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Activation of NFkB and coagulation in lung injury by hyperoxia and excessive mechanical ventilation: one more reason “low and slow” is the way to go?

Jeffrey R. Jacobson and **Konstantin G. Birukov**
Section of Pulmonary and Critical Care Medicine, Department of Medicine, University of Chicago, Chicago, Illinois 60637

Critically ill patients with acute lung injury (ALI) often require mechanical ventilation with the administration of high levels of inspired oxygen to ensure adequate oxygen delivery to tissues and vital organs. In these same patients, derangements in lung compliance may result in excessive lung stretch despite the use of low tidal volume ventilatory strategies meant to be lung protective. We now know that the combined effects of hyperoxia and excessive lung stretch in this setting are injurious in their own right and are associated with among other things activation of the coagulation system.[1]

Disseminated intravascular coagulation in the injured lung is a recognized manifestation of ALI. The combination of increased coagulation and plasminogen activator inhibitor (PAI)-1-dependent suppression of fibrinolysis promotes an intravascular procoagulant state with conversion of fibrinogen into fibrin leading to disseminated micro- and macrovascular thrombosis. Extravascular activation of procoagulant mediators PAI-1 and tissue factor is induced in lung epithelial cells, macrophages, and fibroblasts exposed to bacterial endotoxin, exotoxin, peptidoglycans, and interleukins IL-1β, IL-6, IL-8, and tumor necrosis factor-alpha (TNFα).[2] Bacterial products and inflammatory cytokines also trigger NFκB transcriptional activity, which promotes cytokine production, recruitment, and activation of neutrophils in the lung. In addition, NFκB controls expression of procoagulant molecules PAI-1 and tissue factor.[2]

Separately, the oxidative burst from neutrophil activation is a potent mechanism of innate immunity aimed at killing invading bacteria via the rapid release of reactive oxygen species (ROS). However, sustained oxidant stress may be independently injurious leading to propagation of lung inflammation and endothelial barrier dysfunction. Whether hyperoxia and excessive lung stretch in mechanically ventilated and acutely injured lungs contributes to the deleterious effects of sustained septic oxidant stress remains to be investigated.

PAI-1 also has been identified as a major deleterious mediator of hyperoxic lung injury.[3] Lungs of mice exposed to hyperoxia overproduce PAI-1, and the upregulation of PAI-1 impairs fibrinolytic activity in the alveoli. Moreover, mice genetically deficient in PAI-1 fail to develop intra-alveolar fibrin deposits in response to hyperoxia and are more resistant to the lethal effects of hyperoxic stress.

Recently, Haitsma et al[4] investigated the effect of mechanical ventilation on alveolar fibrin turnover in *Streptococcus pneumoniae* pneumonia. Their results show that low tidal volume

Corresponding address: Konstantin G. Birukov Section of Pulmonary and Critical Care Medicine Department of Medicine University of Chicago 929 E. 57th Street, Room W410 Chicago, IL 60637 Phone: (773) 834-2636 Fax: (773) 834-2683 kbirukov@medicine.bsd.uchicago.edu.

ventilation with positive end-expiratory pressure (PEEP) did not influence alveolar coagulation or fibrinolysis. In contrast, high tidal volume (HTV) ventilation with zero PEEP did intensify the local procoagulant response and increased levels of PAI-1. This mode of ventilation also caused a systemic elevation of thrombin-antithrombin complexes. These studies suggest that mechanical ventilation causing ventilator-induced lung injury increases pulmonary coagulopathy in an animal model of *Streptococcus pneumoniae* pneumonia and may also result in systemic coagulopathy.

These previous reports are important precursors to the current study, which addressed the potential synergistic effects of HTV mechanical ventilation and hyperoxia on the extent and severity of lung injury, NFκB-mediated inflammatory signaling, and PAI-1 activation. In this issue of *Translational Research*, Dr. Liu et al[5] employed a mouse model of HTV ventilation with >95% oxygen and found synergistic effects of hyperoxia and HTV ventilation on the activation of PAI-1, which was dependent on NFκB activation. Their data suggest a positive feedback mechanism of NFκB-dependent PAI-1 generation in this model via augmented NFκB activation by secreted TNFα. Specifically, the authors report that (1) hyperoxia enhanced HTV-induced NFκB and IκB phosphorylation; (2) hyperoxia and HTV synergistically increased TNFα levels in brochoalveolar lavage (BAL) fluid as well as release and activation of PAI-1 that was dependent on NFκB activation; and (3) the use of a TNFα blocking antibody partially inhibited PAI-1 production induced by hyperoxia and HTV.

Previous studies by this and other groups have shown that the NFκB cascade is involved in the mechanisms of lung injury and inflammation induced by HTV. For example, murine ALI induced by HTV is markedly reduced by inhibiting ASK, p38, and JNK stress MAP kinases.[6] It was shown that lung injury in this model is augmented by hyperoxia.[7] The results by Liu et al[5] confirm that this combination indeed represents a two-hit model of ALI.

Interestingly, hyperoxia did not affect the amount of total PAI-1 in the BAL fluid of HTV-treated mice although it did affect additional increases in the levels of activated PAI-1. These results indicate PAI-1 regulation by hyperoxia occurs at a posttranslational level. PAI-1 accumulation was observed mainly in bronchial epithelium, which is consistent with the clinical observations of alveolar coagulopathy and fibrin deposition within the airways in patients with ALI.[2] As cell culture studies have demonstrated the upregulation of both TNFα and PAI-1 in vascular endothelial cells exposed to prolonged cyclic mechanical strain[8]; an interesting question is the relative degrees to which hyperoxia and excessive stretch induce activation of the coagulation system in the lung microvasculature.

Allen et al[9] recently reported that, unexpectedly, neither fibrin nor PAI-1 deficiency was protective in mice with acid-induced ALI. These authors speculated that *in vivo* lung function may be more closely tied to permeability and alveolar protein in general, rather than to being linked specifically to fibrin. These studies emphasize the importance of understanding the specific pathologic mechanisms involved in ALI.

Notably, ROS that appear in inflamed tissues may function as second messengers and control redox-sensitive signaling cascades, including Jnk and p38 stress MAP kinases and NFκB pathways.[10] In addition, high magnitude cyclic stretch promotes ROS formation and ROS-dependent, NFκB-mediated gene expression alone,[11] or may act synergistically with other agonists, thus boosting oxidant stress and barrier-disruptive, proinflammatory redox-sensitive signaling.[12 and 13] In this work by Liu et al,[5] NFκB inhibition abolished additive effects of hyperoxia on TNFα content in BAL. These results support such a synergistic mechanism of redox-sensitive NFκB signaling regulation by hyperoxia and excessive lung stretch.

Finally, the results of Liu et al[5] suggest that strategies aimed at inhibiting NFκB and/or PAI-1 activity may represent novel and effective therapies for patients with ALI who require mechanical ventilation with both high levels of oxygen and increased tidal volumes to ensure adequate gas exchange. Two potential agents in this regard are activate protein C (APC), an anticoagulant that has been shown to confer benefits in patients with severe sepsis,[14] and statins, a class of drugs that is known to both inhibit NFκB activity and downregulate PAI-1 but may also offer other clinically relevant benefits via endothelial barrier protection, the inhibition of ROS generation, and the upregulation of Enos.[15] Indeed, in light of the results presented, the intriguing prospects of both APC and statins in this context certainly warrant future investigation.

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