Vagus Nerve Mediates the Neural Stem Cell Response to Intestinal Injury

Simone Langness, MD, Raul Coimbra, MD, PhD, FACS, Brian P Eliceiri, PhD, Todd W Costantini, MD, FACS

BACKGROUND: Intestinal ischemia and reperfusion injury results in damage to elements critical to maintaining intestinal barrier function, including neurons and glia cells, which are part of the enteric nervous system (ENS). To limit inflammation, the ENS must be restored or replaced, yet the process by which this occurs is poorly understood. Multipotent progenitor cells called enteric nervous stem cells (ENSC) can differentiate into neurons or glia when stimulated. The ability of this cell population to respond to intestinal injury is unknown. In this study, we hypothesized that resolution of intestinal barrier injury would be associated with vagus nerve-mediated expansion of ENSCs.

STUDY DESIGN: Ischemia and reperfusion injury was reproduced in male mice by occluding the superior mesenteric artery for 30 minutes. Abdominal vagotomy was performed in a separate cohort to study the effects of the vagus nerve. Terminal ileum was harvested at various time points after reperfusion and analyzed with histology, flow cytometry, and immunohistochemistry.

RESULTS: Enteric nervous stem cell expansion occurs at 2, 4, and 8 hours after injury compared with sham (4.6% vs 2.1%; $p < 0.001$) and correlated with increased glial fibrillary acidic protein on immunohistochemistry. Vagotomy prevented both ENSC expansion and increased glial fibrillary acidic protein staining after injury. Intestinal permeability was restored to baseline by 48 hours after injury, but remained elevated in the vagotomy group compared with sham and injury alone at 48 hours (3.25 mg/mL vs 0.57 mg/mL and 0.26 mg/mL, respectively; $p < 0.05$).

CONCLUSIONS: Vagal-mediated expansion of ENSCs occurs after ischemia and reperfusion injury and results in improved kinetics of injury resolution. (J Am Coll Surg 2015;221:871−879. © 2015 by the American College of Surgeons)

Ischemia and reperfusion (IR) injury of the intestine can occur after hypotension due to hemorrhagic shock, or in diseases such as mesenteric occlusive disease and necrotizing enterocolitis.[1-3] Inadequate splanchnic blood flow and ensuing local hypoxia result in microvascular injury, production of cytotoxic molecules, and cellular necrosis or apoptosis.[4,5] Several intestinal cell types are vulnerable to damage during IR injury, including epithelial cells[6] and neurons and glial cells, which are part of the enteric nervous system (ENS).[7,8] These structures are critical to maintaining the integrity of intestinal barrier function and this essential role is compromised after IR injury.

Once intestinal barrier integrity has been disrupted, contents that are normally confined to the lumen of the intestine (ie, commensal bacteria, food antigens, proteases, and bacterial products) have the potential to translocate into the gut mucosa and generate a local inflammatory response.[9] This local inflammatory response within the gut mucosa can result in a systemic inflammatory response with deleterious effects on distant organs if the epithelial barrier function is not repaired quickly.[10,11] Therefore, injured cells within the gut epithelium and ENS must be restored rapidly after injury to limit

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From the Division of Trauma, Surgical Critical Care, Burns and Acute Care Surgery, Department of Surgery, University of California, San Diego Health Sciences, San Diego, CA.

Correspondence address: Todd W Costantini, MD, FACS, Division of Trauma, Surgical Critical Care, Burns and Acute Care Surgery, Department of Surgery, University of California, San Diego Health Sciences, 200 W Arbor Dr, #8896, San Diego, CA 92103-8896. email: tcostantini@ucsd.edu

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Abbreviations and Acronyms

BSA = bovine serum albumin
ENS = enteric nervous system
ENSC = enteric neural stem cell
GFAP = glial fibrillary acidic protein
IR = ischemia and reperfusion
Lin = lineage
PGP = protein gene product

persistent gut inflammation and to resume normal gut barrier function.

Although epithelial barrier recovery after injury or inflammation has been studied extensively, the ability of the ENS to regenerate after injury has been a source of debate, as it was believed that all neuronal development was established at birth. However, recent identification of human and murine enteric neural stem cells (ENSCs) that persist into adulthood has challenged this dogma.[12-14] Enteric neural stem cells can be harvested and cultured from fetal and adult intestines to differentiate into neurons and glial in vitro when cultured with neural stem cell proliferation media.[15,16] In addition, isolated ENSCs can be implanted into an aganglionic segment of gut to form complex neuronal networks with phenotypic markers characteristic of the ENS.[17-19]

In this study, we investigated the fate of ENSCs after IR injury. We hypothesized that an expansion of the ENSC population after injury would occur for the ENS to regenerate and ultimately restore gut barrier function. We further hypothesized that this expansion would be a vagal-mediated process, given our earlier work demonstrating the link between the vagus nerve and the ENS.[20]

METHODS

Animal model of intestinal ischemia and reperfusion injury

Male C57BL/6 mice 8 to 10 weeks old (Jackson Laboratories) were placed under general anesthesia using inhaled isoflurane. A ventral incision area was shaved with an electrical clipper and cleansed with Betadine solution. The mice were secured onto a heating pad to maintain appropriate body temperature during anesthesia. A midline laparotomy was performed with subsequent isolation and occlusion of the superior mesenteric artery with an atraumatic vascular clamp (Fine Science Tools) for 30 minutes.[21] Animals were given a subcutaneous injection of 1.5 mL normal saline with buprenorphine for analgesia after injury. The abdomen was closed after reperfusion of the intestine and the animals recovered with the assistance of a heating pad. The animals were given free access to food and water. Sham animals underwent midline laparotomy without occlusion of the superior mesenteric artery. Animal experiments were approved by the University of California animal research committee.

Abdominal vagotomy

Abdominal vagotomy was performed in a separate cohort of animals by dividing the dorsal and ventral vagus nerves

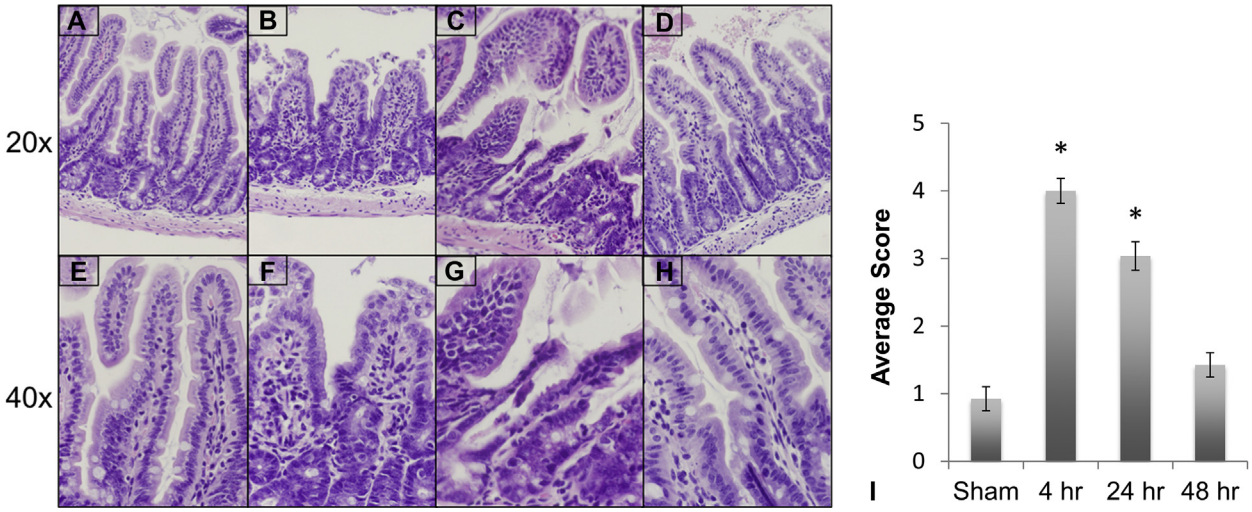

Figure 1. Histologic evaluation of intestinal ischemic injury after superior mesenteric artery occlusion. Terminal ileum sections were harvested and then stained with hematoxylin and eosin. Representative sections of the gut at low (×20) and high magnification (×40) are from sham animals (A, E); animals subjected to 30 minutes of superior mesenteric artery occlusion followed by 4 hours of reperfusion (B, F); 24 hours of reperfusion (C, G); and 48 hours of reperfusion (D, H). (I) Gut histology scores (±SEM) were significantly higher 4 hours and 24 hours after reperfusion when compared with sham. *$p < 0.05$ vs sham.