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Harvest tissue source does not alter the protective power of stromal cell therapy after intestinal ischemia and reperfusion injury

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ABSTRACT

Background: Transplantation of mesenchymal stromal cells (MSCs) may be a novel treatment for intestinal ischemia. The optimal stromal cell source that could yield maximal protection after injury, however, has not been identified. We hypothesized that (1) MSCs would increase survival and mesenteric perfusion, preserve intestinal histologic architecture, and limit inflammation after intestinal ischemia and reperfusion (I/R) injury, and (2) MSCs harvested from different sources of tissue would have equivalent protective properties to the intestine after I/R inury.

Methods: Adult male mice were anesthetized, and a midline laparotomy was performed. The intestines were eviscerated, the small bowel mesenteric root was identified, and baseline intestinal perfusion was determined using laser Doppler imaging. Intestinal ischemia was established by temporarily occluding the superior mesenteric artery for 60 min with a noncrushing clamp. After ischemia, the clamp was removed and the intestines were allowed to recover. Before abdominal closure, 2×10^6 human umbilical cord–derived MSCs, bone marrow–derived MSCs, or keratinocytes in 250 μL of phosphate-buffered saline vehicle were injected into the peritoneum. Animals were allowed to recover for 12 or 24 h (perfusion, histology, and inflammatory studies) or 7 d (survival studies). Survival data was analyzed using the log-rank test. Perfusion was expressed as a percentage of the baseline, and 12- and 24-h data was analyzed using one-way analysis of variance and the Student *t*-test. Nonparametric data was compared using the Mann–Whitney *U*-test. A *P* value of <0.05 was considered statistically significant.

Results: All MSCs increased 7-d survival after I/R injury and were superior to vehicle and keratinocytes ($P < 0.05$). All MSCs increased mesenteric perfusion more than vehicle at 12 and 24 h after injury ($P < 0.05$). All MSCs provided superior perfusion compared with keratinocytes at 24 h after injury ($P < 0.05$). Administration of each MSC line improved intestinal histology after I/R injury ($P < 0.05$). Multiple proinflammatory chemokines were downregulated after the application of MSCs, suggesting a decreased inflammatory response after MSC therapy.

Conclusions: Transplantation of MSCs after intestinal I/R injury, irrespective of a tissue source, significantly increases survival and mesenteric perfusion and at the same time

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limits intestinal damage and inflammation. Further studies are needed to identify the mechanism that these cells use to promote improved outcomes after injury.

Introduction

Acute intestinal ischemia continues to be a life-threatening medical emergency with high morbidity and mortality.[1] Despite the recent therapeutic advances in endovascular techniques for early revascularization, mortality continues to be 30%-68%.[2-4] During intestinal ischemia, the sudden decrease in intestinal blood flow may cause bowel infarction, and, if not discovered, it may rapidly progress to irreversible bowel necrosis, sepsis, multiple organ dysfunction, and ultimately death.[1,5] Of the patients who survive after surgical resection, many are left with short bowel syndrome and consequently require long-term total parenteral alimentation or small bowel transplantation.

Early revascularization continues to be the optimal treatment to salvage the intestine. To date, however, there have been no innovative treatment modalities aimed at recovering the infarcted bowel. Recent studies in the literature have demonstrated reversal of ischemia and reperfusion (I/R) injury and recovery of bowel function with use of bone marrow–derived mesenchymal stromal cells (BMSCs) after I/R injury in animal models.[6-10] BMSCs have also been shown to decrease the inflammatory response through the down-regulation of inflammatory chemokines[11,12] and mitigation of oxidative stress.[13]

Mesenchymal stromal cells (MSCs) are pluripotent, immunomodulatory, proliferative progenitor cells that contribute to tissue repair and regeneration, possibly through the paracrine release of trophic growth factors such as interleukin 6 (IL-6), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), or insulin-like growth factor I (IGF-I).[14-17] They are mobilized in response to tissue injury with the potential to differentiate into adipogenic, osteogenic, chondrogenic, and myogenic cells.[16,18] MSCs are immunogenic, exhibit antioxidant properties,[19] enhance neovascularization,[20] reduce inflammation,[21] and improve functional recovery of ischemic tissues.[22] BMSCs have been shown to ameliorate the destructive effects seen during intestinal ischemia by decreasing intestinal permeability, villus injury, apoptosis, inflammation, and also by promoting recovery of the gut–mucosal barrier after injury.[10,23]

Before stromal cell therapy is used in the clinical setting, the optimal donor source for harvesting these cells must be identified. Although MSCs from different tissue sources have been shown to be similar in morphology and function, subtle differences may exist in their immunomodulatory profile, which may be attributable to microenvironmental niche, method of harvest, or ontogenic age.[24-26] These differences may play a role in MSC reparative properties, and conflicting studies exist.[27-29] We therefore hypothesized that (1) MSCs would increase survival and mesenteric perfusion, preserve intestinal histologic architecture, and limit intestinal inflammation after I/R injury, and (2) MSCs harvested from different tissue sources would have equivalent protective properties to the intestine after I/R injury.

Materials and methods

Cell culture

Three different human cell lines (BMSCs, umbilical cord–derived mesenchymal stromal cells [USCs], and keratinocytes) were used in our experiments. All cell lines were cultured in 225 cm^2 polystyrene culture flasks 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, Grand Island, NY) and passaged to expand primary cultures or used in experimentation. All MSC lines were used between passages 4 and 9 and keratinocytes were used between passages 30 and 35. A fluorescent automated cell counter was used to count cells (Luna Automated Cell Counter; Logos Biosystems Inc, Annandale, VA).

Human BMSCs were obtained from Dr Darwin Prokop at Texas A&M University. His laboratory receives funding from National Institutes of Health to procure, purify, and verify BMSCs from human subjects. All BMSCs were reported to meet MSC defining criteria.[30] BMSCs were cultured in Alpha-Minimum Essential Medium (Life Technologies) with 16% fetal bovine serum (Atlanta Biologicals, Flowery Branch, GA), 1% glutamine (Sigma, St. Louis, MO), and 1% penicillin-streptomycin (MP Biomedicals, Solon, OH).

Human USCs 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. Cells were cultured in Mesenchymal Stem Cell Basal Medium with Mesenchymal Stem Cell Growth Kit–Low Serum (ATCC).

Human keratinocytes (immortalized by overexpression of human telemorase reverse transcriptase) were obtained from Dr Jeffery Travers at Indiana University School of Medicine. These cells were originally purchased from ATCC. Cells were cultured in EpiLife medium with the addition of keratinocyte growth factor (Life Technologies).

In vitro *cell stimulation*

Cells used for *in vitro* experiments were lifted from their flasks using TrypLE Express (Life Technologies). Cells were then pelleted at 400 × *g* for 5 min and resuspended in their respective medias. Cells were counted using an automated fluorescent cell counter (Luna Automated Cell Counter; Logos Biosystems Inc). One hundred thousand keratinocytes, BMSCs, or USCs were plated into each well of a 12-well plate and allowed to adhere to the plastic overnight. Media were changed the following day and cells were exposed to one of two noxious stimuli for 24 h: (1) tumor necrosis factor 50