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## Symposium I: „Endothel – Funktion und Dysfunktion“

### 1 Endothelfunktion und -dysfunktion

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### 2 Endothelial dysfunction – the target for pharmacological intervention

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Healthy endothelium is essential for undisturbed functioning of the cardiovascular system, while endothelial dysfunction leads to its various pathologies. Indeed, endothelial dysfunction precedes clinical symptoms of atherosclerosis, and has diagnostic and prognostic significance in atherosclerosis.

Accordingly, pharmacological reversal of endothelial dysfunction represents a new approach to prevent atherothrombosis. Phenotype of endothelial dysfunction involves: impairment of vasculoprotective mediators (NO and PGI<sub>2</sub>), robust activation of pro-inflammatory phenotype (expression of various cytokines, chemokines, adhesion molecules) as well as activation of pro-thrombotic endothelial mechanisms. Pharmacotherapy of endothelium should aim to reverse all these alterations in endothelial function.

There are number of drugs that modulate endothelial phenotype: inhibitors of angiotensin converting enzyme (ACE-I), inhibitors of HydroxyMethylGlutaryl-CoA reductase (statins),  $\beta$ -adrenolytics, angiotensin receptors antagonists, endothelin receptor antagonists, antiplatelet drugs, aldosteron antagonists, xantine oxidase inhibitors and many others. Surprisingly, ACE-I and statins constitute the forefront of pharmacology of endothelium. Therapeutic effectiveness of ACE-I by far exceeds the benefits expected from their hypotensive effect. Similarly, statins offer cardiovascular protection irrespective of initial LDL cholesterol. These two classes of drugs – apart from their classic mechanisms of action – exert pleiotropic endothelial actions that contributes significantly to their anti-inflammatory, anti-thrombotic, and vasculoprotective actions. In a search of a new candidates for endotheliotropic drug we studied several nicotinamide analogues. We found that 1-N-

methyl-nicotinamide (MNA)- previously known as inactive metabolite of nicotinamide – displayed an interesting endothelial profile of action. Moreover, MNA inhibited atherosclerosis development in mice model of atherosclerosis. Therapeutic potential of endothelial action of MNA merits further studies.

### 3 Expression and localization of lysosomal associated membrane-proteins in cultivated human vascular cells

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**Background.** Lipid infiltration is one of the major mechanisms for arteriosclerosis. Especially low-density lipoprotein (LDL) is associated in the origin of fatty streak formations and creation of atherosclerotic plaques. However, the influence of lysosomes and the released cholesterol in the pathogenesis of arteriosclerosis is unknown.

**Methods.** Human endothelial (EC) and smooth muscle cells (SMC) were originated from the ascending aorta from patients, in whom a Ross-Procedure was performed. Following separation a freeze-fracture replica labeling electron microscopy combined with SDS digestion was performed. Afterwards a cytochemical labeling of the lysosomal membrane proteins Lamp-1 and Lamp-2 was performed with immunogold labeling. Hepatoblastoma cells were used as a control group.

**Results.** Lamp-1 was primary located in the freeze-fracture replica of lysosomes of EC ( $271.8 \pm 6.6/\mu\text{m}^3$ ) followed by SMC ( $93.6 \pm 5.0/\mu\text{m}^3$ ), while Lamp-2 was similar distributed in EC and SMC ( $106.6 \pm 2.3$  and  $116.6 \pm 4.0/\mu\text{m}^3$ ).

**Conclusions.** Both lysosomal membrane proteins occur in different quantity in EC and SMC. The specific functions of Lamp-1 or-2 are not known so far. However, both membrane proteins take part in tight-junctions and might play an important role in the pathogenesis of arteriosclerosis.

### 4 Endothelin-1 augments cytoplasmic and nuclear calcium transients in cardiac myocytes via inositol-1,4,5-trisphosphate signaling

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**Background.** In the heart, endothelin-1 (ET) activates various signaling pathways to alter contractility and to induce hypertrophy. The possible involvement of inositol-1,4,5-trisphosphate (InsP3) signaling in these processes remains elusive. Recent evidence indicates that cardiomyocytes express functional InsP3 receptors that may alter intracellular calcium signaling. Therefore, we tested the hypothesis that ET modulates cytoplasmic and nuclear calcium signaling in cardiac myocytes via InsP3-induced calcium release.

**Methods.** Cytoplasmic and nuclear calcium transients (CaT) were recorded in electrically stimulated (0.7 Hz, 22° C) rabbit atrial myocytes using fast (120 Hz) two-dimensional confocal microscopy and the calcium indicator fluo-4.

**Results.** ET caused concentration-dependent (0.1–50 nM,  $n = 58$ ) biphasic changes in CaT: a transient decrease to ~70% after 2 min, followed by a sustained increase to ~130% of pre-drug control after 10–30 min. Nuclear CaT had a slower time course than cytosolic CaT (time-to-peak:  $169 \pm 5$  ms versus  $121 \pm 4$  ms; time constant for CaT decay:  $336 \pm 30$  ms versus  $228 \pm 15$  ms;  $n = 20$ ,  $P < 0.01$ ). The ET-induced increase in CaT was larger in the nucleus than in the cytosol. The ratio of nuclear to cytosolic CaT increased by  $12 \pm 2\%$  ( $n = 16$ ,  $P < 0.05$ ). The ET-induced increases in cytosolic and nuclear CaT were inhibited by blocking ETA receptors ( $2.5 \times 10^{-7}$  M BQ123,  $n = 4$ ), phospholipase C ( $3 \times 10^{-6}$  M U73122,  $n = 7$ ), or InsP3 receptors ( $3 \times 10^{-6}$  M 2-APB,  $n = 9$ ).

**Conclusions.** ET increases cytosolic and nuclear CaT via InsP3-induced calcium release. Increases in cytoplasmic CaT augment contractility. Increases in nuclear CaT, on the other hand, may contribute to the induction of hypertrophy through stimulation of calcium-sensitive transcription pathways.

### 5 Gender-specific differences in NO-dependent and -independent endothelial function after myocardial infarction

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**Background.** Chronic heart failure is characterized not only by alterations in cardiac function but also by alterations in endothelial function. Studies concerning endothelial dysfunction Post-MI show an incoherent picture. The aim of this study was to analyze NO dependent and independent endothelial function in aortic endothelium after MI.

**Methods.** MI was induced in female ( $n = 8$ ) and male ( $n = 9$ ) Sprague-Dawley rats via ligation of the LAD. Eight female and 7 male rats served as sham control. After 7 respectively 42 day animals were sacrificed, morphologic data recorded. The descending aorta was extracted, cleaned and cut into 6 rings of 3–4 mm width. This rings were mounted with hooks in an organbath filled with Krebs-Henseleit buffer with a tension of 4 g and precontracted with phenylephrine (Phe,  $10E^{-8} - 3 \times 10E^{-7}$  mol/l). Relaxation was induced by cumulative concentrations of acetylcholine (Ach  $10E^{-9} - 10E^{-5}$  mol/l). To test the NO-independent component of relaxation, Ach-induced relaxation was repeated in the presence of L-NAME (300  $\mu\text{mol/l}$ ).

**Results.** The size of MI was comparable in both genders at 7 days ( $32.8 \pm 1.41$  vs.  $35.79 \pm 2.06$ , female vs. male) and 42 days ( $42.47 \pm 2.93$  vs.  $40.69 \pm 3.19$ , female vs male). Neither in early nor in late phase there was a reduced relaxation to Ach compared with sham. MI rats of both genders showed an increased NO-independent relaxation in the early phase. In the late phase only female rats showed an increased NO-independent relaxation compared to sham.

**Conclusions.** Our data suggests (1) there is no impairment in NO-dependent endothelial function at either time-point. (2) there are gender-specific differences in NO-independent endothelial function only in late but not in early phase after MI.

## Symposium II: „Endothel – Ischämie und Reperfusion“

### 6 Ischämie/Reperfusion und Endothel – Entwicklung eines neuen NO-Donors

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Stickoxid (NO) ist ein gasförmiges Molekül, welches unter anderem in Endothelzellen unter normalen physiologischen Bedingungen synthetisiert wird. Die Endothel-abhängige Relaxation von vaskulär glatter Muskulatur ist hauptsächlich auf NO zurückzuführen. Somit bestimmt NO maßgeblich den Gefäßtonus. NO ist in eine Reihe physiologischer und pathologischer Prozesse involviert. So führt NO-Mangel im Ischämie/Reperfusion-Geschehen zu Vasokonstriktion und Ödem-bildung.

In Präparationen von Proteinen mit potentiell freien Thiolgruppen liegen nur 20–35 % tatsächlich in der freien, reduzierten SH-Form vor. Die übrigen 65–80 % sind – insbesondere bei Proteinpräparationen, welche aus Blut gewonnen werden, oder welche im Rahmen ihres Herstellungsverfahrens mit Plasma oder Plasmaderivaten in Kontakt kommen – blockiert, meist durch gemischte S-S Bindungen mit kleinen Thiolgruppen-tragenden Verbindungen, beispielsweise freiem L-Cystein bzw. Glutathion (Katachalski et al [1957] J Am Chem Soc 79: 4096–4099, DeMaster et al (1995) Biochemistry 34: 11494–11499).

Äquimolare Nitrosierung von prozessiertem Human-Albumin entsprechend der frei verfügbaren Thiolgruppen der Proteinkomponente führten zur Entwicklung von S-Nitroso Human Serum Albumin (S-NO-HSA). Es werden Mechanismen besprochen, auf welche Weise S-NO-HSA über „feedback-inhibition“ der konstitutiven NO-Synthase die Entstehung von toxischen Radikalen unterbinden kann. Die Verhinderung des Endothelschadens durch NO-Supplementierung mittels S-NO-HSA werden an Hand eines Ischämie/Reperfusionmodells am Skelettmuskel (Hallström et al (2002) Circulation 105: 3032–3038) oder im hypovolemischen Schock (Int-ravitalmikroskope – Leber; Bauer et al (2004) Shock 21: 165–169) gezeigt.

### 7 Genetic control mechanisms of the inflammatory response in the endothelium

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**Background.** Upon inflammatory stimulation, endothelial cells (EC) express a plethora of molecules including cytokines, chemokines, cell adhesion molecules, components of the coagulation system, and others. They mediate various biological functions, e.g., chemoattraction, adhesion, and transmigration of immune cells. The majority of these molecules is at least in part transcriptionally regulated, however, other regulatory mechanisms may be operative on several levels, and waiting to be discovered.

**Results.** We performed global expression profiling of HUVEC treated with a classical proinflammatory mediator, IL-1. The resulting set of regulated genes allows not only the identification of novel genes with previously undescribed function in EC, but also insights into the types regulatory mechanisms that are operative during inflammation: This includes induced transcription factors, novel transcriptional regulatory mechanisms following large-scale promoter analysis, and the identification of endogenous negative feedback mechanisms.

**Conclusions.** In proinflammatory cytokine-activated EC, a hierarchy of transcription factors exists that may control distinct sets of target genes to exert distinct biological functions. Of special importance are mechanisms of down-regulation of induced genes, which occur on different levels, including mRNA stability and inhibition of kinase cascades, to prevent overshooting and adverse effects the inflammatory response.

### 8 Attenuation of reperfusion damage in experimental myocardial infarction in pigs by Mirococept, a novel complement inhibitor derived from complement receptor type 1

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**Background.** Complement activation associated with ischemia/reperfusion (I/R) injury leads to local endothelial and parenchymal damage of the affected organ. Membrane-targeted application of complement inhibitors, such as complement receptor type 1 (CR1), which intrinsically regulate complement activation on cell surfaces, may ameliorate I/R injury in vivo, circumventing the disadvantages of systemic complement inhibition.

**Methods.** In a closed-chest pig model of acute myocardial infarction (60 min ischemia, 120 min reperfusion), Mirococept (membrane-targeted, myristoyl-tagged construct derived from CR1), the non-tagged CR1-derivative APT154, or

vehicle was administered intracoronarily into the area at risk 5 min prior to reperfusion.

**Results.** Despite similar areas at risk (AAR) in all groups ( $37.1 \pm 9.4\%$ ,  $38.3 \pm 6.5\%$ ,  $36.5 \pm 4.8\%$  of left ventricular mass,  $p > 0.05$  for all comparisons), only Mirococept significantly decreased myocardial infarct size from  $61.9 \pm 6.3\%$  of the AAR for vehicle controls to  $42.5 \pm 4.0\%$  ( $p = 0.0003$ ), whereas APT154 had no effect (infarct size  $60.8 \pm 5.1\%$  of the AAR,  $p = 0.881$  APT154 vs. vehicle controls). Cardioprotection by Mirococept correlated with reduced serum levels of troponin-I, interleukin (IL)-6, IL-8 and endothelin-1, and was associated with reduced myocardial complement deposition and expression of tissue factor. Binding of Mirococept was found specifically in ischemic tissue. Left ventricular enddiastolic pressure and ejection fraction were significantly improved upon reperfusion only in the Mirococept-treated animals (LVEDP  $p = 0.011$ ; EF  $p = 0.027$  for Mirococept vs. vehicle controls).

**Conclusions.** Local cytoprotection and complement inhibition with the cell-membrane targeted complement inhibitor Mirococept prior to reperfusion significantly reduces myocardial I/R injury and may offer a new complement-inhibitory, anti-inflammatory, endothelial-protective treatment strategy in acute myocardial infarction.

## 9 Hypoxia as a mediator of inflammation in human adipose tissue

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**Background.** Obesity acts as an independent cardiovascular risk factor by mechanisms that are not fully understood. Elevated levels of the pro-inflammatory cytokines interleukin(IL)-6, IL-8 and plasminogen activator inhibitor (PAI)-1 are found in obese patients. We could show recently that PAI-1 is increased by inflammatory cytokines IL-6 and oncostatin M in human adipose tissue (Rega et al [2005] *Circulation* 111[15]: 1938-45). Recent studies suggest that inflammation could be an adaptive response to hypoxia within the expanding adipose tissue mass. In this study we investigated the impact of hypoxia on PAI-1, IL-6 and IL-8 regulation in human adipose tissue ex vivo and in vitro.

**Methods.** Primary human preadipocytes and adipocytes were prepared from subcutaneous and visceral adipose tissue. Explants, preadipocytes and adipocytes were cultured under hypoxic conditions. PAI-1, IL-6 and IL-8 antigen were quantified by ELISAs, mRNA levels were determined by Real-TimePCR.

**Results.** PAI-1, IL-6 and IL-8 secretion was significantly increased under hypoxic conditions in subcutaneous and visceral adipose tissue explants. Hypoxia significantly upregulated IL-6 production in preadipocytes and adipocytes up to 7-fold and 44-fold. IL-8 was significantly increased by hypoxia in preadipocytes and adipocytes up to 4-fold and 7.5-fold. PAI-1 was significantly upregulated by hypoxia in visceral preadipocytes and subcutaneous adipocytes. These results were confirmed on the level of mRNA expression.

**Conclusions.** Our data show that hypoxia increases IL-6, IL-8 and PAI-1 production in adipose tissue explants and in cultured human preadipocytes and adipocytes. We therefore hypothesize that hypoxia promotes the pro-inflammatory state seen in obese patients and thus could contribute to the elevated risk for cardiovascular diseases.

## 10 STAT-1 decoy oligonucleotide treatment reduces acute rejection in mouse heart transplantation

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**Background.** During acute rejection leukocyte-endothelial cell interaction fuelled by co-stimulatory molecules such as, e.g. the CD40/CD154 receptor/ligand dyad, causes a disruption in graft microcirculatory blood flow. Down-regulating endothelial CD40 expression by employing a decoy oligonucleotide (dODN) neutralizing the transcription factor STAT-1 may protect the graft.

**Methods.** Heterotopic mouse heart transplantation was performed in the allogeneic B10A2R to Balb/C mouse model. Animals received no additional immunosuppression. Graft coronary arteries were pre-treated with STAT-1 dODN or mutant control ODN (10 mM,  $n = 11$  each) during the period of ischemia. Previous experiments with fluorescence dye-labeled oligonucleotides have demonstrated that the nucleic acids are exclusively taken up by the graft endothelial cells. Vascular rejection, interstitial rejection and pro-inflammatory gene expression, as measured by real time PCR, were evaluated 7 days and 24 hours post transplantation, respectively.

**Results.** Vascular rejection and interstitial rejection scores were significantly reduced in STAT-1 dODN treated animals by 86% ( $p < 0.001$ ) and 71% ( $p < 0.01$ ), respectively. CD40 abundance was markedly attenuated by 82% in STAT-1 dODN treated animals 24 hours post transplantation ( $p < 0.001$ ). Similar effects were seen for monocyte chemoattractant protein-1 and vascular cell adhesions molecule-1 expression.

**Conclusions.** STAT-1 dODN blockade of pro-inflammatory gene expression, including but not restricted to endothelial CD40 expression, effectively blocks vascular and interstitial rejection in mouse heart allografts.

## 11 Capillary endothelia and cardiomyocytes differ in vulnerability to ischemia / reperfusion during clinical heart transplantation

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**Background.** The development of accelerated graft arteriosclerosis is a major cause of late death after orthotopic heart transplantation. The influence and the extent of peritransplant injury, especially of cardiomyocyte or capillary endothelial cell edema is discussed controversially.

**Methods.** A morphometric ultrastructural analysis of myocardial biopsies from 29 donor hearts was performed. Right ventricular biopsies were obtained before cardioplegia (A), immediately following cardioplegia (B) (Custodiol<sup>®</sup>, Dr. F. Köhler Chemie GmbH, Alsbach-Hähnlein, Germany), before implantation (C), after 30 (D) or 60 (E) min of reperfusion and one week after transplantation (F). Mean ischemic time was  $185 \pm 68$  min. A quantitative electron microscopy was carried out on the following parameters: volume density of myofibrils, mean barrier thickness of capillary endothelia.

**Results.** The volume density of myofibrils [Vol.-%] was as follows: (B)  $63.6 \pm 3.2$ , (C)  $61.8 \pm 3.2$ , (D)  $62.9 \pm 3.2$ , (E)  $63.6 \pm 4.5$ . The mean barrier thickness [nm] was as follows: (A)  $353 \pm 21$ , (B)  $376 \pm 59$ , (C)  $416 \pm 71^*$ , (D)  $473 \pm 45^*$ ; (E)  $453 \pm 50^*$ , (F)  $379 \pm 39$ .

**Conclusions.** Apart from a generally accepted edema of cardiomyocytes a relevant capillary endothelial cell edema develops during clinical heart transplantation. In contrast to cardiomyocytes the cell edema of endothelia shows a more pronounced and significant progression during cold ischemia and early reperfusion. Thus, an edema of capillary endothelia probably will trigger inhomogenities in capillary perfusion. Peritransplant injury of endothelia may contribute to the later development of accelerated allograft arteriosclerosis.

## 12 Verbesserte Myokardprotektion in Ischämie-Reperfusion durch Endothelin-A-Rezeptorblockade in der chronischen Herzinsuffizienz

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**Grundlagen.** Die über den Endothelin 1-Rezeptor Typ A(ETA)vermittelte Vasokonstriktion an den Koronararterien spielt eine bedeutende Rolle in der Entstehung der postischämischen vaskulären und myokardialen Dysfunktion. Ziel der Studie war es, den chronischen und akuten Effekt des ETA-selektiven Rezeptorblockers TBC-3214Na in einem ischämischen Herzinsuffizienzmodell an der Ratte während Ischämie und Reperfusion zu ermitteln.

**Methodik.** Durch Ligatur der LAD wurde an männlichen SD-Ratten ein Myokardinfarkt erzeugt. Die Tiere wurden drei Tage nach Infarkt in drei Gruppen randomisiert: Gruppe 1 (n = 5) erhielt TBC-3214Na kontinuierlich (0,45 mg/kgKG/d) mit dem Trinkwasser über 7 Wochen, Gruppen 2 (n = 6) und 3 (n = 6) erhielten Placebo. Sieben Wochen nach Infarkt wurden die Herzen am isolierten, erythrozytenperfundierten Working Heart während Ischämie (60 min) und Reperfusion (30 min) evaluiert. Gruppe 2 erhielt TBC-3214Na (0,45 mg/kgKG) als Zusatz zur Cardioplegie akut während der Ischämie.

**Ergebnisse.** Die Infarktgröße war in allen drei Gruppen mit  $46 \pm 4\%$  des linken Ventrikels vergleichbar. In der Reperfusion kommt es bei konstanter Herzfrequenz (220 bpm) zu signifikant verbesserter postischämischer Erholung des Cardiac Output in beiden mit TBC-3214Na behandelten Gruppen im Vergleich zur Placebogruppe (Gruppe 1:  $91 \pm 10\%$ ; Gruppe 2:  $86 \pm 11\%$  vs.  $52 \pm 15\%$ ;  $p < 0,05$ ), während eine Ver-

besserung des Koronarflows nur in Gruppe 2 (Gruppe 2:  $121 \pm 23\%$  vs. Gruppe 1:  $75 \pm 13\%$ , Placebo:  $64 \pm 15\%$ ,  $p < 0,05$ ) stattfand. Die Evaluierung des Energiemetabolismus am Ende der Reperfusion zeigte eine signifikant verbesserte Präservierung der energiereichen Phosphate (ATP, ADP, AMP und Creatinphosphat) in beiden Behandlungsgruppen gegenüber Placebo, wobei die Verbesserung in Gruppe 2 am ausgeprägtesten war.

**Schlussfolgerungen.** Die Ergebnisse zeigen, dass sowohl die akute als auch chronische ETA-Blockade den Ischämie-Reperfusionsschaden an ischämisch vorgeschädigten Herzen verringert. Eine Verbesserung der Koronardurchblutung ist jedoch nur in der akut während der Ischämie mit TBC-3214Na behandelten Gruppe beobachtbar.

## Symposium III: „Endothelprotektion – Organprotektion“

### 13 Endothelial protection with NO donors

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The endothelium of the coronary arteries has been identified as the important organ that locally regulates coronary perfusion and cardiac function by paracrine secretion of NO and other vasoactive peptides. Therefore, also in cardiac transplantation, the established organ procurement with hypothermic storage in solutions designed to preserve myocytes but not endothelial cells has to be critically discussed.

Heart transplantation is a prestigious high-end treatment for end-stage heart failure patients with promising survival rates: 84% one-year and 65% five-years survival. However, these survival rates are still far away from being satisfying and offer plenty of room for further research in the areas of organ preservation and perioperative management.

I will focus on possible strategies to improve donor and recipient management in regard to a functional endothelium and NO. The following topics will briefly be addressed: (1) NO pathway and effects, to lay the ground for possible therapeutic strategies and interventions. (2) NO and ischemia/reperfusion, to understand the mechanisms that lead to NO depletion and its consequences. (3) NO and hypothermia, to understand the effects of hypothermia on the endothelium. (4) Current status of donor and recipient management, to describe the strategies used today. (5) Possible new approaches: NO-substitution and NO-scavenging, to describe the recent research that is performed in this area including some of our own results. (6) Outlook in donor and recipient management, to give possible new directions, deducted from our current knowledge.

## 14 Free radicals: new strategies of myocardial protection

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Myocardial infarction is the major cause of death in the world. Over the last two decades, coronary reperfusion therapy has become established for the management of acute myocardial infarction (AMI). However, restoration of blood flow to previously ischemic myocardium results in the so-called ischemia/reperfusion (IR)-injury. The different clinical manifestations of this injury include myocardial necrosis, arrhythmia, myocardial stunning and endothelial- and microvascular dysfunction including the no-reflow phenomenon. The pathogenesis of ischemia/reperfusion injury consists of many mechanisms. Herein we provide an overview on the role of free radicals in general and on the role of peroxynitrite in particular. Peroxynitrite, a highly reactive species causes DNA single strand breaks which activates the nuclear enzyme, poly (ADP-ribose) polymerase (PARP). The activation of PARP leads to an energy consuming inefficient repair cycle with subsequent depletion of NAD<sup>+</sup> and ATP pools and necrotic cell death. The present review overviews the pathophysiologic role of the peroxynitrite-PARP pathway in cardiac ischemia/reperfusion injury with special reference to the therapeutic potential of PARP inhibitors in the treatment of this disease.

## 15 S-nitroso human serum albumin attenuates ischemia/reperfusion injury after cardioplegic arrest in isolated rabbit hearts

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**Background.** Depletion of nitric oxide (NO) is associated with ischemia/reperfusion injury. The novel NO donor, S-Nitroso Human Serum Albumin (S-NO-HSA), could bridge NO depletion during reperfusion in cardiac transplantation and minimize ischemia/reperfusion injury.

**Methods.** In an isolated erythrocyte-perfused working heart model rabbit hearts were randomly assigned after assessment of hemodynamic baseline values to receive S-NO-HSA (0.2 μmol/100 mL, n = 8), L-arginine (10 mmol/100 mL, n = 8) or albumin (control; 0.2 μmol/100 mL, n = 8). After 20 min of infusion, the hearts were arrested and stored in Celsius® (4° C) enriched with respective drugs for 6 hours, followed by 75 minutes of reperfusion. Hemodynamic values were assessed and biopsies were taken to determine calcium-ionophore stimulated release of NO and superoxide.

**Results.** During early reperfusion, recovery of cardiac output (75 ± 6% versus 49 ± 5%, p < 0.05), and coronary flow (99 ± 8% versus 70 ± 5%, p < 0.05) were higher and myocardial oxygen consumption was reduced in the S-NO-HSA group compared to controls (4.08 ± 0.46 ml/min/0.1 kg versus 6.78 ± 0.38 ml/min/0.1 kg, p < 0.01). At the end of the experiment cardiac output (53 ± 5% versus 27 ± 5%, p < 0.01) was higher and left atrial pressure (115 ± 9% versus 150 ± 8%, p < 0.05) was lower in the S-NO-HSA group compared to control. NO release was increased (1040 ± 50 nmol/L and 1070 ± 60 nmol/L versus 860 ± 10 nmol/L, p < 0.01) and superoxide release diminished (31 ± 5 nmol/L and 38 ± 5 nmol/L versus 64 ± 5 nmol/L, p < 0.01) in the S-NO-HSA and L-arginine group compared to control.

**Conclusions.** S-NO-HSA improved hemodynamic functions after prolonged hypothermic cardiac arrest by supplementing NO and thereby decreasing I/R injury.

## 16 Multimeric tyrosine sulfate associates with the endothelium and protects from reperfusion-mediated damage in a model of acute myocardial infarction in pigs

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**Background.** Protection of ischemic endothelium and myocardial tissue by local cytoprotection with glycosaminoglycan analogs, such as dextran sulfate, attenuates ischemia/reperfusion (I/R) injury through modulation of the pro-inflammatory and pro-coagulant environment. However, additional, systemic anticoagulation may limit the use of these analogs in certain clinical indications. We therefore developed a novel, fully synthetic cytoprotectant, multimeric tyrosine sulfate (sTyr-PAA), with minimal anticoagulant properties.

**Methods.** The effect of sTyr-PAA was examined in a closed chest porcine model of acute myocardial I/R injury (60 minutes ischemia, 120 minutes reperfusion), whereby local, left-ventricular ischemia was achieved through balloon occlusion of the left anterior descending coronary artery immediately after the first diagonal branch.

**Results.** sTyr-PAA, administered intracoronarily 5 minutes prior to reperfusion into the area at risk, significantly decreased myocardial necrosis. The respective infarct sizes were: 67 ± 12% of the area at risk for vehicle controls, and 39 ± 17% for sTyr-PAA. The areas at risk were similar in both groups (39 ± 12% and 35 ± 9% of left ventricular mass). sTyr-PAA abrogated myocardial complement deposition and substantially decreased vascular expression of pro-coagulant tissue factor in the ischemic myocardium. sTyr-PAA binding, detected using fluorescein-labeled agent, localized to ischemically damaged blood vessels and myocardium. Furthermore, cardioprotection correlated with decreased creatine kinase-MB plasma levels.

**Conclusions.** Targeted cytoprotection of ischemic myocardium with sTyr-PAA significantly decreased infarct size in a pig model of acute myocardial infarction. Local vascular modulation of inflammation without systemic anticoagulation may therefore provide a possibility to attenuate injury in settings where endothelial protection without coagulation inhibition is of importance.

### 17 Addition of the cytoprotectant dextran sulfate to blood cardioplegia solution attenuates myocardial reperfusion injury associated with extracorporeal circulation in a porcine model of cardiopulmonary bypass

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**Background.** Cardiopulmonary bypass (CPB) initiates systemic and local (heart and lungs) inflammation. Contact of blood with artificial surfaces and air (CPB-circuit), but also ischemia / reperfusion (I/R) injury of heart and lungs, activate complement and coagulation systems and lead to endothelial cell damage and -activation. We hypothesized that endothelial-targeted cytoprotection by the complement inhibitor dextran sulfate (DXS, MW 5000) may attenuate CPB-associated myocardial and pulmonary I/R injury.

**Methods.** In 18 pigs total cardiopulmonary bypass was initiated following standard cold blood cardioplegia (BCP). Cardioplegia was repeated after 30 and 60 minutes of ischemia with modified BCP (including either 300 mg DXS, n = 10 or PBS, n = 8). Following 60 minutes ischemia, reperfusion was initiated and the heart weaned off CPB. Experiments were terminated two hours post-CPB, and heart and lungs subsequently analyzed. Cardiac function was monitored by echocardiography and pressure measurements.

**Results.** DXS ameliorated the metabolic situation (lower lactate/pyruvate ratio), reduced complement activation and interleukin levels in coronary sinus blood. Histological and immunochemical analysis revealed decreased focal complement deposition, neutrophil infiltration and hemorrhage in heart and lungs, correlating with decreased myocardial and pulmonary I/R damage. Formation of thrombin anti-thrombin complexes was significantly inhibited by DXS. Despite reduction of pro-inflammatory and pro-coagulant markers, cardiac function post-CPB was not significantly improved by DXS.

**Conclusions.** Addition of DXS to the BCP solution ameliorates post-CPB inflammation as well as the metabolic situation. However, whereas a trend towards an improved diastolic function was observed, differences in heart function between the two treatment groups were not statistically different.

### 18 Role of prostaglandin-mediation of agonist-induced dilations of isolated human coronary arterioles obtained from patients with diabetes mellitus

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**Background.** Previously we found that in an animal model of diabetes mellitus alterations of cyclo-oxygenase-dependent synthesis of vascular prostaglandins contribute to diabetic microvascular dysfunction. In this study we aimed to elucidate the role of prostaglandins in agonist-induced vasodilator responses of human coronary microvessels.

**Methods.** Atrial arterioles were dissected from right atrial appendages (89 ± 15 micron in diameter) obtained at the time of cardiac surgery from patients with or without documented diabetes mellitus. After dissection, vessels were cannulated and pressurized (80 mmHg) under zero flow conditions. Drugs were applied extraluminally and steady-state changes in diameter were measured with videomicroscopy.

**Results.** After spontaneous tone developed, the endothelium-dependent dilator acetylcholine (ACh) elicited dilations of coronary arterioles from non-diabetic patients (at 1 microM: 82 ± 5%), whereas ACh resulted in constrictions in arterioles from diabetic subjects (at 1 microM: 29 ± 7%). In contrast, dilator responses to bradykinin were greater in arterioles from diabetic patients (at 10 nM: 77 ± 10%) compared to non-diabetics (at 10 nM: 38 ± 14%). Arteriolar responses were similar to the endothelium-independent dilator sodium nitroprusside in the 2 groups. In coronary arterioles of non-diabetic patients inhibition of cyclo-oxygenase by indomethacin did not affect ACh- and bradykinin-induced responses. ACh-induced constriction of diabetic coronaries was not affected by indomethacin, however, cyclo-oxygenase inhibition reduced the bradykinin-induced dilations in arterioles from diabetic patients (at 10 nM: 20 ± 4%).

**Conclusions.** We conclude, that isolated human coronary arterioles from diabetic patients exhibit paradox constriction to ACh and enhanced prostaglandin-mediated dilation to bradykinin. Our findings suggest an enhanced dilator prostaglandin synthesis in coronary arterioles in patients with diabetes mellitus.

### 19 High intracellular Na<sup>+</sup> preserves myocardial function at low heart rates in failing hearts

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**Background.** We tested the hypothesis that increased intracellular [Na<sup>+</sup>]<sub>i</sub> in heart failure contributes to preservation of

SR Ca<sup>2+</sup> load which may become particularly evident at slow heart rates.

**Methods and Results.** In SBFI-loaded myocytes from rabbits with pacing-induced heart failure (PHF), [Na<sup>+</sup>]<sub>i</sub> was significantly higher at each frequency as compared to Sham-operated animals. Furthermore, PHF rabbits demonstrated reduced SR Ca<sup>2+</sup>-ATPase protein levels (-37%,  $p < 0.04$ ) but unchanged Na<sup>+</sup>/Ca<sup>2+</sup> exchanger protein levels. At 0.25 Hz, isometric force was similar in cardiac trabeculae from PHF rabbits as compared to control (PHF,  $3.6 \pm 1.3$ , Sham,  $4.4 \pm 0.6$  mN/mm<sup>2</sup>). Unchanged rapid cooling contractures (RCC) indicate preserved SR Ca<sup>2+</sup> load at this frequency. In Sham, isometric twitch force increased with rising frequencies to  $29.0 \pm 2.8$  mN/mm<sup>2</sup> at 3.0 Hz ( $p < 0.05$ ) as compared to 0.25 Hz. RCCs showed a parallel increase by  $186 \pm 47\%$  ( $p < 0.01$ ). In PHF, frequency-dependent increase in force ( $15.8 \pm 4.7$  mN/mm<sup>2</sup> at 3.0 Hz) and RCCs (increase by  $70 \pm 40\%$ ) were significantly blunted.

**Conclusions.** Thus, in PHF in rabbits SR Ca<sup>2+</sup> load is preserved at low frequencies despite decreased SR Ca<sup>2+</sup>-ATPase expression. This may result from [Na<sup>+</sup>]<sub>i</sub>-dependent changes in Na<sup>+</sup>/Ca<sup>2+</sup> exchanger activity.

## 20 S-nitroso human serum albumin, a novel i.v. nitric oxide donor, reduces ischemia/reperfusion injury in an orthotopic heart transplant model in the pig

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**Background.** Prolonged ischemia is a major challenge in clinical transplantation and depletion of nitric oxide (NO) and the production superoxide are major contributors to ischemia/reperfusion (I/R) injury. We hypothesize that NO-substitution with S-Nitroso Human Serum Albumin (S-NO-HSA) could preserve organ function.

**Methods.** Donor pigs ( $n = 12$ ;  $37.1 \pm 6.1$  kg) were monitored and randomized to treatment with (1  $\mu\text{mol/kg/h}$ ) S-NO-HSA ( $n = 6$ ) or HSA ( $n = 6$ ; control) one hour prior to explantation. Hearts were harvested and stored using Bretschneider solution with addition of 10  $\mu\text{mol/L}$  S-NO-HSA or HSA respectively (4 C for 4 hours). Recipient pigs ( $n = 12$ ;  $36.5 \pm 5.9$  kg) were monitored and put on cardiopulmonary bypass (CPB). Donor hearts were implanted followed by 60 min of controlled reperfusion before subsequent weaning from CPB (epinephrine 0.4  $\mu\text{kg/h}$ ) and a follow up of two hours. 30 minutes prior to estimated aortic declamping, infusion with S-NO-HSA or HSA respectively started for 60 min (0.1  $\mu\text{mol/kg/h}$ ).

**Results.** During early reperfusion coronary flow was significantly higher in S-NO-HSA group ( $p < 0.01$ )( $12.4 \pm 5.2$  and  $29.5 \pm 8.9$ ). During follow up period LVP sys was higher in S-NO-HSA group after weaning from CPB becoming highly significant after 60 min ( $p < 0.01$ )( $104 \pm 8.8$  and  $79 \pm 10.8$  mmHg). Hearts of animals of the S-NO-HSA group had a significant better preservation of phosphocreatin ( $p < 0.05$ )

( $28.25 \pm 9.42$  and  $11.82 \pm 6.23$  nmol/mg protein) and significantly more NO ( $p < 0.01$ ) ( $1250 \pm 50$  and  $825 \pm 50$  nmol/l) and significantly less superoxide ( $p < 0.05$ ) ( $48 \pm 16$  and  $77 \pm 13$  nmol/l) were generated.

**Conclusions.** In a pig model of orthotopic heart transplantation with 4 hours of Ischemia S-NO-HSA minimizes I/R injury and improves organ function demonstrated by a superior hemodynamic function, a better energy preservation and an improved NO-synthetase function.

## Symposium IV: „Endothel – Herzinsuffizienz I“

### 21 Herzinsuffizienz und Endothelfunktion

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### 22 Neue Therapeutische Optionen bei diastolischer Herzinsuffizienz

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### 23 Acute functional effects of IGF-1 and Insulin in human myocardium

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**Background.** IGF-1 and Insulin have been shown to exert positive inotropic effects (PIE) in several in-vitro and in-vivo models, but the mechanism of action remains unclear. We examined inotropic responses and signal transduction mechanisms of IGF-1 and Insulin in human myocardium.

**Methods.** Isolated ventricular trabeculae from failing human hearts ( $n = 81$ ), electric stimulation (1 Hz), isometric contractions, 37° C. Simultaneous registration of intracellular Ca<sup>2+</sup>-transients (aequorin-method), SR-Ca<sup>2+</sup>-load x(rapid-cooling contractures, RCCs) or myofilament-Ca<sup>2+</sup>-sensitivity (skinned-fiber-preparations). Protocols: dose-response curves (IGF-1 0.001-0.1  $\mu\text{mol/L}$ ) and single doses of IGF-1 (0.1  $\mu\text{mol/L}$ ) and insulin (0.3 and 3 I.U./L). Blockade of PI-3-kinase (wortmannin 0.1  $\mu\text{M}$ ), IGF-1-receptors (alpha-IR3-antibody), NCX reverse-mode (KB-R7943; 5  $\mu\text{M}$ ) in glucose-containing tyrode solution.

**Results.** IGF-1 and Insulin concentration-dependently increased twitch force to a maximum of  $146 \pm 9\%$  and  $126 \pm 6\%$  of baseline, respectively ( $p < 0.05$  for both) in insulin-free solution the PIE was accompanied by an increase in Ca<sup>2+</sup>-transients to  $151 \pm 12\%$  and  $116 \pm 4\%$ , respectively. SR-Ca<sup>2+</sup>-load (RCC) increased to  $128 \pm 9\%$  and  $115 \pm 5\%$  (both  $p < 0.05$ ). Myofilament-sensitivity was only increased with Insulin.

Blockade of PI-3-kinase almost completely abolished the PIE of IGF-1 and Insulin (to  $112 \pm 4\%$  and  $108 \pm 2\%$ ;  $p < 0.05$ ), and NCX reverse-mode inhibition partially reduced the PIE (to  $115 \pm 7\%$  and  $111 \pm 7\%$ ), while IGF-receptor-blockade only affected IGF-1 induced PIE (to  $108 \pm 4\%$  and  $126 \pm 7\%$ , respectively).

**Conclusions.** IGF-1 and Insulin exert direct PIEs in failing human myocardium. These effects involve PI-3-kinase and reverse-mode NCX activation. The maximum effect is bigger with IGF-1. Insulin-induced PIE is not only  $Ca^{2+}$ -dependent but includes myofilament sensitization and therefore is energetically favourable. These non-genomic functional effects may be of clinical relevance e.g. during insulin-glucose-potassium infusions.

## 24 Value of serial NTproBNP measurements for the prediction of 30-day-mortality in patients with decompensated heart failure and cardiogenic shock

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**Background.** NTproBNP (NBNP) plasma levels are increased in patients with heart failure and are evaluated as a prognostic marker. The predictive value of NBNP in the evaluation of short term-mortality in patients with decompensated heart failure is still elusive.

**Methods.** The study population of this trial was composed of 117 patients with acutely decompensated heart failure of ischemic and non-ischemic origin (73 men, 44 women;  $70 \pm 12$  years;  $EF 40 \pm 13\%$ ; 83 ischemic, 34 non-ischemic). We prospectively tested the predictive value of plasma levels of NBNP (Elecsys, Roche Diagnostics) for 30-day-mortality. NBNP levels were assessed on hospital admission (A), and after 12, 24 and 48 hours.

**Results.** 38 (32.5%) of the 117 patients died within the first 30 days. NBNP plasma levels in non-survivors and survivors are listed in the table.

	All	Survivors	Death 30 Days
N 117	79	38	
NT-proBNP 0 h (median, pg/ml)	3830	2498	7939 **
NT-proBNP 12 h (median, pg/ml)	4907	3271	11686 ***
NT-proBNP 24 h (median, pg/ml)	4627	3458	7958 ***
NT-proBNP 48 h (median, pg/ml)	3164	2141	6415 **

\*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  for deaths vs. survivors

	12h-A	24h-A	48h-A
$\Delta$ (NT-proBNP median (pg/ml))	3372	2967	1490
Death within 30 days	( $p < 0.001$ )	( $p = 0.017$ )	( $p = 0.649$ )
$\Delta$ (NT-proBNP median (pg/ml))	487	208	12
Survivors	( $p = 0.001$ )	( $p = 0.081$ )	( $p = 0.664$ )
p (difference between both groups)	$< 0.001$	0.051	0.367

In patients dying within 30 days NBNP plasma levels were significantly higher on A and at time points 12, 24 and 48 hrs. The closest correlation with 30 day mortality was found for NBNP plasma levels 12 hrs. after admission to the hospital. In addition, the extent of NBNP increase after admission was also predictive of poor prognosis. Receiver operating curve analysis for NBNP at 12 hrs. showed an area under the curve of 0.77 [0.67, 0.87]. A cut-off level of the lowest percentile (1923 pg/ml) demonstrated a sensitivity of 96%, a specificity of 33%, a negative predictive value of 95% and a positive predictive value of 40% for death within 30 days. Of patients who died, only 2.4% were in the lowest NBNP percentile.

**Conclusions.** NBNP plasma levels have prognostic impact in patients with decompensated heart failure. Best predictive values for 30-day-mortality can be assessed by NT-proBNP values 12 hours after admission and by the increase of NT-proBNP values during the early phase of decompensation.

## 25 Regulation of SERCA expression via Akt-signaling in physiological and pathological hypertrophy

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**Background.** The present study examines the hypothesis, that moderate exercise training modulates the expression of SERCA2a, a key calcium handling protein, via the Akt/GSK-3 $\beta$ -signaling pathway.

**Methods.** Over a period of four weeks, 24 adult FVB/N mice (EX) underwent submaximal exercise training on a treadmill (22 m/min,  $2 \times 1$  h/d) and were analyzed in parallel with age-matched sedentary control animals (SG;  $n = 24$ ).

**Results.** Echocardiographically, gravimetrically and histopathologically, a mild but highly significant degree of training-induced cardiac hypertrophy was found (112.2%). Induction of this physiological form of hypertrophy was paralleled by an increase in the activity of Akt in cardiac homogenates (159% phospho-Akt/Akt ratio), whereas relative protein abundance of Akt remained unchanged compared to control animals (SG: 100%). Akt-activation resulted in increased phosphorylation and thereby inactivation of its downstream target glycogen synthase kinase (GSK)-3 $\beta$  (183%,  $p < 0.05$ ). Calcineurin remained unaffected. Interestingly, we also found increased SERCA2 levels in trained animals compared to sedentary controls (133%,  $p < 0.05$ ). These exercise-induced changes were associated with a significant reduction in mortality due to acute Doxorubicin-induced cardiomyopathy

(20 mg/kg) (survival rate EX: 58% vs. SG: 29%,  $p < 0.05$ ). In human heart failure samples, an initial increase in Akt-activation (145%,  $p < 0.05$ ) in "compensated" hypertrophy (LVEDP  $< 16$  mmHg;  $n = 6$ ) is followed by a drop in Akt phosphorylation in terminally failing hearts (53%,  $p < 0.05$ , LVEDP  $> 20$  mmHg;  $n = 6$ ) compared to non-failing myocardium (100%,  $n = 15$ ). This was paralleled by a decrease in GSK-3 $\beta$  phosphorylation and SERCA2 expression levels (73%,  $p < 0.05$ ). Finally, by using IGF-1, a stimulator, and wortmannin, an inhibitor of the PI3-K-/Akt-/GSK-3 $\beta$ -signaling pathway, we show that SERCA-expression is modulated in an Akt-/GSK-3 $\beta$ -dependent manner in cultured adult rabbit cardiomyocytes.

**Conclusions.** Induction of physiological hypertrophy by submaximal exercise training is associated with activation of Akt-dependent cellular survival pathways in the heart, which may be beneficial in restoring depressed SERCA2a levels in human heart failure.

## Symposion V: Moderierte Postersitzung

### 26 Cardiac myocytes and cardiac fibroblasts contribute to regulation of monocyte phenotype

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**Background.** Monocytes and macrophages may play a key role in the repair and scar formation process following myocardial infarction through production of cytokines and growth factors necessary for fibroblast proliferation and neo-vascularization. Macrophage colony stimulating factor (MCSF) is known to facilitate monocyte survival, monocyte to macrophage conversation and macrophage proliferation. Our objective was to determine if human adult cardiac myocytes (HACM) and human adult cardiac fibroblasts (HACF) can contribute to generate an environment favouring monocyte differentiation and macrophage survival under inflammatory conditions.

**Methods.** For this purpose HACM and HACF were isolated from myocardial tissue. They were treated with TNF- $\alpha$  to simulate inflammatory conditions. MCSF protein was detected by a specific ELISA and RT-PCR employing specific primers for MCSF was used to measure mRNA levels. We used a commercially available NF- $\kappa$ B blocker (Parthenolide) and a commercially available JNK blocker to determine the signalling pathway of TNF- $\alpha$ . Signaling protein levels (p50, p65, c-Jun, c-Fos) were determined using specific ELISA.

**Results and Conclusions.** TNF- $\alpha$  increased MCSF production in HACM and HACF on the protein and mRNA level. The promoter of MCSF contains AP-1 binding sites and NF- $\kappa$ B specific binding sites, also the respective signalling proteins were elevated after 1 hour TNF- $\alpha$  treatment in the nucleus of HACM and HACF. Only the NF- $\kappa$ B blocker Parthe-

nolide was able to reduce MCSF expression to basal level. Therefore we suggest that the induction of MCSF through TNF- $\alpha$  is mainly dependent on the activation of the NF- $\kappa$ B pathway. Our in vitro data suggest that HACM and HACF might contribute to generate an environment suitable for monocyte differentiation and macrophage activation under inflammatory conditions.

### 27 Activation of the Igf pathway is protective in pressure overload and prevents heart failure in a model of Gata4 haploinsufficiency

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**Background.** Left ventricular pressure overload (PO) is a clinically relevant form of cardiac stress and regulated in part by the transcription factor Gata4. Mice with haploinsufficiency for Gata4 (G4D) develop heart failure early after PO. Insulin-like-growth factor 1 (Igf1) has known protective effects in conditions such as ischemia and myocardial infarction. We hypothesized that transgenic overexpression of the cardiac Igf1 receptor (IgfR) might have protective effects in PO and influence the pathological phenotype in G4D mice.

**Methods.** Transverse aortic constriction (TAC) and sham operations were performed in mice with the following genotypes: 1) IgfR; 2) Wildtype (WT); 3) G4D; 4) IgfRG4D.

**Results.** 1 week after TAC WT mice showed preserved echocardiographical fractional shortening (FS;  $51 \pm 3\%$ ), presence of fibrosis and Tunel staining revealed increased apoptosis (1.8 fold of sham;  $p < 0.05$ ). In contrast, IgfR mice responded to TAC without significant fibrosis or increase in apoptosis. G4D mice developed overt heart failure after TAC indicative by reduced FS ( $33 \pm 3\%$ ;  $p < 0.05$  vs. WT) and pulmonary congestion. The extent of fibrosis and increase in apoptosis (2.7fold) was significantly higher in G4D mice ( $p < 0.05$  vs. WT). This pathological G4D phenotype was completely prevented in the crossbred line IgfRG4D. Gene expression profiling revealed upregulation of several members of the Tgfbeta pathway in G4D mice which was significantly diminished in IgfRG4D mice.

**Conclusions.** Igf-1 exerts beneficial effects in pressure overload by protection from fibrosis and apoptosis. These effects are able to overcome the detrimental effects in Gata4 haploinsufficiency and might be in part related to prevention of Tgfbeta pathway activation.

## 28 Die Hemmung der p38 MAP Kinase erhöht die dehnungsabhängige Kraftentwicklung im insuffizienten menschlichen Myokard

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**Grundlagen.** Myokardiale Dehnung aktiviert die p38 MAP Kinase (p38). p38 senkt in isolierten Kardiomyozyten die Verkürzungsfraktion, ohne den Kalziumtransienten zu beeinflussen. Wir überprüften daher, ob die Hemmung der p38 die dehnungsabhängige Kraftentwicklung in insuffizientem menschlichen Myokard erhöht.

**Methodik.** Aus insuffizienten (explantierte Herzen) und nicht insuffizienten (nicht verwendete Spenderherzen) humanen Herzen wurden LV und RV Trabekel isoliert. Während isometrischer Kontraktionen (1 Hz, 37° C, 2,5 mM Kalzium) wurde die entwickelte Kraft (eK) gemessen und während gradueller Dehnung die optimale physiologische Länge (Lopt, Frank-Starling Mechanismus) bestimmt. Die Trabekel wurden anschließend für 30 min auf 88 % von Lopt (L88) entdehnt und mit dem p38 Hemmer SB203580 (10 µM) inkubiert. Die Trabekel wurden dann akut auf eine submaximale Länge (98 % von Lmax, L98) gedehnt und für 3 h verfolgt.

**Ergebnisse.** Die Phosphorylierung der p38 (Western Blot) war trendweise höher in insuffizientem als in nicht insuffizientem Myokard (145 ± 26 %, n = 9 vs 100 ± 16%, n = 8, p = 0.16). Die p38 Phosphorylierung in insuffizientem Myokard war nach 30 min Dehnung weiter gesteigert (+75 ± 20 %, p < 0.01). Die eK stieg nach Dehnung auf L98 an, fiel aber über 3 h Beobachtungszeit konsekutiv ab. Nach Hemmung der p38 war der dehnungsinduzierte Kraftanstieg zu allen Zeitpunkten signifikant höher. Weitere Herzmuskelstreifen wurden in Saponin (5 %) inkubiert und die Membran dadurch teilperforiert. In diesen Präparaten erhöhte die Hemmung der p38 die Kalziumsensitivität signifikant.

**Schlussfolgerungen.** Wir schlussfolgern, dass die endogene p38 Aktivierung den dehnungsinduzierten Kraftanstieg im insuffizienten menschlichen Myokard über eine Minderung der Kalziumsensitivität limitiert. Die p38 kann ein therapeutischer Angriffspunkt bei der dekompensierten Herzinsuffizienz sein.

## 29 Impaired contractile performance of human and rabbit myocardium induced by proton pump inhibitors

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**Background.** High intravenous doses of proton pump inhibitors like pantoprazole (PP) are widely used for the treat-

ment of acute upper gastrointestinal bleeding. We investigated the effect of PP on contractile function and Ca<sup>2+</sup> homeostasis in human and rabbit myocardium.

**Methods and Results.** Under physiological conditions (37° C, pH 7.35, 1 mM Ca<sup>2+</sup>) force development was decreased in trabeculae dissected from non-failing human right atria (mean ± SEM: 50 µg/ml PP [n = 15] 2.07 ± 0.45 vs. control [n = 15] 5.35 ± 0.69 mN/mm<sup>2</sup>; p < 0.05). Similar results were obtained in ventricular trabeculae from patients with NYHA-IV heart failure that underwent cardiac transplantation as well as in ventricular trabeculae dissected from healthy rabbits. Measurements on isolated permeabilised rabbit ventricular cardiomyocytes indicated that PP reduced the affinity of the sarcoplasmic reticulum (SR) Ca<sup>2+</sup>-ATPase (SERCA) for Ca<sup>2+</sup> (K<sub>d</sub>: mean ± SEM: 40 µg/ml PP [n = 9] 3.58 × 10<sup>-7</sup> ± 1.45 × 10<sup>-8</sup> vs. control [n = 9] 3.95 × 10<sup>-7</sup> ± 1.18 × 10<sup>-8</sup> mol/l; p < 0.05) without affecting the maximum Ca<sup>2+</sup> uptake rate (V<sub>max</sub>). Intracellular Ca<sup>2+</sup> release was examined in Fura2-loaded voltage-clamped cardiomyocytes. PP (40 µg/ml) dramatically increased diastolic [Ca<sup>2+</sup>] by 33.7 ± 12.3% (n = 13) and reduced the amplitude of the Ca<sup>2+</sup> transient (by 25 ± 7.2%, n = 13) Inward Ca<sup>2+</sup> current (I<sub>Ca,L</sub>) amplitude was also significantly decreased (by 31 ± 5%, n = 13).

**Conclusions.** These data indicate that PP depresses contractile function of human and rabbit myocardium in-vitro. This effect is may be linked to the depression of Ca<sup>2+</sup> signaling. This indicates a potential risk of high dose therapy with PP in patients with reduced cardiac function.

## 30 Stretch-dependent activation of p44/42-, p38-MAPK and p90rsk in failing and non-failing human myocardium

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**Background.** Stretch elicits functional and trophic effects in mammalian myocardium via various mechanosensitive pathways. The relevance of stretch-induced activation of mitogen-activated protein kinases (MAPK) for these effects are not completely understood. Therefore, we tested the effects of stretch on kinase activation and the delayed force response in failing (F) and non-failing (NF) human myocardium.

**Methods.** Trabeculae (n = 108) from F (n = 9) and NF (n = 6) human hearts were stimulated at 1Hz (2.5 mM Ca<sup>2+</sup>, isometric contractions). For analysis of the phosphorylation state of p38-, p44/42-MAPK, and p90rsk, trabeculae were acutely stretched from slack length to 12 mN/mm<sup>2</sup> diastolic tension and shock frozen at various times after stretch. For functional analysis of developed force, trabeculae were stretched from 88% to 98% of optimal length. The resulting slow increase in twitch force (slow force response, SFR) was assessed without and after inhibition of p44/42-MAPK (5 × 10<sup>-5</sup>M PD98059) or p38-MAPK (10<sup>-5</sup>M SB203580).

**Results.** Stretch caused a time-dependent activation of p38-, p44/42-MAPK and p90rsk in both F and NF human myocardium. Maximal activation of all kinases occurred 10-15 min after stretch. The time course of activation for p44/42-MAPK and p90rsk was comparable to the time course of the

SFR. Inhibition of p44/42-MAPK, however, did not affect the SFR. In contrast, p38-MAPK inhibition increased the SFR.

**Conclusions.** Stretch activates p38-, p44/42-MAPK and p90rsk in F and NF human myocardium. This activation may be important for long-term adaptation to stress (via regulation of gene transcription). In addition, p38-MAPK (but not p44/42-MAPK) exerts short-term functional effects on force development by reducing the SFR.

## Symposium VI: „Endothel – Herzinsuffizienz II“

### 31 Prognostische Bedeutung der endothelialen Dysfunktion

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### 32 Das Endothel im Zentrum des therapeutischen Konzepts bei pulmonaler Hypertonie: Vom rechtsventrikulären Energiestoffwechsel bis zur praktischen Anwendung

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### 33 Repetitive intrakoronare Mikroembolisation im Schafmodell führt zur chronischen Herzinsuffizienz und ermöglicht die Erforschung endothelialer Dysfunktion

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**Grundlagen.** Die endotheliale Dysfunktion stellt einen wesentlichen Pathomechanismus in der Entwicklung einer Herzinsuffizienz dar. Ihre Erforschung im Tierversuch erfordert ein valides Großtiermodell, in dem die Entwicklungsstufen der chronischen Herzinsuffizienz simuliert werden. Ziel unserer Untersuchungen war es, zu untersuchen, inwieweit durch repetitive intrakoronare Mikroembolisationen eine chronische Herzinsuffizienz zu etablieren ist.

**Methodik.** Hierzu wurde Schafen ( $n = 6$ ,  $85 \pm 6$  kg) eine 5F-Schleuse in die linke Art. carotis communis implantiert. Das linke Ostium wurde unter Röntgendurchleuchtung kateterisiert und Polyesterolmikrosphären ( $90 \mu\text{m}$ ,  $n = 12.500$ ) in den Hauptstamm injiziert. Koronare Mikroembolisationen (KME) wurden zwei bis dreimal in zweiwöchigen Abständen wiederholt, bis die Tiere begannen, beständige klinische Zei-

chen der Herzinsuffizienz zu entwickeln. Klinische und echokardiographische Daten wurden zu Beginn und 3 Monate (3 Mo) nach der ersten KME analysiert.

**Ergebnisse.** Alle Tiere entwickelten klinische Zeichen der Herzinsuffizienz: signifikant erhöhte Herz- und Atemfrequenzen im Ruhezustand sowie Aszites. Der linksventrikuläre enddiastolische Durchmesser (LVedD) erhöhte sich signifikant, während die EF nur minimal sank. Die histologische Untersuchung ergab eine signifikante Zunahme der Fibroserate ( $> 20\%$  vs. Kontrollgruppe  $12\%$ ), welche inselförmig global im linken Ventrikel verteilt war.

**Schlussfolgerungen.** Repetitive intrakoronare Mikroembolisation führt zu einer bedeutsamen Myokardfibrose und Dilatation der Herzhöhlen und entspricht damit einem Modell der chronischen stabilen Herzinsuffizienz. Es ermöglicht die experimentelle Erforschung der Endothel(dys-)funktion bei der chronischen Herzinsuffizienz sowie die Effektivität einer Ventrikelentlastung durch Implantation von Linksherzunterstützungssystemen.

### 34 Progressive right ventricular failure is not explained by myocardial ischemia in a pig model of pulmonary embolism

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**Background.** Current concepts of acute pulmonary embolism suggest that subsequent right ventricular (RV) dilatation and failure are the consequence of pressure overload-induced RV hypoperfusion and ischemia.

**Methods.** 16 hybrid pigs were instrumented for the measurement of RV and aortic pressure, aortic and right coronary artery blood flow (RCABF), RV oxygen consumption (RV MVO<sub>2</sub>) and RV free wall segment length. The pulmonary artery was constricted (PAC) to increase RV peak pressure acutely 2.5 fold (from  $27 \pm 2$  to  $64 \pm 3$  mmHg,  $n = 9$ ), and the constriction was maintained for 6 h.

**Results.** At 10 min after PAC, a RV work index (RVWI, RV pressure-segment length loops) was increased 2.3 fold, indicating an initial RV adaptation to increased afterload. At 1 h, 3 h and 6 h after PAC, however, RVWI decreased progressively towards control levels, while RCABF and RV MVO<sub>2</sub> continued to increase. The arterial-coronary venous pH difference did not increase throughout the protocol. Arterial troponin T concentration increased from  $0.08 \pm 0.03$  to  $0.80 \pm 0.20$  ng/ml at 6 h after PAC. None of the parameters changed in control animals ( $n = 7$ ).

**Conclusions.** We conclude that in our model RV failure during PAC develops in spite of increased coronary blood flow and MVO<sub>2</sub>. Thus, mechanisms different from ischemia can underly progressive RV failure after pulmonary embolism.

### 35 Six weeks treatment with Si Ni Tang, a Chinese herbal cocktail, attenuates left ventricular dysfunction post myocardial infarction

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**Background.** Recent experimental and clinical data suggests that treatment with SiNiTang, an herbal cocktail used in Traditional Chinese Medicine, might improve survival and cardiac function after myocardial infarction. First data with SiNiTang have shown an anti-platelet and anti-arrhythmic effects and a reduction in MMP activity. Therefore, it is the purpose of our experiments to evaluate whether SiNiTang prevents the adverse effects of ventricular remodeling post MI.

**Methods and Results.** In 16 adult male SD rats MI was induced by coronary ligation, 16 rats underwent sham-operation. The first day rats were randomized to receive either SiNiTang (TeaMI n = 8; TeaSham n = 8) or placebo (MI n = 8; Sham n = 8) over a period of 6 weeks. Prior to sacrifice in vivo measurements of left ventricular function were made by echocardiography (M-mode, ATL 15MHz). At the time of sacrifice there was no difference in body weight, tibia length or infarct size between the groups (mean ± SD). The LV/body weight ratio was significantly reduced in TeaMI compared to MI (2.5 ± 0.42 vs. 3.12 ± 0.2; p < 0.05). Left ventricular end-diastolic (ED) and end-systolic (ES) dilation was less pronounced in TeaMI compared to MI (ED: 9.4 ± 0.4 vs. 10.3 ± 0.1 mm; p < 0.05; ES: 6.4 ± 0.5 vs. 7.8 ± 0.2 mm; p < 0.05). Consequently, fractional shortening (FS) was significantly higher in TeaMI compared to MI (FS: 0.33 ± 0.05 vs. 0.24 ± 0.05%; p < 0.05).

**Conclusions.** It can be concluded that treatment with SiNiTang post MI (1) reduced LV hypertrophy (2) prevented diastolic and systolic left ventricular dilation and dysfunction in a rat model of MI. Therefore SiNiTang could be valuable in the treatment of congestive and ischemic heart failure.

## Symposium VII: „Endothel – Innovative Therapien I“

### 36 Mending the broken Heart: Functional cardiomyogenic differentiation of cardiac side population cells

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**Background.** The cellular mechanism underlying the development of cardiac dysfunction is a decrease in the number of viable cardiomyocytes. Recent observations have suggested that the adult heart may contain a progenitor cell population. Side population (SP) cells, characterized by a distinct Hoechst dye efflux pattern, have been shown to exist in multiple tissues and are capable of tissue-specific differentiation.

**Methods and Results.** In adult mice, we confirm the existence of a cardiac SP cell population. Under physiologic conditions, cardiac SP cells are found to be maintained through local progenitor cell proliferation with little contribution from extracardiac stem cell sources. Following myocardial infarction in adult mice, however, cardiac SP cells are acutely depleted, both within the infarct and noninfarct areas, and are subsequently reconstituted to baseline levels within 7 days after myocardial infarction, through both proliferation of resident cardiac SP cells, as well as through homing of bone marrow-derived stem cells. Furthermore, we determine that cardiac SP cells are capable of both biochemical and functional cardiomyogenic differentiation into mature cardiomyocytes, with expression of cardiomyocyte-specific transcription factors and contractile proteins, as well as stimulated cellular contraction and intracellular calcium transients indistinguishable from adult cardiomyocytes.

**Conclusions.** We, therefore, conclude that cardiac SP cells represent a distinct cardiac progenitor cell population. These cardiac SP cells are maintained through both self-proliferation and selective homing of bone marrow cells, and are capable of cardiomyogenic differentiation into mature cardiomyocytes.

### 37 Tissue Engineering

G. Zünd

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### 38 Rapid endothelialization of synthetic vascular grafts with stem cells

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**Background.** In vitro endothelialization of small diameter vascular grafts for coronary revascularisation has been shown to be clinically feasible for redo patients with no other biological alternative for autografting. However, in vitro endothelialization is a cell culture dependent technique, which takes 3-4 weeks to create an endothelial lining. It is therefore not suitable for acute indications. CD34+ cells have been shown to be able to differentiate into endothelial cells in vitro. In this work we assessed the possibility to seed bone marrow derived CD34+ cells in a single stage procedure onto small diameter vascular grafts in order to create a confluent endothelium.

**Methods.** Human bone marrow was used to utilize CD34+ cells. Cells were resuspended in cell culture medium and seeded on small diameter PTFE vascular grafts by using a rotation device for 5 hours. Prior to seeding, PTFE grafts were precoated with a special solution of fibrinolytically inhibited commercially available fibrin glue. After rotation grafts were further incubated in cell culture medium, containing growth and differentiation factors. Specimens were taken every day and were examined with a vital fluorescence dye, electron microscopy and immunohistochemistry.

**Results.** Immediately after seeding, a multilayer of cells was present on the grafts. Only a fraction of these cells stained positive for CD34. After 1 day in culture, the multilayered structures have disappeared and a confluent monolayer of cells was visible. Some cells still exhibited a rounded shape whereas others appeared to be fully spreaded. These cells stained positive for CD34 and resembled endothelial cells by morphology. Between day 7 and 11 a confluent layer of cells was present which stained positive for CD34, CD31 and F VIII.

**Conclusions.** A confluent monolayer of endothelial like cells can be created on small diameter PTFE grafts within a few days in a single stage procedure. This procedure might be a last alternative for an increasing number of redo patients, when no adequate autografts are available.

### 39 Intramyokardiale versus intrakoronare Applikation von Stammzellen – eine experimentelle Studie mit einem unerwarteten Ergebnis

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**Grundlagen.** Das Ziel der Studie war es, histologisch das Ergebnis der intramyokardialen gegen die intrakoronare Stammzell-Applikation sehr früh nach der Applikation zu vergleichen. Diese Fragestellung wurde bislang nicht untersucht.

**Methodik.** 40 ml Knochenmark wurde von 17 Schweinen (20–25 kg) aus dem Beckenkamm aspiriert. Mononukleäre Zellen wurden mit der Ficoll-Technik isoliert, und mit der Vybrant-Lösung wurde die Vitalität geprüft. Ein Vorderwandinfarkt wurde induziert. Danach wurden fünf Zellportionen zu je 2 × 100 Millionen Zellen in das Zentrum des Infarktes, in die Randzone sowie in das gesunde Myokard appliziert bzw. in die Koronarie distal der Ballon-Okklusion eingebracht. Nach 10, 120 oder 240 Minuten wurden die Herzen extrahiert, und mit einem Fluoreszenzmikroskop wurden die Zellen histologisch in den konsekutiven Schnitten gesucht.

**Ergebnisse.** Nur unmittelbar in den Stichkanälen der Injektionen fanden sich dicht gepackte Ansammlungen von mononucleären Zellen ohne Hinweis auf eine Migration. Nach der intrakoronaren Applikation fanden sich nur nach intensiver Suche einzelne Zellen.

**Schlussfolgerungen.** Es stellt sich die Frage, wie viele Zellen appliziert werden müssen, um ein adäquates Ergebnis zu erzielen. Innerhalb der ersten 240 Minuten kam es zu keiner Migration in das Myokard. Offenbar werden in den klinischen Versuchen beide Methoden doch erst systemisch effektiv, dem widersprechen aber klinische Versuche mit der direkten systemischen Applikation.

### 40 Combined transplantation of skeletal myoblasts and angiopoietic progenitor cells reduces infarct size, apoptosis and improves cardiac function in a model of chronic ischemia

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**Background.** Cellular cardiomyoplasty using skeletal myoblasts or angiopoietic progenitor cells offers a promising approach for the treatment of ischemic heart failure. Although several studies have shown encouraging results in settings of acute and semi-chronic myocardial infarction, the efficacy of

cell therapy on the chronic ischemic heart remains undetermined.

**Methods.** A model of chronic ischemia was created using LAD-ligation in nude rats. Culture medium (C), homologous skeletal myoblasts (SM), human AC-133+ cells (SC) and both homologous skeletal myoblasts and human AC-133+ cells (SM-SC) were injected in the infarct (SM) and peri-infarct area (SC) four weeks after infarction. Assessment of myocardial function was performed using echocardiography 8 weeks after injections. Infarct size, collagen deposits and cardiomyocyte apoptosis were quantified to evaluate the effect cell injections using histology.

**Results.** Echocardiographic studies revealed an amelioration of left ventricular dilatation in animals receiving combined cell transplantation (LVEDD: SM, SC, SM-SC, C:  $7.5 \pm 1.5$ ,  $7.1 \pm 1.6$ ,  $5.7 \pm 0.8$  and  $7.7 \pm 0.09$ ,  $p = 0.003$ ). Left ventricular ejection fraction improved significantly in all three cell therapy groups but no additional benefit was observed in the SM-SC group (SM, SC, SM-SC, C:  $63.5 \pm 13.8$ ,  $63.3 \pm 7.8$ ,  $68.2 \pm 5.6$  vs. control:  $48.6 \pm 8.9$ ,  $p = 0.0017$ ). Quantification of scar tissue showed a significant reduction of infarct area in the SM-SC group (SM, SC, SM-SC, C:  $22.3 \pm 9.1\%$ ,  $19.8 \pm 7.6\%$ ,  $13.2 \pm 5.8\%$ ,  $36.5 \pm 8.2\%$ ,  $p = 0.008$ ). Improvement of myocardial function was associated with reduced apoptotic index in animals after cellular cardiomyoplasty (SM, SC, SM-SC, C:  $3.2 \pm 0.9$ ,  $3.1 \pm 0.6$ ,  $1.8 \pm 0.8$ ,  $10.3 \pm 1.6$ ,  $p = 0.0002$ ).

**Conclusions.** Combined transplantation of skeletal myoblasts and AC-133 angiopoietic progenitor cells leads to improvement of diastolic and systolic left ventricular function, and reduction of scar size and myocardial apoptosis in a model of chronic ischemia.

#### 41 Tissue engineered blood vessels: biocompatibility and remodeling of small-bore, xenogeneic vascular conduits

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**Background.** Many patients, designated for vascular bypass grafting, have no suitable autologous vessels and contribute to the increasing clinical demand for small diameter vascular substitutes. Although the concept of tissue engineering is a promising approach the use of xenogeneic, decellularized bioprosthetic implants is limited by tissue degradation and aneurysmatic remodeling as a result of chronic rejection. We investigated the potential of a new decellularization method to reduce the antigeneic load of xenogeneic scaffolds and to enhance biocompatibility.

**Methods.** Femoral arteries ( $10 \times 1.5$  mm) from juvenile pigs were decellularized (Triton X-100, Na-deoxycholate, Igepal CA-630, ribonuclease treatment). Prostheses were implanted into the abdominal aorta of 30 rats for either 10 days, 4 weeks, 3 or 6 months. Retrieved specimens were evaluated by histology, immuno-histochemical staining, scanning electron microscopy and by isometric tension studies in vitro.

**Results.** The conduits showed repopulation with endothelial cells and myofibroblasts within 10 days. After 6 months, the graft matrix as well as the neointima showed an ingrowth of highly differentiated vascular cells. An early and moderate immunological response (mononuclear cells) in the adventitial layer disappeared within 6 months. Genuine elastic fibers of the conduit were degraded and replaced by newly synthesized fibers. Aneurysm formation occurred in only 6% of the implants. Isometric tension studies showed significant reactivity of the remodeled vessel against thromboxan A2 and prostaglandin E.

**Conclusions.** These results suggest, that implants preserved with this newly developed technique elicit only a moderate immune response which does not interfere with host cell repopulation and vascular remodeling.

#### 42 Expression of adhesion molecules on the endothelialized decellularized porcine matrix

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**Background.** Seeding with autologous cells is an integral part in tissue engineering. We previously reported efficient seeding of the decellularized porcine heart valve matrix and demonstrated endothelialization to effectively abolish thrombogenicity. The aim of this study was to investigate the influence of the acellular porcine matrix on proinflammatory activation of seeded endothelial cells.

**Methods.** Porcine heart valves were decellularized with Triton-X 100, sodium-deoxycholate, IgepalCA-630 and ribonucleases and seeded with human umbilical vein endothelial cells (HUVEC). Expression patterns of E-selectin and ICAM-1 were tested at baseline after seeding, after stimulation with IL-1 $\beta$ , exposure to 60 min of hypoxia followed by a reoxygenation period of 240 min and finally after stimulation with IL-1 $\beta$  and hypoxia/reoxygenation (H/R). Samples were examined using immune scanning electron microscopy.

**Results.** E-selectin expression was not seen in unstimulated HUVEC seeded onto the decellularized matrix but stimulation with IL-1 $\beta$  induced a significant rise of E-selectin expression. Neither H/R alone nor subsequent stimulation with IL-1 $\beta$  led to a significant stimulation of E-Selectin. ICAM-expression at baseline was very low but stimulation with IL-1 $\beta$  resulted in a significant increase of endothelial ICAM-1 expression on the seeded matrix. Although H/R  $\pm$  IL-1 $\beta$  provoked an increase of ICAM-expression in HUVEC attached to coverslips, endothelial cells seeded onto the xenogenic matrix were not influenced.

**Conclusions.** Hypoxia and reoxygenation which would be expected to occur during surgery do not result in proinflammatory upregulation of E-selectin and ICAM-1 expression on endothelial cells grown on a decellularized porcine heart valve matrix.

## Symposium VIII: „Endothel – Innovative Therapien II“

### 43 Gentherapie als Chance für Graft- und Stentfailure

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Die Verwendung von autologen Beinvenen als Bypass-Grafts gehört zum Standard in der koronaren Bypass-Chirurgie. Das perioperative Trauma sowie die nachfolgende Adaptation des venösen Grafts an die geänderten Druck- und Strömungsverhältnisse im arteriellen Kreislauf führen bereits nach wenigen Wochen zu einer intimalen Hyperplasie der Gefäßwand. Dieser Wandumbau bildet die Grundlage für die zunehmende Graft-Atherosklerose mit konsekutiver Verringerung des Gefäßlumens bis hin zum vollständigen Gefäßverschluss. Die Morbidität und Mortalität von Bypass-Patienten werden durch diese pathophysiologischen Mechanismen signifikant reduziert – nach 10 Jahren beträgt die Offenheitsrate der Venen-Bypässe nur mehr 50 %.

Zusätzlich ist die intimale Hyperplasie auch für die Restenose von Koronarstents hauptverantwortlich. Die Inzidenz der Stent-Restenose wird auf 10–30 % geschätzt.

Während die herkömmlichen therapeutischen Möglichkeiten um die Reduktion der intimalen Hyperplasie sehr beschränkt sind, bietet Gentherapie die einzigartige Chance, Umbauvorgänge des Grafts am Ort des Entstehens direkt und langfristig durch die einmalige Gabe eines Therapeutikums zu beeinflussen.

### 44 Uncovering novel markers of heart disease by genome-wide transcript profiling

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**Background.** GeneChip technology provides great potential for the discovery of cardiac disease markers, which could significantly improve clinical diagnosis and stratification of patients with heart failure (HF). Here, we report results from a preliminary study focusing on patients with left ventricular (LV) hypertrophy due to aortic valve stenosis (AS).

**Methods.** Patients with isolated AS undergoing aortic valve replacement have been enrolled and assigned into an early (EF > 50%, n = 3) or end-stage (EF < 30%, n = 3) HF group. Patients with coronary artery disease (CAD), no history of MI, no signs of LV hypertrophy served as non-hypertrophic controls (EF > 60%, n = 3). RNA was isolated from LV septum (AS) and epicardial biopsies (CAD), subjected to cRNA amplification and then analyzed on Affymetrix HG-U133A GeneChips. Data analysis was performed using the GC-RMA algorithm for expression summaries, Bayes t-test for significance analysis, and ArrayTools for class prediction.

**Results.** Expression levels of about 200 transcripts clearly distinguished AS from CAD. Annotation revealed close correlation with hypertrophic response and progressive fibrosis. Amongst AS classifiers, we identified markers like natriuretic peptide precursor A/B as well as connective tissue growth factor or periostin, markers for mechanical stress and ventricular dilation. We further observed that the hitherto unrecognized POMZP3 gene was consistently up-regulated by about 10-fold (P < 0.0005) in patients with AS and EF < 30%, probably representing a novel genetic marker for end-stage HF.

**Conclusions.** Although the numbers of patients was limited in this pilot study, we present several potential candidate genes that could serve as LV hypertrophy markers or even prognostic markers distinguishing compensated from decompensated HF.

### 45 Hybrid mock circuits for mechanical and hydrodynamic testing of vascular specimens and endothelial cells

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**Background.** Exposure to proper mechanical and hydrodynamical forces is essential for a multitude of vascular research topics. Proper design of hybrid artificial-tissue circuits is essential for both diagnostic and culture tasks.

**Methods.** Several mock circuits were designed for seeding and testing endothelial cell cultures, and for investigation of the arterial wall and surgical interventions. Flow profiles were adjusted by adaptation of pumps, tubing elasticity, and staged resistance design, and adapted to the viscosity of culture medium to simulate the blood shear exposure in-vivo. If necessary, gas exchange and oxygen equilibration could be performed with thin-walled silicon tubings, which replaced usual oxygenators. Special soft adaptors were designed for integration of vascular tissue (such as porcine aortae) into the circuit.

**Results.** Several projects were already performed with these devices, including investigation of hemodynamic effects of aortic root prosthesis, stenting of aortic aneurisms, deposition of radioactivated platelets in stenotic regions, and endothelial cell seeding and adhesion testing. With proper selections of components (especially in respect of length, diameter and compliance) and pump and damping adjustment, simultaneously flow and pressure conditions as occurring in the aorta, the distal arterial and the coronary vessels could be simulated. Peripheral pressure and flow conditions could be simulated with a priming volume of only 40cc, coronary conditions with only 10cc. Full gas equilibration could be achieved within 15 minutes without using a separate oxygenator.

**Conclusions.** With proper design of mock, the hydrodynamic and mechanical forces on tissue and cells in vascular walls can be simulated. Depending on the details of the problem, sterile circuits, circuits with minimal priming volume or circuits only using disposables can be established.

## References

- Simon-Kupelik N et al (2002) *Ann Thor Surg* 73: 355–359  
 Schima H et al (2004) *Int J Artif Organs* 27/7: 627 (Abs)  
 Meinhart J et al (2005) *Tissue Engineering* 11

#### 46 Quality improvement of venous bypass grafts by siRNA-mediated adhesion molecule inhibition?

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**Background.** The fate of venous bypass grafts is influenced by the adhesion molecule (AM) expression on endothelial cells. They are stimulated by various causes mediating the adhesion of leukocytes to the endothelial layer and finally their transendothelial migration. Therefore, inhibition of AM expression may be a promising strategy to improve the quality of venous grafts. Liposomal transfection of short interfering RNA (siRNA) is a powerful technique for a post-transcriptional knockdown of activated genes caused by the specific degradation of target messenger RNA.

**Methods.** Primary human venous endothelial cells were isolated from saphenous veins and were transfected using a liposomal reagent with siRNAs targeting either intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule (VCAM) or E-selectin (ES). Further groups were transfected with a sequence-unrelated control siRNA or were left untransfected. TNF- $\alpha$  induced adhesion molecule expression was analyzed by flow cytometry and immunoblotting.

**Results.** In comparison to controls siRNA transfection leads to a significantly reduced receptor expression on endothelial cells. The fractions of ICAM, VCAM or E-selectin positive cells decreased by 86%, 68% and 75%, respectively, after siRNA transfection followed by TNF- $\alpha$  stimulation. Transfection with unspecific siRNA did not affect adhesion molecule expression.

**Conclusions.** siRNAs can specifically suppress AM expression on venous endothelial cells, which is supposed to inhibit the pathological leucocyte-endothelial-interaction leading to a neointimal thickening and graft atherosclerosis, the two most important causes for venous bypass graft failure. Therefore, the application of siRNAs targeting specific AMs may be a novel therapeutic concept for improving the long-term patency of venous grafts.

#### 47 Adenoviral gene transfer in human vein grafts – evaluation of a new transfection technique

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**Background.** Long term patency rates for coronary artery bypass grafting (CABG) using autologous saphenous vein are poor. Effective and proven pharmacologic interventions to prolong vein graft patency are lacking. Gene therapy seems well suited for the prevention or postponement of vein graft failure. However, a major obstacle in gene transfer to human vein grafts is low transfection efficiency due to age, preexisting venous disease, and endothelial barrier.

**Methods.** 26 intact human saphenous veins (age  $68 \pm 7$ ) were cut into rings 15 mm in length. A small piece of each vein was tested for endothelial viability with trypan blue. Individual vein rings were endoluminally incubated with adenoviral vectors expressing a reporter gene (Ad.CMV.lacZ/GFP,  $1 \times 10^{11}$  pfu/mL) with variable pressures. Vein segments were cultured for 7 days after transfection and harvested for cryopreservation. Transfection efficiency was evaluated by X-gal-staining; endothelial integrity and neointima-hyperplasia were evaluated by CD31- and Elastica Van-Gieson-staining.

**Results.** Veins which underwent gene transfer without pressure showed low transfection efficiency ( $12\% \pm 7$ ). In contrast, veins which were pressure-transfected with either 100 or 150 mmHg showed high transfection efficiency throughout the vessel wall ( $45\% \pm 13$  and  $91 \pm 7\%$  respectively,  $p < 0.01$ ). However, CD31 staining revealed a significant loss of endothelium in high-pressure groups, favouring a maximum of 75–100 mmHg during transfection.

**Conclusions.** We here show for the first time that viral transfection using carefully regulated supraphysiological endoluminal pressure greatly enhances transfection efficiency in human vein grafts. Establishing a safe and efficient delivery-method for human cardiovascular gene therapy applications is an important aspect of translation from pre-clinical to clinical gene therapy.

#### 48 Pharmacogenomic evaluation of left ventricular remodeling under endothelin-A receptor blockade in a rat model of myocardial infarction

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**Background.** Endothelin 1 (ET-1), a major player of the neurohumoral defense reaction triggered after myocardial infarction (MI), has been associated with adverse, long-term post-MI remodeling processes. The objective of this study was to evaluate the effects of a novel, highly specific endothelin receptor A (ET-A) blocker on post-MI remodeling by combining classic parameters of cardiac remodeling with pharmacogenomics.

**Methods.** MI was induced in male rats by LAD ligation. At day 3 post-MI, rats were randomized to receive the ET-A blocker, TBC3214Na, or placebo and to survive either 7 or 42 days. Rats surviving for 6 weeks underwent transthoracic echocardiography; hearts from all animals were subjected to morphometric and histological analysis. Total RNAs were extracted from left ventricle (LV) infarct-remote areas, pooled (n = 3), and subjected to gene expression (GE) profiling using GeneChip arrays.

**Results.** At day 42, TBC3214Na significantly reduced hypertrophy (LV-to-body weight ratio) and improved hemodynamics (fractional shortening). GE analysis revealed that the majority of MI-induced changes in GE occurred early after MI and most of early induced genes returned to baseline expression at later stages in the remodeling process. Five days of ET-A blockade resulted in an attenuated expression of 38 MI-induced transcripts (e.g. TnC, Spp1, Sparc, Mmp14) involved in post-MI remodeling.

**Conclusions.** Our findings suggest that the observed changes in GE at an early stage of therapy are responsible for the beneficial effects of TBC3214Na in long-term post-MI remodeling.

### Symposium X: „Endothel – Restenose“

#### 49 Drug eluting stents – perfekt gegen Restenose – schlecht für die Vasomotion

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#### 50 Cardiac therapy: driven by evidence or market mechanisms?

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Coronary artery disease influences survival and quality of life. The impact on survival is most active in the triple vessel and left main disease patient, hardly noticeable in single and two-vessel disease. The investment for society in CABG has therefore focused for years on the triple vessel and left main disease patient, with its well documented long lasting effect. These were documented in mathematical models covering 20 years, allowing patient-specific predictions with very small confidence limits and allowing optimization processes for further improvements as additional arterial grafting. Till today no evidence has been made available showing that PCI even approaches these long-term benefits. CABG has an even stronger impact on the quality of life in the coronary disease patient by transforming the patient into a symptom free individual with absolutely normal quality of life and this for more than a decade at least after the procedure. Progression of disease, as in diabetes and hypertension, has become the only actor in the return of symptoms after the extended use of arterial grafting in the late seventies. Indeed an increase of arterial grafting has postponed dramatically the need for re-intervention. The CABG patient, proposed today, has a mean age that most frequently exceeds 70 years of age, often 80 years. These patients have a natural life expectancy expressed in single digits and frequently not exceeding 5 years. The interaction between the reduced need for re-intervention with additional arterial grafting and the reduced natural life expectancy creates a procedure that covers the complete life-expectancy of the patient in an angina-free quality of life. Cardio-surgical units that have taken an approach towards more extensive arterial grafting and earlier in time, have noticed a total disappearance of repeat coronary surgery. Coronary surgery is therefore a perfect procedure if it were not for the early risk. Indeed the early risk is comparable with PCI, but unacceptable from a patient's perspective. An in-depth re-engineering of CABG is therefore essential to provide to society this nearly perfect service. We have taken this path at K.U. Leuven since 1999. We have been able to annihilate early risk, as well in mortality as in morbidity, in an unselected and consecutive cohort of more than 3000 patients. Through this same re-engineering the resource cost has been lowered way below the cost of PCI even though the hospital stay is still somewhat longer. Will evidence win or market mechanisms? Probably market mechanisms, but the

long-term evidence of CABG could be the guide for an evidence-driven optimization of health care.

### 51 Everolimus attenuates neointimal hyperplasia in cultivated human saphenous vein grafts

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**Background.** Neointimal hyperplasia is the first step of a cascade leading to a reduced patency rate of saphenous vein grafts compared to arterial grafts after coronary bypass grafting. Vascular smooth muscle cells migrate from the media into the intima and proliferate to form a sick neointimal layer. The macrolide immunosuppressant everolimus is a proliferation signal inhibitor, that blocks vascular smooth muscle cell proliferation. We hypothesized, that everolimus attenuates neointimal hyperplasia in cultured human saphenous vein grafts.

**Methods.** Saphenous vein grafts of 10 patients were processed as follows: One piece of vein served as baseline at day 0. One piece of the vein served as control, one piece received 1  $\mu$ M of everolimus solution. Both were cultured for 14 days in RPMI medium. Then all vein-grafts were fixed in formaldehyde, embedded in paraffin-blocks, stained (Elastica van Gieson), and underwent a quantitative histological analysis.

**Results.** Neointima was significantly reduced in the everolimus treated vein-grafts ( $3.7 \pm 1.2 \mu\text{m}$ ) compared to controls ( $10.1 \pm 2.5 \mu\text{m}$ ),  $p < 0.01$ . There was no significant difference between neointima of baseline vein grafts ( $2.8 \pm 1.7 \mu\text{m}$ ) compared to the everolimus group, but statistically significant compared to control,  $p < 0.01$ . There was no statistical difference of the thickness of the lamina media between all groups. A significantly reduced intima/intima+media-ratio was determined in the everolimus group ( $0.10 \pm 0.02$ ) compared to control ( $0.24 \pm 0.07$ ),  $p < 0.01$ . No significant difference was noted between the baseline ( $0.08 \pm 0.05$ ) compared to the everolimus group concerning the intima/intima+media-ratio, but there was a significant difference compared to control,  $p < 0.01$ .

**Conclusions.** Everolimus can inhibit neointimal proliferation in cultivated human vena saphena magna grafts. Therefore, Everolimus has the potential to improve the patency rate of vein-grafts in coronary bypass grafting in a clinical setting.

### 52 Simvastatin verhindert die transiente linksventrikuläre Dysfunktion nach koronarer Mikroembolisation bei Kaninchen in vivo

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**Grundlagen.** Eine koronare Mikroembolisation (ME) tritt spontan bei koronarer Plaqueruptur oder iatrogen bei der koronaren Angioplastie auf und ist mit myokardialer Inflammation

und Funktionsverlust assoziiert. Wir haben ein Modell der koronaren Mikroembolisation bei Kaninchen entwickelt und gezeigt, dass an Tag 2 nach ME eine myokardiale Dysfunktion auftritt, die an Tag 9 nach ME vollständig reversibel ist. Diese myokardiale Dysfunktion ist assoziiert mit einer transienten myokardialen Granulozyteninfiltration, p38 MAP Kinase Phosphorylierung, Tumor Nekrose Faktor alpha Freisetzung und Expression der induzierbaren Stickstoffmonoxidsynthase. Da Statine ausgeprägte antiinflammatorische Eigenschaften besitzen, überprüften wir, ob Simvastatin die myokardiale Dysfunktion nach koronarer Mikroembolisation verhindert.

**Methodik.** Bei anästhesierten Kaninchen ( $n = 14$ ,  $3,4 \pm 0,2 \text{ kg}$ ) wurden unter Röntgendurchleuchtung über einen 3F Mikrokatheter Polysterolmikrosphären (MS,  $45 \mu\text{m}$  Durchmesser,  $n = 10.000$ ) in den linken Hauptstamm injiziert. Ab Tag 3 vor ME bis Tag 9 nach ME erhielten die Tiere Simvastatin (Sim,  $3 \text{ mg/kg/Tag}$ ,  $n = 5$ ) oder Placebo (P,  $n = 9$ ) per os.

**Ergebnisse.** 2 mit P behandelte Tiere starben binnen 12 Stunden nach ME. Troponin T an Tag 1 nach ME war in beiden Gruppen ähnlich erhöht (P:  $1,6 \pm 0,5 \text{ ng/ml}$ , Sim:  $2,1 \pm 0,5 \text{ ng/ml}$ , NS). Während in P die Ejektionsfraktion (EF, Echokardiographie) an Tag 2 nach ME absank ( $45 \pm 3$  vs  $58 \pm 2 \%$ ,  $p < 0,05$ ), war sie in Sim unverändert ( $57 \pm 2$  vs  $57 \pm 2 \%$ ). In beiden Gruppen war die EF an Tag 9 nicht von Kontrolle verschieden (P  $56 \pm 1 \%$ , Sim  $54 \pm 1 \%$ , je NS vs Kontrolle).

**Schlussfolgerungen.** Wir schlussfolgern, dass Simvastatin die transiente, entzündlich vermittelte LV-Dysfunktion nach koronarer ME verhindern kann. Statine könnten bedeutsam sein bei der Behandlung des kardiogenen Schocks und der ischämischen Kardiomyopathie.

### 53 Impact of OGTT on the diagnosis of glucose intolerance in patients who underwent coronary intervention

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**Background.** International studies revealed that among patients with coronary artery disease (CAD) 36% have impaired glucose tolerance and 22% have type-2 diabetes (DM2). CAD patients with impaired glucose metabolism, in particular those who undergo coronary intervention (PCI), have a worse long-term outcome in comparison to patients with a normal glucose metabolism. To investigate to what extent oral glucose tolerance testing (OGTT) is able to detect disturbances of glucose metabolism on top of other methods, e.g. single measurement of fasting glucose status or Hb1Ac.

**Methods.** We studied 68 consecutive eligible CAD patients (76.5 male, mean age  $\pm 11.2$  years) with unknown pre-existing metabolic glucose dysfunction who had undergone successful coronary intervention (PCI). OGTT was performed 3 months after PCI, according to the recommendations of the WHO.

**Results.** Out of 65 patients 51 (75%) had normal fasting plasma glucose (NFG). The remaining 17 patients had impaired fasting glucose (IFG). Among patients with NFG, HbA1c-levels were measured in 47 patients (92.2%). 3 pa-

tients (6.4%) had HbA1c levels  $\geq 6\%$  (Group A) and 44 patients (93.6%) had HbA1c  $< 6\%$  (Group B). OGTT-results of both groups are shown in Table 1. In total, six patients dropped out of this analysis.

**Table 1**

	Group A (n = 3)	Group B (n = 44)
NGT	1 (33.3%)	34 (79.1%)
IGT	1 (33.3%)	8 (18.6%)
DM2	1 (33.3%)	1 (2,3%)

**Conclusions.** HbA1c is not specific enough in order to diagnose a disturbed glucose metabolism. Therefore, OGTT could be essentials as a screening method for patients at high cardiovascular risk.

#### 54 Risk adjusted Evaluation of 30 mortality using EUROSCORE and multiple simulation for comparison

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**Background.** Part of quality control in adult cardiac surgery is the measurement of the 30 day mortality. A VLAD (Variable Life Adjusted Display) to visualise the result does chance not take in consideration. A Method to present the confidence interval 95% for the hypothesis “the observed mortality matches the expected mortality” was developed and implemented in a computer program (Signifigraph).

**Methods.** In a continuous series of patients that undergo consecutive adult cardiac surgery, the logistic EUROSCORE is calculated and the 30 day mortality is collected. The resulting VLAD is compared to the VLADs of a number of simulated series of operations with the same mix of risk and same number of virtual patients. The percentile of the observed data point in relation to that of the simulated data points is calculated and displayed as a graph. The 95% confidence interval for hypothesis “the observed mortality is worse than the expected” is marked as a red line in our computer program. The impact and the time window for taking in consideration less recent data can also be varied. 498 consecutive cases of adult cardiac surgery of one surgeon were entered in the program.

**Results.** In contrast to the conventional VLAD Signifigraph can also give alerts when the VLAD is still showing better than expected results. A change in behaviour of the surgeon can be demonstrated.

**Conclusions.** In contrast o other methods, with our method confidence intervals can be given for every data point and not only for the last calculated value.

## Symposion XI: „Endothel – Graft Failure“

### 55 Perivascular treatment with azathioprine reduces neointimal hyperplasia in experimental vein grafts

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**Background.** Azathioprine is an immunosuppressive and anti-inflammatory drug and it has been shown to induce apoptosis in human T lymphocytes. We investigated whether local treatment with azathioprine can inhibit neointimal hyperplasia in experimental vein grafts.

**Methods.** C57BL6J mice underwent interposition of the inferior vena cava from isogenic donor mice into the common carotid artery using a cuff technique. In the treatment group azathioprine was applied perivascularly. The control group did not receive local treatment. Vein grafts were harvested at 1 and 2 weeks postoperatively and underwent morphometric analysis as well as immunohistochemical analysis for apoptosis (TUNEL).

**Results.** In grafted veins without treatment (controls) neointimal thickness was 10 (6–29)  $\mu\text{m}$ , and 12 (8–40)  $\mu\text{m}$  at 1, and 2 weeks postoperatively. In azathioprine treated grafts the neointimal thickness was 2 (1–5)  $\mu\text{m}$ , and 4 (3–11)  $\mu\text{m}$ . This reduction of neointimal thickness was significant at 1 week ( $p = 0.001$ ) and 2 weeks ( $p = 0.016$ ) postoperatively.

Azathioprine treated vein grafts showed an increased rate of apoptosis in the vascular wall as compared with controls (593 [26–783] vs. 45 [0–106]) apoptotic cells/ $\text{mm}^2$  at 1 week,  $p = 0.063$ , and 656 [327–1270] vs. 19 [0–79] apoptotic cells/ $\text{mm}^2$  at 2 weeks,  $p = 0.016$ ).

**Conclusions.** We conclude that treatment of experimental vein grafts with azathioprine is associated with a reduction of neointimal hyperplasia and an increased apoptosis rate in the vascular wall.

These results suggest that azathioprine may be useful for the prevention of vein graft disease.

### 56 Routine clinical cell transplantation in vascular surgery: A long term follow up

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**Background.** We report on our long term routine clinical experience with endothelial cell transplantation on femoropopliteal bypass grafts.

**Methods.** Synthetic vascular grafts were confluent lined with cultured autologous endothelial cells prior to implantation. In vitro endothelialized grafts were offered to all patients who did not have a suitable saphenous vein available for bypass

grafting. 256 endothelialized synthetic grafts were implanted in the femoro-popliteal position between 1993 and 2005.

**Results.** The median implantation time was 7.7 years. The routine clinical implantation of biosynthetic grafts showed a primary patency rate of 76% after three, 68.0% after five and 60% after 10 years. Seven grafts had to be explanted and could be examined morphologically. In all cases a confluent endothelium was present. In some areas atherosclerotic changes were visible at various degrees. In one graft, a vessel wall like structure, consisting of a tunica intima and a tunica media, divided by an internal elastic membrane, was visible. All explanted grafts were obtained from high risk patients for atherosclerosis.

**Conclusions.** We conclude that biosynthetic grafts have a patency rate which is comparable to that of vein grafts. Grafts can develop atherosclerosis and the formation of a vessel wall is possible. It further proves, that methods employing autologous cell transplantation can be successfully transferred to surgical routine.

### **57 A new concept for in vivo self-endothelialization of vascular prostheses by immobilized DNA-aptamers working as capture molecules for circulating endothelial progenitor cells**

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**Background.** Due to their insufficient biocompatibility and high thrombogenicity, small diameter artificial vascular

prostheses still do not show a satisfactory patency rate. In vitro endothelialization of artificial grafts before implantation has been established experimentally years ago, but, has never been used for routine clinical applications. This study deals with the coating of graft surfaces with capture molecules for circulating endothelial progenitor cells (EPCs), mimicking a pro-homing substrate to fish out EPCs from the bloodstream after implantation.

**Methods.** Aptamers against EPCs were generated by systematic evolution of ligands by exponential enrichment (SELEX), a technique from combinatorial chemistry. They can be selected from a library of 1015 starting nucleotides. We have spotted a defined aptamer onto a hydrogel coated surface, installed in a flow chamber, which allows an online detection of the attachment of EPCs from fresh human whole blood. Finally these cells were cultivated in growth factor enriched medium and fluorescence marked antibodies against CD34, CD 31, von Willebrand factor and VEGFR-2 were used to characterize the cell attachment.

**Results.** After eight SELEX rounds 36 aptamers in total were cloned, sequenced, synthesized and evaluated by flow cytometry. The best binding aptamers were immobilized in a flow chamber. The captured cells were positive for CD34, CD 31, von Willebrand factor and VEGFR-2 and able to differentiate into a confluent endothelial layer.

**Conclusions.** We hypothesize that in vivo self-endothelialization of blood contacting devices by homing factor mimetic capture molecules for EPCs may bring completely new visions to cardiovascular surgery.

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