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Vagus nerve stimulation blocks vascular permeability following burn in both local and distal sites

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ABSTRACT

Recent studies have shown that vagus nerve stimulation (VNS) can block the burn-induced systemic inflammatory response (SIRS). In this study we examined the potential for VNS to modulate vascular permeability (VP) in local sites (i.e. skin) and in secondary sites (i.e. lung) following burn. In a 30% total body surface area burn model, VP was measured using intravascular fluorescent dextran for quantification of the VP response in skin and lung. A peak in VP of the skin was observed 24 h post-burn injury, that was blocked by VNS. Moreover, in the lung, VNS led to a reduction in burn-induced VP compared to sham-treated animals subjected to burn alone. The protective effects of VNS in this model were independent of the spleen, suggesting that the spleen was not a direct mediator of VNS. These studies identify a role for VNS in the regulation of VP in burns, with the translational potential of attenuating lung complications following burn.

1. Introduction

Burn-induced edema and changes in vascular permeability (VP) are a hallmark of severe thermal injury of the skin and secondary organs (i.e. lung, gut, and brain). While established resuscitation protocols exist for the fluid management of burn patients, there remains a significant unmet need to identify novel therapeutic approaches to alter early post-burn injury edema formation in tissues, and minimize the complications of early hypovolemia and massive fluid resuscitation [1–5]. In this study we focused on the VP component of burn-induced tissue edema, and the capacity for the parasympathetic nervous system (PNS) to suppress inflammatory responses in peripheral tissues following full thickness burn to the skin. Although skin is not directly innervated by the vagus nerve, the skin is in fact an important target for non-neural cholinergic anti-inflammatory signaling mediating epidermal innate immune and epithelial barrier function [6], consistent with a central role for the neuroendocrine regulation of immunosuppression [7]. For example, vagal nerve stimulation (VNS) can reduce inflammation through anti-cholinergic signaling [8,9] that reduces the expression of inflammatory cytokines and suppresses circulating immune cell responses [7,10]. The protective effect of VNS on systemic inflammation and secondary organ failure indicates that while the vagus nerve can mediate direct effects on the spleen [11], gut [12] and other organs, VNS can also have anti-inflammatory effects on distal organs [7–10,13–16]. Using a model of cutaneous burn, we show that anti-cholinergic signaling mediated by vagal nerve stimulation affects the vascular integrity (i.e. vascular permeability, VP) of blood vessels in the skin (i.e. margins of the primary injury site), as well as mediating a reduction in the VP of the lung (i.e. a secondary site). Suppressing burn-induced VP through anti-cholinergic signaling presents a novel strategy for the management of burn-related edema.

Significant burn initiates a complex physiologic response that leads to an increase in microvascular VP and an

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accumulation of large amounts of fluid in the cutaneous interstitium both locally at the site of burn and systemically, distal to the burn [17]. Alterations in VP after burns are thought to be mediated through a number of factors. For example, histamine, bradykinin, leukotrienes, substance P, and nitric oxide can all cause endothelial cell activation, which can lead to protein loss, oncotic/osmotic pressure changes and uncontrolled interstitial tissue edema [8–10]. Increased VP in burn models has been studied and established through modalities such as wet to dry ratios, radio-labeled iodine tracers, and Evan's blue dye (EBD) in efforts to identify possible therapeutic targets to prevent massive tissue edema and prevent the morbidity and mortality that ensues following burn [5,18–20]. Here, we use EBD as a tracer that binds to serum albumins to validate our animal model, then focus on the use of fluorescent dextran, which has a superior dynamic range and signal-to-noise, that can be imaged to localize changes in VP.

In the studies described here, we determined first whether VNS affects endothelial barrier integrity in skin adjacent to a skin injury site (i.e. burn margin) and reduces burn-induced VP in the area adjacent to the burn wound (i.e. central burn). We demonstrate that VNS blocks burn-induced VP 24 h post-burn injury (PBI). Burn-induced VP was assessed with a 70 kDa fluorescent dextran tracer to enable non-invasive serial and quantitative imaging of the full thickness burn region as well as a qualitative assessment of skin margins. Second, we examined the translational potential of the VNS-mediated protection of the burn-induced VP response on secondary organs (i.e. lung). We observed that the increase in lung VP following burn could be blocked by VNS, thus supporting the translational potential of VNS in mediating lung edema in this SIRS model.

2. Methods

2.1. Thermal injury model

Male balb/c mice weighing 24–28 g were obtained from Jackson Laboratories (Sacramento, CA). Animals underwent dorsal and ventral fur clipping with an electric clipper, were anesthetized with inhaled isoflurane, and subjected to a 30% total body surface area (TBSA) dorsal steam burn for 7 s using a template designed to estimate 30% TBSA [16]. All animal groups received a subcutaneous injection of normal saline (1 mL) and buprenorphine (100 μL of 12.6 μg/mL) in a non-burned area for fluid resuscitation and pain control. All animal experiments were approved by the University of California San Diego Institutional Animal Care and Use Committee.

2.2. Vagal nerve stimulation

A right cervical neck incision followed by right cervical VNS was performed on select animals immediately prior to the thermal insult. Intermittent stimulation of the vagus nerve was performed using a VariStim III probe (Medtronic Xomed, Jacksonville, FL) at 2 mA for 10 min. Following nerve stimulation, the incision was closed with a running 4-0 silk suture. Sham animals underwent right cervical incision and exposure of the vagus nerve but did not receive electrical stimulation.

2.3. Tissue harvest

Animals were sacrificed at 6 and 24 h following injury and tissues were harvested. Four 8 mm full thickness skin biopsies were obtained from three sites: (1) the central burn zone, (2) the burn margin adjacent to the central burn, and remote zone (chest and abdomen). Skin biopsies were fixed in 10% buffered formalin (Richard Allan Scientific, Pittsburgh, PA) for histologic evaluation and immunohistochemistry.

2.4. Splenectomy

A cohort of animals underwent splenectomy immediately prior to VNS and thermal insult. Prior to the splenectomy, abdomens were shaved and sterilized with 10% betadine and the spleen removed through a small upper midline incision. The vascular pedicles were identified, ligated with 2-0 silk, and the abdomen was closed in one layer with a running 4-0 silk suture.

2.5. Histologic evaluation

Full thickness 8 mm skin biopsies were fixed in 10% buffered formalin, embedded in paraffin, and sectioned. UCSD Histology Core Services performed hematoxylin and eosin staining of skin biopsies. Sections were viewed via light microscopy and the degree of burn evaluated by a pathologist.

2.6. Vascular permeability assay with Evan's blue dye (EBD)

To determine the amount of EBD in the experimental area, 150 μL of a 133 mg/ml EBD solution deionized water was administered through tail vein injection at either 6 h or 24 h following thermal injury. EBD-injected mice were maintained for 30 min and subjected to sacrifice. Two 8 mm full-thickness skin biopsies were excised from: (1) the central burn; (2) the burn margin; and (3) the distal zone of each animal group. A similar procedure and time schedule was used for all animals. Each sample was placed in 500 μL of formamide and incubated for 24 h in a heat plate at 65 °C to extract EBD from the tissue. The amount of tissue EBD was quantified by a spectrophotometer at an absorption of 650 nm [18,21,22]. Three measurements were performed on each sample and the mean value was used for calculations.

2.7. Vascular permeability assay with 70 kDa FITC dextran

Mice were subjected to a tail vein injection with 150 μL (50 mg/ml in PBS) 70 kDa FITC dextran (FD70) (Sigma, St. Louis, MO) and sacrificed 30 min later. Four 8 mm skin biopsies from the central burn, burn margin, and remote skin were obtained. Similarly, lung samples were collected and imaged ex vivo. Skin and lung biopsies were imaged to measure VP to FD70 with a deep cooled CCD imaging system equipped with appropriate fluorescence filter cubes with background subtraction. Images were analyzed using Living Image software version 4.0 (IVIS Spectrum, Caliper Life Sciences, Hopkinton, MA).