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.

A. Gowar; V. Vigorelli; P. Nigro; G. Pampillo
Cardiolog Center Medizin RICC Milan, Milan, Italy

Muscular Dystrophy (MD) is an umbrella term for genetic disorders affecting skeletal and cardiac muscle which arise due to abnormalities in the dystrophin gene. Underlying dystrophin metabolic and structural abnormalities in cardiomyocytes (CMs) which in turn become predisposed to ectopic cell death and fibre-fatty replacement. The Endogenous Cannabinoid System (ECS) is a lipid signaling network present in the cardiovascular system and comprises G-protein coupled receptors (CB1R and CB2R), endogenous ligands (anandamide 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 permits the reprogramming 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 colones (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 cell re-programming and MD cardiac pathophysiology which is of interest to cardiac disease modelling and novel drug discovery.

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

FC. Lewis; T. Teah; E. Domenjo-Vil; T. Theologou; M. Field; W. Awad; M. Yaqrit; B. Naddaf-Girard; GMP-Ellison-Hughes
King’s College London, London, United Kingdom; Liverpool Heart and Chest Hospital, Liverpool, United Kingdom; Barts Health NHS Trust, London, United Kingdom

Background: The adult human myocardium harbors endogenous, multi-potent cardiac stem cells (eCSCs). Manipulation of eCSCs ex-vivo and in situ has opened 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: eCSCs derived from the right atria appendage (~200mg) of different aged patients (12 to 85 years) by enzymatic digestion followed by MACS (Miltenyi). eCSCs were characterised for co-expression of aging/senescence markers (p16INK4a, p53, p21, senesence-associated β-galactosidase) with known stemness/multipotency (Oct-4, Nanog, Bmi-1, TERT, Sox-2) 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 aging/senescence markers 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-senescence’ eCSCs showed limited cloning and growth capacity and impaired cardiac differentiation capacity. Moreover, ‘aged-senescence’ eCSCs expressed increased senescence-associated secretionary phenotype (SASP) factors relative to their younger counterparts. Treatment with the canonical Wnt ligand, Wnt3a significantly increased the proliferation of ‘aged-senescence’ eCSCs to levels observed in younger eCSCs. Conversely a switch to non-canonical Wnt signaling imparted a negative ‘aging’ 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/senescence’ phenotype.

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

G. Foldes; N. Heller; O. Vittay; SE. Harding
Imperial College London, National Heart and Lung Institute (NHLI), London, United Kingdom

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.

Conclusions: The results of this study demonstrate that there is a presence of increased numbers of T cells in endomycardial biopsies from patients with DCM where cardiomyocytes progenitors and dedifferentiated cardiomyocytes were detected too. Exact mechanisms of cardiomyocyte dedifferentiation are poorly understood but our results and evidence that cytokines secreted by T lymphocytes regulate cardiomyocyte dedifferentiation in culture show an emerging role of T lymphocytes in cardiac regenerative processes.
**Purpose:** Our aim was to investigate the role of transcriptional Hippo co-activators YAP and TAZ signaling 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 into cardiomyocytes. Western blotting was used to assess the protein expression levels of YAP, TAZ and TAZ-kinase activity. Immunocytochemistry was used to examine the localization of YAP and TAZ in the cardiomyocytes. **Results:** We found that modulating the YAP/TAZ pathway had different effects on cardiomyocyte function and survival. **Conclusions:** Our findings suggest that the YAP/TAZ pathway plays an important role in cardiomyocyte function and survival.

**515**

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

L. Fuentes1, L. Gomez-Catil1, M. Fernandez-Santos2, S. Suarez-Sanchez1, V. Plessencia1, A. Climent1, R. Sanz-Ruiz1, M. Hedhammar2, F. Atenaza, F. Fernandez-Aviles1

1University Hospital Gregorio Maranon, Madrid, Spain; 2Spiber Technologies AB, KTH School of Biotechnology, Stockholm, Sweden

**Purpose:** The aim of this study was to evaluate the biocompatibility and potential use of a spider silk matrix as a scaffold for cardiac tissue regeneration.

**Results:** The spider silk matrix was found to be biocompatible with cardiac progenitor cells. The cells were able to adhere, spread, and differentiate on the matrix. The matrix was found to promote the expression of cardiac-specific genes and proteins.

**Conclusions:** The spider silk matrix showed promise as a potential scaffold for cardiac tissue regeneration.

---

**517**

**Can NOS/GiGcGiK1 pathway trigger the differentiation and maturation of mouse embryonic stem cells (ESCs)?**

V. Spinelli1, L. DiSalvatore, L. Sartori, A. Vanzo, M. Zanardelli, E. Corbini, P. Falli

1University of Florence, NEUROFARBA (Department of Neurosciences, Psychology, Drug Research and Child Health), Florence, Italy

**Purpose:** The role of the NO/cGMP-dependent protein kinase I (PKG) pathway in adult cardiac cells is extensively studied. Indeed, physiological levels of NO generated by NOS1 and NOS3 or pharmacological treatments with NO-donors promote myocardial contractility and ischemic preconditioning. However, the potential role of the NOcGMP-PKG-I pathway in ESC-derived cardiomyocytes is less defined. Therefore, we investigated NOs/GcGk/GiK1 in the early stage of cardiac differentiation of ESCs, studying its enzymatic activities and expressions during cardiac maturation and the acute and chronic effect of pathway alteration.

**Methods:** Embryoid bodies were differentiated from ESCs. Immunocytochemistry was used to examine the localization of NOS, cGMP, and PKG-I in the cardiomyocytes. Immunofluorescence and Western blotting were used to analyze the expression of NOS and PKG-I in the cardiomyocytes.

**Results:** We found that the expression of NOS and PKG-I increased during the early stage of cardiac differentiation. The activity of PKG-I was detected at the early stage of differentiation, and the activity of NOS increased at the late stage of differentiation.

**Conclusions:** Our findings suggest that the NOcGMP-PKG-I pathway plays a crucial role in the differentiation and maturation of mouse embryonic stem cells.

---

**518**

**Introduction of external iK1 to human-induced pluripotent stem cell-derived cardiomyocytes via iK1-expressing HEK293**

A. Costa1, M. R. Hortogn-Vinagre2, M. Van Der Heyden3, F. Burton4, G. Smith1

1University of Glasgow, Institute of Cardiovascular and Medical Science, Glasgow, United Kingdom; 2University Medical Center Utrecht, Department of Medical Physiology, Utrecht, Netherlands

**Background:** Introducing external iK1 to adult primary cardiomyocytes has been shown to improve cardiac function. However, the use of external iK1 in human-induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) has been limited due to the lack of efficient transfection methods.

**Methods:** In this study, we developed a novel method for introducing external iK1 to iPSC-CMs using a lentiviral vector. The efficacy of this method was assessed by measuring the percentage of cardiomyocytes expressing iK1.

**Results:** We found that the percentage of cardiomyocytes expressing iK1 increased with the duration of transfection. The highest percentage of cardiomyocytes expressing iK1 was achieved with a transfection time of 48 hours.

**Conclusions:** Our findings suggest that introducing external iK1 to iPSC-CMs is a feasible approach to improve cardiac function.
Cardiovascular Research Supplements

594

Abstracts

Results: A uniform monolayer developed using 25k-40k cells/well hiPSC-CMs in the presence of increasing densities of HEK293 cells. Contractility 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 ± 144ms, 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% (975 ± 265 and 522 ± 153ms vs 229 ± 6ms, respectively, n=8 p<0.01), respectively. Earlier and later culture times showed no significant difference in spontaneous contractile activity up day 12. In conclusion, 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 iPSC-CMs but different sources of iPSC-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 cells responded differently suggesting a higher sensitivity to co-culture with IK1 expressing HEK293 cells.

519

Cell therapy of the heart studied using adult myocardial slices in vitro

F. Perbellini1; S. Watson1; M. Scigliano2; S. Tkach1; S. Alayoubi1; SE. Harding1; CM. Terracciano1

Introduction: Cardiac cell therapy is the introduction of stem cells in the heart to repair/replace damaged myocardium. In vivo studies have revealed that this therapy can induce arrhythmias, and the efficiency of improving myocardial function appears limited. A better understanding of the mechanisms involved during cell therapy is required but is suitable representative in vitro model is lacking. Organotypic heart slices are multicellular preparations with preserved structural, biochemical and electrophysiological properties.

Purpose: Here we use heart slices to study the mechanisms of functional integration, proliferation and direct/indirect effects on recipient myocardium of transplanted cells.

Methods: In this study human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) were cultured in vitro on 30μm thick vibratome-cut slices prepared from adult dog left ventricular tissue. Viability and functionality were assessed by force measurement, histology and immunohistochemistry. Calcium transients were recorded by optical mapping.

Results: iPSC-CMs attached to the slices, within 24 hours formed electrical connection with the other grafted cells and beat spontaneously. Their beating activity however could not trigger the activation of the recipient tissue. Some cells, after 3 days in culture, could be paced with field stimulation at 1 Hz and contracted synchronously with the slice. When point stimulation was applied on a distant region of the slice, while the slice contracted, the signal did not propagate to the iPSC-CMs, suggesting a lack of coupling with the recipient tissue. After 9 days in culture some iPSC-CM started to integrate and aligned with the slices myocytes, but others did not and spread into a separate layer as with 2D culture. At this time point however, myocardial slices showed a significant degree of functional deterioration. Slice contractility decreased to 23% by day 6 and this was due to myocytes dedifferentiation and cell death.

Conclusions: Vibratome-cut slices are a viable platform to study cell therapy, particularly in the first few hours. Culture conditions need to be improved to better preserve myocardial slice structure and functionality for long term studies.

520

Enhancement of the paracrine potential of human adipose derived stem cells when cultured as spheroid bodies

C. Sud-Otmane1; HQ. Ly

Montreal Heart Institute affiliated with the University of Montreal, Montreal, Canada

Background: Ischemic heart disease remains a leading cause of mortality and morbidity worldwide. Cardiac cell therapy (CCT) is a promising therapeutic strategy to help in cardiac repair. Multiple cells have been proposed as candidates in CCT. Adipose tissue constitutes an important and accessible reservoir for the stem cells. Both preclinical and clinical data have shown that adipose derived stem cells (ASCs) could improve cardiac function and volumes, mostly through a paracrine mechanism.

Study Aim: The objective of our in vitro study is to characterize and compare the secretion profile as well as the survival of ASCs, when cultured under standard conditions (i.e. as a monolayer (ML)) versus in a three-dimension (3-D) structure (i.e. as a spheroid body (SB)). In vivo, the aim is to compare the anti-inflammatory potential of these two cell structures in periostin in a rat model.

Methods: Human ASCs (hASCs) were expanded in standard culture conditions in a monolayer form. ASCs were characterized according to both surface markers expression (assessed by immunofluorescence) and their ability to maintain multilineage differentiation potential. ASCs were cultured as 3-D structure as spheroid bodies (SBs), by using the hanging drop technique. Lumimex and ELISA assays were conducted to quantify key immunomodulators and angiogenic mediators to compare the two study groups. Western blots were used to study proteins involved in apoptosis.

Results: hASCs expressed similar surface markers as those described for ASCs expanded in a ML form, including CD44, CD105 and CD90. Their ability to differentiate into adipogenic, chondrogenic and osteogenic lineage were unaltered. Paracrine activity of hASCs was enhanced when cultured as hASC-SBs. SBs secreted higher levels of immunomodulatory cytokines such as MCP-1, IL-6, IL-8 and IL-10 in a time dependent manner. Similarly, they exhibited greater pro-angiogenic potential as VEGF levels were increased also compared to hASC-MLs. Activation of caspase 3, shown by its cleaved form, was described in MLs under basic culture conditions and in response to TNFa stimulation, whereas caspase 3 was undetected in SB structures. Moreover, inflammation was reduced in both ASC-ML and hASC-SB treated groups of rats with induced peritonitis compared to the untreated group, with a slight efficacy of SBs over MLs.

Conclusion: hASC represent a promising cell source for stem cell therapy. Their paracrine, therapeutic potential can be optimized. Our findings clearly showed that hASCs cultured as 3D structures (i.e. as SBs) exhibit an improvement in both anti-inflammatory and angiogenic properties associated with resistance to apoptosis. Spheroid body formation thus represents an effective alternative to enhance the therapeutic potential of hASCs.

521

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

NAH. Bassi1; A. Mauretti1; H.H. Van Marioni1; M.C. Van Turnhout1; DW. Van Der Schaft1; CM. Salih1; G. Gouraud2; CVC. Bouten1

1Eindhoven University of Technology, Soft Tissue Biomechanics and Engineering, Eindhoven, Netherlands; 2Leiden University Medical Center, Leiden, Netherlands

Purpose: Cardiomyocyte 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 predifferentiated human CMPCs in a 2D environment, as well as how CMPCs respond to unidirectionally constrained or stress-free (unconstrained) 3D environments. The latter responses were studied using a dual hydrogel platform. CMPCs were cultured in the beating heart chamber. Cyclic strain was applied on a 2D hydrogel layer allowing for interaction of the cells with a sinusoidal strain signal, and strain was quantified with reflective and indirect methods. Tissue mechanics were assessed via optical coherence tomography.
Transcriptional control and RNA species - Heart

525 Gene expression regulation in heart failure: from pathology to bioinformatics
C. Schrøder1, V. Grillmair2, M. Apriola3, R. Esposito3, C. Miatello3, A. Soncini3, V. Colantuoni2, V. Casta2, A. Ciccodicola2, C. Napoli2

1SDN Foundation IRCCS, Naples, Italy; 2Second University of Naples, Naples, Italy; 3Institute of Genetics Biophysics CNR, Naples, Italy; 4A.O. dei Calvi Monaldi Hospital, Naples, Italy; 5Federico II University of Naples, Naples, Italy

Background: Heart failure (HF) syndrome results from abnormalities in multiple biological processes, contributing to the level of the structural gene expression. 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 analysed 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 both for the number of DEGs and the DEG signature. For example, some of the common DEGs, particularly, 35 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 downregulated in both DCM and RCM.

Conclusions: 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
A. Chaloupka1, G. Rowe1, K. Johnson2, Z. Pánička3, F. Del Monte2

1St. Anne’s University Hospital, 1st Department of Internal Medicine, Brno, Czech Republic; 2University of Alabama Birmingham, Division of Cardiologic Disease, Department of Medicine, Birmingham, United States of America; 3Institute of Health and Care Informatics, Prague, Czech Republic

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 Quantrix, based on quantitative real-time polymerase chain reaction (qPCR) for screening of a panel of regulated and putative TFs in the human genome.

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 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.

Conclusions: Quantrix 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 patients 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
F. Forini1, R. D’aurizio2, C. Kuzmic3, G. Nicolini1, M. Baugnart1, H. Groth1, N. Ucciferri1, G. Iervasi1, L. Pito3

1Institute of Clinical Physiology, CNR, Pisa, Italy; 2Clinical Institute of Informatics and Telematics (ITI), CNR, Pisa, Italy; 3Fritz Lipmann Institute (FLI), Jena, Germany

Background: Increasing experimental and clinical evidence indicate that a low T3 state (LT3S) 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 systematic biology approach may help investigating this important issue.

Purpose: The purpose of the study was to unveil putative mitochondria-targeted cardiovascular circuits activated by T3 in the early post IR setting and dependent on the regulation of micro:RNAs.

Methods: To this aim, miRNA profiling and mitochondrial proteome were performed in a model of cardiac IR injury where data were integrated by computational analysis. Briefly, rats developing a low T3 state were treated with T3 (6µg/Kg die) or T3 vehicle for 48h. Then, cardiovascular performance was evaluated through echocardiogram and the rats were sacrificed. Tissue from the LV peri-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-T3S and the proteomic profiling was performed through mass spectrometry.

Results: The presence of a post IR LT3S 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 LT3S in the early post IR and poor cardiac and mitochondrial outcomes, while identifying a beneficial role for T3 treatment possibly through the regulation of miRNA-mediated cardiotoxicity pathways targeted to mitochondria.

528 The effect of ureaemia on the expression of miR-212/132 and the calcineurin pathway in the heart rat
M. Sarkozy1, M. Pizzi2, R. Gaspar1, A. Szakal1, I. Foldesi1, K. Kasi3, P. Bencsik4, T. Thumb1, S. Batáki1, T. Cosor1

1University of Szeged, Faculty of Medicine, Department of Biochemistry, Metabolic Diseases and Cell Signalling Group, Szeged, Hungary; 2University of Szeged, Faculty of Medicine, Cardiovascular Research Group, Department of Biochemistry, Szeged, Hungary; 3Hanover Medical School, Institute for Molecular and Translational Therapeutic Strategies (IMTTS), Hannover, Germany

Background: The prevalence of ureaemia is continuously increasing in developed countries. Uremic cardiomyopathy characterized by left ventricular hypertrophy and diastolic dysfunction is a common cardiovascular complication of uraemia. However, the underlying molecular mechanisms are not clear. The overexpression of miR-212/132 has already been implicated in the development of left ventricular hypertrophy-via modulation of the calcineurin pathway in TAC mice. Purpose. Therefore, here we investigated the effect of ureaemia on the myocardial expression of miR-212/132 and the calcineurin pathway.

Methods: Uremia was induced by 5/6-nephrectomy in male Wistar rats. Eight weeks later serum urea and creatinine levels were measured and transthoracic echocardiography was performed. Then RNA was isolated from left ventricles of nephrectomised and sham-operated rats and expression of miR-212/132 and aegro-1 as well as MCP14, components of the calcineurin pathway, was measured by q-PCR.

Results: In the nephrectomised group, serum urea and creatinine levels were significantly higher proving the development of uremia. 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, aegro-1 showed significant down-regulation and MCP14 showed significant up-regulation in the uremic group.

Conclusion: Myocardial overexpression of miR-212 might play a role in the development of uremic cardiomyopathy by modulating the calcineurin pathway.

Cytokines and cellular inflammation - Heart

531 Lack of growth differentiation factor 15 aggravates adverse cardiac remodeling upon pressure-overload in mice
S.C. De Jager1, JJ. Haan1, L. Bosch1, MAD. Brans1, SM. Van De Weg1, JC. Deddens1, SJ. Lee2, PJ. Sluiter1, G. Pasterkamp3

1University Medical Center Utrecht, Experimental Cardiology, Utrecht, Netherlands; 2Johns Hopkins University of Baltimore, Molecular biology and genetics, Baltimore, United States of America

Introduction: Growth differentiation factor 15 (GDF15) is a distant member of the TGF-β family. Under homeostatic 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

Abstracts S95

Downloaded by guest on November 30, 2016
Cardiovascular Research Supplements

Introduction: We have previously reported an association between low-dose aspirin use and improved long-term outcome in chronic heart failure (HF) patients irrespective of ischemic heart disease. 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 these benefits are likely to be 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 28 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 releasate (PR). 16h) prepared from collagen-activated platelets from the same donor. Finally, primary monocyte/platelet aggregates were incubated with 10 μM aspirin in matrigel-coated invasion transwells (16h) to study the influence on monocyte migration.

Results: Low-dose aspirin was associated with significantly higher overall survival and lower HF hospitalizations over the 3-year follow-up period (HR 0.665, 95% confidence interval, 0.389-0.961). Serum MMP2 and iCD163 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 retropective 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.

S94 Abstracts

S52 Blocking heteromerization of platelet chemokines cc55 and ccx44 reduces inflammation and preserves heart function after myocardial infarction

T. Vagen1; I. Werner2; D. Prohaj1; M. Staudt1; A. Curp1; T. Sohmen1; S. Sмыслыкова3; T. Hacking1; P. Von Hundelshausen1; R. Kienast1; C. Weber1; E. Liehn1
1Cardiovascular Research Institute Maasstricht (CARIM), Beuchochemistry, Maastricht, Netherlands; 2J.W Goethe University, Department of Thoracic and Cardiovascular surgery, University Hospital, Frankfurt am Main, Germany; 3Ludwig Maximilians University, Institute for Cardiovascular Protection (IKP), Munich, Germany; 4RWH University Hospital Aachen, IMCAR, Aachen, Germany

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 threatening 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 CXC4 in the progression of atherosclerosis.

Purpose: To evaluate a specifically designed compound (MKET) that blocks the CCL5-CXCR4 interaction in a mouse model of myocardial ischemia/reperfusion (IR).

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

Results: MKET treatment resulted in a significant decrease in infarct size and preserved heart function as compared to sMKET-treated animals (Figure A, B). Moreover, MKET treatment significantly reduced the inflammatory reaction following IR, as revealed by specific staining for neutrophils, NETs and monocyte/macrophages (Figure C, D). In vivo. Antiplatelet strategies to modulate monocytes may require further, prospective evaluation in HFpEF.

Conclusion: Disrupting chemokine heterodimers during myocardial IR 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.

S53 Is there an association between low-dose aspirin use and clinical outcome in HFpEF? Implications of modulating monocyte function and inflammatory mediator release

N. Olezova1; M. Santos-Martinez2; C. Medina1; C. Watson1; K. McDonald1; J. Gilmer1; M. Ledwidge1
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 improved long-term outcome in chronic heart failure (HF) patients irrespective of ischemic heart disease. 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 these benefits are likely to be 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 28 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 releasate (PR). 16h) prepared from collagen-activated platelets from the same donor. Finally, primary monocyte/platelet aggregates were incubated with 10 μM aspirin in matrigel-coated invasion transwells (16h) to study the influence on monocyte migration.

Results: Low-dose aspirin was associated with significantly higher overall survival and lower HF hospitalizations over the 3-year follow-up period (HR 0.665, 95% confidence interval, 0.389-0.961). Serum MMP2 and iCD163 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 retropective 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.

S54 N-terminal terminated intracellular matrix metalloproteinase-2 expression in diabetic heart.

H.W. Lee1; S.H. Song2; MY. Lee2; MH. Park2; J.C. Choi1; JH. Ahn1; J.S. Park3; JH. Oh1; J.H. Choi1; H.C. Lee1; KS. Chai1; TJ. Hong1
1Pusan National University Hospital, Department of Cardiology, Busan, Korea Republic of; 2Pusan National University Hospital, Medical Research Institute, Busan, Korea Republic of

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 vivo and in vitro connection with activated innate immunity and apoptosis.

Methods: H9c2 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 method. In vivo mouse model was induced with 40mg/kg streptozotocin intraperitoneal injection for 5 days. After sacrificing mice at 12 and 24weeks. 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 H9c2 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.
535
Expression of CD39 and CD73 on peripheral T-cell subsets in calcific aortic stenosis
A. Golovkin¹, I. Kudryavtsev², M. Serebryakova³, A. Malashicheva¹, A. Shakhova¹, E. Zhidulova⁴, O. Moseeva⁴
¹Federal Almazov Medical Research Centre, St Petersburg, Russian Federation; ²Institution of Experimental Medicine, St Petersburg, Russian Federation
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 aorta valve calcification. We examined 24 patients with severe calcific aortic stenosis [average flow gradient 48.3 (46.065.0) mmHg] and 16 healthy volunteers. Mean age was 63 (57.64) years. There were 14 patients with bicuspid (BAV) and 10 with tricuspid aortic 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 naive (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 was measured using flow-cytometry on BAV and TAV. It was found that relative number of Naïve Tcyt (p=0.034) was decreased and the relative number of those in patients with TAV. Taking together achieved results are proving the hypothesis of participation measured using qPCR. A trend towards higher level of TIMP-1, IL-8 was registered in group 1; MMP-9 and uric acid were higher in group 2. Method of logistic regression identified the following factors associated with the presence of the combination of CAD and AH: history of myocardial infarction (OR 0.09 [CI 0.03 0.27], p<0.001); uric acid (OR 0.1 [CI 0.01 0.10], p<0.001); BMI (OR 1.31 [CI 1.18 1.46], p<0.001); MMP-9 (OR 1.03 [CI 1.01 1.05], p<0.001). Conclusion: In patients with CAD and AH the imbalance of MMP-9/TIMP-1 prevailed possibly because of hemodynamic factors in AH and oxidative stress in obesity. It may be associated with the higher risk of cardiovascular events in this group.

540
Pro-inflammatory cytokines as cardiovascular events predictors in rheumatoid arthritis and asymptomatic atherosclerosis
NP. Korzhinskii, KS. Solodorekova, MA. Osadchuk
Imm. Serhiev S. First Moscow State Medical University, Moscow, Russian Federation
Background: Recently rheumatoid arthritis (RA) is considered as an important risk factor of cardiovascular diseases (CVD) and asymptomatic atherosclerosis (AA). In our previous studies we revealed cloners correlation between RA severity, pro-inflammatory cytokines levels and CVD manifestation. Purpose of this investigation was to assess the diagnostic and prognostic value of pro-inflammatory cytokines in the prediction of serious cardiovascular events.
Methods: 112 pts with RA (44 female and 18 male aged 37-74 years) were observed during 5 years. Baseline levels of pro-inflammatory cytokines (TNF-a, IL-1b, IL-6) were determined. Outcomes included cardiovascular death, acute coronary syndrome (ACS) and stroke. We used ROC-analysis to evaluate the validity of determined cytokines in prediction of these CV events and Cox proportional hazard models to calculate the hazard ratios (HRs) for each outcome.
Results: Clinical characteristics of RA were found in 38 pts (45.7%) without history of CHD and stroke. The increased incidence of ACS and CV death was defined in RA pts with history of CVD or with AA. The risk of ACS and CV death was higher in these groups and was associated with elevated levels of IL-1b (HR 1.15, 95% CI 1.06 to 1.19, HR 1.12, 95% CI 1.04 to 1.18, respectively; AUC=0.675, AUC=0.658 b 0.05, respectively) and IL-6 (HR 1.29, 95% CI 1.12 to 1.45, HR 1.32, 95% CI 1.18 to 1.44, respectively, AUC=0.646, AUC=0.628 b 0.05, respectively).
Conclusions: CV risk should be addressed with all pts affected by RA and AA. Elevated levels of IL-1b and IL-6 can be considered as reliable predictors of serious CV events such as ACS and CV death in RA and AA.

541
Characterization of FVBN murine bone marrow-derived macrophage polarization into M1 and M2 phenotypes
M D. E. Santuci, MF. Dutra, FCB. Oliveira, MM. Silva; DG. Passos-Riua, R. Gonçalves; RAS. Santos, RF. Da Silva
University of Fortes de Minas Gerais, Physiology and Biophysics, Belo Horizonte, Brazil
Background: In a range of pathophysiological conditions macrophage activation leads to changes in their functional and phenotypic profiles. Among the wide existing cellular phenotypic spectrum, the subpopulations of classically activated macrophages (M1) and alternatively activated macrophages (M2) represent the two extremes. In vivo, the process of macrophage polarization into the distinct phenotypes is complex and depends, among others, on the local microenvironment. In vitro, macrophage activation can be more easily controlled. However, the actual characterization varies widely in the literature due to incubation time, origin of the cells, animal species and stimuli. Purpose: To characterize the phenotypic profile of FVBN murine bone marrow-derived macrophages polarized into M1 and M2 subpopulations.
Methods: Bone marrow cells obtained from femurs and tibiae of 8-10-week male FVBN mice were cultured for seven days. The cells were divided into three groups: unstimulated, M1 and M2. The four-hour in vitro polarization into M1 phenotype was performed by using lipopolysaccharide and into M2 phenotype, interleukin-4 (n=4 animals; 107 cells per animal). RNA was extracted and after DNAse treatment, qPCR was used to analyze the differential gene expression of M1 markers (IL-12, IL-1b, IL-6) and M2 markers (TNF-a, arginase-2 (Arg2), chymase-C-C, Fizzl (CCL2) and interleukin-6 (IL6), as well as M2 markers mannose receptor (MR) and found in inflammatory zone protein (Fiz1)). Glyceraldehyde-3-phosphate dehydrogenase was used as an endogenous control.
Results: In M1, both TNF-a and IL-12 gene expression increased compared to unstimulated (6.75 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 A.U. and 6.26 0.32 vs 0.14 0.29 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, without reaching significance. Intriguingly, 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 Fiz1 by qPCR was inefficient.
Conclusions: The TNF-a, IL-1b and Arg2 RNA expression profiles observed in M1 are consistent with the current literature on macrophage activation. To confirm that CCL2 gene is important for the characterization of this phenotype in our culture model, the trial number N must be increased. On the other hand, the IL-6 gene, although being a known marker for M1, appears to be inappropriate for this study. In M2, the MR expression profile finds support in the literature, however, changing the Fiz1 primer sequence is necessary to better understand the role of this protein in the characterization of M2 in our cell culture model.
The biological expression and thoracic anterior pain syndrome

D. Plunianu1, CM. Gavrilescu2, CM. Pârvu1, P. Mânescu1, LC. Scară1
1 Clinic CF, Hospital, Medical I, Iasi, Romania; 2 University of Medicine and Pharmacy, Iasi, Romania

The Aim of the Work: The clinical study seeks involvement of oxidative stress and lysosomal dysfunction in chest pain pathology anterior localized (TAP).

Material and Method: It was watched metabolic clinical profile and antioxidant status to a number of 170 patients admitted to the Medical Clinic CF Iasi who have been diagnosed with various disorders with common symptoms: chest pain earlier. The results were obtained compared with the same data from a group of 70 healthy volunteers. Evaluation of patients was made by clinical, laboratory investigations routine (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 biological re-
duced status differs depending on the type of disease or age.

Clinical features of patients with anterior chest pain (TAP)

Table 1

<table>
<thead>
<tr>
<th>Age</th>
<th>Patients under 65 years</th>
<th>Patients over 65 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>isx (mm)</td>
<td>57.40</td>
<td>69.8</td>
</tr>
<tr>
<td>smoking</td>
<td>1/1</td>
<td>3/1</td>
</tr>
<tr>
<td>hta degree</td>
<td>44% Degree II, III</td>
<td>95% Degree II</td>
</tr>
<tr>
<td>obesity</td>
<td>74.4%</td>
<td>11.6%</td>
</tr>
<tr>
<td>association</td>
<td>86.20%</td>
<td>100%</td>
</tr>
<tr>
<td>dyslipidemia</td>
<td>44.4%</td>
<td>75%</td>
</tr>
<tr>
<td>vascular comorbidities</td>
<td>75%</td>
<td>88.8%</td>
</tr>
<tr>
<td>ischemic heart disease</td>
<td>60.4%</td>
<td>39.5%</td>
</tr>
<tr>
<td>HTA</td>
<td>33.3%</td>
<td>16.6%</td>
</tr>
<tr>
<td>peripheral arterial disease</td>
<td>16.6%</td>
<td>0.08%</td>
</tr>
<tr>
<td>Stroke</td>
<td>25.0%</td>
<td>11.1%</td>
</tr>
</tbody>
</table>

Conclusion: Our studies propose that the formation of Hsp90-TGFβRI complex is involved in col-
genesis and further suggest it could be a good target to reduce the damaging myocardial fibrosis.

546

Loss of the inhibitory GalPaO protein in the rostral ventrolateral medulla of the brainstem leads to abnormalities in cardiovascular reflexes and altered ventricular excitation

R. Ang1; A. Abramowicz2; L. Birmbaum3; A. Gourine3; A. Tinker4
1 University College London, Centre for Clinical Pharmacology, London, United Kingdom; 2 National Institute of Environmental Health Sciences, Divison of Intramural Research, Research Triangle Park, United States of America; 3 University College London, London, United Kingdom; 4 Barts and The London School of Medicine and Dentistry, London, United Kingdom

Introduction: The heart is controlled by the sympathetic and parasympathetic limbs of the auto-
nomic nervous system with inhibitory signaling mechanisms recruited in both limbs. This study aimed to determine the role of inhibitory heterotrimetric G proteins in the central mechanisms underlying autonomic control of the heart and its potential role in arrhythmias.

Methods: Mice with conditional deletion of inhibitory heterotrimetric 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 be-
baviour). However, exposure to stressful conditions (novel environment, hypoxia or hypercapnia) in these mice was associated with profoundly exaggerated heart rate responses and an increased bar-
oreflex 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-inhibited 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 cardiovascular responses to stress, altered cardiovascular reflexes and electrical properties of the heart.

547

Selenoprotein P regulates pressure overload-induced cardiac remodeling

Kanazawa University, School of Medicine, Kanazawa, Japan

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 inde-
dendent of established risk factors, the role of SeP during cardiac remodeling is not well understood.

In our 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 com-
pared to WT mice (32.5 % in KO mice (n=39) vs 51.3 % in WT mice (n=40) p<0.05). The echocar-diographic assessment of left ventricular (LV) ejection fraction and LV wall thickness at baseline and at 2 weeks after TAC were significantly smaller in SeP KO than those in WT mice (10.4 %± 0.04 vs 12.7± 0.03 mm, p<0.005; 9.92 ± 0.04 vs 11.2 ± 0.04 mm, p<0.05). LV weight/body weight (BW) ratio and left ventricular (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 (10.4 %± 0.04 vs 12.7± 0.03 mm, p<0.005; 9.92 ± 0.04 vs 11.2 ± 0.04 mm, p<0.05). LV weight/body weight (BW) and left ventricular (LV) wall thickness were significantly smaller in SeP KO than those in WT mice (4.4± 0.13 vs 5.39± 0.22, p<0.05; 6.88 ± 0.47 vs 10.57 ± 0.69, p<0.005). 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 SeP attenuates cardiac hypertrophy and fibrosis by pressure over-
load. In conclusion, SeP is a regulator of cardiac hypertrophy and possibly plays a maladaptive role in progression of congestive heart failure.

548

Study of adenylyl cyclase activity in erythrocyte membranes in patients with chronic heart failure

U. Kamilev1; TOHIRA. Alieva2
1 Republican specialized scientific-practical Medical Center Therapy and Medical Rehabilitation, Tashkent, Uzbekistan; 2 Tashkent medical academy, Tashkent, Uzbekistan

Purpose: study of activity adenylyl 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± 4.6 years) with CHF (NYHA FC III-IV). 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 homogenate was determined by the method of Y. Salomon.

Results: Basal AC activity was less by 31.9% in patients of Group 1 compared to the control group (45.1± 0.14 vs 6 ± 0.19 pmol/min/mg) in patients of Group 2 it was less by 41.6% compared to the control group (33.6± 0.13 vs 6 ± 0.19 pmol/min/mg) 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 (11.3 ± 0.5 vs 6 ± 0.19 pmol/min/mg, P<0.001). In Group 1 patients, the epinephrine-stimulated AC activity was lower about 2 times compared to the control group (5.5 ± 0.19 vs 11.3 ± 0.5pmol/min/mg). In Group 2 patients, this parameter was re-
duced to 3.85 ± 0.19 pmol/min/mg 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
membrane AC to stimulation. Revealed disturbances in ESAC activity in patients with CHF reflect the desensitization of the endothelial system, which is more pronounced in patients of Group 2.

549 Direct thrombin inhibitors inhibit atrial myocardium hypertrophy in a rat model of heart failure and atrial remodeling

C. Juneau; N. Moglenet; M. Dufilho; S. Hatem
Hospital Pitie-Salpe`tre, 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- tractile 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 atrial 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/digibotran or 6 mg/kg/d of another DTI, 539792. Two months treatment with DTIs reduced both left atria dilatation and the duration of burst pacing-induced AF whereas treatments had no effect on venous dilatation and systolic dysfunction. The vitamin K antagonist, Warfarin, had no effect on both atrial and venricular 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, 10nM thrombin upregulated hypertrophic markers and plasminogen activator inhibitor type 1 (PAI-1) while PAR-1 and the Rho/Rho 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, PCI 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 72 h resulted in a hyperphosphorylation of the IGF-1R owing to downregulation of Cav1 transcription. 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 allow 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 aortic aneurysm patients

D.A. Kostina; A.S. Kostina; V.E. Uspensky; O.M. Moiseev; AA. Kostareva; AB. Malashchicha
1Peter the Great St. Petersburg Polytechnical University, St.Petersburg, Russian Federation; 2University of Verona, Verona, Italy; 3Almajaz Federal Heart Centre, Saint Petersburg, Russian Federation

Purpose: Thoricacic aortic aneurysm develops as a result of complex series of events that alter the cellular structure of the aortic wall. It has been shown in our and other previous studies, that patients with defects of left ventricular outflow tract may have mutations in NOTCH1 gene. Notch signaling between endothelial and smooth muscle cells plays an important role for smooth muscle differentiation, which is altered in patients with ascending aortic aneurysm (AAA). The aim of this study was to assess the expression level of Notch signaling components in endothelial and smooth muscle cells derived from aneurysms in patients with bicuspid aortic valve (BAV) and tricuspid aortic valve (TAV).

Methods: Human aortic endothelial cells (HAECS) and smooth muscle cells (SMC) were isolated from tissue fragments of BAV- and TAV-associated thoracic aortic aneurysm patients and from healthy donors used as controls. The baseline level of Notch receptors, ligands and target genes was estimated by qPCR.

Results: Endothelial cells of AoA patients had significantly lower mRNA levels of NOTCH1, NOTCH3, NOTCH4 and DLL4 compared to controls. However the mRNA level of direct Notch target HEY1 was higher in HAEC of AoA patients. On the contrary, SMC of the patients had significantly higher mRNA levels of Notch receptors: NOTCH1, NOTCH2, NOTCH3 comparing to controls. The levels of direct Notch target genes, such as HEY1, HES1, was not changed in SMC of the patients.

Conclusions: Expression level of Notch receptors, ligands and effectors is altered in HAEC of AoA patients. In contrast, in SMC the patients the level of Notch receptor is changed comparing to controls, but not the level of Notch effector genes such as HEY1 and HES1. Our results show that Notch signaling is differently altered in endothelial and smooth muscle patients of AoA patients. This corresponds to the hypothesis that Notch-dependent differentiation of SMC is governed by endothelial cells. We suppose that alterations of key Notch pathway elements in HAEC population may cause an impairment of SMC differentiation in patients with thoracic aortic aneurysm.

552 Frizzled 5 expression is essential for endothelial proliferation and migration

M. Brandt1; C. Ghazi; A. Petrus1; O. Duicu1; L. Kiss2; M. Danila1; I. Baczko2; N. Jost1
1Victor Babes University of Medicine and Pharmacy, Department of Pathophysiology, Center for Translational Research and Systems Medicine, Timisoara, Romania; 2Victor Babes University of Medicine and Pharmacy, Department of Pathophysiology, Timisoara, Romania

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 synthetic benzopyran compounds inhibit human and rodent mitochondrial fatty acid oxidation, and cause mitochondrial DNA depletion.

Methods: The effects of a new synthetic benzopyran (BZP) on mitochondrial morphology, vascular reactivity and H2O2 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.

Results: The effect of K-1487, K-1492, K-1507 (10μM) on endothelium-dependent relax-ation (EDR, assessed in the organ bath system) and H2O2 production (determined by ferrous oxida- tion xylene orange assay) have been studied in diabetic vs. non-diabetic murine and human vascular fragments. Results: We found an important decrease in EDR in diabetic vessels whereas H2O2 gener-ation 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. Conclusion: The novel benzopyran analogues K-1487, K-1492, and K-1507 might be useful in improving vascular function in clinical conditions associated with high oxidative stress and endothelial dysfunction such as coronary artery disease and diabetes.

553 Modulation of vascular function and ROS production by novel synthetic benzopyran analogues in diabetes mellitus

D. Muntean1; A. Sturza1; A. Petrus1; O. Duicu1; L. Kiss2; M. Danila1; I. Baczko2; N. Jost1
1Victor Babes University of Medicine and Pharmacy, Department of Pathophysiology, Center for Translational Research and Systems Medicine, Timisoara, Romania; 2Victor Babes University of Medicine and Pharmacy, Department of Pathophysiology, Timisoara, Romania

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 synthetic benzopyran compounds inhibit human and rodent mitochondrial fatty acid oxidation, and cause mitochondrial DNA depletion.

Methods: The effects of a new synthetic benzopyran (BZP) on mitochondrial morphology, vascular reactivity and H2O2 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.

Results: The effect of K-1487, K-1492, K-1507 (10μM) on endothelium-dependent relax-ation (EDR, assessed in the organ bath system) and H2O2 production (determined by ferrous oxida- tion xylene orange assay) have been studied in diabetic vs. non-diabetic murine and human vascular fragments. Results: We found an important decrease in EDR in diabetic vessels whereas H2O2 gener-ation 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. Conclusion: The novel benzopyran analogues K-1487, K-1492, and K-1507 might be useful in improving vascular function in clinical conditions associated with high oxidative stress and endothelial dysfunction such as coronary artery disease and diabetes.

554 Extracellular matrix and fibrosis - Heart

555 Cardiac fibroblasts as inflammatory supporter cells trigger cardiac inflammation in heart failure

D. Lindner1; F. Goethal1; J. Schor2; M. Schravi1; S. Hinrichs1; S. Blankenberg1; U. Volker2; E. Hammer1; D. Westermann1
1University Heart Center Hamburg, Department of General and Interventional Cardiology, Hamburg, Germany; 2University of Greifswald, Interdisziplinares Institut fuer Genetik und Funktionsforschte Gesamtforschung, Greifswald, Germany
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 chemoactive sentinel cells activated by increasing 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. By 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 flexcell 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 increasing 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 show the close association between fibroblasts and inflammatory cells.

557

A role for galectin-3 in calcific aortic valve stenosis
R. Sabada,1 E. Martinez-Martinez,2 V. Arrieta,1 A. Fernandez-Celis,1 L. Jimenez-Alfaro,1 A. Melero1
V. Alvarez-Asian,2 V. Cachofero,2 N. Lopez-Andres,1
1Hospital de Navarra, Cardiac Surgery, Pamplona, Spain; 2Hospital de Navarra, Cardiology, Pamplona, Spain; 3Complutense University of Madrid, Dept of Physiology, School of Medicine, Madrid, Spain

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 valves from AS (n = 80) and controls (n = 30) were collected and analyzed. Galectin-3 protein levels were measured using western blot and ELISA.

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. Moreover, Gal-3 colocalized with osteogenic markers such as BMP-2, Runx2 and SOX-9 in vitro, MCP treatment decreased the expression of osteogenic markers in differentiated VICs. In rats, MCP treatment prevented the increase in Ga3 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 calcification in AS.

558

Omega-3 polysaturated fatty acids - can they decrease risk for ventricular fibrilillation?
B. Bacovea,1 N. Trubulova,1 G. Wukalish,2 V. Koznetz,2 J. Radosinska,2 M. Baranicki,2
1Slovak Academy of Sciences, Institute for Heart Research, Bratislava, Slovak Republic; 2Max Delbruck Center for Molecular Medicine, Berlin, Germany; 3Slovak Academy of Sciences, Institute of Experimental Pharmacology and Toxicology, Bratislava, Slovak Republic; 4Comenius University, Department of Physiology, Faculty of Medicine, Bratislava, Slovak Republic.

Background: Reports, including ours, indicate that lower omega-3 (ω-3) index accompanied by cardiac remodeling and myocyte conduction disorders (ECG conduction slowing and enhanced spontaneous ventricular activity) to the adrenergic beta-1 receptors (β1-AAB) are implicated in development of heart failure and presenting as an unexplained heart failure in infants and children. One of the postulated causes is the development of coronary artery disease.

Purpose: To study the dynamics of serum levels of matrix metalloproteinases in primary anterior STEMI patients
R. Yarbov1, M. Korcheva1, T. Suvola1, A. Gusakov1, T. Ryaobov2, V. Markov2, R. Karpov2
1Tomsk State University, Translational laboratory of cellular and molecular medicine, Tomsk, Russian Federation; 2Research Institute for cardiology, Emergency cardiology department, Tomsk, Russian Federation; 3Siberian State Medical University, Tomsk, Russian Federation.

Methods: 21 pts with primary anterior STEMI (mean age 60.47 ± 3.5 h. The levels of MMP-2 were decreased in LV samples: 224.95 ± 90.11 (EDP) and (2) elastin-antielastin circulating immune complexes (EA CIC) in sera of patients with CAD. All these parameters agree with the development of coronary artery disease.

Material and Methods: The levels of EDP and EA CIC were studied in sera of 63 patients (mean age 62.5 ± 1.2 years, CAD duration 9.88 ± 3.12 years). Forty-two healthy persons were used as controls (mean age 58.9 ± 7.64). An elasstic-specific ELISA for detection of EDP was used. EA CIC were investigated by a method for immune complexes detection by means of ELISA-type techniques.

Results: Significantly higher levels of EDP (0.196 ± 0.073) than healthy controls (0.015 ± 0.003) p < 0.001. Patients with CAD showed statistically significantly higher levels of EA CIC (0.063 ± 0.027) than healthy controls (0.014 ± 0.004) p < 0.005.

Conclusion: These findings suggest that elevated levels of EDP and EA CIC are associated with the development of coronary artery disease.
Deletion of the alpha-7 nicotinic acetylcholine receptor changes the vascular remodeling induced by transverse aortic constriction in mice.

F. Neto, H. Seemann, TC. Alcântara; M D E C. Santucci, SG. Fonseca, RF. Da Silva
University Federal of Minas Gerais, Physiology and Biophysics, Boto Horizonte, Brazil

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 (0.32 ± 0.0005 cm²) and α7 TAC (0.31 ± 0.02 cm²) groups when compared to their respective controls WT SHAM (0.26 ± 0.02 cm²) and α7 SHAM (0.28 ± 0.012 cm²). A similar pattern was also observed for the area of the lumen, in which the values of WT TAC (43 ± 5.66%) and α7 TAC (38 ± 2.41%) groups were larger when compared to their controls WT SHAM (28 ± 7.01%) and α7 SHAM (29 ± 3.61%). While the WT TAC group had a significant increase in the deposition of collagen type I (7.35 ± 0.89 μm²) and III (2.97 ± 0.76 μm²) 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 TAC 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

M. Lynch1; J. Baraldo-Barrero2; R. Olsz2; M. Fava1; F. Bagi3; Y. Xin1; H. Alabadi2; M. Jabangri2; J. Stoughton2; M. May1
King’s College London, British Heart Foundation Centre, London, United Kingdom; 2Mayo Clinic, Division of Vascular and Interventional Radiology, Rochester, United States of America; 3St Georges Hospital, NHS Trust, London, United Kingdom; 4Harvard Medical School, Division of Vascular Surgery, Boston, United States of America

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 remodeling in human varicose 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 varous tissues and cultured human saphenous vein smooth muscle cells, while sections were processed for histological and immunohistochemical analysis. Matrix proteomics were performed on isolated 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 varicose 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, agrin 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 vascular 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 mirancon, 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.

568 Microtubule-associated protein RPE1 family member 1 modulates sodium channel trafficking and cardiac conduction

V. Porto1; SP. Podlesnaia2; C.C. Veerman3; AOV. Verkerk1; M.K. Klar1; EML. Lodder1; M. Mangarelli1; CRB. Bezze1; CAR. Rennke1

1Academic Medical Center of Amsterdam, experimental cardiology department, Amsterdam, Netherlands; 2Academic Medical Center of Amsterdam, Anatomy, Embryology, and Physiology, Amsterdam, Netherlands

Introduction: Microtubule-associated protein RPE1 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 connexin33 (Cx33), and cardiac sodium and adrenergic ion channels, at the intercalated discs. 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 channels in neurons. We here investigated the effects of EB1 on cardiac sodium channel function and its mode of effect on cardiac conduction.

Methods and Results: eQTL experiments performed on an F2 population of mice of two separate inbred strains carrying a sodium channel mutation (Scn5a+7785insDe+) 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. Over-expression of MAPRE1/EB1 in HEK293 cells together with SCN5A/Nav1.5 led to an increase in sodium current density without affecting kinetic properties, indicating an increased membrane trafficking of the Nav1.5 protein.

Conclusions: The present study demonstrate 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 order to 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

P. Kui1; H. Takacs2; A. Polya1; N. Morvaj1; L. Legran1; T. László1; N. Nagy1; B. Ordog1; A. Farkas1; T. Forster1; A. Varro2; AS. Farkas3

1University of Szeged, 2nd Department of Medicine and Cardiology Centre, Szeged, Hungary; 2University of Szeged, Department of Pharmacology and Pharamacotherapy, Szeged, Hungary; 3University of Szeged, Department of Cardiology, Szeged, Hungary

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 anaesthesia. At the end of training protocol, proarrhythmic sensitivity were tested with dofetilide (50 nM) in Langendorff-perfused rabbit hearts. ECG repolarization parameters and sinus variability of ECG parameters were evaluated. Tissue samples were taken from the left ventricle and messenger RNA (mRNA) expression level of TGF-β3, 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. Dofetilide tended to increase the QTc interval in the ‘Ex’ group in vitro, however, there was no difference in the incidence of pre-ventricular beats between the two groups. RT-qPCR showed significantly greater mRNA expression of TIMP-1 in the ‘Ex’ group.

Conclusions: The increased LVIdd and the decreased heart rate are characteristic 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 sensitivity 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.
miR-1 as a regulator of sinoatrial rhythm in endurance training adaptation

A. Gualdoni1; S. Landi1; M. Borzarelli2; A. D' souza3; B. Boyett2; A. Bucchi1; M. Baruscotti1; O. D’ifrè2, A. Barbuti2

Background: miR-1 is over-expressed in cardiac atrioventricular nodal cells and in regions of the heart, where regulatory mechanisms are responsible for maintaining sinoatrial rhythm. The aim of this study was to assess the effect of endurance training on miR-1 expression and its relationship with the changes in action potential duration (APD).

Methods: Mice were kept on regular (37 °C) or elevated (39 °C, 40 °C) environmental temperature for 3, 7 or 14 days. Pacemaker activity was measured with optical imaging of calcium transients, and the effects of temperature on calcium transients were investigated. Results: In regular temperature, the rate of pacemaker cells (PACs) is lower than the rate of myocardial cells (MCs) at temperature 39 °C and 40 °C. In the experiments with temperature 39 °C, the rate of PACs was lower than the rate of MCs. In the experiments with temperature 40 °C, the rate of PACs was higher than the rate of MCs. The results suggest that there is a temperature-dependent change in the rate of pacemaker activity, which may be related to the changes in APD.

Conclusions: The data suggest that endurance training can affect the rate of pacemaker activity and that this effect is temperature-dependent. The results also indicate that the rate of pacemaker activity is influenced by temperature, which is consistent with previous studies on the effects of temperature on pacemaker activity.
578 The role of HIF-1 alpha, VEGF and obstructive sleep apnoea in the development of coronary collateral circulation

MA. Abe1; AC. Casar1; GZ. Zaher2; EP. Plhala3; PO. Dingli4; SM. Montfort5; RGX. Xuereb5
1Mater Dei Hospital of Malta, Cardiology, Msida, Malta; 2Mater Dei Hospital of Malta, Biochemistry, Msida, Malta; 3Mater Dei Hospital of Malta, Respiratory Medicine, Msida, Malta

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

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

Methodology: A total of 44 patients with reported collaterals 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-1α (HIF-1α 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 pa-
tients were cases or controls.

Results: HIF-1α increased with increasing Rentrop Score (p=0.04), in all patients. VEGF levels were however not significantly higher (p=0.31). HIF-1α levels in moderate and severe OSA patients were significantly higher with higher 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 Ren-
trop 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-1α (p=0.60) and VEGF (p=0.34) in the absence or mild OSA subgroup. There was also no significant difference in the moderate and severe OSA subgroup for both HIF-1α (p=0.825) and VEGF (p=0.454).

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

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

C. Neber1; T. Aschacher1; B. Messner1; E. Eckmair1; W. Mahl2
1Medical University of Vienna, Department of cardiac surgery, Vienna, Austria; 2Medical University of Vienna, Research laboratory for cardiac surgery, Vienna, Austria

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

Material and Methods: 32 open chest pigs were divided sham-operation (n=3); 4 hours Infarct and 1 hour reperfusion (control); 4 hours PCISV in the intact heart (PCISO-A, n=10); PCISO (started 15 min. after ischemia (PCISO-B, n=11). Specimens were taken from: LAD region (infarct), adjacent zones Border1 and 2, Circumflex region remote R, Right ventricle RV. VEGFR1, 2 positive arteries and veins were calculated as percentage of total amount of pixels and Ki67 expression was calculated as total number of pixels using confocal-microscopy.

Results: VEGFR1 was significantly upregulated in arteries and veins in both interventional groups as compared to controls (p<0.05). VEGFR2 expression in arteries was significantly upregulated in ar-
teries of both PCISO groups as compared to control (p<0.05). Significant upregulation could further be found in veins of PCISO groups as compared to control and sham-operated animals (p<0.05). p53 was significantly downregulated in myocardial tissue of pigs from PCISO A group in comparison with control pigs (p<0.05). Ki67 was significantly upregulated in PCISO A in comparison with controls (p<0.05).

Conclusion: Significant upregulation of angiogenic proteins stimulates a creating of new coronary vasculature as a result to temporarily blocking venous drainage, thus activating endothelium. Further-
more the downregulation of p53 is construed as shortage of myocardial damage, usually leading to apoptosis. Whereas upregulation of the proliferating marker Ki67 indicates that a trans-coronary si-
cus catheter intervention enables cell cycle reentry.

In conclusion, this study substantiates the concept that the PCISO catheter displays beneficial effects on pathologically affected myocardium by exciting neangiogenesis and cardioprotection leading to structural repair of the damaged heart.

580 Early adaptation of pre-existing collaterals after acute arteriolar and venular microoclusion: an in vivo study in chick chorioallantoic membrane

W. Xiang1, B. Reglin2, W. Hong3, B. Nitzsche4, M. Maibier1, P. Guimaraes2, A. Ruggeri2, TW. Secomb3
1Mater Dei Hospital of Malta, Cardiology, Msida, Malta; 2Mater Dei Hospital of Malta, Biochemistry, Msida, Malta; 3Mater Dei Hospital of Malta, Respiratory Medicine, Msida, Malta

Introduction: After arteriolar occlusion, outward remodeling of pre-existing arteriolar collaterals occurs due to increased distal resistance stress which has generally been accepted as the driving force. How-
ever, knowledge is lacking on arteriolar collateral adaptation at the early stage post occlusion (PO) and on venular collateral adaptation.

Purpose: To address two questions: (1) What are the morphological and hemodynamic changes of pre-existing arteriolar and venular collaterals from immediately after occlusion up to 24 h PO? (2) What are the differences in those changes between arteriolar and venular collaterals?

Methods: White leghorn chicken eggs were cracked open on embryonic day 3 (E3) and the content was transferred into petri dishes for the development of chick chorioallantoic membrane (CAM). On E4, a 4- vessel-segment ‘collateral unit’ was chosen at an arteriolar or venular anastomosis, a unit comprising the two consecutive vessel segments from each side of the ‘anastomosis’ point. The vessel adjacent to the collateral unit 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.

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; veins: 2 h PO). In parallel, WSR showed an initial increase (arterioles: for 5 h PO; veins for 1 h PO) and then a gradual decrease to the starting values (P>0.05). Maximal collateral enlargement (arteries: 60%; veins: 100%) occurred in the smallest segment before occlusion and maximal WSR increase (arterioles: 230%; veins: 400%) occurred in the second smallest arteriolar and smallest venular collateral segment before occlusion.

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

583 Endothelium

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

A. Plasienko, N. Demiehova, L. Vynnychenko, O. Prystahio
Sunny State University, Medical Institute, Sunny, Ukraine

Endothelial dysfunction is a marker of vascular disease, as well as the development and progression of hypertension in chronic kidney disease is called chronic renocardial 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). Vasoconstriction function analysis showed that the vasoconstrictory response in less then decompression was expressed in patients with night-peaker -5.47 (t0.4, 11.72%) (p<0.0001, compared with the other groups, which is in the group of patients...
with non-diaper was 11.63 (7.76; 18.92)%, diaper 8.94 (7.04; 15.46)%, and over-diaper 7.24 (5.82; 13.37)% (p=0.001), which can act as a squeeze to regulate miRNAs. Due to the exposure of BAV ascending aorta to the non-physiological hemodynamic, we also studied the differential expression of the miRNAs in endothelial cells, obtained from patients, to laminar vs. turbulent shear stress.

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

587

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

H. Morawietz1; S. Gobe1; N. Cockroft2; K. Howitt2; M. Brux1; C. Bruns1
1Dresden University of Technology, Medical Clinic III, Dept. of Vascular Endothelium & Microcirculation, Dresden, Germany; 2British American Tobacco, Group Research & Development, Southampton, United Kingdom

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 (CSE) 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 CSE for 24-48h. CSE 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 CSE 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 CSE in a time-dependent manner. Induction of the NRF2 system by CSE 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 CSE. Low laminar flow induced increased expression of V-CAM1 and SELE compared to high laminar flow. High laminar flow improved endothelial wound healing. This protective effect was inhibited by CSE 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 CSE 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.

588

The prognostic value of anti-connective tissue antibodies in coronary heart disease and asymptomatic atherosclerosis

AR. Babeva1; AA. Tarasov; SI. Davidov; EA. Rznakova
Volgograd State Medical University, Volgograd, Russian Federation

Aims: Since a low-grade immune inflammation may play a role in the pathogenesis of coronary heart disease (CHD), the contribution of anti-connective tissue antibodies in this process should be assessed.

Methods: We studied the association of plasma anti-connective tissue antibodies levels with acute coronary syndrome (ACS) manifestation in chronic CHD and in asymptomatic atherosclerosis (AA). Baseline levels of plasma antibodies against collagen, chondroitin-sulfate and hyaluronic acid were measured in 147 pts with chronic CHD and in 120 individuals with AA. The incidences of ACS in both cohorts during the 5 year period were registered. Statistical analyses include weighted Cox-regression model and ROC-analysis for detection the most informative predictive test.

Results: The association of ACS manifestation with elevated levels of antibodies against chondroitin sulfate was more prominent in chronic CHD (HR=2.57 95% CI: 1.09-5.99). On the other hand in AA the manifestation of ACS was associated with high levels of anti-collagen (HR=5.7 95% CI: 1.6-15.11) and anti-hyaluronat antibodies (HR=4.14 95% 1.54-14.02). According to ROC-analyst the elevated levels of anti-collagen antibodies was more predictive in AA (AUC 0.789).

Conclusions: Anti-connective tissue antibodies levels reflect the manifestation of ACS in chronic CHD and in AA. Evaluation of these antibodies might be used for diagnostic and prognostic purposes.

589

Novel potential properties of bioactive peptides from spanish dry-cured ham on the endothelium

SM. Martinez Sanchez1; A. Tapia Abellan2; D. Angosto Bazarra2; P. Pelegrin Vivancos2; P. Eriksson1; SM. Martinez Sanchez1; A. Tapia Abellan2; D. Angosto Bazarra2; P. Pelegrin Vivancos2; P. Eriksson1
1San Antonio Catholic University, Cardiovascular Risk, Murcia, Spain; 2Hospital Clinica Universitaria Virgen de la Arrixaca, Experimental surgery, Murcia, Spain

Background: Bioactive peptides (BP) showing angiotensin I converting enzyme (ACE) inhibitory capacity have been widely pursued for the management of hypertension and are believed to exert beneficial physiological effects on the endothelium.

Purpose: To test ACE inhibitory capacity of a protein hydrolysate containing characterised BP from Spanish dry-cured ham. Besides, we aimed to evaluate other beneficial properties in the endothelium

Cardiovascular Research Supplements
Lipids
592
Intermediate density lipoprotein is associated with monocyte subset distribution in patients with stable atherosclerosis
KA. Krychtuk1, SP. Kastl1, T. Pongratz2, G. Goliasch2, L. Gaspar3, G. Maurer1, K. Huber4, E. Dostal2, KA. Krychtiuk1; SP. Kastl1; T. Pongratz2; G. Goliasch2; L. Gaspar3; G. Maurer1; K. Huber4; E. Dostal2

Background: Intermediate density lipoprotein (IDL) consists mainly of chylomicron remnants and very low density lipoprotein (VLDL) remnants that are thought to be proinflammatory lipoprotein particles. Atherosclerosis is considered to be an inflammatory disease of the vessel wall in which monocytes and monocyte-derived macrophages are crucially involved. Circulating monocytes can be divided according to their surface expression pattern of CD14 and CD16 into at least three subsets with distinct inflammatory and atherogenic potential. The aim of this study was to investigate whether IDL is associated with proinflammatory monocyte subsets.

Methods: We included 90 patients with stable coronary artery disease (CAD). Monocyte subsets were identified as classical monocytes (CD14++CD16-) and intermediate monocytes (CD14++CD16+) with and without IDL. IDL was determined by flow cytometry and was defined as IDL-correlated CD14++CD16+ cells with a low expression of CD16 and a high expression of CD14.

Results: IDL correlated significantly with the proinflammatory IM (r=0.24; p<0.05) whereas VLDL and low density lipoprotein (LDL) were not associated with monocyte subtypes. LDL was not associated with CM (r=0.18; p=0.09) and NCM (r=0.16; p=0.13) but correlated significantly with the acute phase protein C-reactive protein (r=0.40; p<0.01). The association of IM with IM was independent of cardiovascular risk factors and statin treatment. Patients with IDL median (38mg/dL) showed a significant higher proportion of IM as compared to patients with IDL<38mg/dL. (5.6 IQR 4.3-8.3% vs. 4.1 IQR 2.6-6.2%).

Conclusion: In conclusion, we provide a potential link between elevated levels of IDL and a proinflammatory distribution of monocyte subtypes in patients with stable atherosclerotic disease. This possible proatherogenic role of IDL warrants further studies.

593
The characteristics of dyslipidemia in rheumatoid arthritis
J. Starodubov1, I. Osvipova1, S. Sopotov2
1Altay State Medical University, Barnaul, Russian Federation; 2City hospital 4, Barnaul, Russian Federation

Introduction: Rheumatoid arthritis (RA) and dyslipidemia as the manifestation of atherosclerosis have the same mechanisms of development that causes cardiovascular complications. Purpose: To evaluate the characteristics of dyslipidemia in women depending on the duration of RA.

Materials and Methods: The study included 201 women, 33% of them had early RA lasting less than 1 year. The RA was diagnosed by the criteria ACR/EULAR 2010. Statistical analysis of the results was performed with ‘Statistica 10’ software.

Results: Dyslipidemia was revealed in patients with early RA in 68% (RA in 65%). In addition, the increase of high density cholesterol (HDL) in early RA - 61% (in 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 (3.7) 2.7mmol/L) (p<0.05). Increased level of triglycerides (TG) in early RA was 1.5 times more frequent 51% (in RA - 39%), 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 - 42% (34% in RA), the mean value was 3.2 mmol/L in early RA, (2.6 mmol/L– in RA). Increased high-density lipoproteins (HDL) level was 2.3 times more frequent in early RA-55% and (24% in RA) (p<0.05). Atherogenic coefficient was 63% in early RA (53% in RA) (p<0.05). Erythrocyte sedimentation rate (ESR) was 1.3 times more frequent in early RA (90% (ESR) 70% in RA). CRP, the CRP and between classical monocytes (CD14++) and MDMs (CD14+CD16+) in patients with stable coronary artery disease (CAD). Macrophages are hallmarked by morpho/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 and monocyte-derived macrophages (MDMs) are accepted as a good surrogate. We previously reported that in healthy subjects MDMs spontaneously differentiated in vitro show two dominant morphotypes, spindle and round, with pro- and anti-inflammatory properties respectively. In particular, round MDMs show high effector capacity versus spindle.

Purposes: This study is conceived to delineate the morphological and functional profile of MDMs obtained from CAD patients compared to those of healthy subjects.

Methods: MDMs were isolated from venous blood of 25 healthy subjects (50 ± 15 years) and from 50 CAD patients (61 ± 11 years) and differentiated for 7 days in medium supplemented with 10% autologous serum. The uptake of apoptotic Jurkat T cells, for effector cell assay, was detected by flow cytometry. Transglutaminase 2 (TG2) and tissue factor (TF) were determined by immuno-fluorescence and western blotting. Thrombin generation was evaluated using a thrombinscope.

Results: Morphologically, MDMs of CAD patients show a prevalence of round monocyte-like T cell. Nevertheless, these MDMs displayed less effector capacity compared to control. Impaired effector capacity may be due to the reduced levels of TG2 protein involved in phagosome formation. Moreover, CAD MDMs present higher TF levels that are associated with a quenched thrombin generation.

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

597
Palmityloethanolamide promotes anti-inflammatory phenotype of macrophages and attenuates plaque formation in ApoE-/- mice
P. Pinne; M. Rami; L. Ring; S. Steffens
Institute for Cardiovascular Prevention (IPEK), Munich, Germany

Introduction: The endogenous fatty acid amide palmityloethanolamide (PEA) is a lipid-derived mediator, which does not bind to the cannabinoid receptors CB1 or CB2, but exerts potent anti-inflammatory effects by activating type-a peroxisome proliferator-activated receptors (PPAR-a). PEA has shown to possess therapeutic potential in inflammatory disease models, but the role of PEA and its promise as a therapeutic agent in atherosclerosis remain 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, 6-8 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-a. 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.
Conclusion: Our data show that PEA evokes potent anti-inflammatory effects in cultured primary macrophages, which translate into an anti-inflammatory 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.

598

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

D. Ozkaramanli1, G. Oz;2, S. Gurkar1
1Nevsehir University Faculty of Medicine, Cordigal, Tekirdag, Turkey; 2Nemam Kemal University Faculty of Medicine, Cardiovascular Surgery, Tekirdag, Turkey

Background: Arrhythmias, particularly 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 translated in an anti-atherosclerotic 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.

Methods: Ninety six vascular rings (32 IMA, 32 RA and 32 SV graft samples) obtained from 40 CABG patients were included in the study. Beta blockers and amiodarone were indicated in both prophylaxis and treatment of AF in the perioperative period.

Results: Results showed that both amiodarone and esmolol can safely be used in the perioperative period. Although amiodarone has a class Ila indication for the prophylaxis of AF in CABG patients, present data suggests that it may be the drug of choice due to its more favorable effects on graft patency in this specific patient population.

400

The characteristics of rheumatoid arthritis and systemic inflammation in rheumatoid arthritis

J. Starodubova1, 2, Osipova2, Sapto2
1Atomy State Medical University, Bamoul, Russian Federation; 2City hospital 4, Bamoul, Russian Federation

Introduction: Atherosclerosis is a great contributor to the development of cardiovascular complications in rheumatoid arthritis (RA). Chronic inflammation causes dysproteinemia associated with high atherogenicity.

Purpose: To assess the association between immune-inflammatory markers and atherogenesis in women with rheumatoid arthritis (RA).

Methods and Materials: The study comprised 204 women with RA, including 65 (32%) with early RA lasting less than 1 year. The RA was diagnosed by the criteria ACR/EULAR 2010. The patients' average age in early RA was 55.7 ± 8.9, in RA 54.9 ± 8.4 years. The risk factors of cardiovascular disease, the markers of inflammatory activity, the activity of RA (Visual Analogue Scale (VAS), DAS28) were analyzed, blood lipid profile was studied. Statistical analysis of the results was performed with "Statistics 10" software.

The results of the improvement of total cholesterol level was in 75% of cases with early RA (57% with RA). The mean value of total cholesterol level was 3.5 ± 0.7 mmol/L (2.9 ± 0.7 mmol/L), p=0.055. The increase of low-density lipoproteins in early RA was 63.8% (in RA -36.1), 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 15 ± 0.6 in early RA, (1.8 ± 0.7 mmol/L) mmol/L. Reduced levels of high-density lipoproteins (HDL) was 55% in early RA, (32% in RA) (p<0.05). The conditions associated with atherogenesis 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), transient 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<0.001), C- reactive protein (CRP) (r=0.4, p<0.001), electrolyte sedimentation rate (ESR) (r=0.06, p=0.003), rheumatoid factor (RF) (r=0.09, p<0.001) were revealed. There is correlation between CRP level and blood lipid 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.

601

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

K. Stello1; A. Gass1; K. Stamatopoulou1; L. Piersimoni1; D. John1; F. Lunel2; P. Eriksson1; U. Heber; A. Zaric1; J. Demmel1
1JW Goethe University, Department of Cardiology and Institute of Cardiovascular Regeneration, Frankfurt am Main, Germany; 2Alexandra University Hospital, Department of Clinical Therapeutics, Athens, Greece; 3Karolinska University Hospital, Department of Molecular Medicine and Surgery, Stockholm, Sweden; 4JW Goethe University, Institute of Cardiovascular Regeneration, Frankfurt am Main, Germany; 5Karolinska Institute, Cardiovascular Medicine Unit, Department of Medicine, Stockholm, Sweden; 6JW Goethe University, Department of Cardiology, Center of Internal Medicine, Frankfurt am Main, Germany

Background: Adenosine to inosine (A-to-I) RNA editing is catalysed 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-sequencing (RNA-seq) of human endothelial cells revealed that ADAR1, the main RNA editing enzyme in HUVECs. The vast majority of editing events are detected in pre-established and more advanced atherosclerotic plaques in the early RA and in the late stage of atherosclerosis which is more common in RA than in early RA.

Conclusions: This study shows for the first time that A-to-I RNA editing is a critical modulator of inflammatory gene expression in all stages of atherosclerotic disease development.

599

BMPR1 signaling of fibrocytes, a mesenchymal progenitor cell population, is increased in STEMI and dyslipidemia

T. Hofauer1; A. Mangold1; T. Schier1; A. Parzenbock1; N. Staier1; H. Heidiari1; J. Mueller1; 1LM Medical University of Vienna, Cardiology, Vienna, Austria

Introduction: Inflammation is a hallmark feature of ST-elevation myocardial infarction (STEMI). Fibrocytes, a collagen-I+CD34+CD45+ mesenchymal progenitor cell population accumulate in cardiac tissue of a murine ischemia//reperfusion model. In ACS patients, decreased levels of circulating fibrocytes were found, compared to healthy controls. Bone morphogenetic protein receptor II (BMPRII) is involved in the vascular remodeling of lung and heart. Therefore, we studied BMPR1 expression in fibrocytes at the culprit lesion site (CLS).

Methods: We sampled blood from the CLS and a femoral site in the course of primary percutaneous coronary intervention (PCI) from 50 STEMI patients (n=50, male=78%, mean age=61±15y). Another sample was acquired 72h after PCI (n=51). A cohort of healthy controls (n=20; male=46%, mean age=51±2y) served as controls. Flow cytometry was employed to characterize fibrocytes.

Results: Fibrocytes were increased at the CLS compared to femoral blood (722 [276-1298] vs. 324 [180-589], p=0.0001). 72h after PCI, CLS fibrocyte population was increased compared to healthy controls (244 [151-468] vs. 153 [102-179], p=0.006). Peripheral fibrocytes during PCI were similar to those of controls. No differences were found in BMPR1 expression between coronary and femoral blood of STEMI patients; however, BMPR1 expression was higher in patients than controls (MFI 22106 [13142-34125] vs. 13099 [8944-20231], p=0.0014). In patients suffering from dyslipidemia, BMPR1 on fibrocytes was substantially increased (MFI 26056 [13195-54807] vs. 19913 [13635-22965], p=0.009). 72h after PCI, BMPR1 was significantly upregulated on fibrocytes (MFI 22294 [17937-40142] vs. 31149 [27722-45724], p=0.044).

Conclusions: The more than two-fold increase of fibrocytes at the CLS and subsequent decrease 72h after PCI in peripheral blood supports the concept of an active process. BMPR1 expression is increased in STEMI patients, particularly in patients with dyslipidemia, suggesting lipid-induced inflammation, and the activation of fibrotic vascular remodeling.
We aimed to test the hypothesis that E-selectin-targeted polymers with and without the molecule expressed on activated endothelium that recruits leucocytes to the inflammation site, make novel nanomedicine-based strategy to treat atherosclerosis and to stabilize the vulnerable plaque.

**Conclusion:** E-selectin binding polymers reduce the growth of atherosclerotic lesions. We suggest a final injection, we performed a second vascular US and harvested the aorta for histological analysis. Following the baseline vascular US, mice were randomized into 4 treatment groups: ESBP-Dex, ESBP, N-(2-hydroxypropyl)-meth-acrylamide (HPMA) polymers conjugated with peptides that bind anti-inflammatory drug would prevent inflammation and plaque progression.

**Methods and Results:** To target and modulate vascular inflammation we used novel N-(2-hydroxypropyl)-meth-acrylamide (HPMA) polymers conjugated with peptides that bind bacteria could be latently present in plaques and might have a role in the plaque instability and subsequent thrombus formation leading to an Acute Coronary Syndrome in these patients.

**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 leukocytes to the inflammation site, making it a therapeutic target to interfere with the development and progression of atherosclerosis.

**Purpose:** We aimed to test the hypothesis that E-selectin-targeted polymers with and without the anti-inflammatory drug would prevent inflammation and plaque progression.

**Methods and Results:** To target and modulate vascular inflammation we used novel N-(2-hydroxypropyl)-meth-acrylamide (HPMA) polymers conjugated with peptides that bind E-selectin with high affinity, and with and without dexamethasone 1mg/kg (ESBP-Dex, ESBP), five-month-old ApoE(-/-) mice were fed a high-fat diet (HFD) for 8 weeks. Plaques growth was assessed by vascular ultrasound (US) 4 weeks after onset of HFD. We used a novel Vevo Vasc software used. Plg-RKT levels were also measured on macrophage subsets via flow cytometry. We aimed to analyse the expression of Plg-RKT on monocyte and macrophage subsets. PMBCs were isolated from whole blood samples of healthy donors and were stained with fluorochrome-labelled antibodies against CD 14, CD 16, CD45 and Plg-RKT and uPAR were and analyzed with a flow cytometer. Cells were also incubated with FITC labelled plasminogen and stained and measured as described before via flow cytometry. The same experiments were performed with murine blood samples. However, to identify mouse monocyte subsets, CD11b and Ly6-C antibodies were used. Plg-RKT levels were also measured on macrophage subsets via flow cytometry. 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 matrixmetalloproteinases and are possibly involved in plaque vulnerability and destabilization whereas M2 macrophages are anti-inflammatory and linked to plaque stabilization.

**Conclusions:** E-selectin binding polymers reduce the growth of atherosclerotic lesions. We suggest a novel nanomedicine-based strategy to treat atherosclerosis and to stabilize the vulnerable plaque.
Results: In the media of both acute and chronic MI patients, the number of MCs was significantly higher than in controls. This was also found when evaluating unstable and stable plaques separately. In patients with chronic MI the number of MCs in unstable lesions was significantly higher than in stable lesions. This coincided with a significant increase in the relative number of instable plaques in patients with chronic MI compared with control and acute MI.

Conclusion: The presence of MCs in the media of both stable and unstable atherosclerotic coronary lesions after MI suggests that MCs may be involved in the onset of MI and, on the other hand, that MI serves as receptor for different coxsackie- and adenoviruses. CAR is strongly expressed throughout 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 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. CV4S 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 contraction resulting in the increased embryonic heart beat as recorded for CAR KO embryos. Due to the strong coexpression with CV4x and CV4S 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 CV4x 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.

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

### Table (1): Baseline characteristics of angiographically normaly and ectatic coronary vessels

<table>
<thead>
<tr>
<th>P</th>
<th>Normal (n = 30)</th>
<th>Ectasia (n = 83)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>n (%)</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>0.564</td>
<td>50.35 ± 6.74</td>
<td>54.67 (14)</td>
</tr>
<tr>
<td>0.078</td>
<td>19.8 ± 0.56</td>
<td>54.67 (14)</td>
</tr>
</tbody>
</table>

**Conclusion:** Our data demonstrate that HMW-AGES application acutely reduces ICaL in adult cardiomyocytes.
Postnatal development of cardiac excitation-contraction coupling in rats
A. Zahradnikova Jr, K. Macková, I. Zahradnik, A. Zahradnikova
 Slovak Academy of Sciences, Institute of Molecular Physiology and Genetics, Bratislava, Slovak Republic

As a result of the large size of cardiac myocytes in mammals, excitation-contraction coupling is de-
pendent on spatially distributed calcium release sites working on the calcium-induced calcium release
(CICR) principle. This is enabled by a network of T-tubules that spread the sarcolemma and thus the
excitation signal throughout the cell volume. This morphological feature develops postnatally at early
phases and when myocytes undergo rapid growth and structural remodeling.

This work was aimed at comparison of changes in morphology and growth of T-tubules with the de-
velopment of L-type calcium current amplitude in isolated rat cardiomyocytes.

Ventricular myocytes of neonatal and young rats (ages 2-21 days) were compared with the myocytes of
28 days old rats. Calcium currents recorded in isolated ventricular myocytes using 80 ms depolar-
izations from -50 to 0 mV in whole-cell patch-clamp mode were analyzed for changes in the amplitude
and kinetics. The morphology of T-tubules was assessed in the isolated myocytes as well as in
an intact myocardial tissue using the plasma membrane-specific fluorescent probe FM 4-64 and
laser-scanning confocal microscopy.

Initially, a network of sarcolemmal T-tubules was observed around day 9 (D9) in the form of short mem-
brane invaginations. These progressed into tubular elements with mostly longitudinal orientation and
did not form a regular tubular network. After D14, a loose network containing also transversal elements
formed a loose network containing also transversal elements. After D17, a semi-regular tubular
network was developed. Throughout the observed age interval the cell size, area and membrane
capacitance were increasing and around D28 reached values typical for smaller myocytes of adult rat
heart. Calcium currents showed increase in current density that saturated around D11. Similarly, the rate of Ca-currents increased with age, reaching values typical for adult
myocytes after D10. These data suggest that functional local calcium signaling is present already at
D11, when the T-tubules only start to form. This conclusion is supported especially by the dynamics
of cardiac current decay, which at D11 becomes as fast as in adult cardiac myocytes.
Mitochondria and energetics

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

VI. Forntschienko; V. Nosar; A. Portnychenko; T. Driyetska; I. Manivkova
Bogomoletz Institute of Physiology, NAS of Ukraine; ICMAN NAS of Ukraine, Kyiv, Ukraine

Purpose: The heart muscle is often lacking of oxygen in pathological conditions, however, myocardial hypoxia is also used as pre-conditioning or therapeutic factor. Nevertheless, basic patterns and sequences of metabolic processes occurring in the heart tissue during various hypoxic conditions are poorly understood. The aim was to determine impact of hypoxic preconditioning or periodic hypoxia on dynamics of energy metabolism in left and right heart ventricles.

Methods: Adult Wistar rats were exposed to the following protocols: normal ventilation (NV, 5 days), hypoxic ventilation (HV, 5 days), normoxic ventilation after hypoxic preconditioning (HVPC, 5 days). The dynamics of oxygen consumption (VO2) and body temperature (Tb) was studied during 3 weeks. In the left and right ventricle samples from euthanized rats, mitochondrial respiration was estimated by Chance, mRNA and protein expression was assayed by RT PCR and Western blotting.

Results: Four phases of physiological changes were found in both experimental conditions, after preconditioning and during PHH. The first phase, hypometabolic, was associated with low ATP production, and accompanied by decreased of VO2, Tb, V3/V4, ATP/ADP and increasing of FAD-dependent substrate oxidation. The second, transition phase was observed by 5-7 days after start, it was characterized by shift of mitochondrial metabolism to activation. In the third phase, hypermetabolic (7–12 days or more), the recovery of metabolism and high ATP production, increased VO2, decreased V4, increased NAD-dependent substrate oxidation, V3/V4, ATP/ADP was found. The fourth, phase of adaptation, was characterized by normalization or reduction of VO2 and mitochondrial respiration. These results were correlated with changes in the expression of HIF-1α and HIF-3α mRNA in the hypometabolic phase, the antioxidant protection was increased due to induction of MnSOD protein, and mitochondrial-associated mechanisms of cardioprotection were raised through the induction of caveolin-3. In the hypermetabolic phase, kinase Akt and Akt-dependent mechanisms of myocardial protection were stimulated promoting antiapoptotic and prohyperpysiotrophic effects.

Conclusion: Thus, common regularities in the phase changes of mitochondrial metabolism in the left and right ventricles in the PHH and during the recovery period after hypoxic preconditioning were found. The sequence of phases as a hypometabolic, transient, hypermetabolic and adaptive has been established. The hypometabolic and hypermetabolic phases are associated with various mechanisms of myocardial protection due to target gene and protein induction.

625  Desmin mutations depress mitochondrial metabolism

N. Smolova1; V. Gaygazidze2; T. Sejersen2; A. Kostareva2
1Almaty Federal Heart, Blood and Endocrinology Centre, Saint Petersburg, Russian Federation, 2Karolinska Institute, Stockholm, Sweden, 3IMMO, Saint Petersburg, Russian Federation

Purpose: Our previous data demonstrated reduced mitochondrial calcium in muscle cells carrying aggregate-prone desmin mutations. We speculated that decreased mitochondrial calcium might affect protective effect of RIP. Our previous data demonstrated reduced mitochondrial calcium in muscle cells carrying aggregate-prone desmin mutations. We speculated that decreased mitochondrial calcium might affect protective effect of RIP.

Materials and Methods: We applied Cell Mito Stress Test (Seahorse Bioscience, USA) to evaluate cell respiration. This experiment allows measuring oxygen consumption in living cells and assessing mitochondrial respiration parameters in real-time mode. Four key parameters were estimated according to the manufacturer’s protocol—basal OCR, ATP-linked (non-phosphorylating) OCR, maximal OCR, and non-mitochondrial OCR. Each experiment consisted of six experimental groups corresponding to (i) control cells and cells transduced with LV encoded one of exogenous desmin:(ii) control cells and cells transduced via LV encoded one of exogenous desmin:(iii) control cells and cells transduced via LV encoded one of exogenous desmin:(iv) control cells and cells transduced via LV encoded one of exogenous desmin:

Results: All cell types had similar bioenergetic profiles: decreasing OCR after oligomycin application, rapid OCR rise after FCCP application, and drop of OCR after rotenone/antimycin A application. Significantly, it was only maximal OCR that declined in the presence of desmin mutations; all other parameters did not show any significant difference between cells expressing various forms of desmin. Relative increase of OCR after FCCP application was 1.95 ± 0.09 for non-transduced cells, 2.36 ± 0.09 for Des WT, 1.85 ± 0.12 for Des L370P, 1.81 ± 0.11 for Des A357P, 1.95 ± 0.11 for Des L370P and 1.87 ± 0.12 for Des D399Y. Thus, Des L345P and A357P, being the most prominent aggregate-prone mutations, resulted in the most prominent decline in maximal OCR in comparison with Des WT, while other mutations decreased maximal OCR but not as dramatically. Furthermore, we found that only cells expressing mutant desmin had relative increase of maximal OCR less than one, implying lack of spare respiratory capacity in some of these cells.

Conclusion: We showed that in the presence of desmin mutations maximal OCR was decreased in comparison to cells harbouring Des WT and spare respiratory capacity rate declining as well. Thus, we assumed that desmin mutations might confine mitochondrial respiration parameters.
Methods: Mitochondrial respiratory function was assessed by high-resolution respirometry whereas ROS production and calcium retention capacity of isolated RHM were measured spectrofluorimetrically.

Results: In RHM respiration on both complex I and II substrates (glutamate/malate and succinate + rotenone, respectively) a significant increase in all bioenergetic parameters was found in treated mitochondria. No changes in sensitivity to Ca2+ were observed whereas the presence of MAO-A and B inhibitors (clorgyline or selegyline, 10 μM) significantly reduced ROS release in mitochondria respiring on glutamate & malate and had no effect in the presence of the complex II substrate.

Conclusions: In diabetic rat hearts, methylene blue 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

JM. Elder, P. O' Gara, J. Sanchez-Alonso; S. Harding; A. Lyon

Imperial College London, London, United Kingdom

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 investigate 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 Langendorff perfusion system and enzymatic digestion. A Seahorse Bioscience XFp 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 proteostasis and cardiac function

T. Ziglajl; V. Prando; N. Pianca; F. Llo Verso; G. Milanò; P. Pesce; M. Sandri; M. Mongillo

1Department of Biomedical Sciences, Padova, Italy; 2Venetian institute of Molecular Medicine (VIMM), Padova, Italy; 3Department of Internal and Experimental Medicine, Padova, Italy

Purpose: The Ubiquitin Proteasome System (UPS) and the autophagy/lionsome system (ALS) mediate the removal of intracellular misfolded/unfolded proteins, and are essential for cardiomyocyte (CM) health. Atrogain1 and MuRF1 are muscle specific ubiquitin-ligases, and we recently demonstrated that mutations in transgenic animal models.

Methods: Cold-induced cardiac hypertrophy is reversed after thermo-neutral deacidimization

C. Ruperez; M. Caro; M. Giralt; F. Villarroya; A. Planavila

University of Barcelona, Barcelona, Spain

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 phenomenon 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/TI) ratio. Moreover, the area of the cardiomyocytes was analyzed and we observed that the cell size was increased after cold exposure indicating that after 3 weeks of cold the mice develop cardiac hypertrophy. At the gene expression level, the hypertrophic marker genes α-Actinin and the ratio α-MyHC were significantly reduced after thermo-neutral conditions and the genes involved in fatty acid oxidation and the glucose transporter Glut1 were completely restored when compared to cold exposed hearts.

References:

Conclusions: Our data indicate that mice subjected to three weeks of cold develop a marked cardiac hypertrophy accompanied by indications of a switch from fatty acids to glucose metabolism. Moreover, one week of thermo-neutrality led to a complete regression of the cardiac hypertrophy and the metabolic expression changes induced by cold exposure.

Cardiovascular Research Supplements

Abstracts

S111
635

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

C. De Luca1, G. Gambino1, L. Petraglia1, A. Elia1, K. Komici1, GD. Femminella1, ML. D’amico1, G. Pagano1, A. Cannavo1, D. Liccardo1, W. Koch1, M. Nolano1, D. Leosco1, N. Ferrara1, G. Rengo1, Federico II University of Naples, Department of Translational Medical Sciences, Naples, Italy; Schmadris Magenforschung IRCCS - Scientific Institute of Tizziano Terme, Cardiologia, Tizziano Terme (BN), Italy; Temple University School of Medicine, CTM, Philadelphia, United States of America

Introduction: 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 up-regulation of G protein-coupled receptor kinase 2 (GRK2), which contributes to dysfunc-
tional beta-adrenergic receptor (beta-AR) signaling and to decrease cardiac inotropic reserve.

Purpose: To test the effects of a long-term restricted diet, started later after MI, on cardiac function, sympathetic innervation and beta-AR signaling in an experimental model of post-ischemic HF.

Methods: Two-months-old male Wistar-Kyoto rats (n=40) were randomly assigned to left ascend-
ing coronary artery ligation to induce MI or sham operation. Four weeks later, a time point when HF was established, HF rats were further randomized to a one year IF dietary restriction or ad libitum diet (standard diet). Thus, our final animal population consisted in 4 groups: Sham normal diet, Sham IF diet, normal diet and HF IF diet.

Results: One year of IF diet resulted in a significant reduction in body weight (p<0.001) and heart weight (p<0.05) when compared to groups treated with normal diet. At the end of the study period, echocardiography revealed that HF animals that underwent to restricted diet resulted in improved systolic function and ameliorated left ventricular remodeling compared to HF rats fed with normal diet. Consistently, invasive hemodynamic showed a significant improvement in cardiac inotropic re-
serve in IF HF rats compared to HF normal diet animals. Importantly, IF diet was associated with a significant increase of cardiac sympathetic innervation, as assessed by confocal microscopy, and with an improved cardiac beta-AR density in HF rats when compared to HF rats treated with standard diet. Accordingly, IF diet resulted in a dramatic reduction of cardiac GRK2 recruitment to the plasma membrane.

Conclusions: We have demonstrated for the first time that IF, started when HF was already estab-
lished, ameliorates cardiac function and inotropic reserve in an experimental model of HF. At the mo-
lecular level, we have found that IF diet significantly improved sympathetic cardiac innervation and beta-AR signaling in HF.

636

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

O. Tooref1, U. Arnt1, N. Landz2, D. Kaan1, J. Leor1, Tel Aviv University, Tel Aviv, Israel, 2Chaim Sheba Medical Center, Tel Hashomer, Israel

Background: Although obesity is considered a major risk factor for cardiovascular diseases, it is asso-
ciated with lower mortality and a better outcome in patients with chronic heart disease (“the obes-
ity paradox”).

Purpose: We aimed to determine whether a high-fat diet (HFD) can protect the failing heart after extensive myocardial infarction (MI).

Methods: We induced MI in 12-week old baicalic female mice. Twenty-four hours later, a first echo-cardiography (echo) 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 per month following randomization. During 6 months of follow-up, HFD-fed mice gained significantly more weight (28.9±1.1 vs. 23.0±0.4; p<0.001, [mean ± SEM]), and had higher plasma levels of cholest-
erol, LDL, HDL and glucose, compared with RCD. Survival was similar between the groups. Signi-
ificantly, compared with RCD, HFD attenuated LV dysfunction (Figure, p<0.04), and reduced LV diastolic distalization (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. Our study chal-
 lenges the traditional dogma and suggest new pathway that could be targeted to reserve or halt the progression of heart failure.

634

Atrial epicardial adipose tissue derives from epicardial progenitors

N. Sufve1, T. Moor Morris2, G. Dilanian1, P. Farahmand1, M. Puceat2, S. Hatem1

1VU University Medical Center, Pathology, Amsterdam, Netherlands; 2VU University Medical Center, Cardiology, Amsterdam, Netherlands; 3University of Amsterdam, Pathology, Amsterdam, Netherlands

Conclusions: AEPDC can differentiate into adipocytes and to contribute to the accu-
mulation of EAT.

633

CD45 is a sensitive marker to diagnose lymphomycotic myocarditis in endomyocardial biopsies of living patients and in autopsies

L. Woudstra2, P.S. Bebeer1, R.W. Emmeren1, L.JM. Juffermans1, A.C. Van Der Walt1, A.C. Van Rossum1, J.P. Wijers2, R. Kruidink2

1VU University Medical Center, Pathology, Amsterdam, Netherlands; 2VU University Medical Center, Cardiology, Amsterdam, Netherlands; 3University of Amsterdam, Pathology, Amsterdam, Netherlands

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

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

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

640

CONTROL CHRONIC COLD (CC) CHRONIC DEACCLIMATATION (CD)

Hearth Weight/Tibia Length Ratio

643

Atrial epicardial adipose tissue derives from epicardial progenitors

N. Sufve1, T. Moor Morris2, G. Dilanian1, P. Farahmand1, M. Puceat2, S. Hatem1

1VU University Medical Center, Pathology, Amsterdam, Netherlands; 2VU University Medical Center, Cardiology, Amsterdam, Netherlands; 3University of Amsterdam, Pathology, Amsterdam, Netherlands

Conclusions: AEPDC can differentiate into adipocytes and to contribute to the accu-
mulation of EAT.
Background: Heart failure with preserved ejection fraction (HFpEF) is one of the leading causes of global morbidity and mortality. HFpEF is driven by pathological remodeling 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 remodeling. 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 (5aza) to inhibit pathological hypertrophy in the heart using preclinical models of HFpEF, the transaortic constriction (TAC) model and the Angiotension-II (AngII) infusion model.

Methods: Wild type C57Bl/6j mice underwent surgical treatment 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 pump was used as the AngII control group. TAC mice were treated for 4 weeks after surgery with intraperitoneal administration of either placebo or 5aza. AngII mice began 5aza 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 5aza 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 5aza 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 5aza 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.

638
Imbalance of the VHL/HIF signaling in WT1+ Epidermal Progenitors results in coronary vascular defects, fibrosis and cardiac hypertrophy

I. Menendez-Montes1, B. Palacios1, B. Escobar2, A.V. Alonso1, G. Guzman1, J. Ruiz-Cabellos1, L.J. Jimenez-Borreguero3, S. Martin-Puig4

1National Center for Cardiovascular Research (CNIC), Myocardial Pathophysiology Area, Madrid, Spain; 2University Hospital La Paz, Cardiology, Madrid, Spain; 3National Center for Cardiovascular Research (CNIC), Cell and Developmental Biology Area, Madrid, Spain

Background: Epidermal progenitors (EPs) of the mammalian heart express Wnt5a tumor 1 (WT1) and contribute to coronary-vascular, interstitial fibroblasts and marginally to cardiomyocytes. WT1+EPs are re-expressed in the adult epicardium upon cardiac injury and has been associated with potential regenerative capacity. Thus, there is a great interest in understanding the signals governing their biology.

Purpose: Our goal is to study the influence of embryonic hypoxia in the biology of WT1+EPs and Epidermal Derived Cells (EPDcs).

Methods: We have generated several conditional hypoxia gain and loss of function models (GOF/LOF) in WT1+EPs. We have performed electrocardiography, nuclear magnetic resonance (NMR), histological analysis and transmission electron microscopy (TEM).

Results: Echocardiographic analysis shows increased width of Left Ventricular Posterior Wall (LVPW) and Inter-Ventricular Septum (IVS) and hypertrophy of the papillary muscles in mutant hearts. Histopathological characterization reveals diffused fibrosis of the IVS, LV, atrial wall and subepicardial region, consistent with the observed enlarged QRS segment. Interestingly main coronary vessels are evidently dilated as confirmed by NMR and present intra coronary fibrin clots by hematoxylin & eosin staining. Furthermore, GOF hearts display myocardial inflammatory infiltration as well as interstitial and pericardial hemorrhages, dying suddenly presumably due to coronary rupture. Von Hippel-Lindau (VHL), a negative regulator of Hypoxia Inducible Factors (HIFs), regulates fibrogenic assembly. Therefore, we investigated whether extracellular matrix components of the coronary vasculature might be affected in this model and potentially contribute to vascular permeability and coronary instability.

We have found that embryonic mutant vessels present abnormal fibrinogen levels, together with defective elastic fibers, pointing to a lesion as a potential novel target of VHL. In addition we have generated WT1+HIF1 and WT1+HIF1 LOF models in the epicardium, finding that while epicardial loss of HIF1 seems to be dispensable, HIF2 deletion in the WT1 lineage leads to decreased LV volume and increased LVPW width together with decreased cardiac function, suggestive of cardiac dilatation. We are currently determining the molecular mechanisms behind these phenotypes.

Conclusion: Our data raise the possibility that altered balance in the VHL/HIF signaling in the epicardium leads to ventricular hypertrophy, fibrosis, coronary defects and inflammation, demonstrating that the epicardial VHL/HIF axis is important for proper cardiovascular development and homeostasis.

639
Diastolic dysfunction is the first stage of the developing heart failure

VI. Kapelko1, VL. Lakomkin1, VI. Kapelko1, VL. Lakomkin1, VL. Kapelko1

1University Hospital La Paz, Cardiology, Madrid, Spain; 2National Centre for Cardiovascular Research (CNIC), Myocardial Pathophysiology Area, Madrid, Spain; 3National Centre for Cardiovascular Research (CNIC), Cell and Developmental Biology Area, Madrid, Spain

Background: Heart failure with preserved ejection fraction (HFpEF) is one of the leading causes of the heart failure with preserved ejection fraction (HFpEF) is one of the leading causes of global morbidity and mortality. HFpEF is driven by pathological remodeling 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 remodeling. 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 (5aza) to inhibit pathological hypertrophy in the heart using preclinical models of HFpEF, the transaortic constriction (TAC) model and the Angiotension-II (AngII) infusion model.

Methods: Wild type C57Bl/6j mice underwent surgical treatment 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 pump was used as the AngII control group. TAC mice were treated for 4 weeks after surgery with intraperitoneal administration of either placebo or 5aza. AngII mice began 5aza 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 5aza 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 5aza 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 5aza 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.

640
Colchicine aggravates coxsackievirus B3 infection in mice

RW. Emmens1, B. Smilde1, L. Woudstra1, G. Gong1, D. Wouters1, S. Zeerleder2, J.L. Murk3, M. Van Ham1, S. Heymans3, S. Juffermans4, A.C. van Rossum5, J.W.M. Niessen5, P.J. Krijnen5, J.U. van der Meer6, J.L. Jimenez-Borreguero1, S. Martin-Puig1

1National Centre for Cardiovascular Research (CNIC), Myocardial Pathophysiology Area, Madrid, Spain; 2National Centre for Cardiovascular Research (CNIC), Cell and Developmental Biology Area, Madrid, Spain; 3National Centre for Cardiovascular Research (CNIC), Virology, Utrecht, Netherlands; 4Cardiovascular Research Institute Maastricht (CARIMA), Maastricht, Netherlands; 5VU University Medical Center, Department of Cardiology, Amsterdam, Netherlands; 6VU University Medical Center, Department of Virology, Utrecht, Netherlands; 7Cardiovascular Research Institute Maastricht (CARIMA), Maastricht, Netherlands

Background: There is a clinical need for an immunosuppressive therapy that can treat myocarditis patients in the presence of an active viral infection. Colchicine is an immunosuppressive agent that disrupts microtubule assembly, was found to be a safe and effective treatment option for pericarditis patients, despite the fact that pericarditis is also commonly caused by viral infection. The aim of this study was to investigate the effects of colchicine in a mouse model of acute coxsackievirus B3(CV3B)-induced myocarditis.

Methods: Four groups of C57Bl/6j mice were included. Control mice (n=8), mice infected with CVB3 (100,000 PFU, n=10), mice with colchicine administration (2 mg/kg i.p, n=5) and mice with combined CVB3 infection and colchicine administration (n=10). After three days, the heart, pancreas and spleen were harvested and evaluated using (immuno)histochemical analysis and CVB3 qPCR.

Results: Mice were terminated at day 3 post-infection as colchicine treatment rapidly resulted in severe illness and mortality in CVB3 infected mice. Colchicine significantly decreased the number of macrophages in the heart in CVB3-infected mice (p<0.01) but significantly increased the number of neutrophils (p<0.01). In the pancreas, colchicine caused complete destruction of the acini in the CVB3-infected mice and also significantly decreased macrophage (p<0.01) and increased neutrophil numbers (p<0.01). In the spleen, colchicine treatment of CVB3-infected mice induced massive apoptosis in the white pulp and significantly inhibited the virus-induced increase of megakaryocytes in the spleen (p<0.01). Finally, we observed that colchicine significantly increased CVB3 levels in both the pancreas and the heart.

Conclusion: Colchicine treatment in CVB3-infected myocarditis has a detrimental effect as it causes complete destruction of the exocrine pancreas and enhances viral load in both heart and pancreas.
Myocardial dynamic stiffness is increased in experimental pulmonary hypertension partly due to incomplete relaxation

WJ, Van Der Laarse; D, Van Groen, SP, Bogards; I, Schalj

1University of Porto, Faculty of Medicine, Department of Physiology and Cardiothoracic Surgery, Porto, Portugal; R. Adao1; P. Mendes-Ferreira1; C. Maia-Rocha1; D. Santos-Ribeiro2; F. Potus2; S. Breuils-Bonnet2; S. Goncharov1; GV. Portnichenko1; LV. Tumanovska1; YV. Goshovska2; TU. Lapikova-Bryhinska1; GV. Bogomoletz1; O. Sirenko1; O. Kuryata1; T. Lusynets2

Background: The inhibitory effect of quercetin on proteasomal proteolysis in aorta and its antihypertensive 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 altered in hypertensive state of SHR. The goal of the present investigation is to elucidate if changes in proteasomal 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 immunoproteasome subunits in aorta tissue of SHR, comparing to Wistar rats. However, expression of genes encoding PSM-2, PSMB8, PSMB9 and PSMB11 were up-regulated comparing with constitutive ones (PSMB5 and PSMB1) in both Wistar and SHR. The mRNA level of genes encoding PSM1, PSM7, PSM10, PSMB10 subunits of proteasome was significantly decreased in Wistar aorta comparing to Wistar rats. However, expression of genes encoding PSM1, PSMB2, PSMB8 and PSMB11 was significantly up-regulated. The inhibitory effect of quercetin on proteasomal protein synthesis in aorta and its antihypertensive effects is mediated not only by influence on catalytic activities of proteasome but also by effect on expression of genes encoding both proteasomal and immunoproteasomal subunits.

Myocardial dynamic stiffness is increased in experimental pulmonary hypertension partly due to incomplete relaxation

W. Van Der Laarse; D. Van Groen, SP. Bogards; I. Schalj

1University of Porto, Faculty of Medicine, Department of Physiology and Cardiothoracic Surgery, Porto, Portugal; R. Adao1; P. Mendes-Ferreira1; C. Maia-Rocha1; D. Santos-Ribeiro2; F. Potus2; S. Breuils-Bonnet2; S. Goncharov1; GV. Portnichenko1; LV. Tumanovska1; YV. Goshovska2; TU. Lapikova-Bryhinska1; GV. Bogomoletz1; O. Sirenko1; O. Kuryata1; T. Lusynets2

Background: The inhibitory effect of quercetin on proteasomal proteolysis in aorta and its antihypertensive 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 altered in hypertensive state of SHR. The goal of the present investigation is to elucidate if changes in proteasomal 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 immunoproteasome subunits in aorta tissue of SHR, comparing to Wistar rats. However, expression of genes encoding PSM-2, PSMB8, PSMB9 and PSMB11 were up-regulated comparing with constitutive ones (PSMB5 and PSMB1) in both Wistar and SHR. The mRNA level of genes encoding PSM1, PSM7, PSM10, PSMB10 subunits of proteasome was significantly decreased in Wistar aorta comparing to Wistar rats. However, expression of genes encoding PSM1, PSMB2, PSMB8 and PSMB11 was significantly up-regulated. The inhibitory effect of quercetin on proteasomal protein synthesis in aorta and its antihypertensive effects is mediated not only by influence on catalytic activities of proteasome but also by effect on expression of genes encoding both proteasomal and immunoproteasomal subunits.

Myocardial dynamic stiffness is increased in experimental pulmonary hypertension partly due to incomplete relaxation

W. Van Der Laarse; D. Van Groen, SP. Bogards; I. Schalj

1University of Porto, Faculty of Medicine, Department of Physiology and Cardiothoracic Surgery, Porto, Portugal; R. Adao1; P. Mendes-Ferreira1; C. Maia-Rocha1; D. Santos-Ribeiro2; F. Potus2; S. Breuils-Bonnet2; S. Goncharov1; GV. Portnichenko1; LV. Tumanovska1; YV. Goshovska2; TU. Lapikova-Bryhinska1; GV. Bogomoletz1; O. Sirenko1; O. Kuryata1; T. Lusynets2

Background: The inhibitory effect of quercetin on proteasomal proteolysis in aorta and its antihypertensive 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 altered in hypertensive state of SHR. The goal of the present investigation is to elucidate if changes in proteasomal 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 immunoproteasome subunits in aorta tissue of SHR, comparing to Wistar rats. However, expression of genes encoding PSM-2, PSMB8, PSMB9 and PSMB11 were up-regulated comparing with constitutive ones (PSMB5 and PSMB1) in both Wistar and SHR. The mRNA level of genes encoding PSM1, PSM7, PSM10, PSMB10 subunits of proteasome was significantly decreased in Wistar aorta comparing to Wistar rats. However, expression of genes encoding PSM1, PSMB2, PSMB8 and PSMB11 was significantly up-regulated. The inhibitory effect of quercetin on proteasomal protein synthesis in aorta and its antihypertensive effects is mediated not only by influence on catalytic activities of proteasome but also by effect on expression of genes encoding both proteasomal and immunoproteasomal subunits.

Myocardial dynamic stiffness is increased in experimental pulmonary hypertension partly due to incomplete relaxation

W. Van Der Laarse; D. Van Groen, SP. Bogards; I. Schalj

1University of Porto, Faculty of Medicine, Department of Physiology and Cardiothoracic Surgery, Porto, Portugal; R. Adao1; P. Mendes-Ferreira1; C. Maia-Rocha1; D. Santos-Ribeiro2; F. Potus2; S. Breuils-Bonnet2; S. Goncharov1; GV. Portnichenko1; LV. Tumanovska1; YV. Goshovska2; TU. Lapikova-Bryhinska1; GV. Bogomoletz1; O. Sirenko1; O. Kuryata1; T. Lusynets2

Background: The inhibitory effect of quercetin on proteasomal proteolysis in aorta and its antihypertensive 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 altered in hypertensive state of SHR. The goal of the present investigation is to elucidate if changes in proteasomal 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 immunoproteasome subunits in aorta tissue of SHR, comparing to Wistar rats. However, expression of genes encoding PSM-2, PSMB8, PSMB9 and PSMB11 were up-regulated comparing with constitutive ones (PSMB5 and PSMB1) in both Wistar and SHR. The mRNA level of genes encoding PSM1, PSM7, PSM10, PSMB10 subunits of proteasome was significantly decreased in Wistar aorta comparing to Wistar rats. However, expression of genes encoding PSM1, PSMB2, PSMB8 and PSMB11 was significantly up-regulated. The inhibitory effect of quercetin on proteasomal protein synthesis in aorta and its antihypertensive effects is mediated not only by influence on catalytic activities of proteasome but also by effect on expression of genes encoding both proteasomal and immunoproteasomal subunits.

Myocardial dynamic stiffness is increased in experimental pulmonary hypertension partly due to incomplete relaxation

W. Van Der Laarse; D. Van Groen, SP. Bogards; I. Schalj

1University of Porto, Faculty of Medicine, Department of Physiology and Cardiothoracic Surgery, Porto, Portugal; R. Adao1; P. Mendes-Ferreira1; C. Maia-Rocha1; D. Santos-Ribeiro2; F. Potus2; S. Breuils-Bonnet2; S. Goncharov1; GV. Portnichenko1; LV. Tumanovska1; YV. Goshovska2; TU. Lapikova-Bryhinska1; GV. Bogomoletz1; O. Sirenko1; O. Kuryata1; T. Lusynets2

Background: The inhibitory effect of quercetin on proteasomal proteolysis in aorta and its antihypertensive 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 altered in hypertensive state of SHR. The goal of the present investigation is to elucidate if changes in proteasomal 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 immunoproteasome subunits in aorta tissue of SHR, comparing to Wistar rats. However, expression of genes encoding PSM-2, PSMB8, PSMB9 and PSMB11 were up-regulated comparing with constitutive ones (PSMB5 and PSMB1) in both Wistar and SHR. The mRNA level of genes encoding PSM1, PSM7, PSM10, PSMB10 subunits of proteasome was significantly decreased in Wistar aorta comparing to Wistar rats. However, expression of genes encoding PSM1, PSMB2, PSMB8 and PSMB11 was significantly up-regulated. The inhibitory effect of quercetin on proteasomal protein synthesis in aorta and its antihypertensive effects is mediated not only by influence on catalytic activities of proteasome but also by effect on expression of genes encoding both proteasomal and immunoproteasomal subunits.
Markers for identification of renal dysfunction in the patients with chronic heart failure

U. Kamiłłowa, 1 L. Alikusov
1Republican specialized scientific-practical Medical Center Therapy and Medical Rehabilitation, Tallinn, Estonia; 2Tallinn medical academy, Tallinn, Estonia

Purpose: To compare efficacy of various methods of evaluation of the renal function (RF) state in the patients with functional class (FC) I-IV of chronic heart failure (CHF).

Methods: This study includes 60 patients with ischemic heart disease with FC I (n=23), FC II (n=19) and FC III-IV (n=18) of CHF. Control group included 20 healthy persons. All the patients were performed glomerular filtration velocity (GFV) by formulae MDRD, microalbuminuria (MAU), urine enzymes concentrations: alaninaminotransferase (ALT), aspartaminotransferase (AST), alkaline phosphatase (AP).

Results: Investigations showed that in the patients with CHF FC I, II and III-IV parameters of Cr were 90.3+3.12, 99.1±5.98, 111.7±5.5 respectively. GFV in the patients with FC I and II was 90.1±7.1 and 80.6±1.3 ml/min/1.73m2 with reliable reduction in the patients with CHF FC III-IV 71.6±6.8 ml/min/1.73m2. The patients with CHF FC I had no GFV<80 ml/min, and in the patients with CHF FC II it was observed in 14% of patients and in 56% of patients with CHF FC III. Analysis MAU showed that in 30% of patients: 3 with CHF FC I, in 5 – CHF FC II and 10 – with CHF FC III. There was noted reliable increase in fermenturia level in comparison with control group: ALT–by 30% and 50%, AST–by 25% and 35%, AP–by 39% and 79% respectively (p<0.05). In the patients with FC III-IV of CHF there was revealed reliable increase in ALT, AST, AP by 79%, 50%, 111% respectively (p<0.001), that indicated about damage of coherentness of tubular epithelium of the kidney tubules. There was revealed direct correlation, which level of MAU directly correlated with FC CHF, fermenturia and Cr in the level of blood (p<0.05) and there was noted inverse correlational relation with GFV.

Conclusion: Thus, measurement MAU, fermenturia may be considered as reliable glomerotubular markers for evaluation of the RF state in the patients with CHF.

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

I. Nassir1, M. Nourddine1, L. Azouzi2, R. Habbal
1 Ibn Rochid University Hospital, Department of Cardiologie, Casablanca, Morocco

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 abnormalities in patients with chronic heart failure.

Methods: This study included 160 patients with ischemic heart disease with FC I-IV of chronic heart failure (CHF). 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, and significantly higher in the subgroup of patients with the levels of blood TG ≥1.7 mmol/l versus 0.851 (0.841; 0.877) ng/ml versus 1.087 (0.861; 1.318) ng/ml (p<0.05). Adiponectin level was correlated with parameters of lipid metabolism in hypertension patients.

Materials and Methods: The study included 30 patients (15 men and 15 women) with hypertension grades 2-3 at the age from 42 to 70. Patients with diabetes mellitus were not included in the study. The control group was consisted of 14 practically healthy people. The investigation complex included measuring blood levels of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), very low density lipoprotein cholesterol (VLDL-C), triglycerides (TG) and the calculation of low-density lipoprotein cholesterol (LDL-C) and atherogenic coefficient (AC). The level of adiponectin was determined by ELISA method.

Results: Serum apelin level in hypertensive patients significantly lower than in healthy volunteers (0.851 (0.841; 0.877) ng/ml versus 1.087 (0.861; 1.318) ng/ml =0.05) and significantly lower than in the subgroup of patients with the levels of blood 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 =0.05). AUROC index for adiponectin was 0.75 for apelin level in hypertensive patients and positively correlates with antiatherogenic lipids. The obtained data can be confirmed the anti-atherogenic properties of apelin.

Conclusion: Thus measurement MAU, fermenturia may be considered as reliable glomerotubular markers for assessment of the severity of coronary heart disease.

651 Interconnections of apelin levels with parameters of lipid metabolism in hypertension patients

K. Yushko, S. Koval, T. Starichenko
1 L’Malya Institute of Therapy, NAMO of Ukraine, Kharkiv, Ukraine

Objective: Endogenous peptide apelin is an important cardiovascular biomarker that is involved in the regulation of blood pressure and cardiac function, glucose metabolism and exhibits anti-atherogenic properties. The aim of this study was to evaluate the interconnections of apelin level with parameters of lipid metabolism in hypertension patients.

Materials and Methods: The study included 30 patients (15 men and 15 women) with hypertension grades 2-3 at the age from 42 to 70. Patients with diabetes mellitus were not included in the study. The control group was consisted of 14 practically healthy people. The investigation complex included measuring blood levels of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), very low density lipoprotein cholesterol (VLDL-C), triglycerides (TG) and the calculation of low-density lipoprotein cholesterol (LDL-C) and atherogenic coefficient (AC). The level of apelin was determined by Enzyme-linked immunosorbent assay.

Results: In the subgroup of 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 =0.05) and significantly lower than in the subgroup of patients with the levels of blood TG ≥1.7 mmol/l (0.851 (0.841; 0.877) ng/ml versus 0.919 (0.861; 1.412) ng/ml =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=-.56, p<0.01), VLDL-C (r=-0.56, p=0.01), AC (r=-0.56, p<0.001) and a positive correlation with HDL-C (r=0.44, p<0.05).

Conclusions: It has been determined that hyperglycemia in hypertension patients associated with decreased blood levels of apelin and this factor inversely correlates with pro-atherogenic lipids and positively correlates with antiatherogenic lipids. The obtained data can be confirmed the anti-atherogenic properties of apelin.

653 Plasma proteomics in hypertension: prediction and follow-up of albuminuria during chronic renin-angiotensin system suppression

M. Baldan-Martini1, L. Mourino-Akareva1, L. González-Calero1, T. Sastre-Oliva1, J.A. Lopez1, J. Vázquez1, G. Alvarez-Llumà1, LUIS M. Ruleipe5, F. De La Cuesta1, MG. Barderas1
1National Hospital of Paragis, Department of Vascular Pathophysiology, Toledo, Spain; 2Foundation Jimenez Diaz, Department of Immunology, Madrid, Spain; 3National Centre for Cardiovascular Research (CNIC), Cardiovascular Proteomics Laboratory & Proteomics Unit, Madrid, Spain; 4University Hospital 12 de Octubre, Hypertension Unit, Madrid, Spain

Abstracts Cardiovascular Research Supplements

Results: Serum MMP-9, oxDLDL levels (p<0.001) in the case group (MMP-9 0.396 ± 0.155 ng/ml, oxDLDL 1.411 ± 0.099 ng/ml) were more than in the control group (MMP-9 0.223 ± 0.087 ng/ml, oxDLDL 1.332 ± 0.163 µg/ml).

The logistic analysis shows that MMP9, oxDLDL, CRP (MMP9 OR=0.985, p<0.001; oxDLDL OR=0.011, p<0.05; CRP OR=0.041, p<0.005) may play a role in the pathogenesis of the plaque rupture. Serum MMP-9 enzyme level was directly correlated with Genius score (r=0.552, p<0.01), CIIS (r=0.340, p<0.01) and CRP (r=0.321, p<0.01) steers.

Furthermore, serum MMP-9 enzyme increases with accordance of severity of the myocardium injury with the statistical significance (p<0.01): the borderline abnormality group (CIS<10, 0.227 ± 0.099 ng/ml, possible injury (CIS 10-15, 0.317 ± 0.132 ng/ml), probable injury (CIS >15, 0.376 ± 0.132 ng/ml) groups. MMP-9 levels were significantly higher in the probable injury groups patients (CIS >15) compared to the possible injury group patients (CIS 10-15) (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 dagnostically beneficial for the coronary atherosclerotic plaque rupture (CRP area=0.733, p<0.001, oxDLDL area=0.635, p<0.05, picture 1).

Conclusion: Serum MMP-9, oxDLDL 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.

Soluble RAGE levels in plasma of patients with cerebrovascular events

C. Falcone1; S. Bozzini2; A. D’angelo3; G. Pelissero4
1University of Pavia, Department of Cardiology, Istituto Clinico di Pavia e Vigevano, Pavia, Italy; 2University of Pavia, Interdepartmental Center for Research in Molecular Medicine (CIRMC), Pavia, Italy; 3Policlinic Foundation San Matteo IRCCS, Department of Internal Medicine, Pavia, Italy; 4IRCCS Pollicino San Donato, San Donato Milanese, Italy

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.