Overexpression of MEF2c and IGF-1 in Transplanted Mesenchymal Stem Cells Increases Myogenic Differentiation, Cell Survival and LV Function in Rats

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Background:

We evaluated the synergistic effect of myocyte enhancer factor-2c (MEF2c), which modulates myogenic differentiation, and insulin-like growth factor 1 (IGF-1), which affects cell proliferation and apoptosis, on the efficacy of transplanted mesenchymal stem cells (MSC) for myocardial repair.

Methods and Results:

Female Lewis rats underwent LAD ligation 3 weeks before transplantation with $3x10^6$ male untransfected MSC, MSC transfected with MEF2c (MSC+MEF2c), IGF-1 (MSC+IGF-1), MEF2c+IGF-1 (MSC+MEF2c+IGF-1) or medium without cells (Control). At 2 and 4 weeks MEF2c and IGF-1 expression, donor cell survival and LV function were evaluated. MSC were characterized by flow cytometry prior to transplantation and by immunohistochemistry in the recipient heart.

After 2 weeks, MEF2c expression was greatest in MSC+MEF2c and MSC+MEF2c+IGF-1 ($P<0.001$). IGF-1 expression was greatest at two and four weeks in MSC+IGF-1 and MSC+MEF2c+IGF-1 ($P<0.001$). Donor cell survival was lowest in MSC and MSC+MEF2c, and highest in MSC+IGF-1 and MSC+MEF2c+IGF-1 ($p<0.05$). At 2 and 4 weeks, LVEF was lowest in control, intermediate in MSC and MSC+IGF-1, higher in MSC+MEF2c and greatest in MSC+MEF2c+IGF-1 ($P<0.05$). Immunohistochemistry demonstrated increased expression of both α-SMA and MHC in donor cells in MSC+MEF2c and MSC+MEF2c+IGF-1, indicating myogenic differentiation of the transplanted cells.

Conclusions:

Transplantation of MSC transfected with MEF2c and IGF-I augments myogenic differentiation, improves transplanted cell survival and enhances LV function in infarcted rat hearts. This approach may maximize the efficacy of MSC transplantation for myocardial repair.

Therapeutic Fusion: A Novel Strategy for Reprogramming and Generation of Immune-privileged Cardiomyocytes for Heart Repair

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Background: Some stem cell repair properties originate from their ability to fuse with naturally resident cells in the organs they repair. We hypothesized that *ex vivo* fusion of autologous mesenchymal stem cells (MSCs) and donor cardiomyocytes could provide an invaluable resource of semi-autologous myogenic hybrids with improved regenerative capacity.

Methods and Results: Cell hybrids were engineered by polyethyleneglycol-mediated fusion of fetal mouse cardiomyocytes ($5x10^5$) isolated from LacZ transgenic mice with autologous MSCs ($5x10^5$) from bone-marrow of GFP transgenic mice. Unfused cells were eliminated using specific antibiotics. We used a mixed lymphocyte reaction assay, in which the cells were co-cultured with *GFP* transgenic splenocytes to assess immune response against the hybrids. By lymphocyte proliferation and destruction rate, we showed that MSC-cardiomyocyte cell hybrids were significantly less immunogenic (12% less lymphocyte proliferation). Gene expression analysis by Affymetrix Genechip® array was performed and fused and MSC gene expression were compared. We found that genes related to the major histocompatibilty complex were down-regulated by 2^7 fold. An in vivo experiment was performed in which MI mice were randomized into 5 groups: Cell hybrids (n=10), MSCs (n=10), cardiomyocytes (n=10), mixed MSC and cardiomyocytes (n=9) or saline injection (n=8). Cells were administrated 10 minutes after MI was induced. Echocardiography analysis was performed 3 days and 1 month after the operation and showed that cell hybrids improved left ventricle area change (33.26% ± 5.68 before vs. 39.21% ± 4.87 after, p=0.05) and left ventricle fractional shortening (21.76% ± 5.68 before vs. 27.31% ± 3.82 after, p=0.07).

Conclusion: Our results suggest that the unique power of stem cell fusion can be translated to reprogramming and generation of immune-privileged, therapeutic, hybrid cardiomyocytes.

Acute Decompensated Heart Failure is Associated with Elevated Serum Oxidative Stress

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Background: Oxidative stress (OS) is one of the features of the inflammatory process, a key player in heart failure (HF) pathogenesis. We examined OS serum level in patients who were admitted with acute decompensated heart failure (ADHF), followed OS changes over two months course along with patients' clinical progress and serum NT-pro BNP level.

Methods: We examined sera of 25 (6 females, 19 males, mean age 71) consecutive HF patients who were admitted due to ADHF. Patients' sera were analyzed for OS on admission, discharge and two months after. Simultaneous serum levels of NT-pro BNP were measured as well. We followed patients for HF readmissions. In addition we compared the HF patients OS level to an age and sex matched control group of 36(7 females, 29 males, mean age 74) volunteers with out HF syndrome. Serum OS level was determined using a new technique based on a real time on line assay, thermochemiluminescence (TCL) oxidizability. TCL assay measurement is based on heat induced oxidation of the biological sample and provides a ratio calculating the oxidability measurement in respect to the OS potential. **The lower the TCL ratio, the higher is the OS level.**

Results: The mean OS measurements on admission and on discharge were similar (170±23 and 174 ±25, respectively, p=NS). During the following two months, one patient died and 10 patients were readmitted for ADHF. The OS level was significantly higher in the 10 readmitted patients compared with the 14 patients who were not readmitted (171±15 vs.188±21 respectively, $p<0.05$). The control group mean serum OS level was 193±13. This control group OS level, was significantly better than the OS level of the HF patients measured in the hospital, on both admission and discharge days ($p=0.02$). Two months after hospital discharge , only HF patients who were not readmitted due to ADHF, the OS level was "normalized" , to the same OS level of the control group (188±21 and 193±13 respectively, p=NS). Of note, there was a high correlation between OS values as reflected in TCL ratio measurements and the serum NT-pro BNP values ($r=0.6$, $p<0.01$).

Conclusion: Serum OS level is elevated in ADHF. The OS serum level improves significantly two months after hospital discharge but only in those patients who are not readmitted. Serum OS level correlates with clinical deterioration and with serum NT-pro BNP level. Accordingly, OS is an important factor in acute HF either as a cause or as a reflection of disease severity.

The Clinical Outcome of Patients with Congestive Heart Failure: A Poor Prognosis Despite Preserved Left Ventricular Function

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Background: The clinical outcome of patients with congestive heart failure (CHF) is poor. However, it was presumed that patients with preserved left ventricular function (PLVF) may have a more benign prognosis. Recent data would suggest otherwise.
Objectives: To evaluate the clinical outcome of patients with CHF with PLVF compared to patients with reduced function and the factors affecting prognosis.
Methods: We prospectively evaluated 289 consecutive patients hospitalized with a definite diagnosis of clinical CHF based on typical symptoms and signs. Patients were followed clinically for a period of one year.
Results: More than a third (36%) of the patients had preserved LVF based on echocardiography. These patients were more likely to be older, female, hypertensive and suffer less from ischemic heart disease. The survival rate at 1 year in this group was poor and not significantly different from patients with reduced LVF (75% vs 71%, respectively). The adjusted survival rate by Cox regression analysis was also not significantly different (Figure 1, P=0.25). However, patients with preserved LVF had less re-hospitalizations due to CHF (25% vs 35%, P<0.05). Predictors of mortality in the whole group by multivariate analysis were age, diabetes, chronic renal failure and Sodium < 135mEq/l.
Conclusions: The prognosis of patients with clinical heart failure with or without preserved LVF is poor. Better treatment modalities are needed in these patients.

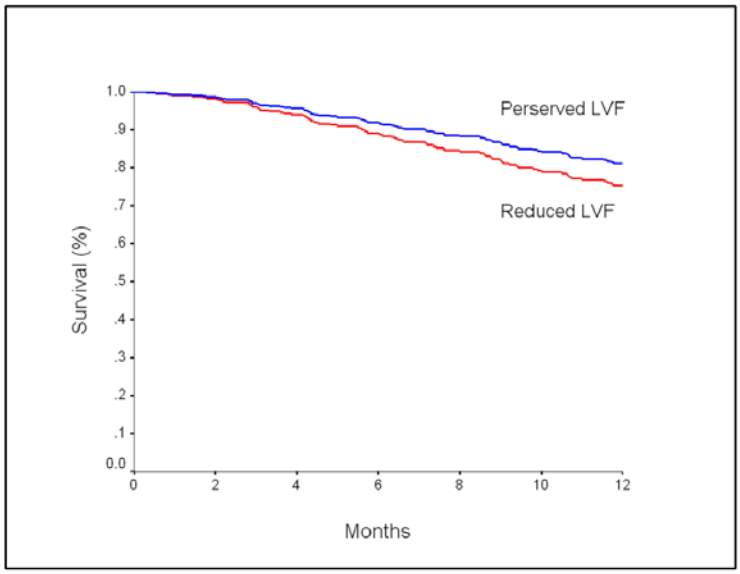

Figure 1. The adjusted survival rate of patients with preserved versus reduced LVF (N=289) by Cox regression analysis. There was no significant difference in survival between the groups.

Hypertrophic Cardiomyopathy with Extreme Left Ventricular Hypertrophy: Risk Profile and Outcome with and without ICD Implant

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Background: Implantation of ICD had been recommended for prevention of sudden death **(SD)** in patients (pts) with Hypertrophic Cardiomyopathy **(HCM)** and extreme (≥ 3.0cm) left ventricular hypertrophy **(ELVH)**.

Objectives: to compare the risk profile and clinical outcome of pts with ELVH who did and did not undergo ICD implantation.

Methods: In the *ISAAC* database, ELVH was diagnosed in 17 pts, aged 1-72 (32.4 ± 17) years, 14 (82%) were male, and follow-up lasted 1-10 (5.3 ± 3.2) years. Presence of major and minor risk factors for SD, survival and type of death were specifically recorded. Major risk factors were considered aborted SD, family history of SD, syncope, nonsustained VT (**NSVT**), and abnormal blood pressure response to exercise, whereas young age at diagnosis, outflow gradient and ischemia on imaging were considered minor risk factors. A Risk Factor Burden was calculated by summation of predefined scores assigned to each risk factor.

Results: The two groups had similar age and similar prevalence of family history of HCM, syncope and minor risk factors for SD. Pts with ICD tended to have a higher NYHA class (2.0 ± 0.7 vs 1.7 ± 1.2, p=0.08) and LV wall thickness. They had a higher Risk Factor Burden due to higher prevalence of family history of SD and NSVT on Holter, as shown in the Table.

	LV Wall thickness	Fam History of SD (n)	NSVT (%)	Nr of Major Risk Factors	Risk Factor Burden
ICD (n=10)	37 ± 6	6	75	1.8 ± 0.6	3.6 ± 1.3
No ICD (n=7)	31 ± 5	0	17	0.9 ± 0.7	1.9 ± 0.9
p	0.08	0.04	0.05	0.02	0.03

Among the patients without an ICD, one died suddenly. One patient had aborted sudden death and had an ICD implanted without further arrhythmic events. In the ICD group one pt died following infective endocarditis and sepsis related to the implant of the device. During the follow up of the 10 pts with an ICD arrhythmia triggered discharges were not recorded.

Conclusions: In our pts with ELVH the decision to implant an ICD was related to presence of additional major risk factors, particularly family history of SD and/or NSVT on Holter. Although SD or aborted SD occurred only in pts without ICDs, one pt died of complications related to device implantation. There is need for further evaluation in a larger population, of the appropriateness of ICD implantation criteria and of the effectiveness of these devices to prevent death in this type of pts.

Immune Cell Function Testing for the Optimization of the Therapeutic Drug Monitoring in Selected Heart Transplant Patients, a Case Series

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Purpose: The Immuknow assay determines the cellular immunity status by quantitative measurement of intracellular ATP level in $CD4^+$ lymphocytes after PHA stimulation. Levels below 225 and above 525 indicate over and under immune suppression respectively. The purpose of the present study was to assess the contribution of Immuknow assay measurement in tailoring the immunosuppressive therapy of heart transplant (HTx) recipients.

Methods and Materials: Between June and August 2007, the immunosuppressive protocol of 15 of the 75 pts transplanted at our center needed re-assessment. Seven pts suffered from infectious episodes (2 recently transplanted with severe fungal infections, 2 pts had 2 episodes of CMV infection despite prophylactic Gancyclovir and 3 had recurrent respiratory tract infections), 5 experienced biopsy proven rejection and 3 pts were started on a CNI free protocol due to CNI side effects. The contribution of the Immuknow assay measurement was assessed.

Results: Mean Immuknow levels were low in the infected pts (235) and high in the pts with rejection (575). During the change to CNI free protocol, mean Immuknow levels were appropriate (426). In the fungal infected pts, the immunosuppressive treatment was diminished to low doses of steroids, lower trough CNI levels, omitting mycophenolate moefetil. The lower Immuknow levels encouraged us to continue the low potency immunosuppression. In the CMV and the respiratory tract infected pts, the Immuknow levels were low despite CNI therapeutic drug levels. Their immunosuppressive regimen was changed to a less potent one. In the pts with rejection, steroids were added and MMF was changed to everolimus. The appropriate Immuknow levels during the change to the CNI free protocol reflect the uneventful protocol change.

Conclusions: Monitoring the Immuknow levels is a valuable and simple tool for immunosuppressive therapy monitoring. It helps decision making in complex situations allowing therapeutic changes that can favorably affect the outcome of HTx recipients.