Cell growth, differentiation and stem cells - Heart

511 The role of the endocannabinoid system in modelling muscular dystrophy cardiac disease with induced pluripotent stem cells.

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Muscular Dystrophy (MD) is an umbrella term for genetic disorders affecting skeletal and cardiac muscle which arise due to abnormalities in the dystrophin gene. Understanding dystrophin metabolism and structural abnormalities in cardiomyocytes (CMs) which in turn become predisposed to ectopic cell death and fibro-fatty replacement. The Endogenous Cannabinoid System (ECS) is a lipid signalling network present in the cardiovascular system and comprises G-protein coupled receptors (CB1R and CB2R), endogenous ligands (arachidonic acid and 2-arachidonoylglycerol) and regulatory proteins (fatty acid amide hydrolase and monoacylglycerol lipase). The ECS has an emerging function in stem cell survival and differentiation, MD skeletal muscle pathology, and cardiovascular diseases in general. Induced Pluripotent Stem Cell (iPSC) technology reprograms the transcriptome of somatic cells (e.g. fibroblasts) into pluripotent stem cells, which can be differentiated into cells from all three germ layers including CMs. In the present study we provide evidence that the ECS is involved in somatic cell reprogramming. Specifically, the CB1R antagonist AM251 prevented the formation of iPSC colonies (p=0.05, vs. control conditions, Newman-Keuls multiple comparison test, n=3). CMs derived from MD patients’ iPSCs (MD-CMs) displayed disease hallmarks such as lack of dystrophin expression, increased expression of Nup153 (a cardiomyopathy-associated protein; p=0.0009, vs. healthy CMs; Student’s unpaired t test, n=3) and increased CM cell death (p=0.0001, vs. healthy CMs; Student’s unpaired t test, n=3). Furthermore, we also provide evidence that the ECS is present in iPSCs and becomes dysregulated in MD-CMs. Our results highlight the dual functionality of the ECS in reprogramming and MD cardiac pathology which is of interest to cardiac disease modelling and novel drug discovery.

512 An emerging role of T lymphocytes in cardiac regenerative processes in heart failure due to dilated cardiomyopathy

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Background/Introduction: Dilated cardiomyopathy (DCM) is a heart muscle disease of varied etiology that is treated with medical therapies, but cardiac function is reduced. DCM exhibit significant cardiomyocyte metabolic and structural abnormalities in cardiomyocytes (CMs) which in turn become predisposed to ectopic cell death and fibro-fatty replacement. The Endogenous Cannabinoid System (ECS) is a lipid signalling network present in the cardiovascular system and comprises G-protein coupled receptors (CB1R and CB2R), endogenous ligands (arachidonic acid and 2-arachidonoylglycerol) and regulatory proteins (fatty acid amide hydrolase and monoacylglycerol lipase). The ECS has an emerging function in stem cell survival and differentiation, MD skeletal muscle pathology, and cardiovascular diseases in general. Induced Pluripotent Stem Cell (iPSC) technology reprograms the transcriptome of somatic cells (e.g. fibroblasts) into pluripotent stem cells, which can be differentiated into cells from all three germ layers including CMs. In the present study we provide evidence that the ECS is involved in somatic cell reprogramming. Specifically, the CB1R antagonist AM251 prevented the formation of iPSC colonies (p=0.05, vs. control conditions, Newman-Keuls multiple comparison test, n=3). CMs derived from MD patients’ iPSCs (MD-CMs) displayed disease hallmarks such as lack of dystrophin expression, increased expression of Nup153 (a cardiomyopathy-associated protein; p=0.0009, vs. healthy CMs; Student’s unpaired t test, n=3) and increased CM cell death (p=0.0001, vs. healthy CMs; Student’s unpaired t test, n=3). Furthermore, we also provide evidence that the ECS is present in iPSCs and becomes dysregulated in MD-CMs. Our results highlight the dual functionality of the ECS in reprogramming and MD cardiac pathology which is of interest to cardiac disease modelling and novel drug discovery.

Conclusions: The results of this study demonstrate that there is a presence of increased numbers of T cells in endomyocardial biopsies from patients with DCM where cardiomyocytes progenitors and dedifferentiated cardiomyocytes were detected too. Exact mechanisms of cardiomyocyte de-differentiation are poorly understood but our results and evidence that cytokines secreted by T lymphocytes regulate cardiomyocyte de-differentiation in culture show an emerging role of T lymphocytes in cardiac regenerative processes.

513 Canonical wnt signalling reverses the ‘aged/senescent’ human endogenous cardiac stem cell phenotype

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Background: The adult human myocardium harbors endogenous, multi-potent cardiac stem cells (eCSCs). Manipulation of eCSCs ex-vivo and in situ has shown new therapeutic avenues for functional myocardial regeneration. However as aging/senescence of eCSCs determines their function and regenerative capacity, regulation of this parameter will impact the efficacy of these therapies, considering the advanced age of the majority of patients in need of regenerative therapy.

Objectives: Our aim is to determine the main factor(s) that determine the ‘aged’ human eCSC phenotype and investigate its potential reversibility.

Methods: c-kit+CD45neg cSCs were isolated from the right atria appendage (~45,000/gram of tissue). eCSCs isolated from young and old hearts showed age-correlated increased expression of TERT, Sox2) and proliferation (Ki67) markers. Telomere length of eCSCs was determined using Q-FISH analysis. DNA damage was assessed using γ-H2AX. The growth (BrDU labelling), clonogenicity and differentiation potential of young and old eCSCs were also evaluated.

Results: The number of eCSCs isolated was similar regardless of age, gender and pathology (~45,000/gram of tissue). eCSCs isolated from young and old hearts showed age-correlated increased expression of TERT, Sox2 and decreased expression of Stemness/multipotency and proliferation markers. Single cell expression analyses revealed heterogeneity within the eCSC population with eCSCs isolated from old hearts harboring a greater proportion of eCSCs with critically short telomeres and increased DNA damage. ‘Aged-senescent’ eCSCs showed limited clonogenicity and impaired cardiac differentiation capacity. Moreover, ‘aged-senescent’ eCSCs expressed increased senescence-associated secretory phenotype (SASP) factors relative to their younger counterparts. Treatment with the canonical Wnt ligand, Wnt3a significantly increased the proliferation of ‘aged-senescent’ eCSCs to levels observed in younger eCSCs. Conversely, a switch to non-canonical Wnt signaling imparted a negative ‘aged’ effect on eCSCs. Importantly, although the cloning efficiency was inversely age-related, single-cell derived eCSC clones obtained from young and old hearts were indistinguishable by their gene expression and differentiation potential, strongly suggesting that eCSC aging is a stochastic process.

Conclusion: eCSCs stochastically develop a senescent phenotype with age impacting their growth and differentiation potential. Manipulation of canonical and non-canonical Wnt signaling pathways reversed the ‘aged/senescent’ phenotype.

514 Hippo signalling modulates survival of human induced pluripotent stem cell-derived cardiomyocytes

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Background/Introduction: Hippo signalling is an evolutionarily conserved pathway that controls organ size by regulating apoptosis, cell proliferation and stem cell self-renewal. Recently, the pathway has been shown to exert powerful growth regulatory activity in cardiomyocytes. However, functional role of this stress- and cell death-related pathway in human cardiomyocytes is not known.

Results: In our study 14 endomyocardial biopsies from patients with DCM were analyzed. The number of CD4+ lymphocytes was 48 ± 8 cells per mm2, the number of CD8+ lymphocytes was 26 ± 7 cells per mm2. The number of cardiomyocytes expressing stem cell markers c-kit, MDR1 and early cardiac transcription factors GATA4, Nkx2.5 was detected in the same samples.

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Conclusions: The results of this study demonstrate that there is a presence of increased numbers of T cells in endomyocardial biopsies from patients with DCM where cardiomyocytes progenitors and dedifferentiated cardiomyocytes were detected too. Exact mechanisms of cardiomyocyte de-differentiation are poorly understood but our results and evidence that cytokines secreted by T lymphocytes regulate cardiomyocyte de-differentiation in culture show an emerging role of T lymphocytes in cardiac regenerative processes.

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Purpose: Our aim was to investigate the role of transcriptional Hippo co-activators YAP and TAZ signalling in human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs), and to test the effects of modulating the pathway on cardiomyocyte function and survival.

Methods: Human iPSC-CMs were differentiated in different conditions or treated with cardiotoxic anthac- 

drines doxorubicin (3-15μM). Gene silencing of YAP and TAZ was achieved by RNAi. The mRNA lev- 
els of YAP and TAZ were measured by real-time PCR, and Cellomics ArrayScan was used to quantify YAP/TAZ nuclear translocation and cell death (with Topro3 nucleosis marker and mitochondrial membrane potential marker TMRE). CellOptiQ optical imaging platform was used to measure cal- 
cium transients in a high throughput manner.

Results: Our results showed that YAP and TAZ genes are abundantly expressed both in iPSC-CM and 

and adult ventricular cardiomyocytes. Nucleolar translocation of nRNA levels of YAP and TAZ were 

increased with decreased cell density (for YAP: p<0.001, TAZ: P<0.01, n=3). Other extracellular 

stress signals such as doxorubicin increased YAP/TAZ translocation (p=0.0004, n=3) and cell loss in 

a dose-dependent manner in iPSC-CM. We showed that transient downregulation of YAP and TAZ 
may increase Topro3-positive iPSC-CM, marking a detrimental effect of their silencing (p<0.0078, n=4). Modulation of YAP/TAZ expression had no effect on iPSC-CM calcium transient and contractility profiles.

Conclusions: Our results suggest that modulation of Hippo cascade may affect cell viability and ne- 
crosis of hiPSC-CM and thereby modulate the survival of human cardiomyocytes.

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Biocompatibility of mesenchymal stem cells with a spider silk matrix and its potential use as scaffold for cardiac tissue regeneration

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One of the problems of using cell therapy for heart diseases is the hostile environment for cell reten- 
tion/cell survival in the area were the cells are delivered. In order to minimize the poor environment on the implanted cells, we are working on using spider silk matrices as cell vehicles for the injured region.

Recombinant spider silk proteins (spidroins) are capable of self-assembling into fibres in aqueous so- 

lutions and can create very thin and resistant matrices with interesting mechanical properties. In this study, we improved both the cytocompatibility and the biological properties of these matrices func- 
tionalizing them with Fibronectin and Vitronectin, two extracellular matrix proteins associated to cell 
adhesion and retention. Thus, we cultured mesenchymal stem cells (MSC) for 8 days in these matri- 
ces: During the first 5 days, we compared the ability of the cells to grow in monolayers and their pro- 
liferation rates. Finally, we determined the impact of cell-matrix interactions in cell behavior by analyzing of the rate of cell migration and changes in cell secretion during the last 48 hours of culture. When comparing the MSC growing curve among the matrices - matrices without functionalization (A), matrices functionalized with fibronectin (B) and matrices functionalized with vitronectin (C) - we observed that the slopes of the regression lines that fitted into the growing curves of functionaliza- 
ted matrices were higher than the slope of the non-functionalized matrix. Moreover, Vitronectin functionalization was the most effective functionalization promoting cell growth: Am=6.37 < 

Bm=10.25 < Cm=12.22. Consistent with this, the analysis of the three croppped secretomes showed that Vitronecin functionalization also enhanced exocytosis (table1).

These results show that Vitronectin functionalized matrices are the best option to be tested as scaffold 

matrices for preclinical studies on small animal models.

Table 1. Summary of the secretion rate of MSC cultured on matrices functionalized with fibronectin or Vitro- 

nectin and compared to non-functionalized matrices (B/A-1 and C/A-1 respectively).

<table>
<thead>
<tr>
<th>Matrix Functionalization</th>
<th>fold change</th>
<th>B/A-1</th>
<th>C/A-1</th>
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<tr>
<td>MSCF</td>
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<td>7</td>
<td></td>
</tr>
<tr>
<td>SEPI</td>
<td>1.2</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>HGF</td>
<td>1.7</td>
<td>168</td>
<td></td>
</tr>
<tr>
<td>MCP1</td>
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<td>13</td>
<td></td>
</tr>
<tr>
<td>VEGF</td>
<td>2.7</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

5 main molecules in MSC culture media

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A snapshot of genome-wide transcription in human induced pluripotent stem cell-derived hepatocyte-like cells (iPSC-HLCs)

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Background/Introduction: Cholesterol metabolism is causally involved in the pathogenesis of ath- 
erosclerosis and cell culture models for studying the pathophysiology of atherosclerosis are needed. However, primary human hepatocytes (PHH), the key cell type involved in cholesterol metabolism, are difficult to acquire and culture due to their tendency to dedifferentiate and lose their liver function in culture. Hepatocytes differentiated from human induced pluripotent stem cells (iPSCs) offer an al- 
ternative for PHH. The validation of the iPSC-derived hepatocytes and the detailed exploration of their transcriptomic profile is an essential step when developing functional hepatocyte cell models for e.g. exploring the lipid metabolism involved in the development of atherosclerosis.

Purpose: Our aim is to differentiate iPSC-derived hepatocyte-like cells (iPSC-HLCs), which recap- 
turate the functionality as well as the transcriptomic profile of primary human hepatocytes as closely 
as possible.

Methods: We produced iPSC lines from dermal fibroblasts of three different patients and character- 
ezize the iPSC lines in detail. We then differentiated iPSCs to hepatocyte-like cells (HLCs). To char- 
acterize the iPSC-HLCs produced we used a wide array of functionality tests and immunocytochemical (ICC) staining. In order to study the genome-wide gene expression, we per- 
formed Global Run-On sequencing (GRO-seq) of the iPSC-HLCs as well as of the iPSCs and PHHs. GRO-seq is a derivative of RNA-seq that aims to measure rates of transcription (in- 
stead of steady state RNA levels) by directly measuring nascent RNA production. In this method, ac- 
tive transcription is allowed to proceed in the presence of actinomycin D and RNA is purified and 

automated using BrUTP-antibody coated beads. The eluted RNA undergoes cap removal and end re- 
pair prior to reverse transcription to cDNA. Deep sequencing of the cDNA provides sequences of 
RNAs that are actively transcribed by RNA polymerase II.

Results: The iPSC-HLCs stain positive for alpha-fetoprotein (AFP), albumin (ALB), LDL receptor (LDLR) and Oil red O. Our principal component analyses of the GRO-seq data suggest that the gene expression profile of the iPSC-HLCs closely resembles the expression pattern of PHHs. For ex- 
ample, the iPSC-HLCs have low expression of pluripotency genes (NANOG, SOX2) and express many liver-specific genes such as apolipoproteins (apoAI, apoAII, apoB), transthyretin (TTR, a pre- 
albumin), as well as liver-enriched transcription factors such as HNF4a and HNF1A.

Conclusion(s): Our study presents a genome-wide transcription map of iPSC-HLCs, with low ex- 
pression levels of pluripotency genes and higher expression of liver-specific genes, closely related to 
PHHs, thus demonstrating the potential of these cells as a laboratory model for human liver.

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Can NOS1/GcGr1K1 pathway trigger the differentiation and maturation of mouse embryonic stem cells (ESCs)?

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Background: The role of nitric oxide synthase (NOS)/soluble guanylyl cyclase (sGC)/ 

gCGRP-dependent protein kinase I (GK1) pathway in adult cardiac cells is extensively studied. Indeed, 

physiological levels of NO generated by NOS1 and NOS3 or pharmacological treatments with NO- 
donor drugs can modulate cardiac contractility and relax coronary arteries. However, cardiomyocytes 

are rapidly recruited during pathological conditions, such as ischemia. In particular, it has been 

recently demonstrated that nitric oxide donor drugs can modulate cardiac contractility and increase 

coronary blood flow throughout the Ser/Thr phosphorylation (GcGr1K1 pathway) and directly by S-nitrosylation of several proteins.

Purpose: Although the effects of NOS1/GcGr1K1 pathway in adult cardiac cells are well known, the influence of this signaling as modulator of cardiac differentiation of embryonic stem cells (ESCs) is less defined. Therefore, we investigated NOS1/GcGr1K1 in the early stage of cardiac differentiation of ESCs, studying i) enzyme expressions and activities during cardiac maturation and ii) the acute and 
chronic effect of pathway alteration.

Methods: Undifferentiated mouse ESCs were cardiac differentiated by Embryoid Bodies formation (EBs). Cardiac maturation was followed for 21 days. Beating EBs were monitored starting from 
7th-10th day. At different stages of maturation, mNOS1, mGr1K1, and mPrkg1 were expressed and protein expressions were de- 
tected by real-time PCR (Q-PCR), western blot analysis and enzymatic activity were used for protein evaluation.

Results: Q-PCR showed that during differentiation enzyme expression increased in a time- 
dependent mode and different time-courses. The peak of mNOS1 expression was measured at d8 and then rapidly decreased. mGr1K1 and mPrkg1 expressions were detected by real-time PCR (Q-PCR), western blot analysis and enzymatic activity were used for protein evaluation.

Conclusions: The time-dependent expression enzyme expression increased in a time- 
dependent manner and different time-courses. The peak of mNOS1 expression was measured at d8 and then rapidly decreased. mGr1K1 and mPrkg1 expressions were detected by real-time PCR (Q-PCR), western blot analysis and enzymatic activity were used for protein evaluation. 

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Results: A uniform monolayer developed using 25k-40kcells/well hiPSC-CMs in the presence of increasing densities of HEK293 cells. Contraction recordings from Cor.4U hiPSC-CMs showed that from day 3 onwards all cultures were spontaneously active. Higher densities of iK1-expressing HEK293 (1:10) lead to an increase in interval time between beats of approximately 60% on day 9 (1972 ± 592 vs 1213 ± 14ms, n=8 p<0.05). Time for relaxation was also significantly prolonged in 1:10 and 1:30 compared with control on day 9, 283% and 128% (875 ± 265 and 522 ± 153ms vs 229 ± 24ms, respectively, n=8 p<0.01), respectively. Earlier and later culture times showed no significant difference in spontaneous contractile activity up day 12. In parallel, Pluricyte hiPSC-CMs were initially quiescent, becoming spontaneous at approximately day 4. Co-culture ratios of 1:10 and 1:30 did not show any spontaneous activity up to day 11.

Conclusions: Co-culturing with iK1-expressing HEK293 may provide a method of adding iK1 conductance to a network of hiPSC-CMs but different sources of hiPSC-CMs respond differently. With Cor.4U hiPSC-CMs higher densities of HEK293 such as 1:10, lead to a slowing of the spontaneous rate and slowing of relaxation time suggesting effects on the electrophysiology of the co-culture. Pluricyte cultures responded differently suggesting a higher sensitivity to co-culture with iK1 expressing HEK293 cells.

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Mechanosensitivity of cardiomyocyte progenitor cells: the strain response in 2D and 3D environments

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Purpose: Cardiomyocytes progenitor cells (CMPCs) are a candidate cell source for cardiac regenerative therapy. To assess their full potential for cardiac regeneration, it is essential to know if and how CMPCs sense and respond to the three-dimensional (3D) environment and mechanical stimuli provided by the beating heart. Therefore, we study the response to cyclic strain of undifferentiated and pre differentiated human CMPCs in a 2D environment, as well as how CMPCs respond to unidirectionally constrained versus stress-free (unconstrained) 3D environments. The latter responses were studied using a hydrogel system that allows for interaction of the cells with a single axis of strain.

Methods: To test mechanosensitivity of CMPCs in 2D and 3D environments, the response of LYTBC CMPCs to uniaxial (cyclic) strain (10% with 0.5 Hz) was investigated. To represent the 3D environment, undifferentiated CMPCs were cultured in unidirectionally constrained and stress-free collagen/ Matrigel hydrogels, where the constrainment provides a static strain to the cells. The cellular mechanoresponse to the applied (cyclic) strain was quantified by cellular re-orientation away from the strain direction (strain avoidance). Next to cellular re-orientation, the effect of strain on cell differentiation was analyzed.

Results: We observe that while undifferentiated cells maintain their original orientation, upon early cardiomyogenic differentiation (pre differentiated) CMPCs exhibit a distinct strain avoidance response during 48hrs of cyclic straining in a 2D environment. In 3D unidirectionally constrained hydrogels, undifferentiated CMPCs retain their cardiomyogenic stem cell profile. CMPCs cultured in 3D collagen/ Matrigel hydrogels respond to static mechanical strains as expected by cell alignment.

Conclusions: Our results suggest that CMPCs respond to the presence of mechanical stimuli, in this research modeled by the application of uniaxial (cyclic) strain in 2D and 3D environments, suggesting that CMPCs are indeed mechanosensitive. Although in 2D environments, mechanosensitivity of the CMPCs is dependent on their differentiation status. Our findings provide the first understanding of the ability of human CMPCs to sense mechanical stimuli, which is the first initial step in mechanotransduction. Mechanotransduction is essential for optimal recruitment, migration, and mechanical integration of progenitor cells into the injured myocardium. Therefore, the presented results can contribute to enhance efficacy of current treatments of cardiac disease, as well as to develop novel regenerative strategies.

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The effect of the vascular-like network on the maturation of the human induced pluripotent stem cell derived cardiomyocytes.

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Introduction: Stem cells and specifically induced pluripotent stem cell derived cardiomyocytes (iPSC-CMs) provide unique alternative to model human cardiomyocyte differentiation, the function of the cardiac cells and the pathology of severe cardiac diseases. However, the differentiated iPSC-CMs are classified to resemble embryonic or fetal like cardiomyocytes due to their gene expression pattern, size, shape and mononuclear nature. Moreover, the lack of i-tubule network has been suggested to be a reason for the slow excitation-contraction coupling and calcium handling. The culture platforms that orientate the cardiac cells could have a positive effect on the overall maturation of the differentiated cardiomyocytes, e.g. patterned biomaterials could induce the orientation and furthermore the more mature phenotype of iPSC-CMs.

Methods: We have utilized natural topography provided by the network formed by endothelial cells and fibroblasts in the maturation of iPSC-CMs. The iPSC-CMs have been differentiated and the beating differentiated cells have been plated to the aforementioned platform and the maturation state of the
Transcriptional control and RNA species - Heart

525 Gene expression regulation in heart failure: from pathology to bioinformatics
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Background: Heart failure (HF) syndrome results from abnormalities in multiple biological processes and contributes to the composite phenotype. Next-generation sequencing technologies revolutionized the analysis of the transcriptome, providing a panoramic view of all the transcriptional activity in a given sample and a powerful tool for the identification of new transcripts.

Purpose: RNA-Sequencing (RNA-Seq) approach was employed to investigate the changes accompanying human HF and to obtain the whole transcriptome of cardiac tissues from transplant recipients with advanced stage of HF. The knowledge of an expression network signature in end-stage HF disease may offer important insights into the complex pathogenesis of advanced cardiac failure, as well as it may provide potential targets for therapeutic intervention.

Methods: RNA from heart tissue explants from dilated cardiomyopathy (DCM) and restrictive cardiomyopathy (RCM) patients and control subjects were analyzed by RNA-Seq. Different informatic tests (edgeR and NOISeq BIO) were employed and compared. Several public tools were used to effect in silico analysis of the specifically differentially expressed genes (DEGs).

Results: The statistical methods adopted, generated different lists of genes for both of the DCM and RCM groups. Among them, the gene symbols VSN1R was utilized to obtain a list with the common genes; particularly, 35 were detected as differentially expressed in failing hearts versus non-failing hearts. Moreover, DAVID functional analysis demonstrated that 5 cytoskeleton-related genes were differentially expressed in DCM. On the other hand, when hearts from RCM patients were compared with non-failing hearts, 19 differentially expressed cytoskeleton-related genes were found. Interestingly, genes encoding ACTA2 and ACTG2 have been associated with HF for the first time in this study. Noteworthy, NMUR1 gene, involved in modulating calcium channels, was particularly down-regulated in both DCM and RCM. Finally, several genes also encode for components of extracellular matrix, including ADAMTS4 and ADAMTS5, belong to the extrapalpated gene list of common DEGs.

Conclusions: Our data revealed a new map of gene expression changes in the DCM and RCM cardiac tissues. Several genes involved in crucial cellular mechanisms were not previously implicated in the molecular phenotype of HF. These new changes may be responsible for alterations found in cardiomyopathies. However, further studies are needed to lead to potential novel biomarkers and targets for therapeutic intervention in these pathologies.

526 Human transcriptome in idiopathic dilated cardiomyopathy - a novel high throughput screening
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Background and Aim: Idiopathic dilated cardiomyopathy (DCM) is the leading cause of heart failure (HF), and the most common indication for cardiac transplantation. DCM is characterized by transcriptional changes, which alter cellular processes, leading to failing phenotype. As the specific molecular mechanisms of DCM are largely unknown, the aim of this study was to develop a novel platform for screening of all human transcription factors (TFs) and to characterize the role of TFs in the molecular mechanisms of DCM.

Methods: Myocardial tissue samples from DCM and control human subjects were analyzed using novel screening platform, called Quantrx, based on quantitative real-time polymerase chain reaction (qRT PCR). The rat heart was used as a model for human gene expression.

Results: We identified 41 differentially expressed TFs. 18 genes were upregulated (fold change > 2, P < 0.05) while 22 genes were downregulated (fold change > 2, P < 0.05) in dilated cardiomyopathy group. The analysis of the differentially expressed genes uncovered TFs affecting important signaling pathways in cardiac development and disease including MAPK and Wnt-signaling pathways and thus allowed the characterization of possible novel regulators that play role in HF.

Conclusion: Quantrx is a new method to screen quickly and effectively all human TFs and provides a valuable resource for further investigation of molecular mechanisms of DCM as well as other diseases. Our data indicate that changes in the expression of 41 TFs affect important signaling pathways, which subsequently alter a number of biological processes in DCM patient's cells and could serve as potential diagnostic or therapeutic targets.

527 A high-throughput approach unveils putative miRNA-mediated mitochondria-targeted cardiovascular circuits activated by T3 in the post ischemia reperfusion setting
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Background: Increasing experimental and clinical evidence indicate that a low T3 state (LT3) in the post cardiac ischemia reperfusion (IR) setting favors long term adverse cardiac remodeling and worsens patients prognosis. We previously reported a cardioprotective role of T3 treatment and suggested the mitochondria as main effectors of this action. Although the regulation of cardiac miRNAs may be the presumable mechanism, a relation of cause and effects has never been demonstrated. A system biology approach may help investigating this important issue.

Purpose: The study was to unveil putative miRNA-targeted cardiovascular circuits activated by T3 in the early post IR setting and dependent on the regulation of micro RNA: Methods: To this aim, miRNA profiling and mitochondrial proteome were performed in a model of cardiac IR. A data were integrated through next generation sequencing. Briefly, rats developing a low T3 state were treated with T3 (5ug/Kg die) or TR3 vehicle for 48h. Therafter cardiac performance was evaluated through echocardiogram and the rats were sacrificed. Tissue from the LV per-infarctual zone was used for miRNA profiling through next generation sequencing. In the same experimental model, mitochondria of the perinfarctual myocardium were purified from rats developing or not the low LT3 and the proteomic profiling was performed through mass spectrometry.

Results: The presence of a post IR LT3 was associated to more serious impairments of cardiac and mitochondrial function and with altered expression of several miRNAs of critical importance for mitochondrial activity and cardiac remodeling, which was reverted by T3 treatment. Also we observed different remodeling of the mitochondrial proteome in the presence or absence of a LT3S, with alterations in groups of proteins that play a key role in energy metabolism, quality control and regulation of cell death pathways. The in silico analysis revealed for the T3 regulated miRNAs several predicted mitochondria targets well fitting with the proteomic results.

Conclusion: Our findings highlight a relationship between LT3 in the early post IR and poor cardiac and mitochondrial outcomes, while indicating a beneficial role for T3 treatment possibly through the regulation of miRNA-mediated cardio-protective circuits targeted to mitochondria.

528 The effect of uraemia on the expression of miR-212/132 and the calcinurin pathway in the rat heart
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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. The underlying molecular mechanisms are not clear. The overexpression of miR-212/132 has already been implicated in the development of left ventricular hypertrophy via modulation of the calcinurin pathway in TAC mice. Purpose Therefore, here we investigated the effect of uraemia on the myocardial expression of miR-212/132 and the calcinurin pathway.

Methods: Uremia was induced by subtotal 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-212/132 and atrogin-1 as well as MCP1, components of the calcinurin pathway, was measured through q-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; however, miR-132 did not change significantly as compared to the control group. Moreover, atrogin-1 showed significant down-regulation and MCP1 showed significant up-regulation in the uremic group.

Conclusion: Myocardial overexpression of miR-212 may play a role in the development of uraemic cardiomyopathy by modulating the calcinurin pathway.

Cytokines and cellular inflammation - Heart

531 Lack of growth differentiation factor 15 aggravates adverse cardiac remodeling upon pressure-overload in mice
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Introduction: Growth differentiation factor 15 (GDF15) is a distant member of the TGF-β family. Under homoasotic conditions GDF15 is not highly expressed, however, upon injury GDF15 levels robustly increase. GDF15 influences many processes including inflammation, apoptosis and fibrosis. In a mouse model of myocardial infarction, GDF15 deficiency results in increased incidence of cardiac.
Cardiovascular Research Supplements

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Background: Myocardial infarction (MI) is among the most common causes of death in developed countries and its incidence is still increasing. Finding new strategies to prevent and treat this threaten- ing clinical event is thus of high priority. Inhibition of CCL5 was shown to have beneficial effects on the outcome of experimental MI in mice, yet might be accompanied by adverse immunologic side effects. In a previous study, we have demonstrated a pathophysiologic relevance for the heteromer formation of CCL5 and CXCL4 in the progression of atherosclerosis.

Purpose: To evaluate a specifically designed compound (MKEY) that blocks the CCL5-CXCR4 interac- tion in a mouse model of myocardial ischemia/perfusion (I/R).

Methods: To examine the effect of MKEY in healing following I/R, 8-week-old male mice were intra- venously treated with MKEY or scrambled control (sMKEY) from 1 day before, until up to 7 days after I/R. Myocardial function was evaluated using echocardiography and intraventricular pressure measure- ments and tissue viability, scar formation, leukocyte infiltration and the formation of neutrophil extracellular traps (NETs) was assessed by histology.

Results: MKEY treatment resulted in a significant decrease in infarction size and preserved heart function as compared to sMKEY-treated animals (Figure A, B). Moreover, MKEY treatment signifi- cantly reduced the inflammatory reaction following I/R, as revealed by specific staining for neutrophils, NETs and neutrophil extracellular traps (Figure C, D).

Conclusion: Disrupting chemokine heterodimers during myocardial I/R might have clinical benefits, highlighting the therapeutic benefit of blocking the interaction of platelet-derived chemokines, and in addition, reducing the inflammatory side effects while maintaining normal immune defense.

S34

N-terminal truncated intracellular matrix metalloproteinase-2 expression in diabetic heart.

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Background: Diabetic cardiomyopathy is a distinct form of cardiomyopathy and can be defined as cardiac damage and ventricular dysfunction which is independent of the concomitant coronary artery disease and/or hypertension. Matrix metalloproteinases (MMPs) are reported to account for increased myocardial collagen content in diabetic cardiomyopathy. Recently reported intracellular type of MMP-2, which is N-terminal truncated (NTT) type, is induced by oxidative stress and reported to account for cardiac dysfunction through activating innate immunity and apoptosis in various conditions.

Purpose: We hypothesized that NTT-MMP-2 is induced in diabetic cardiomyopathy. We aimed to evaluate the expression of NTT-MMP-2 in vitro and in vivo connection with activated innate immunity and apoptosis.

Methods: HR2 cells were cultured with intermediate and high glucose concentration (15, 30mM) for 2, 24, and 48 hours. Cells were analyzed with quantitative reverse transcription polymerase chain reaction (qRT-PCR) and gelatin zymography. AKT and NF-kB expression were also measured with western blot analysis. In vivo mice model was induced with 40mg/kg of streptozotocin intraperito- neal injection for 5 days. After sacrificing mice at 12 and 24 weeks pathological analysis including immunohistochemical (IHC) staining of NTT-MMP-2 were done.

Results: Quantitative RT-PCR showed that there was an expression of NTT-MMP-2 in HR2 cell with glucose exposure compared to negative expression in control group, and it was dose and time- dependent. Also, there was a distinct expression of NTT-MMP-2 in IHC staining from diabetic mouse heart. There was no definite collagen accumulation and fibrosis from light microscopy (LM) evaluation, but there was a mitochondrial damage from electron microscopy (EM) evaluation.

Conclusion: NTT-MMP-2 expression was noted from both in vivo and in vitro model of diabetic cardiomyopathy. Further evaluation of its role in diabetic cardiomyopathy should be followed.

Abstracts

S596

S33

Is there an association between low-dose aspirin use and clinical outcome in HFPEF?

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1University College Dublin, Conway Institute, Dublin, Ireland; 2Trinity College Centre for Health Sciences, Dublin, Ireland; 3St Vincent’s University Hospital, Heart Failure Unit, Dublin, Ireland

Introduction: We have previously reported an association between low-dose aspirin use and im- proved long-term outcome in chronic heart failure (HF) patients irrespective of ischemic heart dis- ease. The majority of community dwelling HF patients present with preserved ejection fraction (HFPEF), a syndrome characterized by inflammation, myocardial extracellular matrix remodeling and diastolic dysfunction. We hypothesized that low-dose aspirin has beneficial effects in HFPEF and that those benefits are likely related to effects on monocyte/macrophage function and cell-cell interactions in the blood.

Methods: In a retrospective analysis of HFPEF patients under the care of a hospital-based HF disease management program, we identified 150 patients taking low-dose (75 mg/ml) aspirin and age- and sex- matched HFPEF controls not taking aspirin. Survival and hospitalizations were assessed over a 3 year follow-up period. From this cohort, we studied 25 HFPEF age- and sex-matched patients (14 aspirin, 14 non-aspirin) using primary monocyte isolation, monocyte qPCR, serum matrix metalloproteinase (MMP) and inflammatory marker assays. Subsequently, primary monocytes were isolated from 6 healthy volunteers and co-cultured with platelet release (PR, 16h) prepared from collagen-activated platelets from the same donor. Finally, primary monocyte/platelet aggregates were incubated with/without 10 μM aspirin in matrigel-coated invasion transwells (16h) to study the influence on mono-ocyte migration.

Results: Low-dose aspirin was associated with significantly higher overall survival and lower HF hos- pitalizations over the 3-year follow up period (HR 0.665, 95% confidence interval, 0.389-0.961). Ser- um MMP2 and -OC16 were significantly reduced in low-dose aspirin HFPEF versus matched HFPEF controls (n=14 per group). Monocyte incubation with PR caused cell activation with increased MMP1, MMP2, MMP9, and MCP1 release. Finally, healthy donor monocyte/platelet invasion was reduced by 50% with low-dose aspirin (p<0.01). Inflammatory cytokines (IL1α, IL1β, CCL17) were reduced in supernatants.

Conclusion: We demonstrate for the first time a retrospective association between the use of low- dose aspirin and better outcomes in HFPEF. We also show that aspirin use is associated with reduced monocyte/macrophage markers in vivo and reduced invasiveness of monocyte-platelet aggregates ex vivo. Antiplatelet strategies to modulate monocytes may require further prospective evaluation in HFPEF.

Expression of NTT-MMP-2 in HR2 cell.

NNT-MMP-2, qRT-PCR

<table>
<thead>
<tr>
<th>Treatment</th>
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<th>SD</th>
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<tr>
<td>Control 2hr</td>
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<tr>
<td>High concentration 24hr</td>
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<td>High concentration 24hr</td>
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<td>High concentration 48hr</td>
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</table>

qRT-PCR, quantitative real time polymerase chain reaction
Expression of CD39 and CD73 on peripheral T-cell subsets in calcific aortic stenosis

A. Golovkin, I. Kudryavtsev, M. Serebryakova, A. Malashicheva, A. Shishkova, E. Zhiduleva, O. Mosseva

Introduction:
Mechanisms and involvement of the immune system into the pathogenesis of aorta valve calcification are still not fully investigated. The aim of the study was to identify possible participation of peripheral T-cell subsets divided using their differentiation status and involvement in adenosine regulation in pathogenesis of aortic valve calcification.

We examined 24 patients with severe calcific aortic stenosis [average flow gradient 48.3 (46.0±6.5) mmHg] and 16 healthy volunteers. Mean age was 63 (57.6±4) years. There were 14 patients with bicuspid (BAV) and 10 with orificial calcified aorta valve (TAV). We did not find significant differences in valve functioning measured using ejection fraction, maximal and average flow gradient on BAV and TAV.

The quantity of circulating CD39 and CD73 of peripheral naïve (N, CD45RA+CD62L+), central memory (CM, CD45RA-CD62L+), effector memory (EM, CD45RA-CD62L-) and terminally differentiated CD45RA-positive effector memory (TEMRA, CD45RA+CD62L-) T-cells were measured using multicolor flow cytometry.

It was found that relative number of Naïve Tcyt (p=0.034) was decreased and the relative number of TH (CD3+CD4+CD73+) and CD73+CD3+CD4+ (Tcyt) cells were measured using multicolor flow cytometry. The relative number of Naïve Tcyt (p=0.034) was decreased and the relative number of TH (CD3+CD4+CD73+) and CD73+CD3+CD4+ (Tcyt) cells were measured using multicolor flow cytometry. The relative number of Naïve Tcyt (p=0.034) was decreased and the relative number of TH (CD3+CD4+CD73+) and CD73+CD3+CD4+ (Tcyt) cells were measured using multicolor flow cytometry.

Conclusion: The relative number of Naïve Tcyt (p=0.034) was decreased and the relative number of TH (CD3+CD4+CD73+) and CD73+CD3+CD4+ (Tcyt) cells were measured using multicolor flow cytometry. The relative number of Naïve Tcyt (p=0.034) was decreased and the relative number of TH (CD3+CD4+CD73+) and CD73+CD3+CD4+ (Tcyt) cells were measured using multicolor flow cytometry. The relative number of Naïve Tcyt (p=0.034) was decreased and the relative number of TH (CD3+CD4+CD73+) and CD73+CD3+CD4+ (Tcyt) cells were measured using multicolor flow cytometry. The relative number of Naïve Tcyt (p=0.034) was decreased and the relative number of TH (CD3+CD4+CD73+) and CD73+CD3+CD4+ (Tcyt) cells were measured using multicolor flow cytometry.

536 Mast cells in the atrial myocardium of patients with atrial fibrillation: a comparison with patients in sinus rhythm

T. Kucera, M. Dursova, M. Blaha, V. Melenovsky, J. Pirik, J. Kastner

Background: Atrial fibrillation (AF) is one of the most frequent arrhythmias and its pathogenesis is still only partially explained. Various morphological and functional alterations have been associated with atrial fibrillation, including signs of inflammation in the atrial myocardium. Such inflammatory process is believed to contribute to structural remodelling and perpetuation of arrhythmia. Mast cells might be one of the inflammatory cell populations involved.

Purpose: Our aim was to characterize and quantify mast cells in the atrial myocardium of patients undergoing an open heart surgery with atrial fibrillation compared to those in sinus rhythm (SR).

Methods: Biopsies from the right and left atrium were obtained during elective open heart surgery. The samples were fixed with formaldehyde and embedded into paraffin. Sections were used to detect mast cells immunohistochemically, using anti-mast cell tryptase antibody. Systematic uniform random sampling was performed for collecting images that were subsequently used for quantification of mast cells in each microscope field of vision.

Results: Markers specific for mast cell tryptase were detected in samples from both patients with AF and SR. No significant differences were found in the number of mast cells between patients with AF and SR when patients with AF and SR were compared. It is unlikely that these cells have any specific role in the development of AF. On the other hand, the IL-6 gene, although being a known marker for M1, appears to be inappropriate for this comparison.

Conclusion: The TNF-α, I L - 1 b and IL-1b RNA expression profiles observed in M1 are consistent with the current literature on macrophage activation. To confirm that CCL2 gene is important for the regulation of the M2 phenotype, interleukin-4 (n=4 animals; 107 cells per animal). RNA was extracted and treated with DNase treatment, qPCR was used to analyze the differential gene expression of M1 markers interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), arginase-2 (Arg2), chemokine C-C motif ligand 2 (CCL2) and interleukin-6 (IL-6), as well as markers M2 like receptor (MR) and RIG-1 in inflammatory zone protein (Fizt1). Glyceroldehyde-3-phosphate dehydrogenase was used as an endogenous control.

Results: In M1, both TNF-α and IL-1β gene expression increased compared to unstimulated (6.76 ± 0.48 and 6.26 ± 0.32 A.U., respectively). Moreover, both genes were more expressed in M1 compared to M2 (6.76 ± 0.48 vs 0.56 ± 0.56 A.U. and 6.26 ± 0.32 vs 0.14 ± 0.12 A.U., respectively). The Arg2 gene expression was also higher in M1 compared to unstimulated (9.98 ± 3.1 vs 2.27 ± 2.51 A.U.). For the CCL2 RNA, a trend towards higher expression in M1 was found, although without reaching significance. Intriguing, the amplification of IL-6 gene was absent by qPCR. In M2, MR gene was more expressed compared to M1 (4.41 ± 1.15 vs 12.37 ± 7.4 A.U.), while the amplification of Fizt1 by qPCR was inefficient.

Conclusions: These results of qPCR analysis in M1 and M2 highlight the potential role of different macrophage genes in the development of AF. The TNF-α, I L - 1 b and IL-1b RNA expression profiles observed in M1 are consistent with the current literature on macrophage activation. To confirm that CCL2 gene is important for the regulation of the M2 phenotype, interleukin-4 (n=4 animals; 107 cells per animal). RNA was extracted and treated with DNase treatment, qPCR was used to analyze the differential gene expression of M1 markers interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), arginase-2 (Arg2), chemokine C-C motif ligand 2 (CCL2) and interleukin-6 (IL-6), as well as markers M2 like receptor (MR) and RIG-1 in inflammatory zone protein (Fizt1). Glyceroldehyde-3-phosphate dehydrogenase was used as an endogenous control.
The biological expression and thoracic anterior pain syndrome D. Dumbaulu1, CM. Gavril1,2, CM. Purasachi3, P. Mrela1, LC. Scarl1
1CIC F.C. Hospital, Medical 1, Iasi, Romania; 2University of Medicine and Pharmacy “Gr. T. Popa”, Medicine of mother and child, Iasi, Romania

The Aim of the Work: The clinical study seeks involvement of oxidative stress and dyslipidemic syndrome in chest pain pathoanterior localized (TAP).

Material and Method: We studied metabolic clinical profile and antioxidant status to a number of 170 patients admitted to the Emergency Medicine Clinic of CIC F.C. hospital. Among them we have diagnosed with various disorders with common symptoms: chest pain earlier. The results obtained were compared with the same data from a group of 70 healthy volunteers. Evaluation of patients was made by clinical, laboratory investigations (blood count, urea, creatinine, glucose, cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, electrocardiography at rest, chest radiography, fundus examination, abdominal ultrasound, echocardiography transthoracic), determination of antioxidant enzymes SOD type, Gpx or measurements of lipid peroxidation (MDA).

Results: - Analysis of obtained data allowed the clinical characteristics of metabolic and biochemical re- dox status differs depending on the type of disease or age.

- Conclusions: - Extensive oxidative stress in aortic-banded mice, in the context syndrome chest pain, shows significant intensification of stress bio-oxidative compared with patients under 60 years, underpinning by the significant reduction of antioxidant enzymes and augmentation significant lipid peroxidation, resulting hypothesis radicals species that play a role in the pathology certainly painful

## Table 1

<table>
<thead>
<tr>
<th>Age</th>
<th>Patients under 65 years</th>
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</thead>
<tbody>
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</tr>
<tr>
<td>smoking</td>
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<td>3/1</td>
</tr>
<tr>
<td>hta degree association</td>
<td>44% Degree II</td>
<td>95% Degree Ii</td>
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<tr>
<td>obesity</td>
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</tr>
<tr>
<td>peripheral arterial disease</td>
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<td>0.08%</td>
</tr>
<tr>
<td>Stroke</td>
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</tbody>
</table>

Clinical features of patients with anterior chest pain (TAP)

Signal transduction - Heart

545 The association of heat shock protein 90 and TGFbeta receptor I is involved in collagen production during cardiac remodeling in aortic-banded mice R. García1, J.M. Gomez1, D. Meroño1, M.A. Hurta1, J.F. Nistal2, A. Airel3, A. Cortetarn1, A. Vilari1
1University of Cantabria, Santander, Spain; 2Hospital Sarmiento, Service of Cardiology, Santander, Spain; 3Hospital Marques de Valdecilla, Santander, Spain

Purpose: We postulate that the association of Hsp90 and TgfBRI in collagen production during damaging myocardial fibrotic events.

Methods: - Left ventricle pressure overload was induced by transverse aortic constriction (TAC group).

Mice (n = 5 to 12 per group) were euthanized at 1 h, 3 h, 7 h, 14 h or 2 weeks after TAC surgery. Levels of expression of TGFβ, Hsp90 and Col1 were determined by qPCR and Western blot. Cardiac fibroblasts were isolated from hearts of mice treated with aortic banding model.

- Conclusions: We have demonstrated the involvement of Hsp90 and TGFβRI in the collagen production during aortic constriction.

546 Loss of the inhibitory Galphao protein in the rostral ventrolateral medulla of the brainstem leads to abnormalities in cardiovascular reflexes and altered ventricular excitation R. Ang1; J. Abramowitz2; L. Bimbamer1; A. Gourine1; T. Tinker1
1University College London, Centre for Clinical Pharmacology, London, United Kingdom; 2National Institute of Environmental Health Sciences, Division of Intramural Research, Research Triangle Park, United States of America; 3University College London, London, United Kingdom; 4Barts and The London School of Medicine and Dentistry, London, United Kingdom

Introduction: - The heart is controlled by the sympathetic and parasympathetic limbs of the autonomic nervous system with inhibitory signaling mechanisms recruited in both limbs. Studies aimed to determine the role of inhibitory heterotrimeric G proteins in the central mechanisms underlying autonomic control of the heart.

Methods: - Mice with conditional deletion of inhibitory heterotrimeric G protein Goα in the rostral ventrolateral medulla oblongata were generated to determine the effect of specific Goα deletions on autonomic control and electrophysiological properties of the heart.

Results: - Goα deletion in the presynaptic area of the rostral ventrolateral medulla (RVLm) was not associated with changes in HR or the arterial blood pressure (BP) at rest (home cage, normal behaviour). However, exposure to stressful conditions (novel environment, hypoxia or hypercapnia) in these mice was associated with profoundly exaggerated heart rate responses and an increased baroreflex gain when studied under urethane anaesthesia. This was associated with a reduced ventricular effective refractory period and lower ventricular tachycardia threshold. This phenotype was reversed by systemic administration of a beta-adrenoceptor blocker atenolol, suggesting that Goα loss in the RVLm increases central sympathetic drive.

Conclusions: - The data obtained suggests that Goα-deleted signalling within the presynaptic circuits of the RVLm contributes to the autonomic control of the heart. Goα deficiency in the RVLm is associated with exaggerated cardiovegetative responses to stress, altered cardiovascular reflexes and electrical properties of the heart.

547 Selenoprotein P regulates pressure overload-induced cardiac remodeling S. Uku1, M. Takamaka2, S. Takahama1, O. Ioue1, H. Misi1, T. Takamura2, S. Kaneko2
1Kanazawa University, School of Medicine, Kanazawa, Japan; 2 Bristol University, School of Medicine, Bristol, United Kingdom

Selenoprotein P (SeP) is a liver-derived secretory protein that induces insulin resistance. Although clinical studies suggest insulin resistance is associated with congestive heart failure incidence independent of established risk factors, the role of SeP during cardiac remodeling is not well understood. In this study, we examine the role of SeP in regulating cardiac hypertrophy and function in response to pressure overload. Transverse aortic constriction (TAC) was applied to SeP knockout (KO) and wild-type (WT) mice. The mortality rate following TAC was significantly decreased in SeP KO mice compared to WT mice (32.5% in KO mice (n=39) vs 51.3% in WT mice (n=40) (p<0.05). The echocardiographic examinations (left ventricular (LV) ejection fraction and LV wall thickness at baseline) were similar between SeP KO and WT mice. Interestingly, both LV septum and posterior wall thickness two weeks after TAC were significantly smaller in SeP KO than those in WT mice (1.04 ± 0.04 vs 1.27 ± 0.03 mm, p<0.05; 1.92 ± 0.14 vs 2.12 ± 0.04 mm, p<0.05). LV weight/body weight (BW) and lung weight/BW were significantly smaller in SeP KO than those in WT mice (4.41 ± 0.18 vs 3.39 ± 0.22, p<0.05; 0.68 ± 0.47 vs 10.57 ± 0.69, p<0.05). mRNA expression of ANF and BNP was significantly reduced in SeP KO compared to WT mice (p<0.05). Furthermore, mRNA expression of collagen 1α1, marker of fibrosis, was significantly decreased in SeP KO compared to WT. These results suggest that the absence of endogenous SeP attenuates cardiac hypertrophy and fibrosis by pressure overload. In conclusion, SeP is a regulator of cardiac hypertrophy and possibly plays a maladaptive role against progression of congestive heart failure.

548 Study of adenyl cyclase activity in erythrocyte membranes in patients with chronic heart failure U. Kamloul1; TOHIRA. Ailev1
1Republican specialized scientific-practical Medical Center Therapy and Medical Rehabilitation, Tashkent, Uzbekistan; 2Tashkent medical academy, Tashkent, Uzbekistan

Purpose: Study of activity adenyl cyclase (AC) in erythrocyte membranes in patients with chronic heart failure (CHF).

Methods: - The study included 56 post-MI male patients aged from 45 to 55 (mean age 51±2.4 years) years with CHF (NYHA FC II-III). All the patients were divided into two groups according to the New York Heart Classification (NYHA) functional class (FC). Group 1 consisted of 30 patients with CHF FC-II and Group 2 consisted of 26 patients with CHF-III. The AC activity in red blood cells homogenates was determined by the method of Y. Saloman.

Results: - Basal AC activity was less by 31.9% in patients of Group 1 compared to the control group (45.1 ± 0.14 vs 61.0 ± 0.19 pmol/min/ml) in patients of Group 2 it was less by 41.6% compared to the control group (33.6 ± 0.13 vs 51.6 ± 0.19 pmol/min/ml) and by 14.2% compared to Group 1 patients. In the control group, we found an increase in the epinephrine-stimulated AC activity of about 2 times in comparison with basal level (113.0 ± 0.5 vs 61.0 ± 0.19 pmol/min/ml, P<0.01). In Group 1 patients, the epinephrine-stimulated AC activity was lower about 2 times compared to the control group (5.2 ± 0.19 pmol/mg/ml vs 11.3 ± 0.5pmol/min/ml). In Group 2 patients, this parameter was reduced to 3.85 ± 0.19 pmol/min/ml and was 65.9% (P<0.05) lower than in the control group and 28.7% lower than in Group 1 patients. A significant increase in the epinephrine-stimulated AC activity of erythrocyte membranes in healthy controls by 85% reflects an adequate response of the
549 Direct thrombin inhibitors inhibit atrial myocardium hypertrophy in a rat model of heart failure and atrial remodeling
C. Jumeau; N. Mougenot; M. Dufillo; S. Hatem
Hôpital Pitié-Salpêtrière, Institut de Cardiologie; ICAN Institute of Cardiometabolism and Nutrition, Paris, France

Atrial fibrillation (AF) is associated with a high risk of stroke due to thrombin formation in poorly con- tractor atria. In addition to its role in thrombus formation, thrombin has pleiotropic effects through the activation of protease-activated receptor-1 (PAR-1). Here we examined the involvement of the thrombin pathway in the atrial remodeling associated with heart failure (HF) and the effects of direct thrombin inhibitor (DTI) on this remodeling process. This study was conducted in a rat model of HF due to myocardial infarction and associated with atrial dilatation and susceptibility to AF. Animals were treated immediately or one month post-M with either vehicle control, 25 mg/kg/d dabigatran or 6 mg/kg/d of another DTI, SM-53972. Two months treatment with DTIs reduced both left atria dilatation and the duration of burst pacing-induced AF whereas treatments had no effect on venricular dilatation and systolic dysfunction. The vitamin K antagonist, Warfarin, had no effect on both atrial and ventricles remodeling. The increase in hypertrophic markers such as brain natriuretic peptide and β-myosin heav- y chain, of the transcription factor NFATc3 observed in vehicle-treated HF rats was suppressed by DTIs. PAR-1 antagonist reproduced the effect of DTI on atrial dilatation and AF susceptibility. In an atrial explant culture model, 10 nM thrombin upregulated hypertrophic markers and plasminogen activator activated through PAR-1 and the Rho/ROK kinase pathway. These results indicate that thrombin is a potent hypertrophic factor of the atrial myocardium and that DTIs and PAR1 inhibitor could prevent the atrial remodeling and AF substrate formation.

550 Tissue factor / FVIIa transactivates the IGF-1R by a Src-dependent phosphorylation of caveolin-1
M. Aberg; A. Siegbahn
Uppsala University Hospital, Department of Medical Sciences, Clinical Chemistry, Uppsala, Sweden

Background: The receptor tyrosine kinase IGF-1R is transactivated and translocated to the nucleus in response to tissue factor (TF)/FVIIa complex formation. This occurs in several cell types including monocytes and aortic smooth muscle cells. Caveolae are well characterized cell membrane signaling compartments, but their role in TF signaling is poorly understood.

Purpose: To clarify the mechanism behind the TF-induced phosphorylation of the IGF-1R, we uti- lized TF-expressing cancer cells to investigate the interaction between IGF-1R and caveolin-1 (Cav1), the principal protein of caveolae.

Methods: Prior incubation with FVIIa, PC3 prostate or MDA-MB-231 breast cancer cells were trea- ted with 500 nM simvastatin, Cav1 siRNA, a peptide corresponding to the Cav1 scaffolding domain, or Src-family inhibitors. The phosphorylations of IGF-1R and Cav1 were determined using the Duo- link In Situ proximity ligation assay (PLA) and western blot (WB), and the nuclear localization of the IGF-1R was assessed by PLA or by WB on fractionated cell lysates.

Results: FVIIa treatment (10 and 100 nM) increased the phosphorylation of the IGF-1R after 30 min- utes and induced a nuclear translocation of the receptor after 2 h. Incubation with simvastatin for 2 h resulted in a hyperphosphorylation of the IGF-1R owing to downregulation of Cav1 expression. The IGF-1R was similarly activated by Cav1 siRNA knockdown. Additional experiments showed that pre- treatment with the Cav1 scaffolding domain peptide completely abolished the effects of FVIIa regard- ing IGF-1R phosphorylation and nuclear translocation. The formation of the TF/FVIIa-complex did not alter Cav1 protein levels but induced a Src-dependent phosphorylation of tyrosine 14 on Cav1 after 10 minutes. Inhibition of Src completely abolished the transactivation of the IGF-1R by TF/FVIIa.

Conclusions: We found the Cav1 scaffolding domain to prevent IGF-1R phosphorylation in resting cell and could connect TF/FVIIa, Src, and Cav1 to the activation and nuclear translocation of the IGF-1R. These results also emphasize the importance of Src-family kinases in diseases characterized by aberrant TF expression such as cancer and atherosclerosis.

551 Notch signaling is differently altered in endothelial and smooth muscle cells of ascending aortic aneurysm patients
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Background: Mitochondria have emerged in the past decade as major therapeutic targets in cardio- vascular pathology. We have previously demonstrated, in isolated rat heart mitochondria, that novel an- giogenic analogues in diabetes mellitus (DM) and mammalian arteries harvested from coronary ar- tery disease patients with and without DM subjected to by-pass grafting.

Methods: The effect of KL-1487, KL-1492, KL-1507 (10 mg/L) on endothelium-dependent relax- ation (EDR), assessed in the organ bath-system) and H2O2 production (determined by ferrous oxida- tion was significantly increased in both humans and rats. Incubation of vascular segments with all inves- tigated compounds attenuated H2O2 production, reduced contractility and partially restored EDR.

Results: In vitro endothelial tubule formation was studied in a 3D coculture system, in which human umbilical vein endothelial cells (HUVECs) and pericytes were cocultured in a collagen matrix. Short interfer- rence RNA (siRNA) based knockdown of Fzd5 in HUVECs resulted in significant reduction of endo- thelial tubule formation. The involvement of endothelial apoptosis as a causative factor for the poor angiogenic phenotype in the Fzd5 knockdown condition was excluded based on TUNEL staining. However, angiogenic proliferative components in AoA patients, was severely inhibited after knockdown of Fzd5. In order to define the molecular cause for the inhibition of angiogenesis after knockdown of Fzd5, known downstream Fzd5/Wnt transcription cascades were studied. No alterations were observed in various parameters of the canonical Wnt/β-catenin pathway and the non-canonical Wnt/Ca2+ pathway after knockdown of Fzd5. qPCR analysis of Fzd5 siRNA- treated endothelial cells did however show a significant up regulation of both Flt1 and Angta2, two important factors in vascular regression. The up regulation of Angta2 could be suppressed by a com- bined knockdown of Fzd5 and the transcription factor Es1-1. These data indicate that the degenerative effect of Fzd5 silencing on vascular structure formation in vitro is dependent on endothelial cells and its inhibition with a combination of Fzd5 knockdown and Wnt7b siRNA was sufficient to reverse vascular regression. The compounds had no effect on angiogenesis in AoA patients. However, knockdown of Fzd5, known downstream Fzd5/Wnt transcription cascades were studied. No alterations were observed in various parameters of the canonical Wnt/β-catenin pathway and the non-canonical Wnt/Ca2+ pathway after knockdown of Fzd5. qPCR analysis of Fzd5 siRNA-treated endothelial cells did however show a significant up regulation of both Flt1 and Angta2, two important factors in vascular regression. The up regulation of Angta2 could be suppressed by a com- bined knockdown of Fzd5 and the transcription factor Es1-1.

Conclusions: The present study was purported to assess the effect of three benzoyl analogues on the vascular reactive and HGOZ production in aortic rings isolated from rats with streptozotocin-induced diabetes mellitus (DM) and mammary arteries harvested from coronary ar- tery disease patients with and without DM subjected to by-pass grafting.

Methods: The effect of KL-1487, KL-1492, KL-1507 (10 μmol/L) on endothelium-dependent relax- ation (EDR), assessed in the organ bath-system) and H2O2 production (determined by ferrous oxida- tion was significantly increased in both humans and rats. Incubation of vascular segments with all inves- tigated compounds attenuated H2O2 production, reduced contractility and partially restored EDR.

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Conclusions: The present study was purported to assess the effect of three benzoyl analogues on the vascular reactive and HGOZ production in aortic rings isolated from rats with streptozotocin-induced diabetes mellitus (DM) and mammary arteries harvested from coronary ar- tery disease patients with and without DM subjected to by-pass grafting.
Purpose: Cardiac remodeling and inflammation are hallmarks of cardiac failure and correlate with outcome in patients. However, the basis for the development of both remains unclear.

We have previously reported that cardiac inflammation triggers transdifferentiation of fibroblasts to myofibroblasts and increase cardiac collagen deposition, one key pathology in cardiac remodeling. Furthermore, our findings reveal that cardiac fibroblasts are chemotactic sentinel cells activated by increased stretch intensities and are able to recruit inflammatory cells into the cardiac tissue, a process known to aggravate prognosis of patients. Here, we investigate the role of fibroblasts in the inflammatory process as well as the cross-talk between fibroblasts and inflammatory cells.

Methods and Results: We address the role of fibroblasts as inflammatory supporter cells in heart failure. Using endomyocardial biopsies from patients with heart failure we created a primary human cardiac fibroblast cell culture system to stimulate the primary fibroblasts we used the flexercell system with increasing stretch intensities or with increasing stretch frequencies. We found that not only increasing stretch intensities mimicking cardiac dilation induce activation of fibroblasts but also increasing stretch frequencies. Both types of mechanical activation lead to up-regulated chemokine production and triggers typical inflammatory pathways in vitro. Furthermore, we investigated the composition of the extracellular proteome of human cardiac fibroblasts using mass spectrometric analysis of the cell culture supernatant. We clearly demonstrate that besides ECM proteins different chemokines could be identified. Next, we used this conditioned medium derived from cardiac fibroblasts to perform co-culture experiments to investigate the cross-talk between fibroblasts and inflammatory cells.

Conclusion: Cardiac fibroblasts serve as supporter cells for cardiac inflammation. Due to different stimuli such as increased mechanical stretch mimicking dilation, increased stretch frequencies mimicking tachycardia, fibroblasts secrete cytokines and chemokines. This might be important in different forms of heart failure and therefore may be one general mechanism specific for fibroblasts. Furthermore, inflammatory cells are further modulated by proteins secreted by activated fibroblasts which shows the close association between fibroblasts and inflammatory cells.

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A role for galectin-3 in calcific aortic valve stenosis

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Background: Aortic stenosis (AS) is a chronic inflammatory disease, and calcification plays an important role in the progression of the disease. Galectin-3 (Gal-3) is a proinflammatory molecule involved in vascular osteogenesis in atherosclerosis.

Purpose: To study whether Gal-3 mediates valve calcification in AS.

Methods: Blood samples and aortic valves (AVs) from 80 patients undergoing aortic valve replacement were studied by histological and molecular analysis. Valvular interstitial (VCs) isolated from adult human AVs were differentiated in the presence or absence of the pharmacological inhibitor of Gal-3, modocit pectin (MCP). In addition, AS rats were treated with MCP (100 mg/kg, i. d.) in the drinking water for 6 weeks.

Results: Gal-3 was spontaneously expressed in the AVs of patients with AS. Positive correlations were found between valvular Gal-3 protein levels and calcification markers. Valvular Gal-colocalized with osteogenic markers such as BMP, Runx and Sox-9. In vitro, MCP treatment decreased the expression of osteogenic markers in differentiated VCs. In rats, MCP treatment prevented the increase in Gal-3 protein levels, as well as the enhanced osteogenic markers found in the AV of AS rats.

Conclusion: Gal-3 appears to play a central role in the process of calcification in AS. Gal-3 could be a new therapeutic approach to delay the progression of valve calcification in AS.

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Omega-3 polyunsaturated fatty acids- can they decrease risk for ventricular fibrillation?

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Background: Reports, including ours, indicate that lower omega-3 (ω-3) index was lower in both SHR. This parameter was significantly increased due to FA intake in both sexes of SHR. cα and 5′SHR also exhibited a significant increase of serum levels of b1-AAB, activity of MMP2, down-regulation and miss-localization of Cx43. It was associated with higher incidence of VF. ω-3 FA intake resulted in significant decrease of b1-AAB levels and MMP2 activity, upregulation of Cx43 and partial elimination of Cx43, its miss-localization in both cα and 5′SHR. Moreover, ω-3 FA decreased incidence of electrically induced VF.

Conclusions: These findings suggest multiple cardio-protective effects of omega-3 intake that can contribute to decreased susceptibility of the hypertensive rat heart to lethal arrhythmias.

Background/Introduction: The vascular remodeling is a response to hemodynamic forces. The mechanical stress causes modifications in the vascular wall, such as reorganization of cellular composition, vascular matrix and vascular inflammatory responses. The α7 nicotinic acetylcholine (α7nAChR) receptor is found in many cell types, including the cells of the immune system. In several inflammatory tissues, the activation of the α7nAChR by acetylcholine inhibits the production of inflammatory cytokines and chemokines through the vagal reflex, thus producing an anti-inflammatory effect. It is not yet the contribution of the α7nAChR for the process of vascular remodeling.

Purpose: The aim of this study was to evaluate the effect of the deletion of α7nAChR in the vascular remodeling developed in response to transverse aortic constriction (TAC).

Methods: We used wild type mice (WT) and knockout mice with deletion of α7nAChR (α7.KO) at 10 weeks of age. Mice were divided into the following groups: WT SHAM, WT TAC, α7 SHAM and α7 TAC. Seven days after TAC, mice were sacrificed and the ascending aorta was isolated for analysis.

Results: The vascular cross-sectional area (VCSA) was increased in WT TAC (0.02 ± 0.0005mm²) and α7 TAC (0.31 ± 0.02mm²) groups compared to their respective controls WT SHAM (0.02 ± 0.002mm²) and α7 SHAM (0.28 ± 0.0102mm²). A similar pattern was also observed for the area of the lumen, in the values of WT TAC (43 ± 0.66%) and α7 TAC (38.4 ± 2.41%) groups were larger when compared with their controls WT SHAM (28 ± 0.71%) and α7 SM (29 ± 3.61%).

While the WT TAC group had a significant increase in the deposition of collagen type I (3.73 ± 0.89μm²) and III (2.97 ± 0.75μm²) when compared to SHAM, the deletion of α7nAChR inhibits this process maintaining the level of both types of vascular collagen as in SHAM and α7 SHAM operated groups. Regarding the density of cells, the α7 TAC group had the highest values.

Conclusion: The results demonstrate that the α7nAChR promotes a positive vascular remodeling in the proximal aorta of both WT and α7nAChR knockout mice. In response to TAC, the vascular deposition of collagen and the density of cells are influenced by this receptor. Further studies are needed to understand the mechanism involved in these processes.

565 Extracellular matrix remodelling in response to venous hypertension: proteomics of human varicose veins

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Background: Extracellular matrix (ECM) remodelling has been implicated in a number of vascular conditions, including venous hypertension and varicose veins. However, to date no systematic analysis of matrix remodelling in human veins has been performed.

Purpose: To assess and provide mechanistic insight into ECM changes in varicose veins.

Methods: Varicose saphenous veins removed during phlebectomy and normal saphenous veins obtained during coronary artery bypass surgeries were collected. Gene expression analysis was performed on RNA extracted from venous tissues and cultured human saphenous vein smooth muscle cells, while sections were processed for histological and immunohistochemical analysis. Matrix proteins were enriched from varicose and control vein tissues and subjected to proteomics analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS).

Results: The proteomics analysis revealed the presence of more than 150 ECM proteins, of which 75 had not been previously detected in human venous tissue and 34 showed significant differences between normal and varicose saphenous veins. ECM in varicose veins was characterised by a loss of several small leucine-rich proteoglycans, aggrecan and a compensatory increase in collagen I and laminin. Chymase and tryptase, two serine proteases commonly attributed to mast cells, were among the up-regulated proteins. Using immunohistochemistry, however, chymase expression was localised to smooth muscle cells in varicose veins. The effect of chymase and tryptase on the venous ECM was explored by incubating normal saphenous veins with recombinant enzymes. Proteomics analysis revealed extensive ECM degradation after digestion with tryptase. In comparison, chymase was less potent and degraded predominantly basement membrane-associated proteins. When human saphenous vein smooth muscle cells were stimulated with transforming growth factor beta (TGF-β), tumor necrosis factor-a (TNF-a) or angiotension II (Ang II), a number of ECM genes differentially expressed in varicose veins, including miceman, changed in response to TGF-β and TNF-a but to a lesser extent to Ang II.

Conclusion: The present proteomics study provides unprecedented insights into the degradation of structural and regulatory components of the vascular ECM in varicosis.

Ion channels, ion exchangers and cellular electrophysiology - Heart

568 Microtubule-associated protein RPE/EB family member 1 modulates sodium channel trafficking and cardiac conduction

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Introduction: Microtubule-associated protein RPE/EB family member 1 (EB1) encoded by the gene MAPRE1 is part of a protein network which binds microtubules at their (+) end extremities underneath the cell membrane. EB1 has been shown to regulate trafficking of connexin43 (Cx43), and importantly, the intercellular diacylglycerol. Furthermore, EB1 is removed from intercalated discs in cardiac hypertrophy, heart failure and in the setting of Cx43 mutations. Recent studies have also demonstrated that EB1 is implicated in the subcellular localization of sodium channel neurons. We have investigated the effects of EB1 on cardiac sodium channel function and its modulation effect on cardiac conduction.

Methods and Results: SOTL experiments performed on an F2 population of mice of two separate inbred strains carrying a sodium channel mutation (Scn5a1798insD+I) showed a strong negative correlation between the expression of the MAPRE1 gene and QRS duration on the surface ECG, suggesting a functional impact for EB1 on ventricular conduction. Co-immuno precipitation experiments confirmed the physical interaction between EB1 and the major cardiac sodium channel Nav1.5. Overexpression of MAPRE1/EB1 in HEK293 cells together with SCN5A/Nav1.5 led to an increase in sodi- um current density without affecting kinetic properties, indicating an increased membrane trafficking of the Nav1.5 protein.

Conclusion: This study demonstrated a functional role for EB1 in cardiac conduction and we highlight its direct regulation of the cardiac sodium channel Nav1.5. We recently produced lentiviruses in knock-down and overexpress EB1 in order to characterize its modulatory effect on ion currents and action potential parameters in hiPSC-CM using the dynamic clamp technique.

569 Investigation of electrophysiological abnormalities in a rabbit athlete’s heart model

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Introduction: 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 (e.g. reduced repolarization reserve) might also lead to life-threatening ventricular tachyarrhythmias. In a new study, the effect of the long-term exercise training was tested on the electrical activity of the myocardium in a new rabbit athlete’s heart model.

Methods: New Zealand white rabbits were randomized into a ‘Sedentary’ and an ‘Exercised’ (Ex) group (n=7). Animals of the Ex’ group were trained during a 12-week long treadmill-running protocol. Electrocardiography and resting ECG recording were performed under ketamine anesthesia. At the end of training protocol, proarrhythmic sensitivity was tested with defibolite (50 μM) 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 (mRNA) expression level of TGF-β, fibronectin-1, collagen-I, -III, MMP-2 and TIMP-1 were quantified with RT-qPCR to determine the collagen metabolism.

Results: Electrocardiography on the 12th week showed significant increase in the internal end-diastolic diameter of the left ventricle (LVIDd) in the Ex’ 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 variability parameters of the RR and Tpeak-Tend intervals in vivo were significantly greater in the Ex’ group. Defibolite tended to increase the QTc interval in the Ex’ group in vitro, however, there was no difference in the incidence of proarrrhythmia between the two groups. RT-qPCR showed significantly greater mRNA expression of TIMP-1 in the Ex’ 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. Greater variability and repolarization parameters may indicate the sensibility of the athlete’s heart to arrhythmia. Increased TIMP-1 indicated structural remodeling in our model. Further investigations are warranted. This work was supported by OTKA (PD 105882) and Bolyai fellowship of Farkas Attila.
Methods: Diabetes was induced in male rats with STZ (60 mg/kg bodyweight, i.p.) and age-matched control rats and experiments were performed 12 weeks after treatment. Real-time RT-PCR techniques were used to measure the expression of genes. Results: Diabetes characterized by a 5-fold increase in blood glucose in STZ vs. control. The effect is attributed to the blockage of the repolarizing rapid delayed rectifier potassium current (Ikr/HERG), that has been characterized in re-expressed channels. Recently, methadone was demonstrated to block the depolarizing cardiac sodium current (INa/Nav1.5), thus exerting an effect opposite to that due to Ikr/HERG channel blockade. Purpose: The aim of this study was to characterize the effect of racemic and levo-methadone on the action potential (AP) profile detected in single human ventricular cardiomyocytes at different driving rates (0.5, 0.5 and 1 Hz). Methods: Single cardiomyocytes were obtained from ventricular samples of patients undergoing septal myectomy or heart transplantation. APs were recorded from cells using the perforated patch technique. Results: Racemem methadone (0.1-10 μM) reduced AP duration (APD) in a concentration-dependent manner. The effect was not prevented by naxolone (1 μM), an opioid antagonist, thus excluding the involvement of cardiac opioid receptors in the reduction of APD. Similarly to the racemic form, levo-methadone (0.1-10 μM) reduced AP of human ventricular cardiomyocytes, showing properties comparable to those of the racemic form. Other AP parameters, including amplitude and maximal diastolic potential were not modified either by racemic and levo-methadone. Conclusion: In our experimental setting racemic and levo-methadone are able to reduce AP of human ventricular cardiomyocytes. Reduction is particularly pronounced in the early phase of AP repolarization, suggesting that, beyond H mingle, other channels, such as INa/Nav1.5, are likely to be affected by methadone. Further studies are ongoing to clarify the molecular mechanism of these effects.

Vasculogenesis, angiogenesis and arteriogenesis

577 Clinical improvement and enhanced collateral vessel growth after monocyte transplantation in mice
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Monocytes are the most important mediators in angiogenesis. Previous results by our group demonstrated the enormous potential of allogenic monocyte transplantation (different mouse strain) for improvement of collateral vessel growth. We investigated the possible beneficial effect of selective NCX inhibition on alterations of left ventricular mechanics occurring during HK and VF. Methods: Experiments were performed on isolated guinea pig and rat hearts. ECG and left ventricular pressure (LVP) were recorded throughout the experiments and analysed off-line. NCX inhibition was achieved with ORIM10962 (ORIM, 1 μM). Results: HK solution increased LVP indicating net cellular Ca gain. Administration of ORIM significantly diminished the HK-induced increase in LVP. ORIM did not affect the ECC parameters. In significant experiments, VF was induced by rapid pacing in isolated rat hearts. Onset of VF resulted in left ventricular contracture, the fast component of which occurred within 5 seconds and was significantly attenuated by ORIM. Similarly, the slowly developing component, evaluated after 3 min of fibrillation, was also decreased in the ORIM-treated hearts. After defibrillation, LVP amplitude showed a mild increase in the ORIM-treated hearts compared to the control group, but this effect did not reach statistical significance. Conclusions: The increased LVP during HK and the myocardial contracture during VF observed in this study support the literature arguing development of elevated Ca load, both in HK and during VF. However, further studies are required to investigate whether these effects can result in decreased arrhythmia propensity in HK and/or reduced defibrillation after successful defibrillation.
578 The role of HIF-1 alpha, VEGF and obstructive sleep apnoea in the development of coronary collateral circulation

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Introduction: Intermittent hypoxia (IH) in obstructive sleep apnoea (OSA) confers cardioprotection by enhancing coronary collateral circulation (CCC) development, thereby decreasing myocardium vulnerability to hypoxia and ischemia. The exact mechanism is as yet unclear. By better understanding of the physiology, one may attempt to replicate these adaptive mechanisms in non-OSA ischemic heart disease (IHD) patients to better augment CCC.

Purpose: The study objective was to assess whether Hypoxia Inducible Factor-1a (HIF-1a) and Vascular Endothelial Growth Factor (VEGF) play a role in the development of CCC in patients with OSA.

Methodology: A total of 44 patients with reported collateral vessels on angiography were selected as cases, with 21 patients not having a CCC recruited as controls. All patients underwent ambulatory polysomnography to test for the presence of OSA. Blood samples for HIF-1a (HIF-1a ELISA Kit, Antibodies-online Inc, Atlanta, GA, USA) and VEGF (Human VEGF ELISA Kit, KHG0111, Invitrogen Corporation, Carlsbad, CA, USA) were collected. The development of CCC was classified according to the Rentrop Score, with the cardiologists interpreting the angiograms blinded as to whether patients were cases or controls.

Results: HIF-1a increased with increasing Rentrop Score (p=0.04), in all patients. VEGF levels were however not significantly higher [p=0.31]. HIF-1a levels in moderate and severe OSA patients were significantly higher with Rentrop Scores (p=0.02). Patients without or mild OSA patients showed no difference with Rentrop Scores (p=0.49). VEGF levels did not differ significantly with Rentrop Score in none of the patient subgroups (no or mild OSA [p=0.23]) and moderate or severe OSA (p=0.29). A separate analysis did not reveal any significant difference between diabetic and non-diabetic patients for HIF-1a (p=0.00) and VEGF (p=0.34) in the absent or mild OSA subgroup. There was also no significant difference in the moderate and severe OSA subgroup for both HIF-1a (p=0.825) and VEGF (p=0.454).

Conclusion: This is the first study to date that links OSA, CCC, and plasma HIF-1a and VEGF levels. Augmented HIF-1a in moderate/severe OSA patients might be an important mediator in the development of CCC, but not in patients with normol OSA.

579 Initiating cardiac repair with a trans-coronary sinus catheter intervention in an ischemia/reperfusion porcine model

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Objective: We analyzed the potential of a trans-coronary sinus catheter intervention activating endothelium to induce angiogenesis and the potential of temporary coronary venous pressure elevation (PICSO) to initiate cardiac repair in an ischemia/reperfusion model.

Material and Methods: 32 open chest pigs were divided: sham-operation (n=3); 4 hours Infarct and 1 hour reperfusion (controls); 4 hours PICSO in the intact heart (PICSO-A, n=10); PICSO (started 15 min. after ischemia (PICSO-B, n=11). Specimen were taken from: LAD region (infarct), left anterior descending (LAD), right coronary artery (RCA), and internal mammary artery (IMA). Specimen were divided in two consecutive vessel segments from each side of the ‘anastomosis point’. The vessel segment adjacent to the collateral was occluded via micro-irradiation. Video recordings were made before occlusion, repeatedly during the first 2 h PO, hourly from 3 to 12 h PO and 24 h PO. Vessel diameter and blood flow velocity of all collateral unit vessels were measured offline from the video recordings. Blood flow and wall shear rate (WSS) were calculated.

Results: Arteriolar and venular collateral diameters did not show a significant increase over 24 hours in the control group (p>0.05). After occlusion, diameter of both arteriolar and venular collaterals decreased and lasted for several minutes and then increased continuously until reaching the maximal (arterioles: 3 h PO; venules: 2 h PO). In parallel, WSS showed an initial increase (arterioles: for 5 h PO; venules: for 1 h PO) and then a gradual decrease to the starting values (p>0.05). Maximal collateral enlargement (arterioles: 60%; venules: 100%) occurred in the smallest segment before occlusion and maximal WSS increase (arterioles: 230%; venules: 400%) occurred in the second smallest arteriolar and smallest venular collateral segment before occlusion.

Conclusions: Niacor® CAM, collateral enlargement occurs minutes after the occlusion, suggesting vasoactive metabolites accumulated during this time might initiate the enlargement. In contrast, collateral adaptation might mainly be driven by an increase in WSS. The differences between arteriolar and venular collateral units in diameter and WSS changes over time as well as the hypothesized cause-effect relations might be useful to develop therapeutic schemes.

Endothelium

583 EDH-type responses to the activator of potassium KCa2.3 and KCa3.1 channels SKA-31 in the mesenteric artery from spontaneously hypertensive rats

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Background: Endothelium-derived hyperpolarization (EDH) type relaxation is NO- and prostacyclin (PGI2)-independent hyperpolarization, with activation of small (KCa2.3) and intermediate conductance (KCa3.1) calcium-activated potassium channels (KCa). Cardiovascular diseases with endothelial dysfunction, e.g. hypertension, are related with impairment of KCa2.3 and KCa3.1. Their activation by SKA-31 increased coronary flow in rats, induced systemic blood pressure in hypertensive mice, normotensive dogs and pigs.

Purposes: Influence of SKA-31 on EDH-KCa2.3/KCa3.1 type responses in spontaneously hypertensive rats (SHR) in endothelium-intact small mesenteric arteries.

Methods: Vascular relaxation was expressed as the percentage of phenylephrine pre-contracted tone. Expression of KCa1.3 and KCa2.1 mRNA was confirmed by RT-PCR.

Results: Concentration-dependent relaxation induced by SKA-31 (0.01-10 µM) was reduced in SHR (p<0.05). EDH-type responses to the activator of potassium KCa2.3 and KCa3.1 channels SKA-31 in the mesenteric artery from spontaneously hypertensive rats (SHR) were investigated in vitro. The addition of SKA-31 (100 µM) was observed to be significantly reduced in SHR when compared with WKY. No significant changes were observed in the respective controls. In SHR we observed significantly reduced expression of KCa3.1 and KCa2.3. In SHR we observed significantly reduced expression of KCa3.1 and KCa2.3.

Conclusion: SKA-31-induced endothelial cell potassium KCa2.3 and KCa3.1 channel activation attenuates relaxation in SHR in endothelium-intact small mesenteric arteries.

584 The peculiarities of endothelial dysfunction in patients with chronic renal syndrome

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Endothelial dysfunction is a marker of vascular disease, as well as the development and progression of hypertension in chronic kidney disease is called chronic renal syndrome.

Objective: To study the characteristics of endothelial dysfunction in chronic renal syndrome.

Results: The study included patients with chronic renal syndrome, hypertension in chronic glomerulonephritis (n = 105, 67 men and 64 women). Vasomotor function analysis showed that the vasodilatory response in less then depensation was expressed in patients with right-peak -5.47 (3.04, 11.72%) (p<0.0001, compared with the other groups, which is in group of patients with the most severe kidney damage).
Abstracts

Methods: Examined 31 men (mean age 56.7 ± 5.6 years) with coronary heart disease and hepatic steatosis. Allocated 3 groups according to BMI: group 1 consisted of 9 (29%) people (BMI 25 to 29.9 kg/m²), group 2 - 13 (42%) (BMI 30 to 34.9 kg/m²), group 3 - 9 (29%) (BMI 35 to 39.9 kg/m²). We studied the levels of leptin in the sample with nitroglycerin r=-0.24 (p=0.017) and normal geometry r=-0.22 (p=0.026), the degree of nocturnal systolic BP r=-0.22 (p=0.030) and the ratio of the velocity of early diastolic filling and atrial r=-0.21 (p=0.037).

Conclusions: In summary, using systems biology approach we have established a hierarchical order of signaling influenced by ncRNAs and miRNAs, which may explain the higher propensity of BAV to develop TAA.


cigarette smoke extract abrogates atheroprotective effects of high laminar flow on endothelial function

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Background/Introduction: Tobacco smoking and local hemodynamic forces are key stimuli in the development of endothelial dysfunction and atherosclerosis. High laminar flow has an atheroprotective effect on the endothelium. This leads to a reduced response of endothelial cells to cardiovascular risk factors compared to regions with disturbed or low laminar flow. The molecular mechanisms controlling the atheroprotective effect of high laminar flow and its effect on the cardiovascular risk factor of smoking is not well understood.

Purpose: We hypothesize that the atheroprotective molecular mechanisms of high laminar flow could be used to prevent the development of endothelial dysfunction by tobacco smoking. Therefore, we exposed human endothelial cells to cigarette smoke extract (CSEex) under different flow conditions and studied gene expression, monocyte adhesion and wound healing.

Methods/Results: Primary human endothelial cells were stimulated with increasing dosages of CSEex for 24-48h. CSEex reduced cell viability in a dose-dependent manner. The main mediator of cellular adaption to oxidative stress NRF2 and its target genes heme oxygenase 1 and NADPH-dehydrogenase (quione 1) were strongly increased by CSEex in a dose-dependent manner. High laminar flow induced elongation of endothelial cells in the direction of flow, activated the PKB/AKT pathway, followed by increased eNOS expression and subsequent NO release. This increase was inhibited by CSEex in a time-dependent manner. Induction of the NRF2 system by CSEex was not further regulated by high laminar flow. In contrast, proatherosclerotic low laminar flow had no effect on eNOS expression and NO release compared to high laminar flow. Proinflammatory adhesion molecule ICAM1, VCAM1, SELE and CCL2 were increased by CSEex. Low laminar flow induced VCAM1 and SELE compared to high laminar flow. High laminar flow improved endothelial wound healing. This protective effect was inhibited by CSEex in a dose-dependent manner. Low laminar flow did not affect wound healing compared to static conditions. Low and high laminar flow decreased adhesion of primary monocytes to endothelial cells. Interestingly, monocyte adhesion was increased by CSEex under low laminar flow, which was not evident under high laminar flow.

Conclusions: In conclusion, our data suggest novel molecular mechanisms that underlie the association between tobacco smoking and the development of endothelial dysfunction. In contrast to low laminar flow, high laminar flow mediates protective effects on tobacco smoke-induced endothelial inflammation and wound healing.
(protein expression and antioxidant activity). Methods: EA.hy 926 endothelial cells were cultured and transiently transfected with a human Ace ORF mammalian expression plasmid containing ACE DNA using Trans-IT X2 reagent (Mirus®). Transfected cells were treated with cultured ham hydrolysate (5-500 μM). ACE activity was tested in cell lysates by spectrophotometry. The effect of BP on cell proliferation and viability was assessed by MTT assay alone and with H2O2 300 μM. Potential mechanisms of action of BP over endothelial function were quantified by RT-PCR after treatment with TNFα 100 ng/ml or H2O2 300 μM and/or BP. Results: BP do not affect cell proliferation but significant ACE inhibition was observed at 50μg/ml BP prevented the TNFα increased ICAM-1 mRNA expression. H2O2 decreases cell viability and BP significantly reversed the H2O2-damage at 37.5 and 100 μM. In the presence of H2O2 they also increased expression of ANOS mRNA although BP alone present an opposite effect. Increased expression of antioxidant enzymes mRNAs such as superoxide dismutase and catalase and reduced NADPH oxidase were found incubating with H2O2 and BP together. Conclusions: BP from Spanish dry-cured ham have important implications over the endothelium and may protect it from oxidative and inflammatory damage. These results implicate that BP may display clinical relevance.

Results: Dyslipidemia was revealed in patients with early RA in 68% (RA in 65%). In addition, the increase in very low-density lipoprotein (VLDL) in early RA–61% (in early RA–55%) was always associated with the disorder of other components of the lipid profile. In early RA the mean value of total cholesterol was higher in 0.2 and a maximum value in 1.4 times than in RA (3.2 (5.5) 10.2 and 3.5 (7.3) 2mmol/L) (p<0.05). Increased level of triglycerides (TG) in early RA was 1.5 times more frequent 51% (in RA–33%), the mean value was 1.9 mmol/L in early RA (1.6 mmol/L– in RA); Increased level of low-density lipoproteins (LDL) was 1.8 times more frequent in early RA–62% (34% in RA); the mean value was 3.2 mmol/L in early RA. Erythrocyte sedimentation rate (ESR) was 1.3 times more frequent in patients with IDL (80%- in RA) than in patients with C- reactive protein (CRP) (70% in early RA, 61% in RA) (p<0.05). CRP was 1.2 times higher in early RA than in RA; the mean value in early RA was 2.93mg/L (19mg/L– in RA). The correlations between total cholesterol and CRP (r=0.23, p<0.05), LDL and CRP (r=0.21, p<0.05) were revealed.

Conclusions: Thus, dyslipidemia in early RA presents the following characteristics: increased blood atherogenicity (the increase of LDL in 1.8 times, TG in 1.5 times, the decrease of HDL in 2.3 times), the average TBC was higher in 0.2 times and a maximum value in 1.4 times in early RA (p<0.05). There is a correlation between the level of TBC, LDL and the markers of systemic inflammation (p<0.05). The contribution of chronic immune-inflammatory processes in the development of dyslipidemia is observed more frequently in early RA in 1.2 times (p<0.05).

Atherosclerosis

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Macrophages differentiated in vitro are heterogeneous: morphological and functional profile in patients with coronary artery disease

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Introduction: Monocytes and tissue macrophages, cells involved in the inflammatory process, play a crucial role at any stage of coronary artery disease (CAD). Macrophages are hallmarkled by morphology and phenotypic heterogeneity described also in atherosclerotic plaque where the presence of a particular macrophage phenotype may have harmful or beneficial functions on CAD development. Tissue macrophages are not easily obtained from site-specific tissue, and macrophages are not considered as high-sensitivity tool for research. Thus, we aimed to investigate the differences in macrophage phenotype depending on CAD.

Methods: Monocytes were isolated from venous blood of 25 healthy subjects (50±15 years) and from 50 CAD patients (41±11 years) and differentiated for 7 days in medium supplemented with 10% autologous serum. The uptake of apoptotic Jurkat T cells, for efferocytosis assay, was detected by flow cytometry. Transglutaminase 2 (TG2) and tissue factor (TF) were determined by immunofluorescence and western blotting. Thrombin generation was evaluated using a thrombinoscope.

Results: Morphologically, MDMs of CAD patients show a prevalence of round morphology respect to spindle. Nevertheless, these MDMs displayed less efferocytic capacity compared to control. Impaired efferocytosis may be due to the reduces levels of TG2, protein involved in phagosome formation. Moreover, CAD MDMs present higher TF levels that are associated with a quidly thrombin generation.

Conclusions: MDMs of CAD patients show a pro-inflammatory and a pro-thrombotic profile characterized by reduced efferocytic capacity and increased of TF levels. MDMs of CAD patients can contribute to platelet aggregation and activation besides to thrombosis formation. Drug handling of different macrophage phenotypes in human disease may provide a basis for new therapeutic strategies able to limit the progression of atherosclerosis.

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Palmitoleylthanolamide promotes anti-inflammatory phenotype of macrophages and attenuates plaque formation in ApoE-/- mice

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Introduction: The endogenous fatty acid amide palmitoleylthanolamide (PEA) is a lipid-derived mediator, which does not bind to the cannabinoid receptors CB1 or CB2, but exerts potent anti-inflammatory effects by ligating type-1 peroxisome proliferator-activated receptors (PPAR-α). PEA has shown to possess therapeutic potential in inflammatory disease models, but the role of PEA and its analogs as a therapeutic agent in atherosclerosis remains unexplored.

Purpose: We aimed to evaluate the therapeutic potential of chronic PEA treatment in atherosclerotic mice.

Methods: The anti-inflammatory efficacy and mechanism of PEA were first investigated in primary bone marrow-derived macrophages (BMDM) under stimulation with lipopolysaccharides (LPS). As an in vivo approach, 8-8 week-old female apolipoprotein E deficient (ApoE-/-) mice on a high fat diet were treated with either vehicle or PEA (3 mg/kg/day) for 4 weeks. Lesion size and macrophage content of plaques were determined in aortic root sections. Furthermore, leukocyte subpopulations and cytokine expression levels at the tissue level were studied by flow cytometry and quantitative PCR, respectively.

Results: In LPS-stimulated BMDMs, PEA reduced the expression pro-inflammatory cytokines in a dose-dependent manner and through the activation of PPAR-α. Without affecting body weight or plasma cholesterol level, chronic in vivo administration of PEA was effective in attenuating atherosclerotic lesion size in ApoE-/- mice. Absolute macrophage-positive area of the lesions was also reduced in PEA-treated mice, but when normalized to total plaque area, macrophage content was comparable between the treatment groups. PEA treatment downregulated the expression of M1-type macrophage markers while enhancing M2 marker expression particularly in the spleen. Unexpectedly, PEA-treated mice had increased levels of classical monocytes in the circulation and aorta, an effect that occurred through a yet unknown mechanism.
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Conclusions: Our data show that PEA evokes potent anti-inflammatory effects in cultured primary macrophages, which translates into an anti-inflammatory protective effect in a model of early atherosclerosis. Future studies will be instrumental to clarify the underlying mechanisms and to evaluate whether this treatment strategy has efficacy also in pre-established and more advanced atherosclerosis.

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Amiodarone versus esmolol in the perioperative period: an in vitro study of coronary artery bypass grafts

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Background: Atrial fibrillation (AF) is a major concern after coronary artery bypass grafting (CABG) surgery. Beta blockers and amiodarone are indicated in both prophylaxis and treatment of AF in the perioperative period.

Purpose: This study is conducted to define and compare the vasoactive effects of esmolol and amiodarone, the two most commonly used beta blockers, on left internal mammary artery (LIMA), radial artery (RyA) and Saphenous vein (SV) grafts in in vitro tissue bath system. Methods: Ninety-six vascular rings (32 LIMA, 32 RA and 32 SV graft samples) obtained from 40 CABG patients (24 male, 16 female; mean age 54.25 ± 7.5 years) were evaluated after testing for vascular functionality of smooth muscle and endothelium. Vascular contractility of graft samples was assessed with phenylephrine, and the presence of a functional endothelium was tested with carbachol. Of 96 functional grafts, half was treated with esmolol and half was treated with amiodarone. The amount of relaxation of phenylephrine-precontracted grafts were assessed in terms of vasodilatation response to increasing doses of drug (amiodarone or esmolol) added to the tissue bath system. The concentration-response curves were constructed after transfer of data via Transfer Acquisition System (MAY IOBS 99, FDT 05, Ankara-Turkey) and MAY-MASTER MP36 analysis software. 

Results: The percentage vasodilatation responses with amiodarone were 71.65 ± 5.87% for RA and 65.07 ± 2.28% for IMA, 58.61 ± 5.18% for IMA, 65.07 ± 5.18% for RA and 58.61 ± 5.18% for IMA respectively. In both groups, IMA samples had more pronounced vasodilatation than RA samples. In addition, percentage vasodilatation responses with amiodarone were 71.65 ± 5.87% for RA and 65.07 ± 2.28% for IMA, 58.61 ± 5.18% for IMA, 65.07 ± 5.18% for RA and 58.61 ± 5.18% for IMA respectively. In both groups, IMA samples had more pronounced vasodilatation than RA samples. Amiodarone treatment of AF in the perioperative period.

Conclusions: The results Increased total cholesterol level was in 75% of cases with early RA (57% with RA). The mean average of total cholesterol levels were 3.5 ± 0.6 and 2.9 ± 0.6 mmol/L (p = 0.05). The increase of low-density lipoproteins in early RA was 68% (in RA - 36%), the mean value was 2.6 ± 0.3 in early RA, (2.1 ± 0.6 in RA) mmol/L. The increased triglyceride level was 51% in early RA, (34% in RA), the mean value was 1.5 ± 0.6 in early RA, (1.8 ± 0.7 in RA) mmol/L. Reduced levels of high-density lipoproteins (HDL) was 55% in early RA, (32% in RA) (p = 0.05). The conditions associated with atherosclerosis in RA are more common in RA than in early RA: ischemic heart disease was 3 times more frequent in RA (χ² = 8.6, p < 0.001), ankle-brachial index < 0.9 in 1.8 times (χ² = 8.5, p < 0.001), intermittent ischemic attack occurred only in RA. The correlation between total cholesterol levels and DAS28 (r = 0.08, p < 0.002), VAS (r = -0.08, p = 0.008), C-reactive protein (CRP) (r = 0.04, p = 0.07), erythrocyte sedimentation rate (ESR) (r = 0.2, p = 0.08), rheumatoid factor (RF) (r = 0.09, p = 0.02) was not revealed. There is correlation between CRP level and blood lipoprotein profile parameters: triglycerides (r = -0.32, p < 0.002), HDL (r = 0.22, p < 0.005).

Conclusions: Thus, atherosclerosis in early RA has the following characteristics: increased blood atherogenicity in early stage of RA occurring in 1.2 time more common, it is associated with immune-inflammatory markers (RF, CRP, ESR), p < 0.005 and the activity of the disease (VAS, DAS28), p = 0.005.

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Role of adenosine-to-inosine RNA editing in human atherosclerosis

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Background: Adenosine to inosine (A-to-I) RNA editing is catalyzed by ADARs (adenosine deaminases acting on RNA) and is an important posttranscriptional regulator of RNA metabolism. Although RNA editing is essential for life, its role in cardiovascular disease is unknown.

Methods and Results: RNA-seq and RNA-sequencing (RNA-seq) of human endothelial cells revealed that ADAR1 is the main RNA editor in HUVECs. The vast majority of editing events are detected in Alu RNA regions due to their ability to form double-stranded structures, a prerequisite for RNA editing. A-to-I RNA-editing of the primary specific A element is observed in 25% of poly(A) + transcripts and in 44% of ribosomal RNA-depleted transcripts. Cathespin S (CTSS), an extracellular matrix degrading enzyme, has 3 Alu elements in its 3’ untranslated region and is among the most extensively edited mRNAs. Interestingly, ADAR1 silencing downregulates CTSS mRNA expression, whereas ADAR1 overexpression results in an increase of both CTSS RNA editing rate and CTSS mRNA expression. Mechanistically, RNA immunoprecipitation (RIP), luciferase reporter and iCLIP assays showed that ADAR1 RNA editing of the Alu elements in its 3’UTR directed to the stabilization of CTSS mRNA and, thus, controls CTSS mRNA stability and expression. CTSS RNA editing is increased under hypoxic or pro-inflammatory conditions in vitro, as well as in patients with coronary or carotid atherosclerotic vascular disease. Further, the extent of CTSS RNA editing correlated with various markers of subclinical atherosclerosis including intima-media thickness and number of atherosclerotic plaques. Importantly, ADAR1 and the extent of CTSS RNA editing correlated with CTSS expression levels in patients’ samples from 6 non-overlapping cohorts of patients with inflammatory vascular diseases (total n=366), including peripheral blood mononuclear cells, carotid atherosclerotic plaques and thoracic aortic aneurysms.

Conclusion: This study shows for the first time that ADAR1 A-to-I RNA editing is a critical modulator of inflammatory gene expression in all stages of atherosclerotic vascular disease development.
Novel E-selectin binding polymers reduce atherosclerotic lesions in ApoE(-/-) mice

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Background: Atherosclerosis is characterized by acute and chronic vascular inflammation and leukocyte infiltration that result in plaque formation, instability, and rupture. E-selectin is the adhesion molecule expressed on activated endothelium that recruits leucocytes to the inflammation site, making it a therapeutic target to interfere with the development and progression of atherosclerosis.

Methods: We aimed to test the hypothesis that E-selectin-targeted polymers with and without the interfering it a therapeutic target to interfere with the development and progression of atherosclerosis. Human monocytes can be divided into a classical (CM, CD14++/CD16-) and a non-classical (NCM, CD14+ CD16+), and an intermediate subset (IM, CD14-CD16+) whereby CM are mainly phagocytes, NCM patrol along the endothelium and IM exhibit proinflammatory properties and are associated with inflammatory diseases such as atherosclerosis. Similar to monocytes, macrophages exhibit distinct heterogeneity. M1 macrophages secrete inflammatory cytokines, reactive oxygen species and matrix metalloproteinases and are possibly involved in plaque vulnerability and destabilization whereas M2 macrophages are anti-inflammatory and linked to plaque stabilization. The plasmogen receptor Plg-RKT might contribute to plaque rupture as it is used together with the receptor of the urokinase plasminogen activator (uPA) uPA to play a role in plaque instability and formation of coronary thrombus. Here we aimed to analyse the expression of Plg-RKT on monocyte and macrophage subsets.

Results: Four different species have been detected in the thrombi aspirates of patients with STEMI. No bacterial DNA was detected in peripheral blood samples of any patient.

Conclusions: Bacterial DNA from four species have been detected in the thrombi aspirates of patients with STEMI. No bacterial DNA was detected in peripheral blood.

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604 Differential expression of the plasminogen receptor Plg-RKT in monocyte and macrophage subsets - possible functional consequences in atherosclerosis

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Methods: ApoE-proteins E gene-deleted mice were fed with a Western-type diet for 11 weeks. Atherosclerotic plaque formation was induced in the carotid artery by a shear stress modifier device, which expose the vessel to distinct patterns of shear stress inducing plaques with distinct composition. Mice were treated with Apelin-13 (2 mg/kg/day) or vehicle for the last 3 months of the protocol.

Results: Apelin-13 treatment did not change the atherosclerotic plaque size in the aorta. Similarly, it did not alter the lipid content of low shear-stress- and oscillatory shear-stress-induced plaques in the carotid. However, Apelin-13 ameliorated plaque stability by increasing intraplaque collagen content, which was associated with a reduction in MMP-9 expression. Furthermore, Apelin decreased cell infiltration (neutrophil and macrophage) and intraplaque reactive oxygen species production. Interestingly, Apelin-13 treatment reduced total cholesterol, LDL levels and free fatty acid serum levels, while HDL triglycerides serum levels were not significantly changed.

Conclusions: Apelin-13 treatment for 3 weeks did not alter the lesion size, but significantly enhanced the stable phenotype of atherosclerotic plaques and improves serum lipid profile. These results indicate that activation of Apelin system decreases plaque vulnerability.

606 Mast cells are increased in the media of coronary lesions in patients with myocardial infarction and favor atherosclerotic plaque instability

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Methods: ApoE-proteins E gene-deleted mice were fed with a Western-type diet for 11 weeks. Atherosclerotic plaque formation was induced in the carotid artery by a shear stress modifier device, which expose the vessel to distinct patterns of shear stress inducing plaques with distinct composition. Mice were treated with Apelin-13 (2 mg/kg/day) or vehicle for the last 3 months of the protocol.

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Conclusions: Apelin-13 treatment for 3 weeks did not alter the lesion size, but significantly enhanced the stable phenotype of atherosclerotic plaques and improves serum lipid profile. These results indicate that activation of Apelin system decreases plaque vulnerability.
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Association of neutrophil to lymphocyte ratio with presence of isolated coronary artery ectasia

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Objectives: Coronary artery ectasia (CAE) has been defined as a dilated artery luminal diameter that is at least 50% greater than the normal artery diameter. Isolated CAE without significant coronary artery stenosis and isolated CAE has more pronounced inflammatory symptoms. Neutrophil to lymphocyte ratio (NLR) is widely used as a marker of inflammation and an indicator of cardiovascular outcomes in patients with coronary artery disease. We examined a possible association between NLR and the presence of isolated CAE.

Study design: In this study, 113 patients who underwent coronary angiography for suspected or known ischemic heart disease were evaluated. Our study population consisted of 83 CAE patients and 30 age- and gender-matched subjects who proved to have normal coronary angiograms. Baseline neutrophil, lymphocyte and other hematologic indices were measured routinely prior to the coronary angiography.

Results: Patients with angiographic isolated CAE had significantly elevated NLR when compared to the patients with normal coronary artery pathology (2.79 ± 1.70 vs. 1.98 ± 0.56; p=0.008). However, there was no statistical difference between both groups as regard to age, gender and common risk factors including hypertension, diabetes, smoking and family history of premature CVD.

Conclusion: Neutrophil to lymphocyte ratio is a readily available clinical laboratory value that is associated with the presence of isolated CAE.

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HMW-AGEs application acutely reduces ICaL in adult cardiomyocytes

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Background: Several studies have shown that advanced glycation end products (AGEs) are associated with adverse cardiac outcome. Growing evidence show that high molecular weight AGEs (HMW-AGEs) play a role as important as the well characterized low molecular weight AGEs (e.g. pentosidine and carboxymethyllysine). Despite their suggested deleterious involvement in chronic situations, HMW-AGEs might also have deleterious effects acutely. However, to date, their effects at the cardiomyocyte level remains unknown.

Purpose: In this study, we investigated whether HMW-AGEs acutely alter ICaL. Methods: HMW-AGEs were prepared by incubating 7 mg/ml bovine serum albumin (BSA) with 90 nM glycidaldehyde dimers in phosphate buffered saline (PBS) (pH 7.4) for 5 days at 37°C. The BSA-modified AGEs sample was validated by SDS-PAGE and fluorescence spectrometry. Single cardiomyocytes from the left ventricle of adult rat males were obtained by enzymatic dissociation through retrograde perfusion of the aorta. Ionic Ca2+ currents were evaluated during whole cell patch-clamp in the presence or absence of HMW-AGEs (200 μg/ml). Perfusion of BSA alone (200 μg/ml) was used as control. Currents were measured in ncells and normalized to cell capacitance. Experiments were performed at room temperature. Data are expressed as mean ± SEM.

Results: The prepared BSA-modified AGEs display high molecular weight and fluorescent proteins, characteristic for advanced glycation levels. After 4 minutes HMW-AGEs application, peak ICaL measured at +10 mV significantly decreased (-5.12 ± 0.59 pA/pF vs -7.72 ± 1.01 pA/pF at baseline, ncells=11; p<0.05). In comparison, 200 mg/ml BSA used a control, did not affect ICaL amplitude (-5.45 ± 0.54 pA/pF vs. -4.69 ± 0.48 pA/pF at baseline, ncells=7). Application of HMW-AGEs did not affect the voltage-dependence of ICaL, which remained bell-shaped and displayed a maximal current at +10 mV, significantly reduced by 33 ± 3% (ncells=6). BSA application in comparison, did not have any effect on Ca2+ currents (ncells=6).

Conclusion: Our data demonstrate that HMW-AGEs acutely alter ICaL and suggest a possible role in altered excitation-contraction coupling in rat cardiomyocytes.

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Calcium fluxes and excitation-contraction coupling

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The cosackase- and adenosine receptor (CAR) regulates calcium homeostasis in the developing heart

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The cosackase- and adenosine receptor (CAR) is a cell adhesion protein of the Ig superfamily which serves as receptor for different cosackase- and adenosine. CAR is strongly expressed throughout the developing heart and in contrast, during adulthood CAR is concentrated found at the intercalated disc. CAR knockout (KO) mouse models show malformation of the heart which leads to death between embryonic day 15.5 and 12.5 indicating an important function of CAR during heart development. Conditional CAR KO mouse models revealed impairment during excitation conduction in the mature heart. The aim of this study was to investigate the physiological function of CAR during early heart beats with regard to intercellular communication and Ca2+ cycling in embryonic cardiomyocytes. By using a global CAR KO mouse model, the investigation of cultivated E10.5 CAR KO cardiomyocytes and E11 CAR KO hearts revealed a significant higher beating frequency. Calcium imaging recordings of spontaneous Ca2+ transients in CAR KO cardiomyocytes showed a significant faster systolic Ca2+ decline compared to wildtype. The analysis of the cardiac Ca2+-extrusion mechanisms revealed a higher activity for NCX and SERCA2 in CAR KO cardiomyocytes. Gene expression and protein level of both NCX and SERCA2 was not changed in CAR KO hearts. Dye spreading studies with lucifer yellow indicated increased cell coupling of cultivated E10.5 CAR KO cardiomyocytes. Ca45 expression was downregulated in CAR KO hearts, however the observed increased cell coupling suggested in CAR KO hearts a remodelling of gap junctions that increases intercellular communication and excitation conduction resulting in the increased embryonic heart beat as recorded for CAR KO embryos. Due to the strong coexpression with Cx43 and Cx45 it can be suggested that CAR is localised in a larger protein complex involved with ZO-1 at the junctional sites. There, CAR may promote correct localisation of Cx43 and ZO-1 and cell-to-cell coupling. Taken together, CAR regulates intercellular communication between embryonic cardiomyocytes, is able to influence spontaneous Ca2+ cycling and is therefore an important regulator for embryonic heart beating.

Table 1: Baseline characteristics of angiographically normal and ectatic coronary vessels

<table>
<thead>
<tr>
<th>P</th>
<th>Normal (n=30)</th>
<th>Mean ± SD</th>
<th>n (%)</th>
<th>Ectatic (n=83)</th>
<th>Mean ± SD</th>
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<tr>
<td>0.564</td>
<td>53.35 ± 6.74</td>
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<td>51.42 ± 8.63</td>
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<td>0.078</td>
<td>19.86 ± 0.56</td>
<td>14 (46.7%)</td>
<td>2.79 ± 1.79</td>
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<tr>
<td>0.008</td>
<td>19.86 ± 0.56</td>
<td>14 (46.7%)</td>
<td>2.79 ± 1.79</td>
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Conclusion: Calcium fluxes and excitation-contraction coupling suggested in CAR KO hearts a remodelling of gap junctions that increases intercellular communication between embryonic cardiomyocytes, is able to influence spontaneous Ca2+ cycling and is therefore an important regulator for embryonic heart beating.
determined by Western blotting. Oxygen consumption by mitochondria was measured using a Clark-type oxygen electrode. The activity of lactate dehydrogenase and succinate dehydrogenase were determined by histochemical method.

**Results:** Experimental animal models showed that development of heart failure (HF) alone led to post-rent contraction depression. The post-rent twitches were not depressed in case of the combination of HF and diabetic myocardium (DM) in vivo. The heart rates in RyK2 levels was smaller than the levels in SERCA2a levels when pathology developed. The development of monopatheologies resulted in a significant decrease in the intensity of energy production. The degree of the energy production activity in diabetic rats was more significant decrease in comparison with postinfarction rats, despite the fact that contractile disturbance of postinfarction rats was more severe. However, the development of the combined pathology resulted in the preservation of the energy production near the control level.

**Conclusion:** Induction of hyperglycemia at early stages of HF resulted in smaller changes in the expression of RyK2 – ATPase and ryanodine receptors and lesser disturbance of the energy metabolism related to glycolysis, Krebs cycle, and oxidative phosphorylation in comparison with monopathies.

### Hibernation, stunning and preconditioning

#### 618 Volatile anesthetic preconditioning attenuates ischemic-reperfusion injury in type II diabetic patients undergoing on-pump heart surgery

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**Background:** Conflicting evidence is existing that whether diabetic myocardium is afforded by ischemic preconditioning (IP) or not. The present study evaluated the cardioprotective effect of sevoflurane and isoflurane on the myocardium of diabetic rats.

**Methods:** A total of 60 patients were recruited. 30 Diabetic Mellitus (DM) patients were randomly selected into two groups (15 patients per each group): DM patients with no AP and DM patients with AP. Another 30 non-DM patients were randomly grouped into non-DM with AP and non-DM with AP. Patients of the AP group received 1% MAC sevoflurane for 15 min washout before three times for three times prior to establishing the CPB. The variation of serum CK-MB and total CK-MB between groups was compared.

**Results:** There were no significant differences regarding preoperative demographic data. Peak of TnI defined at 5 hour postoperatively in the DM group without preconditioning: 2.1 ± 0.93 ng/ml vs. 1.65 ± 0.65 ng/ml in the DM + Sevo group (p < 0.05). In the non-DM group, the group without AP were also observed higher TnI level as 1.62 ± 0.82 ng/ml vs. 1.2 ± 2.45 ng/ml. Total amount of CK-MB released was higher in the group without AP than groups with AP (39.2 ± 3.36 ± 3.42 ng/ml in groups 28.3 ± 0.34 ± 2.46 ± 3.6 non-DM groups respectively; p < 0.01). In the Westen Blot, only phospho-Ser phosphorylation of PKC and total STAT3 showed some difference. Lengths of postoperative intubation, postoperative stay in the ICU were significantly longer in non-AP groups, as well as length of hospital stay.

**Conclusion:** This study showed that sevoflurane-induced preconditioning is provides better cardiot protection and cardiac function postoperatively. The diabetic patients still benefit from preconditi oning of volatile anesthetics. However, larger randomized trials are needed to carry out to clarify the protocol used for AP and follow-up checkups for long-term outcome.

### The effect of early and delayed phase of remote ischemic preconditioning on ischemia-reperfusion injury in type II diabetic rats

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**Introduction:** Phenomenon of remote ischemic preconditioning (RIP) is an alternative strategy of protection which is induced by short episodes of ischemia and reperfusion applied to tissue or organ distant from the heart that results in effective cardiac protection against ischemia-reperfusion (IR) injury. It is well known from experimental studies that hearts of animals with acute diabetes mellitus (DM), except increased sensitivity to ischemia, may also exhibit increased resistance to IR treatment. The role of changes in mitochondrial calcium handling in the pathogenesis of heart failure associated with diabetes remains poorly understood.

**Objectives:** To explore the impact of early (1-RIP) and delayed phase (2-RIP) of remote ischemic preconditioning on IR injury in isolated heart of healthy and pathologically altered diabetic rats.

**Materials and Methods:** We used male Wistar rats; 8-days acute DM was induced by a single dose of STZ (65 mg/kg, i.p.). Diabetic and healthy rats were subjected to RIP induced by 3 cycles of 5-min ischemia / 5-min reperfusion of cuffed occlusion of the right hind limb. Delayed phase was investigated 24-h after the last ischemic impulse. Isolated hearts were perfused following Langendorff immediately after RIP or its delayed phase. After 15 min stabilization, the hearts were subjected to 30 min global ischemia followed by 3-h reperfusion for evaluation of the infarct size (IS, expressed in % of area at risk, AR), contractile function (recovery of LVPD in % of baseline values) and indexes of contraction and relaxation (+dP/dtmax, -dP/dtmax).

**Results:** In diabetic hearts, IS was decreased by 17.8% and LVPD recovery was improved by 56.8% as compared with non-diabetic hearts. In non-diabetic healthy hearts, both 1-RIP and 2-RIP also attenuated postischemic stunning and lethal injury. Early phase significantly reduced ISAR to 11.7 ± 3.2% and delayed phase to 17.8 ± 18% vs. ISAR 33 ± 3% in controls (p < 0.05). In addition, LVPD recovery was increased to 70 ± 11.3% and to 83.4 ± 4%, in 1-RIP and 2-RIP, respectively. On the other hand, when RIP applied in diabetic rats, both 1-RIP and 2-RIP did not convey any additional protective effect.

**Conclusion:** The results indicate that RIP provides an effective protection against IR in healthy myocardium, but does not have any positive impact in the diabetic myocardium. The latter indicates that...
protective effect of HF as may share similar pathways with the mechanisms of increased resistance to ischemia in the acute phase of DM.

620 Post-conditioning with 1668-thioate leads to attenuation of the inflammatory response and remodeling with less fibrosis and better left ventricular function in a murine model of myocardial infarction

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Background: Development of ischemic cardiomyopathy has been associated with inflammation and toll-like receptor (TLR)-signalling. It has been shown that post-conditioning (PCoN) is able to attenuate inflammation and fibrosis in myocardial infarction.

Purpose: The purpose of this study is to investigate whether PCON with the synthetic Cpg-containing TLUR ligand 1668-thioate (CpgT) can modulate the development of inflammation and remodeling in reperfused murine myocardium.

Methods: Thirty min. of LAD-ligation followed by reperfusion was conducted in 12 weeks old male C57Bl/6j mice. Mice where treated with CpgT i.p. 5 min. before reperfusion. Control group received PBS; sham group did not undergo ischemia. After 3, 7 and 28 days M-mode echocardiography and Millar”ll left ventricular (LV) pressure-volume catheter measurements were performed. Hearts where excised and harvested for immunohistochemical analysis. Gene expression (Taqman®-RT-qPCR) was measured after 6 and 24 hrs reperfusion.

Results: Apoptosis markers Caspase 3 and 8, and matrix metalloproteinase (MMP)9 were not induced in CpgT CpgT group compared to high induction in PBS PCon, indicating lesser degree of apoptosis- and extracellular matrix degeneration. However, proinflammatory chemokines CCL2, CCL3 and CCL4, and cytokines TNF-alpha and IL-1beta were significantly up-regulated in CpgT PCon group compared to PBS PCon after 6 hrs. Interestingly, this peak of inflammatory activation was accompanied by significant induction of anti-inflammatory IL-10. Further, after 3 and 7 days significantly lower macrophage density (stained with MAC-2) was observed in the ischemic myocardium of CpgT PCon mice compared to PBS PCon, suggesting that anti-inflammatory and other mechanisms mitigate proinflammatory activity. Total LV collagen area using picrosirius red planimetry was significantly attenuated in CpgT PCon mice after 7 and 28 days compared to PBS PCon mice. CpgT PCon showed significantly lower level of ventricular dysfunction than PBS PCon. TRB1 mRNA was induced in CpgT PCon hearts, while TLUR4 and 9 were not induced. The specific role of the early inflammatory peak, followed by less macrophage infiltration, has to be further elucidated.

Conclusion: Our study suggests a cardioprotective mechanism of Cpg GCP in modulation of remodeling and subsequent development of LV dysfunction in a murine model of reperfused myocardial infarction. This mechanism seems to involve TLR-modulation being associated with early chemokine and cytokine action.

621 Maturation-related changes in response to ischemia-reperfusion injury and in effects of classical ischemic preconditioning and remote preconditioning

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Background: Effect of aging on tolerance to ischemia/reperfusion (IR) injury and adaptation mechanisms has been shown in older human and animal hearts. However, the onset of unfavorable changes has not been explored in details. Results concerning the effectiveness of classical ischemic preconditioning (IPC) also remain controversial since some studies demonstrated preservation of IPC-induced cardioprotection even in the elderly. The purpose of this study was to investigate whether PCon with the synthetic Cpg-containing TLUR ligand 1668-thioate (CpgT) can modulate the development of inflammation and remodeling in reperfused murine myocardium.

Purpose and Methods: We aimed to study the changes in myocardial function, response to ischaemia and changes in adaptation mechanisms related to both, IPC and RIPC, in the hearts of juveniles (15 months), younger adult (3 months) and mature adult (6 months) male Wistar rats, in Langendorff-perfused hearts exposed to 30-min. (120-min. R) or (p) with or prior IP. IPC was induced by one cycle of 5-min/15-min/R, in perfused hearts. RIPC (3 cycles of 5 min/5 min/R) was applied on the hind limb of anesthetized rats (pressure cuff inflation (200 mmHg)/deflation). We measured infarct size (IS, TTC staining), susceptibility to ventricular arrhythmias and recovery of contractile function (left ventricular developed pressure, LVDp).

Results: Maturation did not affect heart function, however, it impaired cardiac injury to lethal IR injury (IS increased by 40% and 65%, respectively, vs. juvenile group) and promoted arrhythmogenesis. IS. IPC reduced occurrence of arrhythmias, IS and improved LVDp recovery in younger animals, while efficacy was attenuated in the mature ones. RIPC also reduced occurrence of arrhythmias, IS and improved LVDp recovery in younger animals. However, different from IPC, cardioprotective effect of RIPC was preserved even in the mature adults.

Conclusion: Early maturation already starts to impair the resistance of rat hearts against IR injury and causes gradual loss in IPC efficiency. On the other hand, RIPC appears more effective and easily performed clinically relevant cardioprotective intervention.

Mitochondria and energetics

624 Phase changes in myocardial mitochondrial respiration caused by hypoxic preconditioning or periodic hypoxic training

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Background: Mitochondrial dysfunction and reactive oxygen species (ROS) generation are critical events in the pathophysiology of type II of diabetes mellitus (DM), the most severe metabolic disease. We have recently reported that monoamine oxidases (MAOs), mitochondrial enzymes with 2 isoforms, A and B, contribute to the oxidative stress in experimental diabetes. Methylene blue (MB) is a redox-drug with widely reported protective effects at mitochondrial levels that also inhibits MAO activity. The present study was purposed to characterize the effects of MB (0.1 μM) on mitochondrial respiration, calcium sensitivity, and MAO-related ROS production in rat heart mitochondria (RHM) isolated from diabetic rats.

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Methods: Mitochondrial respiratory function was assessed by high-resolution respirometry whereas ROS production and calcium retention capacity of isolated RHM were measured spectrophotometrically.

Results: In RHM respiring on both complex I and II substrates (glutamate/malate and succinate + rotenone, respectively) a significant increase in all bioenergetic parameters was found in treated vs. non-treated mitochondria. No changes in sensitivity to Ca++-induced opening of the mitochondrial permeability transition pore were found in the presence of MB. Interestingly, MB elicited a significant increase H2O2 release (Amplex Red assay) in the presence of CI, but had no effect in mitochondria energized with succinate (+ rotenone). Incubation of RHM with MB in the presence of MAC-A and B inhibitors (clocryline or selegiline, 10 μM) significantly reduced H2O2 release in mitochondria respiring on glutamate & malate and had no effect in the presence of the CI substrate.

Conclusions: In diabetic rat hearts, metmyoglobin improved mitochondrial respiratory function regardless the substrates used and elicited a dichotomous, substrate-dependent effect on ROS production. MAO inhibitors mitigated the MB-dependent increase in ROS production for complex I (but not complex II)-supported respiration.

627 Doxorubicin modulates the real-time oxygen consumption rate of freshly isolated adult rat and human ventricular cardiomyocytes

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Introduction: As cancer survival rates improve, cardiotoxicity as a result of the agents used to treat neoplastic disorders has become increasingly clinically relevant. Despite our current knowledge, a full understanding of the early steps and functional changes that lead to cardiac dysfunction remains to be determined. Here, we investigated the acute effect of doxorubicin (DOX) on the real-time oxygen consumption rate (OCR) of ventricular cardiomyocytes.

Methods and Results: Adult rat ventricular cardiomyocytes (ARVC) were isolated using a Langendorf perfusion system and enzymatic digestion. A Seahorse Bioscience XIp instrument was used to measure the OCR of ventricular cardiomyocytes in real-time. To assess the use of ARVC and adult human ventricular cardiomyocytes in a Seahorse assay the Mito Stress Test (Seahorse Bioscience) was performed with compounds that have a known effect on the cellular OCR. The standard Mito Stress Test profile was observed in both cell types in response to the compounds oligomycin, FCCP and a mix of rotenone and antimycin A, indicating that the Seahorse assay is valid to assess the real-time OCR in these cells. To test the acute effect of DOX on the OCR of ventricular cardiomyocytes, following four baseline measurements of the OCR over 20 min, DOX was serially injected into the microchamber at increasing concentrations of 1, 3, 10 and 30 μM. The OCR values are corrected for total protein concentration and normalised to baseline measurements. Acute injection of DOX resulted in a significant concentration-dependent increase in the real-time OCR of ARVC (1 μM DOX 0.974 ± 0.009 vs. control 0.952 ± 0.015; 3 μM DOX 1.18 ± 0.033 vs. control 0.954 ± 0.038; 10 μM DOX 1.64 ± 0.136 vs. control 0.964 ± 0.057; P<0.001; 30 μM DOX 2.44 ± 0.348 vs. control 1.01 ± 0.079; P<0.001, n=6). Serial DOX injections resulted in a similar concentration-dependent increase in the OCR of freshly isolated adult human ventricular cardiomyocytes (n=3).

Conclusions: Serial injections of DOX resulted in an acute concentration-dependent increase in the real-time OCR of freshly isolated adult rat and human ventricular cardiomyocytes. This acute response indicates that DOX has an immediate effect on metabolic function in isolated ventricular cardiomyocytes.

Cardiomyopathies and fibrosis

630 Effects of genetic or pharmacologic inhibition of the ubiquitin/proteasome system on myocardial proteotoxicity and cardiac function

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Purpose: The Ubiquitin Proteasome System (UPS) and the autophagy/lysosome system (ALS) mediate the disposal of intracellular misfolded/unfolded proteins, and are essential for cardiomyocyte (CM) health. Atrogen1 and MuRF1 are muscle specific ubiquitin-ligases, and we recently demonstrated that loss of Atrogen1 impairs the turnover of the ESCRT11 protein CHMP2B, whose accumulation leads to block of autophagy, resulting, during ageing, in hypertrophic cardiomyopathy (1). To address the individual roles and interplay of these ubiquitin ligases in the control of CM proteostasis, we compared the molecular phenotype of MuRF1 KO and Atrogen1/MuRF1 double KO (DKO) mice, and that of mice treated with the UPS inhibitor Bortezomib.

Methods: Heart function and structure were assessed by ECHO, histology, IF and TUNEL assay. Real-time OCR of freshly isolated adult rat and human ventricular cardiomyocytes (n=3).

Conclusions: Serial injections of DOX resulted in an acute concentration-dependent increase in the real-time OCR of freshly isolated adult rat and human ventricular cardiomyocytes. This acute response indicates that DOX has an immediate effect on metabolic function in isolated ventricular cardiomyocytes.

631 Suppression of Wnt signalling in a desmolgin-2 transgenic mouse model for arrhythmogenic cardiomyopathy

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Background: In the desmolgin-2 (DSG2) gene have been identified in patients affected with arrhythmogenic cardiomyopathy (ACM), a progressive heart muscle disease and a leading cause of sudden cardiac death in the young and athletes. The mechanisms underlying DSG2 dysfunction involved in the pathogenesis of ACM remain poorly understood.

Purpose: The aim of the study was to investigate the molecular pathogenesis of ACM due to DSG2 mutations in transgenic animal models.

Methods: Human full-length wild-type and mutant (c.1672C>T; p.Q558*) cDNA sequences for human DSG2 were cloned into a vector containing alpha-MyHC promoter, and tagged at the C-terminus with the Flag epitope. The transgene was delivered into the ES of C57BL/6J mice carrying a C-terminal deletion of the wild-type DSG2 (Tg-hWT) and mutant DSG2 lacking transmembrane and intracellular protein domains (Tg-hQ) were developed. Phenotypic characterization was performed by electrophysiological, histological and electron microscopic analyses. The disease molecular pathology was investigated in transgenic and wild-type heart samples by samples by immunohistochemical and western blot immunostainings.

Results: A proper localization of Flag-tagged human DSG2 to the intercalated discs was demonstrated for Tg-hWT mouse cardiomyocytes, but not for both Tg-hQ10 and Tg-hQ13 cardiomyocytes which showed a cytoplasmic localization of the truncated form of the protein. Whereas Tg-hWT mouse hearts did not detectable histological, morphological, or functional cardiac changes, the Tg-hQ mouse hearts displayed cardiomyopathy and fibrotic ventricular tissue replacement, similar to those of ACM patients. Ultrastructural analysis revealed reduced desmosome number, density, and length at the cardiac intercalated discs, together with swollen cisternae of endoplasmic reticulum, consistent with partial retention of the mutant DSG2, and mitochondrial damage in Tg-hQ mice when compared to Tg-hWT. Despite the unchanged levels of total beta-catenin, Tg-hQ mice at 3-, 6- and 12-months of age displayed reduced active beta-catenin, together with decrease expression of c-Myc, c-fos and Crclin-D1 down-stream targets, and reduction of glycogen synthase kinase 3-beta levels.

Conclusions: These findings suggest that inhibition of Wnt/beta-catenin signalling is involved in the pathogenic mechanism of DSG2-related ACM since early stages of the disease.

632 Cold-induced cardiac hypertrophy is reversed after thermo-neutral deacclimatization

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Introduction: Of the four seasons, winter has the highest mortality and morbidity from cardiovascular complications. Chronic exposure to cold is known to cause hypertension and cardiac hypertrophy, although cold-induced cardiac hypertrophy is independent of elevations in blood pressure. Therefore we aim to study how cold temperatures affect cardiac hypertrophy and whether this phenomena is reversible after return to a thermo-neutral temperature.

Methods: Studies in vivo were performed in two-month old wild-type (WT) mice. Animals were subjected to chronic cold exposure (4°C) for three weeks (CC). After this period animals were put into thermo-neutral conditions (30°C) for one week (CD). Cardiac samples from both groups were obtained and analyzed.

Results: At the morphologic level, we found that chronic cold exposure induced a significant increase in the heart weight/tibia length (HW/TL) ratio. Moreover, the area of the cardiomyocytes was increased in WT compared to animals exposed to chronic cold. Furthermore, the mice exposed to chronic cold displayed an increase in HW/TL ratio. A proper localization of Flag-tagged human DSG2 to the intercalated discs was demonstrated for Tg-hWT mouse cardiomyocytes, but not for both Tg-hQ10 and Tg-hQ13 cardiomyocytes which showed a cytoplasmic localization of the truncated form of the protein. Whereas Tg-hWT mouse hearts did not detectable histological, morphological, or functional cardiac changes, the Tg-hQ mouse hearts displayed cardiomyopathy and fibrotic ventricular tissue replacement, similar to those of ACM patients. Ultrastructural analysis revealed reduced desmosome number, density, and length at the cardiac intercalated discs, together with swollen cisternae of endoplasmic reticulum, consistent with partial retention of the mutant DSG2, and mitochondrial damage in Tg-hQ mice when compared to Tg-hWT. Despite the unchanged levels of total beta-catenin, Tg-hQ mice at 3-, 6- and 12-months of age displayed reduced active beta-catenin, together with decrease expression of c-Myc, c-fos and Crclin-D1 down-stream targets, and reduction of glycogen synthase kinase 3-beta levels.

Conclusions: These findings suggest that inhibition of Wnt/beta-catenin signalling is involved in the pathogenic mechanism of DSG2-related ACM since early stages of the disease.
CD45 is a sensitive marker to diagnose lymphocytic myocarditis in endomyocardial biopsies of living patients and in autopsies

Methods: In hearts of mice with acute viral myocarditis (n=9), controls (n=7) and in the EMB area of the LVPW obtained from autopsy hearts of patients diagnosed with LM (n=18) and controls (n=6) were stained with anti-CD68, -CD3 and -CD45. Subsequently, cells were quantified per mm2. Anti-CD45 cells were also quantified in the remaining LVPW.

Results: In mice with myocarditis the number of CD45+CD68+ cells/mm2 was significantly increased compared to the number of CD3+CD68+ cells/mm2. When applying a threshold of ≥14 leukocytes/mm2, 44% of the mice would be diagnosed for LM with the use of CD3+CD68+. However, 100% of the mice would be diagnosed for LM with the use of CD45+CD68+. In the EMB area of autopsied hearts, using the cut-off value of ≥14 leukocytes/mm2, CD3+CD68 could only confirm 39% of the diagnosis of LM, while the CD45+CD68+ could confirm 56% of the LM cases. Interestingly, a significant increase of CD45+ positive leukocytes/mm2 was observed in the EMB area when compared to the remaining LVPW in LM patients.

Conclusions: The use of the common leukocyte marker CD45 increases the sensitivity of the diagnosis of LM. Furthermore, the inflammatory infiltrate in the EMB area is significantly increased compared to the remaining LVPW, indicating that the sampling area constitutes the highest chance for the histological diagnosis of LM.

Atrial epicardial adipose tissue derives from epicardial progenitors

Background & Aims: The accumulation of the adipose tissue (AT) in the sup- and sub-epicardium of the atrial myocardium is associated with a high risk of atrial fibrillation. Here we addressed the question of the cellular origin of atrial AT.

Methods: Human right atrial specimens obtained during cardiac surgery were used for histological, biochemical studies (n=60) and to harvest epicardial progenitors (n=20). Epicardial progenitor-derived cells (EPDCs) were maintained in culture conditions and characterized using flow cytometry, proteomic and gene expression assays. To study the ability to EDPC to differentiate into adipocyte, progenitors were treated with adipogenic medium from 3 weeks. In order to determine the cellular origin of atrial adipose tissue, we studied a lineage tracing Wt1-Cre-Rosa-tdT+ marker Wilm’s tumor-1 (Wt1) and pre-adipocyte marker pre-adipocyte factor 1 (Pref-1) suggesting that EPDCs could engage in the adipogenic fate. This hypothesis was tested in vitro, using human and mouse EPDCs harvested from atrial samples; atrial EPDC underwent an epithelio-to-mesenchymal transition (EMT) and acquired mesenchymal phenotypes and could differentiate into osteocyte or chondrocyte. When cultured using an adipogenic medium, around 40% of EPDCs cells showed lipid droplet stained with oil red and expressed mature adipogenic markers perlipin, PPARG and CEGBP. These results were supported by the formation of lipid droplet-tomato+ observed in mEPDCs induced by adipogenic medium. To follow the fate of Wt1 we developed a lineage tracing Wt1-Cre-Rosa-tdT+/+ mice model. We found that a number of adipoctyes that compose the atrial EAT could derive from aEPDC through an EMT process.

635 Caloric restriction ameliorates cardiac function, sympathetic cardiac innervation and beta-adrenergic receptor signaling in an experimental model of post-ischemic heart failure

Background: Although obesity is considered a major risk factor for cardiovascular diseases, it is associated with lower mortality and a better outcome in patients with chronic heart disease (the obesity paradox).

Methods: We induced MI in 12-week old balb/c female mice. Twenty-four hours later, a first echocardiography was performed to confirm significant left ventricular (LV) dysfunction. One month post-MI, a second echo was done and mice were randomized into 2 groups: HFD, (n=20) and regular chow diet (RCD). (n=20). Serial metabolic and echo studies were performed once a month following randomization. During 6 months of follow-up, HFD-fed mice gained significantly more weight (20±1 vs. 23±0.4; p=0.001, [mean±SEM]), and had higher plasma levels of cholesterol, LDL, HDL, and glucose, compared with RCD. Survival was similar between the groups. Significantly, compared with RCD, HFD attenuated LV dysfunction (Figure, p=0.04), and reduced LV dastolic dilatation (12.2 ± 2.2% vs. 2.6 ± 2.4%, p=0.003), at 6 months after MI.

Conclusions: High-fat diet attenuates adverse cardiac remodelling and dysfunction after extensive myocardial infarction in mice

636 High fat diet improves cardiac remodelling and function after extensive myocardial infarction in mice

Methods: Restricted diets are effective interventions to enhance cardiovascular function and metabolic profile and are known to improve life span. IF (Intermittent fasting) dietary regimen has a cardioprotective effect in a rat model of myocardial infarction (MI) when diet is started before MI induction. Chronic heart failure (HF) is associated with reduced cardiac sympathetic innervation and with upregulation of G protein-coupled receptor kinase 2 (GRK2), which contributes to dysfunction beta-adrenergic receptor (beta-AR) signaling and to decrease cardiac inotropic reserve.

Hearthe Weight/Tibia Length Ratio

High fat diet attenuated LV dysfunction.
Epigenetic therapy reduces cardiac hypertrophy in murine models of heart failure

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Background: Heart failure with preserved ejection fraction (HfPEF) is one of the leading causes of global morbidity and mortality. HfPEF is driven by pathological remodelling in the heart where there is hypertrophy of cardiomyocytes (cardiac hypertrophy) and an increased accumulation of extracellular matrix proteins in the interstitium (fibrosis). Recent evidence suggests that epigenetic processes such as DNA methylation are involved in the pathogenesis of cardiac remodelling. Inhibition of DNA methylation may yield a novel therapeutic avenue for the treatment of HfPEF.

This study investigated the therapeutic potential of the DNA methyltransferase inhibitor, 5-azacytidine (Saza) to inhibit pathological hypertrophy in the heart using preclinical models of HfPEF, the transgenic contraction (TAC) model and the Angiotension-II (AngII) infusion model.

Methods: Wild type C57Bl6 mice underwent surgical construction of the aortic arch or implantation of a subcutaneous osmotic pump infusing 1000 ng/kg/min angiotensin II (AngII) to induce pressure overload. Sham surgery was used as the TAC surgical control group and a saline infusion was used as the AngII surgical control group. TAC mice were treated with a single subcutaneous injection of either placebo or Saza. AngII mice began Saza treatment every four days after pump implantation. Cardiac structure and function was examined in vivo using non-invasive echocardiography.

Results: Echocardiographic analysis revealed that TAC and AngII mice treated with Saza displayed a significant reduction in the interventricular septal wall and left ventricular posterior wall thickness compared to mice which received placebo treatment. Reduction in left ventricular mass was also evident in both models, even when Saza treatment was initiated in the TAC model after cardiac hypertrophy was established.

Conclusion: Therapeutic options for HfPEF patients are limited. Inhibition of DNA methylation using Saza shows therapeutic potential by reducing cardiac hypertrophy in preclinical models of heart failure and seems to have a beneficial effect even in the setting of established cardiac hypertrophy.

Arterial and pulmonary hypertension

640 Osteopontin as a marker of pulmonary hypertension in patients with coronary heart disease combined with chronic obstructive pulmonary disease

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Background/Introduction: Comorbidity of coronary heart disease (CHD) and chronic obstructive pulmonary disease (COPD) worsens both diseases, leading to the development of complications and, consequently, to a large social and economic burden. Progression of pulmonary hypertension in these patients is one of factors causing mortality. Relationship between Osteopontin (OPN) levels and pulmonary hypertension development in comorbid patients is still debated.

Purpose: The aim of this study was to evaluate the relationship between osteopontin plasma concentrations and pulmonary hypertension levels in patients with coronary heart disease combined with chronic obstructive pulmonary disease.

Methods: 81 patients with known CHD combined with moderate to severe COPD and in regular follow up. 44 patients meeting international guidelines were enrolled in a 6-month-period study. All patients were evaluated by plasma osteopontin level (ELISA kit, Eren Life Science), spiroergometry, echocardiography. The study was approved by our Institutional Ethic Committee. Patients signed an informed consent to take part to the study.

Results: All patients were matched by sex, age (mean age 63.3 ± 8.3) and the severity of the disease. All patients were divided into two groups depending on the development or absence at them pulmonary hypertension. There was no considerable difference between parameters spiroergometry in both groups. Osteopontin level were directly related to mean pulmonary artery pressure (mPAP) (r = 0.25, P < 0.001) and inversely to 6-minutes walk test distance (r = -0.32, P < 0.001). Median OPN value was 43 ng/mL. OPN levels >43 ng/mL remained statistically significant predictor of PH in patients with CHD and COPD (HR = 2.73, 95% CI: 1.06-6.83, P = 0.008).

Diastolic dysfunction is the first stage of the developing heart failure

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Introduction: Cardiac adaptation to ischemic or toxic lesions may result in diastolic or systolic dysfunction, or both. Conditions of their development are not quietly understood.

Methods: The aim of the study is to distinguish conditions determining the form of cardiac dysfunction in rats at ischemic or toxic models of the heart failure.

Methods: The study followed the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (No. 133 of 18 March 1986). The ischemic or toxic models of the heart failure in Wistar rats were created by injections of isoproterenol (ISO) or doxorubicin (Dox), respectively. ISO was injected twice (120 or 180 mg/kg) and doxorubicin (2 mg/kg) was injected intraperitoneally. Rats narcotized by ketamine (100 mg/kg) were subjected to catherization of the aorta and left ventricle (LV) by Millar micromanometers and to echocardiography. Isolated cardiomyocytes were loaded by Fluo-4 and Ca++ transients were detected with Zeiss microscope.

Results: At four weeks after lower ISO dose, the hearts exhibited 2-fold increased LV enddiastolic pressure and twice reduced relaxation constant (RC, 64.9 ± 9.1 vs. 38.1 ± 10.4), while LV systolic pressure, +dP/dt and coronary index (CI, +dP/dt/CI) were only slightly lower. The echocardiographic study of these hearts revealed only slightly increased LV diastolic and systolic volumes and slightly decreased ejection fraction (EF). Rats received higher ISO dose had 2.5 times higher LV diastolic volume and decreased EF by 32%, augmented LV enddiastolic pressure, and decreased CI and RC by 20 and 35%, respectively. Calcium transients showed greatly slowed signal decrement with plateau formation. The comparison of the data at 4 and 10 weeks after start of Dox injections revealed a clear worsening of all indices, namely, EF decreased by 7 and 22%, CI by 13 and 41%, RC by 22 and 55%, respectively (p < 0.05 for all). Calcium transients had lower peak, slowed signal decrement and elevated diastolic Ca++ level.

Conclusion: Diastolic dysfunction with slowed relaxation and increased myocardial stiffness occurs at lower degree of cardiac lesions induced by ISO or Dox while systolic dysfunction with decreased EF and profound fall in CI and RC develops later. Thus, diastolic dysfunction may be considered as the first step of cardiac adaptation to developing cardiomyopathy helping to maintain myocardial contractility and pump function of the heart.
Myocardial dynamic stiffness is increased in experimental pulmonary hypertension partly due to incomplete relaxation

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Inhaled increased myocardial stiffness may cause diastolic dysfunction and explain right atrial dilatation in experimental pulmonary hypertension (PH). We aim to demonstrate that dynamic stiffness of right ventricular papillary muscle is increased in experimental PH, and that increased stiffness is related to basal oxygen consumption.

PH was induced in 15 male Wistar rats (60 mg monocrotaline/kg, i.p.), the 5 controls are untreated. A right ventricular papillary muscle is mounted in an oxygen chamber at 37°C (Wong and colleagues, Am J Physiol H1190-7, 2010). Static passive stiffness is determined from the force—length relation. A sinusoidal length change is imposed to determine dynamic passive stiffness (amplitude ±0.075 Lo at 5 cycles/s, where Lo is the muscle length giving maximum active force). Dynamic stiffness is determined from 0.95 Lo to 2.15 Lo from a sectioned muscle without stimulation. Stiffness is normalized by muscle cross-sectional area (0.925 Lo to allow comparison between muscles. The contribution of cross—bridge cycling to stiffness is estimated using 10μM blebbistatin. Basal oxygen consumption is measured during length changes without stimulation. Static stiffness is 67 ± 17 kPa (mean ± SD) in control and 102 ± 32 kPa in PH (P=0.006). Dynamic stiffness is smaller than static stiffness: 40 ± 19 kPa and 74 ± 37 kPa, respectively (P=0.001). Blebbistatin has no effect on dynamic stiffness in control (38 ± 22 kPa) but reduces dynamic stiffness in PH (to 57 ± 18 kPa, paired t-test P=0.002). Similarly, basal oxygen consumption (before blebbistatin 0.21 ± 0.13 μmol/min and after 0.22 ± 0.13 μmol/min in control) was reduced by blebbistatin only in PH from 0.22 ± 0.07 μmol/min to 0.17 ± 0.06 μmol/min (paired t-test P=0.001).

We conclude that dynamic stiffness in experimental PH is increased and is partly due to cross—bridge cycling caused by incomplete relaxation, leading to increased basal oxygen consumption.

Hypotensive effect of quercetin is possibly mediated by down—regulation of immunotrope and prostatine subunits in aorta of spontaneously hypertensive rats

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Background: Quercetin is flavonoid-based drug that shows antihypertensive and anti-inflammatory effects. Earlier we have shown its ability to inhibit proteasomal activity, however the molecular mechanisms are poorly studied.

It is unknown whether expression of genes encoding proteasomal subunits and proteasomal activities are changed in the aorta of spontaneously hypertensive rats (SHR). The goal of the present investigation is to elucidate if changes in protesosomal subunits gene expression and proteasomal activities are involved in antihypertensive effects of quercetin in SHR.

Methods: Utilizing real-time PCR analysis we have evaluated mRNA levels of proteasome and immunotrope subunits in aorta tissue of Wistar rats, SHR and quercetin-treated SHR. Quercetin (Bcpp, Ukraine) was added to standard diet for 8 weeks in dose of 15 mg/kg. Proteolytic activities (BCPP, Ukraine) was added to standard diet for 8 weeks in dose of 15 mg/kg. Proteolytic activities of the aorta were measured using specific fluorogenic substrates. We also monitored hemodynamic parameters (BCPP, Ukraine) was added to standard diet for 8 weeks in dose of 15 mg/kg. Proteolytic activities of the aorta were measured using specific fluorogenic substrates. We also monitored hemodynamic parameters of the rats (end-systolic pressure, end-diastolic pressure, stroke volume, ejection fraction, cardiac output) with “Millar Instruments” equipment. The structural changes in rat’s aorta were determined by the morphometric analysis and electron microscopy in all experimental groups.

Results: The mRNA level of genes encoding PSM was significantly decreased in SHR rats as compared to Wistar rats. However, expression of genes encoding PSMB8, PSMB9 and PSMB1 were significantly up-regulated. The inhibitory effect of quercetin on proteasomal proteolysis in aorta and its antihypertensive activity is related to subclinical atherosclerosis. The mRNA level of genes encoding PSM was significantly decreased in SHR rats as compared to Wistar rats. However, expression of genes encoding PSMB8, PSMB9 and PSMB1 were significantly up-regulated. The inhibitory effect of quercetin on proteasomal proteolysis in aorta and its antihypertensive activity is related to subclinical atherosclerosis.

Conclusion: This aqueous extract of Buchu may serve as an alternative, cost effective natural therapy for the improvement of hypertension, also causing weight loss and an improved RAS.

Adiponectin level in hypertensive females with rheumatoid arthritis and its relationship with subclinical atherosclerosis

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Cardiovascular risk level in hypertensive (HT) pts with rheumatoid arthritis (RA) do not fully reflect by SCORE and mSCORE. Thus an additional risk factors research is required. Adiponectin may be a biomarker of early atherosclerosis. It little known about the association adiponectin level and subclinical atherosclerosis in HT pts with RA.

We aimed to estimate adiponectin level in HT females with RA and its relationship with subclinical atherosclerosis. The study included 42 HT females with comorbid RA (mean age of 54 [50, 61.5] years) and 20 HT females (control group). The cardiovascular risk was calculated using mSCORE. RA disease activity was measured using DAS28 scale. Carotid ultrasound with stiffness indices detection (ESC 2006) and endothelial-dependent flow mediated vasodilatation (EDV) by D. Celemayer method were performed. The level of adiponectin was measured using ELISA kit test. Serum adiponectin level was significantly higher in the HT females with RA group (13 [12.5, 14.8] mg/ ml) compared to control (p<0.05). HT females with RA and subclinical atherosclerosis were
characterized by significantly higher adiponectin level (p < 0.05). Adiponectin level was correlated with the highest ratio r = 0.34 (p < 0.005), DAS28 r = 0.36 (p < 0.05), cardiovascular risk mSCORE r = -0.33 (p < 0.05), BMI r = 0.79 (p < 0.05) and EDDV r = -0.41 (p < 0.05). AUROC index for adiponectin predictive role in subclinical atherosclerosis develop was 0.78 (95% CI 0.64-0.93; p < 0.05).

Serum adiponectin level determining may be useful additional biomarker for early atherosclerosis develop in hypertensive females with rheumatoid arthritis.

649 Markers for identification of renal dysfunction in the patients with chronic heart failure

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Purpose: To determine the involvement of oxLDL, MMP-9, CRP markers in the pathogenesis of coronary atherosclerotic plaque rupture. Furthermore oxLDL (oxidized Low Density Lipoprotein) is involved in the coronary atherosclerotic plaque rupture (CIIS area=0.73, p=0.001, oxLDL area=0.63, p=0.05; picture 1).

Results: The study included 30 patients with blood levels of TG 1,7 mmol/l levels of apelin were significantly lower than in healthy volunteers (0,851 (0,841; 0,877) ng/ml versus 1,087 (0,861; 1,318) ng/ml) and significantly lower than in the subgroup of patients with blood levels of TG >1,7 mmol/l (0,851 (0,841; 0,877) ng/ml versus 1,087 (0,861; 1,412) ng/ml; p<0.05). Correlation analysis in the whole group of patients with hypertension showed significant negative correlation levels of apelin with blood levels of TG (r= -0.56, p=0.001), VLDL-C (r=-0.56, p=0.01), HDL-C (r=0.56, p=0.05; picture 1). The logistic analysis shows that apelin, oxLDL, CRP (CMR area=0.985, p=0.001; oxLDL CR=0.011, p<0.05; CRP CR=0.041, p<0.05) may play a role in the pathogenesis of the plaque rupture. Serum MMP-9 enzyme level was directly correlated with Geniuns score (r=0.552, p<0.01), CIIS (r=0.340, p<0.01) and CRP (r=0.321, p<0.01) stems.

Conclusions: Serum MMP-9 enzyme increases with accordance of severity of the myocardial infarction with the statistical significance (p<0.01): the borderline abnormality group (CMR<10, 0.227 ± 0.09 ng/ml), possible infarction (CMR 10-15, 0.317 ± 0.132 ng/ml), probable infarction (CMR >15, 0.376 ± 0.132 ng/ml) groups. MMP-9 levels were significantly higher in the probable injury group patients (CMR >15) compared to the possible injury group patients (CMR <10) (p<0.001).

ROC Curve analysis shows that MMP-9 enzyme levels variance (area=0.87, p<0.001) are more than other biomarkers making it a diagnostically beneficial for the coronary atherosclerotic plaque rupture (CMR area=0.73, p=0.001, oxLDL area=0.63, p=0.05; picture 1).

Conclusion: Serum MMP-9, oxLDL and CRP are significantly involved in the pathogenesis of coronary atherosclerotic plaque rupture in the myocardial infarction.

650 cardio-hepatic syndromes in chronic heart failure: North Africa profile

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Purpose: Patients with chronic heart failure (CHF) have a variety of liver abnormalities, known as cardio-hepatic syndromes. The aim of the study was to evaluate the prevalence and importance of liver function tests (LFT) abnormalities in a group of patients with chronic heart failure.

Methods: The study included 140 patients with chronic heart failure consecutively followed from 2010 until end of 2014 in care unit of CHF, departments of cardiology.

Results: The mean age of the patients was 50 ± 13 years. The distribution by sex 930 (66.4%) men and 470 (33.6%) women. Liver function tests abnormalities were observed in patients with chronic heart failure: low albumin in 42% of the patients, total bilirubin in 17%, increased alkaline phosphatase in 13%, increased aspartate aminotransferase in 24%, alanine aminotransferase elevation in 18% of patients. The proportion of patients with reduced ejection fraction (≤ 50%) who had elevations in total bilirubin was 23%. We note baseline abnormalities in bilirubin, alkaline phosphatase and albumin were more common in patients who died.

Conclusions: Mild abnormalities of LFT are relatively frequent in patients with chronic heart failure, with a greater elevation of bilirubin than aminotransferases. Patients with reduced ejection fraction had a higher prevalence of increased bilirubin. Total bilirubin was a predictor of adverse prognosis.

651 To study other biomarkers that assess during myocardial infarction

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Background: When the coronary atherosclerotic plaque becomes vulnerable, a thrombus develops on that ruptured plaque and then occludes the coronary artery, which causes an acute blood deficiency characterized by significantly higher adiponectin level (p < 0.05). Adiponectin level was correlated with the highest ratio r = 0.34 (p < 0.005), DAS28 r = 0.36 (p < 0.05), cardiovascular risk mSCORE r = -0.33 (p < 0.05), BMI r = 0.79 (p < 0.05) and EDDV r = -0.41 (p < 0.05). AUROC index for adiponectin predictive role in subclinical atherosclerosis develop was 0.78 (95% CI 0.64-0.93; p < 0.05).

Serum adiponectin level determining may be useful additional biomarker for early atherosclerosis develop in hypertensive females with rheumatoid arthritis.

Methods: The study was conducted using case-control design. The main inclusion criteria of the 40 cases were that the patients should have a ruptured coronary atherosclerotic plaque, confirmed by clinical symptom, ECG, serum troponin I, and coronary angiography. Also 40 patients with coronary stenosis or chronic occlusion without ruptured plaque were included in the control group. Serum MMP-9 enzyme and oxLDL titers were determined by ELISA according to the manufacturer's recommended protocol. Additionally CRP was measured by full-automated analyzer. We used CIIS (cardiac infarction injury score) by EGG and Genissi score system (Coronary Angiographic Scoring System) for assessing the severity of coronary heart disease.

Results: Serum MMP-9, oxLDL levels (p < 0.001) in the case group (MMP-9 0.396 ± 0.155 ng/ml, oxLDL 1.417 ± 0.099 mg/ml) were more than in the control group (MMP-9 0.223 ± 0.087 ng/ml, oxLDL 1.332 ± 0.163 mg/ml). The logistic analysis shows that MMP-9, oxLDL, CRP (CMR OR=0.985, p=0.001; oxLDL OR=0.011, p<0.05; CRP OR=0.041, p<0.05) may play a role in the pathogenesis of the plaque rupture. Serum MMP-9 enzyme level was directly correlated with Geniuns score (r=0.552, p<0.01), CIIS (r=0.340, p<0.01) and CRP (r=0.321, p<0.01) stems.

Furthermore, serum MMP-9 enzyme increases with accordance of severity of the myocardial infarction with the statistical significance (p<0.01): the borderline abnormality group (CMR<10, 0.227 ± 0.09 ng/ml), possible infarction (CMR 10-15, 0.317 ± 0.132 ng/ml), probable infarction (CMR >15, 0.376 ± 0.132 ng/ml) groups. MMP-9 levels were significantly higher in the probable injury group patients (CMR >15) compared to the possible injury group patients (CMR <10) (p<0.001).

ROC Curve analysis shows that MMP-9 enzyme levels variance (area=0.87, p<0.001) are more than other biomarkers making it a diagnostically beneficial for the coronary atherosclerotic plaque rupture (CMR area=0.73, p=0.001, oxLDL area=0.63, p=0.05; picture 1).

Conclusion: Serum MMP-9, oxLDL and CRP are significantly involved in the pathogenesis of coronary atherosclerotic plaque rupture in the myocardial infarction.
Background/Introduction: Albuminuria is a risk factor strongly associated with cardiovascular disease, the first cause of death in the general population. The search for potential biomarkers identifying patients with sustained and de novo development of albuminuria under renin-angiotensin system (RAS) suppression may represent an effective strategy for adequate intervention. The findings obtained could contribute to a better understanding of the mechanisms involved in the pathogenesis.

Purpose: The application of different proteomic strategies could elucidate specific molecular pathways involved in the pathogenesis and may provide predictors and chronic organ damage indicators.

Methods: In this work, 24 plasma samples of patients with different degrees of renal impairment (normoalbuminuria, de novo albuminuria and sustained albuminuria) were analyzed using a “multi-omic” approach: two-dimensional difference in gel electrophoresis (2D-DIGE) and isobaric tags for relative and absolute quantitation (iTRAQ) labeling followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Significant variations were validated in an independent cohort of 105 subjects using two different methodologies: turbidimetry, an assay focused on clinical diagnostic and selected reaction monitoring (SRM), a proteomic approach with great clinical potential.

Results: Proteomic analysis of plasma has allowed identifying two protein profiles with an important value from a clinical point of view: 1) proteins with predictive value of de novo albuminuria that are related to immune system response and 2) sustained albuminuria indicator proteins related with chronic renal damage.

Conclusions: The study carried out showed two different protein profiles which may be very useful for predicting the development of de novo albuminuria as well as to monitor renal damage. These results highlighted alterations in specific molecular pathways related with immune response and the pathogenesis of organ damage. The possibility of a future strategy based on anti-immune therapy to treat hypertension which could help to prevent the development of albuminuria and hence, the progression of kidney damage.

654 Soluble RAGE levels in plasma of patients with cerebrovascular events
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Background: There is growing evidence implicating the participation of RAGE-ligand interaction in the development and progression of various immune-mediated disorders, including vascular disease.

Purpose: The aim of the present study was to evaluate the sRAGE plasma levels in patients with ischemic stroke or transient ischemic attack in order to identify a biomarker of differentiation in the genesis of these diseases.

Methods: This study included 87 Caucasian subjects (50 males and 37 females) with cerebrovascular event. Plasma levels of sRAGE were determined using a kit for the immunoadsorption enzyme, commercially available.

Results: Our study showed that the plasma concentration of sRAGE is significantly lower in patients with ischemic stroke compared to patients with transient ischemic attack and to controls.

Conclusions: This feedback appears to confirm that transient ischemic attack, in absence of documented organic pathology, does not seem to recognize the atheromasic origin as its primary cause. This analysis contributes information about the pathophysiology of vascular cerebral disease and, in particular, these results reaffirm strong prothrombotic and inflammatory components to the pathophysiology of stroke.