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Harvest Tissue Source Does Not Alter the Protective Power of Stromal Cell Therapy Following 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 following injury, however, has not been identified. We hypothesized that: 1) MSCs would increase survival and mesenteric perfusion, preserve intestinal histological architecture, and limit inflammation following intestinal ischemia and reperfusion injury (I/R), and 2) MSCs harvested from different sources of tissue would have equivalent protective properties to the intestine following I/R.

Methods—Adult male mice were anesthetized and a midline laparotomy performed. The intestines were eviscerated, the small bowel mesenteric root identified, and baseline intestinal perfusion was determined using Laser Doppler Imaging (LDI). Intestinal ischemia was established by temporarily occluding the superior mesenteric artery for 60 minutes with a non-crushing clamp. Following ischemia, the clamp was removed and the intestines were allowed to recover. Prior to abdominal closure, 2×10^6 human umbilical (USCs), bone-marrow (BMSCs) derived MSCs, or keratinocytes in 250μl of phosphate-buffered saline (PBS) vehicle were injected into the peritoneum. Animals were allowed to recover for 12 or 24 hours (perfusion, histology, inflammatory studies), or 7 days (survival studies). Survival data was analyzed using log rank test. Perfusion was expressed as percentage of baseline and 12 and 24 hour data was analyzed using

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one way ANOVA and student's t-test. Non parametric data was compared using Mann-Whitney-U test. A p-value of less than 0.05 was significant.

Results—All MSCs increased seven day survival following I/R and were superior to vehicle or keratinocytes ($P<0.05$). All MSCs increased mesenteric perfusion above vehicle at 12 and 24 hours following injury ($P<0.05$). All MSCs provided superior perfusion compared to keratinocytes at 24 hours post-injury ($P<0.05$). Administration of each MSC line improved intestinal histology after I/R ($P<0.05$). Multiple pro-inflammatory chemokines were down-regulated following application of MSCs suggesting a decreased inflammatory response following MSC therapy.

Conclusion—Transplantation of MSCs following intestinal I/R, irrespective of source tissue, significantly increases survival and mesenteric perfusion while limiting intestinal damage and inflammation. Further studies are needed to identify the mechanism that these cells utilize to promote improved outcomes following injury.

Keywords

Intestinal ischemia; perfusion; human umbilical mesenchymal stromal cells; human bone-marrow mesenchymal stromal cells; survival; mortality; inflammation

INTRODUCTION

Acute intestinal ischemia continues to be a life-threatening medical emergency with high morbidity and mortality [1]. Despite the recent therapeutic advances of endovascular techniques for early revascularization, mortality continues to be 30–68% [2] [3] [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 following 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-reperfusion (I/R) injury and recovery of bowel function with use of bone marrow-derived mesenchymal stromal cells (BMSCs) following I/R injury in animal models [6] [7] [8] [9] [10]. BMSCs have also been shown to decrease the inflammatory response through 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 (IGF) [14, 15] [16, 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 following injury [10, 23].

Prior to stromal cell therapy being 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 histological architecture, and limit intestinal inflammation following intestinal ischemia and reperfusion injury (I/R), and 2) MSCs harvested from different source tissues would have equivalent protective properties to the intestine following I/R.

MATERIALS AND METHODS

Cell Culture

Three different human cell lines (bone marrow derived mesenchymal stromal cells-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), and passaged to expand primary cultures or used in experimentation. All MSC lines were used between passages 4–9 and keratinocytes were used between passages 30–35. 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 lab is NIH funded to procure, purify, and verify BMSCs from human subjects. All BMSCs were reported to meet MSC defining criteria [30]. BMSCs were cultured in Alpha-MEM (Life Technologies, Grand Island, NY) with 16% Fetal Bovine Serum (FBS; 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, Manassas, VA).

Human nTERT keratinocytes were obtained from Dr. Jeffery Travers at Indiana University School of Medicine. These cells were originally purchased from ATCC (Manassas, VA). Cells were cultured in EpiLife Medium with the addition of Keratinocyte Growth Factor (Life Technologies, Grand Island, NY).

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 minutes and re-suspended in their respective medias. Cells were counted using an automated fluorescent cell counter (Luna™ Automated Cell Counter, Logos Biosystems Inc., Annandale, VA). 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 was changed the following day and cells were exposed to one of two noxious stimuli for 24 hours: 1) Tumor Necrosis Factor (TNF) 50 ng/ml, or 2) lipopolysaccharides (LPS) 200 ng/ml. After 24 hours of exposure, supernatants were collected and stored at −20°C.

Assessment of Stromal Cell Paracrine Factors

Cytokines and growth factors produced by stromal cells in culture were quantified using enzyme-linked immunosorbent assay (ELISA) kits specific for human IL-6, vascular endothelia growth factor (VEGF), endothelial growth factor (EGF) and insulin-like growth factor I (IGF-I; R&D Systems, Minneapolis, MN, USA). Assays were performed according to the manufacturer's instructions and diluted if necessary using the dilution buffer provided with the ELISA kits (n=8). Experiments were repeated to ensure accuracy.

Murine Intestinal I/R Model

The experimental protocol and animal use were previously approved by the Indiana University Institutional Animal Care and Use Committee. Wild-type adult male C57BL/6J mice (8–12 weeks, 20–30g; Jackson Laboratory, Bar Harbor, ME) were allowed 48 hours of acclimation to the new environment prior to experiment. They had access to normal chow and were kept in 12 hour light-dark cycle housing.

For surgery all mice were anesthetized with 3% isoflurane and maintained at 1.5% isoflurane intraoperatively. A heating pad was used for murine temperature homeostasis. The abdomen was prepped using a hair removal lotion followed by 70% ethanol and betadine. To account for intra-operative fluid losses one milliliter of 0.9% normal saline was injected subcutaneously prior to surgery.

A midline laparotomy was then performed and the intestines were eviscerated. The root of the superior mesenteric artery was identified and temporary arterial occlusion was accomplished with use of an atraumatic non-crushing microvascular clamp. Ischemia duration was 60 minutes. During ischemia the abdomen was temporary closed using silk suture to reduce evaporative losses. Following ischemia, the abdomen was reopened and the clamp was removed. The abdominal fascia and skin were then closed in a two layer fashion using silk suture. Prior to complete abdominal closure, 250μL PBS vehicle, or 2×10^6 of BMSCs, USCs, or keratinocytes re-suspended in 250μL of PBS were injected into the intraperitoneal cavity based on our previously reported dose response curves [6]. Triple antibiotic ointment was applied to the abdominal incision following complete closure andanalgesia (1mg/kg buprenorphine and 5 mg/kg caprofen) was injected subcutaneously. Murine animals were then allowed to awaken from anesthesia and underwent recovery within a cage placed on a heating pad. Once they recovered they were returned to animal

housing. All animal were monitored post-operatively for signs or symptoms of pain every 12 hours and did not require additional analgesia. Animals that underwent 12 and 24 hour reperfusion studies were re-anesthetized at these time points and the midline incision was reopened. At the conclusion of these studies, animals were euthanized with isoflurane overdose and cervical dislocation.

Survival Analysis

Animals assigned to the survival protocol (N=10 for each group) were treated with: I/R + PBS vehicle, I/R + keratinocytes, I/R + BMSCs, or I/R + USCs. They were monitored twice daily for 7 days following surgery for death, pain, and incisional complications. End points of analysis included animal death or when Laboratory Animal Resource Center veterinarians felt animals were suffering and needed to be euthanized. Survival curves were created based on these end points. Remaining mice were euthanized with isoflurane overdose and cervical dislocation at completion of the 7 day designated time course.

Perfusion Analysis

After the midline incision was made and the bowels were eviscerated, baseline intestinal perfusion was assessed using a scanning laser-Doppler perfusion imager (LDI; Moor Instruments, Wilmington, DE). Three LDI images were taken for each mouse at each time point. Once the images were captured, the region of interest (ROI) was assessed. The boundaries of the ROI were drawn around the entirety of exposed intestines to obtain perfusion within this region. An average perfusion value was calculated based on the mean of all three images. Perfusion images were obtained at baseline, ischemia, and at 12 and 24 hours of recovery (N=6–7 per group, respectively). Perfusion data was expressed as a percentage of baseline (Mean +/−SEM). Animals that died prior to 12 or 24 hour re-perfusion analysis were arbitrarily assigned a perfusion value of zero.

Histology Injury Score

Following euthanasia of experimental groups, terminal ileums were harvested and fixed in 4% paraformaldehyde with subsequent dehydration in 70% ethanol. Paraffin-embedded sections were prepared and stained with hematoxylin and eosin. Histological scoring of the depth of tissue injury was performed as previously described: 0, no damage; 1, subepithelial space at the villous tip; 2, loss of mucosal lining of the villous tip; 3, loss of less than half of the villous structure; 4, loss of more than half of the villous structure; and 5 transmural necrosis [6, 8]. Sections were evaluated blindly by two observers with all scores averaged.

Intestinal Cytokine Analysis

Following euthanasia of experimental groups, mouse intestinal tissues were harvested and snap frozen in liquid nitrogen and stored at −80°C. Once ready for use, intestinal tissue samples were thawed and homogenized in RIPA buffer (Sigma, St. Louis, MO) with protease and phosphatase cocktail inhibitors (1:100 dilution; Sigma, St. Louis, MO) using a Bullet Blender tissue homogenizer (Next Advance, Averill Park, NY). Homogenates were centrifuged at 12,000 rpm to pellet extraneous tissue and supernatants were transferred to fresh Eppendorf tubes for storage at −80°C. Total protein concentration was quantified by

Bradford assay using a spectrophotometer (VersaMax microplate reader; Molecular Devices, Sunnyvale, CA). Intestinal levels of murine interleukin-6 (IL-6), macrophage inflammatory protein 1 alpha (MIP-1α), macrophage inflammatory protein 2 alpha (MIP-2α), and interferon-γ-induced protein 10 (IP-10) were quantified with a Bio-Plex 200 multiplex beaded assay system (Bio-Rad, Hercules, CA) using customizable multiplex plates for murine inflammatory cytokines (Millipore, Billerica, MA). Assays were performed at 1:25 dilution according to the manufacturer's instructions and are reported as nanograms cytokine per gram of total intestinal protein (mean +/− SEM).

Statistical Analysis

Results are expressed as mean ± SEM. Statistical analyses were performed using SPSS 23 software (SPSS, Chicago, IL). Statistical significance for the survival studies was assessed by the Mantel-Cox log rank test and the Gehan-Breslow-Wilcoxon test. Comparison of perfusion at 12 and 24 hours re-perfusion was assessed using one-way ANOVA and student's t-test. Histology and cytokine analysis was performed using the Mann-Whitney U test for nonparametric variables. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Stromal Cells Produce Varying Amounts of Paracrine Growth Factors

BMSCs and USCs produced higher levels of IL-6 at baseline and with LPS and TNF stimulation compared to keratinocytes (Figure 1A). USCs also produced significantly more IL-6 compared to BMSCs. BMSCs produced the highest levels of VEGF, while all USC groups had the lowest production of VEGF(Figure 1B). EGF production was only significantly elevated in USC groups while keratinocytes produced significantly more IGF than both BMSC and USC(Figure 1C,D).

MSCs Increase Survival Following Intestinal Ischemia and Reperfusion Injury

Survival was significantly improved in mice that received MSCs following I/R injury compared to mice that received PBS or the differentiated cellular control (keratinocytes) (Figure 2). In BMSC and USC treated groups, seven day survival was ninety percent. Survival was significantly lower in PBS treated animals (40%, $p<0.05$) and keratinocyte treated animals (20%, $p<0.05$). There was no statistically significant survival advantage observed between mesenchymal stromal cell lines. These data indicate that MSCs significantly improve survival following intestinal I/R and that survival is not significantly impacted by stromal cell tissue source.

MSCs significantly increase intestinal perfusion following I/R injury

Intestinal perfusion was compared in all four treatment groups at 12 and 24 hours after I/R by using LDI (Figure 3A). At 12 hours of reperfusion, PBS treated groups had significantly lower perfusion levels (21.65±10.2%) compared to BMSCs (76.79±4.88%, $p=0.001$) and USCs (95.93±5.35%,$p<0.001$, Figure 3B). There was no difference between keratinocyte and PBS treated groups. BMSC and USC treated groups maintained significantly higher levels of perfusion at 12 hours compared to keratinocytes ($p<0.05$).

After 24 hours of reperfusion, all MSC lines (BMSC: 74.1±10.51%, USC: 67.26±6.46%) maintained significantly higher levels of perfusion compared to both PBS (25.59±6.049%,p<0.05) and keratinocyte treated groups (24.98±11.31%, p<0.05, Figure 3B). No significant differences in perfusion were observed between any of the MSC treated groups at either 12 or 24 hours re-perfusion. These data indicate that MSCs promote better post-ischemic functional recovery compared to differentiated cells, and that differences in stromal cell source tissue does not impact perfusion parameters after intestinal I/R.

MSCs improve histological architecture following I/R Injury

Significant sloughing of intestinal mucosa and destruction of the epithelial layer in the cypt-villous architecture was seen in vehicle and differentiated cell control groups. However, these findings were abated with use of MSCs (Figure 4A). As expected, intestinal histological architecture following I/R injury at both 12 and 24 hours of reperfusion was significantly improved with use of each of the MSCs (Figure 4B).

In the 12 hour reperfusion groups, mean injury score ± SEM was significantly worse in PBS (3.071±0.425) and keratinocyte (3.143±0.4174) groups compared to BMSCs (1.077±0.2646, p=<0.001) and USCs (1±0.9608, p=<0.001). After 24 hour reperfusion, mean injury scores ± SEM were also significantly improved in all MSC treated groups (BMSCs (1.063±0.2322), USCs (0.4375±0.1819)) compared to both PBS (3.5±0.3887, p=<0.001) and keratinocytes (3.5±0.5109, p=<0.001). There were no statistically significant differences in injury scores between keratinocytes and PBS in 12 and 24 hour reperfusion groups. There were no statistically significant differences in injury scores between MSC treated groups at either 12 or 24 hours of reperfusion. These data indicate that MSCs have a significant impact in preserving the intestinal histologic architecture following I/R injury.

Intestinal Cytokine Analysis

Mouse intestinal IL-6 levels were significantly lower in BMSC treated intestines compared to keratinocyte treated groups at 12 and 24 hours of reperfusion. In the 24 hour groups, IL-6 levels were also lower in BMSC treated groups compared to vehicle treated groups(Figure 5-A,B).

Mip-1α levels were significantly lower in USC treated intestines at 12 hours and BMSC treated intestines at 24 hours compared to PBS. No difference was seen in Mip1α levels between MSC treated groups at 12 hours (Figure 5C–D). Mip2α levels were significantly lower in intestines treated with BMSCs at 12 hours and at 24 hours compared to vehicle. Mip2α levels were also lower in BMSC treated intestines at 12 hours compared to keratinocytes. No differences in intestinal Mip2α levels were seen between MSC groups at 12 or 24 hours (Figure 5E–F).

Levels of IP-10 in intestines treated with BMSCs and USCs at 12 hours of reperfusion were significantly lower than vehicle treated groups. Levels of IP-10 were also lower in BMSC treated intestines at 12 hours compared to keratinocytes. No differences between MSCs were observed in intestinal IP-10 levels at 12 hoursand at 24 hours of reperfusion (Figure 5G–H).

DISCUSSION

Intestinal ischemia continues to be a cause of high morbidity and mortality [1]. It is associated with a variety of clinical conditions and can lead to irreversible bowel necrosis, sepsis, massive bowel resection and even death [1, 5]. The ultimate therapeutic goal in patients with intestinal ischemia is to restore blood flow to ischemic tissues prior to the development of necrosis and bowel wall perforation. The use of stromal cell therapy for ameliorating the destructive effects of intestinal ischemia is a novel therapeutic approach to treating this disease. In order to prepare for clinical applications of this therapy, the optimal donor source tissue for MSC harvest needs to be identified. Herein we demonstrated that MSCs from several different tissue sources yielded similar post-ischemic survival results, maintained equivalent improvements in mesenteric perfusion, and significantly improved histological architecture following intestinal I/R.

We have previously demonstrated that MSCs protect the intestines following I/R injury and promote survival [6]. In this study, we observed a distinct survival advantage with the use several MSC lines when compared to differentiated cellular control cells. When this work is compounded with our previous research using adipose-derived stromal cells (ASCs), it further confirms that stromal cell harvest source does not impact intestinal protection following intestinal I/R injury. With ASC therapy, we observed similar improvements in survival (80%), perfusion (71.14±11.85% at 12 hours, 61.13±11.89% at 24 hours), and histological injury scores (0.6875±0.2989 at 12 hours, and 0.5±0.2030 at 24 hours) as compared to therapy with BMSCs and USCs [31]. No significant differences were observed in the protective power of these mesenchymal stromal cells.

With all MSC lines we observed a distinct survival advantage that was associated with improvements in mesenteric vascular perfusion and preservation of intestinal integrity. Improved perfusion restored blood flow and tissue oxygen levels to physiologic levels which likely prevented intestinal mucosal injury, sloughing, and the impending bacterial translocation and sepsis that would likely have ensued. It is unclear though, how the cells promoted improved mesenteric perfusion, but it may be in part to the release of specific paracrine mediators [32, 33] from these cells. In this study, each cell line appeared to have a different cytokine profile, with BMSCs producing higher levels of VEGF, USCs producing higher levels of EGF, and neither stromal cell line producing significant amounts of IGF. It is unlikely that a single paracrine factor mediates the beneficial effects seen, but rather that a combination of factors in the right concentration facilities the observed effects. Future studies are aimed to determine the specific properties of the MSCs that promote improved outcomes.

Although MSCs from bone marrow, adipose tissue, and umbilical cords demonstrated global improvements in survival, mesenteric perfusion, and histology following I/R injury, the levels of proinflammatory chemokines within the intestinal tissues themselves were only moderately affected. MIP-1α, MIP-2α, IL-6 and IP-10 are all pro-inflammatory chemokines which facilitate mobilization of leukocytes to sites of injury [6, 34, 35]. Due to improved perfusion and intestinal integrity, we expected these chemokines to be lower as well in all MSC treated groups. However, only the BMSC treated groups showed consistent decreases

in these proinflammatory chemokines. These data suggest that subtle differences likely exist in MSCs, and that BMSCs may function more effectively to limit inflammation compared to mesenchymal stromal cells from other sources.

Although comparative studies of stromal cells from different sources have been previously performed, we are aware of only one other study in an intestinal ischemia and reperfusion model. The study by Watkins, et al. compared amniotic fluid-derived (AF) MSCs to BMSCs and found that both cell types were equivalent in improving histological injury score and gut permeability following injury [8]. They did note better engraftment of AFMSCs into the intestinal architecture, but these results were likely due to the additional experimental drugs that they were studying. Our combined studies examined ASCs, USCs, BMSCs, and AFMSCs and noted no functional differences in end organ protection with the use of any of these particular cell lines. Therefore, we can conclude that, although subtle differences likely exist in MSCs based on their microenvironment and tissue of harvest, the end organ protection that they provide is quite similar.

LIMITATIONS

This study has several limitations that may affect the impact of the results. First, we only tested two different mesenchymal stromal cell lines in this study, but we did compare it with an additional adipose stromal cell line from a previous study and found no differences in their protective power [31]. Although we demonstrated similar and markedly improved survival, mesenteric perfusion, and histological preservation following injury among all these cell lines, it is possible that other mesenchymal cells from an untested tissue source may provide differing results. Additionally, studied cell lines could have marked differences in their molecular response to injury. Future studies are designed to probe these cells to determine if subtle differences exist at the molecular level.

Furthermore, human cells were utilized in this study as a preclinical assessment in a mouse model of intestinal I/R injury. Cross species transplantation usually does not have effective results in immunocompetent hosts. However, mesenchymal stromal cells, including those of bone marrow, adipose, or umbilical origin, have unique immunomodulatory properties that suppress T-lymphocyte proliferation and allow them to be transplanted across species [6, 36].

An additional limitation exists in the assessment of tissue cytokines. Despite normalizing for total protein concentration, a wide variation of levels was observed both within and between group samples. Although the same relative area of intestine was procured from each subject, it is likely that tissue levels of cytokines are not equivalent throughout even small segments of tissue.

Lastly, multiple mechanisms for stromal cell protective effects have been postulated. One such mechanism surrounds stromal cell engraftment. It is possible that one of the MSC lines may provide better intestinal protection, but may engraft poorly, thereby yielding equivalent results to cell lines that may provide less protection but engraft at a much better rate. Further studies that label and track stromL cells once transplanted could yield further insight into

engraftment possibilities. An additional mechanism of effect surrounds the paracrine release of vital substrates from the cells. It is beyond the scope of this current study to test each of these potential substrates. If one or two substrates could be identified, it is possible to create a drug cocktail that would provide maximum end organ protection without the need for cell therapy.

CONCLUSION

In conclusion, MSC therapy is a viable novel treatment option for acute intestinal ischemia. Herein, we demonstrated that stromal cells from different source tissues provide equivalent protection from intestinal I/R injury. All tested MSCs improved survival and small bowel perfusion, while also preserving histological architecture. BMSCs appeared to have a more profound effect on reducing tissue inflammatory chemokines. Although multiple benefits to stromal cell therapy have been observed, the intracellular mechanisms of end organ protection are still not fully understood. Therefore, further studies to define these mechanisms are required before stromal cell therapy can be applied to widespread clinical use.

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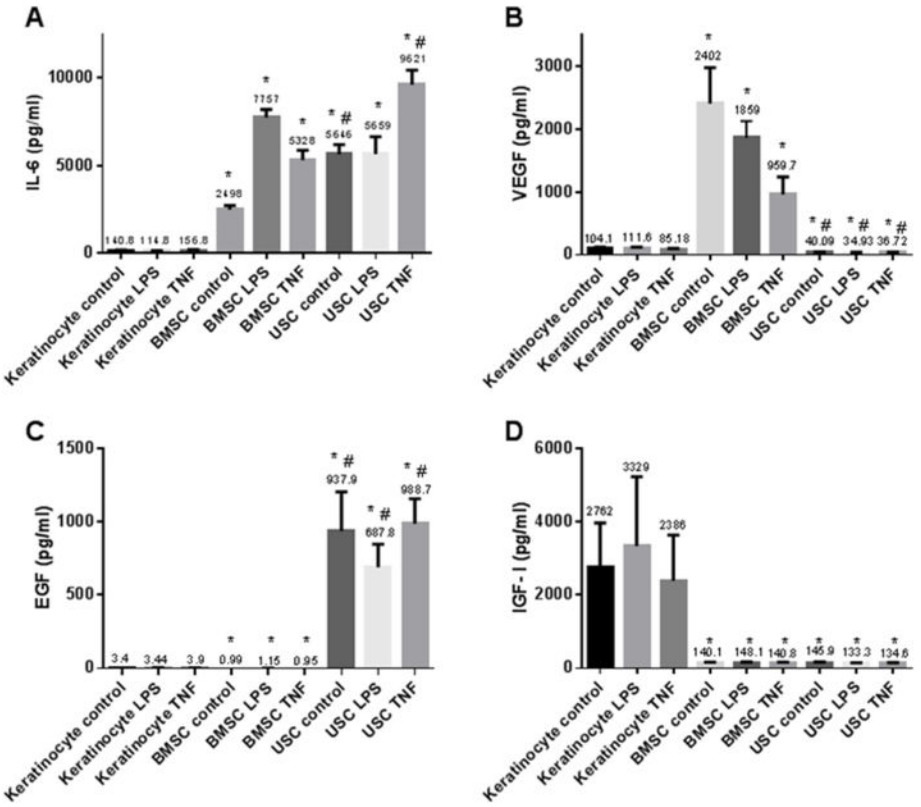

Figure 1.

Supernatant cytokine analysis following *in vitro* experiments with keratinocytes, BMSCs and USCs exposed to noxious stimuli (LPS, TNF). Production of trophic growth factors varied with stromal cell source for IL-6 (A), VEGF (B), EGF (C), and IGF-I (D) *=p<0.05 versus keratinocytes, #=p<0.05 versus BMSCs.

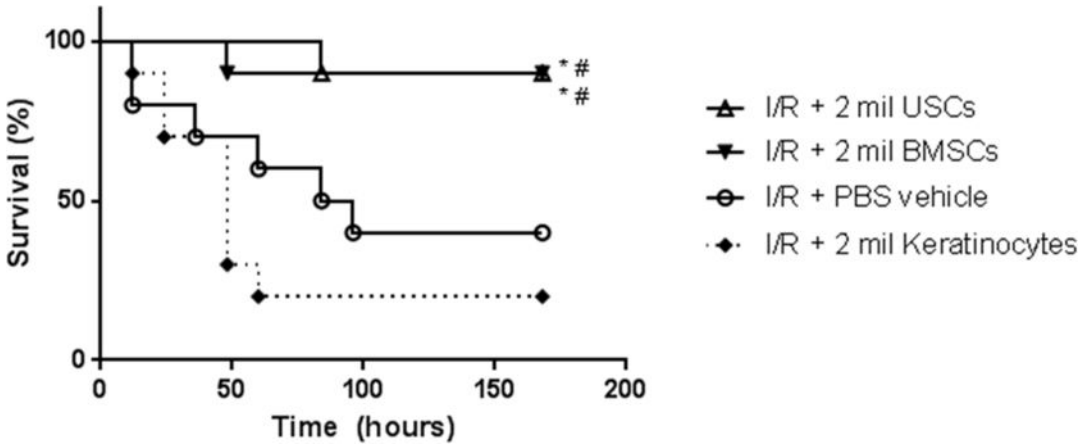

Figure 2.

Kaplan-Meier plots for 7 day survival after ischemia-reperfusion injury of each MSC treatment group. Both mesenchymal stromal cell lines significantly increased 7 day survival compared to PBS vehicle control and keratinocytes ($p<0.05$). No survival benefit was seen with the use of keratinocytes (differentiated cell control). No statistically significant survival difference was observed between mesenchymal stromal cell lines. (*=$p<0.05$ versus PBS, #=$p<0.05$ versus keratinocytes)

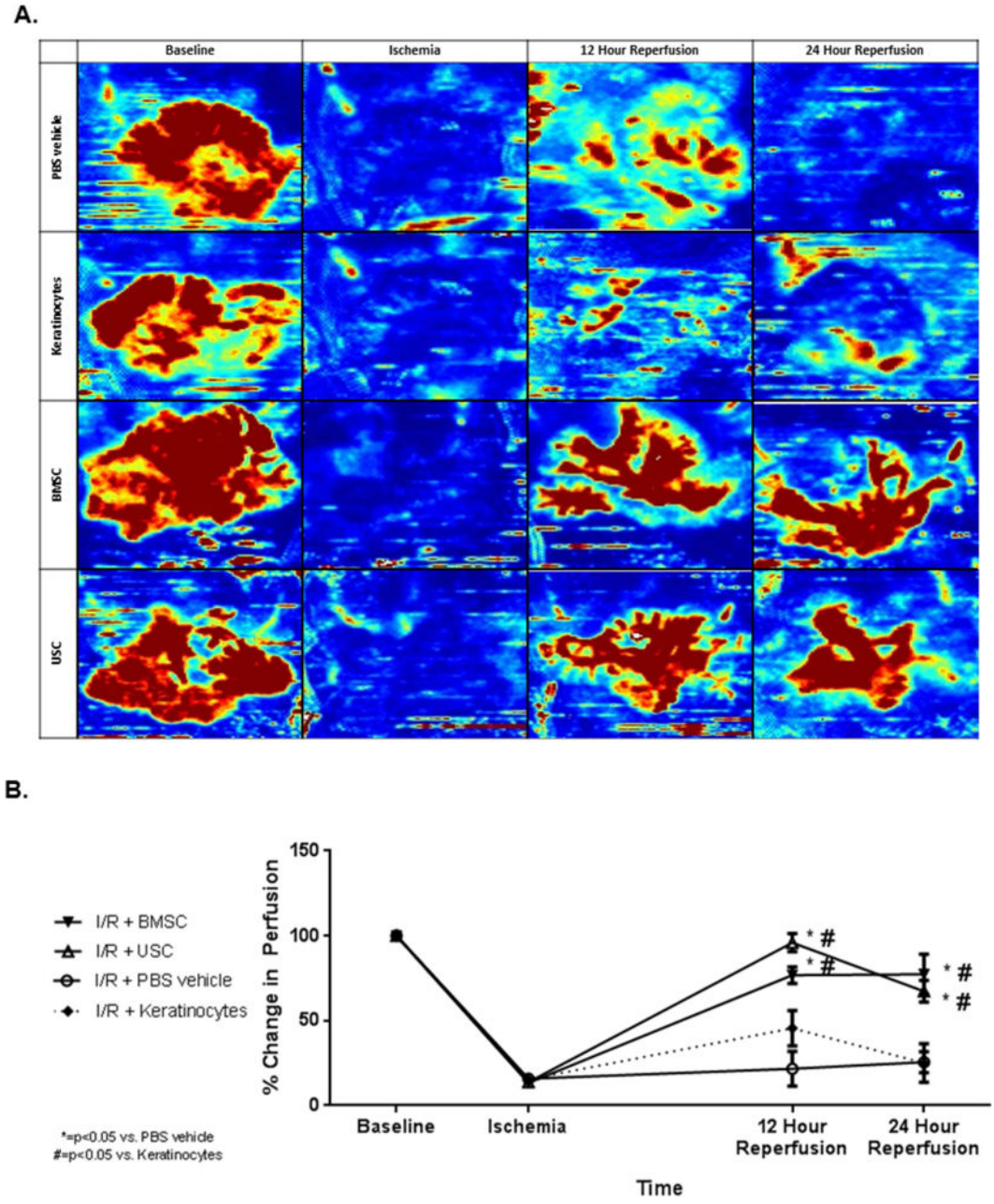

Figure 3.

Mesenchymal stromal cells increase mesenteric perfusion following ischemia. A) Representative images of perfusion in all cells lines at baseline, ischemia, 12 hours reperfusion, and 24 hours reperfusion. B) BMSC and USC therapy significantly increased mesenteric perfusion above vehicle and keratinocytes at both 12 and 24 hours of reperfusion. No differences in mesenteric perfusion were observed between MSC treated groups. (*=p<0.05 versus PBS, #=p<0.05 versus keratinocytes)

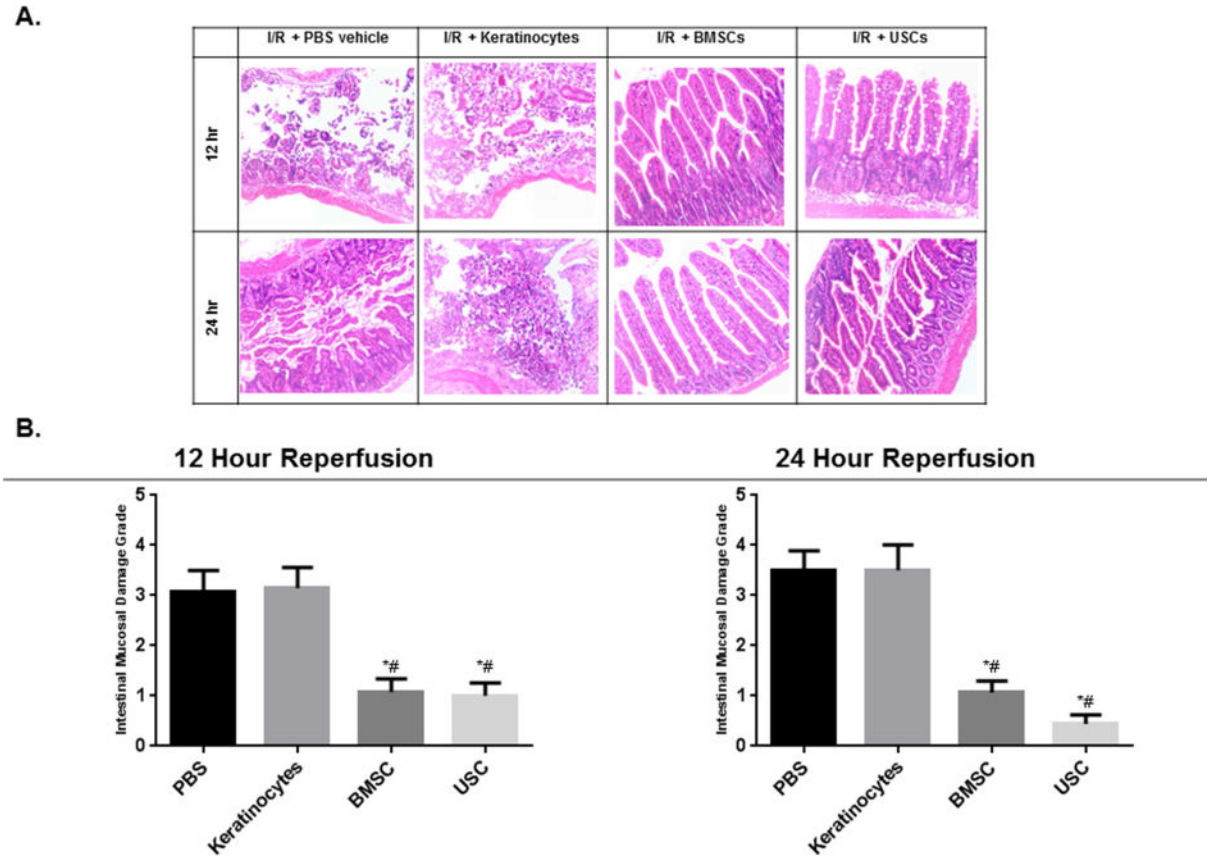

Figure 4.

Histological examination of small intestine following intestinal ischemia (I/R) and MSC treatment. A) Representative histology slides of each treatment group (hematoxylin and eosin stain, ×40). B) Histological scoring of intestinal specimens: 0, no damage; 1, sub-epithelial space at the villous tip; 2, loss of mucosal lining of the villous tip; 3, loss of less than half of the villous structure; 4, loss of more than half of the villous structure; and 5, transmural necrosis. Statistically significant improvement in histological grade in both MSC lines compared to PBS (*) and keratinocytes (#) respectively (*,# p=<0.05).

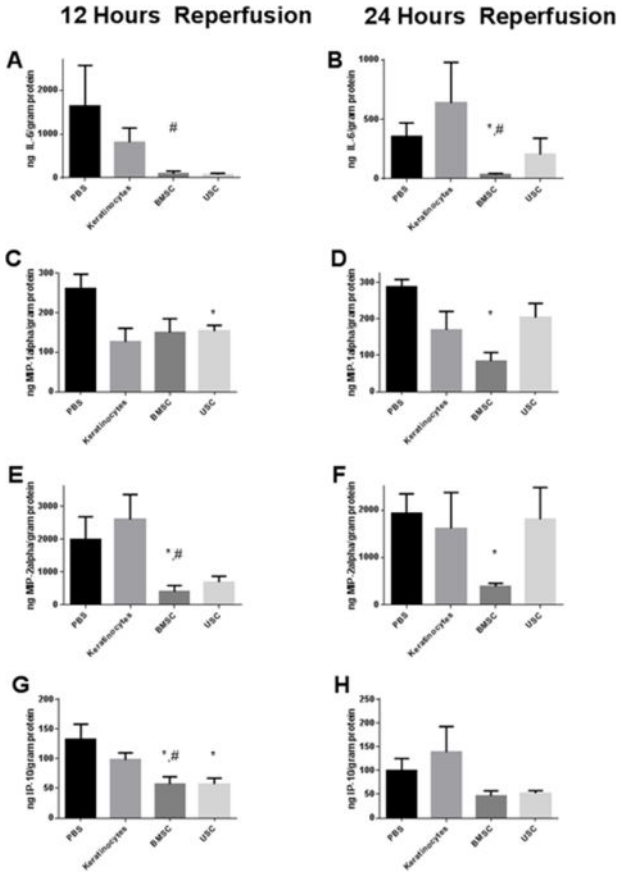

Figure 5.

Cytokine analysis following I/R injury and treatment at 12 and 24 hours. Following I/R mouse intestines were probed for murine expression of IL-6 (A–B), Mip1α (C–D), Mip2α (E–F), and IP-10 (G–H) *=$p<0.05$ versus PBS, #=$p<0.05$ versus Keratinocytes.