Published in final edited form as:

J Mol Cell Cardiol. 2008 March ; 44(3): 473–476. doi:10.1016/j.yjmcc.2007.10.024.

Cell Therapy for Myocardial Infarction: Special Delivery

Alan W. Heldman, MD and Joshua M. Hare, MD

From the Cardiovascular Division and Interdisciplinary Stem Cell Institute, University of Miami, Miller School of Medicine, Miami Florida

The experimental finding that exogenous cell-based therapies stimulate myocardial repair or actual regeneration has revolutionized our understanding of cardiac biology. Traditionally accepted principles – that cardiac myocytes are terminally differentiated; that myocardial infarction (MI) causes irreversible myocardial damage; that healing consists solely of scarring, fibrosis and remodeling – have been replaced by the expectation of new cell-based therapies to repair the damage of MI.

Mesenchymal stem cells (MSCs) are bone marrow-derived non-hematopoietic cells with the potential to differentiate along various lineages depending upon environmental cues from their niche: bone and cartilage¹; fat, tendon, and muscle²; and cardiomyocytes³. These cells were recognized in 1968 by Friedenstein who isolated cells from guinea pig bone marrow which were adherent in culture and which differentiated into bone.⁴ Surface antigens specific for human MSCs were identified in 1992 by Haynesworth.⁵ Although MSCs are rare, representing approximately 0.01% of the bone marrow mononuclear cell fraction, they have attractive features for therapy, including the ability to expand many log-fold *in vitro*,⁶ and unique immune characteristics^{7,8} allowing their use as an allogeneic graft.

The development of MSCs as a therapy for heart damage has followed a logical progression from *in vitro* studies through small animal and large animal studies, and now into clinical trials.⁹ Yet as this sequence has unfolded, questions resulting from the later stage science have pointed out the need to return to cell culture and pre-clinical investigations throughout the process. Rather than being a one-way progression, we have seen that large animal studies are critical to understanding mechanisms of cell therapy, so that the optimal scientific approach involves the parallel conduct of basic, preclinical translational, clinical translational and mechanistic studies.

MSCs have shown a high degree of efficacy in rodent¹⁰, canine¹¹, and porcine models¹², and hold promise as a therapy for ischemic heart disease and acute MI.²⁵ Preclinical studies indicate that their delivery by myocardial injection can reduce infarct size even in chronic infarction.¹³ In a small clinical trial of patients with chronic MI and severe LV dysfunction, bone marrow mononuclear cell injection improved LV function and heart failure symptoms.¹⁴ For cardiac applications, MSCs may be among the most active constituents of the bone marrow. Crucial questions exist in the field, relating in large part to dose, timing, and route of administration. These questions are essential for translational understanding of the therapy, and are related to mechanistic questions. It is in this context that it has become essential to compare delivery methods and their impact on cell retention and subsequent therapeutic efficacy. To date various

Address for correspondence: Alan W. Heldman, MD, 1120 NW 14th St., Room 1119, Miami FL 33136, aheldman@med.miami.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

studies of MSCs have used one of three delivery methods: intravenous infusions, intracoronary infusions, or direct myocardial injection by either transepical or transendocardial needle entry. Head to head comparisons to clarify the balance of risks and benefits of various delivery approaches are relatively lacking, but are essential to guide future clinical trials.

In this issue of the Journal, Perin and colleagues contribute an important set of experimental findings towards our understanding of cell delivery strategies. Using a canine model of myocardial infarction created by 3 hour occlusion of the proximal LAD with ligation of the diagonal branch, they delivered 100×10^6 allogeneic MSCs 7 days after infarction, by either one of two different routes: slow intracoronary infusion through an angiographic catheter, or transendocardial injection using the Biosense NOGA® electromechanical mapping system. Comparing outcomes two weeks after administration they report that the transendocardial approach improved ejection fraction to a greater degree than the intracoronary delivery approach, and that this difference correlated with greater cell retention after the direct myocardial injection. Importantly they also report on the safety of these two approaches, including reporting no ectopic tissue formation in the heart.

We¹⁵ and others including Perin's group have previously reported that transendocardial catheter-based delivery of MSCs after MI is a highly effective therapy, leading to reduced infarct size and improved left ventricular function in animal models. In addition to the greater efficacy of transendocardial delivery, the new work from Perin and co-workers also reports greater safety with this delivery approach. MSCs are large: rounded up cells are ~22–25 microns diameter; and previous animal studies have reported that intracoronary infusion of these cells can cause microvascular obstruction, manifesting as slow coronary flow ("no-reflow"), microinfarction, or death. We noted severe no-reflow in a porcine model with intracoronary MSC infusion (unpublished observations), and Vulliamis and colleagues found microinfarctions in a canine preparation after coronary infusion.¹⁶ Yet in small pilot studies in humans, intracoronary MSC infusion has been better tolerated than the large animal models might have suggested^{17,18}; undoubtedly, cell concentration and other technical components of an intracoronary infusion protocol could influence the ability of cells to pass through the capillary circulation, and to transmigrate into the target tissue. In the acute infarct setting, myocardial factors may favor trafficking of cells to the infarct zone, so that intravenous delivery also may be possible²⁵, despite preclinical evidence for some filtration by the pulmonary circulation.^{19,20} Direct intramyocardial injection of cells bypasses these obstacles for cell delivery, and a small number of laboratories are addressing the challenges of perfecting surgical transepical injection or catheter transendocardial injection approaches.

At the current time there are only a few catheter systems available for clinical and preclinical research applications for transendocardial injection cell-therapy. The Biosense system used in Perin's lab is a remarkable machine, allowing intracardiac navigation, collection of local electrograms and local shortening values, and creation of a 3-dimensional electroanatomical map. A different version of the system used to guide electrophysiology procedures has been widely adopted for clinical use, but the NOGA® system for the left ventricle (LV) has been searching for an interventional application for many years. Perin's group has performed human studies of endocardial injection with other cell types.²¹ Their comparison of transendocardial injection with intracoronary infusion of MSCs is important because there are few other studies specifically addressing this issue. One such study, however, by Freyman and co-investigators, compared intracoronary, transendocardial injection with the Boston Scientific Stilleto® catheter, and intravenous delivery of identically prepared MSCs in a porcine MI model.²² Using an inflated occlusive coronary balloon to deliver the cells into the distal bed (a very different intracoronary technique from Perin's), Freyman found that this protocol for intracoronary delivery was the most efficient technique. Like Perin, however, they also found signs of microvascular obstruction in some animals receiving intracoronary MSCs. The Stilleto®

system allows rapid injection delivery to a number of endocardial sites. A third catheter system, the Biocardia Helical Infusion System,, was used in our studies in porcine models and has been used in one clinical trial.²³ Its steerable deflectable guiding catheter allows access to nearly all parts of the LV, and its helical screw-in needle provides positive engagement into the myocardium.

That the two comparison studies came to different results on the question of the most efficient delivery strategy is not surprising, but it points out a feature common both to scientific revolutions²⁴ and to immature fields of biomedical investigation: the absence of standardized methods. Porcine or canine models? Cell delivery 7 days after infarction, or 15 minutes after? Slow intracoronary infusion of a cell suspension diluted into the coronary blood flow through a simple angiographic catheter, or “stop-flow” delivery of concentrated cell suspension through an occlusive angioplasty balloon? Measure global LV function, regional function, or infarct size?

Three preclinical studies (see Table 1) offer some important lessons and guidance. They indicate that transendocardial injection of cells after MI appears to be technically safe. Mesenchymal stem cells themselves appear to be safe. In particular, large animal studies of MSC delivery to the heart have not indicated any concerns for tumorigenesis, cardiac calcification, or proarrhythmia. These preclinical studies suggest that we should be cautious about infusing large numbers of MSCs directly into the coronary arterial circulation. The varied animal studies also highlight a need for more uniform methodologies in assessing outcome. Time of follow-up, efficacy measures on cardiac performance, tissue and histological findings are key issues, yet vary considerably across preclinical studies.

As the field moves from animal experimentation to clinical trials, practical considerations will prove important for the integration of cell therapy into the health care system. Each delivery strategy has logistical advantages and disadvantages (Table 2). Intravenous delivery of MSCs has been studied in one clinical trial.²⁵ Delivery of MSCs by transendocardial injection catheter will be tested in clinical trials. We have experience with three catheter systems (Biosense, BioCardia, and Boston Scientific), and each has features to recommend it.

The delivery of MSCs into infarcted myocardium by transendocardial injection appears to be one of the most promising new therapies on our horizon. As highlighted here, large animal studies remain invaluable for studying cell types, delivery approaches and other parts of the administration strategies, while contributing importantly to our understanding of potential mechanisms of benefit for cell therapy. Together the studies conducted to date indicate the importance of preclinical models in guiding future human clinical trials, both with regard to safety and efficacy. As with human studies, the value of the preclinical experiments will be enhanced by adopting some uniformity of the questions posed and of the endpoints measured. It is clear from the totality of studies conducted to date that investigators should strive to measure at a minimum cell engraftment, infarct size, and both global and regional LV function. In addition, longer follow-up is to be encouraged so as to correlate early events with sustainability of the treatment effect. The publication of these studies, especially if uniform endpoint measures are chosen, will be invaluable in guiding the design of future clinical trials.

Acknowledgments

The authors gratefully acknowledge helpful comments from Mark Pittenger. The authors are supported by NIH grants R01HL084275, RO1 AG025017 and U54 HL-081028 (Specialized Center for Cell-based Therapy).

References

1. Haynesworth SE, Goshima J, Goldber VM, Caplan AI. Characterization of Cells with Osteogenic Potential from Human Marrow. *Bone* 1992;13:81–88. [PubMed: 1581112]
2. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;284(5411):143–7. [PubMed: 10102814]
3. Makino S, Fukuda K, Miyoshi S, Konishi F, Kodama H, Pan J, Sano M, Takahashi T, Hori S, Abe H, Hata J, Umezawa A, Ogawa S. Cardiomyocytes can be generated from marrow stromal cells in vitro. *J Clin Invest* 1999;103(5):697–705. [PubMed: 10074487]
4. Friedenstein AJ, Petrakova KV, Kurolesova AI, Frolova GP. Heterotopic transplants of bone marrow: Analysis of precursor cells for osteogenic and haematopoietic tissues. *Transplantation* 1968;6:230–47. [PubMed: 5654088]
5. Haynesworth SE, Baber MA, Caplan AI. Cell surface antigens on human marrow-derived mesenchymal cells are detected by monoclonal antibodies. *Bone* 1992;13:69–80. [PubMed: 1316137]
6. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;284:143–147. [PubMed: 10102814]
7. Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringdén O. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp Hematol* 2003;31:890–896. [PubMed: 14550804]
8. Uccelli A, Moretta L, Pistoia V. Immunoregulatory function of mesenchymal stem cells. *Eur J Immunol* 2006;36(10):2566–73. [PubMed: 17013987]
9. Boyle AJ, Schulman SP, Hare JM, Oettgen P. Is stem cell therapy ready for patients? Stem Cell Therapy for Cardiac Repair. Ready for the Next Step. *Circulation* 2006 Jul 25;114(4):339–52. [PubMed: 16864739]
10. Orlic D, Kajstura J, Chimenti S, Bodine DM, Leri A, Anversa P. Transplanted adult bone marrow cells repair myocardial infarcts in mice. *Ann N Y Acad Sci* 2001 Jun;938:221–9. [PubMed: 11458511]
11. Silva GV, Litovsky S, Assad JA, Sousa AL, Martin BJ, Vela D, Coulter SC, Lin J, Ober J, Vaughn WK, Branco RV, Oliveira EM, He R, Geng YJ, Willerson JT, Perin EC. Mesenchymal stem cells differentiate into an endothelial phenotype, enhance vascular density, and improve heart function in a canine chronic ischemia model. *Circulation* 2005 Jan 18;111(2):150–6. [PubMed: 15642764]
12. Amado LC, Schuleri KH, Saliaris AP, Boyle AJ, Helm R, Oskouei B, Centola M, Eneboe V, Young R, Lima JA, Lardo AC, Heldman AW, Hare JM. Multimodality noninvasive imaging demonstrates in vivo cardiac regeneration after mesenchymal stem cell therapy. *J Am Coll Cardiol* 2006 Nov 21;48(10):2116–24. [PubMed: 17113001]
13. Schuleri, Karl H.; Centola, Marco; Zimet, Jeffrey M.; Boyle, Andrew J.; Feigenbaum, Gary S.; Heldman, Alan W.; Lardo, Albert C.; Hare, Joshua M. Intramyocardial Allogeneic Mesenchymal Stem Cells Reduce Infarct Size in a Porcine Model of Chronic Ischemic Cardiomyopathy. *J Am Coll Cardiol* 49(Issue 9 Supplement 1):140A, 907–233.[abstract].
14. Beeres SL, Bax JJ, Dibbets-Schneider P, Stokkel MP, Fibbe WE, van der Wall EE, Schalij MJ, Atsma DE. Intramyocardial injection of autologous bone marrow mononuclear cells in patients with chronic myocardial infarction and severe left ventricular dysfunction. *Am J Cardiol* 2007 Oct 1;100(7):1094–8. [PubMed: 17884369]
15. Amado LC, Saliaris AP, Schuleri KH, St John M, Xie JS, Cattaneo S, Durand DJ, Fitton T, Kuang JQ, Stewart G, Lehrke S, Baumgartner WW, Martin BJ, Heldman AW, Hare JM. Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. *PNAS* 2005;102:11474–11479. [PubMed: 16061805]
16. Vulliet RP, Greeley M, Halloran SM, MacDonald KA, Kittleson MD. Intra-coronary arterial injection of mesenchymal stromal cells and microinfarction in dogs. *Lancet* 2004;363:783–784. [PubMed: 15016490]
17. Chen SL, Fang WW, Ye F, Liu YH, Qian J, Shan SJ, Zhang JJ, Chunhua RZ, Liao LM, Lin S, Sun JP. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow

- mesenchymal stem cell in patients with acute myocardial infarction. *Am J Cardiol* 2004;94(1):92–5. [PubMed: 15219514]
18. Katritsis DG, Sotiropoulou PA, Karvouni E, Karabinos I, Korovesis S, Perez SA, Vordis EM, Papamichail M. Transcatheter transplantation of autologous mesenchymal stem cells and endothelial progenitors into infarcted human myocardium. *Catheter Cardiovasc Interv* 2005;65(3): 321–9. [PubMed: 15954106]
 19. Barbash IM, Chouraqui P, Baron J, Feinberg MS, Etzion S, Tessone A, Miller L, Guetta E, Zipori D, Kedes LH, Kloner RA, Leor J. Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. *Circulation* 2003 Aug 19;108(7):863–8. [PubMed: 12900340]
 20. Chin BB, et al. *Nuclear Medicine Communications* 2003;24(11):1149–1154. [PubMed: 14569169]
 21. Silva GV, Perin EC, Dohmann HF, Borojevic R, Silva SA, Sousa AL, Assad JA, Vaughn WK, Mesquita CT, Belém L, Carvalho AC, Dohmann HJ, Barroso do Amaral E, Coutinho J, Branco R, Oliveira E, Willerson JT. Catheter-based transendocardial delivery of autologous bone-marrow-derived mononuclear cells in patients listed for heart transplantation. *Tex Heart Inst J* 2004;31(3): 214–9. [PubMed: 15562839]
 22. Freyman T, Polin G, Osman H, Crary J, Lu M, Cheng L, Palasis M, Wilensky RL. A quantitative, randomized study evaluating three methods of mesenchymal stem cell delivery following myocardial infarction. *Eur Heart J* 2006 May;27(9):1114–22. [PubMed: 16510464]
 23. de la Fuente LM, Stertzer SH, Argentieri J, Penaloza E, Miano J, Koziner B, Bilos C, Altman PA. Transendocardial autologous bone marrow in chronic myocardial infarction using a helical needle catheter: 1-year follow-up in an open-label, nonrandomized, single-center pilot study (the TABMMI study). *Am Heart J* 2007;154:79. [PubMed: 17584556]
 24. Kuhn, TS. *The Structure of Scientific Revolutions*. Vol. 1. Chicago: Univ. of Chicago Pr; 1962.
 25. Hare, JM. A Double-Blind, Randomized, Placebo Controlled Clinical Trial of Allogeneic Mesenchymal Stem Cells for the Treatment of Patients With Acute Myocardial Infarction; American College of Cardiology Scientific Sessions; March 2007;

Table 1

Transendocardial injection of MSCs appears to be safe and effective.

Study	Animal	Coronary occlusion time	Time between MI & cell delivery	Dose, cell type	Guidance; delivery catheter	Follow-up period after cell delivery	Effect of cells on LV function	Infarct size	Engraftment; histology	Safety findings for delivery technique
Amado et al. 2005	Female Yorkshire swine	Surgical or balloon occlusion of LAD × 60 min	3 days	100–200×10 ⁶ allogeneic porcine MSCs	Fluoroscopic guidance; Stiletto™ endocardial injection catheter or BioCardia™ endocardial injection catheter	8 weeks	P-V loops: Normalized systolic and diastolic function; improved. MRI: EF improved. Restored mechanoenergetics.	Necropsy: infarct reduced from 16% (controls) to 3.4% (MSC-treated)	Labeled cells detected in infarct and border zones up to 8 wks.	No complications
					Intravenous	14 days	Not studied.	Necropsy: No difference among groups	Iridium-labelled cell retention: 0% of administered dose	Lung retention.
					Intracoronary via occlusive balloon catheter				6% of administered dose. MSCs associated with viable myocardium and arterioles. Arteriolar plugging.	Slow-coronary flow in 3/6, without immediate complication but with more fibrosis and thinning.
Perin et al. (editor supply year)	Dog	Surgically occluded LAD × 3hrs; LADD ligated	7 days	100×10 ⁶ allogeneic canine MSCs	Stiletto™ endocardial injection catheter	14 days	Echo: EF did not change after cells	Necropsy: No difference vs. control for either group.	3% of administered dose. MSCs associated with viable myocardium and arterioles.	Lower lung retention of cells compared with IV or IC.
					Intracoronary via angiographic catheter in the LAD				Homogeneous distribution of cells, highest at injury border. Microvascular plugging by MSCs associated with no-reflow.	No-reflow in 1/7 → death
					Electromechanical map guidance; Myostar™ endocardial injection catheter		Echo: EF improved after TE cells. EMM ischemic area reduced compared with controls and IC cells.		High local concentration of cells clustered at injection site.	No complications

LAD: Left Anterior Descending. LADD: Left Anterior Descending Diagonal branch. MSCs: mesenchymal stem cells. Hrs: hours. min: minutes. EMM: electromechanical mapping. IC: intracoronary. IV: intravenous. TE: Transendocardial injection. EF: left ventricular ejection fraction. P-V loops: Pressure-volume loops.

Table 2

Logistical implications of strategies for delivering cells to the heart.

Delivery Approach	Advantages	Disadvantages
Epicardial injection	Could be performed along with coronary bypass. Spatial accuracy of injections.	Requires surgery. Access to interventricular septum may be limited.
Endocardial injection	Access to all territories of the LV. Less invasive than surgery.	Potential for cardiac perforation. May be hard to reach all parts of the LV with some systems. Biosense™ systems require additional cath lab equipment.
Intracoronary infusion	Access to the risk region subtended by the infarct-related artery. Familiar, simple technique.	Microvascular obstruction caused by cell sludging may cause microinfarction.
Intravenous infusion	Simple. Systemic benefits possible.	Low efficiency delivery of cells to heart; filtration by lungs; extracardiac delivery of cells.