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PLENARY LECTURES (PL1 – PL5)

PL1

ANTIPLATELET AGENTS AS A NOVEL THERAPY OF HEART FAILURE DUE TO MYOCARDIAL INFARCTION

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BACKGROUND: Although different antiplatelet agents are used for the prevention of thrombosis and treatment of ischemic heart disease, very little information regarding therapeutic potential of these agents in heart failure is available.

OBJECTIVES: We investigated the effects of some antiplatelet agents such as sarpogrelate (SAR) and cilostazol (CIL) treatments on cardiac dysfunction, cardiac remodelling and subcellular defects in heart failure due to myocardial infection.

METHODS: Heart failure in rats was induced by including the coronary artery for 8 weeks and the drug treatment was started 4 weeks after inducing myocardial infarction.

RESULTS: Marked depression in cardiac output and ejection fraction as well as increases in heart rate, left ventricle (LV) thickness and LV volume in the infarcted animals were attenuated by SAR and CIL. Alterations in myofibril Ca^{2+}-ATPase, as well as myosin isozyme contents and gene expression in the failing heart were reduced by SAR and CIL. Likewise, changes in sarcoplasmic reticular Ca2+-uptake and release activities, Ca^{2+}-pump and Ca^{2+}-release protein content as well as their mRNA levels were attenuated by both drug treatments.

CONCLUSIONS: These results provide evidence that both SAR and CIL delay the progression of heart failure and improve cardiac function by attenuating cardiac remodelling, subcellular defects and abnormalities in cardiac gene expression. It is suggested that antiplatelet agents may prove to be a viable therapy for the treatment of heart failure.

Infrastructure support for this study was provided by the St Boniface Hospital Foundation, Winnipeg, Manitoba

PL2

SYSTOLIC AND DIASTOLIC CA: IN AND OUT OF CONTROL

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Calcium is the master controller of cardiac function. It needs to increase on each beat to trigger the heart to contract to pump blood. It must fall to low enough levels between beats so that the heart can relax to fill again with blood. Heart disease, the major killer world-wide, is associated with abnormal calcium signalling.

In this lecture, I will present an overview of cardiac calcium signalling. Most of the calcium that activates contraction comes from the sarcoplasmic reticulum (SR). I will describe how SR Ca content is controlled. The first half of the talk will concentrate on the simple, yet elegant, mechanisms that regulate systolic calcium. The remainder will focus on the control of diastolic calcium.

PL3

TARGETING CARDIAC FATTY ACID OXIDATION TO TREAT HEART FAILURE

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Heart failure is accompanied by significant alterations in cardiac energy metabolism. This includes a decrease in cardiac energy production due to decrease in mitochondrial oxidative metabolism. Glycolysis increases in heart failure in an attempt to compensate for the decrease in mitochondrial ATP production, although glucose oxidation rates decrease due to the decrease in mitochondrial oxidative capacity. This increase in glycolysis and decrease in glucose oxidation results in an uncoupling of glucose metabolism in the failing heart, resulting in an increased production of protons and a decrease in cardiac efficiency. One potential approach to improve the coupling of glucose metabolism is to inhibit fatty acid oxidation in the heart, which results in a parallel increase in glucose oxidation. One approach to inhibiting fatty acid oxidation is to inhibit malonyl CoA decarboxylase (MCD), which increases myocardial malonyl CoA levels, that results in an inhibition of mitochondrial fatty acid uptake and oxidation. We examined whether MCD inhibition could be used as an approach to inhibit fatty acid oxidation and improve the coupling of glucose metabolism in the failing heart. Sprague-Dawley rats were subjected to a permanent left anterior descending coronary artery ligation, which resulted in a predictable heart failure development over the next 4 weeks. Rats were then treated with the MCD inhibitor CBM-3001106 (100 mg/kg/day for 3 weeks). This significantly improved cardiac function, as reflected by an enhanced left ventricular ejection fraction in vivo, and an increase in cardiac efficiency in ex vivo isolated working rat hearts. The improvement of cardiac function occurred in conjunction with a decrease in fatty acid oxidation rates and a decrease in proton production due to a better coupling between glycolysis and glucose oxidation. We conclude that MCD inhibition improves cardiac energy efficiency in the failing heart by decreasing the uncoupling of glycolysis from glucose oxidation and improving cardiac efficiency.

PL4

ANTIARRHYTHMIC DRUGS IN ATRIAL FIBRILLATION – DO WE KNOW WHAT TO TARGET?

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BACKGROUND: In the wake of demographic changes in Western countries, atrial fibrillation has reached epidemic levels, yet current strategies for drug treatment of arrhythmias lack sufficient efficacy and safety. Novel drug compounds that specifically target atrial functions, with no other cardiac or extracardiac actions, have been developed.

REVIEWED TOPICS: Most such atrial-selective drugs interact with ion channels, especially with potassium (K^+) channels that are either predominantly expressed in the atria or possess electrophysiological properties distinct in atria from ventricles. These channels include the ultra-rapidly activating, delayed outward-rectifying Kv1.5 channel conducting IKur, the acetylcholine-activated inward-rectifying Kir3.1/Kir3.4 channel conducting IK,ACh, the Ca^{2+}-activated K^+ channels of small conductance (SK) conducting ISK, and the two pore domain K^+ (K2P) channels TWIK-1, TASK-1 and TASK-3 that are responsible for voltage-independent background currents ITWIK-1, ITASK-1, and ITASK-3. Direct drug effects on these channels are described and their putative value in treatment of atrial fibrillation is discussed. In addition, indirect drug actions on post-translational regulatory processes, e.g. trafficking or phosphorylation, might have therapeutic potential. Since ion channels are part of macromolecular complexes, they could also be modified indirectly through their partners within the complex.

CONCLUSIONS: Although many potential drug targets have emerged in the process of unravelling details of the pathophysiological mechanisms responsible for atrial fibrillation, we do not know whether novel antiarrhythmic drugs will be more successful when modulating many targets or a single specific one. The answer to this riddle can only be solved in a clinical context.

PL5

MYOCARDIAL AND VASCULAR PROTECTION BY PARP INHIBITORS

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BACKGROUND: Poly(ADP-ribose)polymerase-1 (PARP-1) is an abundant nuclear enzyme. Its stress related over-activation - by altering nuclear NAD+ metabolism and cellular signalling cascades-, contributes to various cardiovascular pathologies.

OBJECTIVES: Our workgroup evaluated the cardiovascular protective effects of pharmacological PARP-1 inhibition against chronic hypertension or ischemia/reperfusion (I/R)-induced oxidative stress in ex vivo and in vivo models.

RESULTS: In Langendorff-perfused hearts, PARP-1 inhibition preserved mitochondrial function and integrity in I/R and isoproterenol-induced oxidative cellular damage, via decreasing ROS release and maintaining proper mitochondrial function. Pharmacological PARP-1 inhibitors can exert protective effect not only against intensive oxidative stress-induced cardiomyocyte death in acute scenarios, but also against chronic low intensity oxidative damage related cellular dysfunction in the cardiac wall and vascular components. In a rat model of chronic hypertension, PARP-1 inhibition suppressed cardiac hypertrophy and the accumulation of fibrotic components, this way attenuated the process of cardiac remodeling. Moreover, PARP-inhibition can slow down the transition of hypertensive cardiomyopathy to manifest heart failure in the same model. The cellular background of these beneficial effects can be the activation of pro-survival AKT node and interference with the stress related MAPK signaling cascade.

CONCLUSION: By preserving cellular NAD+ homeostasis and attenuating stress related signaling events, PARP-1 inhibition is a feasible way to slow down or prevent progression of cardiovascular pathologies connected to altered hemodynamics-induced oxidative stress.

ORAL PRESENTATIONS (O1 – O41)

01

NOVEL MECHANISMS UNDERLYING INSTABILITY OF PLAQUES IN ATHEROSCLEROTIC ARTERIES

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BACKGROUND: Unstable/vulnerable plaques are characterized by a thin cap fibroatheroma and necrotic core, and may result in serious clinical conditions, including transient ischemic attack, stroke, aphasia, and other motor defects. Although inflammation, apoptosis of plaque vascular smooth muscle cells (pVSMCs) and increased degradation of extracellular matrices are the primary events in plaque instability, the precise mechanism of plaque rupture remains to be defined.

OBJECTIVES: Triggering receptor expressed on myeloid cells-1 (TREM-1) expressed on immune cells amplify inflammation. In this study, we examined the role of TREM-1 in the pathogenesis of unstable plaques.

METHODS: We examined the expression of TREM-1, TREM-2, matrix metalloproteinases (MMPs) and collagen in carotid endarterectomy tissues of symptomatic (S) and asymptomatic (AS) patients with carotid stenosis. We also developed the swine model of unstable and stable plaques and examined the role of TREM-1.

RESULTS: We discovered increased expression of TREM-1, MMP-1 and MMP-9 and decreased Col I(α1) and Col III(α1) in unstable compared to stable plaques and their respective pVSMCs from patients with carotid stenosis. Stimulation of pVSMCs with TNF-α further increased the mRNA transcripts of TREM-1, MMPs, Col I(α1) and Col III(α1). TREM-1 antibodies or TREM-1 siRNA attenuated TNF-α induced expression of MMP-1 and MMP-9 and Col I(α1) and Col III(α1) in S compared to AS pVSMCs. Inhibition of NF-kB, JNK and PI3K signaling pathways decreased the expression of TREM-1, MMP-1 and MMP-9 in TNF-α treated pVSMCs isolated from S compared to AS patients. In swine model, we found histological and immunohistochemical parameters similar to unstable and stable plaques in human, including increased expression of TREM-1, high density of CD86+ M1 macrophages and low density of CD206+ M2 macrophages in unstable plaques.

CONCLUSION: Collectively, these data suggest that selective blockade of TREM-1 could be a novel therapeutic approach to stabilize vulnerable atherosclerotic plaques.

O2

BATHING IN CO_2-ENRICHED WATER ALTERS PROTEIN EXPRESSION IN KERATINOCYTES OF SKIN TISSUE

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BACKGROUND: Beneficial effects of balneotherapy using naturally occurring carbonated water (CO_2 enriched) have been known since the Middle Ages. Although this therapy is clinically applied for peripheral artery disease and skin disorder the underlying mechanisms are still not known.

METHODS: Under controlled conditions rats were bathed in either CO2 enriched water (CO_2 content 1200 mg/L) or tap water for 10 min. daily over 4 weeks. Proliferation activity was assessed by Ki67 immunohistochemistry of the epidermis of the abdomen and the capillary density was assessed by immunodetection of isolectin positive cell. Using cryo-fixed abdominal skin epidermis, follicle cells and stroma tissue containing capillaries were separately isolated by means of laser microdissection and subjected to proteomics analysis using label-free technique. Differential expressed proteins were validated with immunohistochemistry.

RESULTS: Proliferation activity of epidermal cells was not significantly different in the epidermis after bathing in CO_2-enriched water and also

capillary density has not been changed. Proteomics analysis revealed in the different tissues analyzed up to 36 significantly regulated proteins. Based on the best expression profiles 10 proteins were selected for immunohistochemical validation. Only one protein, far upstream element binding protein (FBP2), was similarly down regulated in the epidermis after bathing in CO_2-enriched water in both applied techniques. Low FBP2 expression was associated with low c-myc immune-expression in keratinocytes.
CONCLUSIONS: Long-term bathing in CO_2-enriched water showed a cellular protein response of epithelial cells in the epidermis which was detectable by two different methods. However, differences in proliferation activity or capillary density were not detected at least in the normal skin.

O3
ALTERED MYOFILAMENT PROTEIN PHOSPHORYLATION PATTERN CONTRIBUTES TO INCREASED RIGHT VENTRICULAR PASSIVE STIFFNESS IN A RAT MODEL OF POST-ISCHEMIC HEART FAILURE

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BACKGROUND: In a rat model of left ventricular (LV) myocardial infarction (MI)-induced heart failure (HF), recently, we found unchanged cardiomyocyte Ca-sensitivity and increased passive stiffness of RV cardiomyocytes. RV cardiomyocyte mechanical performance seems to be dependent of changes in titin, myosin binding protein C (cMyBP-C) and troponin I (cTnI) phosphorylation.
OBJECTIVES: In this study, we aimed to: 1) investigate the phospho status of titin, cMyBP-C and cTnI 2) correlate it with different kinase activities, 3) investigate the role of collagene as a potential factor in the mechanism of RV remodeling.
METHODS: RV samples from failing rat hearts were studied after 8 weeks of LV MI. Sham-operated animals served as controls. Titin and cMyBP-C phosphorylation were tested on isolated skinned cardiomyocytes, while PKA, CaMKII, PKG and PKC kinase activities were measured in RV homogenates. Collagene 1a1 and 3a1 gene expression was estimated by PCR.
RESULTS: The total phosphorylation of titin and cMyBP-C was comparable in the RV of HF and Sham animals, however hypophosphorylation of titin at S3991, S4080 and S12884, as well as cMyBP-C at S282 were found in the RV of HF animals (by 23%, 20%, 30% and 45% respectively). Total and S23/24 phosphorylation of cTnI was also decreased in HF by 45%. PKA, CaMKII and PKG activities were lower in the HF samples compared to Sham (by 63%, 62% and 52% respectively), while PKC activity doubled. In vitro treatment with either CaMKII or PKG restored RV cardiomyocyte passive stiffness in the HF group. HF did not affect collagene expression in the RV.
CONCLUSIONS: Following LV MI, changes in phosphorylation pattern of titin mediated by decrease in PKA, CaMKII and PKG activity as well as increased PKC activity might contribute to increased passive stiffness in the RV. Collagene expression apparently does not play a role in RV remodelling.

O4
POTENTIAL EFFECTS OF QUERCETIN IN CARDIOPROTECTION

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BACKGROUND: Quercetin is a plant flavonoid that possesses various biological effects including anti-oxidative, anti-inflammatory, anti-coagulation, and oxygen radical-scavenging activities. It is also proposed to exert beneficial effects in cardiovascular system. However, its effects in cardiac ischemia/reperfusion (I/R) injury were not explored extensively.
OBJECTIVES: The aim of the present study was to explore the ability of quercetin to prevent cardiac I/R injury in different experimental settings including its acute and chronic administration. The impact of age and doxorubicin co-treatment was analyzed as well, in the isolated rat heart model of I/R injury. Additionally, involvement of selected intracellular signaling pathways in quercetin action has been investigated.
METHODS: Quercetin was applied either directly to perfusion solution (acute treatment; 15μmol/L), or orally to the rats before the hearts were excised and perfused (chronic treatment; 20mg/kg/day for 4 or 6 weeks, respectively). Chronic treatment was tested in juveniles, adults, and doxorubicin co-treated rats.
RESULTS: Quercetin exerted cardioprotective effects against I/R injury in both acute and chronic settings; in the chronic administration, its cardioprotective effect was age-dependent. Moreover, the duration of treatment has been also shown to be crucial for induction of cardioprotection. Finally, quercetin was cardioprotective also in the hearts of doxorubicin-treated rats. Protective effects of quercetin were manifested mainly by better post-ischemic recovery of heart contractile function in all experiments. Cardioprotective effects of quercetin were linked to activation of Akt/GSK-3β/β-catenin pathway, increased level of connexin-43 and enhanced superoxide dismutase (SOD) activity in the heart tissue.
CONCLUSIONS: Quercetin exerts cardioprotective effect in I/R injury; however, its efficiency depends on the age of animals and duration of the treatment. On the other hand, doxorubicin co-treatment does not abolish its cardioprotective effect. Our data suggest involvement of PI3K/Akt signaling, connexin-43 and SOD activation in the cardioprotective effects of quercetin in I/R injury.
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O5
EFFECT OF STATINS ON VITAMIN D METABOLITES: IMPLICATIONS FOR PHARMACOTHERAPY OF CARDIOVASCULAR DISEASES

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BACKGROUND: Recent studies suggest that vitamin D deficiency plays an important role in the pathogenesis of cardiovascular diseases. Whereas severe vitamin D deficiency resulting in abnormalities of calcium metabolism is rare, mild-to-moderate deficiency is common due to restriction of alimentary fat intake and current lifestyle associated with limited exposure to sunlight. Statins (3-hydroxy 3-methylglutarylcoenzyme A reductase inhibitors) are commonly used in the prevention and treatment of cardiovascular diseases. Previously, we have demonstrated that various statins have different effects on 25-(OH)- and 1,25-(OH)2-vitamin D levels (*Atherosclerosis* 2011; 219: 526-31). Herein, we examine the mechanism of these effects.
METHODS: Male rats received atorvastatin (20 mg/kg/day) or pravastatin (40 mg/kg/day) for 3 weeks. Then, the level of vitamin D metabolites and the expression of enzymes involved in its synthesis/metabolism were measured in the liver and kidney.
RESULTS: Atorvastatin reduced plasma concentration of 25-(OH)-D3 but had no effect on 1,25-(OH)2-D3. In contrast, pravastatin increased blood concentration of both metabolites. The effect of atorvastatin on 25-(OH)-D3 was not reversed by mevalonate suggesting that it was independent of HMG-CoA reductase inhibition. In addition, atorvastatin had no effect on the expression of hepatic vitamin D 25-hydroxylases, CYP27A1, CYP2J3, CYP3A4 and CYP2R1. However, in vitro, atorvastatin interfered with conversion of vitamin D to 25-(OH)-D3 by isolated liver microsomal fraction or recombinant CYP3A4. Pravastatin increased CYP27A1 expression in the liver but had no effect on CYP27B1 (25-(OH)-D3 1-alpha-hydroxylase) in the kidney. In addition, pravastatin infused into the renal artery reduced 24,25-epoxycholesterol (24,25-EC) and increased 1,25-(OH)2-D3 formation, and its effect on 1,25-(OH)2-D3 was reversed by 24,25-EC. In vitro, 24,25-EC reduced conversion of 25-(OH)-D3 to 1,25-(OH)2-D3.

CONCLUSION: Atorvastatin reduces 25-(OH)-D3 synthesis by competing with vitamin D for CYP3A4 in the liver. In contrast, pravastatin increases the expression of CYP27A1 and 25-(OH)-D3 synthesis in the liver. In addition, pravastatin increases conversion of 25-(OH)-D3 to 1,25-(OH)2-D3 by inhibiting renal synthesis of oxysterols being CYP27B1 substrates. These results represent a new mechanism of pleiotropic effect of statins which may be involved in cholesterol-independent effects of these drugs in the cardiovascular system. In addition, despite having similar effects on plasma lipids, various statins have different effects on vitamin D status. The results of this study may be useful in selecting specific statin depending on vitamin D level in a given patient.

O6
CLINICAL EVIDENCE FOR THE BENEFITS OF MEDITERRANEAN-TYPE DIET AND LIFESTYLE MODIFICATIONS IN THE PREVENTION OF CARDIOVASCULAR DISEASES

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The incidences of mortality and disability due to cardiovascular diseases (CVDs) are escalating worldwide. While CVDs and obesity generally manifest in middle age and beyond, it is now recognised that risks of these diseases originate from fetal programming and are manifested in childhood and adolescence. Maternal over nutrition and diabetes are considered risk factors for the offspring obesity, type 2 diabetes and CVDs. Sugar-loaded beverages and excessively salted foods are also potential risk factors in children and adults. Many studies suggest that dietary patterns and lifestyle factors play a significant role in the globalization of obesity, diabetes mellitus, cancer, atherosclerosis, hypertension, myocardial infarction and stroke. There is an overwhelming evidence that Mediterranean-type diets rich in whole grains, fruits and vegetables, legumes, olive oil, fish and omega-3 fatty acids, low-fat dairy products, and moderate red wine consumption are linked with improved serum lipid concentrations and lower incidence of CVDs. Lifestyle modifications such as regular physical activity (about 30 min/day), restriction of caloric and sodium intake, smoking cessation and moderate alcohol consumption are recommended for improving cardiovascular health and quality of life. Emerging evidence suggests that diets containing flavonoids, carotenoids, antioxidants and anti-inflammatory agents decrease oxidative stress and consequently reduce the risk of CVDs, cancer, and chronic diseases multifactorial in origin. This presentation will provide a general overview on the benefits of Mediterranean-type diets, lifestyle changes, and the impact of altered gut microbiota in CVDs.

O7
DEVELOPMENT OF NOVEL THERAPEUTICS FOR CARDIAC FIBROSIS

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BACKGROUND: Cardiac fibrosis – the excessive production of extracellular matrix – arises secondary to numerous cardiovascular disorders including hypertension and diabetes. Fibrosis significantly impairs cardiac contraction and relaxation, facilitates arrhythmogenesis, and increases patient morbidity and mortality. To date, however, no specific treatments have been approved for treatment of cardiac fibrosis.
OBJECTIVES: We previously identified the transcription factor scleraxis as a novel and potent regulator of cardiac collagen production. We therefore determined whether interference with scleraxis function attenuates cardiac fibrosis.
METHODS: In primary cardiac fibroblasts, we assessed the effect of scleraxis knockdown or knockout on basal or TGFβ-stimulated extracellular matrix gene expression and myofibroblast phenotype conversion. We determined the mechanism of scleraxis-mediated gene transcription of putative target genes using electrophoretic mobility shift and chromatin immunoprecipitation assays and site-directed mutagenesis. We examined the effect of scleraxis gene knockout on pressure overload-induced cardiac fibrosis.
RESULTS: Scleraxis was sufficient and required for expression of myriad extracellular matrix genes including collagen 1α2, α-smooth muscle actin and fibronectin, and for myofibroblast conversion. Matrix expression was attenuated by scleraxis knockout, knockdown or mutation of key phosphorylatable amino acids in scleraxis, and TGFβ/Smad3-mediated gene regulation was inhibited by scleraxis loss. Scleraxis regulated gene expression by direct binding to E-boksz sequences within the target promoters. In vivo, scleraxis loss significantly attenuated fibrotic gene expression.
CONCLUSIONS: Scleraxis is required for extracellular matrix gene expression, and works primarily by direct target gene promoter transactivation. Interference with scleraxis activity or expression attenuates basal and fibrotic matrix synthesis. Scleraxis thus represents an exciting new target for the development of anti-fibrotic therapies.
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O8
MECHANISMS OF ATRIAL FIBRILLATION: LESSONS FROM A 20-YEAR EXPERIENCE OF TRANS-CATHETER ABLATION (REVIEW)

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Traditionally, atrial fibrillation (AF) has been thought to be sustained by multiple wavelets as proposed by Moe in the 1950s. Accordingly, the first surgical, then trans-catheter interventions aiming at a long-term maintenance of sinus rhythm (SR) in patients have been designed to compartmentalize both atria by creating long insulating linear lesions thereby precluding a critical number of multiple wavelets to operate simultaneously. With the recognition of focally initiated (or maintained) AF (Haissaguerre et al), the ablation target was shifted to the pulmonary veins as the predilection sites of trigger foci in the majority of cases. The favorable clinical outcome in thousands of patients who underwent pulmonary vein isolation (PVI) procedures proved this concept. However, it has also been realized, that multiple mechanisms may be responsible for different or even for similar clinical manifestations of the arrhythmia and also at different stages of the disease process. Long-term freedom from AF can not be achieved by PVI alone in about 1/3d of of paroxysmal AF and success rates are even lower in the more persistent forms. The concept of rotating reentry without the need for an anatomical obstacle was proposed decades ago and demonstrated in the atria of rabbits by Allessie. Recent clinical data obtained by 3-dimensional electro-anatomical mapping using a basket catheter have suggested that several, spatially stabile rotors in both atria are driving AF and RF ablation of the core of them results in an acute AF termination and long-term freedom from the arrhythmia in 80% of the cases. These initial results await confirmation. Our ability to detect individual disease mechanisms underlying the arrhythmia in individual patients seems to be essential to achieve a significant improvement in trans-catheter treatment of this disease of multifactorial etiology.

O9
PREDICTORS OF PROGRESSION OF CORONARY HEART DISEASE IN THE CLINICAL SETTING: LIFE IS NOT MATHEMATICS

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The importance of conventional risk factors in cardiovascular risk assessment is well known from the Framingham cohorts. Nonetheless, in recent years, researchers consider that some combinations of existing and/or emerging biomarkers could serve as an adjunct to conventional risk assessment, at least in a subset of populations. Multimarker strategies have been evaluated in a number of epidemiologic studies. The predictive performance of inflammatory and hemostatic biomarkers has been evaluated in nested case-control studies or in cohort studies. Five biomarkers were

significantly associated with coronary heart disease after adjustment for conventional risk factors: interleukin-6, D-dimer, factor VII, von Willebrand factor, and homocysteine. Taking into account the intricate pathophysiological mechanisms of atheromatosis, genetic biomarkers have also made their way into predictive algorrhythms, and studies continued to bring in contradictory results.
Although most multimarker studies suggest that biomarkers add relatively little on top of conventional risk factors, there are notable exceptions. Identification of new and more specific biomarkers of cardiovascular risk is a challenging topic of research. This paper has the intention to address following 'burning' questions: 1. Which are the best biomarkers in order to detect additional risk? 2. Which is the best statistical strategy to confirm these choices, given the high costs implied? 3. Can we accommodate the complex variability of life to mathematics, or are we at loose ends in our present-day paradigm?

O10
ROLE OF MITOCHONDRIAL INTEGRITY AND NETWORK DYNAMICS IN REGULATION OF CELL DEATH

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BACKGROUND: Mitochondria actively contribute to cell death through mechanisms including mitochondrial membrane systems' integrity loss, resulting in swelling and disruption of the mitochondria or apoptosis via the release of intermembrane space proteins. These events are often accompanied by fission of the organelle, thus linking mitochondrial dynamics to apoptosis.
OBJECTIVES: We intended to demonstrate that Cyclophylin D (CypD)-regulated mitochondrial permeability transition (mPT) plays critical role in inflammatory reprogramming of gene expression, a major contributor to lethality in septic shock. Additionally, we studied effects of the anti-diabetes drug candidate BGP-15 on mitochondrial fusion.
METHODS: We used the following models; (i) C57BL/6 wild type and CypD deficient mice treated or not with a single dose of intraperitoneal bacterial lipopolysaccharide (LPS; 40 mg/kg), and (ii) hydrogen peroxide and BGP-15 (both 50 μM for 4 h) treated wild type and dynamin-like 120 kDa protein/OPA1 silenced cells.
RESULTS: In the LPS experiment, besides a survival rate of 75% vs. 0% of the wild type, in the CypD deficient mice we found a marked decrease in the expression of 966 genes including that of several transcription factors associated with oxidative stress, inflammation and apoptosis, which were significantly up- or down-regulated in septic shock. We verified our key mRNA findings at the protein and/or functional level. In the mitochondrial network dynamics experiments, we found that BGP-15 attenuated oxidative stress-induced mitochondrial fragmentation, promoted fusion, induced Opa 1 polymerization, facilitated protein kinase B/Akt activation thereby enhanced surface expression of Glut4 glucose transporter. All these effects were abolished in Opa 1 silenced cells.
CONCLUSIONS: Our results suggest a novel role for mPT, namely, massive modulation of inflammatory gene expression. Concerning mitochondrial network dynamics, Opa1 polymerization is a novel mechanism which contributes to cristae membrane stabilization and activates inner membrane fusion, and so it can be advantageous in mitochondria-related diseases.

O11
CELL-BASED PLATFORMS FOR CARDIOPROTECTION

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BACKGROUND: Despite the extensive research in the treatment of reperfusion injury after myocardial infarction, the breakthrough therapeutic intervention is still missing. However, there are several endogenous protective mechanisms, which could minimize the damage. The beneficial effect of both ischemic and pharmacologic conditioning has been established in in vivo and ex vivo animal models with no co-morbidities and co-medication. However, we still do not have cardioprotective drugs on the market, therefore, identification of valid drug targets for cardioprotection is of great importance. Here we provide an overview of in vitro cell-based experimental models that are suitable to study ischemia/reperfusion injury and cardioprotection.
METHODS: A simulated ischemia/reperfusion (SI/R) models were optimized in vitro in: neonatal and adult isolated cardiomyocytes; embryonic stem cell-derived (ESC) and induced pluripotent stem cell derived cardiomyocytes (iPS); 3D engineered heart tissues. SI/R system in neonatal cardiac myocytes was tested with well-known cardiocytoprotective molecules, such as NO-donors and B-type natriuretic peptide, and also with cardioprotective drug candidates such as MMP inhibitors and proteoglycans. Adult cardiac myocytes showed the highest sensitivity to SI/R injury among the tested cardiac myocyte models. The cardiocytoprotective NO donor protected embryonic bodies of ESC-derived cardiac myocytes against SI/R injury, but not iPS cell lines, suggesting that cells from embryonic origin are eligible but iPS-derived cardiac myocytes at this age are not suitable for testing cardiocytoprotective mechanisms. Engineered heart tissue provides 3D model systems, which is suitable for monitoring functional parameters in vitro during SI/R injury and cardiocytoprotection.
CONCLUSION: Cost effective in vitro cardiac myocyte-based experimental models are important for fast testing of potential cardioprotective mechanisms and drug candidates, however, these models needs to be further optimized to provide better predictive value.

O12
GENOME BASED DIAGNOSTICS AND THERAPEUTICS IN CARDIOVASCULAR COMPLICATIONS: ANY LIGHT IN THE TUNNEL?

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In spite of several developments with respect to the medications, management of cardiovascular complications and ineffectiveness of certain prime drugs available for the treatment is still challenging. The use of precision medicine allows the physician to provide a better therapy for patients in terms of efficiency, safety and treatment length, as well as to retardation of the progression of disease and complications. With the advent of state-of-the-art genomic and molecular profiling, it is possible to have biomarkers to determine not only the prognosis but also for the management and getting leads for the new drug development. There has been a remarkable growth in scientific publication on personalized medicine within the past few years but unlike, oncology success in the cardiovascular field has been limited. However, application of personalized medicine into clinical treatment has been very slow. Currently there is a trend to recommend genetic testing by USFDA and provide pharmacogenomic information on the labels of certain drugs. In many countries including India, as such data are scanty and no initiative is there for such recommendations by the drug authorities. Biomarker based development of diagnostics and drug discovery is one of the important, unexplored hidden potentials in such countries because of multi ethnical and genetically variant patient population. We have undertaken studies to develop biomarker panel for diabetic foot ulcer to retard the progression of the disease The development of potential biomarkers will using modern biotechnological tools will enable for early diagnosis of the stage of the diabetic foot ulcers which may lead to better treatment and thus preventing the trauma of amputations.

O13

UTILIZATION OF NEAR INFRARED SPECTROSCOPY IMAGING OF CHRONIC WOUND BY CO2 ENRICHED WATER FOOT BATHING TREATMENT

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BACKGROUND: In recent years, digital imaging analysis has been significantly developed and progressed to help more accurate and to support clinicians' decision in not only transplant surgeries but also chronic wound treatments. Near infrared spectroscopy imaging (Kent^{TM} Camera) has been developed for helping to assess blood flows by tissue oxygen perfusion on the transplanted organs as well as chronic wounds such as diabetic ulcers and bed sores.

OBJECTIVE: Using tissue oxygen perfusion change of before and after the treatment by CO_2-enriched water foot bathing and correlation to the healing progress were examined.

METHODS: Images of the patient's lower extremity or foot wounds were taken using multispectral oximetry imaging with near infrared spectroscopy camera (Kent^{TM} camera). The six patient cohorts consisted of two diabetic and four venous etiology wounds were used for this study as a part of Carbothera^{TM} CO_2-enriched foot bath clinical study at St Boniface Hospital, Winnipeg Manitoba, Canada. Images of tissue oxygen perfusion rates were analyzed before and after the treatment by monthly bases up to 4 months.

RESULTS: Visualization of tissue oxygen perfusion of the chronic wounds helped for the wound healing process and treatment decisions. Also, the area of poor blood flow (poor tissue oxygen perfusion) can be the target area of progressive treatment by debridement. More data accumulation is needed for diagnosis of chronic wound by tissue oxygen perfusion for wounds outcome.

O14

OXIDATIVE STRESS IN EXERCISE: POSSIBLE INTERVENTIONS WITH ANTIOXIDANTS FOR BETTER ADAPTATION

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Exercise increases production of reactive oxygen (ROS) and nitrogen species (RONS) via several mechanisms. Inter alia, increased blood flow during exercise exposes endothelial cells to shear stress, resulting in increased nitric oxide (NO) production. On the other hand, increased oxygen consumption or hypoxia during exercise, as well as, maximal workload induces increased production of ROS and RONS. Furthermore, dynamics of ROS and RONS production during exercise are not fully explored. This abstract is summary of our recent investigations in this field. We tried to examine the effects of long-term engagement in sports with different energy requirements (aerobic, anaerobic, aerobic/anaerobic) on oxidative stress parameters during progressive exercise test. Concentrations of lactates, nitrites (NO_2^-), superoxide anion radical (O_2^-) and TBARS as index of lipid peroxidation were determined in plasma of top level competitors in rowing, cycling and teakwondo. Results showed that sportmen had similar concentrations of lactates and O_2^- in rest. Nitrite concentrations in rest were the lowest in taekwondo fighters, while rowers had the highest levels among examined groups. The order of magnitude for TBARS level in the rest was: bicycling > taekwondo > rowing. During exercise at maximal intensity the concentration of lactate significantly elevated to similar levels in all tested sportsmen and they were persistently elevated during recovery period of 4 and 10 minutes. There were no significant changes in O2.-, nitrite and TBARS levels neither at the maximum intensity of exercise nor during the recovery period comparing to the rest period in examined individuals. Our results showed that long term different training strategies establish different basal nitrite and lipid peroxidation level in sportmen. However, progressive exercise does not influence basal nitrite and oxidative stress parameters level neither at maximal load nor during the first 10 minutes of recovery in sportmen studied. Examination of oxidative status in scuba divers showed significant increases in levels of NO2- and TBARS after the dive, while there were no statistically relevant changes in levels of O_2^-, H_2O_2, SOD and CAT, so we have shown that a dive with these characteristics only slightly disturbs redox homeostasis, without serious intermolecular changes that can lead to prominent oxidative stress.

O15

COMBINED EFFECT OF CLASS III ANTIARRHYTHMIC AGENTS AND CA^{2+}-ACTIVATED K^+ (SK) CHANNEL INHIBITION IN AN ISOLATED HEART MODEL OF ATRIAL FIBRILLATION

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BACKGROUND: Dose is an important parameter in terms of both efficacy and adverse effects in pharmacological treatment of atrial fibrillation (AF). Both of the class III antiarrhythmics dofetilide and amiodarone have documented anti-AF effects. While dofetilide has dose-related ventricular side effects and amiodarone primarily has adverse non-cardiac effects. Pharmacological inhibition of small conductance Ca2+-activated K+ (SK) channels has recently been reported to be antiarrhythmic in a number of animal AF models.

METHODS: In a Langendorff model of acutely induced AF on guinea pig hearts it was investigated whether a combination of the SK channel blocker ICA together with either dofetilide or amiodarone provided a synergistic effect.

RESULTS: The duration of AF was reduced with otherwise subefficacious concentrations of either dofetilide or amiodarone when combined with ICA, also at a subefficacious concentration. At a concentration level effective as monotherapy, dofetilide produced a marked increase in the QT interval. This QT prolonging effect was absent when combined with ICA at nonefficacious monotherapy concentrations.

CONCLUSION: The results thereby reveal that combination of subefficacious concentrations of an SK channel blocker and either dofetilide or amiodarone can maintain anti-AF properties, while the risk of ventricular arrhythmias is reduced.

ICA: N-(pyridin-2-yl)-4-(pyridin-2-yl)thiazol-2-amine.

O16

TETRALOGY OF FALLOT: MOLECULAR DEFECTS IN THE CARDIAC RIGHT VENTRICLE OUTFLOW

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BACKGROUND: Tetralogy of Fallot (TOF) is a cyanotic conotruncal congenital heart defect characterized by maldevelopment of the right ventricular outflow tract (RVOT) that results in infundibular stenosis. Altered development of second heart field cells (SHF) and neural crest cells (NC cells), may be involved in the molecular pathogenesis of Tetralogy of Fallot.

METHODS: In the present study, after obtaining written informed consent, RVOT myocardium were collected from patients with TOF and

donor healthy human hearts intended for transplantation. All patients with genetic syndromes and extra cardiac features such as developmental delay, facial abnormalities etc. which are subtle clinical pointers towards a syndrome were excluded from the study. The expression of candidate genes were analyzed and validated by quantitative RT-PCR. Tissue proteins were subjected to proteomics protein expression analysis by 1D nano- LC MS / MS. Immunoblot and immunohistochemistry experiments were performed to substantiate differential expression of selected proteins.

RESULTS: An increased expression of ISL1, a marker of multipotent progenitor cells that are derived from second heart field (SHF) and neural crest region was observed in cardiac tissues of all the patients. Expression of MEF2c, GATA4 and HAND2 were decreased in the tissues of patients with TOF. On gene expression analysis of retinaldehyde dehydrogenase 2 (ALDH1A2) and retinoid X receptor alpha, decreased expressions were seen indicating aberrant retinoic acid signaling in the myocardium of patients with TOF. In the proteomic analysis, the proteins that were differentially expressed in the infundibular tissues of patients with TOF were those related to muscle contraction, development, calcium signalling and gap junction assembly.

CONCLUSIONS: Premature differentiation of progenitor cells, their ectopic accumulation in the outflow tract and maladaptation of right ventricle to hemodynamic stress could contribute to the increased muscle mass and the resultant RVOT obstruction in TOF.

O17

CARDIOPROTECTIVE EFFECT OF CHRONIC HYPOXIA COMBINED WITH EXERCISE TRAINING

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BACKGROUND: Chronic hypoxia and regular exercise are natural stimuli that confer sustainable cardioprotection against ischemia/reperfusion (I/R) injury but their mechanisms are not fully understood. While the inflammatory response mediated by tumor necrosis factor-α (TNF-α) plays a role in the infarct size-limitation by chronic hypoxia, exercise is associated with anti-inflammatory effects.

OBJECTIVES: This study was conducted i) to determine whether exercise training performed under hypoxic conditions can act in synergy to further enhance myocardial ischemic tolerance and ii) to assess the role of inflammatory and redox signaling.

METHODS: Adult male Wistar rats were adapted to continuous normobaric hypoxia (CNH; 12% O2) for 3 weeks or kept in room air (normoxia). Exercise training (treadmill; stepwise up to 30 m/min for 60 min/day) started the 2nd week of CNH exposure. ELISA and Western blot, respectively, were used to quantify myocardial cytokines and the expression of TNF-α receptors, nuclear factor-κB (NF-κB) and selected components of related signaling pathways. Infarct size and arrhythmias were assessed in open-chest rats subjected to I/R.

RESULTS: CNH increased TNF-α and interleukin-6 levels and the expression of TNF-α type 2 receptor, NF-κB, inducible nitric oxide synthase (iNOS), cytosolic phospholipase A2α, cyclooxygenase-2, manganese superoxide dismutase (MnSOD) and catalase. None of these effects occurred in normoxic trained group, whereas exercise in hypoxia abolished or significantly attenuated CNH-induced responses, except for iNOS and MnSOD. Both CNH and exercise reduced infarct size but their combination provided the same degree of protection as CNH alone.

CONCLUSIONS: Exercise training does not amplify the cardioprotection conferred by CNH. Nevertheless, the high ischemic tolerance of the CNH hearts persists after exercise, possibly by maintaining NO formation and increased antioxidant capacity, despite attenuating TNF-α-dependent protective signalling.

O18

EARLY REPOLARIZATION SYNDROME

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The early repolarization (ER) pattern on the ECG is characterized by ≥ 0.1-mV J-point elevation in 2 contiguous inferior and/or lateral ECG leads. The J-point elevation may manifest as either slurring or notching of the end of the QRS complex. The prevalence estimates for the ER pattern in the general population are highly variable and range between 1% and 13%. Multiple contemporary studies have linked ER with an increased risk of arrhythmogenic sudden death. Early repolarization pattern has been associated with increased risk for ventricular tachycardia/fibrillation (VT/VF), particularly when manifest in inferior leads. Recent clinical studies found that: early repolarization pattern seems to be associated with ventricular tachyarrhythmias in the setting of acute myocardial infarction. Hypothermia has been reported to induce ventricular tachycardia and fibrillation (VT/VF) in patients with early repolarization pattern. The mechanistic basis of ventricular arrhythmogenesis in early repolarization syndrome (ERS) is presently incompletely understood. Furthermore therapy for ER syndrome remains suboptimal. Recent studies have demonstrated that gain of function mutations in KCNJ8, the gene responsible for the pore forming subunit of the ATP-sensitive potassium channel (KATP), is associated with ERS. Loss of function mutations in the α1, β2 and α2δ subunits of the cardiac L-type calcium channel (CACNA1C, CACNB2, and CACNA2D1) have also been identified as causative in patients with ERS. Our data support the hypothesis that ERS is caused by a preferential accentuation of the action potential notch in LV epicardium; this repolarization defect is accentuated by elevated vagal tone. Higher intrinsic levels of Ito account for the greater sensitivity of the inferior LV wall to development of VT/VF; quinidine and isoproterenol exert ameliorative effects by reversing the repolarization abnormality. Hypothermia leads to VT/VF in the setting of ER by exaggerating repolarization abnormalities, leading to development of phase-2-reentry. Quinidine, cilostazol and milrinone suppress the hypothermia-induced VT/VF.

O19

PDE5 INHIBITION IN PROTECTION OF DIABETIC HEART

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BACKGROUND: Obesity and insulin resistance lead to impaired nitric oxide (NO) bioavailability, oxidative stress, chronic inflammation, atherosclerosis and acute coronary syndromes. Hyperglycemia is associated with increased infarct size and higher risk of congestive heart failure in patients. Phosphodiesterase 5 (PDE5) inhibitors including sildenafil, vardenafil and tadalafil protect against myocardial ischemia/reperfusion (I/R) and ischemic cardiomyopathy. Since PDE5 inhibitors increase NO production and improve endothelial dysfunction, we hypothesized that chronic treatment with the long-acting PDE5 inhibitor, tadalafil would protect the diabetic heart against I/R injury.

METHODS: Leptin receptor null (db/db) mice underwent treatment with tadalafil (1 mg/kg) or 10% DMSO for 28 days. The hearts were isolated and subjected to 30 min global ischemia and 60 min reperfusion.

RESULTS: Tadalafil treatment significantly reduced fasting glucose and triglycerides. Infarct size was significantly lower in tadalafil treated mice as compared to the control. Circulating TNFalpha and IL-1beta were reduced following tadalafil treatment. Sirt1 is a histone deacetylase that regulates peroxisome proliferator-activated receptor gamma coactivator-1-alpha (PGC-1alpha) which is a master regulator of mitochondrial biogenesis and co-activator of transcription factors impacting energy homeostasis. Our results showed that tadalafil treated mice had significantly higher plasma levels of NO and increased expression of myocardial Sirt1as well as PGC1alpha. Furthermore, tadalafil treatment attenuated ROS production and improved mitochondrial dysfunction as demonstrated by preservation of oxidative phosphorylation with the complex I substrate, glutamate.

CONCLUSION: Chronic treatment with tadalafil protects against I/R injury in diabetic heart through mechanisms which blunt inflammation

and activate NO-induced Sirt1/PGC-1alpha signalling. We conclude that tadalafil could be an attractive therapy for reducing cardiovascular risk factors while providing cardioprotective effect in diabetic patients.

O20

ROLE OF CNS MR – AT1R SIGNALING IN HEART FAILURE

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In the brain, angiotensinergic sympatho-excitatory pathways do not contribute to acute, second-to-second regulation but play a major role in the chronic regulation of the setpoint for sympathetic tone and cardiovascular homeostasis. Increases in plasma angiotensin II (Ang II), aldosterone, [Na+] and cytokines can directly activate these pathways. Chronically, these stimuli also activate a slow neuromodulatory pathway involving local aldosterone, mineralocorticoid receptors (MRs), epithelial sodium channels, and endogenous ouabain. This pathway increases AT1R and NADPH oxidase subunits, and decreases nNOS, and thereby maintains/further increases activity of angiotensinergic pathways. Blockade of any step in this slow pathway or of AT1R prevents Ang II-, aldosterone-, or salt and renal injury- induced forms of hypertension. MR - AT1R activation in the CNS also contributes to activation of sympathetic activity, the circulatory and cardiac RAAS and the increase in circulating cytokines in HF post MI. These central pathways also play a major role in disease progression post MI. Chronic central infusion of a MR blocker or AT1R blocker prevents a major part of the structural remodeling of the heart and the decrease in LV function post MI. Similar results can be obtained by chronic central infusion of an aldosterone synthase inhibitor, indicating that the MR activation in the CNS post MI depends on aldosterone, locally in the CNS produced. From a clinical perspective, oral treatment with an AT1-receptor blocker at high doses can cause central AT1-receptor blockade and, in humans, lower sympathetic nerve activity. Low doses of the MR blocker spironolactone appear sufficient to cause central MR blockade and decrease sympathetic nerve activity.

CONCLUSION: Integrating the brain and circulating RAAS provides a better framework to understand the pathophysiology of hypertension and heart failure and to direct therapeutic strategies.

O21

CORRELATION OF PULMONARY PRESSURE AND THORACIC IMPEDANCE ASSESSED BY REMOTE MONITORING DEVICES

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BACKGROUND: In chronic heart failure (CHF), an increase in left ventricular (LV) filling pressure may lead to acute decompensation (AHF) by inducing neurohormonal activation and peripheral and pulmonary congestion; the latter, in turn, causes a reduction of thoracic impedance (TI) which can be monitored by ICDs. Recently, a direct appraisal of LV filling pressure has been obtained by measuring diastolic Pulmonary Artery Pressure (dPAP) with telemetric sensors (CardioMems^{TM}).

OBJECTIVES: To investigate the relationship between variations of LV filling pressure and pulmonary congestion.

METHODS: Eleven CHF patients (71±9 years; LVEF 27±4%; NYHA III) with ICDs measuring TI daily and transmitting it every 15 days were implanted with a CardioMemsTM sensor. PAP was transmitted daily: for three months from implantation, however, it was not used to guide treatment. We defined dPAP increase and TI decrease when their mean values in the previous week were higher or lower (respectively) than those of the week before.

RESULTS: We received 691 PAP transmissions. Treatment was adjusted following remote ICDs monitoring in 182 ± 3 episodes of mild AHF. We recorded 47 dPAP increases and 35 TI reductions (of them, 74% were subthreshold, i.e. not inducing impedance alerts by ICDs' algorithms). Increases in dPAP preceded TI reductions by 6.83±3.1 days; a weak relationship was observed (R=0.22; p<0.001). The association of increasing dPAP and absolute dPAP > 22 mmHg predicted the decrease of TI a week later (specificity 59%, sensitivity 42%, PPV 36%; NPV 65%).

CONCLUSIONS: Our observations confirm the correlation between LV filling pressure and pulmonary congestion. The delay between dPAP increase and TI decrease is consistent with the pathophysiological mechanism proposed for AHF. Detectable dPAP increases are mostly followed by subthreshold and delayed TI decreases, justifying the low sensitivity of TI impedance monitoring for detection of impending decompensation.

O22

IMPROVEMENT OF MITOCHONDRIAL FUNCTION BY METHYLENE BLUE IN EXPERIMENTAL DIABETES: A PROMISING CASE OF DRUG REPURPOSING

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Diabetic cardiomyopathy has been unequivocally associated with mitochondrial dysfunction and increased reactive oxygen species (ROS) generation. In the past years, drugs with redox activity have been increasingly used as mitochondrial modulators to enhance energy production and decrease oxidative stress. Methylene blue (MB) is a redox drug with widely reported protective effects in brain mitochondria. We aimed to assess the effects of MB (0.1 μM) on mitochondrial respiration, ROS production, and calcium sensitivity in rat heart mitochondria (RHM) isolated from diabetic vs. control rats. Mitochondrial respiratory function was assessed by high-resolution respirometry whereas ROS production and calcium retention capacity of RHM were measured by spectrofluorimetry. The addition of MB caused an increase in oxygen consumption of RHM energized with complex I and II substrates. MB significantly increased the H_2O_2 release in the presence of CI substrates, but had opposite effect in mitochondria energized with CII substrates. Interestingly, incubation of RHM with MB in the presence of MAO-A and B inhibitors (clorgyline or selegyline, 10 μM) significantly reduced H_2O_2 release in mitochondria energized with CI substrates. In diabetic rat hearts, methylene blue improved mitochondrial respiratory function and elicited a substrate-dependent effect on ROS production that was interfered by MAO inhibition.

O23

POSSIBLE THERAPEUTIC IMPLICATIONS OF THE SELECTIVE CARDIAC NA^+/CA^{2+} EXCHANGER INHIBITION: WHAT CAN WE LEARN FROM THE PHARMACOLOGICAL STUDIES?

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BACKGROUND: The Na^+/Ca^{2+} exchanger (NCX) of the heart has crucial role in the calcium homeostasis providing a main Ca^{2+} efflux mechanism. Therefore, its inhibition theoretically causes intracellular Ca^{2+} accumulation and positive inotropy. In the same time, under certain circumstances such as ischaemia, the NCX can cause intracellular Ca2+ load via reverse mode activity. Thus, theoretically the selective inhibition of the NCX could be a feasible antiarrhythmic strategy as well as a promising novel positive inotropic compound, however the exact analysis of the role of inhibition was seriously hampered by the lack of selective NCX inhibitor.

OBJECTIVES: Our aim was to clarify the possible positive inotropic and antiarrhythmic activity of the NCX inhibition by using a novel selective inhibitor ORM-10962.

METHODS: The ionic currents were measured by the whole cell configuration of the patch clamp technique in isolated canine ventricular

myocytes. The Ca^{2+} transients (CaT) were monitored by the Ca^{2+} indicator Fluo-4AM. The cell shortening (CS) was measured with video-edge detector.

RESULTS: 1 μM ORM-10962 slightly increased the amplitude of the CaT and CS under normal condition. When NCX reverse mode was increased by 70 mM [Na+]out the NCX inhibition decreased the magnitude of CaT and CS in contrary when NCX forward mode was enhanced by 600 nM forskolin, the ORM-10962 caused small further increase. The application of 3 μM forskolin did not caused spontaneous depolarizations, however the subsequently applied ORM-10962 enhanced the instability of the resting membrane potential and in some cases provoked delayed afterdepolarizations.

CONCLUSION: The selective NCX inhibition could be antiarrhythmic during Na^{+}-induced Ca^{2+}-load by inhibiting the NCX reverse mode activity. When NCX forward mode is enhanced positive inotropy is expected, which could turn into proarrhythmic activity when $[Ca^{2+}]i$ is excessively increased.

O24

TRANSGENIC RABBIT MODELS FOR INHERITED ARRHYTHMIA DISORDERS LONG-QT AND SHORT-QT SYNDROME

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The rabbit heart shows pronounced similarities to human hearts in terms of electrical and mechanical features. Therefore, rabbit models are useful tools to investigate human "electrical" disorders and translate the findings on cardiac (patho) physiology gathered with these models into the clinical management of affected patients.

In my presentation, I will give an overview on available transgenic rabbit models for the inherited arrhythmia disorders long-QT and short-QT syndrome – devastating diseases that are characterized by pathologically prolonged or accelerated cardiac repolarization, polymorphic ventricular arrhythmia, syncope and sudden cardiac death in young apparently healthy individuals.

In the first part, I will highlight findings on arrhythmia mechanisms, electro-mechanical dysfunction, and pro- and anti-arrhythmic agents that have been gathered with these transgenic rabbit models - e.g., models for long-QT types 1 (LQT1, KvLQT1-Y315S), type 2 (LQT2, HERG-G628S, loss of IKr) LQT5, and type 5 (KCNE1-G52R, decreased IKs) as well as short-QT type 1 (SQT1, HERG-N588K, gain of IKr). In the second part, I will discuss how these findings may affect future risk stratification and therapeutic approaches of human LQTS and SQTS patients.

O25

PROTECTION OF THE DEVELOPING HEART–POSSIBLE ROLE OF MITOCHONDRIA

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Cardiac tolerance to ischemia changes significantly during ontogenetic development. The mechanisms of the high resistance of the immature heart to oxygen deprivation have not yet been clarified; still unclear is the role of mitochondria. Mitochondrial oxidative phosphorylation is not completely developed in rat heart at birth; cardiac maturation during the first postnatal week is characterized by increasing content and specific activity of cytochrome c oxidase and enhanced flux of adenine nucleotides across the inner mitochondrial membrane. Moreover, in newborn animals, a single population of mitochondria with high mitochondrial membrane potential was observed. Furthermore, we have found significant developmental differences in the role of mitochondrial pore (MPTP) in the I/R injury. Whereas the blockade of MPTP by sanglifehrin had a protective effect on I/R damage in the adult myocardium, it had no effect in the neonatal heart. Furthermore, the Ca-induced swelling of mitochondria from neonatal rats is significantly lower than that from adult animals. All these results support the hypothesis that cardiac mitochondria are involved in the regulation of cardiac tolerance to oxygen deprivation during ontogeny. The neonatal period seems to be critical also for the development of cardiac protection against ischemia: ischemic preconditioning, ischemic postconditioning, or adaptation to chronic hypoxia failed to increase hypoxic tolerance to oxygen deprivation in the newborn rat heart; their protective effect develops only during the early postnatal period. It seems, therefore, that the decreasing tolerance to oxygen deprivation is counteracted by the development of endogenous protection. It may be concluded that the cardiac effect of ischemia is markedly influenced by the age of experimental animals.

O26

NEW VASODILATORS, INOTROPES AND INODILATORS IN THE MANAGEMENT OF ACUTE HEART FAILURE

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BACKGROUND: Restoration of systemic hemodynamics is central in preventing organ failure during acute heart failure (AHF).

OBJECTIVES and METHODS: This presentation aims at the position of new vasodilators, inotropes and inodilators in the management of AHF.

RESULTS: Most AHF patients receive intravenous diuretics and vasodilators, and some receive inotropes or inodilators. Cardiac enlargement compromises myocardial systolic reserve through the exhaustion of the Starling mechanism to a level where an increase in afterload decreases cardiac output. Diuretics alleviate the symptoms of AHF without affecting afterload, while vasodilators can normalize ventricular function through the reduction of afterload. Importantly, there are differences between direct-acting nitrate vasodilators and those that act through receptor-based mechanisms, such as natriuretic peptides and their derivatives. Natriuretic receptor activations mobilize a wide range of potentially beneficial intracellular signalling mechanisms leading to the inhibition of the sympathetic nervous system/renin-angiotensin-aldosteron system etc. in the cardiovascular system. These effects increased clinical awareness of the therapeutic application of natriuretic receptor agonists (e.g. nesiritide). Positive inotropic agents (e.g. dobutamine) have pronounced direct effects on ventricular function curves. Moreover, a combined strategy (e.g. diuretics + vasodilators + inotropes) forecast additive positive effects on circulatory hemodynamics. Nevertheless, in case of conventional beta-mimetic positive inotropes short term benefits are complicated by adverse outcome measures at longer terms. It is currently debated whether alternative positive inotropic approaches through a single drug target (e.g. the direct myosin activator omecamtiv mecarbil), or through the combination of several mechanisms (e.g. the Ca2+-sensitizer levosimendan) offer superior clinical applicability for AHF.

CONCLUSIONS: Symptomatic relief can be achieved by common diuretics, vasodilators and/or inodilators during AHF involving divergent effects on ventricular function and systemic hemodynamics. Organ protection will be the function of improved hemodynamics in combination with characteristic drug effects on regional circulation (venous vs. arterial, pulmonary etc.), and additional pleiotropic effects.

O27

ADDRESSING THE PROBLEM OF HYPERTENSION

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In the year-long FlaxPAD clinical trial, dietary flaxseed generated a powerful reduction in brachial systolic and diastolic blood pressure in patients with peripheral artery disease. Oxylipins were implicated as potential mechanistic mediators. However, the ability of flaxseed to impact central

aortic hypertension, arterial stiffness, or cardiac performance was not investigated. Additionally, the relationship between central blood pressure and oxylipins was not elucidated. Thus, the effects of dietary flaxseed on central blood pressure, oxylipins levels and cardiac function in the FlaxPAD population were studied. Radial tonometry and pulse wave analysis were used to measure central blood pressure and cardiac function in the FlaxPAD population. Plasma oxylipins were analyzed with HPLC-MS/MS. In patients with high blood pressure at baseline, central systolic and diastolic blood pressure decreased in participants ingesting flaxseed versus placebo. Flaxseed did not significantly impact augmentation index or other cardiac function indices. Flaxseed induced a decrease in many oxylipins which corresponded with a reduced risk of elevated central blood pressure. These data extend the anti-hypertensive properties of flaxseed to central blood pressure without cardiac involvement but rather through oxylipins. This study provides further support for oxylipins as therapeutic targets in hypertension.

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O28

(PATHO)PHYSIOLOGY OF ATHLETE'S HEART

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BACKGROUND: Long-term exercise training is associated with characteristic structural and functional changes of the myocardium, termed 'athlete's heart'. However, elevations in cardionecrotic biomarkers and impairment in left ventricular (LV) function have been described after acute exhaustive exercise ('exercise-induced cardiac fatigue').

OBJECTIVES: We aimed at understanding the biochemical, histological and molecular biological alterations in the heart and providing detailed hemodynamic characterization in rat models of athlete's heart and exercise-induced cardiac fatigue.

METHODS: Athlete's heart was induced by long-term swim training in rats (200min/d swimming for 12 weeks). Exercise-induced cardiac fatigue was investigated in rats underwent forced swimming for 3h with 5% body weight (workload) attached to the tail. We performed echocardiography and LV pressure-volume analysis to investigate LV function and mechanoenergetics. Additionally, blood and myocardium samples were harvested for biochemical and histological examination. Gene expression changes were detected by qRT-PCR.

RESULTS: LV hypertrophy was observed in athlete's heart, which was confirmed by echocardiograpy, heart weight data and histomorphometry. Invasive hemodynamic measurements showed unaltered heart rate, arterial pressure and LV end-diastolic volume along with decreased LV end-systolic volume, thus increased stroke volume and ejection fraction, increased LV contractility and improved mechanoenergetics in trained vs. untrained control rats. Despite the significant hypertrophy, we observed unaltered LV stiffness and improved LV active relaxation. In exercise-induced cardiac fatigue, elevated plasma levels of cardiac troponin T were detected. Histological analysis showed sporadic fragmentation of myocardial structure and leukocyte infiltration after exhaustive exercise. We observed increased end-systolic volume, decreased ejection fraction, impaired contractility and mechanoenergetics of LV in the exercised group. Myocardial expression of major antioxidant enzymes were increased along with increased myocardial nitro-oxidative stress. Bax/Bcl-2 ratio and TUNEL staining showed enhanced apoptotic signaling.

CONCLUSIONS: We provided a detailed characterization of functional changes and hemodynamic relations in rat models of athlete's heart and exercise-induced cardiac fatigue.

O29

ACTIVATION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS AS A POTENTIAL MECHANISM OF REMOTE PRECONDITIONING-INDUCED CARDIOPROTECTION IN HEALTHY AND DISEASED HEARTS

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BACKGROUND: Genes encoding enzymes involved in fatty acids (FA) and glucose oxidation as the main energy-producing pathways in the heart are regulated by transcription factors PPAR (peroxisome proliferator-activated receptors). Under conditions of oxygen deprivation, e.g., myocardial ischemia, PPAR-α modulates substrate selection (between FA and glucose) aimed at the adequate energy production. Although loss of innate cardioprotection in pathologically altered myocardium is associated with downregulation of PPARs, their role in protective mechanisms of ischemic preconditioning (IPC) is relatively less investigated. On the other hand, PPAR-α and PPAR-γ have been proposed to play a role in the mechanisms of other, clinically applicable conditioning intervention, „remote" PC (RPC).

OBJECTIVES: We have previously shown that PPAR-α agonists confer PC-like protection linked with activation of pro-survival cascades, antioxidative and antiapoptotic effects, and that all isoforms of PPAR are up-regulated in the acutely diabetic hearts resistant to ischemia/reperfusion (IR) injury. This study aimed to evaluate the involvement of PPAR-α in the mechanisms of non-invasive RPC in adult male Wistar rats.

METHODS: Three cycles of 5-min pressure cuff inflation (200 mmHg)/5-min deflation with or without PPAR-α antagonist MK886 (MK, 3 mg/kg i.p., prior to RPC) were applied on a right hind limb of anesthetized animals. Size of infarction (IS, TTC staining), recovery of function (LVDP) and incidence of ventricular tachyarrhythmias were evaluated in Langendorff-perfused hearts exposed to 30-min global ischemia/120-min reperfusion. In parallel groups, LV tissue was sampled for examination of PPAR-α gene expression (RT-PCR) and PKCε protein levels (WB). RPC was also tested in hypertensive (SHR) and diabetic (STZ i.p., 1 week) rats.

RESULTS: RPC significantly reduced IS, severity of arrhythmias, improved recovery of LVDP, and markedly enhanced mRNA levels of PPAR-α (by 50%) and PKCε protein. All these effects were abrogated in the presence of MK. Protection was also observed in the hearts of SHR rats, while diabetic hearts with higher ischemic tolerance did not exert additional cardioprotective effects.

CONCLUSIONS: The results confirm the effectiveness of RPC in protection against acute IR and suggest the role of PPAR-α as one of potential cardioprotective mechanisms implicating involvement of PKCε.

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O30

PROTECTION OF THE HEART IN SITUATIONS OF INCREASED PRODUCTION OF OXYGEN FREE RADICALS: RADIATION AND REPERFUSION INJURY

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BACKGROUND: Excessive production of oxygen free radicals has been regarded as a causative common denominator of many pathological processes in the animal kingdom. Hydroxyl and nitrosyl radicals represent the major cause of the destruction of biomolecules either by a direct reaction or by triggering a chain reaction of free radicals. Scavenging of free radicals may act preventively or therapeutically. A number of substances that preferentially react with free radicals can serve as scavengers, thus increasing the internal capacity/activity of endogenous antioxidants and protecting cells and tissues against oxidative damage.

OBJECTIVE: Molecular hydrogen (H_2) reacts with strong oxidants, such as hydroxyl and nitrosyl radicals, in the cells, that enables utilization of its potential for preventive and therapeutic applications in situations with excessive production of free radicals including radiation and ischemia-reperfusion.
METHODS: Application of molecular H_2 is relatively simple and effective. Therapeutic application of hydrogen has been performed by different delivery methods including inhalation, drinking hydrogen rich water and/or infusion of hydrogen-saturated solutions.
RESULTS: H_2 rapidly diffuses into tissues and cells without affecting metabolic redox reactions and signaling reactive species. H2 reduces oxidative stress also by regulating gene expression, and functions as an anti-inflammatory and anti-apoptotic agent.
CONCLUSION: Based on the results of animal experiments and clinical observations it is documented that H_2 may represent an effective antioxidant for the prevention of oxidative stress-related diseases. Hydrogen is an important physiological regulatory factor with antioxidant, anti-inflammatory, anti-apoptotic and signal modulatory protective effects on cells and organs. The exact mechanisms of how molecular H_2 operates still needs to be explored.

O31
CELLULAR REPROGRAMMING APPROACHES FOR CARDIOVASCULAR DISEASE

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Heart disease is a leading cause of death in adults and children. We, and others, have described complex signaling, transcriptional and translational networks that guide early differentiation of cardiac progenitors and later morphogenetic events during cardiogenesis. We discovered networks of transcription factors and miRNAs function through intersecting positive and negative feedback loops to reinforce differentiation and proliferation decisions. By leveraging these networks, we have reprogrammed disease-specific human cells in order to model human heart disease in patients carrying mutations in cardiac developmental genes. Deep epigenetic and transcriptome analyses revealed perturbations in pivotal gene networks that contribute to disease that could be corrected by altering dosage of nodal points in the network. We also utilized a combination of major cardiac regulatory factors to induce direct reprogramming of cardiac fibroblasts into cardiomyocyte-like cells with global gene expression and electrical activity similar to cardiomyocytes. The in vivo efficiency of reprogramming into cells that are more fully reprogrammed was greater than in vitro and resulted in improved cardiac function after injury. We are exploring the molecular mechanisms underlying the progressive reprogramming process through the study of DNA-binding of reprogramming factors and the associated epigenetic and transcriptional changes. We have also identified a unique cocktail of transcription factors and small molecules that reprogram human fibroblasts into cardiomyocyte-like cells and are testing these in large animals. Knowledge regarding the early steps of cardiac differentiation in vivo has led to effective strategies to generate necessary cardiac cell types for disease-modeling and regenerative approaches, and may lead to new strategies for human heart disease.

O32
THE ROLE OF CAMP/PKA/EPAC SIGNALLING PATHWAY IN CARDIOPROTECTION

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Recent work from Bristol has shown that consecutive activation of PKA/PKC mediated by isoprenaline/adenosine treatment is cardioprotective. This protection is initiated by an increase in cAMP and is associated with a reduction in both Ca^{2+}-induced mitochondrial swelling and in oxidative stress. cAMP signal transduction pathway has already been implicated in the cardioprotective effects of both ischaemic and temperature preconditioning. However, the precise signalling mechanism of cAMP/PKA-induced cardioprotection has not been fully elucidated and there are issues associated with global cAMP elevation including arrhythmias. Although most biological effects of cAMP in the heart have been assigned to PKA, cAMP also activates Epac (a guanine nucleotide exchange factor directly activated by cAMP). In addition to its involvement in the regulation of different physiological and biochemical processes in a variety of tissues, there is extensive evidence demonstrating that Epac also plays a role in controlling cardiac Ca^{2+} cycling. This is independent of and parallel to cAMP/PKA signalling pathway. One disadvantage of studies involving stimulation of β-adrenergic receptor is that this receptor can be impaired in chronic heart failure. Fortunately, availability of cell-permeable cAMP analogues represents a valuable tool to investigate the involvement of β-adrenergic receptor in cardioprotection and in identifying the role of PKA and Epac signalling pathways. We have used cell permeable cAMP analogues that are selective activators and specific inhibitors of PKA and Epac. Our data using cAMP analogues suggest that cAMP signalling pathways induce a strong cardioprotective effect independently of β-adrenergic stimulation and that simultaneous activation of both PKA and Epac is required for optimal cardioprotection.

O33
A NOVEL FOOT BATHING APPROACH FOR THE TREATMENT OF FOOT ULCERS DUE TO PERIPERAL ARTERIAL DISEASE: A CASE STUDY

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BACKGROUND: It is well known that foot ulceration usually precedes more serious foot complications such as infection, gangrene or amputation.
OBJECTIVES: We are currently evaluating the clinical utility, efficacy and safety of a novel approach to treat diabetic foot ulcers that involves foot bathing in CO_2-enriched water.
METHODS: In this case study, a 65-year-old man with diabetes, peripheral vascular occlusive disease and peripheral neuropathy presented with right foot plantar non-infected ulcers. Foot bathing in CO_2-enriched water (1000-1200 ppm) was conducted at 37±0.5°C, 3/week for 15 min for 2 months. Wounds were assessed using the Silhouette Star camera (Aranz Medical), limb oxygenation determined by near-infra red spectroscopy (Kent Imaging) and blood was collected for routine chemistry at baseline and at the end of each month of the intervention period.
RESULTS: Foot bathing in carbonated water resulted in a significant healing of wounds that was associated with an increase in O_2 saturation levels of the wound area as well as reduced plasma C-reactive protein levels. In addition, a pain reduction as evidenced by the McGill Pain Questionnaire was reported.
CONCLUSION: It is suggested that CO_2 foot bathing could help to accelerate the wound healing process and diminish pain due to peripheral neuropathy.

O34
AN INVESTIGATION ON THE DISTRIBUTION OF ZINC-TRANSPORTERS IN FAILING HEARTS OF MAMMALIANS

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BACKGROUND: Free Zn^{2+} level is low in cytosol for many cells. Its level in cardiomyocytes from mammalian subjects is less than 1 nM, but under pathological conditions, the cytosolic level of Zn^{2+} can be increased, markedly. In this regard, it is very important to have a well-controlled cytosolic free Zn^{2+} homeostasis via proper activitiy of Zn^{2+}-transporters.
OBJECTIVES: Differential expression of Zn^{2+}-transporters is an important component for this regulatory mechanism. The ZIP8 and ZIP14, most closely related two transportersto, are found to be mostly localized in plasma membrane/sarcolemma and carry Zn^{2+} from extracellular to cytosol in different cell types while others including ZIP7, ZnT7 and ZnT8 localize to subcellular compartments with little known about their roles in mammalian

heart. Therefore, in this study, we aimed to examine these transporters in left ventricle from failing human hearts.
METHODS: The hearts from patients with end-stage heart failure undergoing cardiac transplantation due to either dilated or ischemic cardiomyopathy were examined. For validation, Wistar rat hearts mimicked heart failure with induction of chronic hypertrophy via transverse aortic constriction or hypertrophy induced H9c2 rat ventricular cell line with adriamycin incubation were also examined.
RESULTS: In all heart samples, the protein expression levels of ZIP7, ZIP14 and ZnT8 in the left ventricle of failing human hearts are higher than those of the controls with the lower levels of ZnT7 and ZIP8 comparison to their controls. Additionally, in these samples, we observed increased protein levels of the ER stress markers such as GRP78, Calnexin and Gadd133 and significantly decreased BcI/Bax ratio as an apoptotic marker.
CONCLUSIONS: We have impression on possible contribution of differential expression of these transporters as one of the crucial signs of failing heart. Our preliminary results may provide important information related with role of Zn^{2+} transporters for the new therapeutic strategy in diabetic cardiac dysfunction.
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O35
ROLE H2S IN CARDIOVASCULAR REMODELLING

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Hydrogen sulfide (H_2S) has recently been identified as a regulator of various physiological events, including vasodilation, angiogenesis, antiapoptotic, and cellular signaling. Endogenously, H_2S is produced as a metabolite of homocysteine (Hcy) by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3MST). Although Hcy is recognized as vascular risk factor at an elevated level [hyperhomocysteinemia (HHcy)] and contributes to vascular injury leading to renovascular dysfunction, the exact mechanism is unclear. The goal of the current study was to investigate whether conversion of Hcy to H_2S improves renovascular function. Ex vivo renal artery culture with CBS, CSE, and 3MST triple gene therapy generated more H2S in the presence of Hcy, and these arteries were more responsive to endothelial-dependent vasodilation compared with nontransfected arteries treated with high Hcy. Cross section of triple gene-delivered renal arteries immunostaining suggested increased expression of CD31 and VEGF and diminished expression of the antiangiogenic factor endostatin. In vitro endothelial cell culture demonstrated increased mitophagy during high levels of Hcy and was mitigated by triple gene delivery. Also, dephosphorylated Akt and phosphorylated FoxO3 in HHcy were reversed by H_2S or triple gene delivery. Upregulated matrix metalloproteinases-13 and downregulated tissue inhibitor of metalloproteinase-1 in HHcy were normalized by overexpression of triple genes. Together, these results suggest that H_2S plays a key role in renovasculopathy during HHcy and is mediated through Akt/FoxO3 pathways. We conclude that conversion of Hcy to H2S by CBS, CSE, or 3MST triple gene therapy improves renovascular function in HHcy.

O36
CELLULAR ELECTROPHYSIOLOGICAL INVESTIGATION OF THE CHRONIC AND ACUTE EFFECTS OF DESETHYLAMIODARONE IN DOG CARDIAC VENTRICULAR PREPARATIONS

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BACKGROUND: Amiodarone (AMIO) is considered to be one of the most effective antiarrhytmic agents with lower proarrhythmic risk, however, it possesses serious extracardiac adverse effects. It has been suggested that desethylamiodarone (DEA), the active metabolite of AMIO might have similar cardiac profile as the parent compound.
OBJECTIVES: Therefore, the purpose of the present study was to characterize the chronic and acute cellular electrophysiological effects of desethylamiodarone in dog cardiac ventricular preparations.
METHODS: Dogs used to study the chronic effect of desethylamiodarone were treated with 50 mg/kg/day DEA for 4 weeks. Ion current measurements were performed by using whole cell patch clamp technique in canine single ventricular myocytes, and action potentials were recorded from canine ventricular preparations by the conventional microelectrode technique.
RESULTS: The action potential duration (APD) was fond to be significantly longer (by 10.6 %) in the DEA treated group compared to control group and use-dependent block of the maximal rate of depolarization (Vmax) was also observed. The onset and offset kinetics of DEA was also investigated, the time constants for onset and offset of the drug were 5.3 AP and 532 ms, respectively. The rapid (IKr) and slow (IKs) delayed rectifier potassium currents and the late sodium current (INaL) were significantly decreased but other currents – transient outward potassium current (Ito), inward rectifier potassium current (IK1) and the L-type calcium current (ICaL) – were not influenced by DEA treatment. Acute DEA application (10 μM) slightly but significantly lengthened (by 5.5 %) the action potential but Vmax values were not influenced significantly by the drug. 10 μM of DEA inhibited IKr, IKs and INaL currents but did not affect Ito, IK1 and ICaL.
CONCLUSIONS: Chronic and acute desethylamiodarone administration has very similar cardiac electrophysiological effects than amiodarone. This may suggest that chronic DEA application could represent a similarly effective as AMIO but safer therapeutic option for the management of cardiac arrhythmias.

O37
SEPTIC CARDIOMYOPATHY

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BACKGROUND: Septic cardiomyopathy can be classified as a secondary form of cardiomyopathy, the heart being involved in the systemic disease "sepsis". Pathophysiology is much more complex than the pathophysiology of most of textbook heart diseases This complexity is the consequence of the impact of numerous toxins and sepsis mediators on the heart in the course of the disease.
OBJECTIVES: To describe pathophysiology and clinical picture of septic cardiomyopathy.
METHODS: Narrative review of experimental and clinical findings.
RESULTS: Main trigger substances of septic pump failure are endotoxin, TNF-α, IL-1, and NO, which interfere with receptors, inotropic signal transduction pathways, Ca^{2+} transients and the contractile apparatus of the cardiomyocyte. They also impair mitochondrial function of heart cells, with the consequences of cytopathic hypoxia and energy depletion, but also increased production of reactive oxygen species and induction of apoptosis in the organ.
Septic cardiomyopathy is characterized by systolic as well as diastolic dysfunction of both ventricles and – typically – by reversible dilation. Diastolic dysfunction seems to be the prognostically most relevant alteration. For compensation of the sepsis-induced vasoplegia resulting in a fall in blood pressure and in an extensive reduction in afterload, the diseased heart has to pump even more than normal to maintain blood pressure. Thus the impairment of 'afterload-related cardiac performance' (ACP) characterizes the cardiac pump failure in sepsis much better than cardiac index or left ventricular ejection fraction. Septic cardiomyopathy is not a primary ischemic disease, as coronary macrocirculation is not impaired. Drastic alterations are seen in cardiac metabolism, with a strong reduction of free fatty acid fueling. Also chronotropic and bathmotropic dysregulation characterizes septic cardiomyopathy: resting heart rate is high and heart rate variability is reduced, due to cardiac autonomic dysfunction with depressed vagal and sympathetic tone. Moreover, endotoxin interacts with the HCN channels of the pacemaker current in sinus node, thereby contributing to the intrinsic impairment of heart rate regulation.

CONCLUSIONS: The pathophysiology of septic cardiomyopathy is complex, the cardiac alterations can be reversible and the impairments of heart function contribute to the unfavorable prognosis of septic patients.

O38

CLINICAL EXPERIENCES OF CO2 FOOT BATH THERAPY: EFFECTS ON PERIPHERAL ARTERIAL DISEASE OF DIALYSIS PATIENTS WITH DIABETES

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BACKGROUND: The number of dialysis patients with diabetes is increasing year every year in Japan, and dialysis patients tend to suffer from many complications such as cardiovascular disease, cerebrovascular disease, and peripheral artery disease. In our hospitals, about 3000 patients are treated with dialysis per year. In our previous study (Hayashi H et al, *Annals Vascular Diseases*, 1, 111-117, 2008), ulcer prevention effect in patients with CO2 foot bathing after revascularization surgery was suggested, and we usually treat dialysis patients with CO2 foot bathing.

OBJECTIVES: To understand effects of CO2 foot bathing on the dialysis patients with diabetes, course of treatment to some patients were observed in detail.

METHODS: We prepared an appropriate container for foot bath and make necessary amount of carbonated hot water of 1000 ppm at 37-38°C. Patients were treated with CO_2 hot water for 10-15 minutes per session.

RESULTS: In our hospitals, annual mortality rate of acute myocardial infarction was lower than that of average in Japan. Annual rate of amputation of lower limbs was extremely lower than that of average in Japan. Ulcers in some patients were healed. Three months after CO_2 foot bathing twice a day, ulcers of a patient was dramatically improved (Toriyama et al, *Int Angiol* 21, 367-373, 2002), and some other patients were also improved after 3-4 month treatment. A patient treated with CO_2 bathing only is also improved until after 10 months.

CONCLUSIONS: Effects on ulcer of dialysis patients with diabetes will be discussed.

O39

MODULATION OF THE INTRACELLULAR CALCIUM STORE AS AN ANTIARRHYTHMIC ENDPOINT

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Sarcolemmal ion channels are traditionally considered as the elective targets of antiarrhythmic therapy. The contribution to arrhythmogenesis of abnormal Ca^{2+} handling by the intracellular store has been since long recognized for digitalis-induced arrhythmias, but has been, till recently, circumscribed to this specific condition. An increasing amount of evidence points instead to its role as a common cause of arrhythmia, potentially relevant to all arrhythmogenic mechanisms and, thus, to multiple conditions. Cytosolic Ca^{2+}, acting as the signal to couple membrane excitation to sarcomere contraction, is largely released from the sarcoplasmic reticulum (SR). To serve its purpose, SR must be a "stable" store, i.e. it must release Ca^{2+} only upon membrane excitation. When stability of the intracellular Ca^{2+} store is compromised, Ca^{2+} may be released independently from excitation and lead to secondary perturbation of sarcolemmal membrane potential. Indeed, the latter is coupled to cytosolic Ca^{2+} levels through an electrogenic Ca^{2+} transport mechanism (Na^+/Ca^{2+} exchanger) and various Ca^{2+}-sensitive conductances. SR stability is compromised, and contributes to the high incidence of arrhythmias, in acquired as well as in genetic diseases (e.g. heart failure, genetic catecholamine induced arrhythmias); therefore, SR stability may be considered an antiarrhythmic endpoint. Ca^{2+} store stability depends on the interplay between sarcolemmal and SR 'effectors' (ion channels and transports), which are mutually linked by Ca^{2+}-mediated feed-back control; while instrumental to cell homeostasis, such control makes any attempt to modulate SR stability dauntingly complex. In my presentation I will review current knowledge on the factors leading to SR instability, the mechanisms by which SR instability translates into arrhythmias and introduce pharmacological interventions, under investigation in our group, aimed to improve SR stability. Although still at an initial stage of development, such endeavour might contribute novel strategies to antiarrhythmic drug therapy.

040

SCREENING FOR KNOWN AND NOVEL GENETIC VARIANTS IN ION CHANNEL DISEASES WITH NEXT GENERATION SEQUENCING

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BACKGROUND: Ion channel diseases are arrhythmogenic disorders with overlapping phenotypes and a great deal of genetic heterogeneity. Molecular genetics provided essential insights into the pathogenesis of ion channel diseases, but despite extensive investigation of causative genes, only 25-70% of the patients carry mutations in the previously identified genes.

OBJECTIVES: In our work we aimed to identify known and novel genetic variants to cause or to contribute to the disease phenotype in ion channel diseases.

METHODS: Altogether, 108 patients (43 male, 65 female; average age: 28±15 years) with a proven or suspected diagnosis of ion channel diseases, mainly, but not exclusively long QT syndrome (LQTS), were investigated. For targeted resequencing of all the disease-related genes in major ion channel diseases we used a single, in-house developed ion-channel platform, capable to screen for genetic variants in 69 ion channel genes.

RESULTS: Patients carried average 2 variants (range: 0-7 variants). In a subset of 66 patients with LQTS presumably disease-casing variants were identified in 33 patients (KCNQ1: 8; KCNH2: 6; SCN5A: 4; ANK2: 2; KCNE1: 1; KCNJ2: 6, CACNA1C: 1; CAV3: 1; AKAP9: 4). A further 23 patients were found to carry variants in minor ion-channel related genes. Ten LQTS patients carried no genetic variants. In a subset of 8 patients with a clinical diagnosis of catecholaminergic polymorphic ventricular tachycardia 5 RYR2 and 1 SCN5A mutations were found. Patients with familial bradycardia, carrying HCN4 gene mutations, or patients with drug induced QT prolongation, carrying SCN5A or ANK2 mutations were also identified. Analysing genotype-phenotype correlations we found that the average QTc showed a trend to increase in correlation with the number of variants and the average age at the time of first symptoms were lower in patients carrying >1 variant.

CONCLUSIONS: Large scale genotyping of patients with ion channel diseases by targeted resequencing reveals further genetic heterogeneity in ion channel diseases. However, the method has great potential to identify novel disease-causing genes.

O41

THE ASSESSMENT OF STRUCTURAL REMODELING AND PROARRHYTHMIC SENSITIVITY IN A NEW RABBIT ATHLETE'S HEART MODEL

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BACKGROUND: Most sudden cardiac death events in athletes are associated with cardiac muscle structural disorders. However, the underlying cause remains unclear in 3-6% of such death events. Apart from the structural disorders, functional remodeling might also lead to life-threatening ventricular tachyarrhythmias under certain circumstances (e.g. reduced repolarization reserve).

OBJECTIVES: A new rabbit athlete's heart model was characterized and the proarrhythmic sensitivity was assessed in the remodeled hearts.

METHODS: New-Zealand white rabbits were randomized into 'Sedentary' and 'Exercised' groups (n=7). The 'Exercised' group was trained during a

12-week long treadmill-running protocol. Echocardiography and resting ECG recording were performed. At the end of training protocol, proarrhythmic sensitivity was tested with dofetilide (50 nM) in Langendorff-perfused rabbit hearts. ECG repolarization parameters and sinus variability of ECG parameters were evaluated. Tissue samples were taken from the left ventricle and messenger RNA expression level of fibrosis biomarkers were quantified with RT-qPCR to determine the collagen metabolism.
RESULTS: Echocardiography on the 12th week showed significant increase in the internal end-diastolic diameter of the left ventricle (LVIDd) in the 'Exercised' group (17.4±0.3 vs. 14.7±0.8 mm, $p<0.05$) compared to the 'Sedentary' group. Resting heart rate was significantly lower (198±4 vs. 253±8, $p<0.05$), PQ, QT, RR, Tpeak-Tend intervals and the variability parameters of the RR and Tpeak-Tend intervals in vivo were significantly greater in the 'Exercised' group. Dofetilide tended to increase the QTc interval in the 'Exercised' group in vitro, however, there was no difference in the incidence of proarrhythmia between the two groups. RT-qPCR showed significantly greater TIMP-1 level in the 'Exercised' group.
CONCLUSION: The increased LVIDd and the decreased heart rate are characteristics of the exercise-induced athlete's heart. Increased parasympathetic tone of the autonomic nervous system was manifested by the extended PQ and RR intervals and their variability parameters. Repolarization changes and fibrosis may indicate the sensibility of the athlete's heart to arrhythmia, however, further investigations are warranted.

YOUNG INVESTIGATOR AWARD COMPETITION (YIA1 – YIA7)

YIA1

REMOTE ISCHEMIC PRECONDITIONING TRIGGERS CHANGES IN HEART RATE, HEART RATE VARIABILITY, MICROCIRCULATORY BLOOD FLOW AND CARDIAC ENERGETICS

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BACKGROUND: Remote ischemic preconditioning (RIPC) induced by brief ischemia/reperfusion cycles of remote organ (e.g. limb) is cardio-protective. Neural pathways have been implicated in mediating this response.
OBJECTIVES: The aim of this work was to elucidate the involvement of the autonomic nervous system (ANS) in RIPC response by monitoring changes in heart rate, heart rate variability and blood flow.
METHODS: RIPC was induced in anesthetized male C57/Bl6 mice by 4 cycles of 5min of hind limb ischemia using a cuff inflated at 200mmHg followed by 5min of reperfusion. ECG and microcirculatory blood flow in both hind limbs were recorded throughout the RIPC protocol. Heart rate variability analysis was performed at high, low and very low frequency. Hearts extracted at the end of RIPC protocol were used either for Langendorff perfusion to monitor function and injury during I/R or for measurement of myocardial metabolites using HPLC.
RESULTS: Isolated perfused hearts from RIPC animals had significantly less cardiac injury after 30 min index ischemia and 2hrs reperfusion. RIPC protocol was associated with increased heart rate measured both in extracted perfused heart and in vivo. Frequency ratio (low/high) of the Heart Rate Variability spectra was significantly higher during the 3rd cycle of RIPC compared to control. RIPC was associated with a standard hyperaemic response in the cuffed limb but a reduction in blood flow in the uncuffed limb was observed. RIPC hearts had significantly lower phosphorylation potential and energy charge compared to the control group.
CONCLUSIONS: RIPC is associated with changes in cardiovascular activity (heart rate, blood flow, heart rate variability) which in turn triggers mild myocardial ischemic stress that would contribute to protection against index ischaemia and reperfusion.

YIA2

ABLATION OF TLR-4 MITIGATES BLOOD PRESSURE RESPONSE DURING HYPERHOMOCYSTEINEMIA

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BACKGROUND: A significant number of studies have shown a positive correlation between Hcy plasma levels and hypertension. On the other hand pathogen recognition receptor, and in particular Toll-like receptor 4 (TLR-4) is a foreign antigen sensor that plays role in innate immune system activation and has recently gained a significant attention in the field of hypertension.
OBJECTIVE: The objective of this study was to define the mechanisms of homocysteine toxic effect on aortic wall that promote vascular remodeling and hypertension and explore the role of toll- like receptor 4 mutation in alleviation of homocysteine negative effects.
METHODS: For this study we used 5 groups of mice: C57BL/6J, C3H/HEouJ, CBS+/-; C3H/HeJ, and CBS+/-/C3H. For further analysis we used isolated organs: aorta and heart and collected blood. Blood pressure was recorded using noninvasive tail cuff method. Effects of hyperpolarization factor and endothelial-dependent vasodilator on aorta contractility we performed using BIOPAC System Inc. We checked expression of mitochondrial fusion and fission proteins, antioxidant markers and expression of collagen/elastin fragments.
RESULTS: Data showed that there is a trend of increased values of systolic and diastolic pressure in CBS+/- mice in comparing to other groups. C3H/HeJ mice had the decreased levels of both DP and SP in comparing to other groups. The response to hyperpolarization factor and endothelial-dependent vasodilator were blended in CBS+/- aorta, however mitigated in CBS+/-/C3H.
CONCLUSION: The results showed a role of TLR-4 in hypertension during hyperhomocysteinemia.

YIA3

DEPENDENCE OF DIASTOLIC CALCIUM LEVELS ON FREQUENCY AND EXTRACELLULAR CALCIUM CONCENTRATION

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BACKGROUND: The working heart spends half of its time in diastole as the relaxation is required for the normal cardiac function. Impairment of relaxation can lead to diastolic heart failure.
OBJECTIVES: Although much has been learnt about the regulation of systolic calcium concentration, less is known about the regulation of diastolic calcium. The majority of studies use frequencies much lower than physiological, resulting in a situation where diastolic calcium levels can reach resting levels. In this study, therefore, we aimed to investigate the changes of diastolic calcium in relation to various experimental settings.
METHODS: Cardiac myocytes isolated from mice were loaded with Fluo-3-AM. Calcium transients and membrane currents were recorded with the perforated patch clamp technique at various frequencies (0.2 – 3 Hz) or alternatively with altered extracellular calcium concentrations (0.5 – 5 mM).
RESULTS: Increasing the stimulation frequency increased diastolic calcium values (0.2 Hz: 94.4±4.8 nM vs. 3 Hz: 109.8±3.1 nM). These changes were enhanced in the presence of the beta-adrenergic agonist ISO (0.2 Hz: 108.6±12.6 nM vs. 3 Hz: 157.8±11.5 nM). Linear fit of the two curves showed that diastolic calcium increased more steeply with frequency in the presence of ISO ($p<0.001$). Conversely, the systolic calcium transient amplitude decreased with frequency (0.2 Hz: 310.0±103.5 nM vs. 3 Hz: 177.9±90.0 nM). Another set of experiments were performed after applying normal Tyrode's with different extracellular calcium concentrations with pacing the cells at 3 Hz. Compared to 1 mM CaCl2, diastolic, systolic and average calcium levels were decreased in 0.5 mM CaCl2 and increased in 5 mM CaCl2.
CONCLUSIONS: We find that diastolic calcium levels during diastole are markedly altered by changes of stimulational frequency, ISO or by alteration of extracellular calcium concentration. Future work will address the mechanism.

YIA4

CELLULAR ELECTROPHYSIOLOGICAL AND ANTIARRHYTMIC EFFECT OF SODIUM/CALCIUM (NCX) INHIBITION

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BACKGROUND: NCX is an important transmembrane transport system in the heart and considered as a major contributor to the maintenance of the intracellular Ca^{2+} homeostasis. Since NCX is electrogenic, it carries depolarizing or repolarising transmembrane ion currents, which influence cardiac action potentials and in pathological cases it is involved in arrhythmic mechanisms. The direct role of NCX in cardiac repolarization has so far not been investigated experimentally because of the lack of highly selective NCX modulators.

OBJECTIVES: Two new NCX inhibitors, ORM-10103 and ORM-10962 were analyzed on NCX current and their specificity were investigated by measuring the L-type Ca^{2+} current(ICaL), the main repolarizing K^+ currents (Ito,IKr, IKs, IK1), the late sodium current (INaL) and the Na^+/K^+ pump. Functional roles of NCX in the repolarisation and in trigger arrhytmias were also studied.

METHODS: Ion currents were determined by using whole-cell patch clamp technique in canine single ventricular cells. Action potentials were recorded from canine tissue preparations by conventional microelectrode technique. All experiments were performed at 37°C.

RESULTS: ORM-10103 and ORM-10962 inhibited forward (EC50: 780 nM vs. 55 nM) and reverse modes of NCX (EC50: 960 vs. 67 nM). ORM-10962 (1 μM) did not affect INaL, ICaL, Na+/K+ pump, IKr, IKs , Ito, IK1; however ORM-10103 (10 μM) had a 20% inhibition effect on IKr. In Purkinje NCX inhibition by ORM-10962 did not elicit consistent action potential changes and did not affect the amplitude of Ca^{2+} transients in left ventricular myocytes. NCX inhibition induced negative shift of the plateau when forward mode of NCX was augmented and repolarization lengthening when reverse mode of NCX was favoured. In canine Purkinje fibres 10 μM ORM-10103 and 1 μM ORM-10962 also decreased the amplitude of digoxin induced delayed afterdepolarizations (DADs).

CONCLUSIONS: ORM-10103 and ORM-10962 have high NCX-inhibitory activity, but ORM-10962 is the most selective compound. We suggest that NCX plays a moderate role at ventricular repolarization in normal ventricular muscle, depending on the relative magnitude of the forward and reverse mode. NCX inhibition may have antiarrhythmic effects by eliminating triggers for cardiac arrhythmias but its influence on the arrhythmia substrate seems less important.

YIA5

HUMAN-MOUSE CHIMERISM VALIDATES HUMAN STEM CELL PLURIPOTENCY AND CARDIOVASCULAR DIFFERENTIATION

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BACKGROUND: Pluripotent stem cells (PSCs) are defined by their capacity to differentiate into all three tissue layers that comprise the body and to renew themselves through unlimited proliferation. Chimera formation, generated by stem cell transplantation to the embryo, is a stringent assessment of stem cell pluripotency. However, the ability of human pluripotent stem cells (hPSCs) to form embryonic chimeras remained in question.

OBJECTIVES: Our experimental objective was to determine the capacity of hPSCs and their cardiovascular progeny to participate in normal embryo development.

METHODS: We generated a series of novel transplantation assays to the primitive streak and presumptive cardiovascular rudiments in the post-implantation mouse embryo in order to determine the capacity of in vitro derived-human donor cells for interspecies chimera formation.

RESULTS: We show using a stage-matching approach that the two hPSC types, human induced pluripotent stem cells (hiPSCs) and human embryonic stem cells (hESCs), and their in vitro derived cardiovascular progenitors have the capacity to participate in normal mouse development when transplanted into post-implantation mouse embryos. Both hPSCs and their differentiated progeny form interspecies chimeras with high efficiency, whereby they colonize the embryo in a manner predicted from classical developmental fate mapping. Pivotally, hPSCs integrate and differentiate into each of the three primary tissue layers; endoderm, mesoderm and ectoderm. Thus we show that hPSCs have the capacity to participate in normal development, providing in vivo functional validation of hPSC pluripotency.

CONCLUSION: hPSCs and their cardiovascular derivatives can incorporate into mouse embryos in a stage- and location-specific (synchronous, orthotopic) manner. This novel approach enables the study of cell fate decisions and plasticity of tissue specific progenitors during normal development. Faithful recapitulation of tissue-specific fate post-transplantation underscores the functional potential of hPSCs and provides evidence that human-mouse interspecies developmental competency can occur.

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YIA6

EFFECT OF BISPHOSPHONATES ON PARAOXONASE 1 (PON1) ACTIVITY AND GENE EXPRESSION IN VARIOUS TISSUES IN PATIENTS WITH COEXISTING OSTEOPOROSIS AND INCREASED RISK OF ATHEROSCLEROSIS

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BACKGROUND: Bisphosphonates are used for the treatment of osteoporosis, which inhibit bone resorption effectively both in vitro and in vivo. Clinical data suggest that bisphosphonates may have a beneficial in vivo effect on atherosclerotic process and plasma lipid profile, preventing calcification of soft tissues, blood vessels and skin. Homocysteine (Hcy) is linked to the pathogenesis of atherosclerosis through their interactions with the antiatherogenic enzyme paraoxonase 1 (PON1) that has the ability to affect protein N-homocysteinylation process.

OBJECTIVES: The aim of this study was to investigate whether bisphosphonates treatment modulates the effect on PON1 activity, lipid metabolism and the level of protein N-homocysteinylation.

METHODS: The study was performed on Wistar rats. Bisphosphonates were administered for 4 weeks orally: clodronate (3 mg/kg b.w./once a week), alendronate (1 mg/kg b.w./day), risedronate (0.5 mg/kg b.w./day), ibandronate (0.05 mg/kg b.w./day) and intraperitoneally: pamidronate (1 mg/kg b.w./day) and zoledronate (100 μg/kg b.w./twice a week).

RESULTS: We found that bisphosphonates have different effects on PON1 activity in plasma, because drugs such as clodronate, pamidronate, alendronate and ibandronate decreased PON1 activity, the highest for ibandronate. In contrast, risedronate and zoledronate increased PON1 activity. In liver, we found that PON1 activity was increased by alendronate, risedronate and zoledronate. All of bisphosphonates groups had no significant effect on plasma Hcy-thiolactone and N-Hcy-protein levels. Moreover, lipid profile (total cholesterol, HDL-cholesterol and triglycerides) remained unchanged.

CONCLUSIONS: These findings demonstrate that bisphosphonates have different effects on PON1 activity, which may suggest a particular drug selection of this group into the treatment patients with increased risk of atherosclerosis.

YIA7

SARCOLEMMAL CA^{2+}-ENTRY THROUGH L-TYPE CA^{2+} CHANNELS CONTROLS THE PROFILE OF CA^{2+}-ACTIVATED CL^- CURRENT IN CANINE VENTRICULAR MYOCYTES

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BACKGROUND: Ca^{2+}-activated Cl^- current (ICl(Ca)) mediated by TMEM16A and/or Bestrophin-3 may generate a component of the arrhythmogenic transient inward current, leading to delayed afterdepolarizations and cardiac arrhythmias in Ca^{2+} overloaded cells. The true profile of ICl(Ca) during an actual ventricular action potential (AP), however, is poorly understood.

OBJECTIVES: We aimed to study the profile of ICl(Ca) systematically under physiological conditions (normal Ca^{2+} cycling and AP voltage-clamp) as well as in conditions designed to change intracellular Ca^{2+} concentration ($[Ca^{2+}]i$).

METHODS: $[Ca^{2+}]i$ was monitored using Fura-2-AM. The expression of TMEM16A and/or Bestrophin-3 in canine and human left ventricular myocytes was examined. The possible spatial distribution of these proteins and their co-localization with pore forming subunit of L-type Ca2+ channel (Cav1.2) was also studied.

RESULTS: The profile of ICl(Ca), identified as a 9 anthracene carboxylic acid-sensitive current under AP voltage-clamp conditions, contained an early fast outward and a late inward component, overlapping early and terminal repolarizations, respectively. Both components were moderately reduced by ryanodine, while fully abolished by BAPTA, but not EGTA. Setting $[Ca^{2+}]i$ to the systolic level measured in the bulk cytoplasm (1.1 μM) decreased ICl(Ca), while application of Bay K8644, isoproterenol, and faster stimulation rates increased the amplitude of ICl(Ca). Ca^{2+}-entry through L-type Ca^{2+} channels was essential for activation of ICl(Ca). TMEM16A and Bestrophin-3 showed strong co-localization with one another and also with Cav1.2 channels, when assessed using immunolabeling and confocal microscopy in both canine myocytes and human ventricular myocardium.

CONCLUSIONS: Activation of ICl(Ca) in canine ventricular cells requires Ca^{2+}-entry through neighboring L-type Ca^{2+} channels and is only augmented by sarcoplasmic reticulum Ca^{2+} release. Substantial activation of ICl(Ca) requires high Ca^{2+} in the dyadic clefts which can be effectively buffered by BAPTA, but not EGTA.

POSTER PRESENTATIONS (P1 – P39)

P1

HYPERTENSION AWARENESS CAMPAIGN IN THE CITY OF WINNIPEG, MANITOBA, CANADA

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BACKGROUND: 22.6% of Canadian adults have hypertension. In Manitoba, the prevalence of diagnosed hypertension is similar to the national average (20.3%). However, the process of assessing the burden of hypertension in any population is a dynamic process that requires continual monitoring. As a result, a hypertension awareness campaign was designed to determine the prevalence of high blood pressure in an urban centre.

OBJECTIVE: The objectives of the hypertension pressure awareness campaign were to determine the prevalence of high blood pressure in Winnipeg through mobile clinics, increase hypertension awareness and management for the public, and gain knowledge on reasons for lack of therapy adherence.

METHODS: A hypertension awareness team went to workplaces, community events, shopping malls, community centres to measure Blood pressure (BP). BP was measured using the BPTru instrument or with a sphygmomanometer.

RESULTS: 1097 individuals participated in the hypertension awareness mobile clinic. Of the 1097 participants seen, 50% presented with normotension or pre-hypertension. Surprisingly, 2% of participants presented with a hypertensive urgency/emergency. Two-thirds of these individuals reported they had been diagnosed previously with hypertension but were no longer taking anti-hypertensive medications.

CONCLUSION: These data have health implications for the general public by identifying areas that require further attention and provided increased awareness to populations unaware of impending medical need. The mobile clinics offer a strategy for knowledge translation to the public. It is recommended that this type of approach be implemented throughout the country.

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P2

RELATIONSHIP BETWEEN THE MATURATION OF TITIN ISOFORMS AND OXIDATIVE DAMAGE OF CARDIOMYOCYTES

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BACKGROUND: During the perinatal adaptation the N2BA titin isoforms are switched for N2B titin isoforms leading to an increase in sarcomeric passive force (Fpassive). The oxidative modifications of titin isoforms contribute to the postnatal diastolic dysfunction.

OBJECTIVES: Here we attempted to reveal how titin isoform composition and oxidative insults (SH oxidation, carbonylation) influence Fpassive of left ventricular (LV) cardiomyocytes following birth.

METHODS: Investigations were performed at different stages of postnatal development (i.e. at 0, 7 and 21 days; 8 weeks adult) of control Wistar rats. Fpassive was measured in single, permeabilized LV cardiomyocytes. The effects of SH oxidation and carbonylation of titin isoforms on Fpassive were evaluated following in vitro exposures to an oxidative agent, dithiodipyridine (DTDP, 10mM) or Fenton reagents (50μM FeSO4, 1.5mM H_2O_2, 6mM ascorbic acid) in cardiomyocytes. Western-blot analyses were carried out for the semiquantitative determination of SH groups (by biotinylation) and carbonyl groups (by OxyBlotTM) in titin isoforms.

RESULTS: DTDP and Fenton reagents significantly increased Fpassive in 0- and 7-day-old rats, but to a lesser extent in 21-day-old and adult animals (ΔFpassive of DTDP: 99.29±26.02% vs. 64.89±23.2% vs. 38.57±20.9, vs. 18±7%; ΔFpassive of Fenton: 138±33%; vs. 55±13% vs. 27±8% vs. 18±7% $P<0.05$ in 0, 7, 21 vs. adult groups, SL: 2.3μm, n=7-8). The relative extents of DTDP-evoked SH oxidations and Fenton-evoked carbonylations declined with cardiomyocyte age for both titin isoforms (N2BA (DTDP): 80±1%, 71±1%, 64±1%, cannot detect; N2B(DTDP): 74±1%, 62±2%, 53±2%, 32±2%,; N2BA(Fenton): 2.57±0.06 AU, 2.34±0.04 AU, 1.35±0.03 AU, cannot detect; N2B(Fenton): 2.9±0.1 AU, 2.58±0.04 AU, 1.74±0.04 AU, 1.53±0.05 AU in 0, 7 and 21-day-old rats and adult animals, respectively, $P<0.05$, n=4-20).

CONCLUSION: Cardiomyocyte differentiation is accompanied by a gradual decrease in the oxidative sensitivity of Fpassive due to the sarcomeric maturation of N2BA and N2B titin isoforms.

P3

A METABOLITE OF AMIODARONE AS A POSSIBLE ANTI-TUMOR AGENT IN BLADDER CANCER

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BACKGROUND: Bladder cancer (BC) is a common malignancy of the urinary tract having higher frequency at men, and its prevalence ranks 5th in Europe. Metastasis formation and cytostatic resistance are significant

risk factors. Therefore identification of novel drugs killing cancer cells by novel mechanism could be desirable.
OBJECTIVES: Desethylamiodarone (DEA) - metabolite of amiodarone- which occurs at high concentration in human, and can have cytostatic potential in T24 bladder cancer cells.
METHODS: Cell viability assay (MTT), Crystal violet staining, Muse™ Annexin V & Dead Cell Assay, Muse® Cell Cycle Assay, Detecting mitochondrial membrane potential ($\Delta\psi$) by JC-1 die, Western Blot analysis.
RESULTS: DEA activates mitochondrial permeability transition (mPT) showed by the collapse of mitochondrial membrane potential and reduces colony formation of T24 cells indicating its possible inhibitory effect on metastatic potential. DEA induces cell death in T24 bladder cancer cells at physiologically achievable concentration, and cell death is predominantly early and late phase of apoptosis. In addition, DEA induces cell cycle arrest in the G0/G1 phase, which may contribute to the inhibition of cell proliferation and activates apoptotic pathway. DEA inhibits ERK and Akt kinases the major cytoprotective kinases which can participate in autophagy.
CONCLUSION: The high recurrence of bladder cancer is an obstacle in its clinical treatment. Therefore, an effective therapeutic strategy is urgently required. In the current study, we report the ability of an amiodarone metabolite, DEA, to inhibit growth and induce apoptosis through a number of pathways in T24 bladder cancer cells, which indicates its potential as an antitumor agent for the treatment of bladder cancer.

P4

GENOTYPE-PHENOTYPE CORRELATIONS IN LONG QT SYNDROME PATIENTS GENOTYPED BY NEXT-GENERATION SEQUENCING

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BACKGROUND: Long QT syndrome (LQTS) is a genetically determined arrythmogenic disease affecting ion channels of the heart. Several studies suggested that LQTS patients carrying multiple genetic variants may exhibit a more malignant phenotype.
OBJECTIVES: The aim of our study was to examine genotype-phenotype correlations in LQTS patients genotyped for 69 ion channel genes.
METHODS: Thirty-one LQTS patients (9 male, 22 female, avg. age: 28±13 years) were included. Genotyping was done by next-generation sequencing, sequencing 69 ion channel genes in total. Identified rare variants were defined as causative mutations or variants of unknown significance (VUS). Variants were considered causative mutations if literature data proved causation, or predictive models indicated a damaging effect.
RESULTS: A total of 64 variants (17 causative mutation and 47 VUS) were detected in the 31 patient (2,06 variants/patient). The average QTc (QTcavg) showed a trend to increase in mutation carriers (499±46 vs. 489±69 ms, P=0,641), and tended to rise in correlation with the number of variants. The trend for increased QTcavg were also represented in carriers with >1 variants (499±66 vs. 484±38 ms; P=0,497), in carriers with >2 variants (529±85 vs. 481±39 ms, P=0,159) or if the patient carried a causative mutation and multiple VUS (518±67 vs. 479±22 ms; P=0,226). The average age at the time of first symptoms were lower in causative mutation carriers (18±17 vs. 31±13 year; P=0,024); if they carried >1 variant (24±15 vs. 35±15 year; P=0,06), or a mutation and multiple VUS (11±9 vs. 23±16 year; P=0,128). Identical, trend-like differences were observed with regard to maximal QTc and the average age at the time of diagnosis.
CONCLUSION: Our findings suggest that the presence of multiple variants or variants with a dominant effect (i.e. causative mutation) may lead to more severe form of the disease in LQTS patients.

P5

EFFECT OF SPECIFIC DRUGS ON MIRNA EXPRESSION LEVELS IN IRRADIATED RAT HEARTS

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Radiotherapy belongs to the most effective methods to beat cancer diseases. However, irradiation affects also surrounding healthy tissues and may result in unwanted adverse side effects. In the case of thoracic irradiation it can cause radiation-induced heart disease. Therefore, it is necessary to look for ways to minimize this negative impact. The aim of this study was to determine the expression levels of cardiac specific miRNAs (miR-1, miR-15b and miR-21) in the irradiated rat left ventricles (irradiation ratio 6-7 Gy/min, total 25 Gy in a single dose). The rats were treated with selected drugs (Atorvastatin, acetylsalicylic acid (ASA), Tadalafil and Enbrel) for six weeks after irradiation. miRNA levels were measured by quantitative real-time PCR. Down-regulation of miR-1 was observed in many cardiac diseases. MiR-1 was also down-regulated in the rat hearts after irradiation. In tadalafil and atorvastatin treated groups miR-1 expression levels were further decreased compared with irradiated controls. However Enbrel increased miR-1 level in irradiated hearts similarly to non-irradiated untreated group. Increasing of miR-15b is pro-apoptotic in association with ischaemia. In our study, irradiation caused downregulation of miR-15b by more than 26%. In treated groups no significant changes of miR-15b expression levels were found. MiR-21 belongs to the most strongly upregulated miRNAs in response to cardiogenic stress. After irradiation, miR-21 was increased nearly 2-fold compared to control hearts. Our results show that tadalafil has the most protective effect on miR-21 (reduction about 40%) in comparison with irradiated untreated group. Based on the changes in cardiac specific miRNAs our study demonstrated possible protective effect of Enbrel and tadalafil on the heart damaged by irradiation.
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P6

EXPRESSION OF CLASSICAL MEDIATORS IN THE HEARTS OF RATS WITH HEPATIC DYSFUNCTION

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BACKGROUND: Liver cirrhosis is associated with impairment of cardiovascular function including alteration of the heart innervation, humoral and nervous dysregulation, changes in systemic circulation and electrophysiological abnormalities. Choline acetyltransferase (ChAT), enzyme forming acetylcholine, and tyrosine hydroxylase (TH), enzyme participating in norepinephrine synthesis, are responsible for production of classical neuromediators.
OBJECTIVES: The aim was to study the influence of experimentally induced hepatic dysfunction on the expression of ChAT and TH in the heart. METHODS: Hepatic dysfunction was induced by application of thioacetamide (TAA) or by ligation of biliary duct. Biochemical parameters of hepatic injury and levels of peroxidation in the liver and heart were measured. Relative expressions of mRNAs for ChAT and TH were expressed as a ratio of target gene Cq value to Cq value of reference gene.
RESULTS: Liver enzymes measured in plasma were significantly elevated. Level of peroxidation was increased in the liver of the TAA group, while operated animals showed reduction. In the left atrium of operated rats, the expression of TH of animals was lower, while expression of ChAT remained unchanged. In TAA group, no significant differences in the expression of the genes compared to controls were observed.
CONCLUSIONS: Liver injury induced by ligation leads to imbalance in the intracardiac innervation, while chronic administration of TAA does not alter expression of tested mediators in the heart.

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P7
HEMOGLOBIN AND IT'S DEGRADATION PRODUCTS INHIBIT ACE-ACTIVITY

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BACKGROUND: The renin-angiotensin-aldosterone system (RAAS) plays a major role in the salt-water homeostasis and blood pressure regulation of the body. Therefore, dysfunction of RAAS may contribute to the development of several cardiovascular diseases. As a part of RAAS, angiotensin-converting enzyme's (ACE) inhibition is an important therapeutic approach.

OBJECTIVES: Accurate determination of ACE-activity is influenced by several factors (e.g. the albumin-mediated endogenous ACE inhibition), hence in our study we aimed to examine and identify these unknown factors.

METHODS: Using a baculovirus expression system we produced human recombinant ACE in SF9 insect cells. Sera and recombinant ACE-activity was determined with a fluorescent kinetic assay by the cleavage of Abz-FRK-Dnp-P synthetic substrate.

RESULTS: First, we tested the effects of free hemoglobin (reddish samples), bilirubin (yellowish samples) and triglyceride (turbid samples) in human sera. Above 0.53 g/L free hemoglobin concentration (normal serum content: <0.15 g/L) and 64 μM bilirubin concentration (normal serum content <17 μM) we observed significant reduction of the ACE-activity. The turbidity of samples (examined up to 16 mM, normal serum content: <1.7 mM) did not influence the measurements. Using recombinant enzyme we tried to distinguish between interference and real enzyme inhibition. According to biochemical properties of recombinant protein, it appeared to be similar to serum ACE (captopril IC50 values: 1.275 nM, n=3; 1.966 nM, n=3, respectively). Under these conditions hemoglobin and bilirubin significantly inhibited the recombinant ACE-activity as well (IC50 values: 0.23 g/L n=3; 25.15 μM, n=3, respectively).

CONCLUSIONS: In various pathological conditions free hemoglobin and bilirubin can reach these concentrations in the blood (e.g. hemolytic transfusion reaction, immunohaemolytic anaemia, liver cirrhosis), thus RAAS activity may be significantly reduced, in vivo.

P8
ACTIVATION OF PURINERGIC RECEPTORS P2Y11 ALLEVIATED ENDOTHELIAL DYSFUNCTION AND OXIDATIVE STRESS IN AN EXPERIMENTAL MODEL OF ACUTE INFLAMMATION

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BACKGROUND: The contribution of purinergic signaling to pathogenesis of vascular inflammation is complex and partially understood.

PURPOSE: The present study was purported to assess the involvement of purinergic receptors P2Y11 in the regulation of vascular function and reactive oxygen species (ROS) generation in aortic segments isolated from lipopolysaccharide (LPS) treated rats.

METHODS: To this aim rats were treated with a single dose of LPS (8 mg/kg, i.p.). Twelve hours later thoracic aortas were isolated and used for studies of vascular reactivity (organ bath) and ROS measurements (ferrous oxidation xylenol orange/FOX assay).

RESULTS: LPS treatment significantly increased contractility to phenylephrine and attenuated endothelial-dependent relaxation of vascular segments in response to cumulative doses of acetylcholine. An increased generation of vascular hydrogen peroxide (H2O2) also occurred, as expected in the presence of acute inflammation. The P2Y11 activator NF546 (10 μmol/L) reduced the LPS-induced aortic H_2O_2 generation and partially normalized vascular function, i.e. reduced contractility and improved relaxation. The effects were abolished by co-treatment with the P2Y11 inhibitor NF340 (10 μmol/L) and, also, after endothelium denudation. Importantly, the P2Y11 activator NF546 do not act as scavenger for H_2O_2, suggesting that the beneficial effects on vasculature are the direct consequence of P2Y11 stimulation.

CONCLUSION: Activation of P2Y11 purinergic receptors alleviated endothelial dysfunction and ROS generation in experimental acute inflammation elicited by LPS administration. Further studies are required to elucidate whether these receptors are also involved in the chronic, low-grade vascular inflammation associated with atherosclerosis.

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P9
ALTERED MITOCHONDRIAL MORPHOLOGY AND FUNCTION INVOLVED IN THE DELAYED ANTIARRHYTHMIC EFFECT OF SODIUM NITRITE IN ANAESTHETIZED DOGS

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BACKGROUND: We have evidence that the administration of NaNO2 24h before acute coronary artery occlusion and reperfusion significantly attenuates the incidence and severity of ventricular arrhythmias during reperfusion, meanwhile increased survival. We have also shown previously that delayed $NaNO_2$ treatment decreased the tissue peroxynitrite and superoxide production.

OBJECTIVES: There is evidence that changes in the mitochondrial morphology and energetics have cardioprotective effect after NaNO2 administration in rodent models, we have now focused on the changes in the function and phenotype of the mitochondria.

METHODS: Twelve dogs were anaesthetized and received either saline (n=10) or sodium nitrite (n=10) infusion followed by a 25min LAD occlusion and 2min reperfusion 24h later. The sham-operated animals were considered as control group (n=5). Mitochondrial morphology and calcium deposits were characterized by transmission electron microscopy (TEM). The respiratory parameters and the response to calcium overload were measured with Clarke-type oxygen electrode and the ATP production by luminescence assay.

RESULTS: In response to acute myocardial ischaemia and reperfusion, severe morphological damage and elevated mitochondrial calcium were seen on the TEM images which was preserved in the $NaNO_2$ treated animals. The respiratory parameters and ATP production rate were both attenuated 24h after the $NaNO_2$ administration. Furthermore, $NaNO_2$ increased the tolerance of mitochondria to calcium overload.

CONCLUSIONS: $NaNO_2$ inhibits the respiration and ATP production of the mitochondria, which can be especially important at the first few minutes of reperfusion when the burst of ROS production occurs. Consequently, decreased peroxynitrite and superoxide formation were seen. The attenuation of mitochondrial calcium and the increased response to calcium overload can also be cardioprotective, because the mitochondrial respiratory function will be preserved and the ROS burst can also be avoided. Our conclusion is that $NaNO_2$ results in delayed antiarrhythmic protection, in which the preservation of mitochondrial function and morphology may play a role.

P10

POTENTIAL ROLE OF HERG IN THE DEVELOPMENT OF LONG QT SYNDROME 5

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BACKGROUND: Long QT syndrome 5 (LQTS5) is caused by mutations in the gene encoding for minK. A transgenic rabbit model of LQTS5 based on the cardiac over-expression of an LQTS5 allele of minK has recently been developed. MinK is an important regulator of the cardiac slow delayed rectifier potassium current (IKs). Biophysical properties of IKs were different and, surprisingly, kinetic parameters of IKr were also affected in the LQTS5 transgenic animals.

OBJECTIVES: We aim to investigate the possible interaction of wild-type (WT) and the LQTS5 allele of minK with Herg, the pore-forming subunit of IKr channels.

METHODS: Herg was co-expressed heterologously with WT- and LQTS5-minK in CHO cells. Whole cell currents were characterised by the voltage-clamp mode of the patch clamp technique.

RESULTS: Tail current densities decreased in the presence of WT-minK (6.4 pA/pF, n = 13, $p<0.05$), compared to Herg alone (16.5 pA/pF, n=19), while the LQTS5-minK allele had no effect (16.6 pA/pF, n=14). Current densities were also unchanged when both the WT- and LQTS5-minK were present (15.6 pA/pF, n=7). Kinetics of current deactivation was assessed by determining half decay time of tail currents. WT-minK accelerated current deactivation (222.7 ms, n=9, $p<0.05$) compared to Herg alone (563.5 ms, n=17), while the LQTS5-minK allele had no effect (486.4 ms, n=11). On the other hand, current deactivation was accelerated in the presence of both WT- and LQTS5-minK (247.4 ms, n=5, $p<0.05$).

CONCLUSIONS: WT-minK decreased current densities and accelerated current deactivation of Herg. LQTS5-minK displayed dominant negative effect with respect to current densities, but not with respect to deactivation kinetics. These results suggest a possible interaction between minK and Herg, but do not explain IKr changes observed in the LQTS5 transgenic animals.

P11

INHIBITION OF DEVELOPING CHLAMYDIAL INFECTION: CARDIO-PROTECTIVE ASPECT

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BACKGROUND: A strong association exists between chlamydial infection and atherosclerosis, providing the rationale for antibiotic interventions in cardiovascular therapy. Previously, we demonstrated that *Chlamydia pneumoniae* induces proliferation of vascular smooth muscle cells and stimulates arterial thickening. Several different mechanisms must be in place to induce host cell proliferation. Upregulation of Na/H exchanger (NHE) increases proliferation and migration of smooth muscle cells. We hypothesized that chlamydial infection is dependent on the Na^+-translocating NADH:ubiquinone oxidoreductase (Na^+-NQR) and stimulates Na^+ import via NHE, boosting cell proliferation.

OBJECTIVES: To examine whether Na^+-NQR is indispensable for the Chlamydiae infectious process. By using a novel synthetic furanone inhibitor of Na^+-NQR (PEG-2S), we tested the hypothesis that inhibition of Na^+-NQR prevents Na+ accumulation in host cells and could be a valid therapeutic agent preventing chlamydial infection.

METHODS: Infection of HeLa and HEK-293 cells by C. trachomatis was used as a model to assess antichlamydial activity of PEG-2S via immunocytochemistry. Na^+-NQR activity was measured on sub-bacterial vesicles. pHrodoTM Green AM and CoroNa Green Indicators were used to measure intracellular pH and intracellular sodium concentration, respectively.

RESULTS: Infection by C. trachomatis significantly increased first [H+] and then [Na^+] in the host mammalian cells. The Na^+-NQR inhibitor PEG-2S blocked the changes in both [H^+]in and [Na^+]in induced by chlamydial infection, inhibited bacterial growth with a half-minimal inhibitory concentration in the submicromolar range but did not affect the viability of mammalian cells or benign intestinal microflora. At low nanomolar concentrations, PEG-2S arrested Na^+-NQR activity in sub-bacterial vesicles.

CONCLUSIONS: The chlamydia-induced increase in [Na^+] in may represent an additional pathway underlying the known phenomenon of SMC proliferation caused by chlamydial infections. Being a potent inhibitor of Na^+-NQR, PEG-2S prevents infection-driven host cell acidification and subsequent sodium accumulation. PEG-2S may be attractive for clinical use to suppress chlamydial growth and proliferation of infected cells in cardiovascular therapy.

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P12

NOVEL SYNTHETIC BENZOPYRAN DERIVATIVES ENHANCE ENDOTHELIAL FUNCTION AND REDUCE OXIDATIVE STRESS IN VASCULAR PREPARATIONS

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BACKGROUND: Mitochondria have emerged in the past decade as major therapeutic targets in cardiovascular pathology. We have previously demonstrated, in isolated rat heart mitochondria, that novel synthetic benzopyran analogues derived from a BMS-191095, a selective mKATP opener, modulate respiratory function and decrease the generation of reactive oxygen species (ROS) in a dose-dependent manner. Whether the compounds have an effect on vascular function in diseased vessels it is not known.

PURPOSE: The present study was purported to assess the effect of three benzopyran analogues on the vascular reactivity and hydrogen peroxide (H_2O_2) production in aortic rings isolated from rats with streptozotocin-induced diabetes mellitus (DM) and mammary arteries harvested from patients with and without DM undergoing coronary artery bypass graft surgery.

METHODS: The effect of KL1487, KL1492, KL1507 (10μmol/L) on endothelium-dependent relaxation (EDR) assessed in the organ bath and H_2O_2 production (determined by ferrous oxidation xylenol orange assay) have been studied in diabetic vs. non-diabetic murine and human vascular fragments.

RESULTS: We found an important decrease in EDR in diabetic vessels whereas H_2O_2 generation was significantly increased in both humans and rats. Incubation of vascular segments with all investigated compounds attenuated H_2O_2 production, reduced contractility and partially restored EDR.

CONCLUSION: The novel benzopyran analogues KL1487, KL1492, and KL1507 might be useful in improving vascular function in clinical conditions associated with high oxidative stress and endothelial dysfunction such as coronary artery disease and diabetes.

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P13

SYSTEMIC PARP-1 INHIBITION ATTENUATES CAROTID VESSEL REMODELLING AND HAVE PROTECTIVE EFFECTS ON DORSAL HIPPOCAMPUS IN SPONTANEOUSLY HYPERTENSIVE RATS

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BACKGROUND: Chronic hypertension may have detrimental effects due to structural alteration of vascular wall and damage of supplied tissues. OBJECTIVES: We aimed to evaluate effects of systemic PARP-1 inhibition in the Spontaneously Hypertensive Rat (SHR) model.

METHODS: SHRs received (SHR-L) or not (SHR-C) 5 mg/kg/day PARP-inhibitor (L-2286) for 32 weeks. Normotensive age-matched groups were included (WKY-C and WKY-L). Vasomotor responses of isolated carotid artery (CA) rings for cumulative doses of ACh and SNP were measured in a wire myograph. Activation of AKT-1 and the MAPK system was evaluated by Western blot. CA samples were isolated for Masson's trichrome staining and immunolabeling for nitrotyrosine (NT), AIF, MKP-1 and NF-kB. Brain tissues were collected for histological observation of dorsal hippocampus and immunolabeled for oxidative stress markers (NT and 4-HNE).

RESULTS: : L-2286 treatment mitigated the thickening of CA walls in SHR animals without affecting blood pressure and improved relaxation of CA rings of hypertensive rats in the presence of ACh, but not SNP. PARP-1 inhibition enhanced activation of AKT-1, while phosphorylation of ERK 1/2, JNK and p-38 MAPK was decreased in the SHR-L group due to increased MKP-1 expression. We observed lowered accumulation of fibrotic components and NT in CA walls of SHR-L animals. Chronic hypertension induced nuclear translocation of AIF and NF-kB was attenuated by treatment, with elevated MKP-1 levels. In brains of hypertensive animals dilatation of the cerebral ventriculi was apparent, which exerted pressure on dorsal hippocampus, distorting its structure. In SHR animals, accumulation of oxidative markers and cell loss found in Cornu ammonis 1 subfield was attenuated by PARP inhibition.

CONCLUSIONS: PARP inhibition had vasoprotective effects against hypertension-induced remodeling and improved endothelium dependent vasomotor function, via modulation of the AKT-1 and MAPK systems. Applied treatment also attenuated oxidative damage related cell loss in neuronal tissue.

P14

REMOTE ISCHEMIC PRECONDITIONING MAY INFLUENCE VARIOUS ASPECTS OF I/R INJURY IN A DIFFERENT WAY

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BACKGROUND: Remote ischemic preconditioning (RIP) represents a novel form of innate cardioprotection conferred by short episodes of ischemia applied in a distant organ/tissue. Efficiency of RIP in increasing myocardial resistance against ischemia-reperfusion (I/R) injury has been shown in 3-months old male rats with RIP applied either directly prior to I/R or 24-h before ischemia. However, there is no evidence on the effects of RIP in hearts from spontaneously hypertensive (SHR) rats.

OBJECTIVES: This study aimed to investigate the effect of RIP on cardiac tolerance to I/R in male SHR rats of different ages.

METHODS: Rats age of three, five and eight months were anesthetized and RIP was performed on the right hind limb. Its protocol consisted of three cycles of 5-min non-invasive limb occlusion followed by 5-min reperfusion. Subsequently, hearts were excised, Langendorff-perfused and exposed to 30-min global I and 2-h R for the evaluation of reperfusion-induced ventricular arrhythmias, infarct size and recovery of contractile function.

RESULTS: Only hearts of five month-old SHR rats exposed to RIP protocol exhibited significantly improved recovery of contractile function (LVDP). On the other hand, enhanced resistance to myocardial infarction compared to non-preconditioned animals was observed in all experimental groups. Moreover, in three and five month-old animals RIP exhibited antiarrhythmic effect, while its impact on severity of arrhythmias in eight month-old SHR rats was negative.

CONCLUSIONS: RIP may represent an effective protecting stimulus in the hearts of SHR animals. Cardioprotective effects of RIP in SHR rats show partial age-dependency, since in older adult animals, RIP decreased size of lethal injury but failed to improve recovery of contractile function and even worsened arrhythmogenesis compared to younger individuals. RIP may influence various aspects of I/R injury in a different way.

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P15

A POSSIBLE MECHANISM UNDERLYING LOW PENETRANCE IN LONG QT SYNDROME 5

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BACKGROUND: Long QT syndrome 5 (LQTS5) is caused by mutations in the gene encoding for minK, an important regulator of the cardiac slow delayed rectifier potassium current (IKs). An unexpectedly large number of LQTS mutation carriers exist without manifest symptoms but carrying the risk of life threatening arrhythmias. An LQTS5 variant of minK, carrying the G52R mutation suppresses IKs, resulting in decreased current amplitude in vitro. A transgenic rabbit model of LQTS5 based on the cardiac specific over-expression of G52R-minK has recently been developed. IKs amplitudes were unchanged and IKs deactivation was accelerated in LQTS5 transgenic cardiomyocytes compared to wild type cells.

OBJECTIVES: We aim to investigate the possible mechanism of the weak phenotype in the LQTS5 transgenic animals.

METHODS: KvLQT1, the pore-forming subunit of IKS was co-expressed with the regulatory subunits WT- and LQTS5-minK and MiRP2 in CHO cells. Whole cell currents were characterised by the voltage-clamp mode of the patch clamp technique.

RESULTS: The LQTS5-minK allele and MiRP2 decreased tail current densities (9.9 pA/pF, n=21 and 22.6 pA/pF, n = 20, respectively), compared to KvLQT1+WT-minK channels (44.7 pA/pF, n=21). Surprisingly, MiRP2 prevented the dominant negative effect of the LQTS5-minK allele and resulted similar current densities (29.2 pA/pF, n=28) compared to those mediated by KvLQT1+WT-minK+MiRP2 channels. Tail current deactivation was uniquely accelerated when MiRP2 and LQTS5-minK were present simultaneously, compared to any other subunit combination.

CONCLUSIONS: These effects of MiRP2 are in line with the observations made on IKs in the LQTS5 transgenic model. Our findings suggest that MiRP2 may play a critical role in the regulation of IKs function and contribute importantly to the repolarization reserve. As such, MiRP2 may represent a novel, previously unrecognized mechanism for the low penetrance of mutations in LQTS5.

P16

EFFICACY AND SAFETY OF ARTIFICIAL CARBON DIOXIDE FOOT BATHING FOR ISCHEMIC PAIN, ACCORDING TO INITIAL ASSESSMENT OF MICROCIRCULATORY RESPONSES TO THERAPEUTIC SESSIONS

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BACKGROUND: Artificial carbon dioxide foot bathing (CFB) is often used as adjunctive treatment for ischemic symptoms because it increases skin perfusion. However, decreased transcutaneous oxygen pressure

($tcPO_2$) and pain exacerbation occur during CFB in some severe cases. This study investigated the efficacy and safety of CFB for Fontain III patients (rest pain) according to initial assessment of microcirculatory responses to therapeutic sessions and at 1-year follow-up.

METHODS: Seventy-seven limbs of 52 patients with critical limb ischemia (CLI) were studied. All patients underwent revascularization for rest pain (without ulcer or gangrene) before initial assessment. Clinical outcome assessment was based on improvement of symptoms and incidence of adverse events (major or minor amputation). Microcirculatory response was assessed by measuring differences in $tcPO_2$, transcutaneous CO_2 pressure ($tcPCO_2$), and blood flow (BF) values before and after therapeutic sessions (foot immersion in carbonated 37-38°C water for 10 minutes; CO_2: 1,000-1,200 mg/l).

RESULTS: Pain relief was observed within 1-2 weeks of initial assessment and persisted until 1-year follow-up in 87% of patients. Cumulative incidence of adverse events was 7.8% at 1 year. Median differences [interquartile range] for $tcPO_2$, $tcPCO_2$, and BF were −3.0 mmHg [−10.0-1.0 mmHg], 0 mmHg [−1.0-2.0 mmHg], and 2.20 mml/min/100 g [1.25-3.25 mml/min/100 g], respectively. The cut-off value of $\Delta tcPO_2$ was −16.5 mmHg (sensitivity 93%, specificity 67%), and the area under the receiver operating characteristic curve was 0.88 (95% confidence interval 0.77-0.99, $p<0.01$).

CONCLUSIONS: CFB is safe for the treatment of CLI with rest pain, with a high rate of pain relief. Assessing ΔtcPO2 at initial therapeutic sessions may be helpful in identifying non-responders to CFB.

P17

TRANSGENIC LQT2, LQT5 AND LQT2-5 RABBIT MODELS WITH DECREASED REPOLARIZATION RESERVE AS NOVEL TOOLS FOR MORE RELIABLE IDENTIFICATION OF PRO-ARRHYTHMIC MARKERS

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INTRODUCTION: For more reliable prediction of drug-induced pro-arrhythmic side effects, new animal models are needed. Therefore, different transgenic LQTS rabbit models with impaired repolarization reserve were generated (LQT2, HERG-G628S, loss of IKr; LQT5, KCNE1-G52R, decreased IKs; double-transgenic LQT2-5, loss of IKr/decreased IKs).

METHODS: In vivo telemetric ECG and ex vivo monophasic action potential measurements (action potential duration (APD75), triangulation (APD90-APD30), and reverse use-dependence (APD75 at 2Hz - 4Hz)) were performed to assess the effects of potassium channel blockers on cardiac repolarization in wild type (WT), transgenic LQT5, LQT2, and LQT2-5 rabbits.

RESULTS: At baseline, heart-rate corrected QTc (ms) was similar in LQT5 (132.1±6.5) as in WT (135.7±4.8) but was significantly prolonged in LQT2 and LQT2-5 rabbits (163.9±9.2 and 165.4±12.9; $p<0.05$ vs. WT). Slight IKr-blockade by low dose dofetilide (0.02µg/kg, im) prolonged QTc in vivo only in LQT5 (change (ms), +7.7±1.9; $p<0.05$). IK1-blocker BaCl2 (0.3mg/kg, im) prolonged QTc in all groups with a particularly pronounced prolongation in LQT2 (change (ms), LQT2 +15.4±2 vs. WT +7.6±1.3; $p<0.05$). IKs-blocker HMR-1556 (0.1mg/kg, im) alone didn't lengthen QTc. Combined IK1/IKs-blockade by BaCl2+HMR significantly lengthened QTc in all groups. Ex vivo, IKs-blocker HMR-1556 (100nM), IK1-blocker BaCl2 (10µM) or combined IK1/IKs-blockade by BaCl2+HMR prolonged APD75 significantly more in LQT2 and LQT2-5 than in WT or LQT5 (changes (ms), HMR-1556: LQT2 +14.7±2.3, LQT2-5 +12.8±2.9 vs. WT +6.9±1.2; BaCl2: LQT2 +29.4±2.8, LQT2-5 +31.7±4.7 vs. WT +17.7±2.5; all $p<0.05$). Importantly, AP triangulation and reverse use-dependence were also more pronounced upon IK1-blockade or combined IK1/IKs-blockade in LQT2 and LQT2-5 than in WT (changes in APD90-30 (ms), BaCl2: LQT2 +25.8±3.6, LQT2-5 +21.5±3.3 vs. WT +13.2±2; all $p<0.05$ and changes in APD75 at 2Hz-4Hz (ms), BaCl2: LQT2 +22.2±4.7, LQT2-5 +20.1±3.4 vs. WT +10.9±2.7; all $p<0.1$).

CONCLUSION: LQT2 and LQT2-5 models are particularly sensitive to potassium channel blockers demonstrating not only QTc and APD75 prolongation but also increased triangulation and reverse use-dependence. The combined use of different transgenic LQTS rabbits with different extents in reduction of repolarization reserve may provide further insights into pro-arrhythmic mechanisms of potassium channel blocking drugs.

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P18

PROTECTIVE EFFECT OF RESVERATROL IN RIGHT HEART FAILURE

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BACKGROUND: The right heart failure occurs when the heart can't maintain its pump function and deliver enough blood to the lung arteies. The most important energy producing cell organs, the mitochondria have a special role in cell survival and death. These cell organs often go through fission and fusion cycles what results excessive morphological changes; these procedures are organized by membrane bounded dynamic GTPases (FIS1, Mfn1/2, OPA1). The injury of the membrane stability in mitochondria can result the development and progression of several diseases, like pulmonary hypertension causing right ventricle heart disease, and also other illnesses caused by oxidative stress.

OBJECTIVES: Resveratrol is a non-flavonoid polyphenol, found in plants and red wines. Previous studies have shown its favorable effects on cardiovascular system as well as inflammation. We tested the protective effect of resveratrol in monocrotaline induced pulmonary hypertension model.

METHODS: The induction of pulmonary hypertension was induced by monocrotaline subcutan injection. We calculated organ/body mass ratios, analyzed the heart muscle with light microscopy and electronmicroscopy; the biochemical changes with Western blot analysis.

RESULTS: Rats getting resveratrol had significantly smaller heart/body mass ratio and the damage in their heart was reduced. With microscopic methods we demonstrated the protection of resveratrol. The signaling pathways were analyzed with Western blot, the protecting PI3K-Akt, GSK-3β, ERK 1/2 pathways, the p38MAPK, NFκB pathways and the quantitative changes in the mitochondrial proteins (Mfn 1/2, OPA-1, FIS1, TOM20).

CONCLUSION: Resveratrol reduced right ventricle heart failure, and we could detect the beneficial changes in the signaling pathways and the defense of mitochondria.

P19

AMALAKI RASAYANA, A TRADITIONAL INDIAN AYURVEDA DRUG ATTENUATE ADVERSE CARDIAC REMODELING CHANGES ASSOCIATED WITH AGING AND CARDIAC FAILURE AND IMPROVES EXERCISE TOLERANCE IN WISTAR RATS

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RATIONALE: Amalaki rasayana (AR) prepared with fruits of *Phyllanthus emblica*, is considered the best among India's traditionally used rejuvenating 'rasayana' group of drugs. We explored in male Wistar rats the effect of long term intake of AR on cardiac function in aging associated and pressure overload cardiac hypertrophy.

METHODS: In experiment I, three groups (each having 10 rats of 3 months age) were given AR (either 750 or 500 or 250 mg /kg, orally). Another three groups (n=10) were given placebo (either 750 or 500 or 250 mg /kg, orally).

Another 10 rats were left untreated as controls. In experiment II, ascending aorta was constricted to induce left ventricular hypertrophy (LVH) in 30 rats (3 months old). After the rats developed LVH, AR or placebo (500 mg/kg, orally,) was given to two groups of 10 rats. All the rats of experiment I were sacrificed at the end of 21 months and all the rats of experiment II were sacrificed 12 months after initiation of administration of AR. Before sacrifice, electrocardiogram, echocardiogram and exercise tolerance test were done. Heart tissues were collected for histology and protein expression analysis.

RESULTS: LVIDd, LVFS and LVEF were improved ($P<0.05$) and fatigue time in treadmill exercise increased ($P<0.001$) in AR treated group of aging rats, when compared to placebo treated/control rats. AR treatment for 12 months in rats with LVH of experiment II, decreased IVSd, LVPWd ($P<0.05$) and increased LVIDd ($P<0.05$) as well as increased exercise tolerance capacity ($P<0.001$). In heart tissues of AR administered rats of both the experimental groups Myh11, SERCA2, Troponin T, CaM, p53, SOD2, GPx proteins, TCA cycle enzymes and oxidative phosphorylation complex proteins were upregulated, expression of antioxidant genes was increased, and there was decreased phosphorylation of AMPK (Thr172), increased phosphorylation of CREB (Ser133) and increase in mRNA expression of ADRB1 and ADRB2.

CONCLUSIONS: Amalaki rasayana treated rats have improved exercise tolerance capacity, a beneficial cardiometabolic proteome profile, featuring increased TCA, OXPHOS enzymes, antioxidant defense enzymes and increased muscle contraction regulatory proteins.

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P20

ALPHA LINOLENIC ACID PROTECTS CARDIOMYOCYTES FROM PRO-APOPTOTIC OXIDIZED PHOSPHOLIPIDS AFTER ISCHEMIA/REPERFUSION

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BACKGROUND: Alpha linolenic acid (ALA) is a plant derived omega-3 fatty acid, found in high levels within flaxseed. When ALA is ingested it is thought to elicit cardiovascular benefits.

OBJECTIVES: ALA is suggested to be cardioprotective during an ischemic insult however the mechanism(s) by which this protection occurs is unknown.

METHODS: Primary adult rat cardiomyocytes were isolated and exposed to media with or without ALA for 24 hours. Myocytes were then subjected to 60 minutes of non-ischemic control conditions (CTR), simulated ischemia (ISCH) for 60 minutes or 60 minutes of simulated ischemia and reperfusion conditions (IR).

RESULTS: ALA pre-treatment resulted in significant incorporation of ALA within cardiomyocyte phosphatidylcholine (PC). There was an increase in cell death after ISCH and IR in addition the-pro-apoptotic oxidized phospholipids (OxPC), 1-palmitoyl-2-(5'-oxo-valeroyl) sn-glycero-3 phosphocholine (POVPC) and 1-palmitoyl-2-glutaryl-sn-glycero-3-phosphocholine (PGPC). Pre-treating cardiomyocytes with ALA significantly reduced cell death after ISCH or IR. Treatment of cells with ALA significantly decreased apoptosis after ISCH and IR as demonstrated by the reduced the number of tunel-positive myocytes and lower levels of activated caspase-3/7 activity. ALA pre-treatment significantly attenuated the rise of resting Ca^{2+} during ischemia and reperfusion, however it had little effect on the transient Ca^{2+}. The production of PGPC and POVPC after ISCH or IR was significantly decreased following ALA pre-treatment.

CONCLUSIONS: Our results would suggest that ALA provides protection to cardiomyocytes against ISCH damage by inhibiting the production of pro-apoptotic OxPC species.

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P21

EFFECTS OF KISSPEPTIN ON DIABETIC RAT PLATELETS

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BACKGROUND: Hyperglycemia, hyperlipidemia and free radicals result in platelet activation and atherogenesis. Kisspeptin (KP), a member of the arginine-phenylalanine (RF)-amide family, participates in glucose homeostasis, prolongs the bleeding time and lowers the platelet count. Its presence has also been detected in the atherosclerotic plaque.

OBJECTIVES: We examined whether platelet aggregation of diabetic rats depends on the type of the inductor, and how the kisspeptin-13, and its antagonist (RF-9) influences the platelet function.

METHODS: Streptozotocin (i.p.2×65mg/kg) was used to induce diabetes in Wistar-Kyoto male rats. The platelet aggregation was induced with ADP, arachidonic acid, and collagen in the presence of 0-1.25-2.5-5-10×10^{-8} mol/L KP-13 and/or RF-9. We measured the speed (AU/min) and the maximum (AU) of the aggregation, along with the area under the graph (U). For statistical analysis ANOVA and then Tukey post hoc test was used.

RESULTS: Serum glucose, calcium and urine levels of diabetic animals were elevated, while body weights and platelet count were reduced. Collagen was the most effective inductor of all. Using 5×10^{-8}mol/L KP-13 induced comparable aggregation of healthy animals' platelets to that of the control group (97.4±4.1 vs. 78.3±4.2AU). This effect was significantly weaker in the case of the diabetic animals' platelets. 2.5-5-10x10-8mol/L RF-9 heightened the aggregation response of the healthy animals' platelets compared to the control group, while it had no effect on the platelets of the diabetic animals. RF pretreatment changed the effects of 5×10^{-8}mol/L KP-13 in neither animal group.

CONCLUSIONS: Increased in vivo activation and thereby selection of platelets in diabetes could be the reason for the smaller in vitro aggregation response of the platelets, which is supported by the elevated serum calcium and reduced platelet count. In the present experiments we could not demonstrate the antagonistic effect of RF-9 against the kisspeptin fragment.

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P22

ACUTE HEART FAILURE AS FIRST CLINICAL PRESENTATION OF PRIMARY DILATED CARDIOMYOPATHY

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BACKGROUND: Primary dilated cardiomyopathy (DCM) represents 90% of all primary heart muscle diseases in general cardiology practice with incidence 0.73-6.95 pts/100.000 and prevalence 8.3-36.5 pts/100.000. Dilated left ventricle (LV) and depressed LV ejection fraction in absence of congenital heart disease, coronary artery disease, valvular or pericardial diseases are key diagnostic features. Clinically, DCM presents with symptoms of heart failure (HF) in the majority of patients, less frequently with the symptoms of rhythm and conduction disturbances, thromboembolism, and sudden cardiac death (20-50). Acute HF (AHF) may also be the index event in less than 20% of patients. Furthermore, DCM is associated with the high mortality (15-50% in five years), risk for sudden death, and it is a most common indication for heart transplantation and prolonged hospitalization.

METHODS: DCM patients admitted to the Department of Cardiology, University Medical Center, Belgrade, Serbia between January 1997 and august 2003 were analyzed retrospectively. The criteria for entering the study included DCM patients with all forms and manifestation of HF, including AHF.Demographic data, risk factors for development of CVD, family history and history of previous viral infection were obtained. Clinical examination parameters included heart rate, systolic and diastolic blood pressure, gallop rhythm, cyanosis, jugular veins congestion, pulmonary congestion, heart sounds and murmurs, hepatomegaly, cardiac edema and ascites. Laboratory and cardiology work-up methods used were: ECG, chest X-Ray, echocardiography and cardiac catheterization with selective

coronary angiography. The total of 227 parameters were analyzed with cross-section of survival performed in 2004.
RESULTS: A 126 patients with DCM were analyzed, with average age of 54.71±13.23 years, 76.2% men. Average follow up was 91.2 months (4-582 months), average duration of DCM was 63.3 months (4-180 months). AHF as a first clinical manifestation of DCM occurred in 18.4%. Fatal outcome was revealed in 22 patients, 140 patients survived to the end of 2004. The most common cause of death were cardiac arrest (45.5%), followed by progressive HF (27.3%) and respiratory failure (9.2%). Pulmonary embolism, unknown cause of death, lung neoplasm, and diabetes were cause of death in 4.5% of patients. No correlation between AHF and fatal outcome was revealed. Lower systolic and diastolic blood pressure correlated with longer survival in these patients ($p<0.05$ and $p<0.05$ retrospectively). Patients with DCM who had a higher NYHA functional class had significantly shorter survival ($p<0.05$). Atrial fibrillation or flutter statistically significant increased relative risk for fatal outcome ($p<0.05$). Myocardial fibrosis (echocardiography), (excluding septum) was associated with shorter survival of patients ($p=0.02$). High right atrial pressure correlated with shorter survival ($p=0.01$).
CONCLUSIONS: AHF as a first clinical manifestation of DCM occurred in 18.4%. Cardiac arrest and progressive HF were most common causes of fatal outcome. Lower systolic and diastolic blood pressure correlated significantly with longer survival. Higher NYHA functional class, atrial fibrillation or flutter, myocardial fibrosis (excluding septum) and high pressure in the right atrium correlated with shorter survival. No correlation between AHF and fatal outcome was revealed.

P23
FIBROSIS AND COLLAGEN TYPES IN AGEING MOUSE HEARTS

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BACKGROUND: Cardiac aging is characterized by hypertrophy which is paradoxically associated with progressive impairment of heart function. Aging is assocaited with decreased elasticity due to fibrosis (accumulation of collagen) and increased susceptibility to arrhythmias. However, there are several types of collagen that are found in different locations and have differing functions.
OBJECTIVE: To assess the extent of fibrosis in aging mouse hearts and to use proteomics to both identify collagen types and their respective expression.
METHODS: Hearts from adults (2 months old, n=16) and from aged (18 months old, n=10) were collected from male C57BL/6 mice killed by a lethal dose of anesthetic (intraperitoneal (IP) injection of 20mg of pentobarbital sodium). Hearts were extracted and processed for proteomics or fixed and processed for histology. For monitoring collagen content, 5µm sections were subjected to Trichrome staining. Additionally H&E staining was used to identify and measure the lumen of large coronary arteries and assess cardiomyocyte hypertrophy using cell width. Cardiac protein extracts were processed using isobaric tandem mass tagging and analyzed by reverse phase nano-LC-MS/MS as described previously. Statistical analysis (unpaired t-test) was performed to compare differences between adult and aged groups.
RESULTS: Aged hearts showed evidence of myocardial hypertrophy assessed by the width of cardiomyoctes (approximately 1.2-fold increase) and their main ventricular coronary arteries had larger lumen compared to adult (approximately 1.5-fold increase). Trichrome staining revealed that aged hearts exhibited enhanced collagen deposition which was evident around the coronary arteries and within the myocardial tissue. The proteomic analysis revealed the presence of the following types of collagen: I, IV, V, VI, XIV, XV and XVIII and subtypes alpha1, alpha2, and alpha6. Collagen XIV-alpha1 chain (found in tissues containing collagen I) was signifcantly ($P<0.05$) lower in aged hearts compared to adult hearts (0.5-fold). Chains from collagen VI found in connective tissue, including collagen VI-alpha1, collagen VI-alpha2 and collagen VI-alpha6 were more than doubled (2.3-2.5-fold) in aged hearts. Collagen I-alpha1chain and XVIII-alpha1chain were also significantly higher (1.5 & 1.6-fold, respectively) in aged hearts. There was no difference between the two age groups for collagen V alpha1 and alpha2 chains, collagen XV-alpha1chain, and collagen IV-alpha2 chain.
CONCLUSION: This work shows for the first time that increased collagen deposition with age is the resultant of increased collagen types I, VI and XVII, but coincides with decreased collagen type XIV and no change in collagen types IV, V and XV. The importance of these changes in aging-related structural remodelling in the myocardium requires further investigation.

P24
PROGNOSTIC ROLE OF ANTHROPOMETRIC AND OXIDATIVE MARKERS IN A RAT MODEL OF DIET-INDUCED HYPERHOMOCYSTEINEMIA

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Hyperhomocysteinemia (HHcy) is an independent risk factor of cardiovascular disease, but the mechanisms of tissue injury are poorly understood. In the present study, we investigated the effect of HHcy on rat anthropometric characteristics and redox status. We used males Wistar albino rats (n=30; 10 rats per group; 4 weeks old; 100±15g body mass), in which HHcy was achieved by dietary manipulation: methionine-enrichment (HM) or methionine enrichment with B vitamins deficiency (HMLV). Control group (C) were the animals which were fed with standard rodent food. All animals had with free access to food and water. After the treatment for 4 weeks, serum Hcy levels, body weights and food intake were measured at every day during the treatment, prior to euthanasia. High Methionine diet caused significant increase of heart weight/body weight (0.24±0.01 to 0.27±0.01 g/100 g) compared to control conditions. Superoxide production was increased by 2.5-fold in HHcy hearts. Our results confirm the link between elevated homocysteine level and oxidative stress. The successful development and validation of this model have made it a new tool for translational medical research of metabolic disorders-related cardiovascular disease.

P25
NORMALIZED AND INDEXED PARAMETERS OF QT VARIABILITY IN PATIENTS WITH HYPERTROPHIC CARDIOMYOPATHY

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BACKGROUND: Hypertrophic cardiomyopathy (HCM) is primary disease of the myocardium and there is an increased risk of sudden cardiac death (SCD). The reliable assessment of SCD risk in individual HCM patients is critically important. The current SCD risk assessment algorithm in HCM exhibits a low positive predictive value. Different parameters of QT variability may represent a novel marker in SCD risk assessment. OBJECTIVES: The aim of the present study was to investigate different parameters characterizing QT variability in patients with HCM. METHODS: The study population consisted of 37 patients with HCM (21 males, age: 48±15 years). Digitized resting ECGs were recorded for 5 minutes. Using a special software, we evaluated the QT variability normalized for mean QT interval and the time-domain (QTSDNN) and non-linear QT variability parameters indexed for RR variability (QTSD1, QTSD2, QTSD1/QTSD2 determined by Poincaré plot).
RESULTS: All uncorrected parameters of QT variability were found to be

increased in HCM patients. However RR variability parameters did not differ in patients with HCM compared to controls. When normalizing actual QT variability parameters for the mean QT interval, difference between HCM patients and controls were still significant with regard to parameters normalized QTSDNN (0.013±0.003 vs 0.011±0.003, p=0.036) and normalized QTSD2 (0.014±0.005 vs 0.011±0.003, p=0.026). This was caused by a relative stringent correlation between QT variability parameters and the mean QT. Almost all indices, characterising QT variability parameters indexed for RR variability were increased in patients with HCM (QTSDNN: -0.27±0.3 vs -0.46±0.3, p=0.007; QTSD1: -0.19±0.3 vs -0.26±0.3, p=0.333; QTSD2: -0.30±0.3 vs -0.52±0.3, p=0.002; QTSD1/QTSD2: 0.11±0.2 vs 0.26±0.2, p=0.001).

CONCLUSIONS: Majority of the QT variability parameters were increased in patients with hypertrophic cardiomyopathy compared to controls. We need further studies to determine the possible role of these parameters in SCD risk stratification.

P26

DIFFERENT ELECTROPHYSIOLOGICAL EFFECTS OF THE LEVO- AND DEXTROROTATORY ISOMERS OF MEXILETINE IN ISOLATED RABBIT CARDIAC MUSCLE

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BACKGROUND: Racemic mexiletine is a widely used class I.B antiarrhythmic agent which blocks sodium channels. This important drug possesses both cardiac and extracardiac indications.

OBJECTIVES: The effects of R-(-) and S-(+) mexiletine stereoisomers on maximum rate of depolarization (Vmax), impulse conduction time and repolarization have not yet been investigated in isolated cardiac preparations, therefore, the aim of our work was to characterize the cellular electrophysiological effects of the R-(-) and S-(+) mexiletine on rabbit cardiac action potential parameters.

METHODS: We studied the effect of the stereoisomers by using the conventional microelectrode technique. To determine the recovery kinetics of Vmax, extra test action potentials were elicited by using single test pulses (S2) in a preparation driven at a basic cycle length of 1000 ms. The S1-S2 coupling interval was increased progressively from the end of the refractory period.

RESULTS: Both enantiomers significantly depressed the Vmax at fast heart rates (at basic cycle lengths 300 ms – 700 ms). R-(-) mexiletine has more potent inhibitory effect than S-(+) mexiletine. Both R-(-) and S-(+) mexiletine significantly inhibited the Vmax of early extrasystoles measured at 70 ms diastolic interval induced by S1-S2 stimuli. R-(-) mexiletine has more pronounced inhibitory effect than S-(+) mexiletine. The time constant (τ) of recovery of Vmax was found to be τ = 376.0 ± 77.8 ms for R-(-) mexiletine and τ = 227.1 ± 23.4 ms for S-(+) mexiletine which indicates a slower offset kinetics for R-(-) mexiletine from the sodium channels than that of the S-(+) enantiomer.

CONCLUSIONS: R-(-) mexiletine might be more potent antiarrhythmic agent than S-(+) mexiletine. Using lower doses of the probably more potent R-(-) mexiletine in the therapy of different disease conditions (arrhythmias, abnormal hyperexcitability of the myotonic muscles, neuropathic pain, ALS), might result in the reduction of unwanted adverse effects.

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P27

PHARMACOLOGICAL INHIBITION OF THE NATRIUM/CALCIUM-EXCHANGER ATTENUATES THE HYPOKALEMIA-INDUCED ELEVATED CELLULAR CALCIUM LOAD AND DECREASES THE RISK OF ARRHYTHMIAS

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BACKGROUND: Hypokalaemia (HK) increases the risk of cardiac arrhythmias. HK decreases the Na-K-ATP-ase activity, leading to elevated intracellular Na. This may elevate the cellular Ca through activation of reverse mode Na/Ca-exchanger (NCX), which may contribute to the increased arrhythmia risk.

OBJECTIVES: In this study we investigated whether the selective NCX blocker ORM-10962 prevents the HK-induced electrophysiological changes and the associated increase of arrhythmia risk.

METHODS: Left ventricular pressure (LVP) and ECG were recorded in isolated guinea pig and rat hearts, while action potential duration (APD) and cell shortening were studied in isolated rat papillary muscles and ventricular cells, respectively. HK was induced by low K (2 mM) solutions. Since in our model HK alone was not able to induce cardiac arrhythmias, we increased the Ca level to 3 mM in arrhythmia and APD experiments. NCX inhibition was achieved with 1µM ORM-10962.

RESULTS: HK solution markedly increased LVP in isolated guinea pig hearts, indicating net cellular Ca gain. However, administration of ORM-10962 in a separate group completely prevented the HK-induced increase in LVP. In line with this observation, ORM-10962 effectively reduced the HK-induced increase of cell shortening in isolated rat ventricular myocytes. Perfusion of isolated rat hearts with HK solution markedly elevated the total number of arrhythmias compared to the normal solution (n=13 hearts). However, the presence of ORM-10962 in the HK solution significantly decreased the incidence of arrhythmias. HK solution lengthened the APD in rat papillary muscles, which was significantly reduced by ORM-10962.

CONCLUSIONS: Increased arrhythmia propensity in HK may be attenuated by NCX inhibition. Both the increased Ca load and the altered AP morphology may contribute to the arrhythmia generation. Further studies are needed to clarify the exact mechanism of antiarrhythmic protection provided by NCX inhibition in HK.

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P28

THE ACTIVITIES OF CIRCULATING MMP-2 AND MMP-9 IN PATIENTS SUFFERING FROM HEART FAILURE: A CROSS-SECTIONAL STUDY

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BACKGROUND: Extracellular matrix is a subject of extensive study due to its dynamic nature and major role in development of several diseases. Matrix metalloproteinases (MMPs) are responsible for the degradation and remodeling of the extracellular matrix. They are also suggested to play an important role in the pathogenesis of heart failure (HF).

OBJECTIVES: Our aim was to determine the activities of circulating MMP-2 and MMP-9 in patients with HF in respect of gender, occurrence of hypertension and treatment.

METHODS: MMP-2 and MMP-9 activities were determined using gelatin zymography in plasma collected from 51 participants.

RESULTS: We did not reveal any differences in circulating MMP-2 and MMP-9 activities between the patients with HF and without it. However, there was a decrease in activity of MMP-2 in treated hypertensive participants versus healthy ones. In contrast, we observed increased MMP-2 activity in hypertensive participants with coexistent HF versus hypertensive participants without HF. In addition, a decrease in MMP-2 activity was shown in women suffering from HF versus men suffering from HF.
CONCLUSIONS: In conclusion, potential inhibitory effect of antihypertensive treatment on MMP-2 activity was found. Coexistent HF with hypertension probably reduces the inhibitory effect of antihypertensive treatment on MMP-2 activity. Our data also suggest the role of potential cardioprotective factors influencing the activity of MMP-2 in women.
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P29

COMPARISON OF TWO THERAPEUTIC APPROACHES IN PRESSURE OVERLOAD-INDUCED MYOCARDIAL LEFT VENTRICULAR HYPERTROPHY: PHARMACOLOGICAL ACTIVATION OF THE SOLUBLE GUANYLATE-CYCLASE ENZYME VERSUS PRESSURE UNLOADING

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BACKGROUND: Currently, pressure unloading therapy represents the only effective, clinically available therapy in increased afterload-induced left ventricle hypertophy (LVH). However, a growing body of evidence indicates that pharmacological activation of the soluble guanylate cyclase (sGC) - cyclic guanosine monophosphate (cGMP) pathway may also exert anti-hypertrophic effects in the myocardium.
OBJECTIVES: Therefore we aimed to investigate the effects of the sGC activator cinaciguat in a rat model of pressure overload-induced LVH and compare it to the "gold standard" pressure unloading therapy. Furthermore, the potential advantages of the combination therapy were also explored.
METHODS: Pressure overload was induced by aortic banding for 6 or 12 weeks. Sham operated animals served as controls. Pressure unloading was evoked by debanding the aortic constriction after the 6th week (debanded). The experimental groups were treated from the 7th to the 12th postoperative week, with 10 mg/kg/day cinaciguat (Cin) or with placebo (Co) p.o., respectively. The temporal development of LVH was investigated by serial echocardiography. Cardiac function was assessed by pressure-volume analysis. In addition, histological and molecular biological measurements were also performed.
RESULTS: Chronic activation of the sGC enzyme was associated with improved cardiac function (ejection fraction: 47.4±2.7 vs. 63.7±2.4%, $p<0.05$ AB 12week-Co vs. AB 12week-Cin), decreased interstitial fibrosis, restored myocardial cGMP level and attenuated nitro-oxidative stress, however it did not reverse the pre-established LVH (heart weight-to-tibial length ratio [HW/TL]: 0.51±0.02 vs. 0.48±0.02g/cm, $p<0.05$ AB 6week-Co week vs. AB 12week-Cin). In contrast, pressure unloading led to the regression of LVH (HW/TL: 0.51±0.02 vs. 0.39±0.01g/cm, $p<0.05$ AB 6week-Co vs. Debanded 12week-Co). Furthermore, since pressure unloading therapy alone resulted in increased myocardial cGMP content, the combination therapy did not show any additional effects.
CONCLUSION: Our results indicate that both cinaciguat treatment and pressure unloading therapy effectively inhibited the progression of LVH to heart failure, although in a different manner.

P30

THE ROLE OF MIR-212/132 AND CALCINEURIN AXIS IN UREMIC LEFT VENTRICULAR HYPERTROPHY AND DYSFUNCTION

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BACKGROUND: The prevalence of uraemia is continuously increasing in developed countries. Uremic cardiomyopathy characterized by left ventricular hypertrophy and diastolic dysfunction is a common cardiovascular complication of uraemia; however, the underlying molecular mechanisms are not clear. The miR-212/132 cluster has already been implicated in the development of left ventricular hypertrophy via modulation of the calcineurin pathway in TAC mice.
OBJECTIVES: Therefore, here we investigated the effect of uraemia on the myocardial expression of miR-212/132 cluster and the calcineurin pathway.
METHODS: Uraemia was induced by 5/6 nephrectomy in male Wistar rats. Eight weeks later serum urea and creatinine levels were measured and transthoracic echocardiography was performed. Then RNA was isolated from left ventricles of nephrectomised and sham-operated rats and expression of miR-212 and miR-132, as well as components of the calcineurin pathway including atrogene-1 and MCIP1.4 was measured by qRT-PCR.
RESULTS: In the nephrectomised group, serum urea and creatinine levels were significantly higher proving the development of uraemia. In the uremic group, left ventricular anterior and septal walls were significantly thicker, e' was significantly decreased and E/e' was significantly increased referring to left ventricular hypertrophy and diastolic dysfunction. In the uremic group, heart weight/body weight ratio was also significantly elevated as compared to the control group. In the uremic group, miR-212 was significantly overexpressed. Moreover, atrogene-1 showed significant down-regulation and MCIP1.4 showed significant up-regulation in the uremic group.
CONCLUSIONS: Myocardial overexpression of miR-212 and subsequent modulation of the calcineurin pathway might play a role in the development of uraemia induced cardiac hypertrophy and diastolic dysfunction.

P31

EXPRESSION OF ATRIAL NATRIURETIC PEPTIDE IN THE HEARTS OF TWO RAT STRAINS: EFFECTS OF TWO TYPES OF RESTRAINT STRESSORS

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BACKGROUND: Mammalian cardiomyocytes produce atrial natriuretic peptide (ANP) with regulatory role in body fluid homeostasis. ANP induces suppression of the renin-angiotensin as well as the sympathetic nervous system to protect cardiovascular homeostasis, which is also deteriorated by the stress.
OBJECTIVES: We investigated whether ANP mRNA expression in the heart could be affected by stress. The two rat strains, Sprague-Dawley (SD) and Lewis (LE), were used, the latter being known to have a blunted hypothalamic-pituitary-adrenal response.

METHODS: A restraint stressor (immobilization, IS) and IS combined with the immersion of rats in water (ICS) were applied for one hour. Gene expression of ANP was estimated in all heart compartments after one or three hours after stress termination by real-time qPCR (IS1, IS3, ICS1, ICS3).
RESULTS: In control rats of both strains, the expression of the proANP mRNA was higher in the atria than in ventricles. In SD rats, an upregulation of ANP gene expression was observed in the right atrium after IS1, in both atria and the left ventricle after IS3 and in the left atrium and the left ventricle after ICS3. In LE rats with a blunted reactivity of the HPA axis, no increase or even a downregulation of the gene expression was observed.
CONCLUSIONS: Acute stress-induced increase in the expression of the proANP gene is related to the activity of the HPA axis. It may have relevance to ANP-induced protection of the heart.

P32
MODULATION OF NMDA RECEPTORS BY IFENPRODILE AND MEMANTINE IN ISOLATED RAT HEART

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Considering the limited data about the role of N-methyl-D-aspartate (NMDA) receptors in homeostasis maintenance in cardiovascular system and heart, the aim of the present study was to examine the effects of NMDA receptor blockers, ifenprodil tartarate and memantine hydrochloride, in the isolated rat heart. The hearts of male, Wistar albino rats (n = 24, 12 in each experimental group, BM 180–200 g), were retrogradely perfused according to the Langendorff technique at a constant perfusion pressure (70 cmH_2O). The experimental protocol for the first group included the application of ifenprodil tartarate (1 microM) in duration of 5 minutes. In the second group was applied memantine hydrochloride (100 microM) in same duration. The substance application period was followed by wash out period in duration of 10 minutes. Using sensor in the left ventricle we registered the next parameters of myocardial function: maximum and minimum rate of pressure development in the left ventricle (dp/dt max and dp/dt min), systolic and diastolic left ventricular pressure (SLVP and DLVP) and heart rate (HR). Before the ending of application of each substance, coronary flow (CF) was measured flowmetrically. In the coronary venous effluent spectrophotometrically were estimated following oxidative stress biomarkers: TBARS, NO_2-, O_2-, and H_2O_2. Both of applied substances induced reduction of cardiodynamic parameters and coronary flow, as well as some oxidative stress biomarkers.

P33
FERROPTOSIS IN ISOLATED ADULT CARDIOMYOCYTES INDUCED BY OXIDIZED PHOSPHATIDYLCHOLINE (OXPC)

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BACKGROUND: The most abundant class of phospholipids in biological membranes is phosphatidylcholine (PC), highly susceptible to oxidation. Myocardial ischemia/reperfusion (I/R) injury is followed by a large production of OxPCs and concomitant cardiomyocyte loss. Although OxPCs are potent stimulators of cell death, little is known about the effects of OxPCs in cardiomyocytes. Ferroptosis, an iron dependent accumulation of lipid oxidation products, is a possible mechanism of OxPC induced cell death.
OBJECTIVE: The aim of our study was to determine how different OxPCs influence cardiomyocyte viability, and the role of ferroptotic pathway in cell death.
METHODS: Adult rat ventricular cardiomyocytes were isolated and treated with 1-palmitoyl-2-(5'-oxo-valeroyl)-sn-glycero-3-phosphocholine (PONPC), 1-palmitoyl-2-glutaroyl-sn-glycero-3-phosphocholine (PGPC), 1-palmitoyl-2-oxovaleroyl-sn-glycero-3-phosphocholine (POVPC), 1-palmitoyl-2-azelaoyl-sn-glycero-3-phosphocholine (PAzPC), 1-palmitoyl-2-(5-keto-6-octene-dioyl)-sn-glycero-3-phosphocholine (KOdiA-PC), 1-palmitoyl-2-(4-keto-dodec-3-enadioyl)-sn-glycero-3-phosphocholine (KDdiA-PC) and 1-palmitoyl-2-stearoyl-sn-glycero-3-phosphocholine (PSPC) at concentrations of 0.1μM, 1μM, 5μM, and 10μM. Following a 1 h treatment, Live/Dead^{TM} assays were performed, cells were counted and the data presented as the percentage of live vs. total cells. When present, ferrostatin-1, an inhibitor of ferroptosis, was added together with selected OxPCs.
RESULTS: Five OxPCs tested induced a decrease in cell viability at higher concentrations ($p<0.001$), whereas PC and several other OxPCs showed no cardiotoxic effect on isolated cardiomyocytes. Two OxPCs (POVPC and PONPC) induced significant cytotoxic effects at low μM concentrations. When added with POVPC, ferrostatin-1 blocked the cardiotoxic effects of POVPC on its own ($p<0.001$).
CONCLUSIONS: Specific OxPCs can be toxic to cardiomyocytes and may induce cell death through the ferroptosis pathway. These findings implicate specific OxPCs in the process of cell death during I/R injury to the heart. The data may also provide a new therapeutic target for preventing cell loss in myocardial I/R injury.
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P34
PLATINUM(II) COMPLEXES AND OXIDATIVE STRESS IN ISOLATED RAT HEART

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The great success of cisplatin in cancer therapy aroused interest in the development of new PtII complexes, which was developed in order to overcome cisplatin side effects. The main problem in cisplatin usage is its great affinity for sulfur and nitrogen containing biomolecules, such as proteins, enzymes and small molecules. These interaction have been associated with apperance of side effects such as: cardiotoxicity, neprotoxicity, neurotoxicity etc. There is no clear evidence for cellular and molecular mechanisms involved in cisplatin cardiotoxicity, but some experimental and clinical studies support the opinion that increase in oxidative stress may lead to cardiotoxicity. In that sence, the aim of this study was to examine the effects of cisplatin and its analog dichloro-(1,2-diaminocyclohexane) platinum(II) on coronary flow and oxidative stress in isolated rat heart. In this study we used male Wistar albino rats (n = 72, 12 per group, body weight= 180-200 g, 10 weeks old) who were divided, depending on applied complex, in two groups: cisplatin group and dichloro-(1,2-diaminocyclohexane) platinum(II) group. Both groups were divided in three subgroups, depending on administried doses (10μM, 1μM and 0,1μM). Hearts were isolated and retrogradely perfused with tested substances according to the Langendorff technique at a constant perfusion pressure (70 cmH_2O) for 30 minutes. The oxidative stress biomarkers, including thiobarbituric acid reactive substances (TBARS), nitrites (NO2-), superoxide anion radical (O2-), and hydrogen peroxide (H2O2), were each determined spectrophotometrically from coronary venous effluent, and coronary flow were determined flownmetricaly. At the highest applied dose cisplatin induced greater oxidative stress than its analog, while at the middle dose situation was reversed. At the lowest dose both complex induced almost the same changes in oxidative stress biomarkers. These results indicate that administration of platinum complexes induced oxidative stress, and it could be one of the molecular mechanisms in develompent of cardiotoxicity. These results can serve as good basis of further research concenrning the effects of novel platinum compound on the heart, and could be also important for better understanding dose-dependent side effects of anticancer drug.

P35
CHANGES IN SURVIVAL SIGNALLING-RELATED CARDIAC PHOSPHOPROTEOME DURING POSTNATAL DEVELOPMENT: IMPLICATIONS FOR VULNERABILITY TO ISCHEMIA/REPERFUSION

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BACKGROUND: A bell-shaped vulnerability profile to ischemia/reperfusion (I/R) injury was identified over the course of postnatal development, with resistance to I/R injury being greatest in 2 week old rats. We have recently identified changes in the expression of several survival signalling and apoptosis-linked proteins that correlate with vulnerability to I/R with age.

OBJECTIVES: The aim of this study was to identify whether these proteins also show changes at the post translational modification level during postnatal development.

METHODS: Two runs of Tandem Mass Tag 6-plex (TMTsixplex) analysis were performed on cardiac extracts from 14 day (n=3), 28 day (n=3) and adult (n=4) rats, each sample labelled with a unique reporter group (126, 127, 128, 129, 130), before pooling into a single sample (131). Phosphopeptide enrichment was achieved through titanium dioxide (TiO2) bead chromatography, allowing separate elution of the phosphopeptides from non-phosphorylated proteins. Following LC-MS/MS analysis, proteins involved in survival signalling or apoptosis were identified within the resulting phosphoproteomic output.

RESULTS: Phosphoproteomic output revealed 31 phosphorylated proteins that significantly changed in expression with age. Of these, proteomic data for individual phosphosites was available for 25 proteins, including BCLAF1, BNIP2, BIRC6, HSPB6 and YAP1. Analysis of these sites showed general concordance in the degree and pattern of change with age as those displayed by the overall phosphoprotein. Notably, phosphorylation of Akt1 at Ser129 and Beta-Catenin at Ser552 was shown to decrease with increasing age.

CONCLUSION: Our data indicate a strong correlation between expression of these phosphoproteins and the vulnerability profile to I/R during postnatal development. The reported association of their individual phosphorylation sites to improved survival signalling and anti-apoptotic processes highlight a potential mechanism through which cardioprotection may be enhanced in early postnatal development.

P36
INHIBITION OF MONOAMINE OXIDASES ALLEVIATES ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH ADVANCED KIDNEY DISEASE REQUIRING HEMODIALYSIS

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BACKGROUND: A functional arteriovenous fistula (AVF) is mandatory in patients with end-stage kidney disease that require chronic hemodialysis. Failure or dysfunction of the vascular access is frequently encountered in practice and the underlying mechanisms are poorly understood. Oxidative stress has been systematically reported as major culprit in chronic kidney disease but the sources of reactive oxygen species (ROS) are far from being elucidated. We have recently demonstrated that monoamine oxidases, mitochondrial enzymes with 2 isoforms (A and B), that constantly generate hydrogen peroxide (H_2O_2) as by-product, are novel contributors to the oxidative stress-mediated endothelial dysfunction.

PURPOSE: The present study was purported to assess whether MAOs contribute to the endothelial dysfunction in patients with advanced kidney disease with indication of hemodialysis.

METHODS: Fragments of brachial artery collaterals were harvested from ESKD patients during the surgical procedure aimed at creating a brachiocephalic lateral terminal right fistula in the cubital fossa. The samples were placed in Hanks buffer and immediately transferred to the laboratory. The effect of the irreversible MAO-A inhibitor clorgyline (10 μmol/L) and irreversible MAO-B inhibitor selegyline (10 μmol/L) on endothelial-dependent relaxation (EDR) in response to cumulative doses of acetylcholine was studied in isolated phenylephrine-preconstricted rings in the presence of diclofenac (10μmol/L). H_2O_2 production was assessed using ferrous oxidation xylenol orange assay.

RESULTS: Our data showed an impairment of EDR in the vascular segments that has been significantly improved (by 25%) in the presence of the either MAO inhibitor. Also, MAO inhibitors attenuated the ROS production by 50%.

CONCLUSIONS: MAO-related oxidative stress might contribute to the primary dysfunction/lack of maturation of the AVF. MAO inhibition might be a viable therapeutic option for restoring the impaired endothelial function in patients with advanced kidney disease on maintenance hemodialysis.

P37
PARALLEL $I_{K,ATP}$ ACTIVATION AND IKR INHIBITION REDUCES REPOLARIZATION INHOMOGENEITY BUT THE BENEFICIAL EFFECTS ARE LIMITED BY VASORELAXATION

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BACKGROUND: Parallel activation of the cardioprotective $I_{K,ATP}$ current and the inhibition of I_{Kr} , a novel combination could limit the excessive repolarisation prolongation and repolarization heterogeneity in different cardiac tissues caused by I_{Kr} blockers. These beneficial effects could serve as a basis for a safer therapeutic option for management of serious ventricular arrhythmias. It is not known, however, how this combination would affect vascular function.

METHODS: Action potential (AP) measurements were carried out in dog and rabbit ventricular muscle preparations and Purkinje fibres by conventional intracellular microelectrode technique. The I_{Kr} blocker dofetilide (DOF; 12.5nM in rabbits; 50 and 300nM in dogs) and the $I_{K,ATP}$ activators pinacidil (PIN; 20 μM in rabbits; 1 and 3μM in dogs) and P1075 (10 and 75nM in dogs) were used. The vasoreactivity of endothelium deprived isolated rat aortas and porcine coronary arteries were investigated. Arterial rings were contracted with KCl (30mM) and relaxed by PIN (5μM) in the presence and absence of DOF (1μM).

RESULTS: Dofetilide and combinations of DOF+PIN, DOF+P1075 prolonged repolarization at all stimulation frequencies. In rabbits, the AP duration, primarily at slow stimulation frequency (40/min), was increased by combination of DOF+PIN compared to DOF. Slight reduction of repolarization inhomogeneity was detected following the combinations of DOF+PIN or P1075 compared to DOF. Results obtained in dogs were similar. DOF did not influence vasorelaxation induced by PIN in rat aorta or porcine coronary artery (aorta, PIN: 56.8±10.5% vs PIN+DOF: 62.1±9.0%; coronary artery, PIN: 59.0±11.8% vs PIN+DOF: 59.1±10.7%; in % of KCl contraction).

CONCLUSIONS: $I_{K,ATP}$ activation may reduce the proarrhythmic reverse use-dependent and repolarization inhomogeneity increasing adverse effects of I_{Kr} blockers. However, cardioselective $I_{K,ATP}$ activators, not causing reflex tachycardia due to vasodilation, would be preferred.

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MOLECULAR HYDROGEN POTENTIATES CARDIOPROTECTION CONFFERED BY HYPOXIC POSTCONDITIONING AGAINST ISCHEMIA-REPERFUSION INJURY

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BACKGROUND: Postischemic recovery of coronary flow and oxygen supply is related to excessive production of reactive oxygen species within the first minutes of reperfusion. Hypoxic and/or ischemic postconditioning are cardioprotective strategies applied in this critical time are known to reduce oxidative stress, apoptosis, necrosis. Molecular hydrogen (H2) is considered as a selective antioxidant able to react with strong oxidants and preserve cell signaling mediated by NO, •O2-.

OBJECTIVES: This study aimed to verify whether H2 can facilitate benefitial effect of hypoxic postconditioning (HpostC) against ischemia-reperfusion (I/R) injury.
METHODS: Isolated rat hearts perfused with Krebs-Henseleit buffer (KHB) were exposed to 30-min global ischemia/120-min reperfusion. HpostC was induced by 4 cycles of 1-min perfusion with oxygen-free KHB intercepted by 1-min perfusion with normal KHB, while in H2+HPostC group, oxygen-free KHB was enriched with H2. Severity of I/R injury was evaluated by measurement of infarct size (IS) within the area at risk (AR) (IS/AR, TTC staining) and recovery of functional parameters.
RESULTS: IS/AR was markedly reduced in HpostC group to 24.6±0.9% compared with 38.7 ± 1.4% in non-conditioned controls, and even more significantly in H2+HPostC group (16.6 ± 0.8%; P<0.05 vs. both, controls and HPostC). Post-I/R recovery of systolic function (LVDP) was improved in both postconditioned groups (HpostC: 46 ± 11%, H2+HpostC: 53±11% vs. 23±1.6% in controls). However, this difference reached the level of significance (P<0.05) only in the H2-enriched group. End-diastolic pressure (LVEDP) was decreased in both conditioned groups to a similar level (HpostC: 22.1±5.9 mmHg, H2+HpostC: 28.6±5.6, both P<0.05 vs. 55.2±6.9 mmHg in controls).
CONCLUSSION: Application of H2 potentiated the benefitial antiinfarct effect of HpostC, and exerted a significant antistunning effect. Molecular mechanisms behind remain to be elucidated.
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THE EFFECT OF RENIN ANGIOTENSIN ALDOSTERONE SYSTEM INHIBITION IN ISOLATED HEARTS OF STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Renin angiotensin aldosterone system (RAAS) has been proposed as one of key mediator in the pathogenesis of diabetic cardiomyopathy and the effects of chronic hyperglycemia on the tissue. Therefore, the aim of the present study was to estimate acute effects of selective RAAS inihibitors on isolated hearts of streptozotocin-induced diabetic rats. The study was conducted on male Wistar albino rats (200±30 g) divided into six groups: 1. control group (non diabetic rats), 2. control group of diabetic rats, 3. diabetic rats whose hearts are perfused with Zofenopril (1.5 μM), 4. diabetic rats whose hearts are perfused with Spironolactone (0.1 μM), 5. diabetic rats whose hearts are perfused with Valsartan (1 μM), 6. diabetic rats whose hearts are perfused with Aliskiren (1 μM). Diabetes was induced by single dose of streptozotocin (60 mg/kg) i.p. Four weeks after confirmation of diabetes (glycemia > 11.1 mmol/l) hearts were isolated and retrogradely perfused according to Langendorff technique at gradually increased coronary perfusion pressure (40-120 cmH_2O). Control groups were pefused with Krebs-Henseleit solution while diabetic hearts were administered with Zofenopril, Spironolactone, Valsartan or Aliskiren. After insertion the sensor in the left ventricule, following parameters of cardiac function were continuously measured: maximum and minimum pressure in left ventricle (dp/dt max and dp/dt min), sistolic and diastolic pressure in left ventricle (SLVP and DLVP) and heart rate (HR). Coronary flow was measured by using flowmetric method. Oxidative stress markers (TBARS, NO2-, O2- and H_2O_2) were spectrophotometrically determined in coronary venous effluent. Cardiomyopathy was confirmed by routine H/E stain. Our results have shown diminished cardiac function and coronary reactivity in streptozotocin-induced cardiomyopathic hearts, while none of tested RAAS inhibitors failed to improve it. Obtained effects in every group were not mediated via oxidative stress.