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Vagal nerve stimulation modulates gut injury and lung permeability in trauma-hemorrhagic shock

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Abstract

BACKGROUND—Hemorrhagic shock is known to disrupt the gut barrier leading to end-organ dysfunction. The vagus nerve can inhibit detrimental immune responses that contribute to organ damage in hemorrhagic shock. Therefore, we explored whether stimulation of the vagus nerve can protect the gut and recover lung permeability in trauma-hemorrhagic shock (THS).

METHODS—Male Sprague-Dawley rats were subjected to left cervical vagus nerve stimulation at 5 V for 10 minutes. The right internal jugular and femoral artery were cannulated for blood withdrawal and blood pressure monitoring, respectively. Animals were then subjected to hemorrhagic shock to a mean arterial pressure between 30 mm Hg and 35 mm Hg for 90 minutes then reperfused with their own whole blood. After observation for 3 hours, gut permeability was assessed with fluorescein dextran 4 in vivo injections in a ligated portion of distal ileum followed by Evans blue dye injection to assess lung permeability. Pulmonary myeloperoxidase levels were measured and compared.

RESULTS—Vagal nerve stimulation abrogated THS-induced lung injury (mean [SD], 8.46 [0.36] vs. 4.87 [0.78]; $p < 0.05$) and neutrophil sequestration (19.39 [1.01] vs. 12.83 [1.16]; $p < 0.05$). Likewise, THS gut permeability was reduced to sham levels.

CONCLUSION—Neuromodulation decreases injury in the THS model as evidenced by decreased gut permeability as well as decreased lung permeability and pulmonary neutrophil sequestration in a rat model.

Keywords

Vagus; lung permeability; gut barrier

Gut barrier failure is a major contributor to organ failure after hemorrhagic shock.
Hemorrhagic shock disrupts the gut barrier to allow bacteria to cross and invade the sterile

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AUTHORSHIP

G.L., G.V., and E.A.D. conducted the literature search for this study. D.-Z.X., L.U., and E.A.D. contributed to experimental design. G.L., J.E.F., D.-Z.X., W.D., and D.P. collected data, which E.A.D. interpreted. G.L., A.M., and E.A.D. wrote the manuscript. G.L. and L.U. prepared figures.

DISCLOSURE

The authors declare no conflicts of interest.

internal milieu to stimulate systemic inflammation.¹ Although bacteria may remain undetectable in the systemic circulation, the gut is capable of becoming a cytokine-generating organ instigating a strong inflammatory response.² Gut-derived factors increase lung permeability leading to shock induced adult respiratory distress syndrome (ARDS) as well as a systemic inflammatory response syndrome. These gut-derived factors are carried in the mesenteric lymph where they reach the pulmonary system and influence lung injury.^{3,4} Ligation of the mesenteric lymph duct prevents shock-induced lung injury. This confirms that injurious factors leaving the gut can contribute to distant organ injury,⁵ supporting the hypothesis that the hypoperfused gut may be the source of inflammatory mediators that stimulate the systemic inflammatory response.

Direct stimulation of the vagus nerve is able to suppress systemic inflammation and has a protective effect in endotoxemia.⁶ In burn models, vagus nerve stimulation improves gut barrier integrity as measured by occludin protein expression.⁷ The vagus nerve has been implicated as playing an essential role in regulating the inflammatory responses and inhibiting the severity of experimental pancreatitis.⁸ Although there is much information about the role of the vagus nerve in endotoxemia, the extent of available data in trauma-hemorrhagic shock (THS) is limited.

The aim of this study was to measure the effect of vagus nerve stimulation on gut injury and lung permeability after THS. We hypothesize that stimulating the parasympathetic nervous system will protect the gut from injurious factors as well as protect the lung from increased permeability.

MATERIALS AND METHODS

Pathogen-free male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 330 g to 380 g were housed under barrier-sustained conditions and kept at 25°C with 12-hour light/dark cycles. The rats had free access to water and chow (Teklad 22/5 Rodent Diet W-8640, Harlan Teklad, Madison, WI). All rats were maintained in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals, and the experiments were approved by the New Jersey Medical School Animal Care Committee.

Trauma-Hemorrhagic Shock

THS is performed as previously described;⁹ briefly, male Sprague-Dawley rats were anesthetized with pentobarbital (50 mg/kg) intraperitoneally. Using aseptic technique through a midline laparotomy, the intestine is exposed and eviscerated from the abdominal cavity. Intestines are protected by wet gauze in 0.9% saline for 15 minutes, after which the intestines are placed back into the abdominal cavity, and laparotomy incision is closed using 4-0 suture. Femoral artery was dissected and cannulated with PE-50 tubing with anticoagulated saline syringe with Arixtra (Cardinal Health, Swedesboro, NJ) (5 μ L/mL) and attached to a BP-2 blood pressure monitor (Columbus Instruments, Columbus, OH). Right internal jugular vein was dissected out and cannulated for blood withdrawal with Silastic tubing (Dow Corning, Midland, MI) in an anticoagulated syringe as previously mentioned. Trauma sham shock (TSS) animals are prepared as previously mentioned, but no blood is removed.

Blood is withdrawn through the jugular vein line into an Arixtra anticoagulated syringe (3 mg/kg) until the mean arterial pressure reaches 30 mm Hg to 35 mm Hg at a rate of 1 mL per minute and maintained for 90 minutes. At the end of the shock period, the rats are resuscitated with their shed blood at a rate of 1 mL per minute and observed for 3 hours.

Vagal Nerve Stimulation

The vagus nerve was stimulated as previously described.¹⁰ Briefly, once all cannulas were in place, the left side of the animal's neck was subjected to dissection, and the carotid artery was identified. The vagus nerve was carefully dissected off the left carotid artery, and a platinum electrode was placed across the nerve, attached to the stimulation device (STM 150, Biopac Systems, Goleta, CA) controlled by the AcqKnowledge software (Biopac Systems). Vagus stimulation (+VNS) was applied for 10 minutes at 5 V. Sham animals underwent dissection of the left side of the neck, isolation of the left vagus nerve, placement of electrode without stimulation of the electrode and then subjected to hemorrhagic shock or sham shock.

Lung Permeability Assay

Lung permeability was assessed by permeation of Evans blue dye as previously described.⁵ Briefly, on completion of shock resuscitation period, animals were injected with 10-mg Evans blue dye through the internal jugular catheter. After 5 minutes, a 1.5-mL blood sample was drawn from the femoral artery catheter and centrifuged at 1,500 rpm at 4°C for 20 minutes. The plasma was serially diluted to form a standard curve. Twenty minutes after dye injection, the animals were killed, and a cardiopulmonectomy was performed. Modified bronchial lavage was performed on the excised lungs using two small cuts to facilitate fluid removal, and the bronchoalveolar lavage fluid was centrifuged at 1,500 rpm as previously described to remove any cellular debris. The supernatant fluid was assayed with a spectrophotometer at 620 nm for dye concentration and was compared with a standard curve.

Gut Permeability Assay

Gut permeability was measured by in vivo methods as previously described.¹¹ At the end of the shock resuscitation period, midline laparotomy incision was released, and the cecum was identified and protected with moist gauze. A 10-cm segment of terminal ileum was identified, incised distally, and ligated proximally and flushed with 1.5 mL of 0.9% sodium chloride saline to remove feces. Once flushed, the enterotomy was closed, and the distal end of the segment was ligated as well. Fluorescein isothiocyanate dextran (FD4; molecular weight, 4,000 Da; Sigma, St. Louis, MO) in a concentration of 25 mg/mL was prepared. A total of 1-mL FD4 tracer was injected into the 10-cm segment, carefully preventing any spillage onto the external bowel until the bowel segment was slightly dilated. The segment was protected from light under moist gauze and aluminum foil and let to rest, allowing for a circulation of 30 minutes. Once time was completed, 1 mL of venous blood was removed from the internal jugular catheter into a heparinized syringe, protected from light and placed on ice. Plasma FD4 was obtained by centrifuging 3,000 RPFs for 10 minutes at 4°C and compared with a standard curve measured on FLX800 Microplate Fluorescence reader (Biotek) at excitation 485/20, emission 528/20, and a sensitivity of 40.

Myeloperoxidase Assay

Myeloperoxidase (MPO) activity was measured in the pulmonary tissue as previously described.¹² MPO activity was calculated as the amount of enzyme that will reduce 1 μ mol peroxide per minute.

Statistical Analysis

All values were compared statistically with an analysis of variance and Tukey post test comparison with $p < 0.05$ as the cutoff for statistical significance.

RESULTS

Vagus Nerve Stimulation Protects the Lung from Increased Permeability in Hemorrhagic Shock

The results show that THS causes a threefold increase in lung permeability as compared with TSS values (mean [SD], 8.48 [0.36] vs. 2.82 [0.77]; $p < 0.05$). Vagus nerve stimulation (+VNS) protected the lung by decreasing permeability by half as compared with control values (4.87 [0.78] vs. 2.18 [0.6]) (Fig. 1)

Vagal Nerve Stimulation Decreases Neutrophil Sequestration in Pulmonary Tissue

Animals, as described previously, were subjected to THS. On completion of resuscitation, animals were killed, and pulmonary tissue was harvested. Tissue was subjugated to MPO assay and measured with a spectrophotometer. THS caused a significant increase in the MPO activity in the pulmonary tissue when compared with TSS values (19.39 [1.01] vs. 8.16 [1.67]; $p < 0.01$). Vagal nerve stimulation abrogated the response to hemorrhagic shock and decreased the MPO activity in lung tissue almost back to sham shock values (12.83 [1.16] units per gram tissue; $p > 0.05$) (Fig. 2).

Vagus Nerve Protects Against Gut Permeability in THS

After THS, gut permeability was measured with an in vivo plasma FD4 permeability assay. Gut permeability increased significantly in THS as compared with TSS values (4.0 [1.1] $\mu\text{g}/\text{mL}$ vs. 1.5 [0.73] $\mu\text{g}/\text{mL}$; $p < 0.001$). Vagal nerve stimulation reduced hemorrhagic shock-induced increases in gut permeability levels to TSS values (1.8 [0.8] $\mu\text{g}/\text{mL}$ vs. 1.2 [0.4] $\mu\text{g}/\text{mL}$; $p < 0.05$) (Fig. 3).

DISCUSSION

ARDS is a clinically common entity and an early event in the evolution of multiple-organ failure. Studies investigating the pathophysiology of trauma-induced ARDS and multiple-organ failure are a major area of investigation, owing to the high mortality they cause. Acute respiratory distress syndrome is a common consequence of pulmonary and systemic insults characterized by increased permeability and an early sequestration of neutrophils in the pulmonary vasculature.^{13,14} Once in the lung, inappropriately sequestered and activated neutrophils can secrete destructive enzymes and oxidant species, thereby contributing to lung injury after trauma and shock.¹⁵ Although acute lung injury and ARDS may resolve completely for patients after the acute phase, for others, it progresses to alveolitis with persistent hypoxemia and a decrease in pulmonary compliance.¹⁶ Because vagus nerve stimulation has been shown to modulate and abrogate inflammation, in this study, we tested the hypothesis that vagus nerve stimulation will prevent neutrophil sequestration and associated lung injury in the THS model.

The vagus nerve is the longest cranial nerve and innervates most of the peripheral organs. It can modulate the immune response, and it controls inflammation through a nicotinic anti-inflammatory pathway dependant on the $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7\text{nAChR}$).^{17,18} The vagus nerve represents a bidirectional communication between the brain and the immune system. In this paradigm, the immune system activates the afferent sensory fibers of the vagus nerve, which ascend to synapse in the brain. In return, the brain can activate the efferent vagal fibers and thereby modulate the peripheral immune system. Furthermore, earlier studies have shown that electrical or pharmacologic stimulation of the vagus nerve protects against lung injury in murine burn models, and this protection is associated with blunting of the cytokine response and prevention of neutrophil pulmonary sequestration.¹⁹ Vagal stimulation also has been shown to be protective in models of

septicemia²⁰ and pancreatitis;⁸ however, limited data are available in surgical trauma models. Our results add to this body of knowledge by showing that vagal nerve stimulation prevented THS-induced lung injury and neutrophil sequestration. The potential mechanisms by which vagus nerve stimulation protected the lung from THS-induced injury could have involved a direct protective lung effect or could have involved an indirect protective mechanism, such as by preventing gut injury. Due to the abundant evidence in multiple model systems that acute lung injury can be a consequence of gut injury,^{3,19,21} we also investigated and found that vagal nerve stimulation would protect the gut.

Because gut injury potentiates lung injury in conditions associated with splanchnic hypoperfusion, including hemorrhagic shock, it is possible that both gut and lung injuries were prevented by the beneficial effects of vagal stimulation on the gut.^{22,23} There are several potential mechanisms by which vagal nerve stimulation might have reduced THS-induced gut injury. One potential mechanism could involve a direct enterocyte protective mechanism.²⁴ This possibility is supported by a study showing that vagus nerve stimulation limited burn injury-induced gut injury as well as helped maintain tight junction integrity.²⁵ It also noted an association between the gut protective effects of vagal nerve stimulation and glial cell activation. Because intestinal glial cells are neuroimmune cells and have been documented to produce gut barrier-sustaining factors that improve tight junctions, the protective effects of vagal stimulation may involve glial activity.^{26,27} Because our recent studies have indicated that THS-induced loss of the unstirred mucus layer is a critical component of THS-induced gut injury,²⁸ another possible protective mechanism may involve the ability of the vagus nerve to increase mucus production.²⁹ It is clear from gastric mucus studies in vagotomized and vagally intact animals that efferent stimulation of the vagus nerve activates secretion of mucus glycoproteins, which protect against gastric ulceration.²⁹ It is, thus, reasonable to think that a similar mechanism may be present in the distal intestines where vagal nerve stimulation increases mucus production and/or secretion, thereby limiting injury. There are other potential interventional sites where vagus nerve stimulation may confer protective effects on gut injury, such as by preventing degranulation of mucosal mast cells,^{30,31} stimulating the acetylcholine receptors on immune cells and thereby preventing proinflammatory cytokine release,³² as well as by decreasing cellular ischemia.³³

In conclusion, stimulation of the vagus nerve offers a protective effect against the injuries that occur with THS. However, more research is needed to elucidate the mechanisms behind the beneficial effects of the vagal nerve stimulation.

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Figure 1.

Vagus nerve stimulation improves lung permeability. Adult Sprague-Dawley rats underwent trauma-hemorrhagic shock or TSS with or without vagus nerve stimulation. Lung permeability was measured as the amount of systemic Evans blue dye (*EBD*) that permeated through the lung parenchyma. * $p < 0.05$ between THS versus TSS and TSS + VNS ($n = 4$ per group).

Figure 2.

Vagus nerve stimulation prevents neutrophil sequestration in lung tissue. Adult rats underwent THS or sham shock with or without vagus nerve stimulation. Pulmonary tissue was harvested at the end of resuscitation period, and MPO activity was measured and calculated as units per gram lung tissue. * $p < 0.05$ between THS versus all groups ($n = 4$ per group).

Figure 3.

Vagus nerve stimulation protects gut from increased permeability. After THS and resuscitation, in vivo gut permeability was measured with FD4 comparing the circulating plasma levels to a standard curve. * $p < 0.05$ between THS versus all groups (n = 8 per group).