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Symposion I: „Vorstufen des myokardialen Pumpversagens – maladaptive Hypertrophie und Remodeling“

1 Introduction

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2 Sex-specific patterns of gene expression in early and late post-MI remodeling

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Background. Sex-specific differences have been reported in post-MI heart-failure. The cause of this differences might be found in post-MI remodeling. Therefore, we wanted to elucidate sex-specific patterns in gene-expression in post-MI remodeling.

Methods. MI was induced in male ($n = 9$) and female ($n = 9$) sprague-dawley rats via ligation of the LAD. After 7 respectively 42 days animals were sacrificed. Male ($n = 12$) and female ($n = 10$) sham-operated rats served as control. mRNA was isolated from non infarcted left ventricular free wall, pooled ($n = 3$) and analyzed using Affimetrix Gene-Chip® technology. A signal log ratio 2 (SLR2) of greater or equal 1 was taken as significant change (two fold in-respectively decrease).

Results. Infarct sizes were similar in all groups with an infarct size of $47.23 \pm 3.93\%$. At day 7 female rats showed a significant change of 681 targets whereas males showed a change in 479 targets compared to respective sham groups. Only 193 targets were changed in both sexes. Males showed a higher proportion of up-regulation than females (78.5% vs. 67.4%, $p < 0.001$). At day 42 females showed a significant change of 490 targets versus 217 targets in males, 53 targets were changed in both sexes. Females showed a higher proportion of up-regulation than males (81.8% vs. 69.1%, $p < 0.001$). Comparing the two time-points only 101 targets were changed at 7 and 42 days in females and only 49 targets in males compared to respective sham groups.

Conclusions. (1) Sex-specific targets are activated in post-MI remodeling. (2) Specific targets are activated in early and late phase of post-MI remodeling.

3 Intracellular magnesium in isolated guinea pig papillary muscle during acute ischemic heart failure

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Background. Early animal studies and population-based trials have shown that sufficient nutritional magnesium (Mg) supply is pivotal for myocardial function. Mg-free diet leads to myocardial necrosis and death of laboratory animals. Prospective clinical trials investigating possible benefits of supplemental administration of intravenous Mg in patients with ST-elevation myocardial infarction were controversial in outcome. Both intracellular Mg homeostasis and transmembrane Mg transport during myocardial ischemia have not been well understood as yet. Hence, the interpretation of the conflicting results from clinical trials remains difficult.

Methods. Here, we study free, unbound as well as total myocardial Mg during myocardial ischemia using different techniques. In guinea pig papillary muscle, using Mg-selective intracellular microelectrodes, we measure intracellular, free, unbound, biologically active Mg ($Mg^{2+}i$) and, simultaneously, we assess free Mg^{2+} at the surface of the preparation ($Mg^{2+}s$) during the same experiment, also using specific, Mg-selective microelecrodes pressed on the surface of the preparation.

Results. In isolated guinea pig Langendorff perfused hearts, we use atomic absorption spectroscopy in order to measure total Mg (Mgtot) content of the left-ventricular myocardium after 28 minutes of hypoxia and metabolic inhibition with 2-deoxyglucose. In the same Langendorff-perfused hearts, Mg^{2+} is measured in the effluate ($Mg^{2+}e$) using a Mg^{2+}-selective macroelectrode (AVL) during hypoxia and metabolic inhibition. We find that free intracellular Mg^{2+} ($Mg^{2+}i$) rises during myocardial ischemia, while total Mg-content (Mgtot) of the ischemic tissue falls. Furthermore, we see a concomitant rise of Mg^{2+} concentrations at the surface ($Mg^{2+}s$) of the preparation as well as in the effluate ($Mg^{2+}e$) from the ischemic tissue.

Conclusions. We conclude that, during hypoxia, simulated ischemia and metabolic inhibition, Mg^{2+} is liberated from intracellular binding sites and is extruded from the cell.

4 Glukoseabhängige Inotropie von Insulin – akute Verbesserung der Kontraktilität

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Grundlagen. Insulin zeigt an humanem Myokard akute positiv inotrope Effekte (PIE). Wir konnten zuvor zeigen, dass dieser Effekt sowohl Kalzium- als auch Glukose-abhängig ist. Die Signaltransduktion des Glukose-abhängigen Anteils ist bislang nicht beschrieben. Der Glukosetransporter SGLT1 ist im Gegensatz zu den meisten Tierspezies im menschlichen Myokard hoch exprimiert – eine funktionelle Rolle ist bislang nicht beschrieben. Wir untersuchten die Rolle von Glukosetransportern für die Insulin-vermittelte Inotropie am menschlichen Myokard.

Methodik. Isolierte ventrikuläre Trabekel ($n = 45$) aus terminal insuffizienten Herzen und Vorhoftrabekel ($n = 36$ aus 17 Herzen). Bikarbonathaltige Tyrodenlösung (11,2 mM Glukose bzw. 22,4 mM Pyruvat, 2,5 mM Ca^{2+}, 37 °C). Registrierung der isometrischen Kraftentwicklung nach Insulin-Gabe (3 I.E./l). Blockade der PI-3-Kinase (Wortmannin 0,1 µM), des SGLT1 (Phlorizin 200 µM) oder des GLUT4 (Vesikeltransporthemmung mit Latrunculin 250 nM).

Ergebnisse. Insulin zeigt am ventrikulären menschlichen Myokard einen PIE von $24 \pm 4\%$ ($p < 0{,}05$). Dieser ist in Glukose-freier Pyruvat-Tyrode auf $13 \pm 4\%$ signifikant reduziert. Am atrialen menschlichen Myokard zeigt Insulin ebenfalls einen positiv inotropen Effekt von $14 \pm 2\%$. In beiden Geweben ist dieser Effekt durch PI-3-Kinasen-Hemmung reduziert (ventr. auf $9 \pm 3\%$, atrial auf $4 \pm 4\%$; jeweils $p < 0{,}05$). Der PIE von Insulin ist sowohl durch GLUT-4-Inhibition mit Latrunculin (atrial auf $5 \pm 3\%$, ventr. auf $8 \pm 4\%$; je $p < 0{,}05$) als auch durch SGLT1-Hemmung mit Phorizin (atrial auf $6 \pm 3\%$, ventr. auf $7 \pm 5\%$; je $p < 0{,}05$; $p < 0{,}05$) signifikant reduziert.

Schlussfolgerungen. Insulin wirkt am menschlichen Myokard teilweise Glukose-abhängig positiv inotrop. Der Glukose-abhängige Teil ist sowohl GLUT4- als auch SGLT1-vermittelt und könnte positive Effekte von Glukose-Insulin-Kalium(GIK)-Infusionen vermitteln. Damit können wir erstmals die funktionelle Relevanz von SGLT1 am menschlichen Myokard demonstrieren.

5 Verminderte Aktivität des kardialen Na/Ca-Austauschers bei chronischer β1-adrenerger Stimulation in der Maus

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Grundlagen und Methodik. Chronische adrenerge Aktivierung führt zu myokardialer Dysfunktion und erhöhter

Morbidität und Mortalität. In β1-Adrenozeptor transgenen (β1TG) Mäusen führt die chronische β1-adrenerge Stimulation früh (2–4 Monate) zu einem verzögerten diastolischen Abfall des intrazellulären [Ca], noch bevor sich in vivo eine verringerte Kontraktilität nachweisen lässt (Circulation, 2004; 109: 1154). Die Ursachen für den verzögerten Ca-Transport aus dem Zytosol sind derzeit noch nicht geklärt. Wir untersuchten die Ca-Homöostase (Fluo-4AM) in Kardiomyozyten (0,5 Hz Feldstimulation) von β1TG und Wildtyp (WT) Mäusen (3 Monate).

Ergebnisse. In β1TG war das maximale systolische [Ca] höher (585 ± 77 vs. 363 ± 45 nM, $n > 15$/Gruppe; $p < 0{,}05$) und verspätet (143 ± 4 vs. 122 ± 4 ms; $p < 0{,}05$) als in WT. Der diastolische Ca Abfall war verzögert (Zeitkonstante TAUstim: 224 ± 19 vs. 180 ± 12 ms). Der Ca-Gehalt des sarkoplasmatischen Retikulums war in β1TG erhöht (154 ± 6 vs. 70 ± 15 µmol/L Zytosol, $p < 0{,}05$). Die Zeitkonstante des Abfalls des Ca-Transienten in Gegenwart von Koffein (TAUcoff) als Maβ für die NCX Aktivität war in β1TG verzögert (3506 ± 308 vs. 2221 ± 257 ms; $n > 8$/Gruppe; $p < 0{,}01$), die aus TAUstim und TAUcoff berechnete SERCA-Aktivität bei 0,5 Hz jedoch vergleichbar (241 ± 29 ms vs. 198 ± 20 ms). In Übereinstimmung mit einer reduzierten NCX-Aktivität in β1TG war bei Applikation des NCX-Inhibitors Ni (5 mM) die Ni-sensitive Komponente von TAUcoff reduziert ($69 \pm 5\%$ vs. $84 \pm 4\%$ in WT; $n = 6$ bzw. 5; $p = 0{,}059$).

Schlussfolgerungen. Eine verminderte NCX-Aktivität trägt zum verzögerten diastolischen [Ca]-Abfall bei chronischer β1-adrenerger Stimulation in der Maus bei. Die verminderte NCX Aktivität im frühen Stadium der Herzinsuffizienz könnte im Hinblick auf die bekannten kardiotoxischen Effekte einer erhöhten zytosolischen Ca Last für die Entwicklung der Herzinsuffizienz von wesentlicher Bedeutung sein.

Symposion II: Postersitzung „Inflammation und myokardiales Pumpversagen“

6 Hepatocyte growth factor mediates induction of PAI-1, IL-6 and IL-8 in human adipose tissue

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Background. Obesity is associated with an increased risk of developing cardiovascular disease. The pro-atherogenic adipokines plasminogen activator inhibitor-1 (PAI-1), interleukin (Il)-8 and Il-6 are elevated in patients with obesity, insulin resistance and type 2 diabetes. On the other side serum hepatocyte growth factor (HGF) levels are also elevated in patients with atherosclerosis and obesity. In this study we investigate whether HGF regulates the expression of PAI-1, Il-6 and Il-8 in human adipose tissue.

Methods. Primary human preadipocytes were prepared from adipose tissue. Differentiation to adipocytes was induced by hormone-supplementation. Preadipocytes and adipocytes were treated with HGF for 48 h. PAI-1, Il-6 and Il-8 antigen were quantified by ELISA, mRNA levels were determined by RealTimePCR.

Results. HGF significantly up-regulates PAI-1 production in both preadipocytes and adipocytes up to 9-fold and 6-fold, Il-6 production up to 18-fold and 13-fold and Il-8 production up to 6-fold. These results were confirmed on the level of mRNA expression.

Conclusions. Our results demonstrate a significant upregulation of PAI-1, Il-6 and Il-8 expression in human preadiopcytes and adipocytes. If these effects are also operative in vivo one could hypothesize that elevated levels of HGF seen in obese subjects might contribute to the progression of atherosclerosis through upregulation of the adipokines PAI-1, Il-6 and Il-8.

7 Evidence for TRPC3 as a novel marker for the proliferative potential of endothelial progenitor cells

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Background. Recent evidence suggests that human adipose tissues host a significant fraction of somatic endothelial progenitor cells (EPCs). In culture, these cells generate colony forming units (CFUs) that respond to vascular endothelial growth factor (VEGF) with further proliferation and are able to organize in tube-like structures.

Methods. Stromal cells prepared from adipose tissue were immunosorted using magnetic beads coated with CD34/CD133 antibodies. Relative sizes of subpopulations were quantified by flow-cytometry. Cells were cultered under various conditions and analyzed by immuno-histochemical techniques and Fura2-Ca^{2+} imaging.

Results. We observed abundant expression of TRPC3 channel protein in freshly isolated EPCs as well as in early EPC culture (24 h) by flow-cytometry and immuno-histochemical imaging. The population of TRPC3 expressing cells derived from the fresh adipose tissue preparation overlapped with the fraction expressing the selective stem-cell surface marker CD133. After 24 h in culture, TRPC3+/CD133+ CFUs were identified, which showed a heterogenous distribution of TRPC3+ cells within the cell clusters, with TRPC3 expressing cells preferentially located in the outer rim of the colonies. Fura-2 Ca^{2+}-imaging experiments of these cell colonies revealed that VEGF-induced Ca^{2+} entry was significantly larger in TRPC3 expressing ìouter rimî cells as compared to cells in the centre of the clusters.

Conclusions. Thus, TRPC3 is suggested as an element of the VEGF signalling pathway in EPCs, contributing to stem cell proliferation and may be essential for vasculogenesis. TRPC3

may be considered as a possible target that allows for selective interference with vasculogenesis and angiogenesis.

8 A microarray and real time PCR study on the effect of experimental ischemic heart failure upon the expression of the insulin dependent transmembrane glucose transport molecule GLUT4 in human atrial myocardium

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Background. Glucose and high energy metabolism plays a pivotal role in the development of numerous salient characteristics of myocardial ischemia, such as the gating properties of specific ion-channels, intracellular ion-homeostasis, electrical phenomena, contractility and other phenomena. Many of these aspects of myocardial ischemia are linked in one or the other way to transmembrane glucose transport, intracellular glucose metabolism and, in fact, to GLUT4.

Methods. In human cardiac tissue (right auricle), we here investigate to which extent GLUT4 gene expression is altered in experimental ischemia. Using microarray technique we first look at general changes in expression profiles during simulated myocardial ischemia, the behaviour of SLC2A4 (GLUT4, solute carrier family 2 [facilitated glucose transporter], member 4) as well as its regulator gene SLC2A4RG. Then, using Real Time PCR (Light Cycler), we quantify GLUT4 mRNA expression changes in 8 single experiments under ischemic and control conditions.

Results. Using the microarray technique, we find that both the expression of GLUT4 gene (SLC2A4) and its regulator gene remain practically unchanged. In Real Time PCR, the mean ratio for GLUT4 gene expression compared to the house keeping gene G6PDH was under well oxygenated conditions $0.0052 + 0.0203$ and under N2-simulated ischemia $0.0179 + 0.0196$ ($n = 8$; +SEM). No statistically significant difference could be found between the two groups. Results show a trend to a slight increase in expression, however no statistical significance could be seen.

Conclusions. No significant changes are seen in the expression of the GLUT4 gene as well as in its regulatory gene after 30 minutes of N2-mediated experimental ischemia. Similarly, biological processes involved in glucose metabolism are not significantly de-regulated as are others. This, as well as a slight trend towards up-regulation can be interpreted as an attempt of the myocyte to maintain energy metabolism also under hypoxic conditions.

9 An increase of G-CSF is associated with a diminished fibrinolytic activity in patients with coronary artery disease

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Background. There is increasing evidence that G-CSF prevents left ventricular remodeling after myocardial infarction and stem cell mobilization with G-CSF has also been proposed to improve cardiac function. Recent studies suggest that the interaction between stem cells and cytokines may contribute to neo-vascularisation. However, G-CSF therapy was also associated with increased restenosis and may promote the progression of atherosclerotic lesions. The aim of this study was to examine any interaction between G-CSF and the fibrinolytic system in patients with coronary artery disease.

Methods. G-CSF antigen as well as t-PA and PAI-1 active antigen plasma levels were determined with specific ELISA in blood samples from 75 patients taken before and 24 hours after PCI with DES implantation.

Results. PCI significantly increased plasma levels of G-CSF (27 ± 10 vs. 36 ± 18 ng/mL, $p < 0.00001$). G-CSF plasma levels before PCI correlated with PAI-1 active antigen ($R = 0.28$, $p < 0.05$) and showed a significant inverse correlation with t-PA plasma levels ($R = -0.26$, $p < 0.05$).

Conclusions. An increase of G-CSF was associated with a diminished fibrinolytic activity in patients with coronary artery disease. So one might speculate that this could be a new important link between G-CSF and the fibrinolytic system.

10 Thrombin upregulates expression of oncostatin M (OSM) in human macrophages and peripheral blood monocytes

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Background. Hemostatic factors like thrombin play a crucial role in generating thrombotic plugs at sites of vascular damage (atherothrombosis). However, whether hemostatic factors contribute directly or indirectly to the pathogenesis of atherosclerosis remains uncertain. Oncostatin M (OSM) as a member of IL-6 family cytokines is a proinflammatory mediator that is primarily known for its effects on cell growth. We could show recently that OSM induces the expression of plasminogen activator inhibitor-1, an established cardiovascular risk factor, in adipose tissue (Rega et al., Circulation 2005). The aim of the present study was to investigate if thrombin and OSM can act as a link between macrophages, platelets and the development of cardiovascular disease.

Methods. Peripheral blood monocytes (PBMC) were isolated using Ficoll-Paque and magnetically labelled CD14 MicroBeads. For macrophage transformation (MDM) cells

were cultivated for 8–10 days in the presence of human serum. Plaque Macrophages were isolated from atherosclerotic plaques and positive selection of CD14 positive cells was performed employing CD14 antibodies. All Cells were incubated with thrombin at a concentration of 1U/ml. OSM antigen was determined by specific ELISA. OSM specific mRNA was quantitated by RealTime-PCR.

Results. Thrombin increased OSM antigen concentration- and time-dependently up to 20-fold in MDM and up to 8-fold in PBMC. These results could be confirmed on specific mRNA level. In human plaque macrophages stimulation with thrombin leads to a 5-fold increase of OSM mRNA level.

Conclusions. Thrombin induces the expression of OSM in human macrophages in vitro. If this effect is also present in vivo it may be a new link between platelets, macrophages and the development of cardiovascular disease.

Symposion III: „Molekulare und zelluläre Mechanismen“

11 Einführung

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12 Elevated levels of interleukin-1β-converting enzyme and caspase-cleaved cytokeratin-18 in acute myocardial infarction

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Background. Systemic inflammation and apoptosis-specific immune activation play a major role in acute coronary syndromes (ACS) and acute myocardial infarction (AMI). Thrombectomy devices (X-sizer) were introduced in the clinical arena to allow removal of intracoronary thrombotic material in the setting of AMI.

Methods. Plasma samples were obtained from stable angina (SA, $n = 34$), unstable angina (UA, $n = 37$) and patients with AMI ($n = 39$). Coronary plasma was acquired by means of the X-sizer device. The inflammatory and apoptosis-specific soluble proteins IL-1β precursor (IL-1βp) and interleukin-1β-converting enzyme (ICE) and caspase-cleaved cytokeratin-18 (ccCK-18) were determined by ELISA. Group comparisons were evaluated by parametric Tukey test. Multivariate logistic regression analysis was performed to determine predictive values of IL-1βp, ICE and ccCK-18 for occurrence of AMI.

Results. IL-1βp, ICE and ccCK-18 were identified to be altered in the peripheral blood of patients suffering from AMI as compared to controls (both, $p < 0.05$). ROC curves were plotted and revealed that ccCK-18 is a novel sensitive marker for the detection of AMI (AUC 0.925). Moreover, ICE and ccCK-18 were significantly increased at the site of coronary occlusion as compared to plasma samples obtained from systemic blood in AMI (both, $p < 0.001$).

Conclusions. Our results suggest that enhancement of soluble ICE and ccCK-18 are related to ACS. Plasma protein ccCK-18 was identified as novel marker for the occurrence of AMI.

13 Inducible NO synthase (iNOS) expression in endomyocardial biopsies after heart transplantation in relation to the postoperative course

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Background. Infections and acute rejections influence the morbidity and mortality in the postoperative course after heart transplantation. As part of a non-specific defense system, NO is involved in inflammatory processes in the cardiovascular system. This study investigates iNOS-expression in endomyocardial biopsies during the first two weeks after heart transplantation regarding the question if there is a difference in iNOS-expression between patients with an uncomplicated and a complicated course?

Methods. Right ventricular endomyocardial biopsies were obtained from heart transplantation recipients at transplantation and during the first two weeks post-operatively. The recipients were divided into 3 groups depending on the postoperative course during the first year after transplantation: group I compared patients with an uncomplicated post-operative course, patients in group II developed significant signs of postoperative infection, while patients in group III presented with acute rejection ($\leq$ grade 3a ISHLT). iNOS-expression was investigated using the biotin-streptavidin-alkaline-phosphatase-method in paraffin-embedded samples. The expression was analyzed in a semi-quantitative score. As a point/-surface parameter, stained interstitial cells were counted.

Results. iNOS-expression was found in cardiomyocytes, endothelial cells, infiltrating cells and vascular smooth muscle cells. At the time of heart transplantation the expression was significantly increased in the rejection group compared to the other groups. This increase was even more pronounced in week two.

Conclusions. The present study shows that an increased iNOS expression at the time of heart transplantation could precede an acute rejection in the later post-operative course. Thus, measurements of iNOS expression may be of predictive value for an increased rejection risk and therefore offer the possibility of earlier therapeutic intervention.

14 Involvement of nitric oxide in the cardioprotective effect of early ischemic preconditioning in the reperfusion phase in pigs

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Background. We investigated the involvement of myocardial tissue iNOS and cNOS release in the cardioprotective effect of early ischaemic preconditioning (IP) in closed-chest pigs subjected to percutaneous balloon coronary occlusion/reperfusion.

Methods. Catheter-based 90-min occlusion followed by 60-min reperfusion of the LAD coronary artery was performed in 8 pigs (group AMI). IP was applied in 9 pigs (group IP) through 2 cycles of 5-min occlusion and 5-min reperfusion of the LAD before a 90-min occlusion and 60-min reperfusion. Infarct size was expressed as a percentage of the area at risk. Coronary pressure was measured with pressure-wire (PW) placed distally to the occluding balloon. Tissue iNOS/cNOS activities were measured in the infarcted area (iNOSi/cNOSi) and in the border zone (iNOSb/cNOSb) by using of citrullin-assay.

Results. Infarct size was smaller in group IP as compared with group AMI (21.7 ± 4.4 vs $27 \pm 3.4\%$, $p = 0.014$). IP didn't influence the tissue iNOSi/iNOSb activities. A trend towards higher cNOSb activity (49.2 ± 23.2 vs 31 ± 25.1 pmol min^{-1} mg^{-1} protein, $p = 0.142$) was measured in group IP vs AMI, whereas cNOSi didn't differ between the groups. PW measurements distally to occlusion revealed that IP induced incipient collateralization, which was independent from iNOS or cNOS activities. In IP group, significant correlations were observed between cNOSi and PW values ($r = 0.726$, $p = 0.027$), and between cNOSb and PW values ($r = 0.832$, $p = 0.005$) after 60-min reperfusion.

Conclusions. Beneficial effect of cNOS in ischaemia/reperfusion injury after IP results from the improved reperfusion after the release of coronary occlusion, and not from mediation of the incipient collateralization during coronary occlusion.

15 Verminderte dehnungsinduzierte Inotropie im insuffizienten Herzen – Grundlage des akuten Pumpversagens?

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Grundlagen. Myokardiales Pumpversagen geht mit einer akuten Zunahme der ventrikulären Vorlast einher, die zugleich einen ersten adaptiven Mechanismus aktiviert und eine Steigerung der Kontraktilität. Die Dehnung resultiert in einer sofortigen und einer verzögerten dehnungsinduzierten inotropen Antwort. Erstere ist als Frank-Starling Mechanismus (FSM) bekannt und beruht auf einer Sensibilisierung der Myofilamente für Kalzium. Letztere beruht auf einer Aktivierung membranständiger Transportproteine und ist Kalzium-abhängig. Die Bedeutung des FSM am insuffizienten Herzen ist kontrovers, vergleichende Daten zur verzögerten Phase an gesundem und insuffizientem Myokard liegen bislang nicht vor.

Methodik. Muskelstreifen aus 20 terminal insuffizienten (F) und 7 Spenderherzen (NF). 37 °C, 1 Hz, Bikarbonatpuffer. Dehnung von 88% auf 98% ihrer optimalen Länge. Die resultierende sofortige (FSM) und verzögerte Inotropie wurden ohne sowie nach Blockade der NO-Synthase (NOS; 500 mol/l L-NAME) der PI-3-Kinase (PI3 K; 0,1 µmol/l Wortmannin) oder des "reverse-mode" des Na^+/Ca^{2+} Austauschers (rNCX; 5 mmol/l KB-R7493) untersucht. Wir untersuchten zusätzlich $[Na^+]i$ mit SBFI.

Ergebnisse. FSM war in beiden Gruppen vergleichbar (NF vs. F: $226 \pm 16\%$ vs. $232 \pm 14\%$ des Ausgangswertes bei 88%), die verzögerte Phase war im gesunden Myokard ausgeprägter (Anstieg auf $126 \pm 3\%$ (NF) vs. $119 \pm \%$ (F) der Kraft nach FSM, $p < 0.05$). Hemmung der NOS (F $n = 9$; NF $n = 5$) oder der PI3 K (F $n = 6$; NF $n = 7$) zeigten in keiner Gruppe einen Einfluss auf FSM oder die verzögerte Phase. Blockade des rNCX führte zu einer nahezu kompletten Hemmung der verzögerten Phase (NF: $132 \pm 8\%$ vs. $109 \pm 3\%$, $n = 5$; F: $119 \pm 3\%$ vs. $106 \pm 3\%$, $n = 8$; beide $p < 0.05$) ohne Beeinflussung des FSM. Die verzögerte Phase war in beiden Gruppen von einem $[Na^+]i$-Anstieg begleitet. Der Anstieg sowie die Anstiegsgeschwindigkeit war in der Gruppe insuffizienter Herzen trotz geringerer Inotropie größer ($5{,}4 \pm 1{,}3$ mM vs. $1{,}6 \pm 1$ mM bzw. 0,54 mM/min vs. 0,2 mM/min).

Schlussfolgerungen. Der FSM ist im insuffizienten menschlichen Myokard unverändert. Die verzögerte Phase ist hingegen bei qualitativ unveränderter Signaltransduktion reduziert. Dies könnte auf einem gestörten "Na^+-contraction-coupling" im insuffizienten Myokard beruhen.

16 Effect of myocardial ischemia-reperfusion on the constitutive nitric oxide synthase (cNOS). An experimental model of regional ischemia-reperfusion

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Background. Endothelial dysfunction is common after ischemia-reperfusion injury and the change in the production of nitric oxide (NO) plays a critical role. NO is synthesized by different isoforms of nitric oxide synthase (NOS): constitutive isoforms (cNOS) and an inducible isoform (iNOS). The relationship between the ischemic period and the release of NO has not been completely ascertained, nor has the time course of NO release before and after ischemia-reperfusion. Therefore, we performed this study to investigate the effect of temporary coronary artery occlusion on the activity of cNOS and its changes over time.

Methods. In animal model reproducing regional ischemia, 24 male Wistar rats (250–350 g) underwent occlusion of the left anterior descending artery for 30 minutes. The reperfusion was variable in four groups of animal considered (0, 5, 15 and

30 minutes). In all animals ischemic area was identified, sized and then separated from the non ischemic myocardium of the left ventricle. The activity of cNOS was measured in the ischemic-reperfused tissue and in the non ischemic part of the left ventricle as control.

Discussion. The activity of cNOS and particularly the one of the endothelial isoform (eNOS) lowered significantly after ischemia and much more after 5 and 15 minutes of reperfusion. After 30 minutes of reperfusion the activity of cNOS increases again.

Conclusion. The early period of reperfusion (initial 15 minutes) after myocardial ischemia is critical for the endothelial dysfunction. Consequently, interventions aimed at reversing the impairment of NO synthesis in the early period could have important implications for cardiac function.

17 Closing remarks: nitric oxide and NOS isoforms in heart failure

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Symposion IV: „Diagnostische und prognostische Marker“

18 Einführung

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19 Oversizing of a paclitaxel eluting coronary stent is associated with unfavorable results in a porcine coronary stenting model

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Background. Oversizing, high-pressure balloon inflation and overdilation with >10% than the stent nominal size have been introduced to achieve an optimal stent deployment. Limited data exists concerning histopathological and histomorphological changes after gross oversizing (over 10%) of a paclitaxel-eluting stent (PES).

Methods. Twenty-two PESs were implanted randomly in the left anterior descending and left circumflex porcine coronary arteries under general anesthesia. 11 stents with gross oversizing (1.2:1.0 stent artery ratio, group A) and 11 with standard-sizing (1.1:1.0 stent artery ratio, group B), by using appropriate stent balloon inflation pressure. After 1-month follow-up (FUP), control coronary angiography was performed. The histopathological and histomorphological results of the explanted arteries of the 2 groups were compared.

Results. Higher incidence of peri-procedural complications (vasospasm and partial vessel occlusion) were recorded in group A as compared with group B (63.6% vs. 27.3%). At the FUP, coronary angiography revealed significantly lower minimal lumen diameter (1.22 ± 0.53 vs. 2.21 ± 0.5 mm, $p = 0.001$) and reference vessel diameter (2.38 ± 0.27 vs. 2.97 ± 0.72 mm, $p = 0.0001$) in group A compared to group B, indicating an obvious trend towards higher late recoil after oversizing stent implantation. Histopathology showed significantly higher score of intima inflammation (2.09 ± 0.73 vs. 1.40 ± 0.51, $p = 0.027$), and vessel wall necrosis (0.36 ± 0.48 vs. 0 ± 0, $p = 0.031$) in group A compared with group B. Histomorphometry revealed significantly higher neointimal area in group A than group B (2.09 ± 0.96 vs. 0.99 ± 0.51 mm^2, $p = 0.004$), and percent area stenosis (54.1 ± 25.8 vs. $29.9 \pm 18.7\%$, $p = 0.038$).

Conclusions. Implantation of a PES with oversizing balloon dilation is associated with a higher incidence of peri-procedural complications, unfavorable histopathological and -morphological features compared to the standard sizing.

20 Evidence for TRPC3 as a novel marker for the proliferative potential of endothelial progenitor cells

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Background. Recent evidence suggests that human adipose tissues host a significant fraction of somatic endothelial progenitor cells (EPCs). In culture, these cells generate colony forming units (CFUs) that respond to vascular endothelial growth factor (VEGF) with further proliferation and are able to organize in tube-like structures.

Methods. Stromal cells prepared from adipose tissue were immunosorted using magnetic beads coated with CD34/CD133 antibodies. Relative sizes of subpopulations were quantified by flow-cytometry. Cells were cultered under various conditions and analyzed by immuno-histochemical techniques and Fura2-Ca^{2+} imaging.

Results. We observed abundant expression of TRPC3 channel protein in freshly isolated EPCs as well as in early EPC culture (24 h) by flow-cytometry and immuno-histochemical imaging. The population of TRPC3 expressing cells derived from the fresh adipose tissue preparation overlapped with the fraction expressing the selective stem-cell surface marker CD133. After 24 h in culture, TRPC3+/CD133+ CFUs were identified, which showed a heterogenous distribution of TRPC3+ cells within the cell clusters, with TRPC3 expressing cells preferentially located in the outer rim of the colonies. Fura-2 Ca^{2+}-imaging experiments of these cell colonies revealed that VEGF-induced Ca^{2+} entry was significantly larger in TRPC3 expressing ìouter rimî cells as compared to cells in the centre of the clusters.

Conclusions. Thus, TRPC3 is suggested as an element of the VEGF signalling pathway in EPCs, contributing to stem cell proliferation and may be essential for vasculogenesis. TRPC3 may be considered as a possible target that allows for selective interference with vasculogenesis and angiogenesis.

21 Tenascin-C: A possible marker for LV remodeling after myocardial infarction in endothelin therapy

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Background. The matricellular protein Tenascin-C induces production of matrix metalloproteinases (MMPs), inhibits cellular adhesion and mediates cellular de-adhesion. These effects are helpful in the dynamic process of cardiac remodeling. It has been reported that Tenascin-C expression is up-regulated in ventricular remodeling following myocardial infarction (MI) in the border zone between scar tissue and non-infarcted area. Down regulation starts on day 7 post MI and it can no longer be detected on day 21. We analysed the expression of tenascin-C in the post MI infarcted and non-infarcted area after the treatment with a selective Endothelin A (ET_A)-receptor antagonist. Endothelin is a potent vasoconstrictor and growth factor. Blockade of the ET_A-receptor decreases cell proliferation, LV hypertrophy, secretion of proinflammatory mediators and vascular permeability.

Methods. MI was induced in male SD rats by LAD ligation. Three days post MI, rats were randomised to receive either the Endothelin antagonist TBC3214-Na ($n=6$) or placebo ($n=6$), as a control rats were sham-operated without LAD ligation ($n=4$). After 7 days hearts were removed and the scar, peri-infarct and free wall were analysed by western blot using a mAB specifically recognize the EGF like domain of tenascin-C. Tissue was homogenized in urea buffer and protein samples were subjected to 6% polyacrylamide gel SDS-PAGE, transferred on to a membrane and immunostained with the anti-TN-C mAB and anti-mouse alkaline phosphatase AB.

Results. In the placebo group, Tenascin-C was up-regulated in scar tissue and in the peri-infarct area. In the therapy group, Tenascin-C was down regulated in scar tissue and in the peri-infarct area. RT-PCR confirmed our findings.

Conclusions. From these results, we can conclude that (1) tenascin-C regulation is directly influenced by ET_A-blockade and (2) that tenascin-C is a strong marker for LV remodeling after myocardial infarction.

22 Die Prävalenz der diastolischen Herzinsuffizienz steigt mit zunehmendem Alter und geht einher mit erhöhten E/é lat.-Werten als Marker für den linksventrikulären enddiastolischen Druck

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Grundlagen. In den letzten Jahren konnte gezeigt werden, dass ein Großteil der Patienten mit Herzinsuffizienzsymptomatik eine erhaltene Pumpfunktion aufwiesen. Daher untersuchten an einem Risikokollektiv die Prävalenz der diastolischen Dysfunktion/Herzinsuffizienz und welche Parameter (E/é lat., NT-proBNP) dies am besten widerspiegeln.

Methodik. Bei 523 Männer und 614 Frauen (Alter 66,1 ± 7,8 Jahre) mit kardiovaskulären Risikofaktoren wurde neben Anamnese, körperlicher Untersuchung und Blutentnahme eine echokardiographische Untersuchung zur Bestimmung der diastolischen Funktion durchgeführt.

Ergebnisse. Die Prävalenz der diastolischen Dysfunktion (diast. DF) stieg mit zunehmenden Alter an (51–60 Jahre: 59,3%; >80 Jahre: 95,2%). Außerdem wiesen die Patienten altersabhängig zunehmend Symptome einer Herzinsuffizienz auf. 32,6% der 51–60-jährigen Patienten wiesen eine diastolische Herzinsuffizienz (diast. HI) auf, wohingegen bei den über 80-jährigen Patienten 70,0% an einer Herzinsuffizienzsymptomatik litten. Bei einer Analyse der Schweregrade der diast. DF zeigte sich, dass unabhängig vom Alter ∼80–85% der Patienten mit einer diast. HI nur eine milde diastolische Dysfunktion aufwiesen. Des weiteren untersuchten wir als Marker für eine diast. HI E/é lat. und NT-proBNP. Es zeigte sich, dass bei Patienten mit diast. DF sowohl E/é lat. als auch NT-proBNP mit zunehmendem Alter (51–60 Jahre vs. >80 Jahre) signifikant anstiegen (E/é lat.: $9,7 \pm 3,6$ vs. $12,1 \pm 5,5$; $p<0,05$, BNP: $78,8 \pm 7,3$ vs. $307,0 \pm 35,0$; $p<0,001$). Auch innerhalb einer Altersgruppe waren die E/é lat.-Werte bei Patienten mit diast. HI höher als bei Patienten mit diast. DF (51–60 Jahre: $11,9 \pm 1,7$ vs. $9,7 \pm 3,3$ ($p<0,05$); >80 Jahre: $19,4 \pm 10,9$ vs. $11,5 \pm 4,8$ ($p<0,05$)), während die NT-proBNP innerhalb einer Altersgruppe nicht signifikant unterschiedlich waren.

Schlussfolgerung. Patienten mit diast. DF leiden mit höherem Alter zunehmend an einer diast. HI, welche mit erhöhten E/é lat.-Werten einhergeht.

Symposion V: „Innovative pharmakologische und nicht-pharmakologische Behandlungsansätze“

23 Einführung

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24 SiNiTang attenuates ventricular remodeling by influencing matricellular proteins

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Background. Because of lacking experimental evidence, integrating the knowledge of Traditional Chinese Medicine into Western therapy guidelines is difficult. Thus, it was the aim of our study to evaluate a Chinese herbal mixture, SiNiTang, in an experimental model of ischemic heart failure.

Methods. MI was induced in 16 SD rats, 16 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 6 weeks. SiNiTang consists of monkshood, ginger, cinnamon and licorice root, administered by drinking water. In-vivo hemodynamics was assessed by echocardiography.

Results. After 42 days, body weight, tibia length and infarct size have been comparable between the groups (mean $\pm$ SD). Compare to MI, LV/BW-Ratio was reduced in TeaMI (2.5 ± 0.42 vs. 3.12 ± 0.2; $p<0.05$), End-diastolic (ED) and -systolic (ES) dilation was attenuated (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; $p<0.05$) and factional shortening was significantly higher (0.33 ± 0.05 vs. $0.24 \pm 0.05\%$; $p<0.05$). At the protein level, MMP 2 and 9 expression was reduced with a corresponding increase of TIMP 1 and 2.

Conclusions. We could confirm for the first time the positive morphologic and hemodynamic effects of SiNiTang in an experimental model of ischemic HF. The reduced LV dilatation might be due to a favorable influence of SiNiTang on ECM remodeling.

25 Cardiac contractile dysfunction in sepsis: analysis based on in vivo pressure-volume relationships in rats

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Background. Although cellular and mechanistic studies provide insight into the development of sepsis, the clinical applicability can be difficult to ascertain because hemodynamic and cardiac function is difficult to obtain in small intact animals. The purpose of this study was to evaluate the feasibility of using a miniaturized conductance catheter to accomplish measurement of both global hemodynamics and cardiac contractility during sepsis in male SD-rats.

Methods. A laparotomy was performed and the cecum was ligated distal to the ileocecal valve. The cecum was punctured through both walls and bowel content was expressed through the holes (CLP). Rats were randomly assigned for hemodynamic measurements 24 and 48 h after CLP. A pressure-volume conductance catheter connected to a pressure-conductance unit was inserted into the left ventricle. Pressure-volume (PV) loops were recorded during baseline and during unloading by transiently occluding the inferior vena cava (IVC).

Results. Despite decreased preload, strokevolume was preserved and EF was significantly higher than in sham. Load-independent parameters of contractility showed an increase versus all other groups, indicating a hyperdynamic and hypercontractile episode 24 h after CLP. Animals 48 h after CLP showed significant decrease of load-dependent parameters. Contractile reserve was significantly reduced at higher heart rates. After infusion of isoproterenol, CO expectedly increased in the sham group, but this rate-dependent increase was blunted in the 24 h- and 48 h-sepsis group.

Conclusions. The miniaturized conductance catheter allows for effective measurements of hemodynamic function at both steady-state and following IVC occlusion in septic rats and provides measurements that cannot be obtained using other cardiovascular monitoring techniques.

26 Aldosterone receptor blockade in diastolic heart failure (ALDO-DHF) – rationale und design

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Ein Assoziiertes Projekt des Kompetenznetzes Herzinsuffizienz, gefördert vom Bundesministerium für Bildung und Forschung

Grundlagen. Trotz hoher Prävalenz, Morbidität und Mortalität der diastolischen Herzinsuffizienz (DHF) mit enormer klinischer und sozioökonomischer Relevanz fehlen bisher etablierte medikamentöse Therapieschemata. Aldosteron spielt in der Pathogenese der gestörten diastolischen Funktion (Hypertrophie, Fibrosierung, Kollagen-Isoform-Shift, molekulares Remodelling der Kardiomyozyten) über erhöhte Plasmaspiegel und Stimulation des kardialen Aldosteronrezeptors eine zentrale Rolle. Außerdem wurden unzureichende therapeutische Erfolge bisheriger Studien bei DHF, z. B. im CHARM-preserved-Trial (Candesartan), auch mit dem Phänomen des ìaldosterone escapeî erklärt.

Methodik. Design: prospektiv, randomisiert (1:1), plazebokontrolliert, doppelblind, multizentrisch, Phase IIb. Intervention: Spironolacton 25 mg/d ($n=210$) oder Plazebo ($n=210$) für 12 Monate; Subgruppe für 18 Monate. Patienten: 420 Patienten mit DHF. Primäre Zielparameter (12 Monate): maximale Belastbarkeit (peakVO_2), diastolische Funktion (E/é). Sekundäre Zielparameter (12 und 18 Monate): Parameter der Belastbarkeit, der diastolischen Funktion; neuroendokrine Aktivierung, Inflammation, Kollagenumsatz, Lebensqualität, Verträglichkeit der Therapie, Morbidität und Mortalität nach Biometrie: Vergleich Verum vs. Plazebo für beide primären Endpunkte durch Mann-Whitney-U-Test (Typ-I/II-Fehleradjustierung), Konfidenzschätzer der mittleren Differenz bzw. des geometrisch mittleren Quotienten der Behandlungsarme für alle Endpunkte, Korrela-

tionsanalyse für klinische und Lebensqualitätsendpunkte, multiple Regression zur Identifikation der Kovariaten der primären Endpunkte und Adjustierung der Effektschätzer. Einschlusskriterien: schriftl. Einverständnis; Alter $\geq$ 50 Jahre; NYHA II/III; diastolische Dysfunktion, Schweregrad $\geq$ I; LVEF $\geq$ 50% SR; peak $VO_2 \leq 20\,ml/kg/min$.

Schlussfolgerungen. Aldo-DHF wird die erste große klinische Studie über den Einfluss einer Aldosteron-Rezeptor-Blockade auf die diastolische Herzinsuffizienz sein. Es darf davon ausgegangen werden, dass solch eine Studie nicht nur von besonderer Bedeutung für die Behandlung der diastolischen Herzinsuffizienz ist, sondern auch Richtung weisenden Charakter für zukünftige Therapiestudien der diastolischen Herzinsuffizienz hat.

27 Urocortin II verbessert die kontraktile Funktion isolierter Ventrikelmyozyten durch Aktivierung des cAMP-PKA- und des PI3K-Akt-eNOS-Signalwegs

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Grundlagen. Urocortin II (UcnII) ist ein Analog des Corticotropin-Freisetzungsfaktors (CRF), der die kardiale Funktion bei Herzinsuffizienz verbessert. Wir haben die Hypothese untersucht, dass UcnII direkte inotrope und lusitrope Wirkungen auf isolierte Ventrikelmyozyten ausübt, und die zugrunde liegenden Signalkaskaden und Mechanismen charakterisiert.

Methodik. Isolierte Kaninchen-Ventrikelmyozyten ($n = 83$) wurden mit 0,5 Hz elektrisch stimuliert. Zellverkürzungen, [Ca]-Transienten (CaT) und die zelluläre NO-Produktion wurden mittels Edge detection, Fluo-4- und DAF-FM-Fluoreszenz gemessen. Der Phosphorylierungsgrad von Akt und der endothelialen NO-Synthase (eNOS) wurde mit phosphospezifischen Antikörpern mittels Immunoblot bestimmt.

Ergebnisse. UcnII (100 nM) verursachte in Kaninchen-Ventrikelmyozyten einen Ca-abhängigen positiv inotropen und positiv lusitropen Effekt. Hemmung des CRF2-Rezeptors (10 nM Antisauvagine-30) und der Proteinkinase A (PKA; 0,5 µM H89) unterdrückte diese Effekte. Zusätzlich führte UcnII zu einem Anstieg der NO-Produktion (+35% DAF-FM-Fluoreszenz). Dieser NO-Anstieg wurde durch Hemmung der eNOS mit L-NIO (10 µM) reduziert. Immunoblots zeigten, dass UcnII eine Zunahme der Akt-Phosphorylierung (Thr308, Ser473) und der eNOS-Phosphorylierung (Ser1177) verursachte. Die UcnII-induzierte Akt-Phosphorylierung wurde durch Wortmannin (0,3 µM) und LY294002 (10 µM), Hemmstoffe der PI-3-Kinase (PI3K), blockiert, nicht aber durch H89 (5 µM). Im Gegensatz dazu wurde die UcnII-induzierte eNOS-Phosphorylierung sowohl durch Wortmannin als auch durch H89 unterdrückt.

Schlussfolgergungen. UcnII verursacht positiv inotrope und lusitrope Effekte durch eine CRF2-Rezeptor-vermittelte Aktivierung der cAMP-PKA- und der PI3K-Akt-Signalwege. Die beiden Signalwege konvergieren bei der eNOS-Phosphorylierung. Der positiv inotrope Effekt ist cAMP- und Ca-abhängig. Der positiv lusitrope Effekt könnte sowohl cAMP- als auch NO-abhängig sein. Diese direkten Wirkungen auf Ventrikelmyozyten können erklären, wie UcnII die kardiale Funktion bei Herzinsuffizienz verbessert.

Symposion VI: Postersitzung „Freie Vorträge zum Thema myokardiales Pumpversagen“

28 ECMO in a near drowned adult

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Extracorporeal membrane oxygenation (ECMO) is a technique for providing life support, in case the natural lungs are failing and for mechanical circulatory support. We report the case of a 38-year old man who nearly drowned in cold water. He was found with cardiac arrest and after he was rescued within 15 minutes basic life support was begun. Heart action was detected after 10 minutes of advanced life support. Afterwards he was transferred to the nearest hospital. There he developed severe adult respiratory distress syndrome (ARDS) and was admitted to our ICU. He presented with a body core temperature of 34.0 degree centigrade, under continuous inotropic support (Noradrenalin 0.1 µg/ml/h), pulmonary artery pressure (PAP) 26/22 mmHg, pulmonary capillary wedge pressure (PCWP) 15 mmHg, laktat of 10.1 mmol/liter and artery blood gases showed: ph 7.02, pCO_2 68.9 mmHg, pO_2 24.5 mmHg with a fiO_2 1.0. We instituted extracorporeal membrane oxygenation (ECMO) for sufficient oxygenation and for rewarming the patient. Improvement of the arterial blood gases (ph 7.415, pO_2 95.2 mmHg, pCO_2 40.1 mmHg) were noticed shortly after and the patient showed haemodynamic stability without inotropic support (heart frequency 80 bts/min, blood pressure 120/70, PAP 33/25). Three days later ECMO was weaned and assisted ventilation was stopped after 9 days. No neurological deficits were observed.

29 Load dependent regulation of GATA4 in human myocardium

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Background. Pressure overload in animal models activates a hypertrophic program governed by the transcription factor GATA4.

Methods. We examined the regulation of GATA4 in human hearts with chronic overload due to aortic stenosis (AS) versus nonfailing control hearts (NF). Also we tested the effect of acute pre- and afterload (L) versus unloaded state (UL) in isolated atrial trabeculae. Protein expression and phosphorylation at S105 were measured by Western blotting.

Results. GATA4 was upregulated 1.6 fold in protein expression and 1.7 fold in phosphorylation (both $p<0.01$) in AS ($n=20$) versus NF left ventricular specimens ($n=10$). The extent of phosyphorylation in AS correlated inversely with the ejection fraction ($r=-0.55$; $p<0.05$) and cardiac index ($r=-0.85$; $p<0.05$) of AS patients. In isolated atrial trabeculae UL decreased Gata4 expression (to $50\pm11\%$ after 60 min and $49\pm13\%$ after 6 h; $p<0.05$ vs. control). In L this decrease was attenuated and expression versus UL significantly elevated ($186\pm38\%$ after 60 min and $154\pm31\%$ after 6 h ($p<0.05$ vs. UL). Phospho-GATA4 was significantly increased in L versus UL after 1 h ($225\pm35\%$; $p<0.05$) but not after 6 h.

Conclusions. In conclusion GATA4 expression in human myocardium is load dependent and upregulated in chronic overload. GATA4 phosphorylation increases with the degree of myocardial dysfunction. Unloading of the heart ex vivo decreases GATA4 protein level potentially by specific proteasomal degradation. Biomechanical load attenuates this degradation and stimulates GATA4 by transient phosphorylation.

30 Klinische Erfahrungen mit einem nicht-pulsatilen linksventrikulären Unterstützungssystems [INCOR]

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Grundlagen. In den letzten Jahren haben neben den seit einigen Jahren etablierten pulsatilen LVADs nicht-pulsatilen LVADs im klinischen Alltag zunehmend an Bedeutung gewonnen. Vorteile dieser Systeme ist neben der Größe [z. B.: INCOR, Berlin Heart, Berlin (200 g, Länge 12 cm, Durchmesser 30 mm, Leistung 3–4 Watt)], eine wohl auch dadurch bedingte einfachere und schnellere Implantation. Es wird retrospektive unsere klinische Erfahrung mit dem INCOR System berichtet.

Methodik. Zwischen Jänner 2004 und August 2005 wurden 4 männliche Patienten im Alter zwischen 38 und 60 Jahren für eine VAD [INCOR]-Implantation indiziert. Bei einem Patienten bestand eine Kontraindikation für eine HTX [Pulmonaler Hypertonus], drei Patienten befanden sich auf der Warteliste für eine HTX. Die Implantation wurde in allen vier Fällen an der Herzlungenmaschiene durchgeführt. Anastomosierungen für Ein- und Auslasskanüle wurden am Apex des linken Ventrikels sowie an der aszendierenden Aorta durchgeführt.

Ergebnisse. Die Implantation war in allen Patienten elektiv und primär erfolgreich. Zwei Patienten wurde nach durchschnittlich 486,5 [420/553] Tagen am INCOR transplantiert. Ein Patient verstarb nach 570 Tagen am System. Der Tod war nicht korreliert mit dem VAD-System. Ein Patient befindet sich derzeit [Nov. 06] noch unter VAD-Support [128 Tage]. An peri- bzw. postoperativen Komplikationen traten auf: Nachblutung mit operativ Versorgung [1], zerebrovaskuläre Ereignisse [2], sowie Driveline Infektion [1]. Diese wurde oral mit Antibiotika behandelt.

Schlussfolgerungen. Das INCOR wird nun an unserem Zentrum routinemäßig als LVAD-System eingesetzt und hat sich im Langzeitverlauf bewährt [>570 Tage]. Die lange Wartezeit [durchschnittliche Wartezeit deutlich >1 Jahr] bestätigt den Mangel an geeigneten Spenderorganen.

31 Modulation of glycolysis effects vascular response to sulphonylureas: a study on isolated bovine coronary artery rings in simulated ischemia

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Acute ischemic heart failure is counteracted by hypoxic, reflectory coronary artery dilation. In earlier experiments we have shown that the latter can be inhibited by glibenclamide. Here we show that inhibition of oxidative phosphorylation by dinitrophenol (DNP; 1 mM) led to a relaxation of precontracted (26.8 mM KCl) bovine coronary arteries by $58\%+2\%$ ($+$SEM; $n=8$) which could be clearly reduced by various concentrations of glibenclamide. Under conditions of glycolytic inhibition by iodoacetate (IA; IA is an inhibitor of glyceraldehyd-3-phosphate-dehydrogenase; 0.5 mM), precontracted bovine arteries (26.8 mM KCl) relaxed by $98\%+1\%$. This relaxation could not be inhibited by 20 µM glibenclamide or 200 µM ($98\%+2\%$; SEM; $n=8$).

In conclusion, our results show that glibenclamide can inhibit coronary artery dilation induced by hypoxia and blockade of oxidative phosphorylation, whereas coronary dilation secondary to inhibition of glycolysis can not be well prevented by glibenclamide. This observation indicates that glycolytically produced ATP and/or metabolic by-products of glycolysis may modulate the response of KATP-channels to sulfonylureas in bovine artery smooth muscle cells. Seemingly, our findings alude to a further protective mechanism designed to prevent acute ischemic heart failure.

32 Effect of intracellular acidification on pump failure: do protons effect free intracellular Mg^{2+}: An ETH 7025 – Mg^{2+} – ionselective microelectrode study on guinea pig papillary muscle

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Background. The assessment of free, ionised, intracellular Mg^{2+} ($(Mg^{2+})i$) in myocardial tissue is problematic and its metabolism has not been well understood as yet. This makes the design of trials on therapeutic use of Mg (e.g. Limit II and ISIS 4) difficult and the interpretation of conflicting results almost impossible. In order to understand acute ischemic pump failure it would be important to understand more about ischemic components like intracellular acidification and its effect-upon $(Mg^{2+})i$.

Methods. Here, we assess the effect of changing intracellular pH (pHi; measured with pH selective microelectrodes) on cytoplasmatic $(Mg^{2+})i$ using a newly designed Mg^{2+} selective microelectrode with the neutral carrier ETH 7025) and measure $(Mg^{2+})i$ in isolated resting guinea pig papillary muscle (Tyrode, pH 7.4, 36 °C; for details of method see our earlier work (J Physiol 431, 713–41, 1990 and Pflügers Arch 423, 338–342, 1993).

Results. We find that changing extracellular pH for 15 minutes from 7.4 to 6.4 leads to a change of intracellular pH from 7.19 ± 0.03 to 6.81 ± 0.006 ($n = 7$; $\pm$ SEM for all exp.). This change of pHi leads to a small, but detectable rise in (Mg^{2+})i by a maximum amount of 0.19 ± 0.06 mM from an initial value of 0.73 ± 0.08 mM after approximately 7 minutes, followed by a slow decrease of (Mg^{2+})i to almost normal. This would amount to a liberation of 1.5% of the cells total Mg content, assuming that (Mg^{2+})i constitutes $1/17$ of the cells total Mg (J Physiol 224, 121–139, 1972).

Conclusions. In summary, we find that intracellular acidification liberates (Mg^{2+})i from intracellular binding sites. The transient nature of the observed (Mg^{2+})i rise further suggests, that the levels of cytoplasmatic (Mg^{2+})i are well regulated and even small changes in (Mg^{2+})i are adjusted within short time.

Symposion VII: „Chirurgische Therapie bei myokardialem Pumpversagen I: „Rechtsherzversagen und Myokardprotektion“

33 Einführung 1: Physiopathologie des Rechtsherzversagen

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34 Einführung 2: Optimale Myokardprotektion bei eingeschränkter Pumpfunktion

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35 The fibrin-derived peptide B-beta15-42 ameliorates ischemia-reperfusion injury in a rat heart transplant model

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Background. The purpose of this study was to evaluate the protective effect of the fibrin-derived peptide B-beta15-42 on ischemia/reperfusion injury in a rat cardiac transplant model.

Methods. LEW hearts were flushed with chilled (0–1°C) Custodiol preservation solution and either transplanted immediately or stored for 4 or 8 h in the same solution and then transplanted into syngeneic recipients. B-beta15-42 was given i.v. at a dose of 1.2 mg immediately after transplantation or added to the preservation solution prior to harvest. At 24 h and 10 d, graft function was assessed and hearts were retrieved for morphological evaluation. At time of harvest, serum samples were collected for troponin level analysis.

Results. Hearts transplanted immediately or after 4 h of cold ischemia did not show any morphological damage. In contrast, 8 h of ischemia resulted in severe myocardial ischemia associated with an inflammatory response at 24 h. Lesions further progressed at 10 days. Administration of B-beta15-42 resulted in a significant amelioration of myocardial necrosis together with a diminished inflammatory response. A protective effect towards myocyte damage was further underlined by reduced troponin levels in groups receiving B-beta15-42.

36 Endothelin-A receptor antagonist TBC-3214Na added to cardioplegia attenuates ischemia/reperfusion injury in failing hearts

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Background. Ischemia/reperfusion (I/R) injury due to cardioplegic arrest is a problem in patients with reduced LV function. We investigated the effect of chronic versus acute administration of the selective endothelin-A receptor antagonist TBC-3214Na during I/R in failing hearts.

Methods. Male Sprague-Dawley rats underwent coronary ligation. Three days post infarction group 1 ($n = 11$) was administered TBC-3214Na continuously with their drinking water, group 2 and 3 received placebo. Seven weeks post infarction hearts were evaluated on a blood perfused working heart during 60′ ischemia and 30′ reperfusion. In group 2 ($n = 10$) TBC-3214Na and in group 3 placebo was added to cardioplegia during ischemia.

Results. At similar infarct size postischemic recovery of cardiac output (group 1: $91 \pm 10\%$, group 2: $86 \pm 11\%$ vs. placebo: $52 \pm 15\%$; $p < 0.05$) and external heart work (group 1: $90 \pm 10\%$, group 2: $85 \pm 13\%$ vs. placebo: $51 \pm 17\%$, $p < 0.05$) group was significantly enhanced in both TBC-3214Na treated groups while recovery of coronary flow was only improved in group 2 (group 2: $121 \pm 23\%$ vs. group 1: $75 \pm 13\%$, placebo: $64 \pm 15\%$, $p < 0.05$). In addition high energy phosphates were significantly higher and transmission electron microscopy revealed less ultrastructural damage only under acute TBC-3214Na administration.

Conclusions. Acute endothelin-A receptor blockade is superior to chronic blockade in attenuating I/R injury in failing hearts. Therefore acute andothelin-A receptor blockade might be an interesting option for patients with heart failure undergoing cardiac surgery.

37 Brain natriuretic peptide (BNP) verbessert dosisabhängig die kardiale Hämodynamik bei postischämischer Herzinsuffizienz nach Einsatz der Herz-Lungen-Maschine

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Grundlagen. BNP beeinflusst durch seine pharmakologischen Eigenschaften die Vor- und Nachlast des Herzens. Durch direkte Vasodilatation der Koronargefäße wird die myokardiale Perfusion verbessert und die vorhandene Ischämie des Herzen reduziert. Derzeit gibt es wenig Erkenntnisse über die Wirkung von BNP bei kardiochirurgischen Operationen. Ziel der Studie war es, die Wirkung der intravenösen Gabe von BNP bei Operationen unter Einsatz der Herz-Lungen-Maschine (HLM) im Tiermodell zu untersuchen.

Methodik. 29 Schweine wurden (3 Gruppen) unter Einsatz der HLM operiert. Durch Abklemmen der Aorta ascendens und blutkardioplegischen Herzstillstand wurde eine 30-minütige Ischämie simuliert. Die Tiere der BNP-Gruppen erhielten nach Öffnen der Aortenklemme und Freigabe der antegraden Perfusion BNP intravenös als Bolus (Gruppe I: 0,3 µg/kg KG; Gruppe II: 0,6 µg/kg KG), anschließend eine kontinuierliche BNP-Gabe (Gruppe I: 0,015 µg/kg/min und Gruppe II 0,03 µg/kg KG) bis 60 Minuten nach HLM-Ende. Die Tiere der Kontrollgruppe erhielten Plazebo. Perioperativ wurden hämodynamische, klinisch-chemische Parameter sowie die pharmakologische Kreislaufunterstützung dokumentiert.

Ergebnisse. Beide BNP-behandelten Gruppen hatten bei Versuchsende ein signifikant verbessertes Herzzeitvolumen (I: 3,3; II: 3,9 L/min) und Cardiac Index (I: 3,96; II: 4,9 L/min/m^2) gegenüber der Kontrollgruppe (HZV:2,33 L/min; CI: 2,5 L/min/m^2). Der Katecholaminbedarf, die Kreatinphosphokinase, der systemvaskuläre Widerstand und das Laktat waren bei den BNP-behandelten Tieren signifikant niedriger, wobei Gruppe II mit der höheren BNP-Dosis der Gruppe I noch überlegen war.

Schlussfolgerungen. BNP ist hocheffektiv in der Behandlung der postischämischen Herzinsuffizienz nach HLM. Es kommt dosisabhängig zu einer verbesserten Hämodynamik nahezu ohne Katecholamine. Über BNP sind nur geringe Nebenwirkungen bekannt, insbesondere keine arrythmogene Wirkung oder Tachyphylaxie.

38 Der Angiotensin I-Converting Enzym Insertion/Deletion Polymorphismus beeinflusst nicht die systemische, jedoch die pulmonalen Hämodynamik bei koronarer Bypassoperation

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Grundlagen. Der Insertion/Deletion Polymorphismus des Angiotensin-Converting-Enzym (ACE) zeigt drei Genotypen (II, ID, DD) und beeinflusst Mortalität und Morbidität bei D-Allel-Träger nach koronaren Bypassoperationen (ACB) mit Herz-Lungenmaschine (HLM). Untersucht wurde deshalb der Einfluss des Polymorphismus auf hämodynamische Veränderungen bei ACB mit HLM.

Methodik. Bei 110 Patienten mit ACB wurde der ACE-Polymorphismus mittels Polymerase-Kettenreaktion bestimmt. Diese wurden entsprechend ihres Genotyps in zwei Gruppen (II-Gruppe, ID/DD-Gruppe) eingeteilt. Die Hämodynamik wurde mittels Pulmonaliskatheter während HLM, sowie postoperativ 4 h, 9 h und 19 h postoperativ gemessen. Die Messungen beinhalteten Cardiac Index (CI), systemischen und pulmonalen Widerstand (SVRI, PVRI), pulmonalarteriellen Mitteldruck (PAP), zentralvenösen Druck (ZVD) und Adrenalin- sowie Noradrenalinverbrauch.

Ergebnisse. Der Polymorphismus verteilte sich II: 18%, ID: 57% und DD: 25%. Die Patientengruppen unterschieden sich nicht hinsichtlich Alter (II: 66 ± 6, ID/DD: 66 ± 8 Jahre), Body-mass-index (II: 28 ± 2, ID/DD: 29 ± 5 kg/m $\leq$), Geschlecht (II: 16/4, ID/DD: 63/27 m/w) und Euroscore (II: $3{,}1 \pm 1{,}9$, ID/DD: $3{,}5 \pm 2{,}1$). Wir fanden keinen Unterschied in der Mortalität und in der systemischen Hämodynamik. Der PVRI war vor HLM in der ID/DD-Gruppe gegenüber der II-Gruppe signifikant erhöht (II: 227 ± 121, ID/DD: 297 ± 169 dyn*s*m^{2}*cm^{-5}); 4 h nach HLM war dieser Unterschied nicht mehr nachweisbar; 9 h nach HLM fand sich eine Tendenz beim PVRI (II: 247 ± 134, ID/DD: 290 ± 117 dyn*s*m^{2}*cm^{-5}) und ein signifikanter Unterschied beim PAP (II: 19 ± 6, ID/DD: 23 ± 8) in der ID/DD-Gruppe; 19 h nach HLM zeigten sich keine signifikanten Unterschiede.

Schlussfolgerungen. Das D-Allel des ACE-Genpolymorphismus hat keinen signifikanten Einfluss auf die systemische Hämodynamik. Es zeigten sich jedoch signifikante transiente Veränderungen in der pulmonalen Hämodynamik bei Patienten mit ACB und HLM, die auf eine reduzierte pulmonale Aktivität von ACE nach HLM basieren könnte.

Symposion VIII: „Chirurgische Therapie bei myokardialem Pumpversagen II: Revaskularisation und Klappenchirurgie“

39 Alternativer Aortenklappenersatz bei Hochrisikopatienten

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40 Die mitrale Reduktionsanuloplastie bei End-stage Kardiomyopathien – eigene Ergebnisse

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Grundlagen. Die sekundäre Mitralklappeninsuffizienz ist eine signifikante Komplikation der Kardiomyopathie im Endstadium. Es kommt zu einem Anstieg der linksventrikulären Vorlast, zum Anstieg der Wandspannung und des diastolischen Volumens als Folge der ventrikulären Adaptation. Ohne Therapie liegt die 3-Jahres Mortalität über 50%.

Methodik. Das chirurgische Regime verfolgt die Elimination des Insuffizienzvolumens durch die Koaptation der Mitralsegel und der Reduktion der excessiven Arbeitslast. Beides bewirkt die Wiederherstellung der linksventrikulären Geometrie durch die Implantation eines stark unterdimensionierten artifiziellen Mitralkalppenringes (Carpentier Physio).

Ergebnisse. Von den 43 Patienten hatten 16 Patienten eine präop. EF <25%, 27 Patienten hatten eine EF <35%. 19 waren in der NYHA-Klasse III, 24 in der Klasse IV, 14 hatten ein Vorhofflimmern, 32 zusätzlich eine schwere koronare Dreistammerkrankung. Drei Monate postoperativ verbesserte sich die NYHA-Klassifikation auf im Mittel 1,98, die EF verbesserte sich auf im Mittel 38,8% (20–56%). Fünf Patienten verstarben innerhalb der ersten 30 Tage, bei 7 Patienten wurden eine IABP implantiert, ein Patient wurde 2 Jahre später einer HTX zugeführt. Nach einem mittleren Follow-up von 64 Monaten (15–150 Monate) war das 1-, 2- und das 5-Jahres-Überleben 87%, 84% und 57%, wobei das Patientenalter bis zu 81 Jahre betrug.

Schlussfolgerungen. Kombiniert mit einer optimalen medikamentösen Therapie erreicht die Reduktionsanuloplastie akzeptale Langzeitergebnisse mit einer deutlichen Verbesserung der Lebensqualität in einer Patientengruppe, welche früher oft einer Herztranplantation zugeführt wurde.

41 Administration of k-conotoxin PVIIA, a conopeptide interacting with voltage activated K^+ channels reduces ischemia/reperfusion injury in an in vivo rat heart transplantation model

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Background. Ischemia-reperfusion injury is crucial in heart transplantation influencing both short and long term outcome and possibly late graft vasculopathy. The mechanisms underlying this cell damage are not completely understood. Recently it was shown that k-conotoxin PVIIA (k-PVIIA), a peptide from the venom of a marine cone snail known to interact with voltage-gated Shaker K^+ channels reduces the myocardial infarct size in rabbit, rats and dogs when given after the onset of ischemia. In the present study the potential protective effect of k-PVIIA was investigated in a rat heart transplantation model of ischemia/reperfusion injury.

Methods. Male Lewis rats (250–300 g, $n = 3$ in each group) were used. Total cold ischemia was 12 hours, total warm ischemia averaged 25 min. After explantation coronary vessels were perfused either with HTK solution (control [Co]) or 500 nmol k-PVIIA added to the HTK solution. Other groups were treated by administering 500 nmol k-PVIIA systemically 5 min before declamping and the combination of coronary and systemical application. The hearts were allowed 1 hour in vivo reperfusion before histologic evaluation of the percentage of ventricular necrosis and interstitial hemorrhage.

Results. Left and right ventricular necrosis were greatly reduced in k-PVIIA treated hearts compared with control. The combination of coronary vessel perfusion (CP) and systemic application (SA) potentiated this protective effect. In detail: Percentage of left ventricular necrosis: 25.84 ± 4.09 (Co), 3.68 ± 1.57 (CP), 3.49 ± 1.21 (SA), 0.61 ± 0.31 (CP + SA), $p < 0.05$. Percentage of right ventricular necrosis: 23.48 ± 4.19 (Co), 5.46 ± 3.11 (CP), 3.3 ± 1.24 (SA), 0.67 ± 0.027 (CP + SA), $p < 0.05$. Percent of left ventricular interstitial hemorrhage: 33.36 ± 9.3 (Co), 18.60 ± 9.4 (CP), 5.75 ± 2.05 (SA), 4.50 ± 0.82 (CP + SA), $p < 0.05$. Percent of right ventricular interstitial hemorrhage: 32.68 ± 10.5 (Co), 18.64 ± 12.5 (CP), 9.80 ± 5.47 (SA), 4.70 ± 2.32 (CP + SA).

Conclusions. These results demonstrate that k-PVIIA exhibits a highly protective effect for heart cells when administered both before and after ischemia indicating that the activity of voltage activated K^+ channels is important for ischemia/reperfusion induced cell damage during transplantation.

42 Myocardial enzyme release in totally endoscopic CABG on the arrested heart

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Background. Totally endoscopic CABG (TECAB) is a enables coronary surgery without sternotomy or thoracotomy. However longer CPB times and aortic endoocclusion times are currently required as compared with standard CABG operations. We investigated if longer operation times affect the myocardial enzyme release and the postoperative course.

Methods. From 2001–2006 85 patients, aged 58 (31–76) years, underwent TECAB on the arrested heart using the da Vinci™ telemanipulator and remote access perfusion via the femoral vessels (ESTECH™ or HEARTPORT™). The operations intended were LIMA-LAD ($n = 74$), RIMA-RCA ($n = 2$), double vessel LIMA-OM/Cx and RIMA-LAD ($n = 8$), and double vessel LIMA-LAD and SVG-RCA ($n = 1$). TECAB duration was 254 (178–710) minutes, cardiopulmonary bypass time was 114 (57–428) minutes, and aortic endoocclusion time 65 (28–230) minutes.

Results. The postoperative ventilation time was 8 (0–278) hours, the ICU stay 20 (11–389) hours. The postoperative stay

at our department was 6 (4–22) days and we observed no hospital death in this series. Postoperative peak CK-MB levels were significantly associated with: TECAB duration ($r = 0.588$, $p < 0.001$), CPB time ($r = 0.521$, $p < 0.001$), aortic endoocclusion time ($r = 0.400$, $p < 0.001$), ICUstay ($r = 0.432$, $p < 0.001$), ventilation time ($r = 0.517$, $p < 0.001$). CK-MB levels were not associated with gender, body weight, age, or EuroSCORE.

Conclusions. TECAB operations could be performed safely in this series. But an increased myocardial enzyme release was found in longer operations, CPB durations, and aortic endoocclusion times and was associated with a longer ventilation time.

43 Cardiopulmonary and systemic effects of methylprednisolone in patients undergoing cardiac surgery

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Background. Cardiopulmonary bypass (CPB)-related inflammatory response can be attenuated by glucocorticoid treatment, but its impact on postoperative cardiopulmonary function remains controversial. It was investigated whether the systemic and myocardial anti-inflammatory effects of glucocorticoids are associated with improved cardiopulmonary function in cardiac surgery patients.

Methods. Eighty patients undergoing elective coronary artery bypass grafting were randomly assigned to receive a single-shot of methylprednisolone (MP; 15 mg/kg) or placebo (PLA) before CPB. Parameters of myocardial and pulmonary function, and systemic hemodynamics were measured before, 1, 4, 10 and 24 h after CPB. Blood was sampled for measurement of pro- (TNFα, IL-6, IL-8) and anti-inflammatory (IL-10) cytokines (ELISA), troponin T and C-reactive protein. Phosphorylation of IκBα and p38-MAPK was determined in right atrial biopsies before and after CPB (phosphoprotein-assay).

Results. Pre- and intraoperative characteristics of patients were not different between groups. MP attenuated postoperative TNFα, IL-6, IL-8 and C-reactive protein levels while increasing IL-10 release. Myocardial IκBα was preserved with MP ($p < 0.05$ vs. PLA), but p38-MAPK activation occurred in both groups after CPB ($p < 0.05$ vs. pre CPB). MP improved postoperative cardiac index and was associated with decreased troponin T when compared to PLA ($p < 0.05$). Postoperative blood glucose, oxygen delivery index and pulmonary shunt flow were increased in MP group ($p < 0.05$). There was no difference in postoperative oxygenation index, ventilation time and clinical outcome between treatment groups.

Conclusions. Glucocorticoid treatment prior to CPB attenuates perioperative release of systemic and myocardial inflammatory mediators and improves myocardial function suggesting potential cardioprotective effects in patients undergoing cardiac surgery.

Symposion IX: „Mechanische Kreislaufunterstützung“

44 Mechanische Herzunterstützung: auf dem Weg zur chronischen Anwendung

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45 Miniaturized MEDOS® diagonal pump as left ventricular assist device in a sheep model

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Background. This study was performed to investigate the new and miniaturized MEDOS® diagonal pump as left ventricular assist device in a sheep model.

Methods. The new miniaturized MEDOS® diagonal pump has an unique integrated and indwelling motor with magnetic coupling of the rotor. We operated 10 sheep with lateral thoracotomy and implanted the paracorporeal MEDOS® left ventricular assist device atrio-aortal for seven-day-use. The 90-degree angle between inflow and outflow port allows for short coupling cannulae to the left atrium and descending aorta.

Results. At a mean flow rate of 2.5 l/min for seven days five sheep were pumped and no significant thrombus formation occurred in the pump housing or inflow port of the pump. Thrombus formation at the atrial cannula was crucial when wall contact occurred and could be omitted by careful placement of the cannula. No relevant hemolysis was observed and hemoglobin levels remained stable ($7.9 +/- 0.8$ at day one to $7.5 +/- 0.8$ at day eight). Creatinkinase decreased to normal within days ($1033 +/- 507$ U/l postoperatively to $27 +/- 10$ U/l at day eight) and Troponin T remained normal. Only minor emboli could be detected at autopsy.

Conclusions. We conclude that the miniaturized MEDOS® diagonal pump could be run as left ventricular assist device for short to mid-term period.

46 Distinct regulation of a new linear blood pump

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Long-term cardiac support and total artificial heart with displacement or rotary blood pumps is still crucial due to

blood cell damage, hemolysis, thrombus formation or bleeding. We propose a new concept that is not in need of membranes for displacement or impellers for rotating motion of the fluid, but aims to mimic the displacement of an inlet valve against an outlet valve in the natural cardiac cycle. At the end of a tube (diameter 3 cm, length 10 cm) an outlet valve is fixed and a second valve moves within the tube against this valve, driven by a magnetic linear motor. With this design the mean energy loss is calculated to approximately 2.4 W at a flow rate of 4.8 l/min against a load of 120 mm/Hg. As the gap between inner wall of the tube with carbon surface and the moving piston can be reduced to at least 5 m a laminar flow of low viscosity results. The blood pump can be designed for high frequencies and low displacement volumes making cardiac assist for new-borns and infants feasible.

47 Do atrophic changes in the unloaded heart lead to functional impairment?

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Background. Ventricular assist devices (LVAD) are implanted as bridge to transplantation in patients suffering from end-stage heart failure. Recent studies show that ventricular unloading may result in functional recovery. However, benefit from reverse remodeling may be impaired by ventricular atrophy. We aim to characterize, in a time course approach, ventricular atrophy and function induced by mechanical unloading. Atrophic remodeling was induced by heterotopic heart transplantation (HTX).

Methods. Eighty adult Lewis rats underwent HTX for 3, 8, 15, 30, 60 and 90 days. Atrophy and fibrosis of ventricles were assessed by stereological methods. Calcium content was measured by atomic absorption spectrophotometry. mRNA expression of SERCA, TNF-β1, Myogenin-isoforms and caspase-3 were analyzed by RT-PCR. Left ventricular pressure (LVP) was measured on isolated, perfused transplants.

Results. Heterotopic heart transplantation induced a decrease of ventricular volume of 23.9% (SD+/−5.5%) at 3 days up to 68.2% (SD+/−3.6%) at 90 days after transplantation. Stabilization of the weight loss occured after 60 days. Accordingly, cellular atrophy and volume of fibrosis increased. Preliminary data showed a decrease in LVP to 30.4 mmHg (SD+/−1.5), dP/dtmax of 651.6 mmHg s^{-1} (SD+/−48.3) and dP/dtmin of −344.3 mmHg s^{-1} (SD+/−22.6) after 90 days. Apoptosis and cellular remodeling assessed by RT-PCR are under investigation.

Conclusions. We demonstrated that the change in ventricular volume induced by heterotopic transplantation is associated with increased fibrosis and functional impairment. Our results suggest that atrophic changes oppose recovery in relation to the duration of mechanical unloading, which has to be optimally determined in particular when this approach is used toward recovery.

Symposion X: „Regenerative Verfahren“

48 Einführung: Regenerating the broken heart by cell transfer: quo vadis?

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49 The gp130 ligand Oncostatin M contributes to stem cell homing via induction of SDF-1 in cardiac cells

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Background. Stromal Derived Factor 1 (SDF-1) is a CXC chemokine important in the homing process of stem cells to injured tissue. Novel therapies of myocardial infarction include mobilization and transplantation of stem cells, although little is known about the homing potential of the human heart. Growing evidence suggests that the glycoprotein 130 (GP-130) receptor family is involved in survival of cardiac myocytes and in repair processes in the heart. The aim of our study was to determine if GP-130 ligands contribute to stem cell homing via induction of SDF-1 in cardiac cells.

Results. Oncostatin M (OsM) was the only GP-130 ligand capable of inducing SDF-1 in human adult cardiac myocytes (HACM) and human adult cardiac fibroblasts (HACF). Protein expression was up to 7 fold elevated compared to control. The induction of SDF-1 through OsM was dose dependent. Protein data was confirmed by mRNA results. A blocker directed against p38 was able to abolish OsM induced secretion of SDF-1 protein.

Conclusions. Through phosphorylation of p38 OsM induces SDF-1 protein secretion in human cardiac cells. Our in vitro data suggests that OsM via the induction of SDF-1 might play a key role in repair in the human heart and if also operative in vivo might be involved in homing of stem cells to this organ.

50 Increased cell engraftment and neoangiogenesis after combined transplantation of skeletal myoblasts and angiopoietic progenitors in ischemic heart failure

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Background. Combined transplantation of skeletal myoblasts(SM) and AC133 + cells (SC)leads to improved left

ventricular function, reduced infarct size and myocardial apoptosis in a model of chronic ischemia. The aim of this study was to elucidate on the possible mechanisms of increasing cell therapy efficacy in chronic ischemia.

Methods. Heart failure was induced by LAD-Ligation in nude rats. a. Homologous skeletal myoblasts (SM), b. human derived AC133 + cells (SC), c. combination of both cells (comb) and d. culture medium (CM) were injected in the infarct and peri-infarct area, respectively 4 weeks after infarction. Engraftment was detected by fluorescence microscopy and confirmed by immunohistochemistry. Cell survival as well as gene expression levels of VEGF-A, Cardiac Troponin, ACTA2, SDF-1, TGF-beta1 were assessed by RT-PCR.

Results. Both cell types were detected in the injection areas 4 weeks after cell transplantation. SC were identified in both the borderzone and the infarct area, suggesting migration to the scar. Double cell therapy led to increased cell engraftment. This effect was confirmed by PCR analysis. Apoptotic index among engrafted cells was significantly lower in the comb group. SC were positive for smooth muscle a-Actin and vWF suggesting increased vasculogenesis. Evaluation of capillary density revealed increased angiogenesis in the comb group. Neoangiogenesis was assotiated with higher levels of VEGF-A and TGF-beta1 in the injection areas as detected by RT-PCR.

Conclusions. Functional improvement after combined transplantation of SM and SC in ischemic heart failure includes improved survival, lower apoptosis rates of the injected cells and induction of angiogenesis.

51 A role for gp130-ligands in angiogenesis: Oncostatin M regulates Angiopoietin1, Angiopoietin2 and its own receptors

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Background. The angiopoietin-Tie2 system acts as a crucial regulator of vessel maturation and quiescence. Angiopoietin1 (Ang1), which activates the Tie2 receptor stabilizes vessel walls and suppresses angiogenesis. Ang2, mainly expressed by endothelial cells, binds to the same receptor and has been identified as a functional antagonist of Ang1 leading to vessel regression or sprouting. There is evidence that the angiopoietin-Tie2 system is also involved in inflammatory angiogenesis. We asked if there might be a link between the glycoprotein (gp) 130 ligand system, which has been shown to regulate inflammatory events in other tissues, and the angiopoietin-Tie2 system.

Results. OSM increased Ang2 secretion up to 3-fold in HUVEC, HAEC and HHMEC and decreased Ang1 secretion down to 25% of the control level in HUVEC, HAEC and HASMC. Phorbol 12-myristate 13-acetate, but not OSM stimulated the release of stored Ang2 from Weibel-Palade bodies in HUVEC. We could detect the 6 different gp130 ligand-receptors in all cell types and found a significant upregulation of the OSM receptor and gp130 receptor by its own ligand OSM.

Conclusions. We showed for the first time a link between the gp130 ligand system and angiogenesis in human endothelial cells. OSM, mainly produced by activated T-cells and monocytes, is not involved in the rapid release of stored Ang2, but regulates Ang2 secretion at the transcription level. It does not only regulate the secretion of Ang1 and its antagonists Ang2 in a reciprocal manner, but also increased the expression of its own receptors, which amplifies the stimulatory effect which indicates that OSM might be an important contributor to de novo formation of blood vessels.

52 Sequentielle Proteom-Analyse während der Entwicklung einer Myokardhypertrophie durch Konstriktion der Aorta

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Biomechanische Last, wie z. B. die Nachlasterhöhung gehört zu den Hauptstimuli bei der Entwicklung einer Myokardhypertrophoie und letztlich einer Herzinsuffizienz.

Während der letzten Jahre wurden viele funktionelle und molekulare Veränderungen des hypertrophierten Myokards beschrieben. Über die zeitlichen Zusammenhänge der Veränderung von Protein-Expressionsmustern während der Entwicklung der Myokardhypertrophie und letztlich der Nachlast-induzierten Herzinsuffizienz ist jedoch nur wenig bekannt. Deshalb untersuchten wir die zeitabhängigen Veränderungen des Proteoms im Mausmodell der Nachlast-induzierten Myokardhypertrophie nach standardisierter Konstriktion der transversalen Aorta.

Veränderungen des Proteoms wurden untersucht mit 2D-Gelelektrophorese nach einer akuten Steigerung der Nachlast (6 und 48 Stunden), nach einem intermediären Zeitintervall (1 Woche) und nach chronischer Nachlasterhöhung (4 Wochen) mit reduzierter LV-Funktion und erhöhten linksventrikulären Dimensionen im Vergleich zur Sham-Kontrollgruppe.

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