes an increase in pulmonary vascular permeability and lung edema and a decrease in diffusing capacity.

Our ovine smoke inhalation model has shown levels of \( \text{NO}_2 \) and \( \text{NO}_3 (\text{NO}_x) \) after the injury that are two to three times that of baseline levels. Since the lung is the major physiological structure affected by inhalation injury, we have measured lung Arg metabolism in this model by using a stable isotope ([15N]Arg) as a tracer. We have found that, 24 h after a combination injury of smoke inhalation and burns, the lung Arg metabolism is threefold higher than that of baseline levels (Fig. 1). We have shown that this increase is significantly reversed by administering \( \text{N}^\text{G} \)-nitro-L-arginine methyl ester (L-NAME), a nonspecific NOS inhibitor (Fig. 1). These data thus suggest that NOS may be responsible for the increased Arg metabolism.

Hypoxic pulmonary vasoconstriction (HPV) is an essential mechanism that matches lung perfusion to ventilation to optimize pulmonary gas exchange. There is a long history of research on the mechanism of HPV regulation. Recently, it has been shown that pulmonary arterial smooth muscle cells themselves respond to hypoxia. In clinical settings, such as those involving ALL, the loss of HPV is a serious problem, because if the blood flow is diverted from alveoli that are being ventilated to those that are not, the oxygen saturation of the arterial blood will fall (Fig. 2). We have shown that the administration of dopamine had no beneficial effect on restoration of HPV in our smoke inhalation model in sheep. In response to this finding, we have tested the effect of a specific iNOS inhibitor (mercaptoethyl guanidine) on HPV and have found that the inhibition of iNOS can reverse the loss of HPV, suggesting that NO\(^-\) induced by iNOS plays a role in such a loss (5). This study was consistent with the findings by Ullrich et al. (14), who noted that iNOS knockout mice showed attenuation of impaired HPV.

There are a variety of possible sources of NO, including endothelial cells and alveolar macrophages. In addition to these, a recent report (15) has demonstrated that lung epithelial cells express iNOS, a finding that is consistent with what we have observed in immunohistochemistry studies (12). These findings are important because the inhibition of iNOS is beneficial in ALL and myocardial depression after smoke inhalation. We have been able to consistently attenuate the loss of HPV by using several specific iNOS inhibitors in ovine models of inhalation injury. These results offer promising avenues for further study.

There is also a possibility that nNOS plays a role in the pathophysiology of inhalation injury. Recently, we have reported that 7-nitroindazol, an NOS inhibitor, attenuates ALL after smoke inhalation and pneumonia in sheep. Although at present it is still not known which cells express nNOS, we think that nNOS-derived NO plays a role in the inhalation injury. There have been published reports showing that human neutrophils contain nNOS (6). If this is the case, inhibition of nNOS may be another effective treatment strategy in ALL following smoke inhalation injury.

Nonspecific NOS inhibitors such as L-NAME or \( \text{N}^\text{G} \)-monomethyl-L-arginine (L-NMMA) attenuate the gas exchange dramatically by improving the ventilation-perfusion mismatch, but they can have adverse effects on cardiac functions. When L-NAME or L-NMMA are given to the animals after the evident lung injury, in which the oxygen tension of the lung has fallen (P/F ratio < 200), pulmonary arterial pressure rises and cardiac output decreases significantly. Thus the onset of tissue hypoperfusion or ischemia is possible, a fact that prompts careful consideration when these nonspecific NOS inhibitors are given. Another concern regarding treatment strategies using nonspecific NOS inhibitors is that plasma NO levels decrease to lower-than-normal levels in those animals receiving treatment. The control of the synthesis of NO (formation of mRNA for iNOS) is through nuclear factor (NF)\( \kappa \)B.
The activity of NF-κB is inhibited by NO, a process called negative feedback. When NOS is inhibited by these compounds, NO is not formed; thus NF-κB activity is not inhibited; consequently, iNOS mRNA and protein levels could be much higher in NOS inhibitor-treated animals than in vehicle-treated animals (12). This suggests that the rebound reaction might happen when the administration of NOS inhibitors is discontinued. This could cause adverse reactions in patients.

Our animal model has shown that NO induction is not as marked in smoke inhalation injury alone. However, if the inhalation injury is combined with burn injury or pneumonia, NO production has been shown to be significantly higher than with either injury alone. We have found that the inhibition of iNOS is beneficial in both a smoke plus burn model and a smoke plus pneumonia model but that the inhibition of nNOS is beneficial only in a smoke plus pneumonia model. It is important to know in which ways NOS isoforms are involved in the pathophysiology of smoke inhalation injury, especially when considering possible treatment strategies.

NO also plays an interesting role in ONOO$^-\text{induced DNA damage. When a single DNA strand is broken, the nuclear enzyme poly(ADP-ribose) polymerase (PARP) is activated. Although PARP is known to be involved in the DNA-repairing mechanism, it requires a large amount chemical energy in the form of ATP and NAD. As a consequence, the intracellular depletion of these two substances leads to necrotic cell death (8). The release of intracellular contents into the extracellular space in the necrotic process amplifies the inflammatory reaction. Our recent studies have shown that potent PARP inhibitor INO-1001 (Inotek, Beverly, MA) significantly attenuated ALI after burn plus smoke inhalation in sheep. We believe that activation of PARP by ONOO$^-$ is involved in the pathophysiology of ALI after smoke inhalation injury (Fig. 3).

**FIGURE 3.** Poly(ADP-ribose) polymerase (PARP) plays a role in smoke inhalation injury. Smoke inhalation causes peroxynitrite (ONOO$^-\text{formation by polymorphonuclear neutrophil (PMN) activation and NO synthase (NOS) induction. ONOO$^-$ damages DNA single strand. As a consequence, the DNA-repairing enzyme PARP is activated. In the DNA-repairing process, PARP requires large amounts of NAD$^+$ and ATP. Therefore, intracellular NAD$^+$ and ATP are severely decreased and cellular energetic catastrophe leads to necrotic cell death. O$_2^-$, superoxide anion radical.

**Airway obstruction and ventilator-induced lung injury**

Widespread plugging of airways by casting materials is a very severe problem in smoke inhalation injury. In many cases, the cast is solid and hard to remove. The ciliary transport function is damaged by smoke inhalation (7), which is considered to be part of the reason for the airway obstruction. Pathological studies have shown that the obstructing material is mainly composed of infiltrated neutrophils, shed bronchial epithelial cells, mucus, and fibrin. When neutrophils are activated as a consequence of cytokine stimulation or ischemia-reperfusion, most neutrophils accumulate in the lung. There are several possible reasons for this: 1) the pulmonary vascular bed is extremely big; 2) pulmonary capillaries are narrower than systemic capillaries; 3) neutrophils lose their deformability when they are activated, and stiffened neutrophils tend to be trapped in the capillaries; 4) pulmonary capillary pressure is lower than that of systemic; and 5) there is no cytokine production in the lung because of alveolar macrophages.

Activated neutrophils adhere to the activated endothelial cells and injure them, resulting in an increase in pulmonary vascular permeability. The exuded plasma contains coagulation factors such as fibrinogen and/or prothrombin. In addition to the exudation, pulmonary epithelial cells and alveolar macrophages express tissue factor. Tissue factor is an initiator of the extrinsic pathway of coagulation and is known to cause fibrin deposition (clots) in the alveolar space. Fibrin formation in the alveolar space is considered to be a hallmark of acute and chronic lung injury. Fibrin is also known to inhibit surfactant activity. In comparing our various animal injury models, we have found that the obstructing materials contain more fibrin clots in a combination of smoke inhalation plus pneumonia than in smoke inhalation plus burns, suggesting that the airway coagulopathy is more severe if pneumonia is combined with smoke inhalation. Therefore, heparin nebulization has been shown to be effective in the smoke plus pneumonia model but not in the smoke plus burns model.

Ventilator-induced baro/volutrauma is another mechanism of injury after smoke inhalation (Fig. 4). When some parts of the lung are obstructed by cast formation, other parts of the lung will be over-stretched by a ventilator. Generally, low tidal volume ventilation is recommended in the treatment of acute respiratory distress syndrome (13) (6 ml/kg); one of the reasons for this is to prevent ventilator-induced baro/volutrauma. Mechanically over-stretching lung tissue induces chemokines (compounds that cause leukocytes to be attracted to inflamed or injured areas) from epithelial cells, another pro-inflammatory reaction.

Airway pressure increases significantly after smoke inhalation. Sometimes bronchoscopy is needed to remove the obstruction, but the prevention of cast formation is the more important goal of any treatment strategy. The obstructed part of the lung easily gets atelectatic when the patient is ventilated with a high concentration of oxygen. The blood flow to the atelectatic part of the lung will not be oxygenated, contributing to a pulmonary shunt. Thus the ventilation-perfusion mismatch, which results in a severe drop in gas exchange, will be
There are several ways to prevent cast formation. As we have described above, heparin nebulization is a practical treatment. However, heparin itself does not have an anticoagulant property. Because of this, it is necessary for heparin to have a high enough level of antithrombin. In most smoke inhalation cases, the antithrombin level is maintained in the normal range, but in patients with inhalation injury with burns or severe infection, the antithrombin level significantly decreases. In our smoke plus pneumonia model, in which plasma antithrombin activity decreases <50%, supplementation of antithrombin reduces the airway obstruction and attenuates ALI. Since neutrophils are the major component of cast formation, inhibition of neutrophil accumulation is also effective in reducing the airway obstruction. The selectin family of adherence molecules are involved in the early processes of leukocyte adherence to the microcirculation. Among these molecules we studied L-selectin, which is on the neutrophil surface, and P-selectin, expressed on vascular endothelial cells. Our smoke inhalation model in sheep showed that anti-L-selectin antibody significantly attenuated the airway obstruction. However, the anti-P-selectin antibody did not attenuate lung injury, suggesting that the inhibition of the early phase of interaction between neutrophils and endothelial cells is not beneficial. Mercaptoethyl guanidine, an iNOS inhibitor, also inhibits airway obstruction. The inhibition of ONOO− formation by iNOS inhibitor will also reduce the endothelial damage and improve the vascular permeability. NOS inhibitors may also inhibit the activation of PARP and the following necrotic changes in epithelial cells, because ONOO− is a potent activator of PARP.

The activity and number of mucus gland cells (goblet cells) seems markedly reduced by smoke inhalation. We have investigated the effect of aerosolized acetylcysteine, which lysed mucus plugging, in the smoke inhalation plus burn model in sheep and in patients. We have found that the airway obstruction and gas exchange are not attenuated by acetylcysteine nebulization; however, if the acetylcysteine is combined with heparin it is effective in raising arterial blood oxygenation and reducing other markers of lung injury.

Activated leukocyte-mediated tissue injury

Activated neutrophils are involved in the pathophysiology of smoke inhalation. In our ovine model, we depleted leukocytes by giving nitrogen mustard, a bone marrow suppressant. In leukocyte-depleted sheep, smoke inhalation-induced pulmonary edema and vascular permeability were almost completely prevented (3). Also, the elevation of conjugated dienes, an index of lipid peroxidation, was significantly prevented by reducing the number of leukocytes, suggesting that the oxidative stress originated from activated leukocytes. Plasma catalase and glutathione levels were also reported to be decreased in response to smoke inhalation injury. Treatment strategies using antioxidants, such as manganese superoxide dismutase administration or dimethylsulfoxide nebulization, were shown to be effective in our smoke inhalation model. On the other hand, allopurinol, which inhibits O2− formation by inhibiting xanthine oxidase, was not shown to be effective in our inhalation injury model (2). Because xanthine oxidase is activated by ischemia-reperfusion in the tissue, oxygen radicals in injured tissues do not seem to be important in the pathophysiology of smoke inhalation injury.

Vitamins C and E are known to scavenge oxygen radicals. As a result, the effects of both of these on burn and inhalation injuries are currently being investigated in clinical trials. There are several isoforms of vitamin E (tocopherol), designated α, β, γ, etc. We have found that plasma levels and lung tissue content of α-tocopherol are decreased 48 h after smoke inhalation injury. Lung levels of γ-tocopherol were reduced by 50%. We have also noted that, when sheep suffer a combination injury of smoke inhalation and burns, the drop is more severe. In addition to its oxygen-scavenging capacity, γ-tocopherol also has a potent binding capacity for NO. Evidently, the lung is exposed to the oxidative stress as well as excess NO and its metabolites.
Neutrophil elastase is a protease contained in the granules of neutrophils. Neutrophil elastase digests various proteins because of its low enzymatic specificity. When neutrophils are strongly activated, neutrophil elastase is released in the extracellular space. Although there is a mechanism through which the α1 protease inhibitor (α1-PI) immediately binds to elastase and inactivates it, reactive oxygen species, which are also released from activated neutrophils, break α1-PI. Therefore, elastase injures tissue without being inactivated by α1-PI when oxygen radicals coexist.

Gabexate mesilate is a synthetic serine protease inhibitor that attenuates coagulation abnormalities or pancreatitis in animals and humans. We have previously reported that this protease inhibitor attenuates smoke inhalation injury in sheep (10). More recently, it has been noted that gabexate mesilate inhibits elastase release and oxygen radical production (9). We believe that this is one of the mechanisms of action of the drug in reducing smoke inhalation injury. Recently, specific neutrophil elastase inhibitor ONO-5046 (Ono Pharmaceutical, Osaka, Japan) was approved for the treatment of acute respiratory distress syndrome in Japan. Because neutrophil elastase is involved in inhalation injury, this compound would be one possible candidate for the treatment of smoke inhalation.

Other factors

There are several other factors involved in the pathophysiology of smoke inhalation. Cytokines, of course, play an important role. Both IL-1 and -8 are increased after the inhalation injury, because of its low enzymatic specificity. When neutrophils are strongly activated, neutrophil elastase is released in the extracellular space. Although there is a mechanism through which the α1 protease inhibitor (α1-PI) immediately binds to elastase and inactivates it, reactive oxygen species, which are also released from activated neutrophils, break α1-PI. Therefore, elastase injures tissue without being inactivated by α1-PI when oxygen radicals coexist.

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References