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Effects of trauma, hemorrhagic shock, and chronic stress on lung vascular endothelial growth factor

Tyler J. Loftus, MD,[a] Andrew J. Thomson, BA,[a] Kolenkode B. Kannan, PhD,[a] Ines G. Alamo, MD, MPH,[a] Harry N. Ramos, BS,[a] Elizabeth E. Whitley, DVM, PhD,[b] Philip A. Efron, MD,[a] and Alicia M. Mohr, MD[a,*]

[a] Department of Surgery, Sepsis and Critical Illness Research Center, University of Florida Health, Gainesville, Florida
[b] Pathogenesis, LLC, Gainesville, Florida

ARTICLE INFO

Article history:
Received 23 August 2016
Received in revised form
11 October 2016
Accepted 26 October 2016
Available online 2 November 2016

Keywords:
Pulmonary contusion
Lung injury
Inflammation
Pulmonary edema
Vascular endothelial growth factor
Tissue repair

ABSTRACT

Background: Vascular endothelial growth factor (VEGF) and its receptors (VEGFR-1 and VEGFR-2) regulate vascular permeability and endothelial cell survival. We hypothesized that hemorrhagic shock (HS) and chronic stress (CS) would increase expression of lung VEGF and its receptors, potentiating pulmonary edema in lung tissue.

Materials and methods: Male Sprague–Dawley rats aged 8-9 wk were randomized: naïve control, lung contusion (LC), LC followed by HS (LCHS), and LCHS with CS in a restraint cylinder for 2 h/d (LCHS/CS). Animals were sacrificed on days 1 and 7. Expressions of lung VEGF, VEGFR-1, and VEGFR-2 were determined by polymerase chain reaction. Lung Injury Score (LIS) was graded on light microscopy by inflammatory cell counts, interstitial edema, pulmonary edema, and alveolar integrity (range: 0 = normal; 8 = severe injury).

Results: Seven days after LC, lung VEGF and VEGFR-1 were increased, and lung tissue healed (LIS: 0.8 ± 0.8). However, 7 d after LCHS and LCHS/CS, lung VEGF and VEGFR-1 expressions were decreased. VEGFR-2 was also decreased after LCHS/CS. LIS was elevated 7 d after LCHS and LCHS/CS (6.5 ± 1.0 and 8.2 ± 0.8). Increased LIS after LCHS and LCHS/CS was because of higher inflammatory cell counts, increased interstitial edema, and loss of alveolar integrity, whereas pulmonary edema was unchanged.

Conclusions: Elevation of lung VEGF and VEGFR-1 expressions after LC alone was associated with healing of injured lung tissue. Expressions of VEGF, VEGFR-1, and VEGFR-2 were reduced after LCHS and LCHS/CS, and injured lung tissue did not heal. Persistent lung injury after severe trauma was because of inflammation rather than pulmonary edema.

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Introduction

Vascular endothelial growth factor (VEGF) contributes to lung homeostasis by several mechanisms: modulating growth and apoptosis of vascular endothelial cells, increasing vascular permeability, potentiating pulmonary endothelial cell growth and survival, and stimulating type 2 pneumocyte surfactant production.[1,2] VEGF binds to two receptor tyrosine kinases: VEGFR-1 (also known as flt-1) and VEGFR-2 (also known as flk-1).[1] The physiology of VEGFR-1 remains controversial, in

* *Corresponding author.* Department of Surgery, University of Florida, 1600 SW Archer Road, Box 100108, Gainesville, FL 32610. Tel.: +1 352 273 5670; fax: +1 352 273 5683.
E-mail address: alicia.mohr@surgery.ufl.edu (A.M. Mohr).
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http://dx.doi.org/10.1016/j.jss.2016.10.023

part because its signaling properties depend on the animal and cell type.[1,3] VEGFR-2 is the major mediator of VEGF-induced angiogenesis and vascular permeability.[1] Lung VEGF is primarily produced by epithelial cells and also produced in smaller quantities by endothelial cells, macrophages, and neutrophils.[1,4-7] Its major targets are VEGFR-1 and VEGFR-2 on endothelial cells, but also it targets type 1 and type 2 pneumocytes.[8-10]

VEGF is an essential growth and survival factor for pulmonary epithelial and endothelial cells, but also promotes vascular permeability in the lung, which may cause detrimental pulmonary edema.[7,11,12] Therefore, both abnormally low and abnormally high lung VEGF activity may be harmful by different mechanisms. Several studies have investigated VEGF and VEGF receptor function after nontraumatic lung injury, producing inconsistent results, which may be attributable to differences in experimental models, the timing of interventions, and different approaches to measuring the potentially beneficial and adverse effects of lung VEGF.[8,12-16] However, the effects of traumatic lung injury on VEGF are unknown. Pulmonary contusion followed by hemorrhagic shock (HS) and chronic stress (CS) from the intensive care unit environment is a relatively common clinical scenario. Understanding the role of VEGF in lung tissue repair under these conditions may elucidate therapeutic strategies. Based on evidence that hypoxia and hypercatecholaminemia increase VEGF and VEGF receptor expression and function,[8,17-21] we hypothesized that lung contusion (LC) and HS would increase expressions of lung VEGF and VEGF receptors, the addition of daily CS would exacerbate this effect, and that VEGF overexpression would be associated with pulmonary edema and impaired lung tissue healing.

Materials and methods

Male Sprague–Dawley rats (Charles River, Raleigh, NC) aged 8 wk and weighing 300-400 g were housed in pairs and fed *ad lib* with Teklad Diet #7912 (Harlan Laboratories Inc, Tampa, FL) and water during a 1-wk acclimation period. Light and dark cycles were 12 h each throughout acclamation and experimental periods. All animal care was conducted in accordance with University of Florida Institutional Animal Care and Use Committee standards. Animals were randomly allocated to one of four groups: naïve ($n = 8$), LC (1-d model: $n = 5$, 7-d model: $n = 7$), LC followed immediately by HS (LCHS) (1-d model: $n = 5$, 7-d model: $n = 7$), and LCHS with daily chronic restraint stress (LCHS/CS) (1-d model: $n = 5$, 7-d model: $n = 7$). These injury models were chosen to recapitulate common clinical scenarios: isolated blunt chest trauma (LC), blunt chest trauma accompanied by hemorrhage with early recovery (LCHS), and blunt chest trauma accompanied by hemorrhage followed by chronic stressors associated with the intensive care unit environment.

Before LC and HS, animals were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg). Pentobarbital was used to avoid confounding effects on the neuroendocrine response to injury and stress. LC was performed by applying a percussive staple gun (PowerShot Model 5700M; Saddle Brook, NJ) to a 12-mm metal plate applied to the right lateral chest wall 1 cm below the axillary crease. This model has previously been shown to produce a clinically significant and reproducible pulmonary contusion based on histologic findings.[22-24]

Rats that would also undergo HS were then placed on a heating pad, and PE-50 tubing was inserted into the right internal jugular vein and right femoral artery under direct visualization. The arterial catheter was used for continuous blood pressure monitoring with the BP-2 Digital Blood Pressure Monitor (Columbus Instruments, Columbus, OH). Blood was then withdrawn through the venous catheter into a heparinized syringe (10 U/mL) until a mean arterial pressure of 30-35 mm Hg was obtained. This blood pressure was maintained for a 45-min period by withdrawing or reinfusing blood as necessary. After 45 min, shed blood was reinfused at a rate of 1 mL/min.

CS was performed by placing animals in a restraint cylinder (Kent Scientific Corporation, Torrington, CT) for 2 h/d. CS began 1 d after LCHS in the LCHS/CS group. To prevent acclimation during the 2-h period, the cylinders were rotated 180° every 30 min, and alarms were transmitted by speakers placed immediately adjacent to the cylinders for a 2-min period each time the cylinders were rotated. Because animals undergoing CS had no access to food or water while in the restraint cylinder, all other groups were also subjected to a 2-h daily fast. There were no deaths associated with LC alone, and no late deaths attributable to daily CS. HS was associated with approximately 15%-20% mortality within 3 h.

Animals were sacrificed by cardiac puncture after intraperitoneal injection of ketamine (80-100 mg/kg) and xylazine (5-10 mg/kg) on days 1 and 7. Day 1 LC and LCHS rats were sacrificed 1 d after interventions were performed. Day 1 LCHS/CS rats had LCHS, then a single episode of CS occurring 1 d later, and were sacrificed 1 d after CS. Right lung, left lung, and plasma specimens were collected. Lung specimens were initially placed in phosphate buffered saline. Portions of the contused right lung and the noncontused left lung were placed in formalin for hematoxylin and eosin staining and histologic analysis by light microscopy, and a noncontused portion of the right lung was placed immediately in dry ice and then stored at −80°C. Plasma samples were obtained during cardiac puncture by withdrawing 7-10 mL of blood into a heparinized syringe (10 U/mL). Samples were stored at −80°C.

Lung VEGF, VEGFR-1, and VEGFR-2 expressions were assessed by endpoint polymerase chain reaction. The following primers were selected: VEGF forward 5′ gtggacttgagttgggagga and reverse 5′ caaacagacttcggcctctc (product region: 2135-2228, product size: 147 bp), VEGFR-1 forward 5′ agtggctccacgaccttaga and reverse 5′ gaagaccgcttcagttttcgt (product region: 2258-2575, product size: 317 bp), and VEGFR-2: forward 5′ acagcatcaccagcagtcag and reverse 5′ ccaagaactccatgccctta (product region: 3127-3274, product size: 147 bp). Amplifications were performed using a SimpliAmp thermal cycler (Applied Biosystems, Carlsbad, CA) with an initial 4-min denaturation phase at 95°C, followed by 32 cycles with denaturation at 95°C, annealing at 60°C, and extension at 72°C for 45 s each. Products were separated on 1.5% agarose gel stained with Ethidium Bromide (Invitrogen, Carlsbad, CA). VEGF, VEGFR-1, and VEGFR-2 were assessed in the contused

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