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Uncovering the neuroenteric–pulmonary axis: Vagal nerve stimulation prevents acute lung injury following hemorrhagic shock

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ABSTRACT

Aims: Trauma/hemorrhagic shock (T/HS) induced gut injury is known to initiate a systemic inflammatory response which can lead to secondary lung injury. We have shown that vagal nerve stimulation (VNS) protects intestinal epithelial integrity after a severe burn insult. We hypothesize that VNS will protect the lung from injury following T/HS by preventing intestinal barrier failure.
Main methods: Male Balb/c mice were subjected to a T/HS model with and without cervical VNS. Intestinal injury was evaluated by measuring changes in gut barrier function and tight junction protein localization. Lung injury was evaluated using histology and markers of lung inflammation. Using NF-kB-luciferase (NF-kB-luc) transgenic mice, NF-kb–DNA binding was measured by photon emission analysis at 4 after injury.
Key findings: T/HS is associated gut injury characterized by histologic injury, increased epithelial permeability, and altered localization of gut tight junction proteins. Cervical VNS prevented the T/HS-induced changes in gut barrier integrity. Gut injury after T/HS was associated with acute lung injury at 24 h characterized by histologic injury, increased number of MPO positive stained cells and MPO enzymatic activity, and increased ICAM-1 expression in lung endothelium. VNS decreased T/HS-induced lung injury with a marked decrease in lung inflammation compared to T/HS alone. Lungs harvested from NF-kB-luc mice at 4 h post VNS + T/HS demonstrated decreased DNA binding of NF-kB compared to T/HS alone as measured by changes in bioluminescence.
Significance: VNS is effective in protecting against acute lung injury caused by hemorrhagic shock through its ability to prevent gut barrier dysfunction.

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Introduction

Hemorrhagic shock induces a global tissue hypoxia and generalized ischemia that initiates a systemic inflammatory response resulting in end organ injury affecting the liver, intestine, and lung (Barlos et al., 2009; Coimbra et al., 2004; Deitch et al., 1990; Deree et al., 2007b). Intestinal barrier failure plays an important role in the development of post-injury SIRS and acute lung injury (ALI). Post-trauma ALI has specifically been shown to increase morbidity and mortality among trauma patients and although supportive care measures have proved successful in the treatment of ALI, preventive measures are lacking (Bersten et al., 2002; Estenssoro et al., 2002).

The pathophysiologic pathway that results in ALI includes destruction of the pulmonary capillary endothelium by neutrophils and activation of macrophages which results in local production of pro-inflammatory cytokines (Michetti et al., 2003). Pro-inflammatory intracellular signaling cascade activation in the lung is mediated by the transcription regulatory factor nuclear factor kappa-B (NF-κB) in animals after endotoxemia or hemorrhage (Costantini et al., 2010d; Shenkar and Abraham, 1999). NF-κB DNA binding regulates genes involved in the inflammatory response and results in the up-regulation of inflammatory cytokine synthesis such as IL-8. Pro-inflammatory cytokine production in the lung increases neutrophil chemotaxis to specific sites of injury and promotes the inflammatory response (Shenkar and Abraham, 1997). The pulmonary endothelium also reacts to increased local cytokine production and upregulates Intracellular Adhesion Molecule 1 (ICAM-1) expression, facilitating the migration of activated neutrophils which contributes to the pathogenesis of ALI (Gonzalez et al., 2003; Li et al., 2009).

The systemic inflammatory response which results after severe injury is an essential host response to injury; however, unrestrained inflammation can be harmful to host tissues resulting in organ failure and death. Therapeutic interventions designed to limit the cytokine storm which occurs in severe trauma may decrease the late complications of injury. The vagus nerve regulates the systemic inflammatory response by limiting cytokine release through efferent vagus nerve signaling (Tracey, 2002). Previous studies have focused on the ability

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of the vagus nerve to limit cytokine production from immune cells in the spleen (Vida et al., 2011).

Our laboratory has demonstrated the capacity for vagal nerve stimulation (VNS) to prevent intestinal barrier failure and intestinal inflammation in models of severe injury (Bansal et al., 2009; Costantini et al., 2010c; Krzyzaniak et al., 2011b) through the enteric nervous system, a mechanism which is independent of splenic cytokine production, suggesting that the vagus nerve may regulate the inflammatory response through other cell types (Costantini et al., 2012).

We have shown that stimulation of the vagus nerve improves intestinal barrier integrity and the expression and localization of the tight junction proteins occludin and ZO-1 (Costantini et al., 2010a). We have also demonstrated the importance of the neuroenteric axis in altering distant organ injury, showing that VNS prevents secondary ALI in a severe burn injury model (Krzyzaniak et al., 2011c). In this series of experiments, we hypothesized that VNS will attenuate ALI following trauma/hemorrhagic shock (T/HS) which is associated with improved gut barrier function, further demonstrating the capacity of the Vagus nerve to attenuate shock-induced organ injury via the neuro-enteric axis.

Materials and methods

Animal model of trauma/hemorrhagic shock

Male Balb/c mice weighting 20 g to 31 g were obtained from Jackson Laboratories (Sacramento, CA). Animals were anesthetized with inhaled isoflurane prior to beginning the experimental protocol. Animals were subjected to a pressure fixed hemorrhagic shock model (MAP of 35 mm Hg) for 60 min and trauma (2.5 cm median laparotomy with manipulation of intestinal contents). Right cervical VNS was performed by direct stimulation immediately prior to the induction of hemorrhagic shock. Additionally, a cohort of animals underwent abdominal vagotomy (Vx) by severing both branches of the vagus nerve at the gastroesophageal junction prior to VNS and T/HS. The hemorrhagic shock was performed by withdrawing 0.1 mL of blood from the animal through a left arterial femoral catheter over 1 min intervals to maintain a mean arterial pressure (MAP) of 35 ± 5mm Hg. Fluid resuscitation was performed by infusing three times the volume of shed blood using Ringers Lactate solution (Baxter Healthcare Corporation, Deerfield, IL, USA) at 37 °C over 10 min. Every animal's body temperature was maintained at 37 ± 0.2 °C by means of a heating pad using warm water flow. After the procedure, all animals received a subcutaneous injection of buprenorphine (Hospira Inc., IL, USA) at a dose of 0.05 mg/kg for postoperative analgesia. Sham animals were subjected to femoral artery catheterization without withdrawal of blood or resuscitation fluid. Animals were monitored while they recovered from anesthesia while in their cages and were provided food and water ad libitum. These studies were conducted in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The University of California San Diego Institutional Animal Care and Use Committee approved all animal experiments.

Vagal nerve stimulation

A right cervical neck incision was performed and the right cervical vagus nerve exposed. Stimulation of the right cervical vagus nerve was performed using a VariStim III probe (Medtronic Xomed, Jacksonville, FL) at 2 mA, on and off for 1 s, for a duration of 10 min. Immediately following nerve stimulation the neck incision was closed and the animals were immediately subjected to hemorrhagic shock injury as previously described.

Surgical abdominal vagotomy

A cohort of animals was subjected to a surgical abdominal vagotomy performed through a midline laparotomy incision (2.5 cm). The gastroesophageal junction was identified and the dorsal and ventral vagus nerves were visualized on the distal esophagus using an Olympus SZ61 stereo microscope (Leeds Precision Instruments, Minneapolis, MN). Both branches of the vagus nerve were isolated and sharply transected. The abdomen was then closed using continuous running 4–0 silk suture followed by right cervical vagal nerve stimulation as described above. T/HS was performed following Vagotomy and VNS.

Intestinal permeability assay

An in vivo intestinal permeability assay was performed to assess intestinal barrier function ($n = 5$ animals per group). Four hours after injury, animals were anesthetized with inhaled isoflurane. The midline laparotomy incision was reopened, and a 5 cm segment of distal ileum was isolated between silk ties. A solution of 200 μL containing 4 kDa FITC–Dextran (25 mg/mL, Sigma, St. Louis, MO) diluted in phosphate buffered saline (PBS) was injected into the lumen of the isolated segment of intestine. The bowel was returned to the abdominal cavity and the abdomen closed. The animal was maintained under general anesthesia for 30 min, at which time systemic blood was drawn by cardiac puncture and placed in heparinized Eppendorf tubes on ice. Plasma was obtained by centrifuging the blood at 10,000 g for 10 min at 4 °C. Plasma fluorescence was measured with a fluorescence spectrophotometer (FLUOstar Omega, BMG Labtech, Cary, NC) and compared with a standard curve of known concentrations of FITC–Dextran diluted in mouse plasma.

Histological evaluation

Gut specimens were harvested 4 h after injury and lung samples were collected at 24 h after injury and fixed in 10% formalin solution and stored in paraffin. Specimens were section 5 μm thick for histologic evaluation. A pathologist blinded to the experimental groups analyzed multiple fields from sections of lung ($n \geq 3$ mice per experimental condition) imaged at 20× and 60× with a light microscope. The intestinal sections were graded on a scale 1 through 4 (0 = normal, 1 = mild, focal epithelial edema, 2 = moderate, diffuse swelling with villi necrosis, 3 = severe, diffuse pathology, neutrophil infiltration, and 4 = major widespread injury with massive neutrophil infiltration and hemorrhage, as used by others to grade intestinal injury to ischemia/shock (Cuzzocrea et al., 2002)). The lung sections were graded based on a pulmonary scoring system previously used by our laboratory looking at intra-alveolar hemorrhage, pulmonary congestion, edema, and infiltration of inflammatory cells on H&E staining to yield a maximum score of 12. Each lung section was rated on a scale from 0 to 3 ranging from normal to severe injury (Deree et al., 2007a). Histologic injury scores were averaged for each experimental condition.

Confocal microscopy

Segments of distal ileum ($n = 5$ animals per group) were embedded in O.C.T compound and stored at −80°C. Sections of intestine were cut 10 μm thick using a Reichert–Jung Cryocut 1800 at −20°C (Reichert Microscopes, Depew, NY). Sections were fixed onto glass slides with 3.7% paraformaldehyde (Electron Microscopy Series, Hatfield, PA) for 10 min, washed with PBS. Sections were blocked for 1 h in 3% BSA, Sigma. The sections were incubated overnight in the occludin or ZO-1 antibody (Invitrogen), followed by secondary antibody Alexa Fluor 488 (Invitrogen) in 1% BSA for 1 h. Slow Fade (Invitrogen) was added upon placement of cover slips. Images were viewed using the Olympus FluoView™ laser scanning confocal microscope with exposure-matched settings (Advanced Software v1.6, Olympus) at 60× magnification. Investigators blinded to the experimental groups reviewed all images obtained to determine if there were changes in confocal microscopy images between groups.

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