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

Rajesh Dash[1], Ildiko Toma[1], Fumiaki Ikeno[1], Jennifer K. Lyons[1], Shahriar Heidary[1], Marie-Claude Parent[1], I-Ning E. Wang[1], Xiaohu Ge[1], Justin Lam[1], Jaehoon Chung[2], Paul J. Kim[1], Kaori Nakagawa[1], Svetlana Lyalina[1], Grace Do[1], Robert C. Robbins[3], Michael V. McConnell[1,4], Alan C. Yeung[1], Phillip Harnish[5], and Phillip C. Yang[1]

[1]Cardiovascular Medicine, Stanford University Medical Center, STANFORD, CA- CALIFORNIA, United States, [2]Medicine / Cardiology, University of Illinois-Chicago, Chicago, IL, United States, [3]Cardiac Surgery, Stanford University Medical Center, [4]Electrical Engineering, Stanford University, [5]Eagle Vision Pharmaceutical Corporation, Downington, PA, United States

Background:
Although stem cell delivery restores cardiac function after myocardial infarction (MI), it is unclear whether cells survive/engraft in the heart following transplantation. To investigate stem cell viability *in vivo*, we used a Manganese-Enhanced MRI (MEMRI) contrast agent, EVP-1001-1 (Eagle Vision Pharmaceuticals, Inc) in a pig ischemia-reperfusion (IR) injury model. EVP-1001-1 specifically enters live cardiac myocytes, and T1-weighted MRI after EVP-1001-1 injection delineates infarct zones as a MEMRI defect. EVP-1001-1 is also taken up avidly by live stem cells. We tested EVP-1001-1's ability to track human amnion-derived mesenchymal stem cells (hAMSCs) after transplantation into pig hearts post-IR.

Hypotheses:
hAMSC delivery will improve cardiac function, and hAMSC survival will be tracked *in vivo* / longitudinally using MEMRI.

Methods:
Five adult farm pigs underwent 60min LAD coronary IR. One week post-IR, pigs hearts were injected with either hAMSCs (~50 million cells/heart, n=3) or normal saline (NS, n=2) into ~8 peri-infarct and infarct zones, by BioCardia catheter injection (Biocardia, Inc.). Cardiac MRI (CMR) was performed to assess ventricular function (ejection fraction, EF%), infarct % by Delayed Gadolinium Enhancement MRI (DEMRI), and MEMRI with EVP-1001-1 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 (Fig. 1A). One possible mechanism for the improved EF was increased peri-infarct viability with hAMSCs. In support of this hypothesis, MEMRI defect (infarct) volume decreased from d7 to d21 post-IR in hAMSC hearts (60±12% reduction, n=3) more than in NS hearts (38±18% reduction, n=2). MEMRI also identified regions of high contrast-to-noise ratio (CNR) within infarct zones in hAMSC hearts (Figure 2: hAMSC: 8.6±1.4*; NS: 4.9±0.8, n=3, *p<0.05), suggesting increased EVP-1001-1 uptake by live hAMSCs within the infarct zone (Figure 3A). This signal increased from d10 to d17 (data not shown). Human nuclear antigen (hNA) immunostaining (Fig. 3B) revealed intact hAMSC cell clusters in infarct zones at d17 post-transplantation.

Figure 1: Effect of hAMSC injection on Cardiac Function

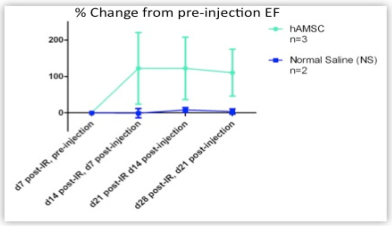


Figure 2: MEMRI CNR of Infarct Zone Higher in hAMSC-treated Hearts

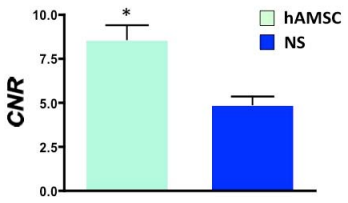
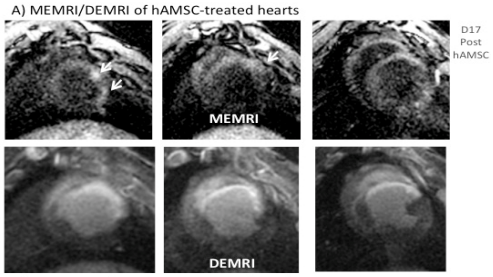


Figure 1. A) hAMSC-injected swine exhibited higher EFs than NS-treated swine at one, two, and three wks post-injection.
Figure 2. MEMRI CNR within the Infarct zone with significantly (*p<0.05, n=3 per group) higher in hAMSC hearts versus NS hearts at 2 weeks post-injection, providing evidence for live hAMSC cells.

A) MEMRI/DEMRI of hAMSC-treated hearts



B) Human Nuclear Antigen Immunohistochemistry

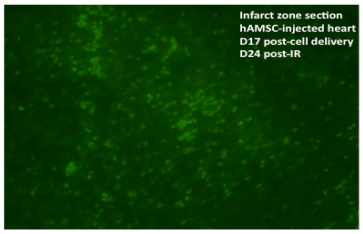


Figure 3. A) Matched short-axis CMR images that show an infarct zone with bright DEMRI signal (bottom row). Discrete foci of bright MEMRI signal (top row, arrows) within islands of MEMRI defect correspond to sites of hAMSC injection. B) hNA staining confirms live hAMSCs in the infarct zone 17d post-delivery.

Conclusions:
Preliminary results demonstrate that hAMSC delivery post-IR improves systolic function compared to control. The mechanism for this functional restoration may be improved peri-infarct viability, as evidenced by a lower MEMRI defect volume in hAMSC-treated hearts. High MEMRI CNR within the infarct zone was associated with positive hNA staining, providing evidence for live hAMSC populations nearly 3 weeks after cell delivery. MEMRI allows both myocardial viability assessment and tracking of stem cell survival/engraftment *in vivo*.