Online Supplement

Comparative Efficacy of Intracoronary Allogeneic Mesenchymal Stem Cells and Cardiosphere-Derived Cells in Swine with Hibernating Myocardium

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Abbreviated Title – MSCs vs. CDCs in Hibernating Myocardium

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Supplemental Methods

Isolation of MSCs and CDCs

Mesenchymal Stem Cell Isolation: Bone marrow was sampled for isolation and expansion of MSCs 2-months after instrumentation as previously described[1]. Approximately 30 ml of bone marrow was aspirated from the sternum under propofol sedation. Samples were heparinized using a 1:10 (v:v) ratio of preservative-free heparin to bone marrow aspirate and placed in a Ficoll gradient (BD Vacutainer CPT with Sodium Heparin) for isolation of the buffy coat. Samples were centrifuged at 2250 RPM (1160 RCF) for 20 minutes at 25ºC and 2 ml of plasma was cryopreserved until MSC implantation. For each sample, 1.5-2 ml of monocyte/lymphocyte layer was removed from the buffy coat layer, washed twice in Hanks Balanced Salt Solution (HBSS), resuspended in 10 ml ADMEM (Invitrogen/Gibco Inc.) with 10% FBS and plated on separate plastic 100 mm tissue culture dishes (BD Biosciences). Samples were incubated in a humidified atmosphere at 37ºC and 5% CO_2 and adherent cells were permitted to attach to the bottom of the plastic dishes. Non-adherent cells were easily detached by successive washings with HBSS at 2 and 4 days after initial plating followed by replacement of fresh media. Upon reaching confluency (5-6 days after initial plating), all cultures were virtually devoid of non-adherent cells and trypsinized (0.25% trypsin in HBSS solution) and expanded in plastic 150 mm tissue culture dishes (BD Biosciences). Culture media was changed every 4-5 days and after trypsinizations. After ~3-weeks of cultivation, cells were collected for intracoronary infusion.

Cardiosphere-Derived Cell Isolation: Left ventricular tissue specimens were obtained by needle biopsies (2-5 biopsies from the LV basal free wall, 20-50 mg total) and cut into 1-2mm pieces [2, 3]. After removal of gross connective tissue, they were washed and partially enzymatically digested in a solution of type IV collagenase for 60 minutes at 37 degrees. Tissue fragments were cultured as "explants" on dishes coated with fibronectin. After ~8 days, a layer of stromal-like cells arose from and surrounded the explants. Over this layer a population of small, round, phase-bright cells migrated. Once confluent, the cells surrounding the explants were harvested by gentle enzymatic digestion. These cardiosphere-forming cells were seeded at 2 to $3x10^4$ cells/mL on poly-D-lysine–coated dishes in cardiosphere medium (20% heat-inactivated fetal calf serum, gentamicin 50μg/ml, 2mmol/L L-glutamine, and 0.1mmol/L 2-mercaptoethanol in Iscove's modified Dulbecco medium). Cardiospheres formed after 4-10 days in culture, detached from the tissue culture surface, and began to slowly grow in suspension. When sufficient in size and number, free-floating cardiospheres were harvested by aspirating them along with media. Cells that remained adherent to the poly-D-lysine–coated dishes were discarded. Detached cardiospheres were then plated on fibronectin-coated flasks where they attached to the culture surface and formed monolayers of CDCs. Cells were subsequently passaged by trypsinization and splitting at a 1:2 ratio until a sufficient number were available.

Supplemental References

1. Suzuki G, Iyer V, Lee TC and Canty JM, Jr. Autologous Mesenchymal Stem Cells Mobilize cKit+ and CD133+ Bone Marrow Progenitor Cells and Improve Regional Function in Hibernating Myocardium. *Circ Res*. 2011;109:1044-54.

2. Suzuki G, Weil BR, Leiker MM, Ribbeck AE, Young RF, Cimato TR and Canty JM, Jr. Global Intracoronary Infusion of Allogeneic Cardiosphere-Derived Cells Improves Ventricular Function and Stimulates Endogenous Myocyte Regeneration throughout the Heart in Swine with Hibernating Myocardium. *PLoS One*. 2014;9:e113009.

3. Smith RR, Barile L, Cho HC, Leppo MK, Hare JM, Messina E, Giacomello A, Abraham MR and Marban E. Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. *Circulation*. 2007;115:896-908.

Supplemental Table I. Effects of icMSCs and icCDCs on Hemodynamics at Rest and During Adenosine Vasodilation in Swine with Hibernating Myocardium. Paired analysis of data at 4-months vs. 3-months for each treatment group

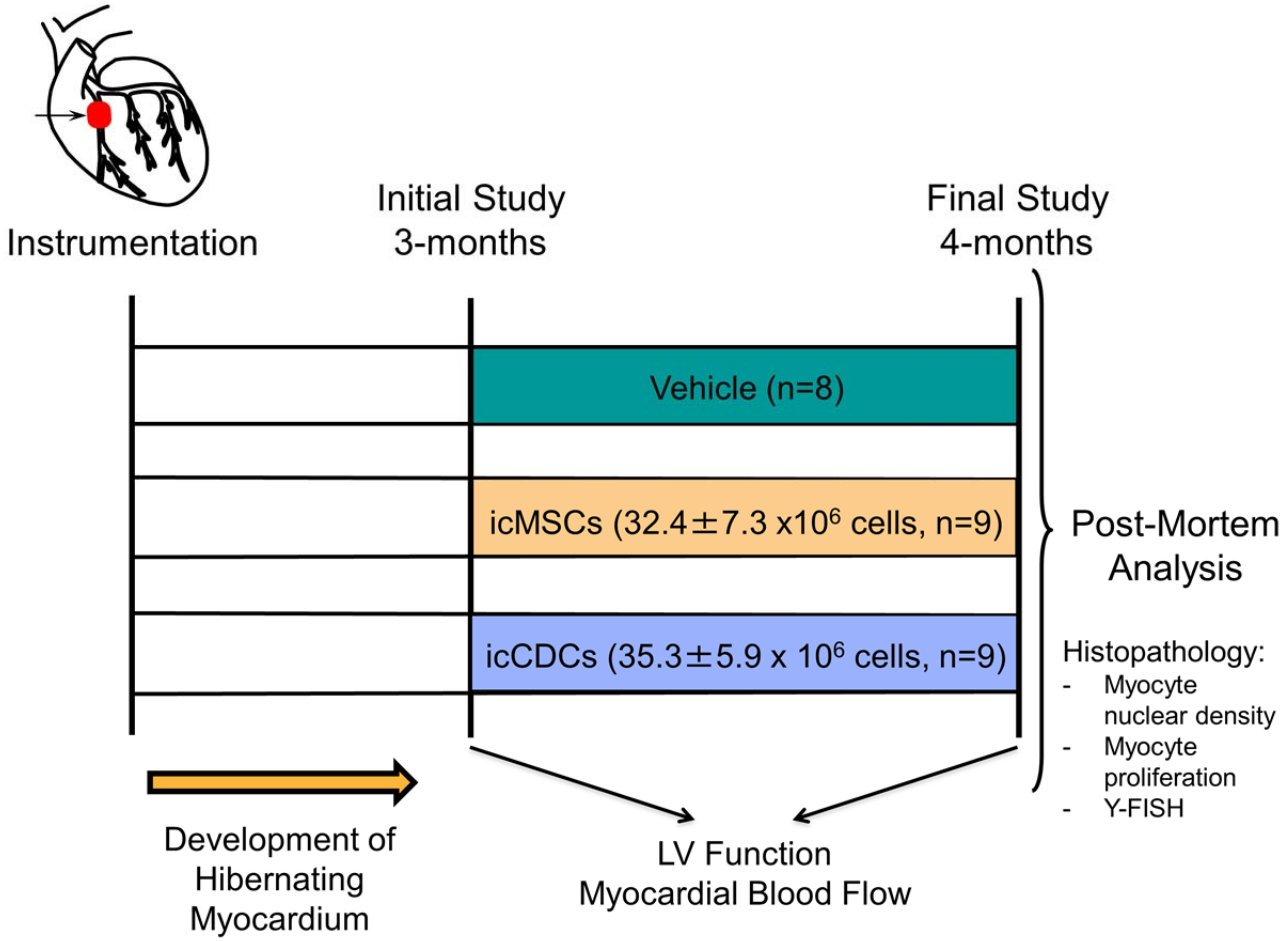
	Systolic Pressure (mm Hg)	Mean Aortic Pressure (mm Hg)	Heart Rate (bpm)	LV dP/dt $_{Max}$ (mm Hg/sec)	LV dP/dt $_{Min}$ (mm Hg/sec)
REST					
Initial					
Vehicle	136±19	109±14	92±15	2367±317	-2745±912
icMSCs	127±19	101±17	102±15	2534±302	-2613±571
icCDCs	138±15	113±16	99±19	2379±328	-2665±478
Final					
Vehicle	143±14	114±12	85±16	2360±317	-2399±242
icMSCs	139±16	110±17	92±13	2224±370	-2437±439
icCDCs	141±14	115±13	93±14	2302±371	-2569±313
ADENOSINE					
Initial					
Vehicle	137±21	95±22	81±15	2163±685	-2109±863
icMSCs	140±14	94±18	90±18	2267±632	-2133±417
icCDCs	136±8	102±8	103±14	2550±383	-2218±464
Final					
Vehicle	141±11	102±15	88±13	2280±401	-1928±362
icMSCs	140±11	99±13	95±12	2287±480	-1889±355
icCDCs	139±13	101±17	95±19	2392±529	-1938±332

Values are mean±SD

Supplemental Table II: Echocardiographic Indices of Left Ventricular Function in Vehicle-, icMSC-, and icCDC-Treated Animals with Hibernating Myocardium.

	LAD Region		Remote Region		Global Function
	%WT (%)	Δ WT (mm)	WT (%)	Δ WT (mm)	LVEF (%)
Vehicle					
3-Months	37.9±12.7	3.6±1.4	77.4±18.4	6.1±1.1	54.7±4.2
4-Months	33.7±9.3	3.5±1.1	59.5±18.1*	5.5±1.7	57.2±9.6
icMSCs					
3-Months	38.1±9.3	3.6±0.9	89.0±20.4	6.2±1.2	58.6±8.2
4-Months	50.7±13.5*#	4.9±1.2*#	88.4±26.1#	7.0±2.1	58.4±8.2
icCDCs					
3-Months	38.1±12.6	3.7±1.2	71.7±19.5	6.1±1.2	57.5±8.4
4-Months	50.9±16.5*#	5.0±1.8*#	94.3±39.3#	7.2±1.5	59.1±10.2

Values are mean±SD; * $p<0.05$ vs. 3-months, # $p<0.05$ vs. Vehicle; LAD – Left Anterior Descending Artery; WT – Wall Thickening; %WT - %Wall Thickening; %WT = 100 * (End Systolic WT – End Diastolic WT)/ End Diastolic WT; Δ WT – End Systolic WT – End Diastolic WT; LVEF = Left Ventricular Ejection Fraction



Supplemental Figure I: Experimental Study Protocol. Juvenile swine were instrumented with a Delrin LAD stenosis to produce hibernating myocardium which consistently developed after 3-months. At that time, a baseline closed-chest study was conducted to assess LV function (echocardiography), myocardial perfusion at rest and vasodilation (microspheres), hemodynamics, and coronary angiography. Subsequently, animals were randomized to receive global intracoronary infusion of vehicle, icMSCs, or icCDCs in a blinded fashion. All animals returned to the laboratory 1-month later for follow-up studies, after which they were euthanized and the heart was excised for post-mortem histopathological analysis.