Sustained Restoration of LV Dysfunction in a Pig Ischemia-Reperfusion Injury Model Using Human Amnion-derived Mesenchymal Stem Cells Tracked by Manganese-Enhanced MRI

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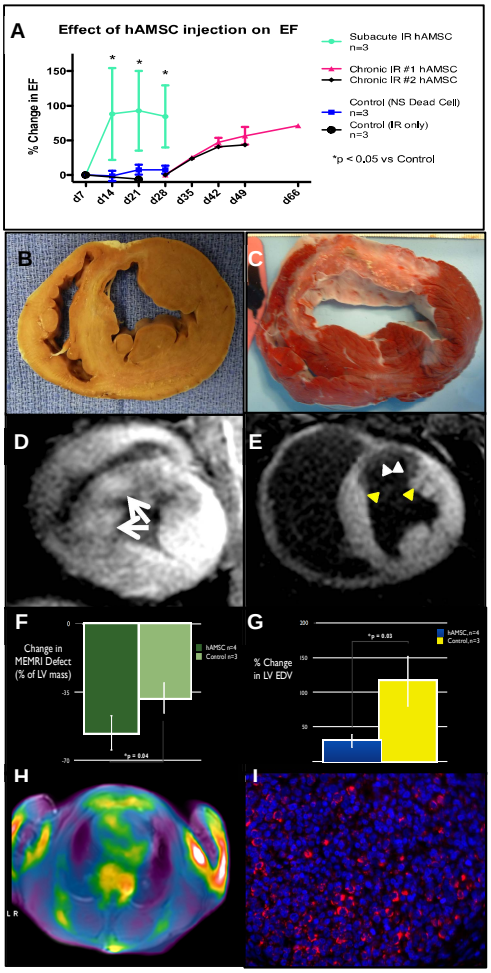
Target Audience: Clinician-scientists with interest in molecular imaging of stem cell therapy for ischemic heart disease

Purpose: It is unclear whether transplanted stem cells can survive and engraft in the heart following transplantation. Human amnion-derived mesenchymal stem cells (hAMSCs) exhibit immunomodulatory cell surface markers that may promote enhanced survival after transplantation. To investigate the viability of transplanted hAMSCs *in vivo*, we used the MEMRI contrast agent, EVP-1001-1 (Eagle Vision Pharmaceuticals, Inc) in a porcine ischemia-reperfusion (IR) injury model. EVP-1001-1 specifically enters the live cells via L-type calcium channels. Following EVP-1001-1 injection, MEMRI delineates infarct zones through T1-signal loss, in contrast to live hAMSCs that exhibit T1-signal gain with MEMRI.

Methods: Nine adult farm pigs underwent 60min left anterior descending coronary IR. One week later, pigs hearts were injected directly with either hAMSCs (~50 million cells/heart, n=6) or normal saline (NS, n=3) into ~8 peri-infarct and infarct zones, using a BioCardia catheter (Biocardia, Inc.). Cardiac MRI (CMR) was performed to assess ventricular function (ejection fraction, EF%), infarct % by delayed gadolinium enhancement MRI (DEMRI), and myocardial viability % by EVP-1001-1 (MEMRI), weekly post-IR. (DEMRI & MEMRI: GE 3T Signa Excite HD: FGRE-irP: RT 4.7ms, TE 1.3ms, FOV 30, TI 200ms, FA 10, ST 10mm, 222x192).



Results: hAMSC and NS EFs were similar at baseline (57±4%, n=5) and 1wk post-IR (24±6%). However, hAMSC injection improved EFs at 1, 2, & 3wks post-hAMSC delivery, compared to NS-injected swine (Figure 1). One possible mechanism for the improved EF was increased peri-infarct viability with hAMSCs. In support of this hypothesis, MEMRI defect (infarct) volume decreased significantly ($p<0.05$) from d7 to d21 post-IR in hAMSC hearts (60±12% reduction, n=3) more than in NS hearts (38±18% reduction, n=3). MEMRI revealed higher contrast-to-noise ratio (CNR) within infarct zones in hAMSC hearts (hAMSC: 8.6±1.4*; NS: 4.9±0.8, n=3, *$p<0.05$), suggesting increasing cellular viability within the infarct and border zones. Moreover signal increased from d7 to d21 after cell delivery. In two swine, 20% of the hAMSCs were transduced with a HSV-tk PET reporter gene, and cardiac PET imaging confirmed co-localizing PET and MEMRI signals, indicating live stem cell populations (Figure 2). Human anti-mitochondrial antibody immunostaining revealed viable hAMSC cell clusters in peri-infarct zones 38 days after transplantation.

Discussion/Conclusions: These results demonstrate that hAMSC delivery in a porcine IR model improves systolic function durably and that hAMSCs survive in vivo for prolonged periods (by PET and MEMRI) with minimal immunosuppression. The mechanism for this functional restoration may be due to improved peri-infarct viability by salvage of the injured cardiomyocytes, as evidenced by a lower MEMRI defect volume and higher MEMRI CNR. MEMRI allows for non-invasive assessment of stem cell survival *in vivo*, without any genetic pre-modification of the transplanted stem cells.

Figure 1. (A) Significant and sustained improvement in cardiac ejection fraction (EF) by CMR upon delivery of hAMSCs to peri-infarct zone in both subacute (7d post-IR) or chronic (28d post-IR) models, whereas saline-injected/controls showed no improvement. (B) Gross hAMSC short-axis section showing increased wall thickness in infarct and peri-compared to control (C). (D) Apical short-axis MEMRI image of hAMSC heart, 38d post-hAMSC delivery, exhibiting high MEMRI CNR within the infarct and peri-infarct zone (white arrows), compared to control (white/yellow triangles)(E). (F) hAMSC hearts exhibited smaller infarct zones by MEMRI Defect quantification and significantly lower LV EDV (G) compared to NS-injected controls. (H) Cardiac PET-CT scan performed 38d post-hAMSC injection, showing increased radiotracer uptake within the inferoseptum and apical segments, indicative of live hAMSC populations. (I) Human anti-mitochondrial antibody stain (red) showing characteristic perinuclear pattern when superimposedon nuclear Hoechst stain (blue). These results confirmation the presence of live hAMSC populations within the peri-infarct zone at 38d post-hAMSC delivery.