Lung endothelial barrier protection by iloprost in the 2-hit models of ventilator-induced lung injury (VILI) involves inhibition of Rho signaling

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Mechanical ventilation at high tidal volume (HTV) may cause pulmonary capillary leakage and acute lung inflammation culminating in ventilator-induced lung injury. Iloprost is a stable, synthetic analog of prostaglandin I_2 used to treat pulmonary hypertension, which also showed endothelium-dependent antiedemagenic effects in the models of lung injury. To test the hypothesis that iloprost may attenuate lung inflammation and lung endothelial barrier disruption caused by pathologic lung distension and coagulation system component thrombin, we used cell and animal 2-hit models of ventilator-induced lung injury. Mice received a triple injection of iloprost (2 μg/kg, intravenous instillation) at 0, 40, and 80min after the onset of HTV mechanical ventilation (30mL/kg, 4h), combined with the administration of a thrombin receptor-activating peptide 6 (TRAP6, 3×10^{-7}mol/mouse, intratracheal instillation). After 4h of ventilation, bronchoalveolar lavage (BAL), histologic analysis, and measurements of Evans blue accumulation in the lung tissue were performed. The effects of iloprost on endothelial barrier dysfunction were subsequently assessed in pulmonary endothelial cells (ECs) exposed to thrombin and pathologic (18%) cyclic stretch. The combination of HTV and TRAP6 enhanced the accumulation of neutrophils in BAL fluid and lung parenchyma, as well as increased the BAL protein content and endothelial permeability judged by Evans blue extravasation in the lung tissue. These effects were markedly attenuated by iloprost. The application of 18% cyclic stretch to pulmonary ECs enhanced the thrombin-induced EC paracellular gap formation and Rho-GTPase–mediated phosphorylation of regulatory myosin light chains and myosin phosphatase. Iloprost markedly inhibited the Rho-kinase–mediated site-specific phosphorylation of myosin phosphatase, and it prevented cyclic stretch- and thrombin-induced endothelial monolayer disruption. This study characterizes for the first time the protective effects of iloprost in the *in vitro* and *in vivo* 2-hit models of VILI and supports consideration of iloprost as a new therapeutic treatment of VILI. (Translational Research 2010;155:44–54)

Abbreviations: ALI = acute lung injury; ARDS = acute respiratory distress syndrome; BAL = bronchoalveolar lavage; cAMP = *cyclic* adenosine monophosphate; CS = cyclic stretch; EBD = Evans blue dye; EC = endothelial cell; GEF = guanine nucleotide exchange factor; HPAEC = human pulmonary artery endothelial cell; HTV = high tidal volume; i/v = intravenous; i/t = intratracheal; IL = interleukin; MLC = myosin light chain; MYPT = myosin-associated phosphatase type 1; PAR1 = protease activated receptor 1; PBS = phosphate buffered solution;

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PGI_2 = prostacyclin; PKA = protein kinase A; TNF-alpha = tumor necrosis factor-alpha; TRAP6 = thrombin receptor activating peptide 6; VILI = ventilator-induced lung injury

AT A GLANCE COMMENTARY

Background

Pathologic lung over-distention is associated with mechanical ventilation at high tidal volumes and compromises the blood-gas barrier, increases lung permeability, and may culminate in VILI and pulmonary edema. This report addresses effects of iloprost on parameters of lung vascular leak and inflammation induced by combined pathologic mechanical ventilation and treatment with thrombin peptide, and links protective iloprost effects in lung vascular endothelium with suppression of Rho-dependent pathway of increase permeability.

Translational Significance

This study shows beneficial effects of iloprost in the two-hit model of acute lung injury. These results suggest utilization of stable prostacyclin analogs as promising strategy for therapeutic treatment of VILI and ARDS.

Although ventilator support is an indispensable treatment for critically ill patients, ventilator-induced lung injury (VILI) with the associated multiorgan dysfunction may lead to significant morbidity and mortality, and thus, it remains one of the most important problems in the management of patients in the intensive care unit.[1] Pathologic lung overdistention is associated with mechanical ventilation at high tidal volumes (HTVs) and compromises the blood-gas barrier, increases lung permeability, and may culminate in VILI and pulmonary edema.[2,3] The activation of coagulation system and elevation of procoagulant and endothelium-activating mediator thrombin is also linked to the development of VILI.[4,5] Clinical investigations and experimental models of acute lung injury (ALI), acute respiratory distress syndrome (ARDS), VILI, and pneumonia show many beneficial effects of anticoagulant therapy.[4-7] A vascular leak observed in VILI patients is associated with increased levels of edemagenic and inflammatory mediators such as thrombin, histamine, tumor necrosis factor-alpha (TNF-alpha), IL-8, and IL-1.[2,8-10] However, the significance of the interactions between the edemagenic agents and pathologic lung mechanical distension in progression of VILI-associated vascular leak and pulmonary edema has been recognized only recently. A model of pulmonary endothelial cells (ECs) exposed to controlled levels of cyclic stretch (CS) and agonist stimulation is a unique tool that reproduces VILI conditions *in vitro*. Using this model, we have described differential effects of physiologic and pathologic CS magnitudes on the agonist-induced EC barrier disruption.[11-13] Previous reports by others and our data suggest that the attenuation of Rho activity and the stimulation of Rac-dependent mechanisms reduces lung vascular leak induced by pathologic CS and inflammatory agents, and it promotes barrier recovery in the *in vitro* and *in vivo* models of ALI/VILI.[11,12,14-16]

Prostacyclin (PGI_2), which is a product of cyclooxygenase, has been implicated in the regulation of vascular function, wound repair, inflammatory processes, and ALI. Aerosolized prostacyclin shows a marked protection against hyperoxic lung injury or lung damage caused by ischemia/reperfusion, and increased levels of prostacyclin stable metabolites have been associated with less severe respiratory distress.[17,18] We have previously demonstrated potent barrier-protective effects of prostacyclin stable analog beraprost in human pulmonary EC.[19] Our study and other studies have shown that the barrier-protective effects of prostacyclin on pulmonary EC are mediated by cAMP-activated protein kinase A (PKA) and cAMP-activated guanine exchange factor Epac, which triggers its effector GTPase Rap1 and downstream signaling to Tiam1/Vav2 and Rac GTPase.[19-21] In turn, Rac activation leads to actin cytoskeletal remodeling and enhancement of adherens junctions.[19]

In the current study, we examined the effects of stable prostacyclin analog iloprost on EC barrier disruption induced by pathologic CS stimulation and thrombin in the *in vitro* model of VILI. The protective effects of iloprost were evaluated *in vivo* in a 2-hit model of lung injury induced by protease-activated receptor 1 (PAR1) receptor ligand thrombin receptor activating peptide 6 (TRAP6) and mechanical ventilation at high tidal volume.

MATERIALS AND METHODS

Reagents. Diphospho-myocin light chain (MLC) and guanine nucleotide exchange factor (GEF)-H1 antibodies were obtained from Cell Signaling (Beverly, Mass); phospho-Thr^{850}- myosin-associated phosphatase type 1 (MYPT) antibodies were purchased from Upstate Biotechnology (Lake Placid, NY). TRAP6 was obtained from AnaSpec (San Jose, Calif). All reagents for immunofluorescence staining were purchased from Molecular

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