Ventilator-induced lung injury: in vivo and in vitro mechanisms

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At the Experimental Biology 2002 meeting in New Orleans, LA, a featured topic presentation was devoted to mechanisms of ventilator-induced and ventilator-associated lung injury. This area has become an important focus of pulmonary research largely because of the compelling clinical evidence that lung protective ventilatory strategies reduce mortality in patients with clinical acute lung injury (1, 4, 10, 14).

Alveolar Epithelial Plasma Membrane Stress Failure

The role of alveolar epithelial plasma membrane stress failure and repair was discussed as an important basic mechanism responsible for mediating mechanical stretch-induced injury in the lung. Cells experience plasma membrane stress failure when the matrix to which they adhere undergoes large deformations. In the lung, such a mechanism may help to explain mechanical ventilation-associated cell injury. There are a number of mechanisms that help preserve plasma membrane tension of deformed cells below lytic levels (11) (Fig. 1). Vlahakis and Hubmayr (11) have shown that deformation induces lipid trafficking (DILT) to the plasma membrane of alveolar epithelial cells, thereby accommodating the required increase in cell surface area. DILT was shown to be a temperature- and energy-dependent process that involves vesicular membrane trafficking. Conditions associated with inhibition of DILT include cold temperature, low cholesterol, and impaired cytoskeletal assembly and disassembly (13). In keeping with a possible cytoprotective role for DILT, Vlahakis et al. (13) recently demonstrated that strain-induced cell wounding is significantly increased when DILT is inhibited. Because the association between inhibition of DILT (a remodeling response) and injury is preserved across the entire spectrum of cell mechanical properties (7), we hypothesized that cell remodel-

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ing was more important for cytoprotection against stress failure than are the strength and connectivity of the cell’s stress-bearing elements.

These observations have been taken from reduced culture systems back to the intact lung and have demonstrated that mechanical ventilation at injurious settings is associated with reversible plasma membrane stress failure. Figure 2 shows confocal images of subpleural alveoli of rat lungs ventilated at injurious settings (tidal volume /H11005 40 ml/kg). The lungs were perfused with a membrane-impermeant label [propidium iodide (PI)] either during or after injurious mechanical ventilation. When PI enters a cell through a membrane defect, it interchelates with DNA and emits a red fluorescence. The image on the left in Fig. 2 was taken from a lung labeled during injurious mechanical ventilation and shows many injured cells (red nuclei), whereas the image on the right in Fig. 2 was labeled after the injurious stress was removed and shows no injured cells. This finding suggests that many of the injured cells had resealed and could no longer be labeled with PI.

In summary, plasma membrane stress failure is a central feature of mechanical lung injury. Active remodeling such as DILT is an important cytoprotective response to deforming stress and deserves to be considered in the search for agents that protect the lung against ventilator-associated injury.

HIGH TIDAL VOLUME-MEDIATED LUNG INJURY MAY BE ATTRIBUTABLE IN PART TO TYROSINE P-DEPENDENT ENDOTHELIAL CELL PROCESSES

Evidence was presented showing that high tidal volume-mediated lung injury may be attributable, in part, to tyrosine P-dependent endothelial cell processes that promote lung leukocyte sequestration (15). In the studies performed, rats were initially ventilated with either a 6- or 12-ml/kg tidal volume ventilation for 2 h. Microvascular endothelial cells were then isolated from the lung. Immunofluorescence labeling of the fresh lung endothelial cells revealed aggregation and colocalization of focal adhesion kinase and the αVβ3-integrin. This result suggested that the higher tidal volume ventilation induces focal adhesions. Immunoprecipitation studies revealed that high tidal volume mediated tyrosine phosphorylation of the focal adhesion protein paxillin. There was also greater expression of P-selectin in the freshly isolated lung endothelial cells. There was an association of paxillin with the immunoprecipitated P-selectin that suggested participation of this focal adhesion protein in the P-selectin expression. Interestingly, genistein, a tyrosine kinase inhibitor, inhibited the high tidal volume-mediated P-selectin expression as well as the coassociation of paxillin with P-selectin. Extravascular lung water content was not different in the lungs ventilated with 6 vs. 12 ml/kg, indicating that the proinflammatory signaling had not yet reached the point where it would alter lung fluid balance. Also, depletion of the lung perfusate of leukocytes blunted the high tidal volume-induced endothelial cell tyrosine phosphorylation. These studies provide new insights into the very early phase of proinflammatory signaling that may occur in lung endothelium with high tidal volume ventilation.

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Fig. 1. Schematic of plasma membrane (PM) responses to deforming stress. [From Vlahakis and Hubmayr (11).]

Fig. 2. Propidium iodide labeling-based cell injury assessment in a rat model of ventilator-induced lung injury. Differences in labeling during vs. after injurious ventilation are attributed to plasma membrane resealing. VT, tidal volume; ZEEP, zero end expiratory pressure. (O. Gajic and J. H. Lee, unpublished observations.)
LUNG EPITHELIAL CELL INJURY

Evidence was presented explaining how lung epithelial cell injury may occur using an in vitro model (3). In these studies, Bilek et al. (3) evaluated the stresses that may occur with airway reopening. Because mechanical stress is hypothesized to be associated with airway reopening, which could cause cellular trauma, reopening conditions were generated using a fluid-occluded parallel plate chamber with lung epithelial cells cultured on a small region of one wall. A column of air was infused that cleared the occlusion. Cellular trauma was assessed using fluorescent exclusion dyes. The controls showed very few injured cells while bubble progression produced significant increases in the density of injured cells for saline-occluded channels. Interestingly, in this model, the injurious stress is not a shear stress, but, rather, a stress that acts normally to the cell layer. For a surfactant-free system, the normal stress gradient introduces a fore-aft pressure difference along the cell of ~350 dyn/cm². This pressure difference appears to be sufficient to cause short-term cell membrane disruption. This pressure was reduced substantially with pulmonary surfactant, which appeared to protect the epithelial cells from damage. The authors (3) concluded that this model could be used to further investigate the airway reopening injury mechanisms and the potential efficacy of specific surfactant replacements.

KERATINOCYTE GROWTH FACTOR CAN ENHANCE ALVEOLAR EPITHELIAL REPAIR BY NONMITOGENIC MECHANISMS

Evidence was presented showing that keratinocyte growth factor (KGF) can enhance alveolar epithelial repair by nonmitogenic mechanisms (2). In these studies, rats were given intratracheal KGF (5 mg/kg) 48 h before alveolar epithelial type II cells were isolated for in vitro alveolar epithelial repair studies. KGF-treated cells have markedly increased epithelial repair compared with control cells. KGF-treated cells also have increased cell spreading and migration at the wound edge but no increase in their in vitro proliferation capacity. KGF-treated cells were also more adherent to extracellular matrix proteins and to polystyrene. Inhibition of the epidermal growth factor (EGF) receptor with tyrosine kinase inhibitors abolished the KGF effect on alveolar epithelial repair. In summary, in vivo administration of KGF augments the epithelial repair rate of alveolar epithelial cells by altering cell adherence, spreading, and migration through stimulation of the EGF receptor. These results provide new insights into the nonmitogenic effects of KGF on alveolar epithelial cells, which may be important in alveolar epithelial repair and may help to explain why KGF treatment ameliorates experimental acute lung injury.

IN VIVO AIRWAY STRETCH CAN INDUCE LOCAL INFLAMMATORY CELL TRAFFICKING

Evidence was presented showing that in vivo airway stretch can induce local inflammatory cell trafficking in the rat microcirculation (8). For these studies, the investigators developed a new application of intravital microscopy in anesthetized, mechanically ventilated rats that provided direct observation of leukocyte trafficking in the tracheobronchial circulation. In these experiments, normal ventilation was maintained at 80 breaths/min with 6 ml/kg tidal volume and 0 cmH₂O positive end-expiratory pressure (PEEP). Increased lung stretch was induced by increasing PEEP to 8 cmH₂O at different time intervals. Normal ventilation did not alter leukocyte rolling velocity or the number of adherent cells. In contrast, the increased level of PEEP for 1 h induced a sharp decrease in blood pressure, which recovered to baseline by 30 min. A
time-dependent reduction in leukocyte rolling velocity occurred that remained reduced even after the excessive PEEP was removed. However, the number of adherent cells reached a maximum at 1 h of observation and returned to baseline by 2 h, suggesting that the endothelium was not irreversibly activated with sustained lung stretch. The authors (8) speculate that leukocyte recruitment is a direct result of excessive lung stretch leading to the local release of inflammatory mediators and the activation of the airway endothelium. To support this hypothesis, data were presented that lung stretch failed to alter leukocyte trafficking in the rat mesentery, despite similar reductions in blood pressure. The data support the hypothesis that excessive lung stretch during mechanical ventilation may have deleterious effects in patients with airway disease.

Evidence was presented showing that N-acetylcysteine (NAC), an antioxidant, decreased lung neutrophil influx in a rat model of ventilator-induced lung injury (9). Rats were ventilated with a tidal volume of either 7 or 21 ml/kg with or without 40 mg/kg of NAC. The animals were ventilated for 2 h and then allowed to recover for 2 h. The NAC-treated animals had a reduced concentration of neutrophils and myeloperoxidase in bronchoalveolar lavage. The authors (9) concluded that lung stretch induces oxidant injury that may play an important role in ventilator-induced lung injury.

N-ACETYLCYSTEINE DECREASES LUNG NEUTROPHIL INFLUX

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ALVEOLAR MACROPHAGES MAY PLAY A CENTRAL PATHOGENIC ROLE IN VENTILATOR-INDUCED LUNG INJURY AND PULMONARY EDEMA

Evidence was presented showing that alveolar macrophages may play a central pathogenic role in ventilator-induced lung injury and pulmonary edema (5). Rats were ventilated for 15 min either at a pressure of 45 cmH₂O without PEEP or with a noninjurious ventilatory strategy. Tracheal instillation of liposomes encapsulated with clodronate eliminates alveolar macrophages. Some rats were also treated with pyrrolidine dithiocarbonate, an inhibitor of nuclear factor (NF)-κB-dependent activation. The data showed that macrophage depletion was accomplished and that static lung compliance was improved with either treatment. Extravascular lung water was also reduced significantly with either treatment. Thus neutralization of alveolar macrophages appears to be an effective experimental method for reducing ventilator-induced lung injury, suggesting that alveolar macrophages and possibly NF-κB-dependent activation may also be an important mechanism for mediating the injury.

IN VIVO STUDIES IN VENTILATED RATS

Finally, the results were presented of in vivo studies in ventilated rats, the results of a recent study published by Frank et al. (6). In these studies, acid-induced lung injury was created in ventilated, anesthetized rats ventilated with a noninjurious ventilation protocol for 2 h. Rats were then randomized to 12, 6, or 3 ml/kg of tidal volume ventilation. As expected, the 12-ml/kg ventilation resulted in a markedly increased extravascular lung water after 4 h. The 6-ml/kg tidal volume ventilation, similar to the lung protective strategy used in the National Institutes of Health clinical protocol (10), markedly reduced lung edema. The 3-ml/kg tidal volume strategy further reduced pulmonary edema (Fig. 3). There was evidence that the additional protective effect of 3 ml/kg was explained by further protection against alveolar epithelial injury, based on both biochemical studies of the alveolar epithelial type I cell antigen as well as better preserved gene expression of surfactant protein C and a nearly normal capacity for alveolar fluid transport. Histology showed less edema and inflammation with the low tidal volume strategies (Fig. 4). Thus there was a protective effect of both ventilatory strategies in reducing injury to the lung endothelium, but the additional protective effect of the lowest tidal volume strategy appears to be explained by limiting injury to the alveolar epithelium.

In summary, the spectrum of issues discussed at this featured topic session illustrates the importance of both in vitro and in vivo studies to understand the basic mechanisms that mediate ventilator-induced and ventilator-associated lung injury. It is apparent from this session as well as other work in the field that there are several important mechanisms that explain the beneficial effect of lung protective ventilatory strategies.

REFERENCES


7 Presented by Fabien Eyal.
8 Presented by Michael A. Matthay.