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Umbilical mesenchymal stromal cells provide intestinal protection through nitric oxide dependent pathways

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ABSTRACT

Background: Umbilical-derived mesenchymal stromal cells (USCs) have shown promise in the protection of ischemic organs. We hypothesized that USCs would improve mesenteric perfusion, preserve intestinal histological architecture, and limit inflammation by nitric oxide–dependent mechanisms following intestinal ischemia/reperfusion (IR) injury.

Methods: Adult wild-type C57BL/6J (WT) and endothelial nitric oxide synthase knock out (eNOS KO) mice were used: (1) WT IR + vehicle, (2) WT IR + USC, (3) eNOS KO IR + vehicle, and (4) eNOS KO IR + USC. Mice were anesthetized, and a midline laparotomy was performed. The superior mesenteric artery was clamped with a nonoccluding clamp for 60-min. Following IR, mice were treated with an injection of 250 μL phosphate buffered saline or 2×10^6 USCs suspended in 250-μL phosphate buffered saline solution. Mesenteric perfusion images were acquired using laser Doppler imaging. Perfusion was analyzed as a percentage of baseline. At 24 h, mice were euthanized, and intestines were harvested. Intestines were evaluated for injury, and data were analyzed using the Mann–Whitney or Kruskal–Wallis tests.

Results: Intestinal mesenteric perfusion was significantly improved in WT mice treated with USC therapy compared with eNOS KOs. Intestinal histological architecture was preserved with USC therapy in WT mice. However, in eNOS KO mice, this benefit was abolished. Finally, the presence of several cytokines and growth factors were significantly improved in WT mice compared with eNOS KO mice treated with USCs.

Conclusions: The benefits of USC-mediated therapy following intestinal IR injury likely occur via nitric oxide–dependent pathways. Further studies are required to define the molecular mechanisms by which USCs activate endothelial nitric oxide synthase to bring about their protective effects.

Introduction

Acute mesenteric ischemia (AMI) continues to be a devastating intra-abdominal emergency with mortality as high as 60%-80% and accounts for about 0.1% of all hospital admissions.[1,2] It is caused by (1) a sudden acute arterial occlusion, (2) a venous occlusion, or (3) a sudden drop in circulating pressure. During hypoperfusion, this insufficient blood flow

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within the mesenteric circulation is unable to meet intestinal metabolic demands, and often, this leads to mesenteric infarction and intestinal necrosis. Patients who remain untreated can quickly decompensate and progress to shock, multi-system organ failure, and death.[3] The most critical factor that continues to impact outcomes in patients is the time to diagnosis and intervention.

If patients survive these ischemic episodes, they are often faced with prolonged hospitalization and long-term parenteral nutrition needs.[4] In addition, reported overall survival at 1, 3, and 5 years following surgery for AMI has been found to be 26%, 23%, and 21% respectively.[5] Early diagnosis and aggressive therapy may significantly reduce the morbidity and mortality of this life-threatening disease. Although clinical studies emphasize diagnostic and therapeutic algorithms to expedite treatment for the diagnosis of AMI, the disease prognosis remains dismal. To that end, noteworthy advancements in the medical treatment of intestinal ischemia within the last decade have been sparse, and a novel therapeutic option is of the utmost importance.

Recent animal studies have demonstrated the ability to reverse ischemic injury and promote recovery of intestinal tissue with the use of mesenchymal stromal cells (MSCs) following ischemia-reperfusion (IR) injury.[3,6-8] Umbilical-derived mesenchymal stromal cells (USCs) are pluripotent, immunomodulatory, and reduce inflammation.[9] They are easily isolated from neonates and obtained from discarded tissue. Of all the types of MSCs (adipose-derived, bone marrow-derived, and so forth), they are the least senescent and have the lowest expression of major histocompatibility complex II, which may enhance cell survival during transplant.[9]

MSCs originally were thought to allow for tissue repair through transdifferentiation or cell fusion. However, several studies have now demonstrated that functional benefits observed with the use of MSCs following injury are most likely related to the secretion of bioactive mediators acting in a paracrine fashion on the affected tissue.[10] Many of these factors include epidermal growth factor, insulin-like growth factor, transforming growth factor beta 1, hepatocyte growth factor (HGF), interleukin 6 (IL-6), interleukin 10 (IL-10), and vascular endothelial growth factor (VEGF).[11,12] Although all of these paracrine mediators have been found to contribute to improved tissue repair and angiogenesis following ischemic injury, it is still unclear how they promote improved mesenteric perfusion following injury.

In this regard, nitric oxide (NO) could be an important downstream contributor to USC-mediated improvements in mesenteric perfusion. NO is known to be a potent vasodilator through relaxation of vascular smooth muscle cells. In the setting of intestinal IR injury, studies have found that endogenous NO production increases, most likely to promote improved perfusion to ischemic organs.[13,14] Within the microvascular endothelial bed, NO is produced by endothelial nitric oxide synthase (eNOS). Therefore, it is possible that USCs may work through eNOS pathways to bring about their protective effects. We hypothesized that: (1) USCs would improve mesenteric perfusion, preserve intestinal histological architecture, and limit intestinal inflammation following intestinal IR injury and (2) the benefits of USC therapy would be mediated through eNOS-dependent pathways.

Materials and methods

Animals

Wild-type adult male mice (C57BL/6J, 8-12 weeks; Jackson Laboratory, Bar Harbor, ME) and endothelial nitric oxide synthase knock out (eNOS KO) mice on a C57BL/6J background (B6.129P2-Nos3tm1Unc/J, 8-12 weeks; Jackson Laboratory, Bar Harbor, ME) were used for all animal experimentation. Animals underwent at least 48 h of acclimation to the new environment prior to experimentation. Twelve hour light/dark cycle housing and normal chow and water were provided to all animals. All experimental protocols were approved by the Institutional Animal Care and Use Committee at Indiana University.

Cell culture

The human USCs used for experimentation were purchased from ATCC (Manassas, VA). Cells were positive for CD29, CD44, CD73, CD90, CD105, and CD166 and negative for CD14, CD31, CD34, and CD45.[15] Cells were cultured in 225 cm^2 polystyrene culture flasks in Mesenchymal Stem Cell Basal medium with Mesenchymal Stem Cell Growth Kit—Low Serum (ATCC, Manassas, VA) at 37°C in a humidified atmosphere of 5% CO_2 in air. Once cells reached 90% confluency they were lifted from the flask with TrypLE Express (Life Technologies) and passaged to expand primary cultures or used in experimentation. USCs were used between passages 4-9. A fluorescent automated cell counter was used to count cells (Luna Automated Cell Counter, Logos Biosystems Inc, Annandale, VA).

Ischemia/reperfusion model

For all experimental groups, mice were anesthetized using 3% isoflurane followed by maintenance at 1.5% isoflurane in oxygen. Temperature homeostasis was maintained intraoperatively through the use of an animal heating pad. All murine abdomens were prepped using hair removal lotion followed by sterile preparation with 70% ethanol and betadine. Prior to surgery, all animals were subcutaneously injected with 1 mL of 0.9% normal saline solution to account for intraoperative fluid loss. Perioperative analgesia included 1 mg/kg buprenorphine and 5 mg/kg carprofen administered by subcutaneous injection.

Using a previously described sterile technique, a midline laparotomy was performed, and the intestines were eviscerated. Using an atraumatic microvascular clamp, the superior mesenteric artery was clamped to cause intestinal ischemia. The intestines were then returned to the abdominal cavity, and the abdomen was closed temporarily for a total of 60 min to prevent evaporative losses. Following the ischemic period, the abdominal cavity was reopened, and the atraumatic