P606 Hemodynamic compromise following high-dose pantoprazole infusion in mice

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Background: It has been shown that proton pump inhibitors reduce contractile force in isolated rabbit and human myocardium by reducing calcium transients and myofilament calcium sensitivity. This study was planned to investigtate the in vivo hemodynamic effects of pantoprazole in healthy myocardium and in the setting of heart failure.
Methods: Pressure volume loops were recorded in sham operated mice and mice 4 weeks after myocardial infarction. The hemodynamic effects of intravenous infusion of Pantoprazole were recorded.
Results: Infusion of 10mg/kg/min pantoprazole induced bradycardia (heart rate 82±2% of baseline, n=9, $p<0.01$), decreased myocardial contractility (dp/dtmax normalized to blood pressure 86±4% of baseline, preload adjusted maximal power 43±7%, n=9, $p<0.01$ each) and reduced arterial elastance (78±6% of baseline, n=9, $p<0.01$). These changes led to a decrease of systolic blood pressure to 84±3% of the value before pantoprazole infusion (n=9,$p<0.01$).
The described effects were fast, beginning immediately with the infusion and usually reaching a plateau after 2 or 3 minutes of infusion. After stopping the continuous infusion the effects were at least partially reversible with a relatively fast kinetics. The effects were of comparable size in healty mice and mice with MI. However in 4 out of 13 mice with MI infusion of 3-10mg/kg/min pantozole rapidly lead to pump failure which was lethal in 2 of the animals. The 2 other mice recovered after the infusion was stopped immediately.
Conclusion: At higher infusion rates pantoprazole is able to induce negative hemodynamic responses. Especially in the setting of heart failure these effects can lead to significant impairment of cardiac function. Therefore high infusion rates of pantoprazole should be avoided especially in heart failure patients. As the observed effects are rate-dependent and reversible lower infusion rates seem to avoid the possible problems.

BASIC PROGRESS IN CARDIOVASCULAR REPAIR

P608 Dual stem cell therapy after myocardial infarction works specifically by the cxcr4-sdf1 axis and stimulates myocardial perfusion and resident cardiac stem cells

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Background: Dual stem cell therapy comprising G-CSF based stem cell mobilization and stabilization of cardiac SDF-1 by DPP-IV-inhibition may revolutionize therapy of myocardial infarction. It finally leads to improved cardiac homing of stem cells, enhanced heart function and increased survival. However, it remains unclear, whether this new approach works specifically by the SDF1-CXCR4- axis, stimulation of resident cardiac stem cells and improved myocardial perfusion.
Methods: We induced AMI in 10 weeks old male C57BL/6 mice using surgical occlusion of the left descending artery (LAD). Mice were then treated with G-CSF +/- the DPP-IV inhibitor Diprotin A. Saline treated animals served as controls. Antagonization of CXCR-4 was performed by application of AMD3100. FACS analyses were used to determine stem cell populations in the myocardium after 6 days. Histology was performed to examine capillary density after 6 days and infarct size 30 days after AMI. Cardiac function parameters were assessed using a Millar-Tip catheter system. Survival was analyzed by Kaplan-Meier-method for 30 days (n=20 in each group). Myocardial perfusion was measured by single photon emission computed tomography (SPECT).
Findings: First, we established in a titration scheme the optimal dosage of AMD3100 (1,25mg/kg) that is sufficient to block CXCR-4 but is not mobilizing stem cells in a relevant extent. Based on these findings, AMD3100 effectively inverted the beneficial effect of G-CSF and Diprotin A concerning the homing of circulating stem cells, cardiac remodeling (infarct size), heart function and survival (Kaplan-Meier curves). Secondly, G-CSF and Diprotin A application significantly enhanced neovascularization (represented by CD31 positive capillaries in the borderzone). Besides, dual stem cell therapy significantly enhanced myocardial blood flow (SPECT) which was antagonized by AMD. Finally G-CSF + Diprotin A administration effectively stimulated the pool of resident cardiac stem cells (FACS) which was reversed by AMD3100 as well.
Interpretation: Dual stem cell therapy mainly works by the SDF1-CXCR4-axis. This gives final proof that the extent of stem cell homing which is associated with an increase of myocardial perfusion and enhancement of resident cardiac stem cells is decisive for the success of this new therapeutic approach.

P609 Human embryonic stem cell-derived cardiomyocytes improve cardiac function after myocardial infarction: paracrine effects outweigh the contribution of active contractile force

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Purpose: Transplantation of human embryonic stem cell-derived cardiomyocytes (hESC-CM) has been shown to improve function of the rodent heart one month after myocardial infarction. Since the mechanistic basis and optimal delivery strategies are unclear, we investigated the influence of the number of injected cells, resulting graft size and possible paracrine mechanisms in this process.
Methods: Myocardial infarction was induced in non-obese diabetic severe combined immunodeficient (NOD-SCID) mice (n=84) followed by injection of hESC-CM at different dosages, hESC-non-CM derivatives, culture medium or no injection at all. Serial high resolution (9.4 T) magnetic resonance images were acquired after 2 days, 4 weeks and 12 weeks to assess cardiac function (n=70). Graft sizes were quantified, identified by epifluorescence of a transgenic GFP-marker and characterized by immunofluorescent co-staining. Possible paracrine contributions of the donor cells were investigated using (immuno-)histochemical staining.
Results: Transplantation of either hESC-CM or other differentiated hESC-derivatives improved short, mid- and long term cardiac performance and survival, although cardiomyocyte-containing populations provided additional functional benefit above other cells at mid-term (4 weeks). Cardiomyocytes were also the only cells present in significant numbers in grafts 12 weeks after injection. The temporary cardiomyocyte-specific enhancement was associated with elevated vascular density around the graft and attenuated compensatory remodeling. However, increasing the number of hESC-CM for injection failed to enhance heart function further. Moreover, we observed that small graft size was associated with a better functional outcome.
Conclusions: Our results showed that hESC-CM increased myocardial vascularization and improved heart function in an immunodeficient mouse model of myocardial infarction but that their functional advantage over differentiated hESC-non-CM was lost at the long term. Since doubling graft size did not further enhance cardiac function, active contraction of donor cells is not likely to be the mechanism behind the observed functional improvement in this model; paracrine effects including stimulation of neovascularization appear more important.

P610 Safety and efficacy of autologous mesenchymal stem cells for the treatment of end-stage dilated cardiomyopathy - a comparison of intracoronary and direct intramyocardial injection

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Background: Bone marrow stem cells may improve cardiac function following heart attack. Mesenchymal stem cells (MSC) from bone marrow can differentiate into cardiomyocytes, vascular smooth muscle and endothelial cells. They also exhibit immune-modulatory and paracrine effects to augment cardiac repair. However their safety, optimal cell number and route of administration are not determined for patients with severe dilated cardiomyopathy.
Objective: To demonstrate the safety and efficacy of autologous MSC treatment for patients with ischemic or non-ischemic dilated cardiomyopathy via direct intramyocardial and intracoronary injection.
Methods: Twenty patients were screened. Eight patients were excluded due to presence of significant viable myocardium amenable to revascularisation while two patients were referred for biventricular pacing instead. Of the remaining patients (all male, mean age 58 years) five had ischemic cardiomyopathy deemed unlikely to benefit from CABG alone. Two patients had previous revascularization that remained patent, and three had non-ischemic dilated cardiomyopathy. MSC expansion using animal-free culture media achieved required numbers within three weeks. Patients who had not been revascularised (n=5; IM group) received CABG with concurrent intramyocardial injection of 1.0×10^6 MSC/kg body weight while patients with patent vessels (n=5, IC group) received intracoronary injection of 2.0×10^6 MSC/kg b.w. via coronary catheterization.
Results: All patients tolerated either procedure well (mean follow up 1 year). There were no ventricular arrhythmias, pericardial bleeding or coronary occlusion post-treatment. There were significant improvements from baseline to six and twelve months in functional score (NYHA 3.8;1.5,1.0), left ventricular ejection fraction (26.5%, 50.1%, 63.5%), end diastolic and end systolic volumes and diameter and interventricular septal wall thickness. IM group showed greater improvement than IC group. The magnitude of improvement in each group is larger than that reported for conventional therapy alone. Scar reduction was noted in both groups by 12 months.
Conclusion: Autologous bone marrow mesenchymal stem cells are safe for severe dilated cardiomyopathy and appear to be beneficial, whether as adjunctive

treatment to revascularization in ischemic cardiomyopathy or for non-ischemic dilated cardiomyopathy. The cell number required were appropriate for respective route of administration. Larger randomized multicentre studies are now warranted.

P611 Adipose-derived mesenchymal stem cells markedly attenuate brain infarct size and improve neurological function in rats

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Purpose: This study tested the therapeutic effect of adipose-derived mesenchymal stem cells (ADMSCs) on brain infarction area (BIA) and neurological status in a rat model of acute ischemic stroke (IS).
Methods and Results: Acute IS of left middle cerebral artery territory was induced by occluding distal left internal carotid artery in adult male Sprague Dawley (SD) rats (n=30) that were then categorized into group 1 (IS only) and group 2 (IS plus intravenous ADMSCs (2.0 x 106 at 0, 12 and 24h after the procedure). The rats were sacrificed and brain tissues were harvested for analysis on day 21 after the procedure. The results showed that BIA was larger in group 1 than in group 2 ($p<0.001$). The sensorimotor functional test (corner test) identified a higher frequency of turning movement to left in group 1 than in group 2 ($p<0.05$). The mRNA expressions of Bax, caspase 3, interleukin (IL)-18, toll-like receptor-4 and plasminogen activator inhibitor-1 were higher, whereas Bcl-2 and IL-8/Gro were lower in group1 than in group 2 (all $p<0.05$). Western blot demonstrated a lower CXCR4 and stromal-cell derived factor-1 (SDF-1) in group 1 than in group 2 (all $p<0.01$). IHF staining showed lower expressions of CXCR4, SDF-1, von Willebran factor and doublecortin, whereas the number of apoptotic nuclei on TUNEL assay was higher in group 1 than in group 2 (all $p<0.001$). IHC showed that cellular proliferation and number of small vessels were lower but glial fibrillary acid protein was higher in group 1 than in group 2 (all $p<0.01$).
Conclusions: ADMSC therapy significantly limited BIA and improved sensorimotor dysfunction after acute IS.

P612 tPA-mediated plasmin generation is crucial for kinetic and angiogenesis ability of EPCs in response to ischemia through modulating activities of MMP-9 and chemokines

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Purpose: By applying ischemic models to different genetically modified animals, including metalloproteinase knock out (MMP-9-/-) mice and B6 wide-type, this study attempted to clarify the upstream signaling of tissue plasminogen activator (tPA) in manipulating the downstream signaling of chemokine [stromal cell-derived factor (SDF)-1α] and proteolytic enzyme (MMP)-9) to regulate EPC kinetics and angiogenesis.
Methods and Results: Compared to only culture-medium group, tPA-treated the culturing human umbilical vein endothelial cells (HUVECs) and mouse endothelial cells (SVEC 4-10 cell line) markedly enhanced the SDF-1α, KDR and vascular endothelial growth factor expressions in both surface of these cells and in supernatant levels (all p values <0.01). After acute limb-ischemic induction (left femoral artery and vein to be ligated, cut, and excised), circulating level of SDF-1α, VEGF and EPCs (CD34+, CD133+, KDR+ Sca-1+ and C-kit+ cells) were markedly enhanced in B6 mice with then in without tPA treatment (all p values <0.01). Additionally, in the ischemic condition, these EPC chemokine levels were remarkably higher in B6 mice than in MMP-9-/- mice (all p values < 0.05). Also, in ischemic condition, tPA treatment notably increased circulating level of SDF-1α and EPCs (CD34+, CD133+, KDR+ Sca-1+ and C-kit+ cells), whereas this treatment significantly decreased bone-marrow levels of SDF-1α and EPCs in B6 mice (all p values < 0.001). However, these alternations were less obvious and less consistent in MMP-9-/- than in B6 mice. Moreover, by day 28, the angiogenesis determined by immunohistochemical stain and blood flow assessed by laser Doppler in ischemia area was substantially higher in B6 than in MMP-9-/- mice.
Conclusion: tPA therapy promotes kinetic activity of EPCs in response to ischemic stimulations though manipulating plasminogen/plasmin-mediated MMP-9 and chemokine activity.

P613 Long term functionality and growth of tissue-engineered living, autologous vascular grafts in a large animal model: the final step towards human application?

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Purpose: Living autologous vascular grafts with the capacity for regeneration and growth may overcome the limitations of contemporary artificial prostheses. Particularly in congenital cardiovascular surgery, there is an unmet medical need for growing replacement materials. Here we investigate long-term growth capacity, functionality and safety of tissue-engineered living pulmonary arteries in a growing lamb model.
Methods: Vascular cells were sequentially seeded on biodegradable scaffolds (diameter 18±1mm) and were grown in vitro for 21days using biomimetic conditions. Next, the produced tissue-engineered vascular grafts (TEVGs) were surgically implanted as main pulmonary artery (PA) replacements in lambs (n=15) and followed up for 220 weeks.
Results: All procedures were performed uneventfully. Trans-oesophageal echocardiography at 20, 50, 80, 100 and 220 weeks displayed excellent functionality and CT–angiography did not detect any signs of degeneration such as thrombus formation, calcification, stenosis or aneurysm. In regard to wall tension, shear stress and flow velocity, 3D-CT analysis showed sufficient and stable results over the whole follow-up period. Functional growth was confirmed by help of CT volume measurements which displayed a significant volume increase of the TEVG from an initial volume of 6.4ccm early after implantation up to 13.2ccm after 240weeks. Histology showed tissue formation reminiscent of native PA.

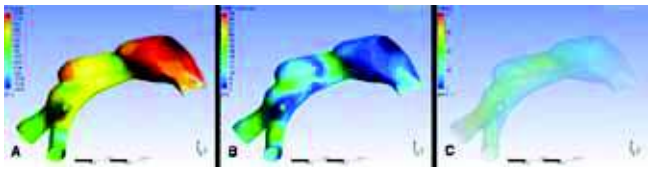

Figure 1. 3D-CT analysis displays normal systolic wall pressure (A), low wall shear stress (B) and normal velocity-coded streamlines (C).

Conclusions: Our results provide systematic evidence of growth, functionality and safety of TEVG in a full growth animal model over a long-term period. These findings provide the experimental basis to enter into future clinical trials.

P615 Caffeine induces endothelial tissue factor expression through inhibition of phosphatidylinositol 3-Kinase

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Background: Tissue factor (TF) is a key activator of coagulation and involved in acute coronary syndromes. Caffeine is often reported to increase cardiovascular risk; however, its effect on cardiovascular morbidity and mortality is controversial. Hence, this study was designed to investigate the impact of caffeine on endothelial TF expression.
Methods and Results: Caffeine concentration-dependently enhanced TF protein expression and surface activity of human endothelial cells stimulated by tumor necrosis factor (TNF)-α or thrombin. Tissue factor pathway inhibitor (TFPI) expression was not altered under these conditions. Caffeine inhibited Phosphatidylinositol 3-Kinase (PI3K) activity and this effect was comparable to that of the known PI3K inhibitor LY294002. Consistently, treatment of endothelial cells with LY294002 enhanced TNF-α induced TF expression to a similar extent as caffeine, and adenoviral expression of the active PI3K mutant (p110) reversed the effect of both caffeine and LY294002 on TF expression. Caffeine and LY294002 increased DNA binding capacity of the transcription factor NFkB, whereas the activation pattern of mitogen-activated protein kinases (MAPK) remained unaltered. Luciferase reporter assay revealed a caffeine dependent activation of the TF promoter, and RT-PCR revealed a dose dependent increase in TF mRNA levels when stimulated with caffeine in the presence of TNF-α. In THP-1 cells and VSMC TF expression remained unaltered by caffeine.
Conclusions: These observations indicate that PI3K signalling mediates caffeine induced TF expression leading to activation of NFkB and upregulation of TF. Since the caffeine concentrations applied in the present study are within the plasma range measured in humans, our findings indicate that caffeine enhances the prothrombotic potential of endothelial cells and underscore the importance of PI3K in mediating these effects.