POSTER SESSION

903

Stem Cells

Sunday, March 12, 2006, 9:00 a.m.-12:30 p.m.
Georgia World Congress Center, Hall B1
Presentation Hour: 10:00 a.m.-11:00 a.m.

903-215 Intramyocardial Bone Marrow Stem Cells Transplantation for Non-ischemic Dilated Cardiomyopathy: 4 Months Follow-up

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Background: A study on safety, feasibility and efficacy of transthoracic intramyocardial mononuclear autologous bone marrow stem cells transplantation for end-stage non-ischaemic dilated cardiomyopathy (NIDC) is being conducted. This paper reports preliminary results at 4 months follow-up.
Methods: From Dec/04 to Jun/05, 6 patients(pts) (2 fem), age 42.3±14,1 years, NYHA Class III/IV and (LVEF)<35% were enrolled. All pts underwent clinical, echocardiographic(ECO) and nuclear magnetic ressonance (NMR) assessment. Cells were collected from the iliac bone, isolated, washed and suspended, resulting in a mean of 9.6±2.6 x 10^7 (1.5±0.7% CD34+) cells in 5ml saline. These were injected, through 5-6cm thoracotomy at the 5th left intercostal space, at 20 points of the free left ventricular wall with a 21F Butterfly needle. Pts were reviewed at 2 and 4 months of follow-up.
Results: There were no immediate major complications. One pt required placement of 2 stitches for epicardial bleeding control and other needed lidocaine IV administration for persistent ventricular arrhythmia on touching the heart. There was improvement in NYHA functional class after 2 and 4 months. The baseline x 2 months x 4 months follow-up results were: NYHA Functional Class= IV-2, III-4 to I-5, II-1 and I-3, II-2 pts, respectively. Quality of life evaluated by Living with Heart Failure Questionaire (mean±SE)=65.4±7.6 x 27.0±22.4 x 24.0±23.0 points (p=0.03; p=0.02). ECO: LVEF=26.7±3.9 x 36.0±7.9 x 31.7±4.7% (p=0.05 and 0.19, respectively); LV short´n fract=13.8±4.7 x 17.9±4.1 x 15.5±2.5% (p=0.06; p=0.24); end diastolic volume (EDV)=223.6±110.5 x 161.1±51.1 x 161.9±47.9ml (p=0.12; p=0.137); end systolic volume (ESV)=168.5±99.6 x 106.1±47 x 111.5±38ml (p=0.07; p=0.12). NMR (baseline x 2 months): LVEF=23.2±16.5 x 30.2±15.6% (p=0.04); EDV=415.6±205.9 x 381.2±236.9ml(p=0.14);ESV=339.1±218.4x292.2±232.3 ml(p=0.04);stroke volume=76.2±34.1 x 89±11.7ml (p=0.36)
Conclusions: This study suggests that transthoracic intramyocardial bone marrow stem cell transplantation is feasible and safe through a minithoracotomy. Early improvements on symptoms, quality of life and LV performance encourage further studies.

903-216 Bone Marrow-Derived Cells Do Not Transdifferentiate Into Cardiomyocytes After Myocardial Infarction

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Background: The paradigm that cardiac myocytes are non-proliferating and terminally differentiated cells has recently been challenged since some studies reported the ability of bone marrow derived cells (BMC) to transdifferentiate into cardiomyocytes. However, these results were discussed controversially and could not be reproduced by others. We therefore examined the contribution of BMC in post myocardial infarction repair mechanisms.
Methods: Mice were sublethally irradiated and bone marrow from eGFP transgenic mice was transplanted. Coronary artery ligation was performed 3 months after engraftment. The infarct size was quantified using magnetic resonance imaging. The hearts were studied 7 days (n=13) and 21 days (n=12) after myocardial infarction. Immunohistochemical staining was performed using titin and connexin 43 antibodies to identify cardiomyocytes, vimentin for fibroblasts, vimentin and α-smooth muscle actin for myofibroblasts, α-smooth muscle actin for smooth muscle cells, and F4/80 for macrophages. Endothelial cells were stained by BS-1 and CD31. Additionally, anti-eGFP immunostaining was used to exclude autofluorescence. Sections were analyzed using fluorescence microscopy and confocal laser microscopy.
Results: Bone marrow transplantation was successful as FACS analysis showed 84±5% eGFP expressing leukocytes. The occlusion typically resulted in infarct sizes of 41±6% of the left ventricle. In 25 examined hearts, only 3 eGFP positive cardiomyocytes were found. CD45+/eGFP+ inflammatory cells were found frequently after 7 days and to a lesser degree after 21 days. Numerous BMC derived fibroblasts and myofibroblasts were found in the periinfarct and infarct area. BMC contributed sporadically to scar tissue neoangiogenesis but not to angiogenesis in the periinfarct region or in the remote zone.
Conclusion: Transdifferentiation of BMC into viable cardiomyocytes is a negligible event in the normal repair processes after myocardial damage. Correspondingly, no BMC-induced neoangiogenesis could be documented. However, scar tissue formation after myocardial infarction largely involves BMC-derived cells.

903-217 TGF-β1 Enhances Cardiomyogenic Differentiation Potential of Adult Primitive Cells

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Culture conditions are crucial for lineage commitment of pluripotent stem cells (PSCs) and the optimal medium for cardiac differentiation remains to be established. We compared the efficacy of four cytokine/growth factor combinations in the induction of cardiomyogenic differentiation in murine skeletal muscle (SM)-derived PSCs. Sca1+/CD45-/c-kit-/Thy-1+ (SM+) and Sca1-/CD45-/c-kit-/Thy-1+ (SM-) cells were differentiated in basic medium (BM) or BM supplemented with bFGF, oxytocin (OT), or TGF-β1. The expression of cardiac-specific transcription factors (TFs) and intracellular antigens (IAs) was evaluated by confocal microscopy (Fig. 1A). After 28 days of differentiation, BM alone, BM+bFGF, BM+OT, and BM+TGF-β1 induced cardiac differentiation in 12.9%, 11.6%, 9.1%, and 14.9% of cultured SM+ cells, respectively, that expressed cardiac-specific TFs and/or IAs. In cultured SM- cells, BM alone, BM+bFGF, BM+OT, and BM+TGF-β1 induced cardiac differentiation in 16.6%, 15.8%, 11.8%, and 25.2% cells, respectively. Compared with SM+ cells, SM- cells exhibited a relatively higher potential to differentiate into cardiomyocytes irrespective of the medium. We conclude that supplementation of culture medium with TGF-β1, a growth factor known to modulate cardiac gene expression, enhances cardiomyogenic differentiation in SM-derived PSCs. This strategy may potentially be utilized to culture and test the cardiomyogenic potential of other adult tissue-derived primitive cells.

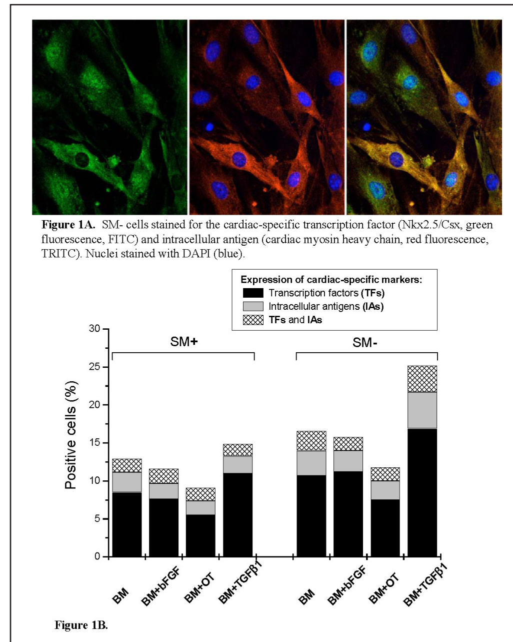

Figure 1A. SM- cells stained for the cardiac-specific transcription factor (Nkx2.5/Csx, green fluorescence, FITC) and intracellular antigen (cardiac myosin heavy chain, red fluorescence, TRITC). Nuclei stained with DAPI (blue).

Figure 1B.

903-218 Tracking of Intra-Coronary Delivered Mesenchymal Stem Cells Using Magnetic Resonance Imaging in a Porcine Model of Myocardial Infarction

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Background -Optimization of stem cell therapy for myocardial infarction (MI) is facilitated using non-invasive imaging in large animal models. We present initial results using magnetic resonance imaging (MRI) for cell tracking in porcine myocardium and reticuloendothelial system.
Methods -Anterior MI was induced in swine with a 90 minute balloon occlusion of the left anterior descending artery (LAD). On day 7 post MI, 2-3 x 10^6 canine mesenchymal stem cells (MSCs) were incubated with Cell Tracker Orange (Molecular Probes) and iron-fluorescent microspheres (Bangs Laboratories) and delivered into the LAD in two 2ml aliquots via an end-hole balloon catheter (Guidant). Spatial patterns of myocardial T2*-weighted hypointensity, delayed hyperenhancement and gross fluorescence (Kodak Multi-model Imager) were evaluated. An experimental sequence depicted remote cell engraftment as a positive contrast.
Results -Hypointense regions characteristic of the cells' magnetic label were observed predominantly in the infarct border rather than within the infarct itself (fig. a, b). Bilateral posterior hyperintense signal indicated remote cell sequestration intra-abdominally(fig. c). Post-mortem fluorescent imaging displayed concordance of fluorescence and MRI hypointensity (fig. d).
Conclusion - MRI visualized intra-coronary infused MSCs, predominantly in the infarct border but also intra-abdominally. Further work using autologous cells will evaluate long-term cell fate and functional benefit.