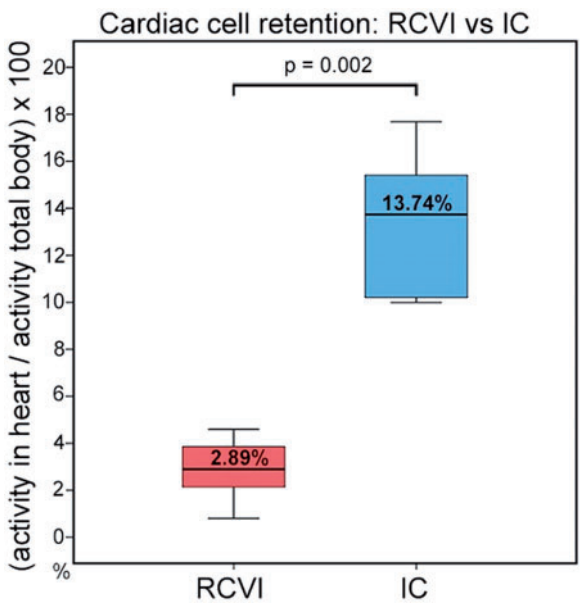
after, and 24h post cell delivery. In vitro bioluminescence signal was used to identify tissue samples containing GFP-Luc-MSCs. Myocardial tissue matrix metalloproteinase 2 (MMP2) (index of ischemic/oxidative stress) and CXCR4 receptor expression (index of homing signal) were measured in bioluminescence positive and negative myocardial areas one day post cell transfer. Biodistribution of the implanted cells was quantified by using Luciferase assay and confirmed by fluorescence immunochemistry. Global left ventricular ejection fraction (LVEF) was measured at baseline and one month post cell therapy using MRI.
Results: AMF decreased immediately after intracoronary cell delivery, while no change in tissue perfusion was found in the IM group. Intracoronary delivery led to a significant increase in myocardial MMP2 expression and decreased expression of CXCR4. Fluorescence immunochemistry indicated a higher expression level of a variety of homing (tenascin and cadherin) and angiogenic factor (FGF-2 and VEGF) in the IM group. LVEF increase was also significantly higher in IM group at the 1-month follow up.
Conclusions: Intracoronary stem cell delivery decreased AMF, increased myocardial expression of MMP2, and lead to reduced CXCR4 expression with enhanced biodistribution and diminished functional recovery post-infarction.

P111

Retention of mesenchymal stem cells in the heart is lower after retrograde coronary venous infusion compared to intracoronary infusion in a porcine model of chronic myocardial infarction

WA. Gathier; M. Van Der Naald; BR. Van Klarenbosch; AE. Tuinenburg; JLM Bemelmans; JPG Sluijter; FJ. Van Slochteren; PA. Doevendans; SAJ Chamuleau
University Medical Center Utrecht, Cardiology, Utrecht, Netherlands

Funding Acknowledgements: This research is part of Cardiovasculair Onderzoek Nederland (grant number: CVON2011-12)
Background: An important aspect of cell therapy in the field of cardiac disease is safe and effective delivery of cells. Commonly used delivery strategies such as intramyocardial injection and intracoronary (IC) infusion both present with advantages and disadvantages. Therefore, alternative delivery routes are explored, such as retrograde coronary venous infusion (RCVI).
Purpose: The aim of this study is to compare cardiac cell retention between RCVI and IC infusion. The secondary endpoint is safety of RCVI.
Methods: Myocardial infarction (90 minutes LAD occlusion) was induced in 16 female, landrace pigs. Four weeks later, the surviving 12 pigs were randomized to receive a median of 3.1 million [interquartile range 2.6 – 3.5] bone marrow-derived porcine mesenchymal stem cells (MSCs) in 10 ml phosphate buffered saline, labeled with the radioactive isotope Indium-111 either via RCVI (n=6) or IC infusion (n=6). In case of RCVI, 40ml of sodium chloride was infused on top of the 10ml of cell suspension in order to fill the coronary venous system and prevent cells from only staying in the coronary sinus. Four hours after cell administration, nuclear imaging was performed to determine the amount of cells retained in the heart as a percentage of cells retained in the whole body of the pig.
Results: A significantly lower percentage of MSCs is retained in the heart after RCVI compared to IC infusion (RCVI: median 2.89% [interquartile range 2.14 – 3.86] vs IC infusion: median 13.74% [interquartile range 10.20 – 15.41]) as presented in figure 1. Retention of cells in other organs did not significantly differ between RCVI and IC infusion, although a numeric difference in retention was seen in the lungs (RCVI: median 35.45% [interquartile range 26.53 – 45.22] vs IC infusion: median 22.07% [interquartile range 20.36 – 29.22]). RCVI led to development of pericardial fluid and hematomas on the frontal wall of the heart in three cases. Dissection of the coronary venous system after RCVI was seen in three pigs. IC infusion led to no-flow in one pig.
Conclusion: RCVI is significantly less efficient in delivering bone marrow-derived MSCs to the heart compared to IC infusion. RCVI led to more safety issues than IC infusion in this study, with multiple cases of venous dissection and development of hematomas and pericardial fluid collections.



Abstract P111 Figure.

P112

Epithelial-to-mesenchymal transition is required for a therapeutic effect of epicardial-derived cells after myocardial infarction

AM. Smits; AMD Vegh; T. Van Herwaarden; MJ. Goumans
Leiden University Medical Center, Department of Molecular and Cellular Biology, Leiden, Netherlands

Funding Acknowledgements: This study is funded by The Netherlands Organization for Scientific Research (NWO) VENI and a LUMC Research fellowship
Introduction: The cells of the epicardium -the epithelial layer covering the outside of the heart-, are activated after ischemic injury. Upon damage these cells undergo epithelial-to mesenchymal-transition (EMT). The epicardial-derived cells (EPDCs) contribute to repair either via migration and differentiation, or via a paracrine mechanism. Previous research revealed that direct transplantation of cultured human mesenchymal EPDCs into the infarcted mouse heart attenuated remodelling, increased vascularisation and improved cardiac function.
We recently developed a cell culture protocol for human EPDCs in their cobblestone-like epithelial state (cEPDCs) allowing us to compare EPDCs prior to and post-EMT. cEPDCs are more plastic with the ability to differentiate into a range of mesenchymal cell types including smooth muscle cells, cardiac fibroblasts, and possibly to cardiomyocytes and endothelial cells. Therefore, we questioned whether cEPDCs have a better therapeutic potential compared to mesenchymal spindle-shaped EPDCs (sEPDCs) after myocardial infarction (MI).
Purpose: to investigate the importance of EMT in EPDCs prior to transplantation in preserving cardiac function after MI.
Methods: Epicardium was isolated from human atrial appendages and processed into a single cell suspension. After plating, cEPDCs were cultured with either ALK4/5/7 kinase inhibitor, or treated with TGF-β to induce EMT. Immediately after induction of MI in NOD-SCID mice we injected either sEPDCs, cEPDCs, or PBS into the border zone at 2 sites. Cardiac function was assessed for 6 weeks via ultrasound and hearts were isolated at 3 days and 6 weeks post-MI. Infarct size, human collagen deposition, number of engrafted cells and vascularization of the border zone were determined after 6 weeks. Short-term cell engraftment and immune response are determined at 3 days post-MI. The angiogenic secretome of EPDCs was determined with a human angiogenic antibody array, and confirmed by ELISA.
Results: Six weeks after transplantation only sEPDCs were able to partially preserve cardiac function compared to cEPDCs and PBS control. This coincided with a smaller infarct size in the sEPDC group. Immunostaining for human cell markers revealed that low numbers of EPDCs were present at six weeks in either group, suggesting a paracrine role rather than a cellular contribution. The secretome of the conditioned medium of cultured sEPDCs and cEPDCs showed angiogenic potential, however, the vascularization of the border zone at six weeks was similar in all groups. We observed a higher deposition of human collagen in the sEPDC group. We are currently investigating the cell survival and effect on immune response at 3 days post-MI.
Conclusion: sEPDCs ameliorated cardiac function, likely via a paracrine mechanism. Although more plastic, cEPDCs do not contribute to repair. These data will help in understanding the potential of EPDCs as a local source for endogenous cardiac repair.

P113

Levels of anti-elastin IgA antibodies are associated with high risk of atherosclerosis in diabetics with essential hypertension

A. Nikolov[1]; M. Tsekova[1]; A. Blazhev[2]
[1]Medical University Pleven, Department of Propedeutics of Internal Diseases, Pleven, Bulgaria; [2]Medical University Pleven, Department of Biology, Pleven, Bulgaria

Purpose: Thickening of basement membrane in capillaries and small vessels is a well-known finding and important in the progression of diabetic microangiopathy. Patients with diabetes mellitus and arterial hypertension are at higher risk for development of atherosclerosis.
Methods: Serum levels of antibodies to elastin (AEAbs) IgG, IgM and IgA were measured using an ELISA method of 93 patients with type 2 diabetes mellitus (T2DM) and arterial hypertension (AH) (mean age 61,4±11,3 years, diabetes duration 9,88±3,12 years; hypertension duration 9,28±4,98). These values were compared to serum AEAbs in 42 age and sex matched controls. Diabetics were divided in two groups according to presence- Group 1 (n=67) or absence- Group 2 (n=26) of microangiopathy.
Results: AEAbs IgA levels in patients with T2DM and AH were statistically significantly higher than these in healthy controls 0,338(0,133÷0,452) vs. 0,006(0,052÷0,068) (KW=19,54; P<0.0001). Patients with microvascular complications (Group 1) showed significantly higher levels of AEAbs IgA than Group 2- 0,353 (0,173÷0,471) vs. 0,235 (0,098÷0,377) (KW=3,36; p=0.05) and controls 0,353 (0,173÷0,471) vs 0,006 (0,052÷0,068) (KW=20,37; p<0,0001). Patients without vascular changes also showed higher levels of AEAbs IgA than controls 0,235 (0,098÷0,377) vs. 0,006 (0,052÷0,068) (KW=8,54; P=0.003) The highest levels of AEAbs IgA were found in patients with vascular damage. Serum levels of AEAb IgG and IgM in patients with T2DM and AH were lower than these in controls, but the differences are not statistically significant. AEAbs IgA showed correlation with insulin dose (r=-0.35); (p=0.01), systolic blood pressure (r=0.31); (p=0.001), HbA1c (r=0.21); (p=0.04), BMI (r=0.22); (p=0.01).
Conclusion: Our study showed a relationship between elevation of serum levels of AEAbs IgA in diabetics and development of vascular changes. The elevation of AEAbs IgA can be related with later clinical manifestation of atherosclerosis. We suggest that AEAbs IgA can be useful method for identfying a high atherosclerotic risk in diabetic patients.

P115

Vascular wall stiffness parameters as risk factors of cerebrovascular complications in hypertensive patients with abdominal obesity

K. Avdeeva; TI. Petelina; LI. Gapon; NA. Musikhina; LN. Kopylova; LA. Dobrynina
Tyumen Cardiology Center, Tyumen, Russian Federation

Introduction: several of major studies has shown that increasing the stiffness of the arteries is an independent predictor of cerebrovascular disease and mortality. The vascular inflammatory reaction are of great importance in the process of vascular remodeling.