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Transvenous Intramyocardial Cellular Delivery Increases Retention in Comparison to Intracoronary Delivery in a Porcine Model of Acute Myocardial Infarction

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Abstract

Background—Clinical trials using intracoronary (IC) delivery of cells have addressed efficacy but the optimal delivery technique is unknown. Our study aimed to determine whether transvenous intramyocardial (TVIM) approach was advantageous for cellular retention in AMI.

Methods—Domestic pigs (n=4) underwent catheterization with coronary angiography and ventriculography prior to infarction and pre- and post-cells. Pigs underwent 90 minute balloon occlusion of the left anterior descending artery (LAD). After one week they were prepared for IC (n=2) or TVIM (n=2) delivery of bone marrow mononuclear cells (MNC) labeled with GFP. IC infusion used an over-the-wire catheter to engage the LAD and balloon inflation to prevent retrograde flow. Venography via the coronary sinus was used for TVIM delivery. The anterior interventricular vein was engaged with a guide wire allowing use of the TransAccess™ catheter, which is outfitted with an ultrasound tip for visualization. Animals were sacrificed one hour after delivery and tissue was analyzed.

Results—Procedures were performed without complication and monitoring was uneventful. 1×10^8 MNC's were isolated from each BM preparation and 1×10^7 MNC delivered. Ventriculography at one week revealed wall motion abnormalities consistent with an anterior AMI. TVIM and IC delivery revealed mean 452 cells/section and 235 cells/section on average, respectively in the infarct zone ($p = 0.01$).

Conclusion—We have demonstrated that TVIM approach for cell delivery is feasible and safe. Moreover, this approach may provide an advantage over IC infusion in retention of the cellular product, however, larger studies will be necessary.

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INTRODUCTION

Cardiovascular disease is the number one cause of death globally with 17.5 million deaths around the world in 2005 and projected to remain the leading cause of death [1]. There have been significant strides in the reduction of morbidity and death in acute coronary syndromes but there is a lack of significant therapeutic improvement in patients that are refractory to revascularization intervention [2]. Recent studies have demonstrated improvement in myocardial function after targeted repair in infarcted myocardium via implantation of progenitor cells, whether derived from bone marrow (BM) [3,15,16,17,18] or umbilical cord blood (UCB) [4,19,20,21,22]. Although various strategies for delivery of these cells have been investigated including intracoronary (IC) [5,6,10,11,14], intravenous (IV) [6,14], intramyocardial (IM) [7,11,14], endomyocardial (EM) [6,13,14], retrograde coronary venous (RCV) [8,11,13,14] and transvenous intramyocardial (TVIM) [9,12,14] approaches, the optimal avenue for cell delivery is yet to be determined.

Our study aimed to determine the use of a TVIM approach using the TransAccess™ catheter system (Medtronic Vascular, Santa Rosa, CA) in comparison to IC delivery for cellular retention after acute myocardial infarction (AMI) in the pig model. The composite catheter system allows visualization with intravascular ultrasound (IVUS) for stable access and injection with a nitinol needle for cell delivery [Figure 1]. Although, this system was found to be safe and feasible in a healthy pig model [9], the safety and feasibility of its use for delivery of progenitor cells in an acute myocardial infarction model has not been previously evaluated.

METHODS

Animal procedures

All animal procedures were approved by the Case Western Reserve University Institutional Animal Care and Use Committee. Animals were housed in the Animal Resource Center Health Science Animal Facility. Four female Yorkshire pigs, weighing approximately 55 kilograms each, were selected to test the safety and feasibility of TVIM delivery of cells in a model of acute myocardial infarction. Anesthesia was induced with intravenous ketamine (100 mg/kg) and acepromazine (10 mg/kg). Anesthesia was maintained with mechanical ventilation (Narkovet VCII Series One) using inhaled isoflurane (5% bolus followed by 1–2% maintenance) with 1–2 L/h of oxygen. All animals received cefazolin (1g) intravenously prior to all procedures and 0.5% bupivacaine (5 mL) at incisional sites at the conclusion of all procedures.

Bone marrow harvest and mononuclear cell isolation

Animals were anesthetized as described above. The ventral pelvic region was shaved, prepped and draped in sterile fashion. The distal portion of the femur just superior to the knee was located and a 5–10 millimeter incision in the skin directly over the selected harvest site was made. A bone biopsy needle was advanced perpendicular to the femur and marrow (40–60 mL) was aspirated into a sterile syringe with heparin (400 IU per 20 mL marrow). Mononuclear cells (MNC) were isolated by Ficoll-Paque (GE Healthcare, Tampa, FL)

density gradient centrifugation and transfected with green fluorescence protein (GFP) using an Amaxa™ transfection technology (Amaxa, Gaithersburg, MD). The yield of MNC from each BM aspirate was approximately 1×10^8 cells.

Coronary angiography and contrast ventriculography

Animals were anesthetized as described above. The right groin was shaved, prepped, and draped in sterile fashion. A sagittal incision was made in the right groin for direct exposure of the femoral artery. An 8 French (Fr) sheath was introduced into the artery, aspirated and flushed with sterile saline. All animals received intravenous unfractionated heparin (50 units/kg), magnesium sulfate (1–2 g), and lidocaine (50–100 mg 2% solution) at the start of the procedure. All cardiac catheterization procedures were performed using a Philips 6028 Cath Lab Fluoroscopy Unit (Philips, Andover, MA). All animals underwent quantitative coronary angiography (Camtronics, Hartland, WI) and contrast ventriculography both at baseline, following infarction, and prior to injection. A 7 Fr pigtail catheter (SciMed, Maple Grove, MN) was used to cross the aortic valve and enter the left ventricle. Contrast ventriculography was performed using a power contrast injector (Medrad Medmark V, 514V, Indianola, PA) in standard right anterior oblique (RAO) and left anterior oblique (LAO) views. A 7 Fr AL2 catheter (Boston Scientific, Natick, MA) was advanced over a 0.035 inch guide wire to selectively engage the left and right coronary arteries. Digital cineangiograms were taken in RAO and LAO views. Following all coronary diagnostic and cell injection procedures, the sheath site was repaired in multiple layers.

Myocardial infarction via balloon occlusion of LAD

Following the left coronary injection, an over-the-wire balloon catheter (Maverick 3.5 × 25 mm, Boston Scientific, Boston, MA) was advanced over a 0.014 inch guidewire (Choice Standard, SciMed, Maple Grove, MN) into the left anterior descending artery (LAD). Infarction was attained by 90 minute balloon occlusion of the artery in the mid vessel after the first septal perforator branch [Figure 2]. Anterior infarction was confirmed by ST-segment elevations on ECG monitoring and anterior wall motion abnormality on subsequent ventriculography.

Cell Delivery

One week post-infarction, all four animals were prepared for either IC (n=2) or TVIM (n=2) delivery of GFP transfected BM-MNC. Approximately 40% of total cells were transfected with GFP vector, determined by GFP expression under fluorescence microscope 16 hours post-transfection. Animals were anesthetized and femoral sheaths placed percutaneously as previously described.

Following a left coronary injection, an over-the-wire balloon catheter (Maverick 3.5 × 9 mm, Boston Scientific, Boston, MA) was advanced over a 0.014 inch guide wire (PT Graphix Intermediate, SciMed, Maple Grove, MN) into the LAD. BM-MNC (1×10^7 cells) were delivered into the mid vessel after balloon expansion to prevent retrograde washout of cells.

The coronary sinus (CS) was accessed through a femoral venous sheath using a 7 Fr Porcine 3 catheter (Medtronic Vascular, Santa Rosa, CA). An exchange length, 0.035 inch hydrophilic angled wire (Radiofocus Glidewire, Terumo, Somerset, NJ) with J-tip was advanced into the CS, through the great cardiac vein (GCV), and into the anterior interventricular vein (AIV). The diagnostic catheter was withdrawn with the guide wire in place, and a 14 Fr CS guiding catheter (Medtronic Vascular, Santa Rosa, CA) and introducer were placed with conventional over-the-wire technique. The TransAccess™ catheter (Medtronic Vascular, Santa Rosa, CA), which is a 6 Fr monorail catheter system combining IVUS and an extendable 24-gauge nitinol needle (IntraLume), was advanced over the 0.014 inch guide wire and into the AIV for myocardial access [Figure 3a]. After confirmation of position within the coronary vein in the territory of the infarct, the nitinol needle was extended into the myocardium for injection [Figure 3b]. A series of five injections were performed using the pullback technique to allow contiguous depots of cell delivery in the infarcted myocardium. The BM-MNC were prepared at a concentration of 2×10^6 cells per milliliter and delivery was administered at 1 milliliter per injection for a total of 1×10^7 cells.

Post-mortem protocol

One hour after cell delivery, animals were euthanized by intravenous injection of 200 mg/kg sodium pentobarbital (Fatal-Plus, Vortech Pharmaceuticals, Dearborn, MI). Hearts were removed and pressure-perfused with normal saline followed by 10% formalin for one week. Thirty sections of the heart were dissected around the infarct zone [Figure 4] and placed in bottles containing 10% formalin for an additional 2 days. These sections were further trimmed, placed in labeled cassettes, and fixed for one additional day before processing.

Tissue processing and embedding

Using an automated tissue processor (Jung Histokinette, Leica Inc, Deerfield, IL), the cassettes were transferred through increasing grades of alcohol for complete dehydration and xylene for clearing. The specimens were embedded in paraffin using stainless steel cassette moulds and sections 5 microns thick were obtained using a microtome. These sections were floated on a warm water bath to smooth out the creases and placed on a glass slide to dry before staining.

Cell staining and counting

The retained BM-MNC were observed under fluorescence microscopy after incubation with monoclonal anti-GFP primary antibody followed by secondary antibody which was cross-linked with fluorescent dye, Alexa Fluor 488 or Alexa Fluor 647 (Invitrogen, Carlsbad, CA) for green or red stains respectively. Counter staining for nucleus was performed with DAPI, which fluoresces blue under microscope. The cells were counted using an automated measuring program (AxioVision 4.5, Carl Zeiss MicroImaging, Thornwood, NY) which allows objects to be identified and counted based on color and size, along with the tools for manual addition or deletion of objects. Cell counting was performed under $10 \times$ magnification by a blinded investigator.

Statistical analysis

Data were reported as mean $\pm$ standard deviation. Statistical significance was assessed using analysis of variance (ANOVA) and deemed significant when $p < 0.05$.

RESULTS

BM-MNC were isolated from each animal and 1×10^7 cells prepared for delivery. Myocardial infarction was induced in both animals and ventriculography at one week confirmed wall motion abnormalities consistent with an anterior AMI. IC and TVIM delivery was performed successfully without complications.

Myocardial sections were obtained and cell counts performed as described earlier. Immunofluorescence microscopy revealed variable retention of BM-MNC in the infarct zones dependent on mode of delivery (TVIM [Figure 5] versus IC [Figure 6]). Cell counting confirmed greater retention with TVIM compared to IC approach both with total cell counts (13579 ± 586 TVIM versus 7049 ± 302 IC; $p = 0.04$) and mean cell counts per section (453 ± 107 TVIM versus 235 ± 55 IC; $p = 0.01$) [Figure 7a]. Regional cell counts, accounting for distance from infarct zone, also revealed farther penetration of injected cells with TVIM compared to IC delivery (5665 ± 346 TVIM versus 3336 ± 228 IC; $p = 0.01$) [Figure 7b].

DISCUSSION

Cardiac regenerative therapy using progenitor cells is under intense investigation due to its potential to improve myocardial perfusion and function after AMI. Various delivery platforms for cellular therapy have been studied; however, the optimal approach is yet to be determined.

The advantages of a percutaneous RCV delivery system has been previously established [23,24,25] since the coronary sinus drains the anterior left ventricular wall, the posterior wall in the majority of patients, and even the right ventricular wall in a third of patients. This allows selective regional myocardial treatment by retrograde penetration of the venocapillary vasculature in the setting of total coronary arterial occlusion creating a reservoir of potentially bioactive material which exerts a local effect over the time that it is retained. It has also been shown that complete balloon occlusion of the AIV makes little difference to LAD flow due to diversion through alternative venous channels [23]. The limitations of this approach have been described as variations in coronary venous anatomy between animal models and humans and discrepancy in the area of myocardium served by a given coronary vein. In addition, although coronary venous delivery has slower washout compared to IC delivery, the dwell times appear to be longer for IM approach which offers direct local delivery into the interstitium with minimal washout [11]. However, the difficulty of direct IM approach in patients makes this mode of delivery less appealing.

The TVIM approach combines the advantages of the RCV and IM strategies while overcoming some of the described limitations of both techniques. It allows a percutaneous RCV approach with penetration of multiple regions of myocardium along with the long dwell times of IM delivery. The TVIM approach utilizes a catheter system that employs

IVUS and fluoroscopy to allow direct visualization thereby minimizing any procedural complications of IM delivery. Moreover, the use of a nitinol needle allows deep penetration into the myocardial tissue thereby delivering cells into the periphery of the infarct zones. Our study showed better cell retention and distal penetration with the TVIM strategy compared to IC.

However, we recognize the limitations of our study. The sample size (n=4) is underpowered for making broad assumptions but the findings were consistent and confirmed across all our endpoints reaching statistical significance. Secondly, myocardial infarction was attained by 90-minute balloon occlusion rather than ligation, which may not represent a true AMI model, but it does solidify our findings that the TVIM approach was superior despite restoration of coronary blood flow. Finally, the animals were sacrificed within one hour of cell delivery, which may not address durability of cell transfer but does apply to our question of cell penetration immediately following delivery. Future studies will need to address the above limitations in larger groups prior to widespread acceptance of TVIM as a valid delivery technique.

In conclusion, we found the TVIM strategy to be a safe, feasible and valid approach for cell delivery. In comparison to the IC approach, TVIM was found to be superior in cellular retention both in the infarct zone and in the periphery, however, the study is limited by small sample size. Further studies need to be performed before consideration of this technique of cell delivery in clinical trials for patients post myocardial infarction.

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References

1. World Health Organization. Mortality Data. 2005. Statistical Update. www.who.int
2. Marzilli M. Recurrent and resistant angina: is the metabolic approach an appropriate answer? *Coron Artery Dis.* 2004; 15(suppl I):S23–7. [PubMed: 15179125]
3. Orlic D, Kajstura J, Chimenti S, et al. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc Natl Acad Sci USA.* 2001; 98:10344–9. [PubMed: 11504914]
4. Leor J, Guetta E, Feinberg MS, et al. Human umbilical cord blood-derived CD133+ cells enhance function and repair of the infarcted myocardium. *Stem Cells.* 2006; 24:772–80. [PubMed: 16195418]
5. Bhakta S, Greco NJ, Finney MR, et al. The safety of autologous intracoronary stem cell injections in a porcine model of chronic myocardial ischemia. *J Invasive Cardiol.* 2006; 18:212–8. [PubMed: 16670448]
6. Freyman T, Polin G, Osman H, et al. A quantitative, randomized study evaluating three methods of mesenchymal stem cell delivery following myocardial infarction. *Eur Heart J.* 2006; 27:1114–22. [PubMed: 16510464]
7. Ott HC, Kroess R, Bonaros N, et al. Intramyocardial microdepot injection increases the efficacy of skeletal myoblast transplantation. *Eur J Cardiothorac Surg.* 2005; 27:1017–21. [PubMed: 15896611]

8. Suzuki K, Murtuza B, Fukushima S, et al. Targeted cell delivery into infarcted rat hearts by retrograde intracoronary infusion: distribution, dynamics, and influence on cardiac function. *Circulation*. 2004; 110(suppl II):II-225–30. [PubMed: 15364867]
9. Thompson CA, Nasser BA, Makower J, et al. Percutaneous transvenous cellular cardiomyoplasty: a novel nonsurgical approach for myocardial cell transplantation. *J Am Col Cardiol*. 2003; 41(11): 1964–71.
10. Musialek P, Tracz W, Skotnicki AB, et al. Transcoronary stem cell delivery using physiological endothelium-targeting perfusion technique: the rationale and a pilot study involving a comparison with conventional over-the-wire balloon coronary occlusions in patients after recent myocardial infarction. *Kardiol Pol*. 2006; 64:489–98. [PubMed: 16752333]
11. Hou D, Youssef EAS, Brinton TJ, et al. Radiolabeled cell distribution after intramyocardial, intracoronary, and interstitial retrograde coronary venous delivery: implications for current clinical trials. *Circulation*. 2005; 112(suppl I):I150–6. [PubMed: 16159808]
12. Siminiak T, Fiszer D, Jerzykowska O, et al. Percutaneous trans-coronary-venous transplantation of autologous skeletal myoblasts in the treatment of post-infarction myocardial contractility impairment: the POZNAN trial. *Eur Heart J*. 2005; 26:1188–95. [PubMed: 15764613]
13. Baklanov DV, Moodie KM, McCarthy FE, et al. Comparison of transendocardial and retrograde coronary venous intramyocardial catheter delivery systems in healthy and infarcted pigs. *Catheter Cardiovasc Interv*. 2006; 68(3):416–23. [PubMed: 16892441]
14. Perin EC, Lopez J. Methods of stem cell delivery in cardiac diseases. *Nat Clin Pract Cardiovasc Med*. 2006; 3(suppl I):S110–3. [PubMed: 16501616]
15. Kamihata H, Matsubara H, Nishiue T, et al. Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. *Circulation*. 2001; 104:1046–52. [PubMed: 11524400]
16. Fuchs S, Baffour R, Zhou YF, et al. Transendocardial delivery of autologous bone marrow enhances collateral perfusion and regional function in pigs with chronic experimental myocardial ischemia. *J Am Coll Cardiol*. 2001; 37:1726–32. [PubMed: 11345391]
17. Hou M, Yang KM, Zhang H, et al. Transplantation of mesenchymal stem cells from human bone marrow improves damaged heart function in rats. *Int J Cardiol*. 2007; 115(2):220–8. [PubMed: 16889848]
18. Makela J, Ylitalo K, Lehtonen S, et al. Bone marrow-derived mononuclear cell transplantation improves myocardial recovery by enhancing cellular recruitment and differentiation at the infarction site. *J Thorac Cardiovasc Surg*. 2007; 134(3):565–73. [PubMed: 17723800]
19. Henning RJ, Burgos JD, Ondrovic L, et al. Human umbilical cord blood progenitor cells are attracted to infarcted myocardium and significantly reduce myocardial infarction size. *Cell Transplant*. 2006; 15(7):647–58. [PubMed: 17176616]
20. Hu CH, Wu GF, Wang XQ, et al. Transplanted human umbilical cord blood mononuclear cells improve left ventricular function through angiogenesis in myocardial infarction. *Chin Med J*. 2006; 119(18):1499–506. [PubMed: 16996002]
21. Kim BO, Tian H, Prasongsukarn K, et al. Cell transplantation improves ventricular function after a myocardial infarction: a preclinical study of human unrestricted somatic stem cells in a porcine model. *Circulation*. 2005; 112(9 suppl):I96–104. [PubMed: 16159872]
22. Ma N, Stamm C, Kaminski A, et al. Human cord blood cells induce angiogenesis following myocardial infarction in NOD/SCID mice. *Cardiovasc Res*. 2005; 66(1):45–54. [PubMed: 15769447]
23. Herity NA, Lo ST, Oei F, et al. Selective regional myocardial infiltration by the percutaneous coronary venous route: a novel technique for local drug delivery. *Catheter Cardiovasc Interv*. 2000; 51:358–63. [PubMed: 11066126]
24. Hou D, Maclaughlin F, Thiesse M, et al. Widespread regional myocardial transfection by plasmid encoding Del-1 following retrograde coronary venous delivery. *Catheter Cardiovasc Interv*. 2003; 58:207–11. [PubMed: 12552546]
25. Youssef EAS, Zhang P, Rogers PI, et al. Enhancing myocardial plasmid expression by retrograde coronary venous delivery. *Catheter Cardiovasc Interv*. 2005; 65:528–34. [PubMed: 16003788]

Figure 1.

The TransAccess™ catheter system is delivered via access into the coronary venous system by engaging the coronary sinus. The catheter incorporates an intravascular ultrasound (IVUS) to guide an extendable nitinol needle into the myocardium. Remote areas of the myocardium can then be accessed via targeted injections from the coronary venous system. (Figures adapted from Thompson CA et al, JACC 2003)

Figure 2.
Fluoroscopy demonstrating myocardial infarction via balloon occlusion of the mid LAD.

Figure 3. Fluoroscopy demonstrating access into the coronary sinus and delivery of the TransAccess™ catheter system.

Figure 4. Schematic showing the thirty sections taken from the infarct and peri-infarct zones of pig heart for immunohistochemical analysis.

Figure 5. GFP-labelled BM-MNC in the infarct zone demonstrating cellular retention after TVIM delivery. Cells were detected by using anti-GFP antibody in an immunofluorescence staining. Nucleus of cells was stained with DAPI.

Figure 6. GFP- labeled BM-MNC in the infarct zone demonstrating cellular retention after IC delivery. Cells were detected by using anti-GFP antibody in an immunofluorescence staining. Nucleus of cells was stained with DAPI.

Figure 7.

Histograms demonstrating cellular retention of BM-MNC in the infarct [7a] and peri-infarct [7b] zones after cell delivery via IC and TVIM approaches. Data are reported as mean with error bars representing standard deviation within each group.