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Global Contractility Increment in Nonischemic Dilated Cardiomyopathy After Free Wall Only Intramyocardial Injection of Autologous Bone Marrow Mononuclear Cells: An Insight Over Stem Cells Clinical Mechanism of Action

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Introduction: Bone marrow mononuclear cells (BMMC) have been used in experiments and small clinical trials to treat non-ischemic dilated cardiomyopathy (NIDC). Left ventricular myocardial contractility improvements occur, but doubt remains about their mechanism of action. We compared contractility changes in BMMC treated (free wall) and non treated areas (septal wall), in selected patients who had showed significant ventricular improvement after free wall only intramyocardial stem cells injection. **Methods:** From 15 patients with NYHA functional class III/IV and LVEF inferior to 35%, who received 9.6±2.6 × 107 BMMC divided in 10 points over the left ventricular free wall, 7 (46.7%) showed LVEF relative improvement greater than 15%. Those patients were selected for further contractility study. BMMC were collected from iliac bone and isolated with Ficoll-Hypaque. Magnetic resonance imaging was used to measure the systolic thickening of the septal (non treated) and free wall (treated) before injection and 3 months post-operatively. **Results:** Mean systolic septal wall thickening increased from 0.46 to 1.23 mm (an absolute 0.77±1.3 mm and relative 167.4% increase) and the free wall increased from 1.13 to 1.87 mm (an absolute 0.74±1.5 mm and relative 65.5% increase). There was no difference in the rate of absolute (p=0.866) or relative (p=1.0) systolic thickening between the two walls, when cells were injected only in the left ventricular free wall. **Conclusion:** BMMC transplantation in NIDC can improve ventricular function by an overall effect, even in areas that are not directly injected. This finding favours the existence of a diffuse mechanism of action, rather than a local effect and should be reminded when the pathophysiology of stem cells is considered.

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Sphingosine Kinase-1 Protects Transplanted Mesenchymal Stem Cells and Improves the Performance of the Infarcted Heart

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Although mesenchymal stem cells (MSCs) have been tested experimentally and clinically for cardiac repair, poor survival rate greatly restrict their therapeutic efficiency. Sphingosine kinase 1 (SPK1) is a key enzyme in modulating programmed cell death. Sphingosine-1 –phosphate, as one of its key product, mediates angiogenic responses. The main objects of this study are to identify whether SPK1 modification could offer cytoprotective effects on MSCs in vitro and in vivo in a myocardial infarction rat model. MSCs carrying green fluorescent protein (MSCs/GFP), SPK1 (MSCs/SPK1) or with firefly luciferase genes (MSCs/GFP/luc and MSCs/SPK1/luc) were obtained and functionally identified. The in vitro protective effects of SPK1 on MSCs were evaluated after exposure to serum-deprivation and cobalt chloride stimuli. Cells (1×10^6) were injected intramyocardially around the infarcted zone and the fate of the transplanted cells was traced by SPK1 and luciferase assessment in the ischemic myocardium. The survival of the remaining myocardiocytes was evaluated by in situ TUNEL assay 72 hours after cell transplantation. The morphological and functional features of the injured heart were observed with echocardiography, hemodynamic and histological examinations. The results showed that SPK1 protected MSCs both in vitro and in vivo. MSCs/SPK1/luc implantation elevated SPK1 activities in the ischemic myocardium, which peaked on day 3 and reduced to the baseline on day 7. Compared with MSCs/GFP/luc, luciferase activity was significantly higher in MSCs/SPK1/luc-injected myocardium ($p<0.01$ on days 3 and 5 post-injection). The percentage of TUNEL-positive cells in the ischemic area was significantly lower in MSCs/SPK1 (%, 15.5±2.3 vs. MSCs/GFP 23.1±4.9, $p<0.05$). Concordantly, the parameters including fractional shortening (%, 29.33±2.94 vs. MSCs/GFP 23.29±2.86, $p<0.05$), ejection fraction (%, 60.35±4.96 vs. 51.99±5.16, $p<0.05$), left ventricular end-diastolic pressure (15.3±3.6 vs. 18.2±3.3 mmHg, $p<0.05$) and blood vessel density (number per field: 33.82±5.45 vs 23.06±4.01, $p<0.01$) were greatly improved in MSCs/SPK1, though those of the infarct size, collagen deposition in non-infracted area and the spherical indexes of the hearts were comparable between two cell treatment groups. In conclusion, MSCs/SPK1 improve the performance of the infarcted hearts by providing prosurvival signals to the transplanted MSCs and myocardiocytes.

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Combination of Ischemic Postconditioning and Mesenchymal Stem Cells Confers Long-Term Benefit to Cardiac Performance After Ischemia-Reperfusion in Rats

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Introduction: Ischemic postconditioning has been well demonstrated to acutely protect against myocardial ischemia-reperfusion injury (MIRI). Attenuated MIRI may also been acutely conferred by mesenchymal stem cell conditioned medium. We hypothesized that the combination of ischemic postconditioning and mesenchymal stem cells may confer long-term benefit to cardiac performance following MIRI. **Methods:** The left main coronary arteries of Sprague-Dawley rats were occluded for 30 min followed by sustained reperfusion for 72 hours or 28 days. All rats were randomly allocated to five groups (n=16 per group): Control without other intervention, Post (ischemic postconditioning with 3 cycles of 10 sec reperfusion and 10 sec ischemia before reperfusion), BMSC given an intramyocardial injection of bone marrow-derived mesenchymal stem cells prepared from rats 120 min after reperfusion, Post+BMSC, and Sham without myocardial ischemia. Plasma tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-10 were evaluated by enzyme linked immunosorbent assay, B-cell leukemia-lymphoma (BCL)-2 and Bcl-2-associated X protein (BAX) by Western-blot, matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) by Real-time PCR and immunohistochemistry, and cardiac function by hemodynamics. **Results:** Compared with Control, at 72 h reperfusion, Post, BMSC and Post+BMSC attenuated the levels of TNF-α, IL-1β and BAX, and enhanced the expression of IL-10 and BCL-2. At both 72 h and 28 d reperfusion, the maximum and minimum first derivative of left ventricle peak systolic pressure (±dp/dtmax) in Post, BMSC and Post+BMSC were improved relative to Control [+dp/dtmax at 72 h, (6163±718) mmHg/s, (5965±694) mmHg/s, (7085±799) mmHg/s vs. (5033±890) mmHg/s, $P<0.05$; +dp/dtmax at 28 d, (6746±667) mmHg/s, (6880±593) mmHg/s, (7647±629) mmHg/s vs. (5414±640) mmHg/s, $P<0.05$; -dp/dtmax at 72 h, (5111±349) mmHg/s, (5468±914) mmHg/s, (6250±640) mmHg/s vs. (4389±518) mmHg/s, $P<0.05$; -dp/dtmax at 28 d, (5460±902) mmHg/s, (5782±343) mmHg/s, (6533±984) mmHg/s vs. (4458±531) mmHg/s, $P<0.05$]. At 28 d reperfusion, the gene and protein expression of MMP-9 in Post, I/R+BMSC and Post+BMSC is lower and TIMP-1 higher compared with Control (gene expression, MMP-9, 0.61±0.08, 0.53±0.07, 0.21±0.04 vs. 1±0.07, $P<0.01$; TIMP-1, 1.91±0.17, 1.84±0.28, 2.21±0.27 vs. 1±0.13, $P<0.01$). The above effects of Post+BMSC were more beneficial than Post and BMSC ($P<0.05$). **Conclusion:** The combination of ischemic postconditioning and mesenchymal stem cells improves long-term functional recovery after ischemia-reperfusion following MIRI, with attenuation in inflammatory responses, apoptosis and matrix metalloproteinase expression.

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In vitro on Biological Character of Embryonic Hepatic Stem Cells

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Aims: (1) To improve the method for isolation, purification and cultivation of mouse EHSCs; (2)To compare the characteristics of mouse EHSCs in different embryo duration; (3)To investigate the possibility to make mouse EHSCs differentiate along cardiac lineage; (4)To compare the differentiation states of EHSCs which are in different conditions. **Methods:** 1. We examined the isolation, purification, expansion and compared the characteristics of mouse embryonic hepatic stem cells in different embryo duration (ED 13.5 day, ED 16.5 day and ED 19.5 day). Collagenase and EDTA digestion and the method of adhering to culture plastic in different time were used to isolate EHSCs from mouse fetal liver which were then cultivated by L-DMEM containing 15% fetal bovine serum. 2. We investigated the possibility to induce EHSCs to differentiate along cardiac lineage and compared the differentiation states of EHSCs which are in different conditions. Cells from passage 3–4 were planted at the density of 1.5×104 /cm^2 and were treated with the combination of 5-azacytine (5-aza) and DMSO in different doses for different hours. **Results:** 1. The cells of the least difference in morphology, the best situation of growing and the most evident characteristics of stem cells were obtained from ED13.5 day group and the morphological difference between cells and the ALB and CK19 expression increased yet growing situation decreased gradually with the increasing embryo duration. 2. Cells, treated with 5-aza of 5 μmol/L and DMSO of 0.8% for 24 hours, cultured at 37 OC, 5%CO2 and 20%02, showed the sign of cardiac differentiation and stained positive for troponin T and α-actin 4 weeks after treatment. **Conclusion:** 1. It is suggested that a majority of primordial progenitor cells exist in early age of fetal liver and differentiate into ALB+CK19+ stem cells gradually and a large quantity of EHSCs whose properties were stable were obtained by the improved methods. 2. EHSCs have the potential to differentiate along cardiac lineage; The stimulus for the cardiac differentiation of mice EHSCs is different from other source.

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Reduced Cardiopulmonary Exercise Capacity in Patients with Chronic Heart Failure: Impact of Left Ventricular Systolic Dysfunction

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Introduction: Impaired exercise capacity is one of the most common clinical manifestations in patients with chronic heart failure (CHF), and Cardiopulmonary exercise testing (CPX) is a new method to evaluate the cardiopulmonary exercise capacity. The aim of the current study was to evaluate the exercise capacity in patients with chronic heart failure by CPX. **Methods:** Cardiopulmonary exercise testing on the bicycle ergometer were performed in 74 patients age and gender and BMI matched, 37 having congestive heart failure (LVEF<0.45) and the other 37 having heart diseases but without heart failure (control group, LVEF>0.55). VO_2AT, VO_2 Peak, Load AT, Load peak, VE/VCO_2 slope were documented. **Results:** (1) VO_2AT, VO_2Peak, Load AT, Load peak all were significantly reduced in patients with CHF compared with controls[VO_2 AT: (11.34± 2.26) ml.kg^{-1}.min^{-1} vs (12.61±2.43) ml.kg^{-1}.min^{-1}, $p=0.025$; VO_2peak: (15.16± 4.28) ml.kg^{-1}.min^{-1}vs (17.26±3.55) ml.kg^{-1}.min^{-1}, $p=0.025$; Load AT: (25.16±18.82) J.s^{-1} vs (43.89±18.94)J.s^{-1}, $p=0.000$; Load peak: (54.86±22.50)J.s^{-1} vs (77.92±22.60)J.s^{-1}, $p=0.000$]. (2) VE/VCO_2 slope were increased in patients with CHF compared with controls[(36.74±6.74)vs (30.73±5.09), $p=0.000$]; (3) VO_2AT, VO_2Peak, Load AT, Load peak, VE/VCO_2 slope all not correlate with LVEF ($r=0.054$, $P=0.76$; $r=0.03$, $P=0.858$; $r=0.310$, $P=0.089$; $r=0.174$, $P=0.304$; $r=-0.203$, $P=0.229$). VO_2AT, VO_2Peak,