Hydroxyethyl starch reduces high stretch ventilation-augmented lung injury via vascular endothelial growth factor

LI-FU LI, CHUNG-CHI HUANG, YUNG-YANG LIU, HORNG-CHYUAN LIN, KUO-CHIN KAO, CHENG-TA YANG, and SHUEN-KUEI LIAO

TAOYUAN AND TAIPEI, TAIWAN

Disruption of epithelial and endothelial barriers found in patients with acute lung injury often results in the need for the support of mechanical ventilation. High tidal volume (V_T) mechanical ventilation can increase lung damage through lung inflammation, but the mechanisms are unclear. We hypothesized that a colloid supply with hydroxyethyl starch would decrease neutrophil infiltration, lung edema, and vascular endothelial growth factor (VEGF) production in mice exposed to high V_T mechanical ventilation. Male C57BL/6 mice, weighing 20 g to 25 g, were exposed to high V_T (30 mL/kg) mechanical ventilation with room air for 1 h to 5 h and infused with 15 mL/kg/h normal saline or hydroxyethyl starch intravenously at the beginning and every 30 min during ventilation. Evans blue dye, lung wet-to-dry weight ratio, histopathologic grading of lung tissue, myeloperoxidase, and inflammatory cytokine were measured to establish the extent of lung injury. Knockdown of VEGF by short interfering RNA (siRNA) was used to explore the role of VEGF. High V_T ventilation induced the increases of microvascular permeability, neutrophil influx, expressions of VEGF mRNA and VEGF, production of VEGF protein, positive staining of VEGF in epithelium, and apoptotic epithelial cell death. Lung injury induced by high V_T ventilation was attenuated with the supply of hydroxyethyl starch and pharmacologic inhibition of VEGF expression by siRNA. We conclude that hydroxyethyl starch reduces high V_T mechanical ventilation-induced lung injury and neutrophil infiltration through an inhibition of VEGF expression. (Translational Research 2011;157:293–305)

Abbreviations: ALI = acute lung injury; ARDS = acute respiratory distress syndrome; BAL = bronchoalveolar lavage; DAB = diaminobenzidine; EBD = Evans blue dye; IL = interleukin; GAPDH = glyceraldehydes-phosphate dehydrogenase; H&E = hematoxylin and eosin; MIP-2 = macrophage inflammatory protein-2; MPO = myeloperoxidase; RT-PCR = reverse transcription-polymerase chain reaction; siRNA = short interfering RNA; TNF-α = tumor necrosis factor-α), TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling; VEGF = vascular endothelial growth factor; VEGFR = VEGF receptor; VILI = ventilator-induced lung injury; V_T = tidal volume

From the Division of Pulmonary and Critical Care Medicine, Chang Gung Memorial Hospital; Chang Gung University; Department of Respiratory Therapy, Chang Gung Memorial Hospital, Kweishan, Taoyuan, Taiwan; Chest Department, Veterans General Hospital; National Yang-Ming University, School of Medicine; Department of Cancer Immunotherapy, Taipei Medical University Hospital, Taipei, Taiwan.

Supported by National Science Council 98-2314-B-182A-084-MY3.

Submitted for publication November 1, 2010; revision submitted December 20, 2010; accepted for publication December 20, 2010.

Reprint requests: Li-Fu Li, Chang Gung Memorial Hospital, Division of Pulmonary and Critical Care Medicine, 5 Fu-Hsing Street, Kweishan, Taoyuan, Taiwan; e-mail: lfp3434@adm.cgmh.org.tw.

1931-5244/$ - see front matter

doi:10.1016/j.trsl.2010.12.009

AT A GLANCE COMMENTARY

Li L, et al.

Background

Increase of microvascular permeability and capillary leakage related with the loss of epithelial and endothelial integrity found in patients with acute lung injury (ALI) often results in the need for the support of mechanical ventilation. High tidal volume mechanical ventilation can increase lung damage, but the mechanisms are unclear. Hydroxyethyl starch may attenuate increased capillary permeability, and vascular endothelial growth factor (VEGF)-mediated signaling regulates various pathologic processes.

Translational Significance

The data of this study have added to the understanding of the protective mechanism of hydroxyethyl starch related to the reduction of VEGF. Additional studies targeting this signaling may afford a potential diagnostic and therapeutic strategy involved in ALI.

Acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) is characterized by an alteration of microvascular permeability and capillary leakage because of a loss of epithelial and endothelial integrity that facilitate the passage of fluid containing inflammatory cytokines and activated inflammatory cells into the interstitial tissues and alveoli.[1] Pathologic lung overdistension may occur in the healthy parts of the lungs in patients with ARDS employing ventilator support. The use of high tidal volume (V_T) in normal animals mimics this overdistension of the normal lung. The phenomenon of overdistension of alveoli causes ALI (ventilator-induced lung injury, VILI) characterized by an inflammatory response that is morphologically and histologically similar to that caused by a lung previously injured by bleomycin or bacterial lipopolysaccharide.[2,3] The recruitment of inflammatory cells with high V_T ventilation is initiated by an enhanced production of inflammatory mediators, including murine macrophage inflammatory protein-2 (MIP-2), plasminogen activator inhibitor-1, and vascular endothelial growth factor (VEGF).[4,5]

The major sources of VEGF are alveolar type II epithelial cells, alveolar macrophages, and neutrophils.[6,7] Induction of VEGF has been described *in vivo* and *in vitro* in response to several stimuli, including lung stretch, reactive oxygen species, and inflammatory cytokines.[6] Systemic overexpression of VEGF may cause ALI with the development of pulmonary edema.[8] VEGF is critical in the regulation of both vascular permeability and endothelial cell survival. An experimental model of septic shock in dogs showed that an infusion with hydroxyethyl starch improved capillary permeability, pulmonary edema, and the concentration of VEGF. [9] The relationship among VEGF, hydroxyethyl starch, and VILI has not been well characterized.[10-12]

Early goal-directed therapy in the management of sepsis and septic shock, emphasizing early and adequate fluid therapy, may improve the outcomes in patients with ALI.[13,14] Our previous study in patients with ARDS showed that hydroxyethyl starch, a colloid synthesized by partial hydrolysis of amylopectin plant starch, resuscitation significantly improved pulmonary vascular permeability.[15] Hydroxyethyl starch may attenuate increased capillary permeability by directly plugging leaky endothelial cells and by exerting antiinflammatory effects, including inhibition of neutrophil recruitment and proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, VEGF, and IL-6.[9,16] The mechanism of the effect of hydroxyethyl starch on the inflammatory response of ALI has not been characterized.

In this high V_T ventilation-induced ALI model in mice, we demonstrated that VILI was accompanied with VEGF production, with an attenuation of VILI through inhibition of VEGF with short interfering RNA (siRNA) targeted to VEGF, and with a reduction of VEGF production by hydroxyethyl starch. We hypothesized that a colloid supply with hydroxyethyl starch would decrease neutrophil infiltration, lung edema, and VEGF production in mice exposed to high V_T mechanical ventilation.

METHODS

Experimental animals. Male C57BL/6 mice, aged between 6 and 8 weeks, weighing between 20 and 25 g, were obtained from Jackson Laboratories (Bar Harbor, Maine) and the National Laboratory Animal Center (Taipei, Taiwan). The study was performed in accordance with the animal experimental guidelines of the National Institutes of Health and with the approval of the local research committee.

Experimental groups. Animals were randomly distributed into the following 7 groups in each experiment: group 1, control, nonventilated wild-type mice with normal saline (n = 6 each for western blot/VEGF mRNA, Evans blue dye [EBD] assay, immunohistochemistry/terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling [TUNEL]/lung injury assay, cell counts/bronchoalveolar lavage [BAL] total protein/VEGF, and myeloperoxidase [MPO]/lung water);