



CARDIOVASCULAR RESEARCH DAYS

1st–3rd MARCH 2018
DEBRECEN, HUNGARY

Scientific Program & Abstracts





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Dear Colleagues, Dear Friends,

On behalf of the Organizing Committee, it is our great pleasure to welcome you at the **CARDIOVASCULAR RESEARCH DAYS 2018** meeting.

The 1st **CARDIOVASCULAR RESEARCH DAYS** (17 – 20 January. 2006, Weissensee, Austria with the support of the Austrian, German and Swiss Societies of Cardiothoracic Surgery and Cardiology) was a great success, and hence it prompted the establishment of a tradition for subsequent meetings on a similar basis. Over the years **CARDIOVASCULAR RESEARCH DAYS** gradually addressed more and more scientists (also from Italy and Hungary), and thus it became a well-respected forum for cardiovascular scientists of Central Europe. Not only the scientific contents of excellent presentations, but perfect organizations filled with friendly atmosphere made these events truly memorable. Scientific programs of prior meetings concentrated on several important aspects of clinical/experimental cardiology/cardiac surgery ranging from “Endothelial Dysfunction” (2006) through “Myocardial Dysfunction” (2007), “Repair, Regeneration and Protection” (2008), “The Old and Multimorbid Patient” (2010), “Remodeling and Reverse Remodeling in Valvular Heart Disease” (2012), “Organ Preservation and Regeneration from Basic Science to Clinic” (2014) to “Novel Techniques - Crazy Ideas” (2016). These mottos clearly reflect the strong translational character of **CARDIOVASCULAR RESEARCH DAYS** where practicing cardiologists, cardiac surgeons and experimental cardiologists aim at narrowing the gap between “bed” and “bench”, together. The **motto for CARDIOVASCULAR RESEARCH DAYS 2018** became somewhat longer than before, i.e.: “**Translational Efforts to Understand Life Threatening Situations in Cardiovascular Medicine**” not only to emphasize the above incentives, but also to stress our concerns for critically ill patients.

The 8th **CARDIOVASCULAR RESEARCH DAYS** meeting takes place between the **1st and 3rd of March, 2018** at the “**Kölcsey Convention Center**” of **Debrecen, Hungary** where the “Debrecen Cardiology Meeting” organized by the Hungarian Society of Cardiology for Hungarian cardiologists is to be held as a parallel congress. This arrangement aided organizational efforts and promotes scientific and personal exchanges among a wide range of cardiology specialists.

The organizers are most honored by your participation at the CARDIOVASCULAR RESEARCH DAYS 2018 meeting.

Several important details on the congress can be found at our website:

<http://cardiovascularresearchdays.eu/>

We wish you successful participation at **CARDIOVASCULAR RESEARCH DAYS 2018** and an enjoyable stay in **Debrecen**.

Zoltán Papp
Meeting Organizing Chairman

Bruno Podesser
Meeting Organizing co-Chairman

Scientific Program

THURSDAY, March 1, 2018

13:30	Opening of the Symposium <i>Bruno Podesser, Zoltán Papp</i> László Mátyus, Dean of the Medical Faculty of the University of Debrecen Zoltán Papp, chairman of Cardiovascular Research Days 2018
14:00 - 17:20	AFTERNOON SESSIONS
14:00 - 15:20	Session 1: From bench to bed <i>David Bernhard, Giuseppe Faggian</i>
20min	High intensity interval training in cardiac rehabilitation: A randomized controlled trial investigating platelet function (<i>Stefan Heber</i>)
20min	Heart failure and mechanical assist devices (<i>Angela Maria Rajek</i>)
20min	Myocardial protection in pediatric and congenital cardiac surgery (<i>Giovanni Battista Luciani</i>)
20min	Patient-specific 3D-Printing of cardiovascular anatomies for in-vitro testing (<i>Maximilian Grab</i>)
20min	Vein graft thrombi, a niche for smooth muscle cell colonization – a hypothesis to explain the asymmetry of intimal hyperplasia (<i>Isaac Blaas</i>)
15:40 - 16:00	Coffee Break and posters
16:00 - 17:20	Session 2: From bed to bench <i>Stefan Chłopicki, Nazha Hamdani</i>
20min	The Metabolic Road of Co-morbidities to Understanding the Pathophysiology of Heart Failure: The Role of Inflammatory Signaling Pathways in Obesity and Diabetes (<i>Nazha Hamdani</i>)
20min	Early inhibition of retinoic acid signalling upon myocardial infarction restores cardiac function and prevents cell, tissue, and animal death (<i>David Bernhard</i>)
20min	Endothelial profiling in vivo (<i>Stefan Chłopicki</i>)
20min	Cardioprotection by remote ischemic conditioning and its novel signaling mechanisms (<i>Attila Kiss</i>)
18:00	Bus will leave to Tokaj

FRIDAY, March 2, 2018

09:00-12:30 MORNING SESSIONS

09:00 - 10:20 Session 3: Cardiac surgery of the 21st century

Bruno Podesser, Tamás Szeráfin

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| 20min | "High-tech" cardiac surgery to optimize postoperative outcome: focus on mitral valve and ventricular reconstruction. (<i>Gábor Szabó</i>) |
| 20min | Computer assisted decision making in cardiac surgery: from 3D preoperative planning to computational fluid dynamics in the design of surgical procedures (<i>István Hartyánszky</i>) |
| 20min | Biological and synthetic scaffolds for cardiovascular tissue engineering and regenerative strategies - Many ways leading to one goal? (<i>Nikolaus Thierfelder</i>) |
| 20min | Nitric oxide administration to the gas inflow of oxygenator improve cerebral perfusion and neuroprotection during ECMO (<i>Daniele Linardi</i>) |

10:20 - 11:00 | *Coffee Break and posters*

11:00 - 12:20 Session 4: The cardio-renal axis

Jennifer Pollock, József Balla

- | | |
|-------|---|
| 20min | Regulation of vascular and valvular mineralization in chronic kidney diseases (<i>József Balla</i>) |
| 30min | Endothelin, salt intake, and the molecular clock (<i>David Pollock</i>) |
| 30min | Childhood Adversity and Adult Cardiovascular Disease Risk (<i>Jennifer Pollock</i>) |

12:20- 14:30 | *Lunch Break*

14:30 - 18:00 AFTERNOON SESSIONS

14:30 - 15:50 Session 5: Novel translational concepts

Nikolaus Thierfelder, Johann Wojta

- | | |
|-------|--|
| 20min | Placental growth factor-based therapy for ischemic cardiomyopathy (<i>Péter Pokreisz</i>) |
| 20min | Monocyte subsets and their role in cardiovascular pathologies (<i>Johann Wojta</i>) |
| 20min | MicroRNAs and myocardial protection (<i>Bruno Podesser</i>) |
| 20min | P21-activated kinase inhibits vascular reactivity via inhibition of MLCK (<i>Karen Uray</i>) |

15:50 - 16:30 | Coffee Break and posters

15:50 - 18:30 Session 6: Poster Session

160min | Moderated poster presentations

19:00 | Party together with the Hungarian Society for Cardiology

SATURDAY, March 3, 2018

09:00-12:30 MORNING SESSIONS

09:00 - 10:20 Session 7: Positive inotropy

Gerhard Pözl, Piero Pollesello

- 20min Cardiovascular effects of the novel myosin activator omecamtive mecarbil: positive inotropy and beyond (*Árpád Kovács*)
- 20min Omecamtiv mecarbil causes the alternation of myocardial excitation-contraction coupling at high pacing frequencies (*Balázs Horváth*)
- 20min Drug discovery and development for acute heart failure drugs: are expectations still too high? (*Piero Pollesello*)
- 20min Repetitive use of inodilators in advanced heart failure (*Gerhard Pözl*)

10:20 - 11:00 | Coffee Break and posters

11:00 - 12:20 Session 8: Risky business

Seth Hallström, Tamás Radovits

- 20min Myocardial reverse remodeling: can we heal a broken heart? (*Zoltán Papp*)
- 20min Role of cGMP-signalling in the prevention of heart failure with preserved ejection fraction (*Tamás Radovits*)
- 20min The role of the renin-angiotensin system in cardiovascular diseases (*Attila Tóth*)
- 20min Reduction of cardiovascular risk with functional foods (*Miklós Fagyas*)

12:20-12:45 | Closing remarks and farewell, Carl Apstein Prize 2018

Bruno Podesser, Zoltán Papp

Bruno Podesser

Zoltán Papp

Moderated Poster Session

Session 1

Johann Wojta, Zoltán Papp

P01	Beáta Mészáros	Human voltage-gated proton channels in chorion derived mesenchymal stem cells
P02	Viktória Csató	Oxidant Activation of PKG controls vascular tone by regulating Ca^{2+} spark frequency
P03	Dénes Kiss	Contribution of late sodium current to the electrophysiological characteristics of canine left ventricular cardiomyocytes
P04	Florina Zákány	Determining the target of cholesterol on the gating of voltage-gated potassium channels
P05	Ágota Csóti	Characterization of novel high-selectivity KV1.3 inhibitor peptide
P06	Csaba Dienes	TRPM4 inhibitor 9-phenanthrol blocks Na^{+} and K^{+} but not Ca^{2+} currents in canine ventricular myocytes
P07	Andrea Orosz	Analysis of cardiac repolarization parameters and risk factors of sudden cardiac death in patients with hypertrophic cardiomyopathy
P08	Roland Veres	Omecamtiv mecarbil prolongs the contraction and relaxation in isolated left ventricular cardiac myocytes
P09	Orsolya Vörös	The C-terminal HRET sequence of Kv1.3 regulates gating rather than targeting of Kv1.3 to the plasma membrane
P10	Bettina Kurtán	Role of calcium/calmodulin-dependent protein kinase II activation in beta-adrenergic stimulation of potassium currents in canine ventricular cardiomyocytes under action potential clamp conditions

Session 2

Attila Kiss, Attila Tóth

P11	Márta Sárközy	Selective heart irradiation induces cardiac overexpression of the pro-hypertrophic miR-212/132 cluster
P12	Andrea Sümegi	A novel splice-site indel alteration in the EIF2AK3 gene is responsible for the first cases of Wolcott-Rallison Syndrome in Hungary
P13	Boglárka Balogh	Functional polymorphism of innate immunity pattern recognition receptors do not constitute the risk of bacterial infections other than spontaneous bacterial peritonitis and also not the progressive disease course in patients with cirrhosis
P14	Imre Barabás	Medical engineering as a patient-specific planning support system in complex cardiac surgical cases - Case series
P15	Bálint András Barta	Comparison of speckle-tracking echocardiography with invasive hemodynamics for the detection of characteristic cardiac dysfunction in type-1 and type-2 diabetic rat models
P16	Bernhard Winkler	TRITON artificial tissue graft for small diameter application in cardiovascular procedures - preliminary results

P17	Elena Kuster	Challenging procedural parameters in pericardial decellularization -The influence of pre-processing storage and treatment temperature
P18	Simone Müller	Pulmonary artery root decellularization - Significant improvement of established protocols by modification of physical and procedural factors
P19	Christian Doppler	Metabolomic and MALDI profiling of thoracic aortic aneurysms and dissections – Implications for pathophysiology and biomarker discovery

Session 3

Gerhard Pölzl, Gábor Szabó

P21	Beáta Bódi	The phosphodiesterase-5A inhibitor vardenafil prevents the development of diabetes mellitus-associated cardiomyocyte dysfunction
P22	David Santer	Improved myocardial protection with hypothermic St Thomas' Hospital polarizing blood cardioplegia
P23	Mária Lódi	Prevention of ultrastructural myocardial injury in a rat model of doxorubicin induced cardiomyopathy
P24	Attila Oláh	Electrophysiologic investigation of exercise-induced cardiac hypertrophy in a rodent model of athlete's heart
P25	Árpád Kovács	Improved diastolic function through reduced oxidative stress and cardiomyocyte titin-based stiffness due to chronic stimulation of soluble guanylyl cyclase in heart failure with preserved ejection fraction
P26	Evelyn Kienle	Sterilization of bovine pericardium – A big challenge for clinical application
P27	Katalin Szabó	The effect of fenugreek (<i>Trigonella foenum - graecum</i>) seed flour and diosgenin on the endothelium - dependent relaxation of abdominal aorta in a rat model of metabolic syndrome
P28	Muhammad Azeem Jalil Umar	Direct Measurement of Factor Xa Activity in Human Blood
P29	Boglárka Balogh	IgA antibodies against filamentous actin are frequently detected in patients with cirrhosis and indicate a progressive disease course
P30	Tamás Debreceni	Incidence, survival and determinants of mortality following mesenteric angiography and continuous intra-arterial prostaglandin E1 perfusion for non-occlusive mesenteric ischaemia in cardiac surgery

Session 4

Seth Hallström, Tamás Radovits

P31	Hamza Ouafa	Closed chest animal model of ischemic mitral regurgitation in the pig
P32	Alexandra Csongrádi	Endogenous ACE inhibition caused by cell free haemoglobin can lead to the development of hypotension after coronary artery bypass grafting

P33	Tamás Gáll	Heme induces endoplasmic reticulum stress in human aortic smooth muscle cells
P34	Kata Csekő	Integrative characterization of chronic cigarette smoke-induced cardiopulmonary comorbidities in a mouse model
P35	Klaudia Bottyán	Angiotensin converting enzyme activity measurement in human lung tissue
P36	Katalin Éva Sikura	Targeting H-ferritin to mitigate valvular mineralization
P37	László Potor	Hydrogen sulfide abrogates hemoglobin-lipid interaction in atherosclerotic lesion
P38	Nóra Lampé	Effects of BGP-15 on the contractility of human right atrial myocardium
P39	Viktor Bánhegyi	Tissue expression and regulation of angiotensin converting enzymes (ACE, ACE2) in the human lung
P40	Erzsébet Zavacki	Hydrogen sulfide inhibits calcification and osteoblastic differentiation of vascular smooth muscle cells

Session 5

Nikolaus Thierfelder, Péter Pokreisz

P41	Ines Goncalves	Tenascin C role in ischemia/reperfusion injury, and chronic hypertension - a potential miRNA targeting approach in an in vitro and in vivo rat model
P42	Áron Üveges	Discordant resting and hyperemic pressure gradients in relation to the coronary flow reserve
P43	Isaac Blaas	Vein graft thrombi, a niche for smooth muscle cell colonisation
P44	Patrick Pilz	Repeated remote ischemic conditioning enhances Neuregulin-1/ErbB2/3/4 expression following myocardial infarction in rats
P45	Kálmán Benke	Pharmacological stimulation of the NO/sGC/cGMP pathway reduces ischemia/reperfusion injury and improves donor organ function in a rat model of heterotopic heart transplantation
P46	Felix Nagel	Tenascin-C deficiency attenuates abdominal aortic aneurysm progression in mice
P47	Éva Szőke	The effect of doxycycline administration and the role of matrix metalloproteinases in a chronic cigarette smoke-induced cardiopulmonary comorbidity mouse model
P48	Mihály Ruppert	Effect of gender on myocardial reverse remodeling in a rat model of banding and debanding of the abdominal aorta
P49	Nikolett Oláh	Different mechanisms for heart failure progressions in male and female mRen2 rats

Functional polymorphism of innate immunity pattern recognition receptors do not constitute the risk of bacterial infections other than spontaneous bacterial peritonitis and also not the progressive disease course in patients with cirrhosis

B. Balogh

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Introduction: Innate immunity pattern recognition receptors (PRRs) recognize distinct pathogen-associated molecular patterns (PAMPs) on the cell surface or in the cytoplasm. Functional polymorphisms of various PRRs have been established to contribute to susceptibility to spontaneous bacterial peritonitis (SBP). However, their role in the development of cirrhosis-associated bacterial infections (BI) beyond SBP remains unknown.

Aims: We aimed to investigate the link between PRR gene variants, pathological bacterial translocation (BT) and thus the development of cirrhosis-associated complications.

Material and methods: 349 patients with cirrhosis (stable outpatients: 243 and acute decompensation: 106) were genotyped for the common *NOD2* (p.R702W, p.G908R and c.3020 insC), *TLR2* (g.6686T/A), *TLR4* (D299G) and *CD14* (c. 159C/T) gene variants. In the stable outpatients (male: 116, age: 56±11 yrs, alcohol: 62.6%, ascites: 36.2%, MELD score: 11) incidence of BI, decompensating events (ascites, variceal bleeding and hepatic encephalopathy) and liver-related death were prospectively assessed in a 5-year follow-up observational study. BT was assessed based on the presence of anti-microbial antibodies (anti-OMP Plus IgA and/or endotoxin core IgA antibody [EndoCab]) or increased serum level of lipopolysaccharide-binding protein (LBP).

Results: Ninety-four (38.7%) patients encountered at least one episode of BI. Distribution of bacterial infections were as follows: urinary tract infection (39.4%), SBP (27.7%), pneumonia (13.8%) and miscellaneous (24.5%). 5.3% of the cases were multifocal. PRR genetic profile was not associated with prior BI episode and also not with the risk of overall BI or infection-related death during the follow-up. *NOD2* variants, however, were associated with an increased cumulative probability of SBP in patients with ascites (n=88) as compared to wild type (76.9% vs. 30.9%, $p_{\text{LogRank}}=0.047$). Frequency of anti-microbial antibodies and LBP levels did not differ between various PRR genotypes. Correspondingly, PRR genetic profile was not able to predict the long-term disease course in cirrhosis.

Conclusions: In a Hungarian patient cohort with cirrhosis, common *NOD2* gene variants but not the other PRR polymorphisms enabled to improve identification of patients with high risk for SBP. None of the examined PRR gene variants influenced the risk of other types of BI or the long-term disease course. They were also not associated with serological markers of BT.

The work/publication is supported by the EFOP-3.6.2-16-2017-00006 project. The project is co-financed by the European Union and the European Social Fund.

IgA antibodies against filamentous actin are frequently detected in patients with cirrhosis and indicate a progressive disease course

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Introduction: Gut barrier failure and pathological bacterial translocation (BT) are characteristic features of cirrhosis and play an important role in the progression of liver disease.

Aims: To investigate, whether hallmarks of gut barrier failure are associated with accelerated development of complications and liver-related death in cirrhosis.

Material and methods: Sera from 260 outpatients with cirrhosis (male: 129, age: 56±11 yrs, alcohol: 167 [64.2%]) and from 155 healthy subjects were assayed for antibodies against filamentous actin [AAA IgA and IgG] and gliadin [AGA IgA and IgG]) and for intestinal fatty acid-binding protein (I-FABP) by ELISA. Association of gut failure markers with disease specific characteristics was assessed at baseline. BT was assessed based on the presence of anti-microbial antibodies (anti-OMP Plus IgA and/or endotoxin core antibody IgA [EndoCab]). We evaluated decompensating events (ascites, variceal bleeding, hepatic encephalopathy, bacterial infections) and liver-related death in a 5-year follow-up, observational study.

Results: Elevated concentrations of the gut failure markers IgA-AAA (62.7 vs. 4.4%) and IgA-AGA (27.7 vs. 2.6%) were more often observed in cirrhosis as compared to healthy controls ($p<0.001$ for both). In addition, serum I-FABP was increased in cirrhosis as compared to controls (741 vs. 244 pg/mL, $p<0.001$) and correlated with serum levels of IgA-AAA and IgA-AGA. IgA-AAA positivity was associated with alcoholic liver disease, liver disease scores and decompensated clinical stage (all $p<0.001$). Serological markers of BT were more often found in patients with elevated IgA AAA compared to those without (72.3 vs. 13.5 % for IgA-EndoCab and 85.2 vs. 20.5% for IgA anti-OMP, $p<0.001$ for both). In patients with compensated disease stage ($n=131$) the risk of decompensation was higher in patients with elevated IgA-AAA (HR [95%CI]: 1.85 [1.06-3.24]), as was the risk of liver-related mortality (HR: 2.66 [1.27-5.56]). Such associations were not observed for IgG-AAA and IgA/IgG-AGAs. In the overall cohort, IgA-AAA remained an independent predictor of liver-related death (HRadj: 1.96 [1.08-3.55]) when adjusting for important clinical variables (MELD score, etiology, clinical stage, see Table 1).

Conclusions: Presence of IgA antibodies against filamentous-actin indicate patients with an unfavourable outcome in cirrhosis, which may be related to intestinal damage beyond being related to bacterial translocation. IgA-AAA might be consider as a novel serologic marker of disease progression.

The work/publication is supported by the EFOP-3.6.2-16-2017-00006 project. The project is co-financed by the European Union and the European Social Fund.

Tissue expression and regulation of angiotensin converting enzymes (ACE and ACE2) in the human lung

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The renin angiotensin aldosterone system (RAAS) plays a pivotal role in cardiovascular pathophysiology and represents a key pharmaceutical target in cardiovascular diseases. Textbooks state, that the angiotensin converting enzyme (ACE) is produced in human endothelium related tissues, primarily in the lung.

The goal of our work was to investigate the endothelium related enzymes (ACE, ACE2) in human lung tissue samples.

Lung tissue- and blood samples were collected from patients undergoing lung surgery at the Department of Thoracic Surgery, University of Debrecen (n=71). Fluorescent kinetic assays were applied for ACE, ACE2 activity measurements and ACE concentration was measured by ELISA. In addition, the I/D genotype of the ACE enzyme was determined, and the medical history was recorded. ACE secretion was also tested in cultured primary Human Aortic Endothelial Cells (HAOEC).

We found that ACE I/D genotype had a profound impact on circulating ACE activities: (ID: 9.645 ± 0.4223 U/ml, n=36, P=0.0043; DD: 11.20 ± 0.6203 U/ml, n=26, P=0.0005; vs. II: 6.966 ± 0.5166 U/ml, n=9). Surprisingly, there were no genotype related differences in the ACE activities of the lung tissues from the very same patients (ID: 3.034 ± 0.1996 U/ml, n=36 p=0.6421; DD: 2.709 ± 0.2495 U/ml, n=26, p=0.7920; vs. II: 2.833 ± 0.3179 U/ml, n=9). Furthermore, signs for endogenous tissue ACE inhibition were also found.

The direct administration of ACE specific substrate Abz-FRK (Dnp) to our HAOEC cell culture resulted 299.6 U/ml ACE activity which was inhibited over 90% by the ACE inhibitor captopril (at 200 nM). ACE2 specific activity was not detected in HAOEC.

Our data suggests that the genotype dependent regulation of ACE for circulating ACE is different from that of the lung. Moreover, our data implicate that ACE activity is regulated by endogenous inhibition in vivo. Furthermore, HAOEC cell culture provides a good model system for the investigation of the mechanism of endothelial ACE secretion.

The work/publication is supported by the EFOP-3.6.2-16-2017-00006 and GINOP-2.3.2-15-2016-00043 projects. The project is co-financed by the European Union and the European Social Fund.

Medical engineering as a patient-specific planning support system in complex cardiac surgical cases - Case series

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Semmelweis University, Heart and Vascular Center, Department of Cardiac Surgery, Budapest, Hungary

The increasingly rapid progression of new technology fosters an environment of innovation and change that must lead to some new personalized surgical opportunities in surgery. The bridge between the computer imaging techniques and surgical specialties is the quantitative modeling with possibility of analyzing the predicted postoperative results before the first incision. We report the application of patient-specific 3D models as a surgical planning support to plan the treatment of complex cardiac surgical cases. Clinically acquired images were used to set up patient-specific anatomical and computational models for finite element (FE) and computational fluid dynamics (CFD) analyses.

The 3D geometry was reconstructed from computed tomography or magnetic resonance images in specific cases. Although every surgeon cogitates in 3D and use their stereopsis ability during preparation for operation but simply two dimensional records were pretended by the routine computer tomography and magnetic resonance images. To resolve this problem 3D models and computational calculations were performed before every complicated cases in our practice. The short, and long-term results of this type of surgical interventions - based on 3D reconstruction and/or computational calculations - proved excellent clinical accuracy. Our method was used to assist in clinical decision-making, training, and consultation between medical specialties.

Three different type of surgical cases are presented in our poster. The first is a left ventricle aneurysm case after the Computer Assisted Ventricle Engineering (CAVE) procedure. The second is post-deep sternal wound infection complication case where the 3D printed model was used during the surgical intervention to separate the pseudoaneurysm from sternum closure wire. In the third case, a synovial sarcoma is presented in position of anterior leaflet of mitral valve. Extension and accurate localization of the sarcoma was calculated with our developed Matlab script, using MR Dicom files. Considering the calculation, a 3D model was printed with a two-armed printer, where the tumor mass is show-through the external surface, taking into account of tumor's border. Based on the planning, the synovial sarcoma was removed completely without recurrence within the following one year.

Comparison of speckle-tracking echocardiography with invasive hemodynamics for the detection of characteristic cardiac dysfunction in type-1 and type-2 diabetic rat models

B. A. Barta, Cs. Mátyás, A. Kovács, B. T. Németh, A. Oláh, Sz. Braun, M. Tokodi, K. Benke, M. Ruppert, B. K. Lakatos, B. Merkely, T. Radovits

Semmelweis University, Heart and Vascular Center, Budapest, Hungary,

Introduction: Measurement of systolic and diastolic function in animal models is challenging by conventional non-invasive methods. Therefore, we aimed at comparing speckle-tracking echocardiography (STE)-derived parameters to the indices of left ventricular (LV) pressure-volume (PV) analysis to detect cardiac dysfunction in rat models of type-1 (T1DM) and type-2 (T2DM) diabetes mellitus (DM).

Methods: Rat models of T1DM (induced by 60mg/kg streptozotocin, n=8) and T2DM (32-week-old Zucker Diabetic Fatty rats, n=7) and corresponding control animals (n=5 and n=8, respectively) were compared. Echocardiography and LV PV analysis were performed. LV short-axis recordings were used for STE analysis. Global circumferential strain (GCS), peak strain rate values in systole (SrS), isovolumic relaxation (SrIVR) and early diastole (SrE) were measured. LV contractility (preload recruitable stroke work, PRSW), active relaxation (Tau) and stiffness (end diastolic pressure volume relationship, EDPVR) were measured by PV analysis.

Results: In T1DM, contractility and active relaxation were deteriorated to a greater extent compared to T2DM (relative impairment in T1DM vs. T2DM; PRSW: -45.7 ± 4.4 vs. $-21 \pm 4.8\%$; Tau: $+36.6 \pm 8.5$ vs. $+14.2 \pm 4.6\%$). In contrast, diastolic stiffness was impaired in T2DM (EDPVR: $+72.1 \pm 3.8$ vs. $+25.9 \pm 14.3\%$). Correspondingly, STE described more severe systolic dysfunction in T1DM (SrS: -45.9 ± 2.9 vs. $-17.1 \pm 4.1\%$). Among diastolic STE parameters, SrIVR was more decreased in T1DM (SrIVR: -55.4 ± 1.9 vs. $-22.2 \pm 6.1\%$), however, SrE was more reduced in T2DM (SrE: -31.9 ± 2.8 vs. $-23.1 \pm 2.9\%$). In T1DM, SrS correlated with contractility (SrS and PRSW $r = -0.785$, $p < 0.01$), SrIVR with active relaxation (SrIVR and Tau: $r = -0.636$ $p < 0.05$), while in T2DM SrE was related to cardiac stiffness (SrE and EDPVR: $r = -0.8$, $p < 0.001$), cardiomyocyte diameter (SrE and CD: $r = -0.698$, $p < 0.01$) and fibrosis (SrE and percentage of fibrotic area: $r = -0.642$, $p < 0.05$).

Conclusions: Strain and strain rate parameters can be valuable and feasible measures to describe the dynamic changes in contractility, active relaxation and LV stiffness in animal models of T1DM and T2DM. STE corresponds to PV analysis and also correlates with markers of histological myocardial remodeling.

Pharmacological stimulation of the NO/SGC/cGMP pathway reduces ischemia/reperfusion injury and improves donor organ function in a rat model of heterotopic heart transplantation

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Introduction: Despite the fast evolution of mechanical circulatory support devices, heart transplantation (HTX) remains the definitive therapy of end-stage heart failure. Ischemia-reperfusion (I/R) injury occurring during transplantation is a primary determinant of long-term outcome of HTX and primary graft insufficiency. The most important pathobiochemical changes induced by reperfusion in the myocardium of the donor organ are increased production of reactive oxygen species (ROS), intracellular Ca²⁺ overload, energy deficit and acidosis. Modification of the nitric oxide (NO)/soluble guanylate cyclase (sGC)/cyclic guanosine monophosphate (cGMP) signaling pathway appears to be the most promising among the pharmacological interventional options developed recently. The first clinically applicable member of this group is the sGC stimulator riociguat. We aimed at characterizing the cardio-protective effects of this drug in a rat model of heterotopic heart transplantation.

Methods: Donor Lewis rats were treated orally with either riociguat (10mg/BWkg) or placebo for two days. Hearts were stored for an hour in cold preservation solution (Custodiol) following explantation, then were transplanted heterotopically. One hour after initiation of reperfusion, left ventricular (LV) pressure-volume relations and coronary flow were recorded in order to assess post-transplant graft function. Molecular biological measurements and histological examination were also completed.

Results: LV contractility (LV systolic pressure at 120μl of LV volume: 117±13 vs. 48±5mmHg, $p<0.001$; dP/dt_{max}: 2963±221 vs. 1653±159mmHg, $p<0.001$) and active relaxation (dP/dt_{min} at 120μl of LV volume: -2014±305 vs. -1063±177mmHg, $p=0.019$) improved significantly after an hour of reperfusion, while alteration of coronary flow standardized to heart weight (2.52±0.34 vs. 1.67±0.22ml/min/g, $p=0.06$) was a trend following pretreatment with riociguat. Myocardial expression of antioxidant markers were significantly improved after heart transplantation.

Conclusions: Pharmacological preconditioning with riociguat decreases I/R injury and improves donor organ function in our small animal model of heart transplantation. The observed cardio-protective effect might be attributed to the stimulated sGC and increased myocardial cGMP-signaling. Riociguat therefore might be a potential cardio-protective agent in the inventory of heart transplantation surgery and during cardiac surgical procedures requiring sustained ischemia.

Vein graft thrombi, a niche for smooth muscle cell colonization – a hypothesis to explain the asymmetry of intimal hyperplasia.

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Background: Autologous saphenous veins are widely used in coronary artery bypass grafting; however, 10 years after surgery, 40% of grafts are completely occluded, and another 30% show reduced blood flow.

Objective: In the past, the central processes and signaling pathways responsible for this loss of patency have been identified. However, one central finding in the process of graft failure is so far not understood: the asymmetric character of intimal hyperplasia. It was the goal of the present study to address this aspect.

Methods: By the use of a cuff technique-based vein interposition mouse model with a new anticoagulation regime, alterations in vein grafts were analyzed 1 h, 1 day, 2 days, 3 days, 7 days and 21 days after reperfusion by means of immunolabeling, histochemistry, and high-resolution ultrasound.

Results: The novel and major finding of this study is that the vein graft thrombus may serve as a niche that is infiltrated and colonized by smooth muscle cells (SMCs). Fibroblast growth factor-1 and platelet-derived growth factor-B may be the SMC-attracting factors in the thrombus. The focal character of early thrombi may define the focal and asymmetric character of vein graft intimal hyperplasia.

Conclusions: Inhibiting the formation and reducing the size of early thrombi is an old concept for reducing vein graft failure. However, in light of the present new findings obtained under a clinic-like anticoagulation regime, early vein graft thrombus prevention/size reduction should be revisited in the prevention of graft failure.

The phosphodiesterase-5A inhibitor vardenafil prevents the development of diabetes mellitus-associated cardiomyocyte dysfunction

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Introduction: Patients with diabetes mellitus exhibit diastolic dysfunction with decreased nitric monoxide production (diabetic cardiomyopathy). Elevated intracellular cyclic guanosine monophosphate (cGMP) levels can confer cardioprotection in different heart diseases. Here we investigated the effects of vardenafil, a phosphodiesterase-5A (PDE-5A) inhibitor in a rat model of diabetic cardiomyopathy on cardiomyocyte function.

Materials and methods: Experiments were performed in male Zucker Diabetic Fatty (ZDF) and ZDF Lean (ZDFL) rats (as controls). Seven weeks old animals received either vehicle (ZDFLV) or 10 mg/bwkg vardenafil per os (ZDFV). Functional measurements were performed at the age of 32 weeks. Permeabilized left ventricular (LV) cardiomyocytes were used during isometric force measurements. Maximal Ca^{2+} -activated active force production ($F_{\max}$), its Ca^{2+} -sensitivity (pCa_{50}), and Ca^{2+} -independent passive force (F_{passive}) were monitored. Western immunoblotting was applied to assess site-specific phosphorylation status of cardiac troponin-I (cTnI) and cardiac myosin binding protein C (cMyBP-C). Total phosphorylation status of titin protein was probed by a ProQ Diamond phosphoprotein staining kit.

Results: No significant differences were observed among $F_{\max}$ values of the four groups. The pCa_{50} and F_{passive} values were significantly higher in the ZDF than in the ZDFL, ZDFLV or ZDFV groups (pCa_{50} and F_{passive} : ZDF: 5.88 ± 0.03 and 1.98 ± 0.12 kN/m²; ZDFL: 5.76 ± 0.01 and 1.02 ± 0.12 kN/m²; ZDFLV: 5.78 ± 0.03 and 1.03 ± 0.14 kN/m²; ZDFV: 5.76 ± 0.02 and 1.40 ± 0.13 kN/m², $P < 0.05$, $n = 6-7$, mean $\pm$ SEM). In ZDF rats cTnI phosphorylation levels at Ser22/23, Ser43 and Thr143 sites were significantly lower than those in the ZDFV group (ZDF: 0.77 ± 0.05 , 0.77 ± 0.08 and 0.68 ± 0.06 ; ZDFV: 1.01 ± 0.08 , 1.35 ± 0.18 and 1.35 ± 0.16 , in relative units, respectively, $P < 0.05$, $n = 4$). No significant differences in the site specific phosphorylation status of cMyBP-C at Ser282 and in the total phosphorylation status of titin between the ZDF and ZDFV groups were observed.

Conclusion: Vardenafil prevented the development of diabetes mellitus-associated cardiomyocyte alterations.

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Angiotensin converting enzyme activity measurement in human lung tissue

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The renin angiotensin aldosterone system (RAAS) plays a key role in the cardiovascular physiology and pathophysiology. Central elements of the RAAS are the angiotensin II producing ACE and eliminating ACE2. Recently we found that circulating ACE2 is increased in hypertension and heart failure. Here we made an effort to identify its source.

All textbooks state that the primarily source of ACE is the lung. Therefore, we tested ACE2 expression in the human lung and ACE2 activity in the sera of patients undergoing lung surgery at the Department of Thoracic Surgery, University of Debrecen (n=110). To aid ACE protein extraction, we administered Triton X-114 detergent to the homogenization phase during tissue processing. Enzyme activities of ACE and ACE2 were determined using fluorescently labeled substrates in kinetic assays.

Triton X-114 enhanced the extraction of angiotensin converting enzymes, but inhibited their activities (50-70%). For this reason, Triton X-114 was removed from the solution after extraction by Detergent Removal Resin and Spin Columns (DRSC). DRSC effectively eliminated the detergent, with a minimal loss of protein (95% removed, while the protein recovery was >90%). Interestingly, the high tissue ACE2 activity was in contrast to the very low activity in the sera determined by the fluorescent substrate Abz-Ser-Pro-Tyr(NO₂)-OH. The measured tissue ACE2 activity was inhibited by the ACE2 inhibitor MLN-4760 (90% inhibition). Surprisingly, the low serum ACE2 activity could not be verified by Mca-APK(Dnp) substrate, showing lower specificity in tissue samples than that for Abz-Ser-Pro-Tyr(NO₂)-OH, but high serum ACE2 activities.

The use of Triton X-114 combined with detergent removal procedure provided an improvement in ACE2 extraction from human lung tissue samples over the published methods. Abz-Ser-Pro-Tyr(NO₂)-OH is a novel tissue specific fluorescent substrate for ACE2 activity measurements. Our data serves with a new tool in the research of ACE2 shedding contributing to elevated ACE2 levels in the sera in patients suffering from cardiovascular diseases.

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Oxidant activation of PKG controls vascular tone by regulating Ca²⁺ spark frequency

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We have recently shown that oxidant activated Protein Kinase G (PKG) contributes to small artery contractility by regulation of the Ca²⁺ spark/Large conductance Ca²⁺-activated K⁺ (BK) channel axis.

To explore characteristics of this signalling pathway in more detail, a transgenic mouse model was used, in which PKG is resistant to oxidant induced dimerization. Vascular function was examined in vitro with pressure myography. Ca²⁺ sparks and caffeine-induced Ca²⁺ transients (indicative of sarcoplasmic reticulum (SR) Ca²⁺ load) were imaged with high-speed spinning-disc confocal microscopy. Western blot protocols examined phospholamban and ryanodine phosphorylation.

In wild type (WT) arteries, Ca²⁺ spark frequency increased with intraluminal pressure but reached a 'Ca²⁺ spark frequency-ceiling' at 80 mmHg. There was no effect of pressure on the Ca²⁺ spark frequency in oxidant-resistant (OR) arteries. Similarly, exogenous hydrogen-peroxide (H₂O₂) increased Ca²⁺ spark frequency in WT arteries at low pressures but did not have this effect at higher intraluminal pressures. Conversely, caffeine-induced Ca²⁺ transients showed very little relationship with intraluminal pressure, although at higher pressures SR Ca²⁺ content was reduced in WT compared with OR arteries. Both exogenous H₂O₂ and the BK agonist NS11021 vasodilated pressure-constricted mesenteric arteries equivalently between WT and OR arteries. H₂O₂ caused phosphorylation of the ryanodine receptor but not phospholamban.

Oxidant activated PKG appears to target the ryanodine receptor to increase Ca²⁺ spark frequency and maintain BK function during pressure induced constriction. However, once activated, further increases pressure or exogenous oxidants cannot additionally increase the activity of this vasodilatory pathway, despite only low-level activity of the BK channel.

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Integrative characterization of chronic cigarette smoke-induced cardiopulmonary comorbidities in a mouse model

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Cigarette smoke-triggered inflammatory cascades and consequent tissue damage are the main causes of chronic obstructive pulmonary disease (COPD). There is no effective therapy and the key mediators of COPD are not identified due to the lack of translational animal models with complex characterization. This integrative chronic study investigated cardiopulmonary pathophysiological alterations and mechanisms with functional, morphological and biochemical techniques in a 6-month-long cigarette smoke exposure mouse model. Respiratory alterations characteristic of emphysema (decreased airway resistance: RI; end-expiratory work and pause: EEW, EEP; expiration time: Te; increased tidal mid-expiratory flow: EF50) were detected in anaesthetized C57BL/6 mice, unrestrained plethysmography did not show changes. Typical histopathological signs were peribronchial/perivascular (PB/PV) edema at month 1, neutrophil/macrophage infiltration at month 2, interstitial leukocyte accumulation at months 3-4, and emphysema/atelectasis at months 5-6. Emphysema was proven by micro-CT quantification. Leukocyte number in the bronchoalveolar lavage at month 2 and lung matrix metalloproteinases-2 and 9 (MMP-2/MMP-9) activities in months 5-6 significantly increased. Smoking triggered complex cytokine profile change in the lung with one characteristic inflammatory peak of C5a, interleukin-1 α and its receptor antagonist (IL-1 α , IL-1ra), monokine induced by gamma interferon (MIG), macrophage colony-stimulating factor (M-CSF), tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) at months 2-3, and another peak of interferon- γ (IFN- γ), IL-4, 7, 13, 17, 27 related to tissue destruction. Transient systolic and diastolic ventricular dysfunction developed after 1-2 months shown by significantly decreased ejection fraction (EF%) and deceleration time, respectively. These parameters together with the tricuspid annular plane systolic excursion (TAPSE) decreased again after 5-6 months. Soluble intercellular adhesion molecule-1 (sICAM-1) significantly increased in the heart at month 6, while other inflammatory cytokines were undetectable. This is the first study demonstrating smoking duration-dependent, complex cardiopulmonary alterations characteristic to COPD, in which inflammatory cytokine cascades and MMP-2/9 might be responsible for pulmonary destruction and sICAM-1 for heart dysfunction.

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Endogenous ace inhibition caused by cell free haemoglobin can lead to the development of hypotension after coronary artery bypass grafting

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BACKGROUND: Hypotension is a common intra-, and postoperative complication in patients undergoing coronary artery bypass graft (CABG) surgery. During prolonged cardiopulmonary bypass (CPB) red blood cells are frequently damaged causing intravascular haemolysis therefore increasing the level of circulating plasma haemoglobin. We hypothesized that cell free haemoglobin can decrease the activity of the angiotensin converting enzyme (ACE) thereby contributing to the reduction of systemic blood pressure during and after CPB.

PURPOSE: In this study, we examined the effects of free haemoglobin on ACE-activities of sera and recombinant ACE, as well as on tissue bound ACE using saphenous vein rings.

METHODS: Serum derived and recombinant ACE-activities were measured by a fluorescent kinetic assay. The inhibition of tissue ACE-activity was tested in unused saphenous vein rings (n=20) that remained after CABG surgeries (n=14) using an isometric myograph system.

RESULTS: We found that free haemoglobin decreases ACE activities in the serum and of recombinant ACE (IC₅₀= 1.7 nM; 1.3 nM, respectively) in vitro. The mechanism of haemoglobin dependent inhibition was identified by Lineweaver-Burk double reciprocal plot as a non-competitive inhibition (common X-axis intersection point). During saphenous vein experiments where contractile responses elicited by angiotensin I in the presence (n=10 rings) and absence of 0.5 g/L haemoglobin (n=10 rings) were followed, contractile force was significantly decreased in the haemoglobin treated group in contrast to the control group (relative strength compared to norepinephrine: 39.49±7.14%, 79.25±5.70%; P=0.0004, respectively). Significant differences between the kinetics of the contractions (control: 0.17±0.04mN/sec; 0.15±0.04 mN/sec, P=0.6849) and desensitizations (control: 5.10±1.04 µN/sec; 6.48±0.96 µN/sec, P=0.3445) were not found.

CONCLUSIONS: Our findings illustrate haemoglobin as non-competitive inhibitor of the ACE enzyme. In addition, we propose that free haemoglobin can suppress tissue ACE-activity thereby contributing to the development of hypotension during and after CABG surgery in association with haemolysis.

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Characterization of novel high-selectivity KV1.3 inhibitor peptide

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Ion channels expressed by T lymphocytes play key roles in the regulation of the membrane potential and calcium signalling. The physiological function of T lymphocytes can be modulated by blocking of Kv1.3 channels using peptide toxins. They have a significant therapeutic potential in the treatment of autoimmune diseases, so the discovery of new, selective toxins is highly motivated. Based on the importance of voltage-gated K⁺ channels in the cellular processes in many other cell types especially in excitable cells, therapeutic application of non-selective inhibitors have high potential risk of developing side effects.

Vm24 is a novel Kv1.3 specific peptide isolated from the venom of scorpion *Vaejovis mexicanus smithi*. The Vm24 inhibits Kv1.3 with high affinity ($K_d = 2.9\text{pM}$). However we examined several voltage-gated potassium channels, and hKCa3.1, mKv1.1, and hKv1.2 were partially blocked by the peptide at 10nM, whereas it was unaffected on other channels. A synthetic Vm24 analogue sVmKTx has been generated by amino acid substitution to improve the selectivity of Vm24 on Kv1.3.

Using whole-cell patch-clamp technique we tested the affinity of the sVmKTx in different concentrations on Kv1.3 ion channel. Kv1.3 currents were half-blocked by 770pM sVmKTx. In contrast, we could not observe significant effect of sVmKTx (100 nM) on currents of the following K⁺ channels: hKv1.1, hKv1.2, hKv1.4, hKv1.5, rKv2.1, hKCa3.1, and hKCa1.1 and Nav1.5.

Our results confirm the higher selectivity of the novel peptide toxin, sVmKTx on Kv1.3 as it was expected, but its affinity also decreased.

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Early inhibition of retinoic acid signalling upon myocardial infarction restores cardiac function and prevents cell, tissue, and animal death

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Aims: Retinoids are generally considered to be cardioprotective agents and inhibit remodelling of the adult heart when applied systemically. Physiologically, following myocardial infarction (MI) retinoid levels increase in the infarcted area. The role and function of these locally increased levels of retinoids in the MI zone are not known to this day.

Methods and Results: Using a human cell culture model for hypoxia and an MI model in rats, we analysed the impact of locally increased levels of all-trans retinoic acid (ATRA) on cell signalling, cell viability, tissue survival, heart function, and MI-induced death in rats. Moreover, we aimed to rescue the ATRA-dependent MI phenotype by the addition of 5'-methoxyleolin (5ML), a new cardioprotective and potentially retinoid signalling interfering compound. The results of this study show – for the first time – that locally increased levels of retinoids (ATRA) in the MI zone significantly worsen the outcome of MI in rats (2.5-fold increased mortality compared to control). On molecular level, ATRA signalling causes induction of TXNIP, a potent inhibitor of the physiological antioxidant thioredoxin (TRX1) and sensitizes cells to necrotic cell death upon hypoxia, which could be prevented by 5ML. Moreover, we found that 5ML reduced cellular ATRA uptake following a strong reduction of ATRA-dependent gene expression as well as a restoration of thioredoxin (TRX1) function. This was accompanied by reduced ROS formation and cell death. The cardioprotective effect of 5ML and molecular signalling were confirmed by our MI rat model in vivo, also suggesting that high local retinoid levels dramatically worsen the outcome of MI.

Conclusions: In summary, we provide important evidence, that physiologically and locally increased levels of retinoids in the infarction area worsen disease outcome and that a local reduction of retinoids effects using 5ML may constitute a novel treatment strategy.

Incidence, survival and determinants of mortality following mesenteric angiography and continuous intra-arterial prostaglandin E1 perfusion for non-occlusive mesenteric ischaemia in cardiac surgery

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Background: Non-occlusive mesenteric ischaemia (NOMI) is a rare but severe complication. Early recognition of NOMI is difficult due to its aspecific symptoms and the lack of specific laboratory parameters. Prevention, diagnosis and optimal therapy are under examinations.

Methods: Our aim was to evaluate the incidence of NOMI, to analyse results of treatment and to identify variables associated with mortality. Hospital records and clinical data of patients treated for NOMI during the last 17 years were reviewed. Clinical outcomes and factors influencing mortality were studied. Statistical analysis was performed to determine the significance of risk factors on mortality with Fisher exact test or χ^2 test in categorial variables. Wilcoxon rank test was used to test the continuous variables.

Results: Between 7/2001 and 1/2018 NOMI developed in 95 cases (0.5%). Mean age of patients was 68.6 ± 7.7 year. First symptoms occurred 2.5 ± 2.1 days after the operation. Mesenteric artery angiography was performed in 90 patients (95%). After confirmation of mesenteric vasospasm, a continuous infusion of $2.4 \mu\text{g/h}$ of prostaglandin E1 (PGE1) was administered in the superior mesenteric artery. The 30-day mortality of NOMI after PGE1 treatment was 61%. Logistic regression analysis of risk factors revealed preoperative NYHA stadium ($P < 0.02$), presence of internal carotid artery stenosis ($P < 0.05$), prolonged CPB and aortic cross clamp time ($P < 0.01$), postoperative arrhythmias ($P < 0.05$), reoperation ($P < 0.03$), use of arterenol and/or IABP ($P < 0.05$), serum lactate level ($P < 0.01$), length of ventilation ($P < 0.001$) and number of transfused red blood cell units ($P < 0.05$) were independent predictors of mortality.

Conclusions: Mesenteric ischaemia in cardiac surgery remains a devastating complication. A high index of suspicion with prompt diagnostic evaluation may reduce the delay prior to intervention. Early mesenteric angiography and intraarterial PGE1 infusion seems to be useful to improve survival.

TRPM4 inhibitor 9-phenanthrol blocks Na⁺ and K⁺ but not Ca²⁺ currents in canine ventricular myocytes

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Background: 9-phenanthrol has been used for suppression of TRPM4 channels in various cardiac preparations, however, the selectivity of the compound to these channels is uncertain. Therefore, the present study was designed to investigate the concentration-dependent effects of 9-phenanthrol on the action potential morphology and on the major cardiac ionic currents in enzymatically dispersed canine ventricular cells.

Methods and results: Ionic current measurements were carried out in the whole cell configuration of the patch-clamp technique, where the pipette solution contained 10 mM BAPTA in order to prevent the Ca²⁺-dependent activation of TRPM4 channels during the measurements. 9-phenanthrol (10 and 30 μM) significantly suppressed the transient outward K⁺ current, the rapid delayed rectifier K⁺ current and the inward rectifier K⁺ current with the blocking potency for $I_{K1} < I_{Kr} < I_{to1}$. These effects of 9-phenanthrol were partially reversible. L-type Ca²⁺ current was not affected by 9-phenanthrol up to the concentration of 30 μM. In addition to these effects, 9-phenanthrol induced a steady outward current at potentials positive to -40 mV. The amplitude of this current was larger at more positive voltages and increased with the concentration of 9-phenanthrol.

Action potentials were recorded using sharp microelectrodes. The maximal rate of depolarization, phase-1 repolarization and terminal repolarization were significantly decreased and the plateau potential was depressed by 9-phenanthrol (3-30 μM) without alteration in the resting membrane potential. These changes in action potential morphology are congruent with the observed alterations of ionic currents.

Summary: In conclusion, 9-phenanthrol is not selective to TRPM4 channels in canine ventricular myocardium, therefore its application as a TRPM4 blocker can be appropriate only in expression systems but not in native cardiac cells.

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Metabolomic and MALDI profiling of thoracic aortic aneurysms and dissections – Implications for pathophysiology and biomarker discovery

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Background: A deeper understanding of the pathogenesis of ascending thoracic aortic aneurysms (ATAA) and dissections is highly demanded for a better risk prediction, diagnosis and treatment. Modern clinical imaging cannot sufficiently predict the individual risk for dissection and ruptures; only disease progression can be observed. Metabolomic analyses offer an opportunity to increase our understanding of the ATAA and to find disease and progression markers.

Methods: Using a targeted FIA-MS/MS metabolomics approach, we analysed the metabolic profiles of ascending thoracic aortic wall tissue of age-matched controls (n=8), bicuspid aortic valve-associated aneurysms (BAV-A; n=9), tricuspid aortic valve-associated aneurysms (TAV-A; n=14), and tricuspid aortic valve-associated aortic dissections (TAV-Diss; n=6). All together, the metabolite concentration of 182 single metabolites from various substance groups (sphingomyelins, glycerophospholipids, ceramides, amino acids, acylcarnitines) was determined.

Further, matrix-assisted laser desorption/ionisation (MALDI) imaging was used to investigate metabolite distribution in 60µm diameter microdomains across unfixed cryostat sections of aortic wall tissue.

Results: Out of 182 analysed metabolites only 5 could be used as markers to differentiate between controls, ATAA and dissections. Interestingly, MALDI screening revealed that many metabolites are not distributed equally across the aortic wall; some occurring only in the tunica media or the tunica adventitia, other metabolites show a characteristic distribution pattern within the tunica media.

Conclusions: The analyses of aortic tissue revealed the usability of sphingolipids and glycerophospholipids for the identification and differentiation of ATAAs and dissections. MALDI imaging revealed that the aortas' tunica media is everything but a metabolically homogeneous structure, arguing for distinct functionally different sublayers within the aortic tunica media, which by means of classical histology seems to be a homogenous structure.

Reduction of cardiovascular risk with functional foods

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Angiotensin converting enzyme (ACE) inhibitors have the potential to reduce cardiovascular mortality by up to 40%. We have recently reported that ACE activity is endogenously inhibited by human serum albumin (HSA). We hypothesize that decreased endogenous ACE inhibition associates with development and progression of cardiovascular diseases. Here we aimed to identify and characterize factors influencing HSA-mediated endogenous ACE inhibition.

68 patients with documented cardiovascular diseases were involved into the study. Besides routine laboratory parameters, the extent of endogenous ACE inhibition, free fatty acid (FFA) concentration and composition were also measured.

The extent of endogenous ACE inhibition varied considerably among patients (62-83%, $n=68$), but it had no relation with albumin concentration in the samples ($r^2=0,149$). Hence we postulated, that other factors could also influence the extent of endogenous ACE inhibition. Removal of hydrophobic molecules from the surface of albumin significantly decreased the capacity of albumin to inhibit ACE activity (before treatment: $IC_{50}=9.91\pm1.0g/L$, $n=3$, after treatment: $IC_{50}=35.8\pm2.7g/L$, $n=3$, $p<0.001$). FFA concentrations were measured from the samples and we found no relationship between FFA concentration and the extent of endogenous ACE inhibition ($r^2=0.089$). We concluded that different types of FFAs may modify the endogenous ACE-inhibiting capacity of albumin to various extents. Treatment of FFA free HSA with different types of FFAs confirmed this hypothesis. Among the examined saturated and unsaturated FFAs, C18:3 cis,cis,cis, Δ 6,9,12 had the most potent ACE-inhibitor modifying effect ($IC_{50}=5.5\pm0.4g/L$), while the effect of C10:0 treated albumin ($IC_{50}=32.3\pm1.6 g/L$) was not different from that of FFA free albumin. In case of individuals with identical HSA concentrations, we measured higher degree of endogenous ACE-inhibition in those samples containing higher concentration of FFAs with high ACE-inhibition modifying effect.

Our results revealed that FFA-binding to albumin is essential for endogenous ACE inhibition. There are several FFAs, which are able to increase significantly the ACE-inhibitory effect of HSA. Therefore, intake of these FFAs via functional foods may decrease cardiovascular risk and delay the development of cardiovascular diseases.

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Heme induces endoplasmic reticulum stress in human aortic smooth muscle cells

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Introduction. During their physiological synthesis, the majority of proteins are folded into their native conformations and submitted to further posttranslational modifications in the endoplasmic reticulum (ER). If this process is inhibited or the demand for folding exceeds the capacity of the ER, unfolded protein response pathways are activated provoking ER stress. In pathologic hemolytic conditions, extracellular free hemoglobin is submitted to rapid oxidation which leads to heme release and diffusion to organs. The amphipathic heme is easily taken up by cells and tissues which sensitize them against oxidative injury. Therefore, we raised the question whether there is a direct or indirect relationship between ER stress and heme injury.

Methods. Vascular smooth muscle cells are one of the key players of atherogenesis, therefore it was selected as a model cell to prove the link and cooperation between ER stress and heme injury. Protein and mRNA markers involved in ER stress (phosphorylated eIF2 α /ATF4/CHOP, XBP1, ATF6, GRP78) and heme stress (heme oxygenase-1, ferritin) were quantified via Western blot, real-time polymerase chain reaction and immunocytochemistry on cell cultures, and with immunohistochemistry on human vessel wall samples.

Results. Our results demonstrated that heme can induce not only heme stress but also ER stress as well in a time and dose-dependent fashion. The most important evidence to prove that interaction is that heme scavenger proteins alpha-1-microglobulin (A1M) and hemopexin (Hpx) inhibited ER stress and markedly reduced heme stress as well. Consistent with these in vitro findings, elevated expression of the ER stress marker protein grp78 was observed in atheromas and complicated lesions compared to healthy aortas.

Discussion. In conclusion, it is highly plausible that heme triggers ER stress in a time and dose-dependent manner in aortic smooth muscle cells. A1M and Hpx hampered heme and ER stress, revealing a potential novel therapeutic approach to reverse the deleterious effects of heme.

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Tenascin C role in ischemia/reperfusion injury, and chronic hypertension – a potential miRNA targeting approach in an in vitro and in vivo rat model

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Background: Tenascin C (TN-C) upregulation following myocardial infarction (MI) and chronic pressure overload is associated with an increase in myocardial fibrosis and impaired left ventricular (LV) function. However, the mechanisms for TN-C upregulation and the impact of hypoxic and hypertrophic stimuli on TN-C expression have not been investigated yet.

Purpose: Therefore, we aimed to 1) clarify the effect of hypoxia and hypertrophic agents on TN-C and mir-335 expression, as a potential regulator of TN-C in H9c2 cardiomyoblasts; 2) investigate TN-C role on rats subjected to MI.

Methods: H9c2 rat cardiomyoblasts were incubated 1) for 0-24 hours with glucose and oxygen deprivation (OGD); 2) with 1 μ M Angiotensin II; and 3) with 1 and 10 μ g/mL of human TN-C. Viability was assessed by annexin V/PI staining and FACS analysis. RT-qPCR was performed to assess the expression of hypertrophic, fibrotic markers and mir-335. In addition, ELISA quantified TN-C in hypoxia treated cells and rat plasma. OFA-1 male rats were subjected to MI by ligation of the left anterior descending artery and transthoracic echocardiography was used to assess LV function prior to and 7 days after MI.

Results: Tnc mRNA expression was markedly increased by hypoxia and hypertrophic agents compared to controls ($p < 0.05$). Cell viability was substantially decreased after 16 hours of OGD, registering the highest TN-C protein expression. Accordingly, TN-C mRNA expression was markedly increased following 6 hours OGD ($p < 0.05$). Moreover, prolonged periods of hypoxia dysregulated the expression of mir-335, being inversely regulated to TN-C formation. Furthermore, the administration of human TN-C significantly increased the expression of BNP, Mmp2 and β -MHC after 48h ($p < 0.05$) as well as time and dose dependently modified the expression of integrin $\alpha 6$ and integrin $\beta 1$. Finally, seven days after MI, ejection fraction was declined, associated with a marked increase of plasma TN-C expression ($p < 0.0001$).

Conclusions: We first time demonstrated that hypoxic and hypertrophic stimuli markedly increased TN-C expression in cardiomyoblasts, which showed inverse regulation with mir-335 expression. These results indicate that targeting TN-C by mir-335 might provide a novel therapeutic approach to improve cardiac function following myocardial infarction and chronic pressure overload.

Patient-specific 3D-Printing of cardiovascular anatomies for in-vitro testing

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Background & Aims: With the increasing range of indications for the TAVI procedure, the prevention of postoperative complications, for instance paravalvular leakage and AV-blockage, is gaining importance. We tried to establish an in-vitro TAVI testing system using patient specific data and 3D-printing technology.

Methods : Anonymized pre-operative CT-data of 101 TAVR patients were analyzed. The datasets were evaluated and classified, based on the severity and distribution of calcification in the aortic valve. To create 3D-printable models, the aortic root, leaflets including calcifications, and the lower part of the ascending aorta were extracted using thresholds based on Hounsfield-units. To ensure laminar flow conditions, the in- and outflow tract of the volume models were adapted to fit the mount of the bioreactor. The finalized models were 3D-printed using liquid photopolymers and afterwards tested manually and in an in-house developed bioreactor.

Results: During characterization, it was possible to determine six distinct groups with different calcification volume and distribution patterns. Furthermore, the data analysis showed a correlation between calcification distribution and the occurrence of AV-blocks. Based on these results, datasets were selected for further processing and manufacturing. The accordance of the models was analyzed by overlapping the 3D-data of the model with the underlying CT-Data. The manufacturing process showed to have no negative impact on the models level of detail. During in-vitro testing, the models displayed no structural weakness, creating an environment mimicking physiological conditions.

Conclusion: The introduction of 3D-printing offers great potential to analyze pre-interventional complication risks. While showing great accordance to the underlying CT-data, the models can further be adapted to the mechanical properties of aortic tissue by choosing different material combinations.

The Metabolic Road of Co-morbidities to Understanding the Pathophysiology of Heart Failure: The Role of Inflammatory Signaling Pathways in Obesity and Diabetes

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The development of effective treatments for heart failure (HF) with left ventricle (LV) diastolic dysfunction (DD) is currently limited by poor understanding of the underlying pathophysiology. Abnormal diastolic LV function with impaired relaxation and increased diastolic stiffness is characteristic of HF with preserved ejection fraction; (HFpEF). HFpEF accounts for more than 50% of all cases of HF in Western societies and is closely associated with co-morbidities and gender. To date, all large multicentre trials of HFpEF treatments have produced disappointing results. This outcome suggests that a “one size fits all” approach to HFpEF may be inappropriate and supports the use of tailored, personalized therapeutic strategies with specific treatments for distinct HFpEF phenotypes. Recent evidence suggests that co-morbidities common to HFpEF promote a systemic inflammatory state that contributes to endothelial dysfunction, cardiomyocyte dysfunction, altered extracellular matrix, reactive oxygen species production, nitrosative stress, and which affect the pathophysiology of HFpEF by modulation of LV stiffness, at least partly the giant protein titin and extracellular matrix. Titin isoform transitions and post-translational modifications such as phosphorylation and oxidation are major modulators of titin-based stiffness and contribute to diastolic stiffness. We think our recent findings may help to assess specific treatment strategies in an attempt to develop tailored HFpEF therapy.

Totally percutaneous model with echoguidance of ischemic mitral regurgitation in the pig

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Background: Development of translational animal models of cardiovascular disease is crucial to understand the disease mechanism and pathophysiology and provide a unique platform to test novel therapies and devices. The European heart survey showed that 49% of patients with severe symptomatic mitral regurgitation were denied surgery. This patient population was characterized by one particularly recurrent parameter: Secondary mitral regurgitation. Surgical treatment of secondary mitral regurgitation remains a subject of controversy and still doesn't show a clear impact on the mortality. In addition, there is unmet need to establish less invasive approaches in patients with secondary mitral regurgitation. **Aims:** therefore, the aim of the present study was to establish a clinically reliable large animal model of mitral valve regurgitation.

Methods: Young female domestic pigs were used for this model establishment (n=9). The induction of mitral valve regurgitation was performed by localized posteromedial papillary muscle (PMPM) myocardial infarction. The PMPM irrigating branches are first identified by selectively injecting contrast media in the circumflex branches while performing echocardiography. Then a 2ml of pure Ethanol are injected in the identified branches. The evaluation of the mitral valve regurgitation and cardiac function was assessed by echocardiography.

Results: 7 pigs survived during the 6 weeks follow up period. One pig was euthanized after 3 weeks and another after 2 because of refractory pulmonary edema. Ethanol injection resulted in postero-inferior wall and PMPM dyskinesia. Significant left ventricle enlargement was noticed (End diastolic diameter at baseline: $50,04 \pm 4.34\text{mm}$ vs at 6 weeks $62,12 \pm 3.92\text{mm}$; $p<0.001$) as well as left atrium enlargement (left atrium area at baseline: $7.75 \pm 0.95\text{cm}^2$ vs at 6 weeks $17.65 \pm 3.2\text{cm}^2$; $p<0.001$). Mitral regurgitation jet area significantly increased over the 2 weeks follow up period (jet area at baseline $0.03 \pm 0.015\text{ cm}^2$ vs at 6 weeks $3.22 \pm 0.53\text{cm}^2$). A significant tenting area developed over the follow up period (Tenting area at baseline $0.35 \pm 0.21\text{cm}^2$ vs $2.17 \pm 0.63\text{cm}^2$ at 6weeks; $p<0.001$)

Conclusion: Our results clearly provided significant evidence about a totally percutaneous clinical relevant model of ischemic mitral valve regurgitation in pigs.

Computer assisted decision making in cardiac surgery: from 3D preoperative planning to computational fluid dynamics in the design of surgical procedures

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Although the surgical specialties utilized static model but by the evolution of dynamical planning method and practical usage of computer simulations created the possibility of introduction of dynamical parameters in cardiac surgery.

Our aim was the amelioration and application of 3D models in cardiac surgical practice wherewith the prediction of fluid dynamical variables and remaining ventricle shape, volume and function in surgical ventricle restoration cases.

Using own developed script, the raw Dicom files were imported, the dilated left ventricle was modeled and fluid dynamical parameters simulated, such as flow kinematic and profile analysis, turbulence calculation and myocardial response to shear stress. Then step-by-step simulation of the surgical ventricle restoration procedure was accomplished and the calculated variables were imbedded in silico model of the left ventricle reconstruction. The extension and length of resection lines were modified based on the previous computer simulation. Optimal resection of the myocardium was applied during the operation, considering the all feasibility.

The sphericity and conicity indexes were improved significantly in postoperative period (0,42vs. 0,67 és 0,36vs. 0,72, $p < 0,05$, Student t-test). The occurred shear stress at endocardium decreased 83% due to the normalization of flow kinematic pattern of the ventricle in postoperative period (54 ± 12 vs. 32 ± 9 $p < 0,02$, Student t-test). The postoperative turbulent flow pattern - based on Reynolds number - significantly decreased, according to our computational method (2712vs. 1823, $p < 0,0001$, Student t-test).

With our method, the standardization of the surgical ventricle reconstruction was achievable and the surgical steps were predictable. Therefore, a new decision making support system was established in cardiac surgery for high risk patients. Consequently, a personalized surgical technique was offered our patients, improving their life expectancy and quality of life.

High intensity interval training in cardiac rehabilitation: A randomized controlled trial investigating platelet function

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QUESTIONS: Exercise training is a cornerstone of cardiac rehabilitation (CR) programs. However, the exercise-intensity eliciting maximal beneficial adaptations remains controversial. Since platelets play a key role in atherosclerosis, the aim of this study was to compare effects of high-intensity interval training (HIT) with moderate-intensity continuous training (MCT) on platelet function.

METHODS: At the beginning of CR, patients with coronary artery disease were randomized to either HIT or MCT performed on bicycle ergometers, with identical net energy expenditure. Both groups performed 4 training sessions per week over a period of 12 weeks. Maximal oxygen consumption ($\dot{V}O_2\text{max}$) and parameters of platelet function were assessed before training, after 6 and 12 weeks. Primary endpoint was platelet reactivity measured as the half-maximal effective dose (EC_{50} in μM) of platelet agonist TRAP-6 in terms of P-selectin expression after 6 weeks of training, quantified by flow cytometry.

RESULTS: 70 patients were randomized to HIT or MCT. There were no significant baseline differences between groups regarding $\dot{V}O_2\text{max}$ (HIT vs. MCT: 22.9 vs. 23.1 ml/min/kg, $p > 0.5$) or platelet reactivity (6.59 vs. 6.63 μM , $p > 0.5$). The overall increase of $\dot{V}O_2\text{max}$ after 6 weeks was 2.5 ml/min/kg ($p < 0.0001$) without any group differences ($p > 0.5$). However, HIT had greater effects on parameters of platelet function than MCT, including the primary endpoint: The EC_{50} of TRAP-6 (P-selectin expression) was higher after 6 weeks of HIT (7.60 vs. 6.74 μM , $p < 0.01$), indicating lower platelet reactivity in response to HIT compared to MCT.

CONCLUSIONS: HIT seems to be more effective than MCT in reducing platelet reactivity in patients undergoing cardiac rehabilitation.

Omecamtiv mecarbil causes the alternation of myocardial excitation-contraction coupling at high pacing frequencies

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Background: Omecamtiv mecarbil (OM) is a myosin activator agent developed for the treatment of heart failure. OM was reported to increase left ventricular ejection fraction and systolic ejection time, but little is known about the effect of heart rate on the action of OM. On the cellular level the present study was designed to investigate the frequency-dependent effects of OM on the elements of excitation-contraction coupling of the left ventricle (LV): action potential (AP) morphology, intracellular calcium transients (CaT), and unloaded cell shortening. To test the in vivo effects of OM on LV function at high heart rates, rats were chosen as experimental models, having resting heart rates around 350-450 beats per minute.

Methods: During our in vitro experiments, effects of 1 μ M OM were tested on isolated canine LV cardiomyocytes at pacing frequencies of 1-5 Hz. APs were recorded through an intracellular microelectrode, cell length was monitored by an optical edge detector and CaTs were visualized using Fura-2. In vivo effects of OM on LV function of Wistar-Kyoto rats were assessed by echocardiography. OM was administered through a jugular venous canule in increasing doses between 200-1200 μ g/kg.

Results: 1 μ M OM did not change the overall AP configuration or the average CaT. OM however significantly reduced both diastolic and systolic cell lengths, increased fractional cell shortening and increased the overall time of contraction. Accordingly, OM improved LV systolic function (ejection fraction and dP/dtmax), but impaired diastolic function (decreased E/A ratio and increased isovolumetric relaxation time).

With increasing stimulation frequency, alternating APs, CaTs and cell shortening could be seen in 1 μ M OM. Such behavior could not be observed in the absence of OM at any stimulation rates, or even in the presence of OM at low pacing rates (1 Hz and 2 Hz). In line with these observations, echocardiography revealed that 1200 μ g/kg OM evoked alternating LV contractions in the majority of cases: every effective contraction was followed by an ineffective one.

Conclusions: Our results suggest that supratherapeutic concentrations of OM may impair the overall LV function especially in tachycardic patients.

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Sterilization of bovine pericardium – A big challenge for clinical application

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Objectives: Decellularized materials offer great potential in the field of regenerative medicine. Next to ensuring total cell removal, providing a sterile product is essential. Thus, the aim of this study was to assess different sterilization methods for decellularized pericardium.

Methods: Decellularization of bovine pericardium was performed with SD/SDS 0.5% in PBS for 16h. We evaluated five different chemical (ethanol 70%, 30min; peracetic acid 0.1%, 3h; octenidine dihydrochloride 0.1%, 1h; benzalkonium chloride 2%, 15min; povidone iodine 7.5%, 10min), one biological (10.000 U/ml Penicillin G + 10mg/ml Streptomycin + 25mg/ml Amphotericin B, 24h) and two physical (UV-exposure 15W for 3h/side; Gamma-irradiation, 3kGy) sterilization methods. Scanning electron microscopy, tensile testing and histology (H&E, DAPI, Pentachrome and Picrosirius Red staining) were used for assessment. Sterilization effectiveness was validated by incubation of treated materials with two different culture mediums (Thioglycollate Medium, Trypticase Soy Broth) for seven days. Exposure of the sterilization mediums (three dilutions) to endothelial cells for 24h, 48h and 72h was used to evaluate cytotoxicity of the chemicals. According to the obtained results we tested two combinations of octenidine dihydrochloride and peracetic acid. Treatment was modified in order to minimize detrimental effects and to ensure sterility.

Results: Preservation of collagenous and elastic fibres after sterilization could be proved by histological staining. DAPI and H&E validated successful decellularization except some stained particles in the center of a few samples. Specimen treated with povidone iodine and antibiotic-antimycotic solution showed comparable tensile-strength to decellularized pericardium. Sterilization methods using octenidine dihydrochloride, povidone iodine or antibiotic-antimycotic solution caused no observable damage. However sterilization effectiveness of these methods was insufficient. Benzalkonium chloride preserved the pericardial structure and sterilized effectively but was proven toxic to endothelial cells. Ethanol, peracetic acid and the physical methods showed detrimental effects on the surface morphology and tensile strength. Supplemental combination methods ensured sterility while maintaining samples ultrastructure and biomechanical strength.

Conclusion: Individually, none of the evaluated sterilization methods led to optimal results. Therefore we recommend a combination of octenidine dihydrochloride (0.5%, 60min) and peracetic acid (0.1%, 60min) to achieve sterility and preserve samples structure.

Cardioprotection by remote ischemic conditioning and its novel signalling mechanisms

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Cardioprotective strategies aim to salvage myocardium from ischemia/reperfusion (IR) injury and to reduce infarct size as well its consequences such as the development of heart failure. There is large body of evidence that short episodes of IR insult at sites remote from the heart initiates cardioprotection on the heart in a setting of myocardial ischemia/reperfusion insult. This phenomenon called ischemic remote conditioning. The efficacy of remote conditioning of the heart has been established in preclinical studies, although recent large clinical trials provided conflicting results and failed to demonstrate the beneficial effect of remote ischemic preconditioning in patients undergoing elective cardiac surgery. More recently, it has been demonstrated that repeated remote ischemic conditioning over a number of days has the potential to augment the protective process to improve cardiac and vascular function in patients with heart failure. However, the underlying cardioprotective mechanisms are not fully understood. This study focuses on the current evidence base on four levels of remote and repeated remote conditioning and its resulting profound cardioprotection: 1) remote ischemic conditioning for improvement myocardial protection in a setting of acute myocardial infarction and elective cardiac surgery, 2) the effectiveness of repeated remote conditioning on infarcted myocardium and in heart failure in different animal models and patients and 3) novel mediators and signalling mechanism from the cardioprotection by repeated remote conditioning. Nevertheless, further elaborated studies are warranted to precise mechanical understanding of the cardioprotection by remote condition and translate more successfully to patients with various cardiovascular disease.

Contribution of late sodium current to the electrophysiological characteristics of canine left ventricular cardiomyocytes

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Background: The late sodium current $I(\text{Na,L})$ plays pathophysiological role in several heart diseases. Despite this fact, the contribution of $I(\text{Na,L})$ to the cardiac ventricular action potential (AP) has not yet been characterized under physiological circumstances. We wanted to visualize $I(\text{Na,L})$ during a canine ventricular AP and to determine the effect of $I(\text{Na,L})$ blockade on the AP shape and on the short term variability of the AP duration (APD).

Methods: Experiments were performed in isolated canine left ventricular myocytes. We used the AP voltage clamp technique to visualize $I(\text{Na,L})$ during the AP by 1 μM GS-458967 (GS). Conventional microelectrode technique was used to determine the effects of $I(\text{Na,late})$ blockade on AP morphology.

Results: Under the AP, the GS sensitive current showed a peak at the AP upstroke (likely because of early sodium current ($I(\text{Na,E})$) inhibition), and then showed a sustained, gradually decreasing current due to $I(\text{Na,L})$.

At 1 s pacing cycle length (PCL) GS reduced the maximal rate of depolarization (v_{max}) by 35% and AP amplitude (APA) by 15%. These effects are likely due to the small $I(\text{Na,E})$ blocking effect of GS. GS shortened the APD by 14% measured at 90% of repolarization and depressed the mid-plateau membrane potential by 6 mV. GS decreased short term APD variability by 18%. At shorter PCL, effects of GS on APA and v_{max} were more pronounced, indicating a stronger $I(\text{Na,E})$ blockade under these conditions. Effects of GS on APD, mid-plateau potential and short term APD variability were less prominent with shorter PCL.

Conclusion: In our experiments the profile of $I(\text{Na,L})$ was successfully recorded under physiological conditions. The blockade of $I(\text{Na,L})$ has been shown to affect AP morphology and APD short term variability. Based on these results, $I(\text{Na,L})$ significantly contributes to the electrophysiological characteristics of cardiac ventricular myocytes.

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Cardiovascular effects of the novel myosin activator omecamtiv mecarbil: positive inotropy and beyond

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Background: The cardiac myosin activator omecamtiv mecarbil (OM) improves the contractility of the failing heart via increased number of acto-myosin interactions and prolonged systole. These promising cardiotonic effects of OM have been reported in preclinical and phase I/II clinical investigations. However, there is limited preclinical information on OM.

Purpose: Here we investigated the alternative cardiac mechanisms of action of OM beyond positive inotropy.

Methods: In vivo effects of OM were studied on Wistar-Kyoto rats by left ventricular (LV) hemodynamic analysis (n=10), echocardiography (n=10) and blood pressure measurement by carotid artery catheterization (n=10). OM was administered through a jugular venous canule in increasing doses from baseline (BASE) up to 200, 600 and 1200 µg/kg cumulative doses. In vitro effects of 0.1 and 1 µM OM were tested on isolated LV myocytes. Action potential characteristics, calcium transients and unloaded cell shortening were studied on isolated canine (n=9), and force generation was studied on human donor (n=9) cells.

Results: LV systolic function was improved upon treatment with OM (ejection fraction, %: BASE: 70±2; 200 µg/kg: 72±4; 600 µg/kg: 78±5; 1200 µg/kg: 82±8; and dP/dtmax, mmHg/s: BASE: 9102±597; 200 µg/kg: 9633±533; 600 µg/kg: 10683±594; 1200 µg/kg: 11055±437). At the same time, we found dose-dependent deleterious effects of OM on diastolic function: decrease of E/A ratio (BASE: 2.0±0.1; 200 µg/kg: 1.7±0.1; 600 µg/kg: 1.4±0.1; 1200 µg/kg: non measurable) and dP/dtmin (mmHg/s: BASE: -11622±607; 200 µg/kg: -10548±710; 600 µg/kg: -10704±810; 1200 µg/kg: -9751±679), and increase of isovolumetric relaxation time (ms: BASE: 22.3±1.3; 200 µg/kg: 31.7±2.3; 600 µg/kg: 35.1±1.8; 1200 µg/kg: 50.7±4.2) and tau (ms: BASE: 9.2±0.4; 200 µg/kg: 10.4±0.5; 600 µg/kg: 12.6±0.1; 1200 µg/kg: 15.4±0.7). These results are in accordance with the dose-dependent effects of OM on skinned myocytes enhancing not only Ca²⁺-dependent active force, but Ca²⁺ sensitivity (pCa₅₀) and Ca²⁺-independent passive force of the contractile machinery. In vivo at 1200 µg/kg OM evoked an electromechanical dissociation in 76.6% of cases (n=23 of total 30): with continuous ECG every effective contraction was followed by an ineffective one. In line with this phenomenon, at the myocyte level, we found alternating action potential, calcium transient and cell shortening upon administration of 1 µM OM at high pacing frequencies (4 and 5 Hz). Finally, the above mentioned cardiac alterations at high (1200 µg/kg) dose led to a significant decrease of blood pressure (vs. BASE: systolic: -59±9%; diastolic: -63±11%).

Conclusion: OM improves LV systolic function, but induces diastolic dysfunction even at low doses in the rat. In addition, OM can evoke periodical electromechanical dissociation beyond myosin sensitization.

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Improved diastolic function through reduced oxidative stress and cardiomyocyte titin-based stiffness due to chronic stimulation of soluble guanylyl cyclase in heart failure with preserved ejection fraction

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Background: Heart failure (HF) with preserved ejection fraction (pEF) is characterized by diastolic dysfunction, increased myocardial stiffness and high oxidative stress.

Purpose: A hypothetical benefit of stimulated nitric oxide/soluble guanylyl cyclase/cyclic guanosine monophosphate/protein kinase G (NO/sGC/cGMP/PKG) signalling was investigated in a HFpEF model.

Methods: Chronic sGC stimulation was studied on 15-week-old male Dahl/Salt Sensitive rats. Age-matched male SS-13^{BN} rats served as controls (CTRL). Rats were randomized in 4 groups (n=11-12/group): HFpEF; CTRL; and both HFpEF and CTRL treated with 1.5 mg/kg/day BAY 41-8543 via drinking water for 4 weeks.

Results: Left ventricular (LV) diastolic dysfunction was found in HFpEF but not in CTRL animals (E/A: 1.17±0.04 vs. 1.81±0.11; IVRT: 35.4±1.8 ms vs. 28.0±0.9 ms; Tau: 11.8±0.6 ms vs. 9.7±0.4 ms, respectively). Diastolic dysfunction improved upon BAY 41-8543 treatment (1.59±0.06; 26.6±1.2 ms; 9.3±0.4 ms, respectively). HFpEF but not CTRL rats showed a high LV end-diastolic pressure (11.9±2.9 mmHg vs. 4.2±0.6 mmHg) and an upward and leftward shift of the LV end-diastolic pressure-volume relationship, indicative of LV diastolic dysfunction. This latter was also improved upon treatment with BAY 41-8543. Arterial elastance was increased in HFpEF rats, and reduced upon treatment with BAY 41-8543. The above results were in accordance with *ex vivo* vascular experiments showing arterial stiffening and endothelial dysfunction in the HFpEF group, which was corrected after BAY 41-8543 treatment. High oxidative stress level and inflammation were reduced after BAY 41-8543 treatment, which in turn could correct the low NO level observed in HFpEF rats. This improvement resulted in increased cGMP concentration and PKG activity in HFpEF rats after BAY 41-8543 treatment. Total and PKG-mediated site-specific Ser-4080 hypophosphorylation of titin in HFpEF animals (relative to CTRL: ~41% and ~33%, respectively) were greatly improved by BAY 41-8543 treatment (~182% and ~210%, respectively). Accordingly, HFpEF cardiomyocytes showed increased stiffness, which was reduced upon BAY 41-8543 treatment.

Conclusion: Chronic stimulation of the NO/sGC/cGMP/PKG signalling pathway improved diastolic function via restored endothelial function, and reduced oxidative stress and cardiomyocyte titin-based stiffness. Our data suggest that chronic stimulation of the cardiovascular sGC may be a promising treatment option for HFpEF patients.

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Role of calcium/calmodulin-dependent protein kinase II activation in beta-adrenergic stimulation of potassium currents in canine ventricular cardiomyocytes under action potential clamp conditions

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Introduction and purpose: Acute β -adrenergic receptor (β -AR) stimulation shortens the ventricular action potential (AP). This effect is mainly regulated by the β -adrenergic stimulation of the cardiac potassium currents. Our aim was to investigate the extent of calcium/calmodulin-dependent protein kinase (CaMKII) involvement in mediating the effect of β -AR activation on the most important potassium currents.

Methods: We carried out our experiments on isolated cardiomyocytes originating from canine left ventricles. 4 potassium currents (rapid and slow delayed rectifier potassium current – I_{Kr} , I_{Ks} ; transient outward current – I_{to} and inward rectifier potassium current – I_{K1}) were measured under a “canonical” AP under action potential voltage clamp conditions. Data were collected in four study groups: [1] Tyrode solution (CTRL), [2] after beta-adrenergic stimulation with 10 nM isoproterenol (ISO), [3] inhibition of CaMKII with 1 μ M KN-93 (KN-93), [4] after beta-adrenergic stimulation with inhibited CaMKII (KN-93+ISO).

Results: Neither I_{to} nor I_{Kr} differed significantly in the four groups studied. I_{Ks} was prominently larger in ISO than under CTRL or KN-93 conditions having an about 6-fold larger current amplitude and carrying about 8 times as much total charge. In the KN-93+ISO group, I_{Ks} showed an about 2.5 times smaller amplitude and carried roughly half as much total charge compared to the ISO group.

I_{K1} current amplitude did not differ among the studied groups, the total carried charge however was significantly, about 25 % larger in the ISO group compared to CTRL, and about 15 % larger compared to KN-93+ISO. Under beta-adrenergic stimulation, I_{K1} starts to activate earlier during the AP plateau. I_{K1} density was about 3 times greater both at +20 mV and at 0 mV membrane potential under the command “canonical” AP in ISO compared to CTRL. Similarly, I_{K1} density was about 60 % and 90 % larger at +20 mV and at 0 mV, respectively, in KN-93+ISO compared to KN-93.

Conclusion: Based on the results of our researches the CaMKII activation plays an important role in β -adrenergic stimulation of I_{Ks} and I_{K1} potassium currents.

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Challenging procedural parameters in pericardial decellularization – The influence of pre-processing storage and treatment temperature

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Objectives: Decellularized bovine pericardium is a highly promising tissue engineered scaffold in the field of reconstructive heart surgery. Decellularization (DC) protocols include different physical, chemical and enzymatic factors. Although there are several published papers concerning the optimization of decellularization protocols, this study is first in investigating the influence of temperature, as a physical factor, on DC efficiency of bovine pericardium.

Methods: An established DC procedure for bovine pericardia, based on a stream induced whirling in a solution of 0.5% SD and 0.5% SDS for 16 hours was applied. Six groups (n=5) were treated at different thermal conditions (4°C, 13°C, 21°C, 29°C, 37°C, 56°C). Due to the lack of automatized decellularization processes and time-consuming protocols it may be unavoidable to start the DC a day after slaughtering. Thus, the influence of storage of the pericardia for 24h at 4°C before DC was additionally investigated and compared to the directly treated group. The efficiency of cell removal was analyzed by DAPI as well as H&E staining. Scanning electron microscopy, pentachrome- and pikrosirius-red staining as well as tensile tests were used for a comprehensive analysis of the specimens.

Results: Results showed a high correlation between successful DC and the temperature of the DC detergent. Successful DC is defined as total cell removal in combination with preserved structural integrity and high ultimate tensile strength (UTS). The correlation displays a parabolic pattern, with best results achieved at 21°C. Higher as well as lower temperatures lead to a decreased efficiency in DC. In addition, more damage to the structural integrity of the ECM was observed via SEM analysis as well as via tensile testing. Furthermore, a loss of collagen fibers and a loss of the collagen structure with increasing temperatures was detected. Storage of the pericardia before DC had neither positive nor negative effects in regard to cell removal but negatively influenced the UTS.

Conclusion: It could be shown, that the applied DC process is most effective at 21°C if ECM integrity is also taken into account. The decrease of UTS by pre-processing storage showed the importance of smart and conscious handling of biological tissue.

In conclusion, it has become evident that thermodynamics have a significant influence on DC and have to be carefully considered in all DC procedures.

Effects of BGP-15 on the contractility of human right atrial myocardium

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Cardiovascular (CV) mortality is high worldwide carrying great socioeconomic burden. Improper lifestyle choices leading to stress and evolution of CV risk behavior (smoking, sedentary lifestyle, improper diet and excessive alcohol use) result in the impairment of the CV system. Beyond prevention, therapy of CV diseases must be addressed, opening new avenues for the development of new therapeutic agents that are more efficacious and/or have limited adverse effects. BGP-15 (O-(3-piperidino-2-hydroxy-1-propyl)nicotinic acid amidoxime; N-Gene Ltd) has known cytoprotective and insulin sensitizing effects in humans, and was shown to be well-tolerated. Nevertheless, BGP-15 has a significant structural similarity with the classic β -blocker propranolol, hence it may affect the CV system.

In the current *ex-vivo* study, we evaluated the effect of BGP-15 on the contractility of human atrium, with samples taken from open heart surgeries. Samples were pre-stimulated with isoproterenol (administered at its EC₅₀). Propranolol was used for control.

Our results show that the effect of BGP-15 at low concentration (< 10 μ mol/l) was not significant in the isoproterenol-stimulated samples, while it elicited substantial negative inotropy at higher concentration (> 1 mmol/l). In the intermediate range (10 μ mol/l<<1 mmol/l), effect of BGP-15 depended on the atrial responsiveness to isoproterenol. Only samples exhibiting strong positive inotropic response to isoproterenol produced considerable negative inotropic response to BGP-15. In contrast, propranolol's action was not significantly influenced by the β -adrenergic responsiveness of the atria. The maximal effect (E_{max}) was similar for BGP-15 and propranolol, while potency was smaller for BGP-15 than for propranolol (EC₅₀ of BGP-15 was greater).

These findings show that BGP-15 has rather indirect than direct negative inotropic effect, which develops only at higher concentrations. Furthermore, regarding the indirect negative inotropic effect, BGP-15 is less potent than propranolol, whereas they have similar efficacy.

Nitric oxide administration to the gas inflow of oxygenator improve cerebral perfusion and neuroprotection during ECMO

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Introduction: The haemolytic product free-hemoglobin reduces nitric oxide (NO) bioavailability during extracorporeal membrane oxygenation (ECMO) and may be a possible factor in the pathogenesis of impaired cerebral blood flow and oxidative stress in cerebral damage.

This study was conducted to investigate whether NO administration in the ECMO circuit can improve neuroprotection during ECMO support.

Methods: Venoarterial ECMO was instituted for 60 minute in male Sprague-Dawley adult rats (450-550g), after 10 minutes of cardiac arrest. They were randomised to receive 20 ppm NO to the gas inflow of the ECMO oxygenator or standard conduct of ECMO.

Results: Inflammatory response measured with IL-1, IL-18 and TNF- α through Western blot was decreased in NO treated rats. Oxidative stress expressed with Reactive Oxygen Species production and Malondialdehyde was reduced with NO 20 ppm administered through the oxygenator. Furthermore pro-Survival pathways were activated and apoptosis was reduced in NO treated rats. Some rats underwent MRI and an enhancement of cerebral blood flow and reduction of cerebral edema was evident in treated rat.

Conclusion: In conclusion NO administered after cardiac arrest during reperfusion through ECMO can attenuate cerebral perfusion impairment, reduce neuronal damage and possibly could ameliorate neurologic outcome.

Prevention of ultrastructural myocardial injury in a rat model of doxorubicin induced cardiomyopathy

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Background: Doxorubicin (DOX) induced adverse myocardial remodeling and corresponding ultrastructural changes have been well characterized by electron microscopy. These chemotherapy induced subcellular changes introduce irreversible myocardial injury leading to a reduced left ventricular ejection fraction. If untreated, the left ventricular dysfunction may progress to heart failure compromising the patients' quality of life and clinical outcome.

Purpose: We set out to investigate whether a prophylactic or a conventionally scheduled heart failure therapy is more effective in preventing the DOX induced ultrastructural injury using a rodent model of doxorubicin induced cardiomyopathy.

Methods: 12-week-old male Wistar rats were divided into 4 subgroups: negative controls receiving intravenous (iv.) saline (CON), positive controls receiving iv. DOX (6 times; D-CON), and DOX treated animals receiving either prophylactic (PRE, started 1 week before DOX exposure) or conventionally applied (POST, started 1 month after DOX exposure) combined heart failure treatment of bisoprolol, perindopril and eplerenone. Following in vivo echocardiographic investigations, at 2 months the animals were sacrificed, their hearts were excised and deep frozen. A small piece of the left ventricular free wall was fixated and prepared for electron microscopy. 50 nanometer (nm) ultrathin sections originating from 3 different layers (surface, 50 µm and 100 µm depth) were cut and stained with uranyl-acetate and lead-citrate. Smaller (x3000) and higher magnification (x8000) micrographs displaying the ultrastructure of the myocardium were acquired. Analysis of the images were performed by blinded visual scoring, background corrected densitometry and mitochondrial contour mapping using the Image J and Neurolucida softwares.

Results: Survival of the D-CON animals was significantly worse than of CON animals which was associated with pronounced ultrastructural myocardial damage represented by myofibrillarolysis, mitochondrial shrinkage and disintegration, chromatin fragmentation and lysosome formation. While the prophylactic treatment substantially reduced most of the above abnormalities, appearance of the myocardium of the POST animals was very similar to that of the D-CON group. Contour measurements revealed significant mitochondrial shrinkage in the D-CON group, evident from a smaller mitochondrial area and perimeter, which could not be prevented by any drug intervention. The ultrastructure of the 4 groups was in line with the in vivo functional measurements as well: left ventricular ejection fraction was: significantly reduced in the D-CON group, preserved in the PRE group, and slightly reduced in the POST group.

Conclusions: In contrast to a conventionally applied treatment, prophylactic heart failure therapy effectively prevents DOX induced myocardial damage.

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Myocardial protection in pediatric and congenital cardiac surgery

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Perioperative myocardial dysfunction is still the leading cause of morbidity and mortality after infant and complex adult congenital cardiac surgery. Unlike adult patients with acquired cardiac pathology, the population of children and adults with congenital heart disease presents with heterogeneity of anatomic, physiologic and pathophysiologic conditions. In addition, history of multiple prior cardiac operations or procedures is very common. This complexity explains the paucity of studies on myocardial protection in the pediatric and adult congenital population. It is a paradox that these very same patients would be the ones to benefit the most from improved strategies of myocardial protection in terms of reduction of morbidity and burden of hospital care. The evidence on myocardial protection during repair of congenital heart lesions will be reviewed, including innovative approaches to limit or avoid myocardial ischemia.

Human voltage-gated proton channels in chorion derived mesenchymal stem cells

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The voltage-gated proton channels (Hv1) are widely expressed; they are present in numerous immune cells such as neutrophils, eosinophils, basophiles, B-lymphocytes, as well as cancer cells (glioblastoma multiform and Jurkat T cell lines etc.), osteoclasts and alveolar epithelial cells. The H⁺ extrusion mechanism is able to avoid acidification-related cell death via hHv1 and the proton channels may play a part in the cell migration and proliferation. Mesenchymal stem cells (MSCs) can differentiate into various cell types (osteoblasts, chondrocytes, adipocytes etc.) and the application of MSCs can be a novel therapeutic strategy for/in the regenerative medicine. Based on the widespread expression of Hv1 and its versatile physiological functions we hypothesized that this channel may be present in MSCs as well.

To confirm the hypothesis we carried out the analysis of the expression of Hv1 on mRNA level using RT-PCR. Single-cell electrophysiology were used to determine biophysical and pharmacological hallmarks of Hv1 (pH- and voltage-dependence, 5-chloro-2-guanidinobenzimidazole (ClGBI) and arachidonic acid (AA) sensitivity). The cell viability were tested by MTT reduction assay. For the induction of mineralization were used two different methods: the “classical inducing cocktail” and the pathological pathway by inorganic phosphate. Calcium deposition was examined with Alizarin Red S staining. Analysis of cell migration was carried out by scratch wound assay.

In this study, we demonstrate the presence of hHv1 in the chorion-derived mesenchymal stem cells (cMSCs) using RT-PCR, we confirm the voltage- and pH-dependent gating of these channels using electrophysiological measurements, and its sensitivity to 5-chloro-2-guanidinobenzimidazole and arachidonic acid. Our results report that the inhibition of voltage gated proton channels significantly decreases the cell viability and the mineral matrix production of cMSCs induced by both pathological and classical osteogenic pathway, moreover the migration of these cells was declined using ClGBI treatment in the wound healing assays.

Physiological mineralization is necessary for the bone formation, but the pathological or ectopic mineralization leads to many disorders or in serious/severe case to death. Nevertheless, the migration of MSCs to the cancer cells may be the disadvantage of the stem cells therapy, so we propose that hHv1 might be a new target or control point in the regulation of therapeutic application of MSCs.

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Pulmonary artery root decellularization - Significant improvement of established protocols by modification of physical and procedural factors

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Objectives: Decellularization (DC) is a promising technique to produce acellular pulmonary artery roots for cardiac surgery. The aim of this study was to evaluate and optimize four published detergent based DC procedures in regard to their decellularization efficiency as well as extracellular matrix (ECM) preservation.

Methods: Porcine pulmonary valves were treated exactly according to four different published DC procedures (n = 5 valves): 0.5% SD-SDS, 24h¹; 1% SD, 24h²; 1% SDS+ 1% TritonX100, 6h³; 0.05% IGEPAL/Triton X 100/ SD, 48h⁴. After evaluation, processes were modified (e.g. cyclic DC, ultrasonic exposure) and repeated using a different experimental setup (circulatory system). An enzymatic (DNase and α -galactosidase) incubation step was added after the detergent incubation. The efficiency of each procedure was analyzed by histological evaluation, scanning electron microscopy, quantification of DNA and glycosaminoglycan as well as tensile tests.

Results: The scaffolds of the SD/SDS treated group were completely decellularized, however showed severe ECM damages. Therefore, the length of the procedure was halved, which resulted in successful DC and a preserved ECM. The published SD based procedure was not able to produce acellular valves. A changed DC setup with a cyclic incubation scheme was necessary for a successful procedure. The published SD/Tx treatment did not lead to full DC as well. Alterations to a cyclic protocol and a circulatory DC setup resulted in degeneration of the ECM. Further modifications and reduction of the detergent concentration (0.5% SDS/Triton X 100), finally resulted in acellularity while the ECM was preserved. All newly developed protocols showed significantly reduced DNA concentrations - especially after nuclease treatment. The protocol using low concentration SD/Tx/IGEPAL was not able to produce acellular scaffolds. As it showed only minimal treatment effects, no modifications were investigated.

Conclusion: None of the reproduced protocols led to a successful DC and preservation of the ECM as it was published. Therefore, we strongly recommend a comprehensive and stringent quality control for all DC processes. However, after optimization the investigated protocols produced acellular pulmonary valves with a preserved ECM.

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Tenascin-C deficiency attenuates abdominal aortic aneurysm progression in mice

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Introduction: Tenascin-C (TNC) is a matricellular protein produced mainly by vascular smooth muscle cells (VSMC) as well as fibroblasts and plays a role in various pathological remodeling processes, including abdominal aortic aneurysms (AAA). The aims of this study are to evaluate 1) whether TNC deficiency could attenuate AAA formation and 2) whether TNC influence VSMC phenotypes.

Methods: Male A/J TNC ^{-/-} and A/J wildtype (WT) mice were used. After laparotomy and preparation of the infrarenal aorta, AAA were induced by periaortal CaCl₂ at 0.5M application for 15 minutes. The sham-operated groups were treated identically with saline solution. The external diameter of the infrarenal aorta was measured both prior to AAA induction and before organ harvesting at 3 and 10 weeks. Aortic samples were stained with Elastica Van Gieson for elastin structure evaluation and further qualitative scoring. Additionally, in vitro human VSMC were incubated with either TNF- α (5ng/ml) or TNC (3 μ g/ml) for 4 and 24h. The relative expression of SM22- α and TNC were evaluated by quantitative real-time PCR.

Results: Mice with CaCl₂ induced AAA showed significantly higher diameter ratios than the sham groups (3w: $p < 0.0001$; 10w: $p < 0.0001$). Whereas, no significant changes in diameter ratios were found in sham groups, TNC knockout (KO) mice with AAA showed significantly lower diameter ratio compared to the wildtype group 3 weeks (TNC KO: 1.39 ± 0.25 , WT: 1.67 ± 0.22 $p < 0.05$) and 10 weeks (TNC KO: 1.51 ± 0.47 , WT: 1.98 ± 0.55 $p < 0.05$) after AAA induction. Additionally, WT mice with AAA showed a more disrupted Elastin structure than TNC KO mice 10 weeks after AAA induction. VSMC exposed to TNF and TNC markedly reduced the expression of TNC and SM22- α , respectively. Although, after 24h incubation, expression of TNC showed an upregulation tendency, while the expression of SM22- α was significantly upregulated (TNF- α : 195.18 ± 43.79 , TNC: 8.96 ± 1.91 , Control: 0.22 ± 0.06 $p < 0.001$).

Conclusion: Our results are a first evidence that TNC might play a role in the formation and progression of AAA as well as in changes of VSMC. These results might indicate that targeting TNC is a potential therapeutic approach in AAA.

Electrophysiologic investigation of exercise-induced cardiac hypertrophy in a rodent model of athlete's heart

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Purpose: Although regular exercise training is associated with cardiovascular benefits, cardiac remodeling induced by long-term, intense exercise training is also related to increased risk of arrhythmia. We aimed at providing electrophysiologic investigation of exercise-induced myocardial hypertrophy in a rat model of athlete's heart and determining sex-specific differences.

Methods: Age-matched young adult rats were divided into female exercised (FEx), female control (FCo), male exercised (MEx) and male control (MCo) groups. After exercised animals completed a 12-week-long swim training protocol, echocardiography was used to confirm exercise-induced hypertrophy. In vivo electrophysiologic characterization was performed by programmed stimulation with an octapolar catheter inserted into the right atrium.

Results: Myocardial hypertrophy was verified by left ventricular mass (echocardiography) and post-mortem heart weight data in both exercised groups. We found signs of atrial remodeling in female exercised rats, increased P-wave duration and amplitude, as well as prolonged right atrial effective refractory period (RAERP 43.5 ± 2.2 ms FEx vs. 36.7 ± 2.0 ms FCo, $p < 0.05$). We also observed increased T-wave amplitude and QT interval in female swim-trained rats. Hearts of male exercised rats were primarily associated with increased RR duration and Wenkebach cycle length (WCL 109.8 ± 4.7 ms MEx vs. 97.3 ± 2.1 ms MCo, $p < 0.05$) compared to control ones. Exercise training was related to increased R wave amplitude and QRS duration in both genders and we could induce non-sustained atrial flutter in two exercised animals by double extrastimulation.

Conclusions: Our data suggests that exercise-induced cardiac hypertrophy might hold an increased risk of arrhythmia. In male individuals elevated parasympathetic tone, while in female ones marks of atrial remodeling could be the characteristic alterations.

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Different mechanisms for heart failure progressions in male and female mREN2 rats

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Introduction: The effectiveness of renin-angiotensin-aldosterone system (RAAS) inhibitors in heart failure (HF) therapy indicates that RAAS plays a crucial role in the pathomechanism of HF. Transgenic rats (mREN2) harboring an extra copy of renin gene develop fulminant hypertension at an early age which progresses into HF.

Purpose: We aimed to investigate hypothetical gender dependent differences in the pathomechanisms in the mREN2 model of HF.

Methods: Blood pressure measurement and echocardiography was performed in mRen2 and control rats. Internal non-transgenic wild type rats (WT) served as controls. To reveal the mechanisms contributing to HF progression, levels of RAAS activity in isolated tissues were studied in vitro.

Results: Mean arterial pressure (MAP) of mRen2 rats were high in both females (138.5 ± 11.7 mmHg) and males (168 ± 5.8 mmHg). Male rats had higher mortality till 1 year of age (survival rate: males: 23% versus females: 75%). At 1 year rats exhibited signs of mixed systolic and diastolic cardiac dysfunctions, indicating the progression of hypertension to HF (EF in females: WT: 68.28 ± 2.1 versus mRen2: 68.26 ± 2.3 ; males: WT: 74.7 ± 6.1 versus mRen2: 60.2 ± 4.9 ; E/A: in females: WT: 1.74 ± 0.03 versus mRen2 1.47 ± 0.052 ; males: WT: 1.56 ± 0.01 versus mRen2: 1.06 ± 0.038). In parallel, a dysregulation of the tissue RAAS was observed. In particular angiotensin converting enzyme (ACE) activity was higher in male mREN2 left ventricles (9.5 ± 0.8 U/mg) than in those of their WT littermates (5.5 ± 0.2 U/mg), while no similar differences were observed in the lungs (71 ± 21 versus 76 ± 9 U/mg) and in any of the above parameters in females. Activities of angiotensin 2 eliminating ACE2 enzymes were similar in the left ventricles, lung, kidney of WT and mRen2 animals irrespectively of gender.

Conclusion: Our work illuminated important gender differences in the progression of hypertension to HF. In particular, our data implicate that left ventricular ACE activities increase in males more than in females. This is in accordance with the higher clinical effectiveness of ACE inhibitors and the higher HF risks in males than those in females.

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Analysis of cardiac repolarization parameters and risk factors of sudden cardiac death in patients with hypertrophic cardiomyopathy

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Background: Hypertrophic cardiomyopathy (HCM) is a common inherited disease of the myocardium, associated with increased propensity for ventricular arrhythmias and increased risk of sudden cardiac death (SCD). The identification of patients with high risk for SCD is considered incomplete. Previously we observed that ECG repolarization parameters are increased in patients with HCM, and these parameters may represent a novel marker in SCD risk assessment. In this present study we investigated the correlation between different ECG parameters characterizing ventricular repolarization and SCD risk factors in patients with HCM.

Methods: We examined 62 HCM patients (35 males, age: 48±14 years). From 5-minute digitized ECG recordings the following parameters were determined: frequency corrected QT interval (QTc), QT dispersion (QTd), T wave peak-to-end distance (Tpeak-Tend) and short-term QT variability (QT-STV). SCD risk was determined as the number of traditional risk factors or as the 5-year risk of SCD calculated by the HCM-Risk-SCD Calculator. Patients were categorized as having low or high risk (<1 vs. ≥2 risk factors or <4% vs. >6% 5-year risk of SCD).

Results: Low or high SCD risk patients had a similar age and sex distribution. Patients with ≥2 risk factors had higher value of QT dispersion (QTd: 44±20 vs. 49±10 ms; P=0.3912) and lower values of other repolarization parameters (QTc: 507±66 vs. 467±63 ms, P=0.085; Tpeak-Tend: 112±33 vs. 102±30 ms; P=0.3819; QT-STV: 0.0048±0 vs. 0.0045±0 s; P=0.568), but the difference was not significant. However, patients with >6% 5-year SCD risk had a non-significantly increased values of the repolarization parameters (QTc: 493±59 vs. 522±60 ms; P=0.3247; Tpeak-Tend: 109±31 vs. 114±41 ms; P= 0.7260; QT-STV: 0.0044±0 vs. 0.0048±0 s; P=0.3059).

Conclusion: There is a non-significant increase of ECG parameters in HCM patients with high 5-year risk for SCD. The lack of significance might be due to the relatively low number of patients.

Myocardial reverse remodeling: can we heal a broken heart?

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Myocardial remodeling impairs cardiac performance and limits life expectancy of patients with cardiovascular disorders. Pathologic alterations develop both at macroscopic and microscopic levels. Left ventricular chamber dilation associates with a thinned ventricular wall, reduction in cardiomyocyte number, alterations in the composition of the extracellular matrix and pathologic changes in the biological functions of cardiomyocytes (e.g. contractility, metabolism, gene expression). Reverse remodelling, i.e. normalization of left ventricular function and macroscopic structure has been documented in response to a number pharmacological and device related therapies. Mechanical unloading appears to be a central driver of recovery in left ventricular pressure-volume relations and ejection fraction. Nevertheless, biochemical processes mobilized during therapeutic interventions are only partially understood. Moreover, restoration of cardiomyocyte signaling, its transcriptome, metabolism and the extracellular matrix is mostly found to be partial. It is therefore more appropriate to speak about remission than recovery during reverse remodeling. In addition, it is instructive to introduce a term as Heart Failure with improved Ejection Fraction (HFief) for patients presenting with major degrees of reverse remodelling following life threatening myocardial depressions. Myocardial pathologies associating with significant cardiomyocyte loss, alterations in extracellular matrix and/or major genetic alterations have limited chances for full recovery. Future therapeutic approaches with novel strategies may improve the efficacy of myocardial reverse remodelling.

Repeated remote ischemic conditioning enhances Neuregulin-1/ErbB2/3/4 expression following myocardial infarction in rats

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Introduction: Adverse left ventricle (LV) remodelling following myocardial infarction (MI) plays a key role in the progression of congestive heart failure (HF). Recombinant human Neuregulin-1 (rhNRG-1) has been demonstrated to have both anti-fibrotic and anti-inflammatory effects. Chronic administration of rhNRG-1 markedly improved LV ejection fraction (LVEF) and coronary microcirculation in patients with HF. Repeated remote ischemic conditioning (RIC) is considered as a potential clinically approach to improve cardiac function following MI, however the mechanisms are not fully elicited.

Purpose: The aim of the present study was to (1) clarify the effects of a brief period of RIC on LV hemodynamic function and coronary flow (CF) and (2) to assess the expression of NRG-1, ErbB2/3/4 expression following MI.

Methods: Male Sprague-Dawley rats were subjected to permanent left coronary artery (LCA) occlusion and allocated to two groups: (1) MI (n=7) and (2) MI+RIC (n=5). Repeated RIC was started at the 3rd day after MI once a day for 5 days by 3 cycles of 5 min of unilateral hindlimb ischemia and 5 min of reperfusion. Cardiac functional parameters were assessed by transthoracic echocardiography at baseline and at days 3 and 8 following MI. Coronary flow (CF) and LV systolic pressure (LVSP) were evaluated on an isolated erythrocyte-perfused working heart model at day 8 following MI. The alterations in CF primarily reflect alterations in coronary resistance, allowing evaluation of microvasculature function in this experimental setup. The expression of plasma level of NRG-1 was measured by ELISA and mRNA expression of ErbB2/3/4 was accessed by RT-qPCR.

Results: Short term duration (5 days) of RIC enhanced LVEF as compared to MI group ($63\pm1\%$ vs. $58\pm2\%$ on day of 8th following the induction of MI, $p=0.074$). This was accompanied by preserved LV systolic function in rats with RIC as compared with MI (LVESD: 5.9 ± 0.06 mm and 6.4 ± 0.2 mm, $p=0.064$). Results were obtained from the isolated working heart system showed that CF and LVSP were markedly enhanced in rats with RIC as compared to MI (CF: 4.3 ± 0.2 vs 3.1 ± 0.2 ml/g heart weight and LVSP: 109 ± 2 mm Hg vs 119 ± 4 ; mm Hg; $p<0.01$, respectively). Both plasma and tissue expressions of NRG-1 were significantly elevated by RIC in comparison to MI group (plasma: 10.6 ± 1.7 μ g/ml vs 19.4 ± 3.3 μ g/ml and LV tissue: 0.53 ± 0.09 vs. 3.16 ± 0.9 1/18S; $p<0.05$). Similarly, the mRNA expression of ErbB2/3/4 showed at least partly significant differences between the groups (ErbB2: 1.0 ± 0.2 vs. 2.08 ± 0.4 1/18S, $p<0.05$; ErbB3: 1.07 ± 0.3 vs. 2.4 ± 0.4 1/18S, $p<0.05$; ErbB4: 0.46 ± 0.12 vs. 0.64 ± 0.23 1/18S; n.s) in LV tissue samples, taken from the infarcted zone.

Discussion: RIC preserves systolic LV function and markedly enhances basal CF following MI in rats. These results were accompanied by with a marked increase in NRG-1 levels in plasma and myocardial tissue samples indicating enhanced cardioprotection. Therefore, repeated remote RIC is a potential therapeutic approach for improved post-MI remodelling.

MicroRNAs and myocardial protection

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In cardiac surgery, the number of elderly and multimorbid patients has dramatically increased over the last 20 years and is associated with elevated perioperative mortality. Aging also increases cardiac apoptosis and decreases ventricular function after ischemia-reperfusion. Therefore, constant efforts to improve intraoperative myocardial protection are essential to optimize postoperative outcome. Cardiopulmonary bypass and cardioplegic arrest remain the most popular techniques in open heart surgery. However, both can directly or indirectly result in cardiac morbidity following surgery.

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression through a process called RNA interference. MicroRNAs are important regulators of many cellular functions, and loss/gain of function has been shown to affect tissue function and facilitate the onset and progression of cardiovascular disease such as myocardial infarction, atherosclerosis, heart failure as well as ischemia/reperfusion (IR) injury. It is considered to be known that miRNAs such as miR-1, miR-133a, miR-499 and miR-208 are contributed to IR injury in patients with acute myocardial infarction by altering key signaling elements, thus making them potential biomarkers and therapeutic targets. However, there are only few studies investigated changes in microRNAs profiles under “protected ischemic event” such as cardiopulmonary bypass surgery. We will address the role of microRNAs in the development of acute myocardial infarction and during ischemia/reperfusion injury. A particular focus will be made on key signaling elements. We further will address the different expression in circulating and tissue-derived miRNAs using next generation sequencing for the qualitative assessment of all possible miRNAs existing in LV tissue samples. Thereby we hope to make miRNAs to potential biomarkers and/or therapeutic targets.

Drug discovery and development for acute heart failure drugs: are expectations still too high?

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The development of drugs for acute heart failure (AHF) over the last two decades has resembled a particularly scary roller coaster ride for the researchers: great enthusiasm, hard work, important investments, great expectations, and, alas, many disappointments.

A search of the literature for clinical trials in AHF identifies over 30 medium-to-large double-blind trials (phases IIb and III) on new chemical entities published after 2000 (see Figure updated from Pollesello et al.¹). The reader will certainly recognize names such as dopamine, istaroxime, milrinone, enoximone, levosimendan, and omecamtiv mecarbil in the inotrope/inodilators group; cinaciguat, clevidipine, tezosentan, nesiritide, ularitide, and serelaxine among the vasodilators; and the diuretics tolvaptan and rolofylline. Very few drugs made to the market.

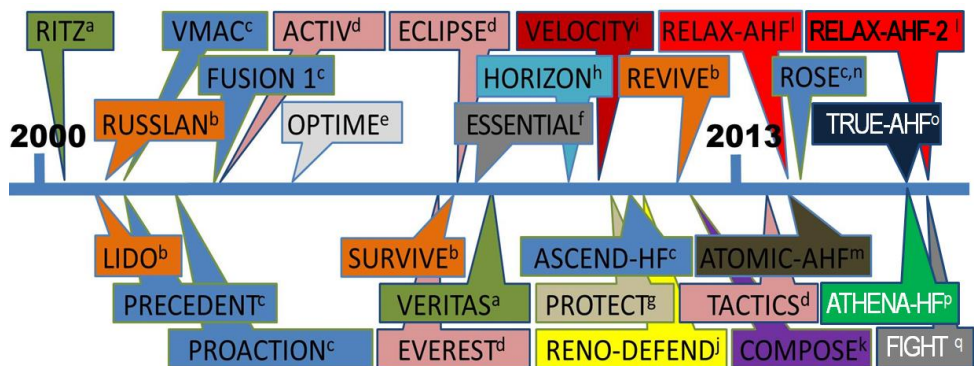
From my position in the industry I feel that the development of novel treatments for AHF is not going as smoothly as it might. The five most tenacious problems contributing to this state of affairs are:

- The definition of AHF is still evolving. Typical symptoms and signs of heart failure are still considered of limited diagnostic value,
- No new families of drugs in sight. Cheap generic inotropes, vasodilators, and diuretics on the market (often introduced without a proper development process).
- Difficulties in justifying a premium price to institutional payers with rising budget limitations,
- Harsh competition for R&D financing between therapy areas within pharmaceutical industry,
- Rising costs of clinical trials. Need for long term mortality data. Difficulties in showing advantages vs. placebo on top of existing standard of care,
- Risk of running clinical trials on populations which do not fully represent the real patients.

Striving after a magic bullet in AHF has been the downfall of much research in the past. As an example levosimendan was developed as an inotrope and meant to be used independently of the SBP value of the patient. Its vasodilatory effect very soon became evident, however, and nowadays its use is restricted to patients with SBP >100 mmHg.²

Pharma industry seems to have, alas, surprisingly short memory. As it regards Omecamtiv mecarbil, for example, it appears that the earlier discussion on sarcomere-active drugs has been forgotten. Decades ago, molecules which prolong the contractility transient were ditched because potentially harmful in case of ischemic conditions.³ Is it time to be rethinking the strategy?

Conflict of Interest: the author was among the inventors of levosimendan, one of the drugs cited in the editorial, and is currently employed by the company which has the global rights for this drug.



Legend to the figure

Around 30 large/medium-large sized double-blind trials on new chemical entities (phases IIb and III) were run on Acute Heart Failure from year 2000. The acronyms of the trials are marked as follows: trials on (a) tezosentan, (b) levosimendan, (c) nesiritide, (d) tolvaptan, (e) milrinone, (f) enoximone, (g) rolofylline, (h) istaroxime, (i) clevidipine, (j) SLV320, (k) cinaciguat, (l) serelaxine, (m) omecamtiv mecarbil, (n) dopamine, (o) ularitide, (p) high dose spironolactone, (q) liraglutide. Levosimendan is the only the drugs which is currently authorized for sales as treatment of AHF and still under active research program.

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Endothelin, salt intake, and the molecular clock

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High salt (HS) intake is a powerful stimulus for ET-1 production both within the kidney and vascular tissue. Recent evidence suggests that the cell autonomous molecular clock maintains circadian control of ET-1 production by the kidney. Accordingly, we recently observed that loss of ET_B receptor function is associated with a time of day dependent impairment in the ability to excrete an acute salt load. Circadian dyssynchrony either within or between organ systems can lead to increased risk of cardiovascular disease. Therefore, our current study was designed to determine whether HS intake leads to circadian dyssynchrony within the kidney, and whether the renal endothelin system contributes to control of the renal molecular clock. We observed that HS feeding led to region-specific alterations in circadian clock components within the kidney. For instance, HS caused a significant 5.5 hour phase delay in the peak expression of *Bmal1* and suppressed *Cry1* and *Per2* expression in the renal inner medulla, but not the renal cortex, of control rats. The phase delay in *Bmal1* expression appears to be mediated by endothelin (ET-1) because this phenomenon was not observed in the endothelin B receptor (ET_B) deficient rat. In cultured inner medullary collecting duct cells, ET-1 suppressed *Bmal1* mRNA expression. Furthermore, *Bmal1* knockdown in these cells reduced epithelial Na⁺ channel expression consistent with molecular clock regulating Na⁺ conserving pathways. These data reveal that HS feeding leads to intra-renal circadian dyssynchrony associated with salt-sensitive hypertension, potentially through activation of ET_B receptors within the renal inner medulla.

Childhood Adversity and Adult Cardiovascular Disease Risk

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Childhood adversity or early life stress (ELS) is an under-appreciated, independent cardiovascular disease (CVD) risk factor. The CDC childhood adversity project elucidated that childhood psychological stressors are a greater risk factor for ischemic heart disease than known CVD risk factors. We reported that young adults who experience childhood adversity have higher pulse wave velocity, increased total peripheral resistance, and elevated diastolic blood pressure when compared to young adults with no childhood adversity. We utilized a mouse model of ELS, maternal separation with early weaning (MSEW), to further evaluate possible mechanisms of ELS-induced vascular dysfunction. We found that mice exposed to MSEW exhibit endothelial dysfunction with increased aortic superoxide production as well as higher expression of pro-inflammatory mediators and increased aortic macrophages resulting in elevated vascular inflammation compared to normally reared mice. Our studies demonstrate that young adults with childhood adversity have significantly greater circulating ET-1 levels when compared to young adults with no childhood adversity. Furthermore, adult mice exposed to MSEW also have increased circulating ET-1 levels compared to normally reared mice. Reports show that over-expression of endothelial-derived ET-1 exacerbates atherosclerosis with increased vascular oxidative stress and elevations of aortic pro-inflammatory macrophages in mouse models of CVD. Early in life or during disease development, the cellular balance of acetylation and deacetylation critically influences gene expression and cell signaling pathways. Histone deacetylase (HDAC) enzymes regulate deacetylation of lysine residues on histones and non-histone cellular proteins leading to modifications in gene expression and cell signaling. We found that mice exposed to MSEW have increased aortic HDAC activity. MSEW-induced aortic endothelial dysfunction is reversed with HDAC inhibition, but does not affect endothelial function in normally reared mice. We found that HDAC9 isoform is upregulated in MSEW mice. HDAC9 is expressed in endothelial cells, smooth muscle cells, and macrophages. Investigations show that higher HDAC9 expression in macrophages is pro-atherogenic. Further, our studies in endothelial cells reveal that HDAC9 regulates ET-1 production. Reports demonstrate that HDAC9 is a possible causative factor in atherosclerosis in mice and show a role for HDAC9 in the early stages of atherogenesis. The HDAC9 SNP, rs2107595, is strongly associated with large artery stroke, coronary artery disease, and common carotid intima media thickness. The rs2107595 SNP is the strongest risk locus reported to date for stroke. The rs2107595A risk allele is associated with a functional up-regulation of HDAC9. *We propose that early life stress or childhood stress induces HDAC9 and ET-1 up-regulation mediating endothelial dysfunction, vascular inflammation, and CVD risk.*

Hydrogen sulfide abrogates hemoglobin-lipid interaction in atherosclerotic lesion

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Atherosclerosis-related morbidity and mortality are closely associated with the presence of the complicated lesion. Vascular lesions are called complicated where due to an infiltration of red blood cells (RBC). Inside the plaque RBC lyse and the released hemoglobin (Hb) is oxidized to ferrylHb which shown pro-oxidant and pro-inflammatory function. Cystathione-gamma lyase (CSE)-derived hydrogen sulfide (H₂S) has been suggested to possess diverse anti-atherogenic behaviors.

We showed that, expression of CSE was upregulated mainly in macrophages, foam cells and myofibroblasts of human atheromatous plaque derived from carotid artery specimens of patients. Similar pattern was detected in apolipoprotein E knock out mice fed with high-fat diet. We identified some triggers for provoking CSE expression in macrophages and human aorta smooth muscle cells including heme, ferrylHb, plaque lipids, oxidized low-density lipoprotein, tumor necrosis factor- α and interleukin-1 β . In the interaction between Hb and atheroma lipids, H₂S significantly diminished oxidation of Hb preventing the formation of ferrylHb derivatives, therefore providing a novel function as a heme-redox-intermediate-scavenging antioxidant. By inhibiting Hb-lipid interplays H₂S lowered oxidized Hb-mediated expression of adhesion molecules in human endothelial cells and the disruption of endothelium integrity. Exogenous H₂S inhibited heme and Hb-mediated lipid peroxidation of human atheroma derived lipid and human complicated lesion.

In conclusion, the present study provides evidence that high vascular expression of CSE defines atheromatous plaques and complicated lesions, both in human carotid atherosclerotic lesions and in hyperlipidemia-induced atherosclerotic mouse model. Based on our in vitro results, we suggest that the raised CSE expression might serve as a compensatory atheroprotective reply, in which the formed H₂S inhibits the formation of pro-oxidant and pro-inflammatory lipid intermediates and Hb forms and subsequent endothelial responses provoked by these species.

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Role of cGMP-signalling in the prevention of heart failure with preserved ejection fraction

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Background: Heart failure with preserved ejection fraction (HFpEF) has a great epidemiological importance. The role of cyclic guanosine monophosphate (cGMP) signaling has been intensively investigated in the development of HFpEF. Elevated levels of cGMP have been shown to exert cardioprotective effects in various cardiovascular diseases, including diabetic cardiomyopathy. We investigated the effects of long-term preventive application of vardenafil (inhibitor of the cGMP-degrading phosphodiesterase-5A (PDE5A) enzyme) in diabetic cardiomyopathy-associated HFpEF.

Methods: Zucker Diabetic Fatty (ZDF) rats were used as a model of HFpEF. ZDF Lean rats served as controls. Animals received vehicle or 10mg/kgBW PDE5A-inhibitor vardenafil per os from the 7th to 32nd weeks of age. Cardiac function, morphology was assessed by left ventricular (LV) pressure-volume analysis and echocardiography at the 32nd week. Cardiomyocyte force measurements were performed. The key markers of cGMP signaling, nitro-oxidative stress, apoptosis, myocardium hypertrophy and fibrosis were examined.

Results: ZDF animals showed diastolic dysfunction (increased LV/cardiomyocyte stiffness, prolonged LV relaxation time), preserved systolic performance, decreased myocardial cGMP level coupled with impaired protein kinase G (PKG) activity, increased nitro-oxidative stress, enhanced cardiomyocyte apoptosis, hypertrophic and fibrotic remodeling of the myocardium. Vardenafil effectively prevented the development of HFpEF by maintaining diastolic function (decreased LV/cardiomyocyte stiffness and LV relaxation time), by restoring cGMP levels and PKG activation, by lowering apoptosis and by alleviating nitro-oxidative stress, myocardial hypertrophy and fibrotic remodeling.

Conclusions: We reported that vardenafil successfully prevented the development of diabetes mellitus associated HFpEF. Thus, PDE5A inhibition in a preventive manner might be a promising option in the management of HFpEF patients with diabetes mellitus.

Effect of gender on myocardial reverse remodeling in a rat model of banding and debanding of the abdominal aorta

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Background: Gender differences have been intensively investigated during the development of pressure overload-induced left ventricular (LV) myocardial hypertrophy (LVH). However, it is less clear whether female sex also affects the regression of LVH after pressure unloading therapy.

Purpose: In the present study we aimed to investigate the effect of gender on myocardial reverse remodeling following pressure unloading in male and female rats utilizing a rat model of banding and debanding of the abdominal aorta.

Methods: Pressure overload of the left ventricle was induced in male (M) and female (F) Sprague-Dawley rats by abdominal aortic banding (AB) for 6 or 12 weeks. Sham operated animals served as controls. Pressure unloading was evoked by removing the aortic constriction after week 6 (debanding). Serial echocardiography and LV pressure-volume analysis was performed to assess the morphological and functional alterations. In addition, histological and molecular biological measurements were also carried out.

Results: In male (M) and female (F) AB groups, development of LVH at a similar extent was confirmed by increased left ventricular mass, heart weight-to-tibial length ratio (HW/TL) and cardiomyocyte diameter (CD). On the functional level, LVH was associated with diastolic dysfunction in both genders. However, impairment of systolic function could be only detected in male aortic-banded rats. In contrast to the sex-dependent differences in LVH, removal of the aortic constriction resulted in a similar morphological (HW/TL: 0.38 ± 0.01 debanded-M vs. 0.47 ± 0.01 AB-M, $p < 0.05$; 0.28 ± 0.01 debanded-F vs. 0.36 ± 0.02 AB-F, $p < 0.05$), histological (CD: 16.4 ± 0.5 debanded-M vs. 18.2 ± 0.6 AB-M, $p < 0.05$; 14.7 ± 0.4 debanded-F vs. 17.7 ± 0.6 AB-F, $p < 0.05$) and functional reverse remodeling in both genders.

Conclusion: Our results provide evidence that pressure unloading therapy at a relatively early time point leads to myocardial reverse remodeling at a comparable degree in male and female rats.

Improved myocardial protection with hypothermic St Thomas' Hospital polarizing blood cardioplegia

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Objective(s): Patients undergoing cardiac surgery are currently older and sicker with increased prevalence of cardiovascular disease. Aging is associated with impaired cardiac functional recovery following ischemia reperfusion; ultimately, more effective cardioprotective regiments are warranted. This study was aimed to compare the cardioprotective efficacy of low-dose blood-based St Thomas' Hospital Polarizing cardioplegia (STH-Pol-B: esmolol, adenosine, magnesium) with conventional hyperkalemic blood-based St Thomas' Hospital cardioplegia (STH2-B) in a porcine model of cardiopulmonary bypass (CPB) and to describe direct effects of STH-Pol-B during on-pump reperfusion and, consecutively, show potential hemodynamic effects during off-pump reperfusion.

Methods: Pigs were subjected to normothermic CPB and hearts arrested via antegrade cold (4°C) blood cardioplegia (either low-dose STH-Pol-B: n=7 or STH2-B: n=6). Ischemia (60 min) was followed by 60 min of on-pump reperfusion and an additional 90 min (off-pump reperfusion) with constant hemodynamic monitoring. Left ventricular (LV) tissue samples were taken for analysis of high-energy phosphate content, ultrastructural changes (electron microscopy) and expression of microRNAs.

Results: On-pump reperfusion revealed reduced arterial and coronary CK-MB ($p<0.05$, respectively) in STH-Pol-B vs. STH2-B. During off-pump reperfusion STH-Pol-B showed improved systolic arterial pressure ($p<0.01$), increased left ventricular systolic pressure ($p<0.01$). Off-pump reperfusion revealed higher stroke volume ($p<0.01$), increased external heart work ($p<0.01$) and dp/dt_{max} ($p<0.05$) compared to STH2-B. In addition, wedge pressure was significantly lower in STH-Pol-B vs. STH2-B ($p<0.05$) during off-pump reperfusion. Energy charge was comparable in both groups. Overall 238 microRNAs were expressed in LV tissue samples; only miR-708-5p and miR-122 showed different expression between groups ($p<0.05$, respectively).

Conclusions: Polarized cardiac arrest (with STH-Pol-B) improves functional recovery and reduces myocardial ischemic damage in a porcine CPB model. Additionally, differential expression of microRNAs indicates promising new candidates for markers of ischemia-reperfusion injury. These results indicate efficacy of the novel STH-Pol-B cardioplegia, suggesting potential clinical feasibility.

Selective heart irradiation induces cardiac overexpression of the pro-hypertrophic miR-212/132 cluster

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Introduction A deleterious, late-onset side effect of thoracic radiotherapy is the development of radiation-induced heart disease (RIHD). It is characterized by left ventricular hypertrophy and diastolic dysfunction and often manifests as heart failure with preserved ejection fraction. The miR-212/132 family is a crucial regulator for pathologic cardiac hypertrophy and miR-132 has been proposed as a therapeutic target for heart failure. Therefore, our aim was to investigate whether the miR-212/132 cluster and its hypertrophy associated targets play a role in the development in RIHD.

Methods RIHD was induced in a clinically relevant chronic rat model. A single dose of 50 Gy was delivered to the whole heart of the animals and 19 weeks later, cardiac function was assessed by transthoracic echocardiography and tissue samples collected for histology and molecular analysis.

Results Echocardiography and histology revealed left ventricular hypertrophy with preserved ejection fraction, diastolic dysfunction and interstitial fibrosis in the irradiated group. The miR-212/132 cluster was overexpressed and FOXO3 mRNA was repressed in the irradiated hearts. In contrast, total FOXO3 protein level failed to decrease in response to heart irradiation. However, cardiac phospho-FOXO3 level and phospho-FOXO3/total FOXO3 ratio showed a non-significant increase in irradiated hearts. Cardiac total AKT level and the phospho-AKT/total AKT ratio failed to change in the irradiated hearts as compared to controls.

Conclusions Cardiac overexpression of the miR-212/132 cluster might play a role in the development of cardiac hypertrophy in RIHD. The development of cardiac hypertrophy seems to be independent from the AKT/FOXO3 mediated pathways in RIHD.

Targeting H-ferritin to mitigate valvular mineralization

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Objective: Valvular heart disease is a prominent finding in elderly and patients with the end-stage renal disease. Here we investigated mechanisms involved in the pathogenesis of this disease and identified potential targets for future therapeutics.

Approach and Results: Our results indicate that cultured interstitial cells (VIC) of stenotic aortic valve (AS) from patients undergoing valve replacement exhibited significant susceptibility to mineralization/osteoblastic transdifferentiation in response to phosphate and calcium compared to those harvested from isolated insufficient aortic valve (AI). This process was abrogated by iron and ferritin as reflected by lowering alkaline phosphatase expression (ALP) and osteocalcin secretion, increasing pyrophosphate generation, preventing extracellular calcium accumulation, and averting nuclear translocation of runt-related transcription factor 2 (RUNX2). The beneficial effect was more pronounced in VIC from AI compared to AS valve. 3H-1, 2-dithiole-3-thione (D3T) mimicked such a benefit in VIC via induction of H-ferritin. H-ferritin inhibited cellular phosphate uptake and lysosomal phosphate accumulation. Accordingly, expression of phosphate channels was decreased. Furthermore, localization of lysosomal H-ferritin occurred with high phosphate binding capacity. Histological analysis revealed higher levels of H-ferritin expression in AS compared to with AI. In regions where H-ferritin positive cells were present lack of calcium deposition, and ALP staining was not observed. Conversely, in mineralized and ALP positive tissue, H-ferritin positive cells were not detected.

Conclusions: Our results indicate that H-ferritin in VIC is a stratagem in mitigating valvular mineralization/osteoblastic differentiation. Utilization of D3T to induce ferritin expression maybe a potential strategy for the prevention of valvular calcification.

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A novel splice-site indel alteration in the EIF2AK3 gene is responsible for the first cases of Wolcott-Rallison Syndrome in Hungary

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Background: Wolcott-Rallison Syndrome (WRS) is a rare autosomal recessive disease that is the commonest cause of neonatal diabetes in consanguineous families. WRS is caused by various genetic alterations of the Eukaryotic Translation Initiation Factor 2 Kinase 3 (EIF2AK3) gene.

Subjects and Methods: Two patients in a family where the parents are fourth-degree cousins showed the typical clinical features of WRS, therefore Sanger sequencing of the coding and splicing regions of the EIF2AK3 gene was performed. The effect of the observed splice-site mutation was further tested by sequencing the cDNA of Patient 1. This cDNA was used for the cloning and expression of the mutated kinase domains of the EIF2AK3 protein, tested by Western-blotting.

Results: The genetic background of the disorder is a novel alteration in the EIF2AK3 gene involving the splice site of exon 11 - intron 11-12 boundary: 12 nucleotides are deleted that is combined with the insertion of TG bases resulting in the development of a new splice site. Due to the altered splicing extra nucleotides of intron 11-12 are inserted to the transcribed mRNA. The combination of these genetic events resulted in a frame-shift and the development of an early termination codon. The truncated protein is functionally inactive based on in vitro cloning and expression studies.

Conclusions: Here we report the genetic examination of the first WRS cases in Hungary, caused by a unique, novel indel mutation resulting in altered splicing, frame-shift, early termination and a non-functional truncated protein.

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The effect of fenugreek (*Trigonella foenum-graecum*) seed flour and diosgenin on the endothelium-dependent relaxation of abdominal aorta in a rat model of metabolic syndrome

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Fenugreek is a widely used medicinal herb and spice in the Asian countries that contains several bioactive components including diosgenin. In this study, the effect of dietary fenugreek seed flour and diosgenin was investigated on the endothelium-dependent relaxation of abdominal aorta isolated from rats receiving high-fat high-sugar diet (HFHSD).

60 male Wistar rats were randomly divided into 6 groups: (i) a negative control fed conventional rat chow; (ii) a positive control fed HFHSD; (iii) a test group fed 2 g/kg bw/day fenugreek seed flour (containing 10 mg/kg bw/day diosgenin) + HFHSD; (iv) three test groups fed 1, 10 and 50 mg/kg bw/day diosgenin + HFHSD. Treatment lasted for 6 weeks. The abdominal aorta was isolated, 2 mm wide rings were cut off and mounted at a resting tension of 10 mN in organ baths containing Krebs solution (36 °C) exposed to 95% O₂ and 5% CO₂. After 60-min incubation, a norepinephrine concentration-response (E/c) curve was generated to determine their half-maximal effective concentration (EC₅₀) value. After 60-min wash-out, a pre-contraction with norepinephrine EC₅₀ was made, followed by an acetylcholine E/c curve. Plasma glutathione levels, glutathione-handling enzyme activities and blood antioxidant capacities were also determined.

We found that HFHSD significantly decreased the endothelium-dependent relaxation evoked by acetylcholine, and it increased plasma glutathione levels, which effects were significantly reversed by fenugreek seed flour, furthermore by 10 and 50 mg/kg bw/day diosgenin.

Our results show that both dietary fenugreek and diosgenin can counteract the deleterious effects HFHSD has on endothelial function and vascular redox balance, rendering fenugreek seed flour a promising functional food.

The effect of doxycycline administration and the role of matrix metalloproteinases in a chronic cigarette smoke-induced cardiopulmonary comorbidity mouse model

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Cigarette smoke-induced inflammatory processes and consequent tissue damage play a major part in the pathogenesis of chronic obstructive pulmonary disease (COPD). Several data support the involvement of matrix metalloproteinases (MMPs) in chronic inflammatory cascades and pulmonary structural destruction, however their role in the development of smoking-induced cardiac impairment is yet to be investigated. We previously optimized and characterized the pathophysiological mechanisms of a COPD mouse model with complex methodology, and demonstrated elevated MMP-2/-9 activities in the lung. In our present study we aimed at investigating its functional relevance.

We exposed male C57Bl/6J mice to whole-body cigarette smoke for 10x30 min weekly for 6 months. To elucidate the role of MMPs in the pathophysiological mechanisms, separate groups of animals were orally treated with non-selective MMP inhibitor doxycycline dissolved in drinking water (subantimicrobial dose: 0.5 mg/ml); serum doxycycline level was measured with HPLC. Cardiac function was measured by echocardiography, pulmonary function with restrained whole body plethysmography. Cardiac MMP-2 activity was determined by gelatin zymography, while cardiac and pulmonary MMP-2/-9 relative gene expressions were measured by qPCR at the end of the 3rd and 6th months.

Six months of cigarette smoking significantly decreased the left ventricular ejection fraction, deceleration time and tricuspidal annular plane discursion time indicating both systolic and diastolic dysfunctions. Although cardiac MMP-2 activity did not change, cardiac MMP-2 and pulmonary MMP-9 relative gene expressions were significantly elevated. Despite in vitro inhibition of MMP activity by doxycycline based on its in vivo plasma levels significantly decreased MMP-9 activity in lung samples but not MMP-2 activity either in lung or in cardiac homogenates, all the above cardiac functional parameters showed significant improvement in doxycycline-treated mice after 6-month smoking. Cigarette smoke deteriorated pulmonary functions that were improved upon doxycycline administration, although it was not statistically significant.

Therefore, we conclude that systemic doxycycline treatment has beneficial effects on COPD-related chronic cardiac dysfunctions, but it seems not to be directly related to MMP-2 activity inhibition in the heart.

Biological and synthetic scaffolds for cardiovascular tissue engineering and regenerative strategies – Many ways leading to one goal?

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Introduction: Around 30 years after the rise of tissue engineering (TE), countless approaches in cardiovascular application have been investigated. However, today only a few therapeutic options are available in the clinic. In a comprehensive study, we tried to identify benefits and drawbacks of decellularized and electrospun scaffolds for cardiovascular application.

Methods: Medical grade polyurethane was electrospun into sheets and heart valve like shapes. Additional post-production modification by extracellular matrix coating was investigated. As decellularized biologic materials, porcine valves and vessels, bovine pericardium as well as homograft valves were evaluated. Different detergents, treatment protocols, additional physical methods (e.g. ultrasound) and various sterilization methods were used to improve the decellularization processes. Scanning electron microscopy, standard and immunological staining methods, tensile testing, nucleic acid and glycosaminoglycan quantification as well as biocompatibility assays were used for scaffold evaluation. Additionally, application simulation of cell seeded scaffolds for a TE approach was performed.

Results: Production of extracellular matrix coated synthetic scaffolds was possible. Cyclic procedures, ultrasonic exposure and treatment at room temperature enhanced decellularization efficiency significantly. Sterilization of the biologic scaffolds was challenging and only a combination of peracetic acid and octenidine resulted in sterile samples. Tensile tests revealed high variations within the native and processed biologic scaffold groups. All investigated scaffolds showed biocompatibility and were successfully cell seeded. TAVI-simulation of cell seeded valve scaffolds resulted in an inflammatory reaction and severe damage of the cellular coating.

Conclusion: Natural variation and final sterilization of biologic tissues are big challenges for a successful scaffold production. Cell seeded TE scaffolds showed a high vulnerability. Therefore, we recommend a stringent quality control for biologic scaffolds and the focus on synthetic (surface modified) materials for a regenerative application.

The role of the renin-angiotensin system in cardiovascular diseases

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Activation of the renin angiotensin aldosterone system (RAAS) is a well described feature in cardiovascular diseases, such as hypertension and heart failure. Inhibition of the elements of RAAS, such as angiotensin converting enzyme (ACE) delays the progression of heart failure, improves 5-year mortality by about 20%.

We have studied the role of RAAS in mREN2 rats and in human. The mREN2 rats develop a fulminant hypertension at an early phase of life (at about 10 weeks). Signs of cardiac hypertrophy and diastolic dysfunction were detected by echocardiography at the age of 15 weeks. Ageing of the rats was accompanied by the appearance of systolic dysfunction in 1 year old rats. This was paralleled by a high mortality rate in male rats. Measurement of the RAAS in these conditions revealed an increase in circulating ACE2 activities, and a decrease in heart tissue located ACE2 activities, suggesting a re-distribution of this enzyme.

These animal data were tested in human. ACE2 activity was higher in hypertensive patients than that in normotensive individuals. Moreover, the activity of ACE2 further increased by the occurrence of systolic left ventricular dysfunction in human.

ACE2 was considered to be a passive partner in the RAAS, until recently, when we have showed that the ACE is under endogenous regulation. ACE was found to be inhibited the serum albumin, similarly to ACE inhibitory medication. This finding suggested that the slow angiotensin 1-8 formation by ACE may be further limited by fast conversion to the inactive angiotensin 1-7 by ACE2.

The balance of ACE and ACE2 determines the activity of the RAAS. Limited ACE activity is maintained by endogenous inhibition of the enzyme. However, the angiotensin 1-8 inactivating ACE2 becomes dysregulated in heart failure, potentially contributing to the development and progression of the disease.

Our data suggest that dysregulation of ACE2 expression and shedding is the first step in the pathological activation of the RAAS in heart failure. Further directed studies may identify ACE2 dysregulation as a new pharmaceutical target in heart diseases.

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Direct measurement of Factor Xa activity in human blood.

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Clotting Factor Xa is the first member of the common pathway of the coagulation cascade. It is activated by factor IX, VII and their co-factors. Once activated it cleaves Prothrombin to Thrombin which in turn converts soluble Fibrinogen into insoluble Fibrin which binds platelets to form the clot. Specific Factor Xa inhibitors such as rivaroxaban and apixaban have been developed and are used in the clinical practice.

Here we made an effort to set up a method to directly measure Factor Xa activity, to aid the clinical application of its inhibitors.

The assay consists of two stages. In stage one Factor X is activated by Russle's viper venom (RVV) in the presence of Calcium. In the second step the activated Factor X (FXa) hydrolyses the chromogenic substrate Z-D-Arg-Gly-Arg-pNA (S-2765), thus liberating the chromophoric group pNA (p-nitroaniline). The kinetics of conversion is followed at 405nm for 1 hour 40 minutes using varying dilutions of patient plasma.

Plasma was collected from seven patients with hypothyroidism, high risk pregnancy, hepatorenal syndrome, uraemia with syncumar therapy, syncumar therapy alone and patients taking rivaroxaban.

The Factor X activity was in the range of 0.5-1 AU/min in all patients at 32-fold dilution, except the patients with direct Factor X inhibitors, where the activity was 0.3-0.5 AU/min. In all cases the activity increased with further dilution. In particular, the Factor X activity was in the range of 0.9-4.3 AU/min at a dilution of 4096-fold.

Our data suggest that direct measurement of Factor X activity can be used as an objective measure of clinical effectiveness of medical therapy.

P21-activated kinase inhibits vascular reactivity via inhibition of MLCK

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Changes in vascular reactivity contribute to the pathology of a number of different diseases including heart failure, hypertension, severe trauma, and sepsis. We have shown that P21-activated kinase 1 (PAK1) can alter smooth muscle contractile activity, and P21-activated kinase has been implicated in altering vascular reactivity.

The objective of this study was to determine the mechanism by which PAK1 regulates smooth muscle contractility to alter vascular reactivity. The effects of PAK activation and inhibition on basal and agonist-induced aortic contraction in aortic rings was measured. We also measured the effects of PAK activation on the phosphorylation of myosin light chain (MLC) and the myosin targeting subunit of MLC phosphatase (MYPT1), and on MLC kinase (MLCK) activity in primary human aortic smooth muscle cells (ASMC). Finally, the effects of increased mechanical stimulation on PAK activation, MLC phosphorylation, and MLCK activity were determined in ASMC.

PAK activation inhibited both basal tone and agonist-induced contraction of aortic rings. Conversely, PAK inhibition increased agonist-induced contractions. In ASMC, PAK activation inhibited MLC phosphorylation and PAK inhibition increased MLC phosphorylation. However, PAK activation increased MYPT1 phosphorylation in ASMC. Increased mechanical stimulation of ASMC increased PAK activation and decreased MLC phosphorylation and MLCK activation.

These data suggest that PAK inhibits vascular reactivity via inhibition of MLCK. These findings are in contrast to our previous findings in intestinal smooth muscle cells where PAK inhibits MLC phosphorylation via decreased phosphorylation of MYPT1.

Discordant resting and hyperemic pressure gradients in relation to the coronary flow reserve

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Background: Fractional flow reserve (FFR) is considered as an appropriate tool for the selection of epicardial coronary lesions with intermediate severity for stenting, while coronary flow reserve (CFR) is considered as the true indicator of myocardial ischemia. Recently, non-hyperemic distal to proximal pressure ratios (RPR: resting pressure ratios) challenged the need for vasodilatation for clinical decision making.

Aims: We aimed to calculate CFR using the 3D coronary angiography parameters and the measured pressure gradients on the basis of fluid dynamic equations and comparison the hyperemic FFR and the basal RPR values with regard to the calculated CFR values.

Methods: FFR measurements were performed on 32 coronary arteries. The lumen of the interrogated vessel segments was reconstructed in 3D using a dedicated 3D QCA software package. The components of the pressure gradients due to laminar and “turbulent” flow were modelled by fluid dynamic equations. CFR was calculated on the basis of these equations and the measured pressure gradients.

Results: A close correlation was found between FFR and RPR values ($r=0.91$, $p<0.001$), however 8/32 cases demonstrated discordant results. There were also significant correlations between FFR and CFR values as well as between RPR and CFR parameters, but the latest demonstrated stronger relation ($r=0.33$, $p=0.066$ and $r=0.52$, $p=0.002$, respectively).

The diagnostic ability of RPR for the prediction of myocardial ischemia defined by $CFR<2$ was significantly higher than that of FFR in the receiver-operating characteristic analysis: the area under the curve was 0.85 vs 0.67 ($p=0.003$), respectively. The CFR was calculated to be higher in 5 cases with $RPR>0.90$ and $FFR<0.80$ than that in the discordant 3 cases with $RPR<0.90$ and $FFR>0.80$ (2.50 ± 0.71 vs 1.30 ± 0.07 , $p=0.05$).

Conclusion: We suggest that comprehensive evaluation of the resting and hyperaemic pressure gradients helps the appropriate classification of the functional state of an individual coronary vessel. Further studies warranted to clarify the relevance of the discordant FFR/RPR phenomenon.

Omecamtiv mecarbil prolongs the contraction and relaxation in isolated left ventricular cardiac myocytes

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Background: Left ventricular contractility is reduced in systolic heart failure. Omecamtiv mecarbil (OM) is a promising drug candidate, which is intended to be used in the treatment of systolic heart failure. OM directly enhance myocardial myosin ATPase activity, therefore increases cardiac contractility without any change in intracellular calcium levels. The aim of our experiments were to examine the effects of OM on contractility and calcium transients of canine left ventricular myocytes.

Methods: All experiments were performed at 37 °C on cardiomyocytes enzymatically isolated from canine left ventricle. Cell shortening measurements were used to assess contractility. Intracellular calcium levels were examined by Fura-2 dye.

Results: 10 µM OM did not change calcium transients evoked by 1 Hz stimulation. In our cell shortening experiments, OM concentration-dependently decreased the diastolic length of cardiac muscle cells. 1 µM OM increased cell shortening in all examined cycle lengths (2000, 1000 and 500 ms). 10 µM OM did not change fractional cell shortening, moreover in the case of 500 ms cycle length OM reduced this parameter. 1 and 10 µM OM prolonged the contraction and relaxation in all examined cycle lengths. In measurements at 500 ms cycle length some contractions have already begun prior to a complete relaxation.

Conclusion: Based on our measurements, OM did not change calcium transients. Our results indicate that OM concentration of 1 µM significantly increased the extent of cell shortening, which shows the positive inotropic effect of the agent on cellular level. However OM significantly reduced the resting cell length and prolonged both contraction and relaxation. These results indicate that OM may have an adverse effect on the diastolic ventricular filling, especially at higher heart rates. Therefore the therapeutic use of OM can be questionable.

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The C-terminal HRET sequence of Kv1.3 regulates gating rather than targeting of Kv1.3 to the plasma membrane

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Voltage-gated Kv1.3 channels are expressed in several cell types including immune cells, such as T lymphocytes. The targeting of Kv1.3 to the plasma membrane is essential for T cell clonal expansion and assumed to be guided by the C-terminus of the channel. In many Kv channels it has been described that the conserved HRET sequence right after the C-terminus regulates the membrane targeting of the channel and the channel conductance.

In this study we used wild-type (WT) and two point mutant (A413V: fast inactivation kinetics, and H399K: tetraethylammonium-insensitive) phenotypes of Kv1.3 with full-length and truncated C-terminus, containing both extracellular FLAG-epitope and EGFP-tag. All mutations were introduced with the flanking PCR method. Ionic currents were measured in whole-cell and outside-out configuration of voltage-clamp patch-clamp technique. The membrane expression of the channels was verified by immunocytochemistry, i.e. labeling extracellular FLAG-epitope.

Using two point mutants of Kv1.3 with remarkably different features compared to the wild-type Kv1.3 we showed that both Kv1.3 channel variants target to the membrane when the C-terminus was truncated right after the conserved HRET sequence and produce currents identical to those with a full-length C-terminus. The truncation before the HRET sequence (NOHRET channels) resulted in membrane-targeting but non-functional phenotypes. NOHRET channels did not display gating currents, and coexpression with wild-type Kv1.3 did not rescue the NOHRET-A413V phenotype, no heteromeric current was observed. Interestingly, mutants of wild-type Kv1.3 without or with five alanine substituted for the HRET(E) motif expressed current indistinguishable from the wild-type.

These results demonstrate that the C-terminal region of Kv1.3 immediately proximal to the S6 helix is required for the activation gating and conduction, whereas distal region of the C-terminus is not required for trafficking of Kv1.3 to the plasma membrane.

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TRITON artificial tissue graft for small diameter application in cardiovascular procedures - preliminary results

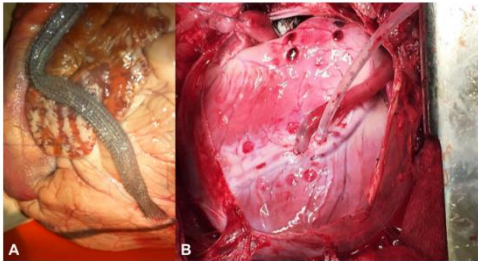
B. Winkler

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Background: An increasing number of people (up to 30% according to WHO report on cardiovascular diseases 2016) who require cardiac or vascular surgery or even a dialysis shunt cannot be provided with suitable autologous bypass material due to pre-existing diseases or already used bypass material. Existing artificial vascular prostheses have serious limitations. The demand for small-diameter (<4.5 mm) artificial grafts is very high. Aim of this project is to design, evaluate a novel technique of an alloy based scaffold- anti-thrombogenic composite tissue graft for replacement strategies in cardiac surgical procedures.

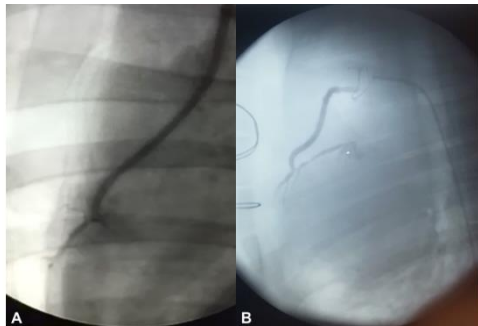
Materials and Methods: Mechanical issues: Implantation of artificial vascular graft with supporting 4.0 and 4.5 mm mesh scaffolds with a length of 150mm and burst strength > 800 mm Hg. All grafts were autoclaved according to hospital standards (University Hospital of Basel – USB) and stored pre-implantation 3 months in sterile bags

with normal saline solution (NS) at room temperature ranging from 20 to 22 °C (68 to 72 °F). Transport in same environment (Figure 1. A). Implantation: In-vivo porcine models were anaesthetized and intubated. The artificial vascular grafts were implanted off-pump without the use of extracorporeal circulation to the left coronary artery (LAD- left anterior descending) with proximal anastomoses to the ascending aorta (Figure 1. B). Standard off pump dose of heparin, protamine reversal, standard sternal and wound closure.



Results: Coronary angiographies postoperative: Initial postoperative angiography of the artificial graft bypass to

the LAD showing patency with distal outflow and excellent results without dissection or no narrowing at the distal anastomoses (Figure 2. A). Clinical course: All porcine models recovered fully within 24h hours, taking solid food and water 24h after intervention. Despite the inert anti-thrombogenic scaffold material for the first series aspirin is administered in usual dosage. No bleeding or signs of infection over 4 weeks. Follow-up: Coronary angiography at 4 weeks after implantation revealed patent flow in artificial graft bypass graft without any signs of burst or dissection (Figure 2. B).



Immuno-histological staining and graft examination: The explanation with macroscopic and histological analysis was performed 4 weeks after the initial implantation. All grafts were excised with attached heart and aorta segments and transferred in OCT. No signs of burst or dissection and no haematomas were discovered.

Conclusion: The provision of artificial grafts, in particular small-diameter artificial grafts, is highly desirable, in order to provide means of an optimal therapeutic artificial vascular graft, which can be used for a cardiovascular bypass operation for patients lacking suitable autologous bypass material. It is an object of the present project to improve on the mentioned state of the art (saphenous vein graft), in particular to provide safe and efficacious artificial grafts, which could be used instantly after unpacking, without the limitations of the existing artificial grafts.

Placental growth factor-based therapy for chronic ischemic cardiomyopathy.

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Introduction: The prevalence of chronic heart failure increases with better survival rate of acute coronary events and demographic aging. Limitations of current revascularization and device therapies for chronic ischemic myocardial dysfunction necessitate alternative strategies. Placental growth factor (PIGF) has pleiotropic effects on endothelial, myeloid progenitor and smooth muscle cells during collateral growth. Albeit PIGF does not affect quiescent vessels in healthy organs, angiogenic therapy carries a risk of stimulating atherosclerotic plaque growth. We investigated whether sustained infusion of recombinant human (rh) PIGF splice variant 2 improves myocardial perfusion and left ventricular (LV) function in a porcine model of chronic ischemic cardiomyopathy (ICMP) and evaluated the risk-benefit ratio of rhPIGF2 administration in atherosclerotic mice with ICMP.

Methods: In domestic pigs, myocardial ischemia was induced using a flow-limiting stent in the LAD. Animals with confirmed myocardial dysfunction were randomized to receive continuous systemic infusion of rhPIGF2 or vehicle (VEH) for 14 days. At 8 weeks, we measured hemodynamics, contractile function and regional perfusion at rest and during stress using MRI and evaluated neovascularization post mortem.

Apolipoprotein E-deficient mice were maintained on a high cholesterol diet and myocardial infarction was induced by transient LAD ligation at 4 weeks. At 8 weeks, we assessed LV function and randomized mice to receive rhPIGF2 or VEH subcutaneously for 28 days. At 12 and 20 weeks cardiac function was evaluated using echocardiography and invasive pressure-volume measurements followed by extensive histopathologic analyses.

Results: RhPIGF2 administration increased PIGF serum levels in pigs more than 63-fold without adverse effects. At 8 weeks, rhPIGF2 significantly improved the global function and enhanced perfusion in the ischemic region at rest and during adenosine hyperemia. Regional contractile function improved concomitantly at rest and during dobutamine stress. RhPLGF2 increased vascular density in the ischemic region.

In mice, sustained rhPIGF2 administration was tolerated without adverse hemodynamic or inflammatory side effects. RhPIGF2 did not increase plaque area, composition, or vulnerability in the aortic arch, but significantly improved ejection fraction and reduced end-diastolic volume indices with a concomitant increase in capillary and arteriolar density in ischemic myocardium.

Conclusions: In a clinically relevant porcine model of chronic ICMP, systemic rhPIGF2 administration significantly enhanced regional myocardial perfusion and induced a prominent recovery of cardiac function. In a murine ICMP with advanced atherosclerosis, rhPLGF2 improved LV function and remodeling without inducing plaque growth or instability. Development of PIGF-based therapy may represent a promising strategy in chronic ICMP.

Determining the target of cholesterol on the gating of voltage-gated potassium channels

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Introduction: Membrane lipids can affect the gating of voltage sensitive ion channels through different non-specific and specific mechanisms. It has been shown that cholesterol has remarkable effects on the gating of Kv1.3 (shift in voltage-dependency of activation and a slower rate of activation) but it is not clear whether these effects are mediated by the actions through the voltage sensor domain (VSD) or the pore domain (PD). Our aim was to investigate whether the major target of the action of cholesterol is the VSD, PD or the coupling between these two domains. To test the specificity of the effect we carried out our measurements on voltage-gated potassium channels with differing structures and gating mechanisms: Kv1.3 and the non-domain-swapped Kv10.1.

Methods: Current recordings were performed with voltage-clamp fluorometry (VCF) technique using a *Xenopus laevis* oocyte expression system. To monitor the movement of the VSDs of the channels the specific cysteine residues on the S3-S4 extracellular linker were labeled with MTS-TAMRA. The determination of single-channel conductance and open probability was carried out using patch-clamp technique. Cells were loaded with cholesterol complexed with methyl-beta-cyclodextrin.

Results: By VCF measurements we determined typical current parameters of the channels and F-V curves from the fluorescent signal in control and sterol loaded cells. In the oocyte expression system we were able to successfully reproduce the shift in voltage-dependence of activation and a slower rate of activation in the case of Kv1.3 and as a novel finding we obtained similar results with Kv10.1. We found no voltage shifts in the F-V curves paralleling the G-V shifts in either ion channel, only the slopes of the F-V curves in the case of Kv1.3 were decreased. We could not observe any differences between the kinetics of the fluorescent signals of control and sterol loaded cells.

Conclusions: These results suggest that cholesterol exerts its effect by acting directly on the PD and/or the coupling mechanism, instead of influencing the VSD.

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Hydrogen sulfide inhibits calcification and osteoblastic differentiation of vascular smooth muscle cells

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Introduction: Vascular calcification is associated with cardiovascular morbidity and mortality, in which osteoblastic differentiation of vascular smooth muscle cells (VSMC) is implicated in the mineralization process. Phosphate (Pi) uptake through a sodium-dependent phosphate co-transporter, Pit-1, is essential for VSMC calcification and phenotypic modulation in response to elevated Pi. This process involves upregulation of core binding factor alpha-1 (CBF- α 1), a key regulatory transcription factor critical for the differentiation of osteoblasts, and its downstream transcript proteins including alkaline phosphatase and osteocalcin. Hydrogen sulfide (H₂S) has been recognized as a gas with important functions in the cardiovascular system produced endogenously by cystathionine γ -lyase in VSMC.

Methods and Results: We investigated the role that H₂S may play in VSMC mineralization and transition of VSMC into osteoblast like cells induced by high Pi. H₂S inhibited calcium deposition in the extracellular matrix of VSMC in a dose responsive manner, providing a significant inhibition at concentrations of ≥ 50 μ mol/L. In addition, H₂S also suppressed the induction of genes involved in osteoblastic transformation of VSMC; alkaline phosphatase, osteocalcin and CBF- α 1. Moreover, Pi-uptake and phosphate-triggered upregulation of the Pit-1 were also prevented by H₂S. More importantly, reduction of endogenous production of H₂S by inhibiting cystathionine γ -lyase enzyme activity in VSMC resulted in increased mineralization and phenotype transition of VSMC into osteoblast like cells. Low plasma levels of H₂S, associated with decreased cystathionine γ -lyase enzyme activity, were found in patients with chronic kidney disease receiving hemodialysis.

Conclusions: We conclude that H₂S is an inhibitor of calcification and osteoblastic differentiation of human VSMC via suppressing Pi-uptake by cells.

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