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Image Guided Transarterial Directed Delivery of Human Mesenchymal Stem Cells for Targeted Gastrointestinal Therapies in a Swine Model

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Abstract

Purpose: To evaluate the feasibility of catheter-directed intra-arterial stem cell delivery of human mesenchymal stem cells (MSCs) to the small bowel in a porcine model.

Materials & Methods: The cranial mesenteric artery of six Yucatan minipigs was selectively catheterized under fluoroscopic guidance following cut-down and carotid artery access. A proximal jejunal branch artery was selectively catheterized for directed delivery of embolic microspheres (100–300 μm) or MSCs (0.1–10 million cells). Subjects were euthanized after 4 hours and specimens were collected from the proximal duodenum and the targeted segment of the jejunum. The Chiu/Park system for scoring intestinal ischemia was used to compare H&E stained sections of jejunum to duodenum.

Results: Successful delivery of microspheres or MSCs in a proximal jejunal branch artery of the cranial mesenteric artery was achieved in all subjects. Radiopaque microspheres and post-delivery angiographic evidence of stasis in the targeted vessels were observed on fluoroscopy following delivery of embolics. Preserved blood flow was observed after MSC delivery in the targeted

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vessel. The Chiu/Park score for intestinal ischemia in the targeted proximal jejunal segments were similar for microspheres (4, 4; N=2) and MSCs (4, 4, 4, 3; N=4), indicating moderate ischemic effects that were greater than control duodenal tissue (3, 1; 0, 0, 3, 3).

Conclusion: Selective arteriographic deployment of MSCs in swine is feasible for study of directed intestinal stem cell delivery. In this study, directed therapy resulted in intestinal ischemia.

Introduction:

Inflammatory bowel disease (IBD) is a chronic, life-long disorder. Inflammation and ulcerations of the intestine result in malnutrition and intestinal failure, and often progress to fibrosis – leading to complications such as obstruction, fistulas, and bowel perforations. As a result, IBD results in substantial morbidity and mortality for patients. Standard anti-inflammatory regimes such as corticosteroids and 5-ASA are the primary available treatments to attenuate the underlying inflammation, but neither halt the progression of the disease or change the natural history of IBD. As a result, research has focused on the evolution of novel biological immunomodulators, including stem cells. [1–3]

A well-known obstacle to stem cell therapies is the first pass effect. The majority of systemically administered cells through peripheral veins are sequestered within the pulmonary circulation. [4] The concept of image guided directed MSC delivery affords three distinct potential benefits over systemic administration: 1) bypass of the first pass effect, 2) decreased dose requirements, and 3) enhanced local paracrine effects. [4–17]

Interventional radiologists are uniquely suited to develop roles for directed stem cell therapy in a variety of conditions, including IBD. Animal and translational models are needed to evaluate and optimize parameters for directed delivery using image guidance, be it percutaneous, intra-arterial, or other. One potential avenue for image guided directed delivery of cell therapy is via supplying arteries, thereby circumventing the pulmonary first pass effect. [18–22]

In the present study, we evaluated the following parameters regarding study of directed intraarterial MSC delivery to the bowel in a swine model: 1) the feasibility of selectively delivering MSCs directly to jejunal branch arteries, 2) the shear stress effects on cells during catheter delivery *in vitro*, and 3) target tissue ischemia following delivery of cells vs. microspheres vs. control.

Materials and Methods:

Animal Subjects:

This study was approved by the local Institutional Animal Care and Use Committee and procedures were performed in accordance with institutional guidelines. A total of six Yucatan female minipigs (28–35 kg) were obtained from Sinclair Bio-Resources and housed per protocol by the local Division of Animal Resources Center.

MSC Isolation and Culture:

Human MSCs were isolated from bone marrow aspirates collected from the iliac crest of consented humans as previously described. [23] Briefly, bone marrow aspirates were diluted 1:2 with phosphate-buffered saline and layered onto a Ficoll-Paque density gradient to isolate mononuclear cells. The cells were centrifuged at 400 g for 20 minutes and thereafter plated in complete MSC medium (α-MEM, 10% human platelet lysate or Fetal bovine serum, 100 U/ml penicillin/streptomycin) at 200,000 cell/cm^2. Non-adherent hematopoietic cells were removed by changing the medium after 3 days of culture and MSC allowed to expand for 7 days. Thereafter, the cells were passaged weekly and reseeded at 1000 cells/cm^2. Prior to delivery, hSMCs were trypsinized and suspended in 10 mL plasma-lyte-A for infusion. Bone marrow derived MSCs were confirmed for its identity through International Society for Cell Therapy-defined surface markers (CD45-, CD73+, CD105+, CD90+, CD44+) (Figure S1). [24] All experiments were performed between 2–4 total MSC passages.

In Vitro Shear Stress Testing:

Human MSCs were suspended at 0.5×10^6 cells/ml in Plasma-Lyte-A and collected following infusion through a multi-lumen catheter (Translational Research Institute, Nabil Dib Infusion Catheter ND100–02) terminating in six 0.006 inch inner-diameter lumens. Controlled flow rates were achieved with an in-line pressure transducer (Cook Medical, CompassCT CCTP001) to generate low (<500 mmHg), medium (<999 mmHg), and high (>999 mmHg, maximum achievable) shear stress conditions. Viability, apoptosis, and cell recovery rates were measured for each condition by means of trypan blue and annexin V/propidium iodide staining. The samples were then assessed by cell count or via flow cytometry, respectively. A more detailed description of these methods can be found in the Supplementary Data.

Targeted Delivery via the Cranial Mesenteric Artery:

Animals were placed under general anesthesia to obtain sheath access via carotid artery cut-down. The needle was exchanged over a 0.018” guidewire for a 5 French introducer sheath (Terumo, Pinnacle RSS503), through which a 0.035” guidewire was advanced under fluoroscopic observation to the abdominal aorta (Figure 1–A). Iohexol contrast (Omnipaque 350) was injected, and a digital subtraction aortogram performed. Using a 5F catheter (Terumo, Glidecath CG507), the cranial mesenteric artery – supplying the small bowel, ascending colon, and proximal transverse colon – was selectively catheterized and a contrast run performed confirming location. Subsequently, the first or second jejunal branch was selectively catharized (Figure 1–B) and radio-opaque embolic microspheres (BTG, LC Bead LUMI, 100–300 um, ½ vial) (N=2) or MSC (0.1 million cells, N=1; 1 million cells, N=2; 10 million cells, N=1), were administered to anesthetized minipigs over the course of 1 minute. Embolic delivery was performed per established clinical techniques and cell dosage calculated according to previous reports. [18] A final digital subtraction angiogram (DSA) run was performed for evaluation of potential stasis and blood flow following injections.

Histologic Analysis of Intestinal Ischemia:

At the completion of the 4-hour time course following delivery of microspheres or MSCs, the minipigs were euthanized with barbiturates per institutional protocol. Immediately following euthanasia, the necropsy begun with initial dissection to the aorta and subsequent identification of the cranial mesenteric artery. The caudal duodenopancreatic, right colic, and ileocolic branches of the cranial mesenteric artery were identified. Images from the time of administration were used to identify and enumerate the targeted jejunal branch at the time of necropsy (Figure 1–B). Next the appropriate first or second jejunal branch artery was identified (Figure 1–C) and selected with harvest of a 15 cm segment of jejunum supplied by the vessel. The first segment of the duodenum was then harvested. The duodenal and jejunal specimens were then immediately fixed in 10% neutral buffered formalin, routinely processed, paraffin-embedded, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E). Each section was scored for degree of ischemia based off the Chiu/Park system. The duodenum, which served as the internal negative control, and jejunum were removed and processed for histological analysis, particularly embolic sequela. The standardized Park/Chiu histology score system was used to evaluate sections for ischemia as follows: 0 = normal mucosa, 1 = subepithelial space at villus tips, 2 = extension of subepithelial space with moderate lifting, 3 = massive lifting down sides of villi, some denuded tips, 4 = denuded villi, dilated capillaries, 5 = disintegration of lamina propria, 6 = crypt layer injury, 7 = transmucosal infarction, and 8 = transmural infarction. [25, 26]

Results:

Embolic Microsphere Delivery:

Catheter placement was successful in all animals. Following delivery of particles, stasis was observed in the proximal aspect of the cranial mesenteric artery distribution (Figure 2). Pre- and post-microsphere delivery DSA demonstrated successful embolization of targeted jejunal branches. (Figure 3).

Images from the time of administration were used to identify and enumerate the targeted jejunal branch at the time of necropsy. Specimens from the harvested proximal duodenum and proximal jejunum fed by the targeted artery of each animal were sectioned and stained with H&E. (Figure 4–A,B) Each section was scored for degree of ischemia based off the Chiu/Park system. (Table S1)

Chiu/Park 4 ischemic effects were observed in each of the targeted segments of jejunum. In a control segment of duodenal tissue, which did not receive embolic materials, Chiu/Park Scores of 3 and 1 were observed (Figure 4–C, Table S1).

MSC Delivery:

A proximal jejunal branch of the cranial mesenteric artery was successfully selectively catheterized under fluoroscopic guidance (Figure 5–A). With the catheter held in place, a suspension of human MSC (N=4, Table S1) was administered over the course of 1 minute. DSA following delivery of the MSCs demonstrated preserved blood flow in the targeted artery in each of the 4 subjects (Figure 5–B).

Following a 4-hour time course, an analogous necropsy was performed to obtain duodenal and jejunal specimens. Chiu/Park Scores of 3–4 were observed in each of the targeted jejunal segments. The duodenal tissue, which was not targeted with MSCs, produced Chiu/Park Scores of 0 and 3 (Figure 4–C). The jejunal ischemia scores were greater than the duodenal scores despite gross preservation of blood flow immediately following MSC delivery (Figure 5–B).

Shear Stress Testing.

Suspended MSCs were exposed to TWSS of 68.5±1.8, 134.0±4.8, and 792±55 dyne/cm^2 and stained with trypan blue (Figure S2) and annexin V/propidium iodide (Figure S2) to assess MSC viability and membrane integrity following delivery. There was no significant loss of cell viability or membrane integrity up to TWSS values of 792±55 dyne/cm^2 (Figure S2 – S4).

Discussion:

Mesenchymal stem cells (MSCs) have emerged as an effective therapeutic option for many immune related disorders, including conditions such as inflammatory bowel disease. [1–3, 27, 28] At present, stem cell therapies for IBD are administered systemically via peripheral venous access, and many of the cells undergo first-pass sequestration in the pulmonary vasculature. [5, 29] As a result, other routes of administration are being investigated for purposes of potentially increasing the concentration of cells at the therapeutic target site. For example, MSCs injected intraperitoneally – but not intravenously – have been shown to migrate to the bowel and ameliorate colitis in rats, lending support to the significance of mechanically, geographically directed cellular therapy for IBD [30].

Porcine models of inflammatory bowel disease and transarterial anatomy and intervention are well established, [31–35]including a framework for the study of MSC therapy for enteritis in pigs. [36] This study evaluated a swine model for future study of directed intraarterial delivery of MSCs to the bowel by analyzing three distinct parameters: feasibility of angiographic cell delivery, effect of the shear stress related to catheter injection on the cells themselves, and target tissue ischemia as compared to microspheres and controls.

In all of the cases, a 5 Fr catheter over a 0.035 microwire were readily directed to the supplying artery of interest following carotid access and sheath placement per established techniques. Angiograms were performed, and both embolic admixtures and designated cell concentrations were deployed under direct fluoroscopic observation with subsequent digital subtraction imaging without difficulty.

Regarding cell viability and shear stress during injection, MSC infusion via 5F catheter over the course of 1 minute corresponds to a transcatheter wall shear stress (TWSS) of 17 dyne/cm^2. Our *in vitro* investigation on the influence of TWSS on MSC quality following delivery revealed no significant loss of cell viability or membrane integrity up to TWSS values of 792±55 dyne/cm^2. Therefore, the delivery of intact MSCs to an artery is feasible through a 5F catheter with preserved viability and integrity.

Third, evaluation of the targeted jejunal tissue demonstrated early histological ischemic effects following cell delivery that were similar to the microsphere delivery. This was despite obvious differences in angiographic blood flow immediately after microsphere and MSC delivery, suggesting that immediate post-delivery DSA does not accurately predict the degree of ischemia after 4 hours. This important downstream effect of cell delivery despite the absence of stasis on angiography warrants further investigation as intra-arterial options evolve.

Several limitations should be noted regarding these results. Vascular access in these studies was removed and the surgical incision closed shortly after the start of the 4-hour time course to minimize risk of infection for the animal, as per institutional guidelines. As a result, angiography was not performed immediately prior to euthanasia. It may be the case that the targeted jejunal branch vessel thrombosed during the time course which resulted in the observed ischemic effects. Alternatively, the roughly 30-minute time period between euthanasia and sample recovery may have similarly contributed to enhanced ischemic changes on histology. To this point, a small number of duodenal control samples also showed ischemic changes, supporting a methodological limitation of histological analysis. Finally, the small sample size precludes inter-subject comparisons with regard to different concentrations of MSCs leading to ischemia.

Going forward, the information from this study may be used as a foundation for optimizing delivery parameters to reduce ischemia following intra-arterial delivery. One potential adjustment that may decrease ischemic effects is titrating the volume of MSC delivered. A reduction in the infusion volume may reduce the time that the intestinal segment is subjected to reduced blood flow related to the presence of the catheter. Similarly, a coaxial approach and smaller catheter would avoid the same potential stasis. A more proximal delivery site or more dilute admixture may still allow for bypass of the first pass effect and directed delivery to the bowel, resulting in improved efficacy and decreased dose, but with less selectivity than attempted in this model. Lastly, methods to detect cells delivered intra-arterially to relatively larger landscapes (e.g., bowel vs. brain or intervertebral disc) will aid in answering questions regarding the importance of proximity and residence in this setting.

In conclusion, a porcine model to study directed intra-arterial delivery of stem cells for potential management of IBD is feasible, and may be used to optimize intra-arterial delivery parameters so that target tissue ischemia is reduced, target site cell concentration is increased, and efficacy of MSC therapy is improved over current systemic administration routes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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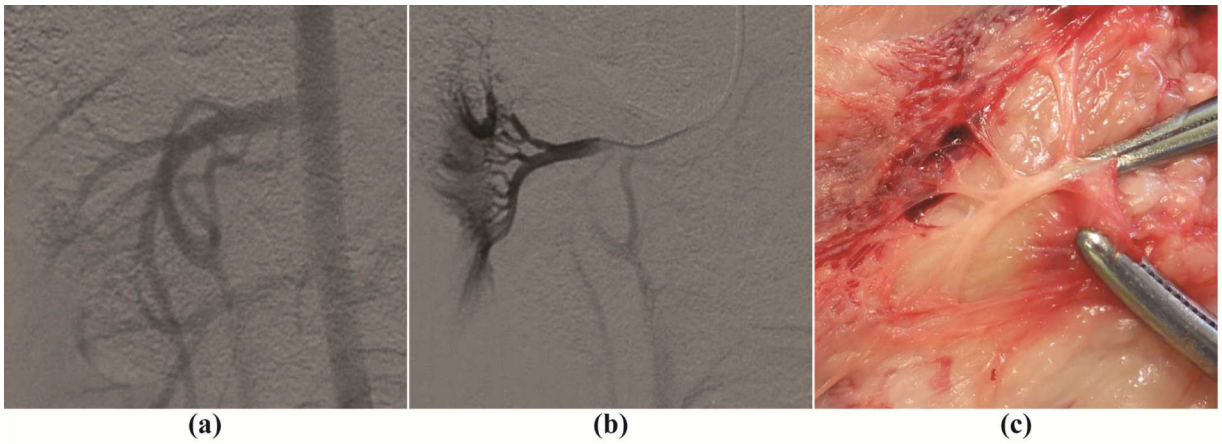

Figure 1: Radiologic-Anatomic Correlation to Guide Targeted Jejunal Specimen Harvest.
A) Aortogram demonstrating abdominal vascular anatomy. B) Selected jejunal branch (first) of the cranial mesenteric artery. C) Isolation of the first jejunal branch during necropsy.

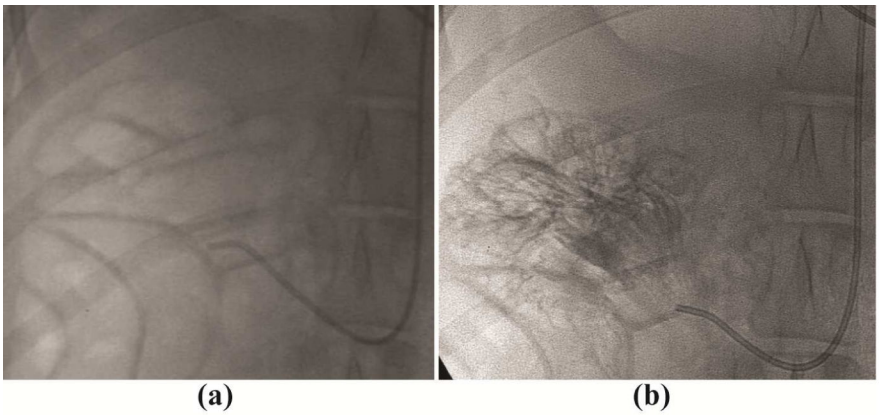

Figure 2: Spot Digital Fluoroscopy Demonstrating Retention of Radiopaque Microspheres.
A) Fluoroscopy demonstrating catheter positioning in a jejunal branch of the cranial mesenteric artery prior to delivery of radiopaque microspheres. B) On repeat fluoroscopy following microsphere delivery, retention of radiopaque materials was observed in the tissue supplied by the proximal portion of the cranial mesenteric artery.

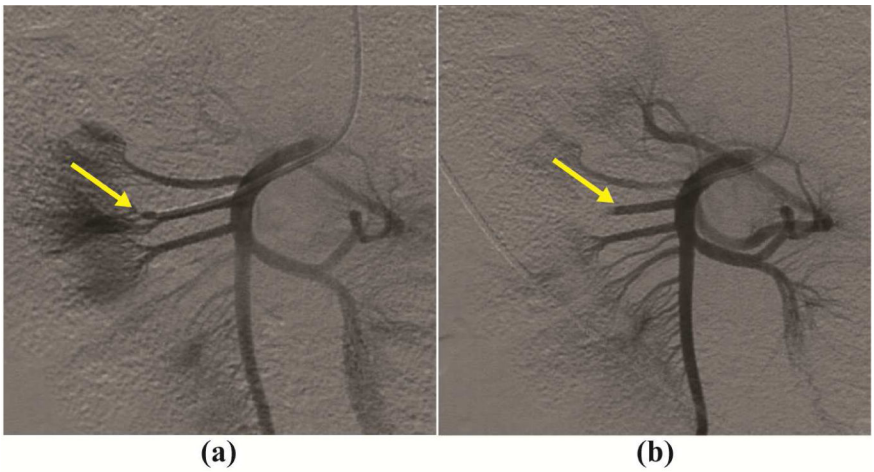

Figure 3: Selected DSA Frames Demonstrating Super Selective Arterial Embolization.

A) Arterial distribution is shown on DSA with the catheter in position for microsphere delivery. The 5F angled catheter is advanced into the 3rd jejunal branch artery of the cranial mesenteric artery with infusion of dilute contrast and reflux into adjacent vasculature. B) On repeat DSA following microsphere delivery, the catheter was withdrawn to the level of the cranial mesenteric artery and maintained proximal to the 3rd jejunal branch. A marked reduction in flow was observed in the target vessel (yellow arrow).

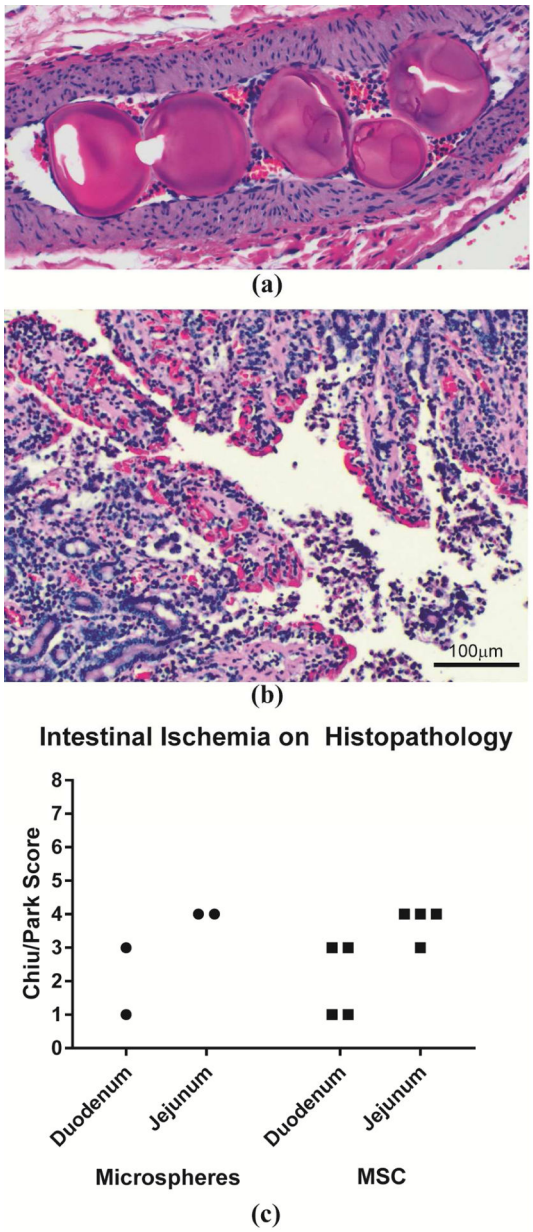

Figure 4: H&E Staining Demonstrating Microspheres and Ischemia in Bowel Sections

A) Arterial section from Animal #1 demonstrating arterial occlusion with microspheres surrounded by inflammatory cells. B) Jejunal section from Animal #1 demonstrating superficial villar denuding and hemorrhages consistent with a Chiu/Park Score of 4. C) Dot plot of Chiu/Park Intestinal Ischemia Scores in duodenal (square) and jejunal (circle) sections for microsphere (N=2) and MSC (N=4) groups.

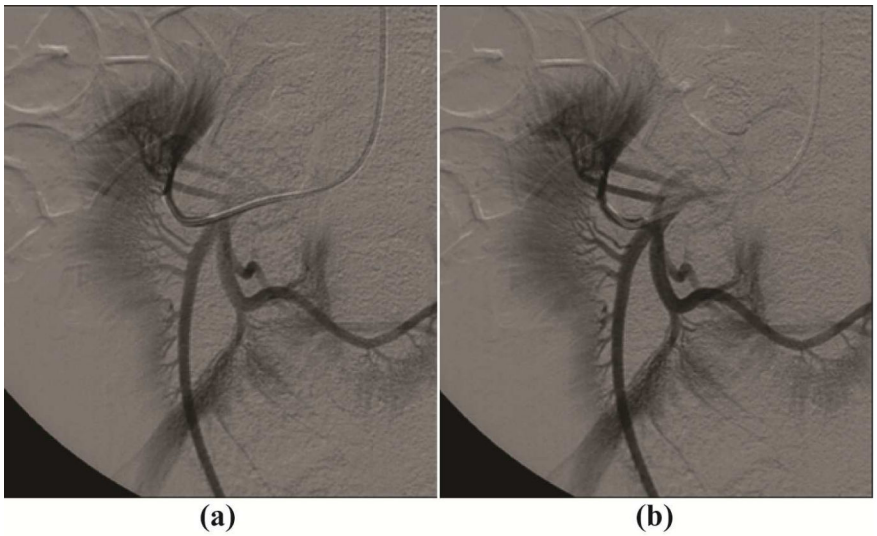

Figure 5: Selected DSA Frames Demonstrating Preserved Blood Flow Following MSC Delivery.
A) Arterial distribution is shown on DSA with the catheter in position for MSC delivery. The 5F angled catheter is advanced into the 3rd jejunal branch artery of the cranial mesenteric artery with infusion of dilute contrast and reflux into adjacent vasculature. B) Re-demonstration of arterial distribution on repeat DSA following MSC delivery.