Morphogenetic mechanisms

290
Mir-133 regulates retinoic acid pathway during early cardiac chamber specification
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Introduction: We have previously demonstrated the ability of retinoic acid to regulate the expression the atrial specific markers AMHC-1 and Tbx5 during early cardiac chamber specification. However, the molecular mechanisms responsible for this process still remain unclear. At present, microRNAs represent a novel layer of complexity in the regulatory networks controlling gene expression during cardiovascular development.

Methods: Our model is focused on developing chick at gastrula stages by in vitro electroporation of mir-133, a microRNA which is has been shown expressed at the level of linear cardiac tube.

Results: Our results show the mir-133 expression at the level of the primitive heart tube. Moreover, or work reveals that overexpression of mir-133 suppresses AMHC-1 and Tbx5 expression.

Conclusion: These data support that mir-133, a putative microRNA that targets RARB 3UTR, regulates the early cardiac chamber specification via retinoic acid pathway.

Developmental genetics

294
Association of deletion allele of insertion/deletion polymorphism in alpha 2B-adrenoceptor gene and hypertension with or without type 2 diabetes mellitus
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Background: Emerging evidence that the cannabinoid type 1 receptor (CB1) and its endogenous ligands, endocannabinoids, involved in regulation of feeding behavior and body weight. Over-activation of ECS is associated with metabolic diseases as dyslipidemia and insulin resistance involved in CAD and diabetes.

Objectives: The aim was to determine whether G1359A polymorphism of CNR1 associated with CAD with and without T2DM, and with T2DM patients free of CAD and elucidate the association of CNR1 polymorphism with CAD risk factors.

Patients and Methods: The study was carried on 50 patients with CAD (25 patients with and 25 patients without T2DM), 25 patients with T2DM free of CAD and a group of 20 healthy subjects as a control group. Coronary artery angiography for patient group, serum lipid profile (TG, TC, LDL and HDL) and assessment of G1359A polymorphism of CNR1 by RFLP method were done.

Results: CAD patients with and without T2DM had significantly higher age, fasting blood glucose, systolic and diastolic blood pressure, male gender, smoking, and body mass index (BMI) compared with control. GG genotype and G allele of G1359A polymorphism were significantly associated with CAD patients with T2DM (p<0.05). G allele increased risk of occurrence of CAD with diabetes by 5.22 (OR) 95% CI (1.32-20.54). GG genotype was significantly associated with higher TC (p=0.01), HDL (p=0.001) and BMI (p=0.001).

Conclusion: Association of G1359A polymorphism with BMI and disordered lipid may explain in part its association with CAD patients with T2DM and may encourage use of cannabinoid receptor antagonist in treatment of these disorders.

Cell growth, differentiation and stem cells - Vascular

298
Gamma-secretase inhibitor prevents proliferation and migration of ductus arteriosus smooth muscle cells: a role of Notch signaling in postnatal closure of ductus arteriosus
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Objectives: Patient ductus arteriosus (PDA) is a common congenital heart disease that can cause morbidity and mortality in infants. Vascular remodelling, characterized by proliferation and migration of
smooth muscle cells (SMCs), is an essential process for postnatal closure of ductus arteriosus (DA). Sonic Hedgehog (SHH) is a key regulator of SMC proliferation and homoeostasis, but its role in DA remodeling is unclear. Therefore, this study was to explore the role of Notch signaling in proliferation and migration of DASMCs induced by angiotensin-II (Ang II).

Methods: DASMCs were cultured from neonatal Wistar rats within 1 hour after birth. Proliferation and migration of DASMCs were measured by MTT assay and Boyden chamber assay, respectively. DNA synthesis and cell cycle arrest were determined by BrdU assay and flow cytometry, respectively. Underlying mechanisms including, Ca2+ influx, reactive oxygen species (ROS) production, signal transductions of Akt and MAPK were examined. Further, the effects of DAPT on Ang II-induced nuclear translocation of intracellular domains of Notch1 and Notch 3 receptors were assessed by the confocal microscopy. Finally, expressions of down-stream target genes including Hes1/2/5 and Hey1/2/3 were investigated by RT-PCR.

Results: We found that DAPT inhibited Ang II-induced DASMCs proliferation and migration dose dependently. DAPT also inhibited DNA synthesis, arrested the cell cycle progression in the G0/G1-phase, and attenuated calcium load and ROS production caused by Ang II. Moreover, Ang II-activated ERK1/2, JNK and Akt were also counteracted by DAPT. Finally, DAPT inhibited Ang II-induced nuclear translocation of Notch3 receptor intracellular domain, with down-regulations of its target genes Hes1, Hey2 and Hey3.

Conclusions: Notch inhibition by DAPT prevents Ang I-induced DASMCs proliferation and migration. These effects are potentially mediated by attenuated calcium load, reduced ROS production, and deactivations of MAPK and Akt signal transduction, through the Notch3-Hes1/2/5 pathway. Therefore, Notch signaling has a role in DA remodeling and may provide a target strategy for therapeutic intervention of PDA.

299 Mesenchymal stromal-like cells (MLCs) derived from induced pluripotent stem (iPS) cells: a promising therapeutic option to promote neovascularization

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Background: Stem cell therapy is a promising therapeutic option for treating ischemic events. However, it has been shown that the presence of metabolic disorders, such as obesity and diabetes, negatively impacts on several cell populations that have been suggested to promote neovascularization, such as endothelial progenitor cells, bone marrow-derived stem cells, and mesenchymal stromal cells derived from adipose tissue (A-MSCs). This would compromise the efficiency of autologous cell therapy in these patients, precisely the main potential candidates to be treated. Therefore, new strategies are required to offer these patients a viable therapeutic option.

Purpose: IPSCs can be differentiated to mesenchymal stromal-like cells (MLCs), but the functional equivalence of MLCs and bona fide MSCs is subject of debate. Specifically, little is known about the angiogenic potential of MLCs. Thus, in a first approach to explore the usefulness of MLCs to treat ischemic vascular disease, here we analyzed the endothelial properties and pro-angiogenic potential of iP-SMCs in comparison with A-MSCs.

Methods: IPS-derived MLCs and A-MSCs, both from healthy controls, were cultured and characterized by flow cytometry. Using in vitro assays the endothelial cell differentiation and capacity to form capillary-like structures in in vitro functional assays after endothelial differentiation. Gene expression analysis confirmed endothelial differentiation from both IPS-MSCs or A-MSCs with increased expression levels in the former for CD31 (p < 0.01), ENG (p < 0.01), FLK1 (p < 0.05), KDR (p < 0.001) and TGFβ1 (p < 0.01). When we analyzed the ability to stimulate the formation of capillary-like tubes by HUVECs, only CM from EC-MSCs significantly enhanced the endothelial network formation compared with negative control culture medium (p < 0.01) and CM from EC-A-MSCs (p < 0.01). When HUVECs were incubated with CM from expansion cultures, we observed an antiangiogenic effect of CM obtained from A-MSCs compared to the positive control medium (p < 0.03). In agreement with the functional studies, A-MSCs and ENG were significantly higher expression of the angiogenic molecule thrombospandins-1 (p < 0.001).

Conclusions: Our results show that IPS-MSCs display higher angiogenic potential than A-MSCs. Further studies in patients with metabolic diseases will be required to confirm whether IPSCs could be a better choice to promote neovascularization in this group of patients.

300 Sonic Hedgehog promotes mesenchymal stem cell differentiation to vascular smooth muscle cells in cardiovascular disease

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Background: The morphogen Sonic Hedgehog (Hh) and its signaling pathway components are significantly up-regulated within arteriosclerotic vessels in mice coconitement with enhanced accumulation of SMCs; an effect that is attenuated in vivo following Hh receptor, Ptc1 depletion.2 There is evidence supporting a role for stem cell derived-vascular smooth muscle cells (VSMCs) in contributing to arteriosclerotic vascular disease. In this context, Hh signaling may be an important regulator of stem cell self renewal and differentiation to SMC in vitro.

Aims: Determine the effects of sonic hedgehog on bone-marrow derived mesenchymal stem (MSC) differentiation to vascular smooth muscle cells (SMC) in vitro.

Methods: Murine CD44+ bone-marrow derived mesenchymal stem cells (MSCs) were examined for their capacity to differentiate to SMCs before and after treatment with sonic hedgehog for 7 days in the presence or absence of Hh inhibitors, cyclopamine and HPI-4. The transition to SMC phenotype was determined by examining intermediate (calponin1) and late (myosin heavy chain, Myh11) SMC differentiation marker expression by western blot analysis and immunocytochemistry, respectively.

Results: Hh stimulates mesenchymal stem cell growth in vitro concomitant with a significant increase in SMC differentiation marker expression. Specifically, combinatoric sonic Hh (Shh) increased SMC differentiation marker protein expression (calponin1 |CMN1| and smooth muscle cell myosin heavy chain, (Myh11)) by western blot analysis and immunocytochemistry, an effect inhibited by Hh inhibition with smoothened inhibitors, cyclopamine and HPI-4.

Conclusion: Hedgehog may control mesenchymal stem-like cell differentiation to SMC in vivo.

301 Proinflammatory cytokine secretion and epigenetic modification in endothelial cells treated LPS-Gingivitis

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Introduction: The discovery that the oral stem cells are capable of differentiating into endothelial cells raises the exciting possibility that these cells can be an autologous source for vascular network and represent a model for the study of endothelial disease. Porphyromonas gingivalis-induced endothelial cell apoptosis has been identified as one of the major pathways involved in the progression of periodontal disease, causing chronic inflammation in oral cavity and affect the systemic health in human particularly in the cardiovascular system is related. In fact, recent studies have demonstrated that periodontitis is associated with cardiovascular disease and to epigenetic modification of histones. The purpose of this study was to induce periodontal ligaments stem cells (hPDLCs) toward endothelial differentiation and investigate the role of LPS-Gingivitis (LPS-G) in terms of: i) cell growth, ii) proinflammatory cytokines secretion and iii) epigenetic modulation of histones.

Methods: In vivo endothelial differentiation human periodontal ligament stem cells (hPDLCs) were cultured with endothelial growth medium (EGM-2MV) supplemented with vascular endothelial growth factor (VEGF). Control and induced cells were treated with Sucgil of LPS-G for 24h. Cell growth was evaluated through a MTT test. Subsequently, proinflammatory cytokines were evaluated through a QuantiArray assay (RayBio) and the DNA methylationtransformation (DNMT) was assayed through RT-PCR.

Results: hPDLCs induced to endothelial differentiation expressed endothelial markers CD31, CD34 and formed tube-like structures when cultured on matrigel. MTT assay shows a decrease of cell growth in treated cells and QuantiArray array revealed an increase of IL-6, IL-8 and MCP-1 in endothelial cells treated with LPS-G. RT-PCR showed downregulation of DNA methyltransferases.

Conclusion: Our results demonstrated that the hPDLCs induced to endothelial differentiation express CD31, CD34 markers and from tube-like structure. The secretion of IL-6, IL-8 and in particular of MCP-1, that increases macrophage infiltration, inflammation and insulin resistance in transgenic mice, and contributes to atherosclerosis by attracting monocytes into the subendothelial in union with the down regulation of DNMT involved in the histone modification could suggest the strategic role in the process of pathobiont Porphyromonas gingivalis-induced cardiovascular disease.

Cell death and apoptosis - Vascular

304 Mitophagy acts as a safeguards mechanism against human vascular smooth muscle cell apoptosis induced by atherogenic lipids

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Objective: Mitophagy is a critical cellular process that selectively targets damaged mitochondria for autophagosomal degradation both under baseline conditions and in response to stress to prevent oxidative damage and cell death. Recent studies have linked dysfunctional mitochondria function and reduced autophagy with the development of age-related pathologies. However, the significance of mitochondria autophagy in vessel wall in response to atherogenic lipid stressors is not known. We aimed to investigate the role of mitophagy on human vascular smooth muscle cells (VSMC) apoptosis induced by oxidized low-density lipoproteins (LDL).

Methods and Results: Using a variety of complementary techniques, we reported for the first time that the engulfment of defective mitochondria by autophagosomes occurred in human VSMC in response to oxidized LDL. The molecular mechanism mediating mitophagy in human VSMC involved Drp1-mediated mitochondrial fission, accumulation of PTEN-induced putative kinase 1 (PINK1) and the recruitment of the E3 ubiquitin ligase Parkin to the mitochondria. Likewise, we found increased mitochondrial proteins ubiquitination and LC3 association. Using flow cytometry assay, we showed that PINK1 and Parkin silencing impaired mitophagy flux and enhanced oxidized LDL-induced endothelial cell apoptosis although overexpressed PINK1 and Parkin were protective by limiting cell death. Moreover, reduced Bax levels found in human VSMC expressing Parkin indicated cross talk among mitophagy and mitochondrial apoptotic signaling pathways.

Conclusions: Altogether these data demonstrate that mitophagy is a safeguards mechanism against human VSMC apoptosis induced by atherogenic stressors and highlight mitophagy as a potential target to stabilize atherosclerotic plaque.
Background: Long noncoding RNAs (lncRNAs) are non-protein coding RNAs recently emerging as key players in gene expression. Although for some lncRNAs a relevant role in hypoxic endothelium has been shown, the regulation and function of lncRNAs is still largely unknown in the vascular physiopathology.

Purpose: Transcriptomic changes induced by endothelial cell exposure to hypoxia were investigated by next generation sequencing techniques.

Methods: Paired-end sequencing was performed using polyadenylated RNA derived from human umbilical vein endothelial cells (HUVECs) exposed to 1% O2 or normoxia. Results: More than 2000 differentially expressed genes were identified by bioinformatics analysis, including 122 lncRNAs. Validation was performed extensively by both microarray and qPCR. Among the validated lncRNAs, H19, HNRPS, H2RAS, HMG, MALAT1 and HNRPG were also induced in a mouse model of hindlimb ischemia. To investigate the functional relevance of lncRNAs in endothelial cells, knockdown of H19 expression was performed. H19 inhibition decreased HUVEC proliferation, inducing their accumulation in G1 phase of the cell cycle; accordingly, p21 (CDKN1A) expression was increased. Furthermore, H19 knockdown also diminished HUVEC ability to form capillary-like structures when plated on matrigel.

Conclusion: A high-confidence expression profile of lncRNAs modulated by hypoxia in HUVECs identified a specific and significant impact of H19 lncRNA was shown.

310

Specific circulating microRNAs levels associate with hypertension, hyperglycemia and dysfunctional HDL in acute coronary syndrome patients

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Purpose: Our aim was to identify the effect of hypertension, obesity or hyperglycemia on circulating microRNAs levels in sera and HDL from acute coronary syndrome (ACS) patients.

Methods: microRNAs levels were determined in sera and HDL from 35 stable angina (SA) and 72 ACS patients with unstable angina or at one month after myocardial infarction, with/without hyperglycemia, and 30 healthy subjects by using a cDNA-based screening array and individual TaqMan assays.

Results: From the analyzed CAD patients, over 70% were hypertensive, 48% were obese and 26% were hyperglycemic. Results of the screening and individual analysis showed that microRNA-223, microRNA-92a, microRNA-486, microRNA-125a and microRNA-146a levels were higher in ACS compared to SA group, being increased in hyperglycemic ACS sera compared to the normoglycemic ones. A multiple linear regression model, the body mass index (BMI), serum levels of glucose, HDL-cholesterol, apoA-I and paraoxonase 1 (PON1) activity correlated with the variance of the serum levels of microRNA-223, microRNA-146a, microRNA-125a and microRNA-486 (all, p<0.001).

In a binary logistic regression model, all above microRNAs levels significantly discriminated the risk for ACS with an area under the receiver operating characteristic index of 0.845 (p<0.001), after adjustment for hypertension, obesity, serum glucose, HDL-cholesterol, apoA-I levels and PON1 activity. MicroRNA-122 and microRNA-92a levels together with a high blood pressure had the most significant individual contribution to the above risk prediction. MicroRNA-223, microRNA-486 and microRNA-92a levels in HDL discriminated between ACS and SA patients; their levels were increased in HDL from hyperglycemic ACS patients versus normoglycemic ones.

Conclusions: The levels of specific microRNAs associated with HDL discriminate between ACS and SA patients. The high blood pressure together with two microRNAs, microRNA-122 and microRNA-92a, known to be associated with liver dysfunction and with endothelial dysfunction, respectively, are important contributors to the prediction of the risk for ACS.

311

Phosphodiesterase5A up-regulation in vascular endothelium under pro-inflammatory conditions: a new diagnostic and therapeutic strategy for the omega-3 polyunsaturated fatty acid docosahexaenoic acid

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Phosphodiesterase (PDE) 5A (PDE5A) catalyzes the hydrolysis of cGMP into GMP, thus curtailing NO signaling and favoring inflammation. The ω-3 fatty acids docosahexaenoic (DHA) and eicosapentaenoic (EPA) are considered health-promoting nutrients. However, molecular mechanisms underlying their final effects remain incompletely understood. We therefore investigated whether inflammatory stimuli known to be involved in inflammatory endothelial dysfunction and angiogenesis affect endothelial PDE5 expression, and whether cell exposure to DHA alters such expression.

Methods: Human umbilical vein endothelial cells (HUVECs) were treated with increasing concentrations of the inflammatory and pro-angiogenic stimuli interleukin (IL)-1β, tumor necrosis factor (TNF)α, IL-6, and vascular endothelial growth factor (VEGF) for 24-60 hours. At this time, PDE5a protein and mRNA expression were assessed by Western and qPCR, while the activation of Nuclear factor (NF)-κB and Activator Protein (AP-1, in terms of Rela, c-Fos, c-jun, fos-pho-c-jun, Fos-B-, and Fra-1-DNA binding, were assessed by transactivation assays. To evaluate DHA effect on PDE5A expression, HUVECs were treated with 0.50 μM DHA for 48 hours before stimulation.

Results: PDE5a protein and mRNA expression increased significantly after stimulation with 10 ng/ml IL-1β and TNFα (P<0.01 vs control). DHA treatment of HUVECs for 48 hours before cytokine stimulation reduced PDE5A induction at the protein and mRNA levels (P<0.05 vs IL-1β and TNFα) and, correspondingly, DNA binding of Rela, c-Fos, fos-pho-c-jun and Fos-B- induced by IL-1β (for all, P<0.05 vs IL-1β). CONCLUSIONS: Pro-inflammatory, but not pro-angiogenic stimuli, induce PDE5A expression in the endothelium, with the involvement of inflammatory signaling. DHA reduces PDE5A induction interfering with both NF-κB and AP-1 activation. Since PDE5A inhibitors are now approved for use in erectile dysfunction and pulmonary hypertension and have a potential in treating other

309

Long noncoding RNA landscape of hypoxic endothelial cells

C. Voellenkle

Cardiovascular Research Supplements
314 Cardiovascular risk modifying with extra-low dose anti-cytokine drugs in rheumatoid arthritis

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Background: Rheumatoid arthritis (RA) is characterized by increased cardiovascular risk (CVR), as- sociated with erosive arthritis, high activity and positive rheumatoid factor (RF). These features are considered as additional risk factors for CV events. In our previous studies we defined the efficacy of anti-TNFα antibodies in combination with anti-IFNγ antibodies (both in extra-low dose) in pts with RA.

Aims: to assess the influence of combination of anti-tumor necrosis factor (anti-TNFα in extra-low dose) and area- teron (anti-IFNγ in extra-low dose) on CV factors in pts with RA in opened placebo-controlled study.

Methods: 68 pts with active RA enrolled into investigation were divided randomly on two cohorts. The 1st cohort (38 pts) was treated with combination of anti-tumor necrosis and anaferron, which was added to standard therapy. The 2nd one (30 pts) received placebo of these drugs and standard therapy. We monitored the clinical and laboratory parameters of RA activity, CVR and registered the incidence of CV events during the 3 years period.

Results: All RA pts met the criteria of high/very high CVR. There were no significant differences in demographic and clinical characteristics of compared cohorts as well as in baseline laboratory data. We defined the significant impact of combination of anti-tumor necrosis and anaferron on RA activity: disease activity score (DAS28) and plasma levels of IL-1β, IL-6, TNFα decreased more dramatically in the 1st cohort than in 2nd one. The target blood pressure was reached in 70.6% pts from the 1st cohort (24 pts from 34 pts with AH), whereas in the 2nd one only 32% pts (9 from 28 hypertensives) reached and maintained target BP. The incidence of CV events (unstable angina, severe hypertensive crisis, deteri- oration of chronic heart failure) was fewer in 1st cohort (15.2%) than in 2nd (36.7%).

Conclusions: Combination of two anti-cytokines, containing extra-low doses of antibodies against TNFα and IFNγ can improve the efficacy of RA therapy and can decrease CVR.

315 Conversion of human M-CSF macrophages into foam cells reduces their proinflammatory responses to classical M1-polarizing activation

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Background: Atherosclerosis lesions, cholesterol-laden macrophage foam cells are formed and exposed to M1 and M2-polarizing factors; however, their effects on the proinflammatory and the anti-inflammatory potential of foam cells are not known.

Purpose: To investigate the effects of M1- and M2-polarizing factors on the expression of inflamma- tion-related genes in human macrophage foam cells.

Methods: Human monocytes were differentiated into macrophages in the presence of M-CSF, and then converted into cholesterol-loaded foam cells by incubation with ac-LDL. Finally, the generated foam cells were polarized into M1 phenotype by classical activation with LPS and IFNG or into M2 phenotype by alternative activation with IL-4.

Results: The non-loaded M1- and M2-polarized macrophages were characterized by typical upregu- lation and down-regulation of several key proinflammatory genes, respectively. However, the proin- inflammation response of the foam cells to the classical M1 activation was weaker than that of non- loaded macrophages, as demonstrated by reduced upregulation of TNFA, IL1B, CXCL1 and COX2, reduced TNFα secretion, and tendency for lower NF-kB activation. In contrast, alternative M2 activation of either non-loaded or cholesterol-loaded macrophages failed to suppress the gene expression of proinflammatory cytokines.

Conclusions: Conversion of cultured human macrophages into foam cells suppresses their proinflammatory responses to M1-polarizing factors. Thus, in M1-polarized macrophages, IL-4 and IL-10 reduce the proinflammation and down-regulation of several key proinflammatory genes, respectively. However, the proinflammatory responses to classical M1-polarizing activation remained.

316 Lymphocytic myocarditis coincides with increased plaque inflammation and plaque hemorrhage in coronary arteries, facilitating myocardial infarction

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Background: Lymphocytic myocarditis coincides with increased plaque inflammation and plaque hemorrhage in coronary arteries and as such might play a role in MI induction.

Methods: The three main coronary arteries were isolated at autopsy of patients with LM but without MI (LM; n=10), patients with LM and acute MI of less than 6 hours old (LM+MI; n=11) and controls (n=5). Tissue sections were made of coronary segments and were stained with antibodies against lymphocytes, macrophages, neutrophils and mast cells. Subsequently, inflammatory cells were quantified in the coronary wall layers. Additionally, plaque stability, intraplaque hemorrhage (IPH) and thrombosis were determined by (immuno)histological criteria.

317 Serum osteoprotegerin level predicts declined numerous of circulating endothelial-derived and mononuclear-derived progenitor cells in patients with metabolic syndrome

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Background: Osteoprotegerin (OPG) is a secreted multi-functional glycoprotein belonging to the tumor necrosis factor receptor superfamily and secreted by activated mononuclear cells. Elevated level of OPG was recently found in patients with metabolic syndrome (MetS) and type 2 diabetes mel- litus (T2DM). However, the exact molecular mechanisms mediated OPG to RANK/RANKL axis and vascular complications in MetS have not yet elucidated.

Aim of the Study: To investigate whether an elevated level of osteoprotegerin (OPG) predicts imbalance between different phenotypes of circulating endothelial (ECs) and mononuclear (MPCs) progenitor cells in MetS patients.

Methods: Forty seven patients with MetS and 35 healthy volunteers were prospectively evolved in the study between February 2013 and November 2013. We enrolled dysmetabolic disorder sub- jects without known CV disease including arthrosis pectoris, asymptomatic atherosclerosis (negative contrast-enhanced multiparametric tomography angiography). All patients have given their informed writ- ten consent for participation in the study. MetS was diagnosed based on the National Cholesterol Education Program Adult. Treatment Panel III criteria.

Results: The mean serum level of OPG was significantly higher among entire MetS patients’ cohort compared to the healthy volunteers (1142 ± 186 pg/mL in MetS group vs. 245 ± 75 pg/mL in con- trol group; p < 0.001). Patients with MetS were divided in to two subgroups depending on serum level of OPG using mean value as cutoff point. Subjects with OPG level <1142 pg/mL were included in cohorts with lower (n=18) and higher (n=29) OPG level, respectively.

Multivariate regression analysis adjusted age, sex and BMI has shown that OPG related negatively with numbers of CD14+ /CD309+ cells (r = -0.559, P = 0.001), CD14/CD309/Tie2+ (r = -0.510, P = 0.001), CD68/CD309+ cells (r = -0.298, P = 0.001), triglycerides (r = -0.22, P = 0.001), hs-CRP (r = -0.24, P = 0.001), LDL cholesterol (r = -0.283, P = 0.002), soluble receptor activator of nuclear factor kappa-b ligand (r = -0.303, P = 0.001), serum uric acid (r = -0.218, P < 0.001), and positively related with galectin-3 (r = 0.41, P < 0.001), HOMA-IR (r = 0.36, P = 0.001). The MetS Z score at >1 SD (r = 0.262, P = 0.001), Framingham risk score (r = 0.254, P = 0.001) in multivariate logistic regression analysis we found that OPG, dyslipidemia, galectin-3, and HOMA-IR were independent predictors for depletion in number of circulating ECs and MPCs, alone, as well as combined variable. ECs and MPCs. The comparison of predictive models based on several biomarkers including dyslipidemia, galectin-3, and HOMA-IR has shown a lack of advan- tages of these models versus predictive model constructed on OPG alone.

Conclusions: We found the higher predictive value of elevated OPG level in MetS patients without known CV disease and beyond traditional CV risk factors.

Growth factors and neurohormones - Vascular

320 Effect of gastrin-releasing peptide (GRP) on vascular inflammation

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Gastrin-releasing peptide (GRP), one member of the bombesin-like peptide family, acts as a novel trophic factor to various vascular endothelial cells and the aortic endothelium by induction of cell adhe- sion molecules, ICAM-1 and VCAM-1/mRNAs and proteins. And we found that GRP-mediated inductions of ICAM-1 and VCAM-1 are regulated by nuclear factor-κB (NF-κB) or cAMP response element-binding protein (CREB). Moreover, the antagonist of GRP receptor regulated the prolifer- ation and migration of vascular smooth muscle cells. Taken together, our findings provide a potential role for GRP-GRPR receptor in pathogenesis of vascular dysfunction.

Signal transduction - Heart

323 A new synthetic peptide regulates hypertrophy in vitro through means of the inhibition of mTOR

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Background: GRK5 is part of the family of G protein coupled receptors kinases which are known to regulate GPCR through phosphorylate of effects. It has been shown that the amino-terminal region of GRK5 (GRK5NT) is able to regulate cardiac hypertrophy both in vitro and in vivo, through the in- hibition of the transcription factor NFkB, by means of the RH domain. We have identified the
potential minimum sequence (10 amino acids) which is able to inhibit NfkB and we synthesized a new peptide (mimics the reverse sequence of RGS3L peptide).
Purpose: The aim of this study is to identify the minimum effective region of Rf, evaluate its effectiveness on NfkB activity in cardiac myocytes in response to hypertrophic stimuli and evaluate its effectiveness on the regulation of calcium-calmodulin dependent cardiac transcription factors.
Methods: We developed a novel transgenic mouse model that enabled inducible and conditional deletion of p38α in fibroblasts in vivo by crossing C57BL/6 mice expressing fibroblast-specific tamoxifen-inducible Cre recombinase (Cor1α2-CreERT) with those expressing a modified p38α gene flanked by loxP sites. Deletion of p38α in fibroblasts in vivo was achieved by i.p. injection of tamoxifen (100 mg/kg/day) for 5 days at 3 weeks of age. Time-matched injected male mice (both Cre-negative control and Cre-positive knockout) were subjected to myocardial injury at 10-12 weeks of age by subcutaneous mini-aortic pump infusion of 30 mg/kg isoproterenol (ISO) or saline control for 2 weeks, followed by removal of pumps and recovery for 1 week. Cardiac function was then assessed by Millar conducting partial volume catheter. Heart samples were weighed and analysed for mRNA expression by real-time RT-PCR.
Results: ISO infusion in control mice promoted overt cardiac hypertrophy and dysfunction: increased end systolic volume (ESV) (ISO=17.7 ± 1.8 μl, saline=9.9 ± 1.3 μl; P<0.001), reduced ejection fraction (EF) (ISO=43.7 ± 3.6 %, saline=70.6 ± 4.0, P<0.001), increased heart weight/body area ratio (H/W) (ISO=1.9 ± 0.1 mm/g, saline=1.1 ± 0.1 mm/g; P<0.001) and up-regulation of mRNA for cardiomyocyte hypertrophy markers ANF (P<0.05) and β-MHC (P<0.001). Fibroblast-specific p38α knockout mice exhibited remarkable protection against myocardial injury: ESV (ISO=10.6 ± 1.6 μl, saline=9.9 ± 0.7 μl, P<0.05, n=7-8), EF (ISO=63.9 ± 4.8 %, saline=47.4 ± 3.1 %, P<0.05), H/W (ISO=1.3 ± 0.3 mm/g, saline=1.8 ± 0.33 mm/g; P<0.001). Iso-induced increases in ANF and β-MHC mRNA were also abrogated in fibroblast-specific p38α knockout mice. Conclusion: Selective deletion of p38α MAP kinase in fibroblasts prevents cardiac dysfunction and cardiomyocyte hypertrophy after ISO-induced myocardial injury. 326 Binding to RGS3 and stimulation of M2 muscarinic acetylcholine receptors modulates the substrate specificity of p190RhoGAP in cardiac myocytes M. Levy, T. Wieland Institute of Experimental Pharmacology and Toxicology, Medical Faculty Mannheim, Unih. of Heidelberg, Mannheim, Germany RGS3L (Regulator of G protein signaling 3, long form), which is known to be higher in expression in heart failure, is not only a GTPase activating protein (GAP) for the β-subunits of Gi/o and Gq/11 proteins, but also is able to switch the activation of monomeric GTPase Rac1 to the activation of RhoA after M2 muscarinic acetylcholine receptor (M2/M3/M4) stimulation. The mechanism of this switch is still unclear: p190RhoGAP (p190) is one of the most important regulators of the Rac1/Rho balance in the cell. We have previously found, that a complex formation of p190 with RGS3L, eNOS and caveolin-3, which occurs most likely in caveolae, is important for the appearance of a carbobacth-induced Rac1 activation in rat ventricular myocytes. Therefore we aimed to investigate whether the complex formation between p190 and RGS3L is able to modulate the GAP activity of p190RhoGAP. The functional activity of p190 was analyzed by a pulldown assay using constitutive active Rac1Q46L-GST or RhoA36L-GST covalently linked beads. This assay is based on the principle that the active form of p190 binds only to the GTP-bound form of monomeric GTPases. To verify the effectiveness of RGS3L and p190 a co-expression of RGS3L and p190 causes a switch from RhoGAP to the RacGAP activity of p190, which is likely responsible for the previously described RGS3L dependent decrease in Rac1- and concomitant increase in RhoA activation in cardiac myocytes after M2/M3/M4 stimulation. Therefore, our data reveal an interesting new regulatory mechanism, which could explain the M2/M3/M4RhoGAP induced RhoA activation and increased cardiotoxicity seen in experimental heart failure. 327 Cardiac regulation of post-translational modifications, parylation and deacetylation in LMNA dilated cardiomyopathy mouse model N. Vigier, C. Macquart, M. Chatzifragkoukos; A. Evans; G. Bonne; A. Muchir Hopital Pitié-Salpêtriere, INSERM U974-Institut of Myology, Paris, France The pyridine nucleotide NAD+ , an important player in maintaining the cardiac function in addition to being a major player in redox reactions is also a product of enzymes involved in multiple cellular processes. In particular, two families of enzymes, poly(ADP-ribose) polymers (Parp) and sirtuins (Sirt), consume NAD+ to regulate proteins post-translational modifications, parylation and deacetylation respectively. Parylation is mainly associated with the cellular response to DNA damage but also with regulation of apoptotic factors such as AIF and cleaved caspase-3. Sirtuin’s deacetylation is an important player in the control of energetic metabolism, which regulates mitochondrial biogenesis and function. Furthermore crosstalk between Parp and Sirt is involved in the response to cardiac remodelling. Mutations in the lamin A/C gene (LMNA), encoding nuclear envelope proteins, cause dilated cardiomyopathy by mechanisms that remain incompletely understood. LMNA dilated cardiomyopathy is characterized by an increase in both myocardial mass and volume. Despite current strategies to manage LMNA cardiomyopathy, the disorder remains a common cause of heart failure. The aim of this study was to decipher the post-translational modifications linked to NAD+ in a mouse model of dilated cardiomyopathy. We found that NAD+ was significantly decreased (25%) in total heart tissues from a mouse model of LMNA cardiomyopathy (LmnaH222P/H222P mice, H222P) compared to control mice (WT). We quantified Parp1 and Sirt1 mRNA and protein expression level by RT-PCR and immunoblotting in H222P and WT hearts. Parp1 mRNA and protein levels were both decreased (35% and 50% respectively) in mutants compared with WT, whereas Sirt1 mRNA and protein levels were unchanged. Furthermore, immunoblotting revealed decreased (66%) parylation level and increased (80%) deacetylation level of total proteins in H222P hearts compared with WT hearts. We then evaluated the impact of these changes on apoptosis and mitochondrial function by quantifying the expression levels of AIF, cleaved caspase 3 and mitochondrial complexes I and IV. AIF and cleaved caspase 3 were decreased (65%) and (70%), while mitochondrial complexes I and IV were unchanged. All together, our results suggest, that availability of NAD+ plays a major role in maintaining apoptosis in the heart of a mouse model of LMNA cardiomyopathy but have no effect on mitochondrial function. This study opens new perspectives to explore potential therapeutic targets, as increasing NAD+ synthesis.

Cardiovascular Research Supplements
331 Oxidative stress-induced milk-200c disrupts the regulatory loop around SIRT1, FOXO1 and eNOS
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Introduction: Reactive oxygen species (ROS) play a pivotal role in different pathologic conditions including ischemia, diabetic and age-related vasculopathies. We previously showed that ROS increase milk-200c in human umbilical vein endothelial cells (HUVEC), causing apoptosis and senescence. Herein, we dissect the interaction among milk-200c and three related proteins that modulate endothelial cell (EC) functions: SIRT1, eNOS (endothelial nitric oxide synthase) and FOXO1 (forkhead box O1). SIRT1 promotes endothelial cell survival by deacetylating eNOS-derived nitric oxide (NO) bioavailability, NO in turn, enhances SIRT1 mRNA and protein stability, modulates EC functions and induces vascular relaxation. FOXO1 transcription factor and p33 are SIRT1 deacetylation targets; FOXO1 acetylation inhibits its transcriptional activity decreasing SIRT1 and ROS scavengers transcription; acetylated p33 exhibits enhanced transcriptional activity, apoptosis induction and increased ROS production. Methods and Results: HUVEC were infected either with a lentivirus expressing milk-200c or with a control virus. We found that milk-200c-over-expression decreases SIRT1, eNOS and FOXO1 protein expression. Treatment of HUVEC with 200 nM H2O2 for 18h, 16h, 24h down-modulates SIRT1, eNOS and FOXO1 proteins and anti-miR-200c transfected transfection rescued H2O2 induced protein down-modulation. We demonstrated that milk-200c targets directly the 3’UTR of SIRT1, eNOS and FOXO1. Moreover, HUVEC over-expressing milk-200c display NO decrease and an increase in acetylation of its targets, FOXO1 and p33. In keeping, we previously showed that HUVEC over-expressing milk-200c display apoptosis increase and a p33-mediated milk-200c transcription upon H2O2 treatment. Moreover, milk-200c over-expression in HUVEC inhibited FOXO1 transcriptional activity; accordingly, ROS scavengers were down-modulated and ROS production increased. These results were validated in two in vivo models of oxidative stress i.e. skin fibroblasts from old donors and a mice model of hindlimb ischemia. In both cases milk-200c was higher vs control and its targets i.e. ZEB1, SIRT1, eNOS, FOXO1, were down-modulated.

Conclusion: Using miR-200c disrupts SIRT1/Foxo1/eNOS regulatory loop. This event promotes cell senescence, apoptosis and ROS production, and may contribute to endothelial dysfunction under conditions of increased oxidative stress.

332 Antioxidant therapy prevents oxidative stress-induced endothelial dysfunction and Enhances Wound Healing
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Background: Antioxidant strategies induce a clinical advantage in patients with endothelial dysfunction and atherosclerosis and protect from oxidative damage. Impaired wound healing represents a high cost for health care systems and endothelial dysfunction characterizes dermal microangiopathy and contributes to delayed wound healing and chronic ulcers. Endothelial dysfunction impairs cutaneous microvascular blood flow by inducing an imbalance between vasorelaxation and vasoconstriction as a consequence of reduced nitric oxide (NO) production and the increase of oxidative stress and inflammation. The aim of the present study was to analyze the effects and mechanisms of action of antioxidant regimens on endothelial function Propionyl-L-carnitine (PLC) is a natural derivative of carnitine that has been reported to ameliorate post-ischemic blood flow recovery.

Methods and Results: Antioxidant efficacy of N-acetylcysteine, ascorbic acid and propionyl-L-carnitine (PLC) was evaluated in serum-deprived and TNF-a-stimulated HUVEC and human dermal microvascular endothelial cells (HDMECs) in vitro. Antioxidant pretreatment restored serum-deprived and TNF-a-activated mitrophosphoryl pl-oxidation by reducing flavin adenine dinucleo-
tide level and counteracting increased CAM and NOx+ expression, leukocyte adhesion and inflammatory cytokines secretion. Moreover, Antioxidant pretreatment ameliorated endothelial dysfunction by increasing iNOS, PIGF, VEGER receptors and expression of NOS in HDMECs, vascular endothelial growth factor (VEGF), placental growth factor (PIGF) and reduction of NADPH-oxidase 4 (Nox4) expression. HDMEC dysfunction was prevented by Nox4 inhibition and pl-oxidation inhibition counteracted beneficial effects of antioxidants.

Conclusion: Antioxidant strategies prevented oxidative stress-induced endothelial dysfunction by modulating NOx and pl-oxidation in vitro and improved rat skin flap viability and wound healing by stimulating dermal angiogenesis in vivo. Antioxidant targeting of endothelial dysfunction may represent a promising tool for the treatment of delayed wound healing or chronic ulcers.

Cytoskeleton and mechanotransduction - Heart
336 Novel myosin activator, JSH compounds, increased myocardial contractility without chronotropic effect in rats
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Purpose: The aim of this study is to discover novel preclinical candidate and back-up compounds in the area of cardiac ionotrope through the optimization of JSH lead compounds, which show positive inotropic activity through myocardial activation by enhancing myosin ATPase catalytic activity.

Methods: To optimize JSH lead compounds, JSH family members were studied with the primary emphasize to vascular modeling. More than 100 JSH compounds were tested the inotropic effect in rats using echocardiography (Veo2100, VisualSonics Inc. 25 mL), which were performed by an echocardiographer who is blind on the JSH compound. Sprague Dawley rats was anesthetized by isoflurane and the JSH compound was infused via jugular vein from baseline to the rate of 2, 4, 8, 16 mcg/kg/min in every 5 minutes. The ejection fraction and fractional shortening were compared with unanesthetized. At the end of each rat test, dobutamine was infused as a positive inotropic control.
Ablation of Toll-like receptor 9 causes cardiac rupture after myocardial infarction by attenuating proliferation and differentiation of cardiac fibroblasts

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Background: Toll-like receptor 9 (TLR9)-mediated inflammatory response is involved in the genesis of heart failure. However, its role in myocardial infarction (MI) has not been elucidated. Purpose: This study is performed to elucidate the role of TLR9 signaling pathway in the development of cardiac remodeling after MI.

Methods and Results: TLR9 knock-out (KO) and C57Bl/6 wild-type (WT) mice were subjected to left coronary artery ligation to achieve MI. TLR9-KO mice started to experience cardiac rupture 4 days after MI, and as a result, the survival ratio of TLR9-KO mice was significantly lower than WT 2 weeks after MI (36.4% versus 88.9%, p < 0.05). We analyzed the hearts 3 days after MI. There was no difference in MI size, left ventricular (LV) volume, and LV function analyzed by cardiac magnetic resonance imaging. There was a difference in cardiac fibrosis between the both groups evaluated by Masson’s trichrome stain. The inflammatory cells infiltrated into infiltrated myocardium in the both groups. However, TLR9 deletion did not affect the extent of inflammatory cell infiltration. Although the mRNA levels of collagen and pro-inflammatory cytokines were increased after MI, the extent of the increases showed no differences between the injured hearts of WT and TLR9-KO by RT-qPCR. This result of RT-qPCR was consistent with the histological findings. Tissue inhibitor of metalloprotease (TIMP) mRNA level was upregulated in WT hearts, but not in TLR9-KO 3 days after MI compared to that 1 day post-operation. Gelatin zymography revealed that matrix metalloproteinase 2 (MMP2) activity was increased in TLR9-KO hearts compared with WT hearts after MI. Alpha-smooth muscle actin (αSMA)-positive myofibroblasts and αSMA/Kit-8-double-positive proliferative myofibroblasts were increased in WT injured hearts but not in TLR9-KO.

Conclusions: This study suggests that TLR9-mediated signaling pathway did not affect acute inflammatory responses in injured hearts, but the pathway can ameliorate the occurrence of post-infarct cardiac rupture by promoting differentiation and proliferation of cardiac fibroblasts. The increased MMP2 activity observed in TLR9-KO hearts might contribute to post-infarct cardiac rupture. TLR9 might be a possible therapeutic target for post-infarct cardiac rupture, which is the major cause of sudden death after MI.

Altered vascular remodeling in the mouse hind limb ischemia model in Factor VII activating protease (FSAP) deficiency

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Background: The factor VII activating protease (FSAP) is a multifunctional circulating plasma serine protease. Human genetic studies and investigations in FSAP-/- mice suggest a role for FSAP in stroke, thrombosis and atherosclerosis. This study was designed to investigate the role of FSAP vascular remodeling processes related to arteriogenesis and angiogenesis in the mouse hind limb ischemia model.

Methods: Femoral artery ligation was performed on FSAP-/- mice and C57Bl/6 mice. FSAP vs control (cонтrol) proteases were injected intra muscularly into the upper hind limb after occlusion of the femoral artery and Laser Doppler Perfusion Imaging was performed up to 3 weeks. Furthermore, immune histochemistry and morphometric analysis were done to quantify angiogenesis and arteriogenesis.

Results: A significant delay in blood restoration was still determined after one week (FSAP i.m. 0.60 ± 0.08 vs. control 0.87 ± 0.14, p < 0.05). 2 weeks after the FSAP 1mg group had reached the plateau of the control group demonstrating the same perfusion index (FSAP 0.90 ± 0.03 vs. control 0.91 ± 0.06, n=5). While the capillary density (cells/mm²) within the gastrocnemius muscle, reflecting angiogenesis, remained nearly unchanged in the control group, we observed a compelling twofold increase of capillary density for the FSAP treated animals within the first week after ligation (1720 ± 250 vs. 395 ± 150 capillaries/mm², n=11, p<0.001). Perfusion was not different between the genotypes but there were 2.5-fold more collateral arteries in the adductor muscle of FSAP-/- mice (p<0.05) which was associated with a higher infiltration of monocytes (p<0.05). Capillary density in the gastrocnemius muscle was not altered.

Conclusions: In the absence of endogenous FSAP arteriogenesis is enhanced and this is associated with a greater infiltration of monocytes but angiogenesis is unchanged. Exogenous FSAP had the opposite effect of arteriogenesis. Thus, apart from regulating coagulation processes in the blood FSAP is a regulator of vascular wall remodeling.

Vasculogenesis, angiogenesis and arteriogenesis

343

Pro-angiogenic effects of proly-hydroxylase inhibitors and their potential for use in a novel strategy of therapeutic angiogenesis for coronary total occlusion

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Introduction: Up to 30% coronary chronic total occlusions (CTO) cannot be revascularised by conventional angioplasty. Therapeutic angiogenesis to enhance existing collateral circulation and improve antegrade flow may offer a novel solution for such cases. We have explored the use of proly-hydroxylase inhibitors (PHI), to target a pro-angiogenic ‘master-switch’, the transcription factor hypoxia inducible factor-1α (HIF-1α), with the aim of stimulating a local angiogenic signalling cascade to enhance collateral vasculature and antegrade blood flow and thus provide symptom relief for difficult CTO.

We describe in-vitro and early in-vivo studies on the pro-angiogenic potential of two PHI compounds, FG-2216 and FG-4592 (already in clinical trials for renal anaemia).

Methods: Western blotting was used to assess expression of HIF-1α protein in (1) Human umbilical vein endothelial cells (HUVECs), after treatment with 5-500μM FG-2216 or FG-4592 for up to 24 hours and (2) ex-vivo fresh aorta tissue incubated with 5-100μM FG-4592 for 2 hours. To assess angiogenesis, HUVEC were cultured on growth factor reduced Matrigel, with significantly enhanced tubule formation compared to control (1.2-5 fold increase, p<0.05 vs. ctrl). Capillary density in the gastrocnemius muscle, reflecting angiogenesis, was measured by image analysis of photomicrographs (image J). Quantitative real-time RT-PCR (qPCR) was used to examine the expression of vascular endothelial cell growth factor (VEGF) mRNA from treated HUVEC (up to 24 hours). Cobalt chromium stents were spray-coated with FG-2216 or FG-4592 in a range of programmable elution polymers (PEP), and elution of each PHI compound measured. An in-vivo model of ischemia in which to test the PHI-eluting stents was developed.

Results: Western analysis demonstrated accumulation of HIF-1α protein in human EC within 1.2 hours of treatment with FG-2216 or FG-4592 (Fig1a). A similar effect was observed in ex-vivo aorta treated with 5-50μM FG-4592. Both PHI had a dose response, pro-angiogenic effect on EC cultured on Matrigel, with significantly enhanced tubule formation compared to control (1-2.5 fold increase, p<0.005 Fig1b). qPCR showed a significant maximal 2.4 fold increase (p<0.05) in mRNA for the main angiogenic target of HIF-1α, vascular endothelial growth factor (VEGF), after treatment with these PHI. An optimal PEP blend was established for each PHI compound. An adaptation of the hind-limb ischemia model with stent deployment in the iliac artery was successfully developed.

Conclusions: These novel proof-of-concept investigations confirm that FG-2216 and FG-4592 have a pro-angiogenic effect in-vitro. As part of our strategy for the local, sustained delivery and release of PHI as an effective treatment for difficult CTOs, stent coating and elution studies have been conducted and we have developed proprietary programmable eluting polymers for optimal elution of each drug. FG-2216 and FG-4592-eluting stents are now being evaluated for efficacy and safety in in-vivo studies.

Cardiovascular Research Supplements
344

Nrf2 drives angiogenesis in transcription-independent manner: new function of the master regulator of oxidative stress response

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Background: Nrf2 is a transcription factor, which is a master regulator of oxidative stress response in our cells. Our data demonstrate that angiogenesis driven by stromal cell-derived factor-1 (SDF-1) depends on Nrf2 presence in all-or-none manner.

Purpose: The aim of study was to define a molecular mechanism of SDF-1-induced Nrf2-dependent angiogenic response.

Methods: The study was done on primary human aortic endothelial cells (HAECs) transfected with siRNA against Nrf2 or subjected to transduction with adenoviral vectors overexpressing Nrf2. SDF-1 is a chemoattractant that induces integrin αvβ3 expression in endothelial cells, and the integrin component that interacts with the aPC receptor was investigated in our model. We used ex vivo experiments using aortic rings isolated from Nrf2–/– or wild-type mice. The involvement of Nrf2 in this process was confirmed by stimulation of HAECs with sAPPa, an αPC activator, and by overexpression of αPC with adenoviral vectors. Angiogenesis induced by SDF-1 was evidenced to be independent of Nrf2 transcriptional activity, what is in line with lack of acetylation and delayed nuclear translocation of Nrf2 driven by SDF-1. We demonstrated that a role of Nrf2 transcription factor in angiogenic response of HAECs is related to cytoplasmatic compartmentation of the cells and relies on involvement in actin cytoskeleton rearrangements.

Conclusion: Nrf2 transcription factor regulates SDF-1-induced angiogenesis independently of its transcriptional activity, being involved in actin cytoskeleton rearrangements instead.

345

Angiogenic gene therapy, despite efficient vascular growth, is not able to improve muscle function in normoxic or chronically ischemic rabbit hindlimbs - role of capillary arteriolarization and shunting

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Methods: To study the effects of angiogenic vascular endothelial growth factor (VEGF) on muscle function in normoxic or chronically ischemic rabbit hindlimbs - role of capillary arteriolarization and shunting. Muscle function was measured by guest on December 30, 2016 Downloaded from Cardiovascular Research Supplements 2009 Abstracts S63

346

Effect of PAR-1 inhibition on vessel growth in the murine hind limb model

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Background: inflammation triggers the formation of angiogenesis. It is known that activated protein C (aPC) has anti-inflammatory effects in addition to antiangiogenic effects, and these anti-inflammatory effects are mediated by the protease-activated receptor 1 (PAR-1). Since the extent to which aPC’s anti-angiogenic effects can be modified by PAR-1 have not been fully characterized, we seek to use the aPC transgenic mouse model to examine PAR-1 inhibition on collateral formation. We ligated the right femoral artery of C57Bl/6 transgenic mice, which overexpress activated protein C (aPC-high-mouse). After 3, 7, and 14 days, a selective PAR-1 inhibitor (PAR-1 INH, SCH79797, 44 μM/mL) was injected into the tail vein. Laser Doppler perfusion imaging was used to detect hind limb perfusion pressure post-operatively, after 7, 14 and 21 days, and to calculate the perfusion index (PI, ligated/unligated side).

Results: In the absence of Nrf2, the involvement of Nrf2 in this process was confirmed by stimulation of HAECs with sAPPa, an aPC activator, and by overexpression of aPC with adenoviral vectors. Angiogenesis induced by SDF-1 was evidenced to be independent of Nrf2 transcriptional activity, what is in line with lack of acetylation and delayed nuclear translocation of Nrf2 driven by SDF-1. We demonstrated that a role of Nrf2 transcription factor in angiogenic response of HAECs is related to cytoplasmatic compartmentation of the cells and relies on involvement in actin cytoskeleton rearrangements.

Conclusion: Nrf2 transcription factor regulates SDF-1-induced angiogenesis independently of its transcriptional activity, being involved in actin cytoskeleton rearrangements instead.

347

Quaking is a key regulator of endothelial cell differentiation, neoangiogenesis and anagioinvasion

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Background: Endothelial cell differentiation has a major role in wound healing and is of relevance in pathobiochemical processes.

Methods and Results: In this study, using the model of iPS cells differentiation towards ECs, the RNA binding protein QKI was found to be an important regulator of the VE-cadherin stabilisation and VEGF2 transcriptional activation during the EC differentiation process. IPS cells have been generated based on a highly efficient approach, fully characterized and forced to differentiate towards ECs. The role of QKI has been further elucidated in EC differentiation derived from IPS cells. QKI was found to be induced during EC differentiation from IPS cells, and its expression was shown to be maintained at high levels in mature ECs. Notably, QKI overexpression induced the activation of ECs, whilst knockdown of QKI suppressed EC differentiation. It has been demonstrated that QKI plays a role in the induction and stabilization of VE-Cadherin and activation of VEGF-regulatory binding sites AP1 and STAT3 and STAT3 phosphorylation. Importantly, QKI modulated the transcriptional activation of VEGFR through STAT3 signalling. The notion that QKI indeed played an important role during EC differentiation was further supported from additional data which clearly demonstrated that knockdown of QKI resulted in induction of angiogenesis in vivo, followed by ECs derived from iPS overexpressing QKI improved neoangiogenesis and blood flow recovery almost 100% in a hind limb ischemia model by showing an enhanced engraftment capacity when compared to non-modified iPS-ECs or PBS control groups. Notable, human IPS cells overexpressing QKI induced angiogenesis on Matrigel plug assays in vivo only seven days after subcutaneous injection in SCID mice.

Conclusions: These results demonstrate that QKI holds a key role in EC differentiation highlighting a clear functional benefit in neoangiogenesis, blood flow recovery and angiogenesis.

348

“Emerging angiogenesis” in the chick chorioallantoic membrane (CAM). An in vivo study

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Introduction: In ischemic conditions such as myocardial infarction, new vessels are generated by emerging angiogenesis. The capability to derive ECs from induced Pluripotent Stem (iPS) cells holds huge therapeutic potential. Elucidation of the molecular mechanisms underlying EC differentiation will ultimately advance stem cell regenerative therapy towards reality which would represent a paradigm shift in the treatment of cardiovascular diseases.

Methods and Results: In the ex ovo CAM model, microvasculature was recorded on embryonic day 11 using time-lapse intravital video microscopy for up to 24 hours. A theoretical model of vessel diameter...
349 Exosomes from cardiomycocyte progenitor cells and mesenchymal stem cells stimulate angiogenesis in vitro and in vivo via EMMPRIN

[33x390]Conclusions: Both cell types secrete vesicles with typical characteristics of exosomes, which are efficiently via secreted paracrine factors and among others affect angiogenesis. Among these secreted factors are

Interestingly, a lot of evidence emerged that transplanted cells do have beneficial effects in their environment

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Background: To date, cellular transplantation therapy has not yet fulfilled its high expectations for true cardiac repair. A major limiting factor is the lack of long-term engraftment of the transplanted cells. In literature, there is a lot of evidence emerging that paracrine effects of the transplanted cells via secreted paracrine factors and among others affect angiogenesis. Among these secreted factors are

Conclusions: Exosomes from exosomes are curved and located on the mesh level, at later stages they straighten and sink to a lower level finally getting covered by a reconstituted mesh network cells.

352 Reciprocal regulation of GRK2 and bradykinin receptor stimulation modulate Ca2+ + intracellular level in endothelial cells

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Background: Bradykinin (BK) is an important modulator of the cardiovascular system by regulating cardio-vascular and vascular responses. Several endothelial mediators are under its control, through the G-protein coupled receptors B1 and B2, using Ca2+ + as second messenger. The G-protein coupled receptor kinases GRK2 can modulate B1 and B2 receptors through desensitization. Purpose: To verify the effects of GRK2 inhibition in regulating BK signaling. Methods: In bovine aortic endothelial cells (BAEC) we evaluated BK2 expression as well as intracellular Ca2+ + level in endothelial cells. Endothelial cell migration and vessel formation were all stimulated in the presence of EMMPRIN or MSC exosomes in different in vivo models (scratch wound, spherical sprouting and tube network formation), mediated via ERK/Akt signalling. Additionally, these exosomes in-
Methods and Results: Here we found that H2S, produced through CSE activity upregulated by Zo- nepro, caused endothelium-dependent contractions induced by interleukin-1 beta (IL-1β)- or tumor necrosis factor alpha (TNF-α)-induced HUVECs, es- pecially the NF-κB/cyclooxygenase-2/prostacyclin biochemical pathway. The pre-incubation with Zo nepro/H2S prevented IL-1β-induced paracellular permeability through the control of ex- pression and localization of cell-leaflet junctional markers ZO-1 and VE-cadherin. Moreover, Zo nepro/ H2S reduced the expression of the endothelial markers CD40 and CD31, involved in the recruitment of circulating mononuclear cells and platelets.

Conclusions: These in vitro data document the anti-inflammatory activity of Zo nepro/H2S on vascular endothelium, reinforcing the cardiovascular protective effect of this multitasking drug.

356 A new class of glycomimetic drugs to prevent free fatty acid-induced endothelial dysfunction
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Background: Carbohydrates play a major role in cell signaling in many biological processes. We have developed a set of glycomimetic drugs that mimic the structure of carbohydrates and represent a no- vel source of therapeutics for endothelial dysfunction, a key initiating factor in cardiovascular complications.

Purpose: Our objective was to determine the protective effects of small molecule glycomimetics against free fatty acid-induced endothelial dysfunction, focusing on nitric oxide (NO) and oxidative stress pathways.

Methods: Four glycomimetics were synthesized by the stepwise transformation of 2,5-dihydroxy- benzoic acid to a range of 2,5-substituted benzoic acid derivatives, incorporating the key sulfate functional groups to mimic the interactions of heparan sulfate. Endothelial function was assessed using acetyl- choline-induced, endothelium-dependent relaxation in mouse thoracic aortic rings using wire myo- graph. Human umbilical vein endothelial cell (HUVEC) behavior was evaluated in the presence or absence of the free fatty acid, palmitate, with or without glycomimetics (1μM). DCFH-DA and H2DCF-DA assays were used to determine nitric oxide (NO) and reactive oxygen species (ROS) production, respectively. Lipid peroxidation colorimetric and antioxidant enzyme activity assays were also carried out. RT-PCR and western blotting were utilized to measure Akt, eNOS, Nrf-2, NQO-1 and HO-1 expression.

Results: Ex vivo endothelium-dependent relaxation was significantly improved by the glycomimetics under palmitate-induced oxidative stress. In vitro studies showed that the glycomimetics protected HUVECs against the palmitate-induced oxidative stress and enhanced NO production. We demon- strate that the protective effects of pre-incubation with glycomimetics occurred via upregulation of Akt/NOS signaling, activation of the Nrf2/ARE pathway, and suppression of ROS-induced lipid peroxidation.

Conclusion: We have developed a novel set of small molecule glycomimetics that protect against free fatty acid-induced endothelial dysfunction and thus, represent a new category of therapeutic drugs to target endothelial damage, the first line of defense against cardiovascular disease.

357 Endothelial progenitor cells to apoptotic endothelial cell-derived microparticles,ratiation differentiantessae preserved from reduced ejecction fraction heart failure
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1Smooth muscle and pericytes

Abstracts

362 CXCR1 positive myeloid cells regulate vascular smooth muscle tone by inducing calcium oscillations via activation of IP3 receptors
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Background: CXCR1+ myeloid cells (CMC) are reported to contribute to pathological vascular remodeling. CMC are closely associated with blood vessel lumen and exhibit patrolling behaviour, however the physiological influence of the patrolling CMC on regulating vascular tone is not known.

Purpose: We tested the hypothesis that patrolling CMC due to their close association with vascular lumen, regulate vascular tone.

Methods: Single cell contractions coupled with imaging, arterial ring contractions in tissue-baths and fluorescence activated cell sorting (FACS) techniques were used to study the vascular smooth muscle contractions and isolate cells respectively. A novel FACS based protocol was developed to study cal- cium dynamics in Flu-o-4 loaded cells stimulated with vasoconstrictors (Phenylnephrine and Angioten- sin II) in absence or presence of freshly isolated CXCR1+ myeloid cells from bone marrow.

Results: Single cell and arterial ring contraction assay, presence of myeloid cells significantly (p < 0.01) enhanced the Phenylnephrine (24.5 ± 4.51% and 81.62 ± 12.45 % respectively) and Angio- tension II (Ang II; 28.39 ± 3.25% and 68.42 ± 9.61 % respectively) induced contractions. The intra- cellular calcium levels in vascular smooth muscle cells (VSMC) at baseline was significantly (p < 0.05) increased by 12.29 ± 3.87 % in presence of myeloid cells. This increase in intracellular calcium levels was specifically derived from extracellular calcium source as we did not observe any difference in intra- cellular calcium levels under extracellular calcium free condition. Stimulation of VSMC with 100 nM Ang II increased intracellular calcium levels by 30.19 ± 9.18 % and 21.79 ± 9.74 % in absence or presence of myeloid cells respectively. Although the effects of Ang II on intracellular calcium levels in VSMC was blocked by 10 μM infｕdipine (an L-type calcium channel blocker), this was however not so in presence of myeloid cells. Interestingly in presence of myeloid cells, following 100 nM

Smooth muscle and pericytes

Cardiovascular Research Supplements

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Ang II stimulation, we observed continuous calcium oscillations in VSMC up to 20 minutes, which was insensitive to 10 μM nifedipine but sensitive to 10 μM 3-amino-phenylphosphonate (APAP), indicating the involvement of IP3 receptors.

Conclusion(s): We report here a novel mechanism of arterial smooth muscle tone regulation by CX3CR1+ myofibroblasts. Further understanding of this cellular influence on vascular tone may help identify novel targetable myofibroblast specific factors regulating vascular tone.

363

A novel function of PI3Kg on eNOS regulation, role in arterial wall hyperplasia through modulation of smooth muscle cells proliferation

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Arterial fibrosis and stiffness are characterized by vascular smooth muscle cells (VSMC) proliferation. These hyperplasic processes involved inflammation as well as disrupted flow contributing to vascular remodeling. We investigated in aortic smooth muscle cells molecular mechanism involved. We showed that forskolin induced higher elevation of cAMP in PI3Kg-KO genotype compared to PI3Kg-KD and WT indicating that PI3Kg could modulate degradation of this nucleotide through a catalytic independent function of PI3Kg in non-immune cells promotes IH development. In addition a flow in-duced arterial remodeling model shown that PI3Kg-KO mice present a lower medial hyperplasia compared to PI3Kg-KD mice or WT mice, demonstrating a catalytic independent function of PI3Kg. We investigated in aortic smooth muscle cells mechanical function involved. We showed that PI3Kg deletion induced a decrease in phosphodiesterase (PDE) 4B activity in WT, PI3Kg-KD and PI3Kg-KO cells suggesting a PI3Kg-dependent PDE 4B control. These data suggest the involvement of nongenomic pathway in the mechanism of ALDO-induced platelet activation.

Conclusion(s): These data provide evidence for a novel function of PI3Kg on cAMP regulation, role in arterial wall hyperplasia through modulation of smooth muscle cells proliferation.
in the ferric chloride model, the thrombus is mainly composed of platelets, while thrombus that occur after stenosis mainly consist of fibrin and red blood cells.

Conclusion: After validation of the mouse thrombosis models, we are ready to start testing the newly synthesized 111In-DTPA–A14 probe. We hypothesize that the probe will bind better to the thrombus in the stenosis model, as the fibrin content is higher than in the Ferric Chloride model.

370 The antiplatelet effects of structural analogs of the taurine chloramine

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Introduction: Antiplatelet agents, which produce a chemical modification of targets, are employed for an inhibition of platelet functions in clinical practice and scientific researches. The platelet functions are suppressed by chloramine derivatives of amino acids and taurine (2-aminoethanesulfonic acid). These chloramines modify chemically the free cysteine and methionine residues that are located near a protein surface. Such residues are available in the platelet chemoreceptor for ADP (purine receptor PY212) whose activation is an important step in arterial thrombus formation.

Purpose: This work was aimed at a design of structural analogs of chlorotaurine which are inner stable and simultaneously possess a reactive specificity towards the free cysteine or methionine in protein targets. Also, we intended to explore the selective action of compounds studied on platelets in whole blood.

Methods: Rate constants for the reactions between glutathione (or methionine) and the studied chloramines were determined with uv-spectrophotometry. The ratio of the glutathione rate constant to the methionine rate constant was a measure of the chloramines reactive specificity. We used bovine serum albumin (BSA) as a model for a detection of the protein modifications. Rabbit blood was treated with the 0.25 mM investigated chloramines. Platelet aggregation was induced with ADP and measured with whole blood aggregometer.

Results: Several structural analogs of n-chlorotaurine were synthesized, including n-methyl, n-isopropyl, n-acetyl analogs. The above-mentioned ratio was equal approximately 0.2 for n-methyl-n-chlorotaurine. The ratio comes up to several orders in the case of n-chloromethane (data received by other authors). The BSA tryptophan fluorescence was diminished by 5-10 % under addition of n-chlorotaurine, n-acetyl-n-chlorotaurine or n-methyl-n-chlorotaurine at molar proportion 1:1. This effect represents a change of BSA tertiary structure. n-Acetyl-n-chlorotaurine and n-methyl-n-chlorotaurine also inhibited the platelet aggregation in whole blood. We observed a pronounced decrease in the aggregation (about 40 %) at the 0.25 mM chloramine. Judging by haemolysis absence, the essential erythrocyte damage did not occur.

Conclusion: Our results demonstrate the antiplatelet effect of the structural analogs of taurine chlorination has a selective character, i.e. at the significant suppression of platelets aggregation, changes of red cells do not take place. The structural analogs of taurine chloramine may be usable in the investigations of thrombus formation with platelet participation.

371 The influence of heparin anticoagulant drugs on functional state of human platelets

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Introduction: Heparin drugs are widely used in clinical practice for therapy and prevention of thrombotic events. Efficiency of heparin therapy is limited by the risk of thrombocytopenia and local rethrombosis. Monitoring of platelet functional state is necessary for safe using of heparin anticoagulant drug. The aim of this work was to study influence of unfractionated heparin (UH), low molecular weight heparin (LMWH) and synthetic pentaacetyldiphenylophosphine on platelet aggregation and secretion of serotonin and histamine on platelet aggregation. rabbit plasma.

Methods: Human platelets were isolated from blood of healthy volunteers. Aggregation was studied in preparations of platelet rich plasma (PRP) and washed platelets. Activity of platelet PAI-1 was determined in human plasma by ELISA method.

Results: PAI-1 activity in human plasma from patients treated with LMWH and UH was increased, while PAI-1 activity in blood plasma can be prerequisite for further thrombotic complications during heparin therapy. Therefore, the determination of platelet PAI-1 level can be considered as additional diagnostic test if there is a risk of thrombus formation.

Conclusion: The obtained results showed pleiotropic character of heparin anticoagulant drugs. The increase of PAI-1 activity in blood plasma can be prerequisite for further thrombotic complications during heparin therapy. Therefore, the determination of platelet PAI-1 level can be considered as additional diagnostic test if there is a risk of thrombus formation.

372 Regulation of platelet aggregation and adenosine diphosphate release by dimer in acute coronary syndrome (in vitro study)

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Background: Platelets glycoproteins IIb/IIIa and lb, the receptors for fibrinogen and von Willebrand factor are also known as fibrin substrates, raising the question of whether activation of fibrinolysis directly modulates the formation of the primary platelet plug. In several clinical studies it has been shown that platelets may initiate thrombotic reocclusion of blood vessels following successful thrombolytic therapy. The role of platelet function as it relates to fibrinolysis needs further investigation.

Purpose: The purpose of this study was to examine the effects of D dimer concentration on adenosine diphosphate (ADP)-induced platelet aggregation and ADP release in vitro.

Methods: The study included normal patients admitted for acute coronary syndrome (ACS) and 10 practically healthy volunteers (PHV). Blood samples of patients were obtained within 6 hours since ACS manifested and before thrombolytic therapy start or primary PCI performed. Assessment of ADP-induced platelet aggregation and ADP release was performed by impedance and luminescence aggregometry. All samples were preincubated with D dimer samples with increasing concentrations (0, 190, 452, 853 and 1365 ng/ml). Results: Preincubation of citrate whole blood samples with D dimer alters subsequent ADP release in response to ADP and platelet aggregation (measured as maximal amplitude of impedance). Preincubation with 452 ng/ml D dimer significantly inhibited ADP release in PHV and patients with ACS (24.6 % or 43.9 %, p < 0.05). Both in PHV and patients with ACS there was Li shaped dependence between D dimer concentration and ADP release (fig. 1: full line - ACS, dotted line - PHV), statistically significant as to its extremums (ANOVA Chi Squ. = 11.96 p = 0.018; coeff. of concordance = 0.59; aver. rank r = 0.49). Maximal rate of ADP release in PHV was at 691.9 ng/ml vs 1303.7 ng/ml D dimer. In PHV ADP release minimum was associated with 412 ng/ml D dimer, while in patients with ACS it was 593.6 ng/ml.

Conclusions: These data show that D dimer concentration can modulate platelet function in vitro. Received results suggest presence of a feedback mechanism impaired in patients with acute coronary syndrome.

Oxygen sensing, ischaemia and reperfusion

375 Sirtuin 5 mediates brain injury in a mouse model of cerebral ischaemia-reperfusion

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Background: Ischemic stroke is one of the leading causes of mortality and morbidity in industrialized countries and, due to the progressively aging population, its incidence is predicted to increase further. Sirtuin 5 (SIRT5) protects from ischemia/reperfusion injury in the heart (Boyston et al. 2015). However, whether SIRT5 displays similar properties in ischemia/reperfusion-induced brain injury still remains unknown.

Aim (s) or purpose: To investigate the role of sirtuin 5 (SIRT5) in a murine model of cerebral ischaemia-reperfusion (IR) induced by middle cerebral artery occlusion (MCAO).

Design & methods: Male 12- to 14-week old C57B6/6 mice were randomly divided into SIRT5 transgenic (SIRT5) or scrambled siRNA control (siC57) treated groups and subjected to transient middle cerebral artery occlusion (MCAO) for 45 min followed by 24 h of reperfusion. Knockdown of SIRT5 was performed 24 h before MCAO using small interfering RNA. Final infarct volumes were analyzed by TTC (2,3,5-triphenyl-2H-tetrazolium chloride) staining at 24 h after ischemia. Neurological deficit was determined by Bederson index and rotorad performance test at 2 h, 24 h and 48h post-MCAO.

Results: siSIRT5 treated mice displayed smaller infarct volumes compared to siC57 (24.15 ± 2.61 vs. 31.2 ± 1.50 % p=0.03, Figure 1). In line with the above, siSIRT5 mice demonstrated blunted neurological deficit as compared to siC57 at 48h post-MCAO as assessed by the Bederson index (0.68 ± 0.14 vs 1.27 ± 0.19, p=0.036). In addition, there was a tendency for increased latency to fall in the rotarod test in siSIRT5 compared with siC57 (81.3 ± 2.51 vs 69.0 ± 6.21 s, NS, data not shown) nonetheless, this difference did not reach statistical significance.

Conclusions: Knockdown of SIRT5 improves stroke outcome including stroke size and neurological deficit in mice indicating a deleterious role of this histone deacetylase in these experimental settings. Additional experiments will be performed to elucidate the underlying mechanisms of this effect.
Protective effects of ultramicronized palmitoylethanolamide (PEA-um) in diabetic cardiomyopathy.

Introduction: Abscisic acid (ABA) is a phytohormone which lies at the interface between abiotic stress and metabolic signaling in plants. We previously showed that ABA is also a mammalian hormone involved in glycemia homeostasis. Plasma ABA (ABAP) increases after a glucose load in healthy (Brunzzone et al., 2012), but not in diabetic subjects (Amer et al., 2015), and ABA stimulates the disposal of glucose directly via production of glucose uptake by skeletal muscle cells and adipocytes (Brunzzone et al., 2012), and indirectly by stimulating insulin secretion (Brunzzone et al., 2008). The functional effects of ABA on animal cells are mediated by its receptor LANCL2 (Sturla et al., 2009; Bruzzone et al., 2012).

Methods: Rat H9c2 cardiomyoblasts were cultured in 95% N2 and 5% CO2 for 15 hours and ABA release was measured. NO release from human vascular endothelial cells (HUVEC), from H9c2 cells and from neonatal ventricular cardiomyocytes (NVMC) isolated from wild-type or from LANCL2/-/- mice, cultured with or without ABA, was measured with the fluorescent probe 2,3-diaminonaphthale (DAN) (Misko et al., 1993). Glucose uptake by H9c2 cells in the presence of ABA was evaluated with [14C]-2-deoxy-D-glucose (Bruzzone et al., 2012).

Results: Hypoxia stimulated ABA release from H9c2 cardiomyoblasts (panel A). ABA stimulated NO production in HUVEC and H9c2 cells (panel C). Finally, ABA stimulated glucose uptake by H9c2 cardiomyoblasts (panel B).

Conclusions: These in vitro results suggest a role for endogenous ABA, through its receptor LANCL2, in the protection of cardiomyocytes against oxygen depletion, by stimulating NO production and glucose uptake. Dysfunctional ABA production in DM may thus be involved in the dysregulation of both glycemia homeostasis and cardiac protection. This hypothesis warrants further investigation aimed at the identification of a possible new target for therapeutic intervention in diabetic cardiomyopathy.

Abstracts

376 Abscisic acid: a new player in cardiomyocyte protection from ischaemia?

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Introduction: Abscisic acid (ABA) is a phytohormone which lies at the interface between abiotic stress and metabolic signaling in plants. We previously showed that ABA is also a mammalian hormone involved in glycemia homeostasis. Plasma ABA (ABAP) increases after a glucose load in healthy (Brunzzone et al., 2012), but not in diabetic subjects (Amer et al., 2015), and ABA stimulates the disposal of glucose directly via production of glucose uptake by skeletal muscle cells and adipocytes (Brunzzone et al., 2012), and indirectly by stimulating insulin secretion (Brunzzone et al., 2008). The functional effects of ABA on animal cells are mediated by its receptor LANCL2 (Sturla et al., 2009; Bruzzone et al., 2012).

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378 Identification of stem cell-derived cardiomyocytes using cardiac specific markers and additional testing of these cells in simulated ischemia/reperfusion system

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Introduction: Cardiomyocytes have developed to a leading cause of death in modern society. We focused on an accessible model, which provides a reliable and convenient cell source that can be used for study of cardiac cell pathophysiology as well as disease modeling, cardiac cell replacement therapy and for pharmaceutical investigations. In this project, we aimed to identify cardiomyocytes in embryonic bodies via detection of the cell surface antigen VCAM-1 and intracellular cardiac specific marker Troponin I. Furthermore we subjected these cells into simulated ischemia-reperfusion by the aim to examine the hypoxic sensitivity of the cells.

Materials and Methods: As we wanted to reveal the age specific differences of cardiac specific markers, we worked with two stages of embryonic bodies (EBs) 8 and 16 days old. EBs were grown on gelatin coated coverslips. After the cells digested into a single cell suspension, the identification of cardiac cells was performed by using fluorescent activated cell sorting method. For another group of cells we performed simulated ischemia-reperfusion experiment to model the effects of a heart attack and evaluate the rate of cell survival. Mouse EBs (from H1M embryonic induced and incubated pluripotent stem cells, iP-3.4, iP-4.1) were subjected to a 150 min ischemic period, which was followed by a 120 min reperfusion. Cell viability of EBs was evaluated using propidium iodine viability assay.

Results: The intracellular antigen cTnI was remarkably expressed in both day-8 and -16 with all types of digestions. The highest cTnI expression was registered on day-8 (75% of total cells) with application of trypsin. The cell surface antigen VCAM-1 was not detectable after trypsin digestion. However, the application of collagenase type IV on day-16 resulted in the highest ratio of VCAM-1 positive cells (41%). IPS (line 3.4) EBs and VCAM positive cells were more sensitive to SI/R injury than iP-4.1 cell line. Interestingly, SNAP protected full EBs from SI/R, but not immunopositive cardiac myocytes isolated from the EBs.

Conclusion: We conclude that the cardiocyteprotective NO donor protects full EBs of H1M cells against SI/R injury but not iP-4.1 EBs. Suggesting that cells from embryonic origin are eligible but iP- derived cardiac myocytes at this age are not suitable for testing cardioprotective mechanisms.

379 Single-dose intravenous metformin treatment could afford significant protection of the injured rat kidney in an experimental model of ischemia-reperfusion

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Background: Despite novel therapeutic strategies, renal ischemia-reperfusion (IR) injury still remains an unresolved problem in pharmacotherapy. Recent studies have shown that metformin, an oral antidiabetic agent, also posses anti-inflammatory and antioxidant effects. Purpose: We aimed to analyze the acute effects of a single-dose intravenous metformin administration as three different times in the experimental model of IR kidney injury in rat.

Methods: Male adult Wistar rats (n=57; b.w. 250-300 g) were acclimatized and then subjected to bilateral renal ischemia (45 min) followed by reperfusion with saline lasting 4 hours. This study was carried out in strict accordance with the Animal Welfare Act of the Republic of Serbia (Official Gazette of the Republic of Serbia No. 41/09) and “Principles of laboratory animal care” (NIH Publication No. 85-23 revised 1985). Metformin was administered in doses of 3 mg/kg iv. and 10 mg/kg iv., 30 min before I, 30 min before R and 5 min before R. Selected biochemical (uric, creatinine and fractional excretion of sodium) and pathohistological parameters (tubular necrosis, interstitial edema, loss of brush border, casts formation and total histological score), KM-I staining score and parameters of oxidative stress (MDA, SOD, CAT, GPx) were followed in the Sham-operated animals and rats subjected to IR injury and pretreated with saline or chloroquine. These markers were obtained from the appropriate serum, urine or tissue samples at the end of reperfusion period.

Figure 1. Impact of SIRT5 silencing on cerebral lesion width

A. Cardiomyocytes were cultured in 95% N2 and 5% CO2 for 18 hours. Total ABA content was measured by ELISA.

B. Hypoxia (85%) relative to normoxia, by t-test. BB MYC cells were cultured without or with 10 µM ABA for 20 min and uptake of [3H]-deoxyglucose was measured. *P<0.01 vs control, t-test. HC2 and H9c2 cells (panel C) or neonatal ventricular cardiomyocytes from wild-type or from LANCL2/-/- mice (panel B) were cultured for 30 min without (control) or with 10 µM ABA. The expression of cTnI was measured using the fluorescence probe SARA. **P<0.01 relative to untreated cells, by t-test. (measure2D from 4 experiments).


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Results: Both doses of metformin (3 mg/kg i.v. and 10 mg/kg i.v.) were protective regarding biochem- ical and histological markers of I/R injury (serum creatinine and fractional excretion of sodium, as well as total histological score, tubular necrosis score and KIM-1 staining score) (P < 0.05 vs. corre- sponding controls, i.e. rats subjected to I/R injury and treated with saline only). The protective effects of the lower dose of metformin were more profound. Time-related differences between pretreat- ment and post-treatment were not observed (P > 0.05, all). Metformin (3 mg/kg i.v.) significantly reduced levels of MDA and GSH-Px regardless of the time of injection (P < 0.01). However, metformin in dose of 10 mg/kg, i.v. reduced, but did not completely abolished the effects of I/R injury (P < 0.05).

Conclusion: Our study shows for the first time that a single dose of metformin (3 mg/kg i.v.) could afford significant protection of the injured rat kidney.

380 Cardiotoxicity of long acting muscarinic receptor antagonists used for chronic obstructive pulmonary disease

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Chronic obstructive pulmonary disease (COPD) is a progressive, inflammatory condition of the air- ways due to the pathogenesis of emphysema and bronchitis. Muscarinic receptor antagonists, such as Ipratropium, (short acting - SAMAs) and long acting Acldinium and Tiotropium (LAMAs) are used to alleviate symptoms associated with COPD. These drugs target muscarinic receptors involved in the modulation of airway bronchoconstrictors; thus blocking muscarinic receptors to result in distal effects. However, recent clinical studies have correlated the use of aminosugars with an increased risk of cardiovascular events, including stroke and myocardial infarction.

The aim of the current study was to assess the cardiac safety profiles of selected LAMAs used in vitro models of myocardial ischaemia-reperfusion (IR) injury. Following 20 minutes of stabilisation, Langen- dorff hearts, from male Sprague Dawley rats, were subjected to regional ischaemia (35 minutes) and subsequent reperfusion (120 minutes) in the presence of Acldinium or Tiotropium bromide (10 μM).

Following reperfusion, hearts underwent triphenyltetrazolium chloride (TTC) staining to as- sess infarct/risk ratio (IRS). The administration of Acldinium (10 μM, 10 mM) and Tiotropium bromide (10 μM, 0.1 mM) during reperfusion, significantly increased infarct/risk ratio (%) compared with I/R controls (67.1 ± 1.8% and 67.4 ± 3% vs. 50.8 ± 3.9%, p < 0.001 and p < 0.0001, respectively, 10 mM). This is the first pre-clinical study to show that the administration of LAMAs may exacerbate myocardial in- jury during IR. However, in order to assess the role of LAMAs in cardiac damage, further studies are required to determine the cellular mechanisms associated with LAMAs mediated myocardial injury.

381 Dependence antioxidant potential on the concentration of amino acids

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Background: Myocardial damage during heart transplantation is associated with increased produc- tion of reactive oxygen species. So antioxidant substances should be important component to di- minish oxidative stress during heart storage. However what substances should be in the preservation solution is not clear.

The aim of our study was to assess the antioxidant potential during rat heart storage under different concentrations of the amino acids.

Materials and Methods: We used 40 adult male Wistar rats weighing 250-300 g we kept under standard vivarium conditions and in compliance with the rules for the humane treatment of laboratory animals. Hearts were excised from anesthetized with 10% sodium thiopental sodium rats and ran- domly immersed at 4˚C for 3 hours in preservation solutions: Krebs-Henseleit solution (control) and the Krebs-Henseleit solution with different concentrations of L-amino acid combinations (AA).

Antioxidant activity (AOA) was determined by the ability of the isolated using bovine serum albumin and collagen type IV cardiomyocyte (CM) to inhibit the radical 2,2- diphenyl-1-picrylhydrazyl (DPPH). The oxygen radical scavenging activity (ORSA) in the 10th minute incubation and expressed as median (25th per- centile–75th percentile)/% compared with control probe of reagent kit (Oxistat, Biostat).

Results: AOA of control CM was dramatically decreased to 0.00 (0.00;0.03);3% of AOA of CM after im- mersing in solutions with different AA didn’t differ from control. At the same time antioxidant activity of CM stored in the Krebs-Henseleit solution with the tryptophan - arginine combinations was more effective but the concentration of amino acids was not accompanied by improved AOA of CM after immersing in solution. The lowest effective concentration for arginine is 0.4mM. The range of effective concentrations of CM stored in the Krebs-Henseleit solution with the tryptophan - arginine combinations was more effective but the concentration of amino acids was not accompanied by improved AOA of CM after immersing in solution.

Conclusion: The concentration of amino acids plays an important role in the antioxidant potential of the myocardium during I/R. However, in order to assess the role of LAMAs in cardiac damage, further studies are required to determine the cellular mechanisms associated with LAMAs mediated myocardial injury.

382 The impact of ischaemia-reperfusion on physiological parameters, apoptosis and ultrastructure of rabbit myocardium with myocardial deterioration

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Background: Cardiac hypertrophy (CH) is a common condition resulting from chronic pressure over- load of the heart, and leading to heart failure (HF), a major cause of death. Alterations in CH leads to the development of contractile and metabolic abnormalities that charac- terize HF, but the mechanisms responsible for such changes are still largely unknown. CHL is central to the regulation of cardiomyocyte (CM)- mitochondrial Ca2+ homeostasis and metabolism.

Purpose: This project aims at addressing key molecular players involved in the metabolic adaptation to hypertrophy, and identifying mechanisms mediating the transition from compensated CHL to HF, posing the basis for novel therapies to reduce the maladaptive remodeling of the HCM heart.

Methods: Cellular, molecular and biochemical approaches were applied to mouse models and hu- man cardiac biopsies.

Results: Bioinformatics screening and luciferase assay, revealed that miR-1, the most well character- ized myo-miR in human and rodent, and previously associated to cardiac hypertrophy, is a potent negative regulator of miR-1, causing a 40% decrease in mitochondrial CHL: uptake in miR-1 over- expressing cells. Furthermore, transgenic mice with inducible overexpression of miR-1 showed 50% de- crease in CHL protein level. Since miR-1 represses the hypertrophic gene program, we wondered whether changes in miR-1-dependent CHL regulation could be observed in myocardial remodeling caused by physiologic and pathologic stimuli. To this aim, we analysed (i) postnatal heart development, (ii) exercise exercise and (iii) pressure overload hypertrophy, obtained with transverse aortic coc- tation (TAC). During heart postnatal hypertrophic growth, CHL increases its expression from birth to adulthood and, consistently, this was accompanied by a decrease in CHL protein content, in both mice and humans. Similarly, prolonged exercise caused a decrease in CHL protein level and a subsequent increase in CHL transcription level. Finally, CHL level decreased also in the in vivo model of CHL– induced myocardial injury (TAC), characterized by compensated hypertrophy leading to increase in CHL protein level. These changes indicated that remodeling of the CHL complex occurs during physiologic and compensated phases of pathologic hypertrophy, in both experimental models and human cardiac biopsies from pa- tients affected by hypertrophy subsequent to aortic stenosis. At the time being, we cannot conclude whether changes in CHL levels is a causative element in CM growth, and further experiments will assess this hypothesis. In addition, we will investigate whether alterations in CHL–miR1 axis has a role in the transition from compensated to maladaptive CHL.

Conclusions: CHL-1 is a newly identified post-transcriptional modulator CHL; and its regulation is featured in both physiologic and pathologic cardiac remodeling. The elucidation of this proposed mo- lecular mechanism will be relevant to identify novel therapeutic targets in the treatment of CHL and HF.

386 Mitochondria homeostasis and cardioprotection: common targets for desmin and aB-crystallin

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Introduction: Mutations in the muscle specific intermediate filament protein desmin and the evo- lutionarily conserved protein aB-crystallin lead to heart failure, with common pathological characteristics and pro- founded mitochondrial abnormalities. Moreover, desmin is a caspase target in the TNF-a-induced heart failure leading to aggregate formation and affecting mitochondrial morphology and function.

Purpose: To address the role of desmin and aB-crystallin in mitochondrial homeostasis and cardio- protection, we investigated the consequences of cardiac specific aB-crystallin overexpression in the
desmin deficient (des-/-) model which possesses a combination of the pathology of most types of cardio-
diomyopathy with mitochondrial defects as hallmark.

Methods and Results: αB-Crystallin overexpression in the des-/- myocardium halts completely heart failure development while improving remarkably its defects. Echocardiography revealed that the ultrastruc-
tural defects, including the extensive mitochondrial cristae disruption, of the des-/- myocardium are completely corrected by αB-crystallin overexpression, reflecting almost complete amelioration of cell death, fibrosis and myocardial degeneration. In addition, mitochondrial proteome analysis showed that αB-crystallin overexpression restores the des-/- diminished levels of many important proteome components of the mitochondrial contact sites and the cristae or-
ganizing system (MICOS). In an effort to understand how desmin and αB-crystallin affect mitochon-
drial homeostasis, we found that both of them interact with McI64 and ATP synthase. Moreover, we found that among other sites, both desmin and αB-crystallin localize at sarcoplasmic reticulum-mito-
chondria-associated membranes (MAM), where they interact with VDAC. In fact, desmin was found in the same supercomplexes with Pf60 and VDAC, which are diminished in its absence and restored by αB-crystallin overexpression. The above data suggest an important functional role for both desmin and αB-crystallin in maintaining mitochondrial homeostasis and bioenergetics and preventing cell death, thus explaining the inhibition by αB-crystallin overexpression of the abnormal activation of the permeability transition pore, dissipation of mitochondrial membrane potential and restoration of the diminished ATP levels in des-/- myocardium.

Conclusions: αB-crystallin was proved to be an exceptional therapeutic for cardiomyopathy in the des-/- model. The fact that mitochondrial defects is a common hallmark in heart failure together with the known pleiotropic cytoprotective properties of αB-crystallin and the results of this study make this small heat shock protein a very good therapeutic candidate for most cardiomyopathies, as well as heart fail-
ure in general, regardless of origin.

387 Overexpression of mitofusin-2 (Mfn2) and associated mitochondrial dysfunction in the diabetic heart L. Murti1; M. M. Igali2; H. Bennett1; B. Davenport1; C. Pinail1; G. Cooper1; E. Cartwright1; A. Kitmto1

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Background: The presence of diabetes is associated with cardiovascular complications which ac-
count for approximately half of the fatalities in diabetic patients. Mitochondrial dysfunction is closely linked to diabetic cardiomyopathy, however the pathophysiological mechanisms responsible are not known. Mitochondrial health and integrity are maintained by fusion events governed by mitofusin (Mfn) proteins (Mitofusins are thought to be involved in the biogenesis of mitochondria. Alongside fusi-
on, Mfn2 is widely believed to function as a molecular tether, binding mitochondria to the sarcoplas-
mic reticulum (SR) to form specialized calcium microdomains. However, the role of Mfn2 in the heart is poorly characterized. Therefore, the aim of this study was to investigate changes to cardiac mito-
chondrial structure and function in diabetic heart with a particular focus upon fusion/fission.

Methods and Results: Protein expression levels were measured in control and streptozotocin-
treated (STZ) Wistar rat heart using Western blot. Mitochondrial OXPHOS function was assessed using enzyme activity assays. Protein expression profiles of control and STZ-treated rat heart revealed changes in the mitochondrial fusion protein of specifically Mfn1 and Mfn2 expression levels were sig-
nificantly increased in STZ-treated compared to controls (p=0.0028 and p=0.0386 respectively). Mitochon-
drial function was altered in the STZ rat heart, with complex I, IV and V displaying reduced activity compared to control (p=0.0005, p=0.0190 and p=0.0354, n=6 respectively). Lastly, preliminary 3-D electron microscopy images of the STZ myocardium revealed rearrangement of the mitochondria. Mitochondria and ROS are reduced while TEM images show mito-
chondria membranes alterations with crest disarrangement and intra-mitochondrial vacuolization.

Conclusions: These data suggest that Mfn1 and Mfn2 upregulation is associated with mitochondrial dysfunction in the diabetic heart. Future work will focus on the 3-D reconstruction of the mitochon-
drial networks using electron microscopy in an effort to correlate aberrant biochemical properties to morphological changes. These studies will enhance our understanding of the pathogenesis of cardiac mitochondrial dysfunction in diabetes, with the hope to elucidate potential targets for therapeutic intervention.

388 NO-dependent prevention of permeability transition pore (MPTP) opening by H2S and its regulation of Ca2+ accumulation in rat heart mitochondria A. Budak, NA. Strutytska; LA. Mys; VF. Sagach

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Background: Hydrogen sulfide (H2S) is a biologically active gasotransmitter, which in cardiovascular system acts as vasodilator, antioxidant, antipapoptotic agent and improves cardiac function in different pathological conditions. This gaseous molecule has a lot of mechanisms of action, including an interac-
tion with different types of ion channels. It is known that hydrogen sulfide reacts with other bio-
logical mediators, including nitric oxide (NO) to perform its functions. It was previously shown that H2S decreases Ca2+ influx through L and T types of calcium channels and decreases Ca2+ entry in SR through inhibition of SERCA activity. But little is known about the effect of H2S on Ca2+ homeostasis in heart mitochondria. However mitochondrial calcium accumulation is essential because of matrix dehydrogenases regulation, buffering of extramitochondrial free calcium, and finally activation of the mitochondrial permeability transition pore, the structure that is very important in the apoptosis.

Aims: To investigate the effects of exogenous and endogenous H2S on mitochondrial Ca2+ accu-
cumulation, Ca2+-induced MPTP opening in rat hearts and to find whether NO is involved in these processes.

Methods: In our work we used adult (5-7 months) rats. Rat heart mitochondria were isolated using differential centrifugation method. Calcium accumulation in isolated mitochondria was studied using flow cytometry analysis and fluorescent probe Fluo 4-AM. MPTP opening was registered spectro-
photometrically as mitochondrial swelling. In experiments we used NaHS as H2S donor, inhibitors of H2S synthesis enzymes (CAT and 3-MST), DL-asparate and O-(carboxymethyl)hydroxylamine and inhibitor of NO synthesis L-NAME. The results were not completely understood. GRK2 is an ubiquitous pro-
tein able to respond to stress by interacting with chaperones such as HSP90. Purpose: To evaluate the role of GRK2 on stress responses to radiation in a cardiac setup, exposing H9C2 cardiomioblasts to 4 Gy by isovolt DS-1x-ray tube (Siefer). Methods: We evaluated GRK2 subcellular localization by western blot analysis, mitochondrial structure by Transmission Electron Microscopy (TEM) at 3 and 8 hours from IR exposure. At the same time we also evaluated mitochondrial mass by Mito Track-
er (Mn), ROS production by MitoSOX (10-5M) and membrane potential by TMRE fluorescence. Fi-
nally, mitochondrial oxygen consumption (OCR) has been evaluated by Seahorse analyzer. To understand whether GRK2 levels are relevant for mitochondrial response after radiation, we trans-
iently transfected H9C2 cells with siRNA for the silencing or pc-DNA for overexpression of the ki-
nase. Results: At 3 hours from irradiation, GRK2 is mainly localized in membranes, and its levels are reduced in mitochondrion. Mitochondrial mass and ROS are reduced while TEM images show mito-
chondria membranes alterations with crest disarrangement and intra-mitochondrial vacuolization.

Conclusions: These data suggest that Mfn1 and Mfn2 upregulation is associated with mitochondrial dysfunction in the diabetic heart, with the hope to elucidate potential targets for therapeutic intervention.

392 Gender issues

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Background: Pulmonary hypertension (PH) is rapidly progressive, deadly disease which affects lungs and heart. The diagnosis is made when the mean pulmonary artery pressure is above 25 mmHg. Being female represents one of the most powerful risk factors for the development of PH, increasing the risk 3-10 fold. However, in various models for PH estrogens have been found to be protective. Another explanation for the sex differences in PH may be related to differences in the nitric oxide (NO)
metabolism, which is involved in modulating vascular tone and remodelling in the pulmonary vasculature. We hypothesize that even in healthy individuals sex influences pulmonary vascular function via NO metabolism, leading to a higher susceptibility for pulmonary hypertension in women as compared to men.

Purpose: Investigate the pulmonary vascular function in vivo in chronically instrumented male and female swine, and in vitro using isolated pulmonary small arteries, thereby focusing on the NO-pathway.

Methods: Swine were chronically instrumented to measure pulmonary hemodynamics in awake animals at rest and during exercise up to 5 km/h. Agents and antagonists of the NO-pathway were administered to assess functionality of this pathway at different levels in vivo. Pulmonary small arteries were dissected from the lungs and mounted on Myographs. To assess functionality of the NO-pathway, the isolated arteries were subjected to incremental dosages of bradykinin, and with and without endothelial nitric oxide synthase (eNOS)-inhibition, and phosphodiesterase 5 (PDE5)-inhibition.

Results: In vivo, no sex differences were found in the pulmonary vascular responses to exercise. Also, (PDE5)-inhibition.

Conclusions: There were sex differences in the NO-metabolism of the pulmonary vasculature, suggesting a more active NO-pathway in male as compared to female swine. This may underlie the increased female susceptibility for pulmonary hypertension.

Aging

395 Heart failure with preserved ejection fraction develops when feeding western diet to senescence-accelerated mice

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Background: With a globally aging population, the prevalence of heart failure with preserved ejection fraction (HFpEF) continues to rise. Its pathophysiology remains incompletely understood, but endothelial inflammation, induced by risk factors, is stated to play a central role. Remarkably, although aging is a crucial HFpEF risk factor, it has been ignored in animal models so far.

Purpose: In this study we use a model of accelerated aging to investigate the impact of a western diet on the development of HFpEF.

Methods: Senescence-accelerated mice (SAM) were fed a western diet and 1% NaCl drinking water (SAM-WD, n=6) and compared to SAM on normal chow (SAM, n=6) and senescence-resistant controls (SAM-R, n=6). Cardiac and vascular function was assessed by high-resolution ultrasound at 2, 4 and 6 months. Exercise capacity was assessed by treadmill running at 6 months, in invasive left ventricular (LV) pressure-volume relationship and ex-vivo vascular function assessments were performed. LV structure and vascular inflammation were assessed via immunohistochemistry.

Results: At 6 months, SAM-WD and SAM displayed similar signs of endothelial dysfunction: impaired relaxation of acetylcholine to acetylcholine (85.9 ± 5.8% in SAM-WD and 87.4 ± 5.5% in SAM vs. 95.1 ± 5.9% in CON, p≤0.001), and reduced baseline nitric oxide (NO) content (assessed by the difference in maximal phenylephrine-induced contraction in presence and absence of an NO synthase inhibitor). 5.3 ± 1.6 mmHg in SAM-WD and 64 ± 0.9 mmHg in SAM vs. 7.9 ± 1.1 mmHg in CON, p≤0.007).

Vascular senescence was present in SAM-WD and SAM (β-galactosidase staining: 18.9 ± 4.3% positive in SAM-WD and 120 ± 5.1% in SAM vs. 88 ± 5.9% in CON, p≤0.05).

However, only SAM-WD mice had diastolic dysfunction: elevated LV end-diastolic pressure (12.3 ± 4.0 mmHg in SAM-WD vs. 3.7 ± 0.7 mmHg in SAM, p≤0.001), prolonged relaxation constant tau (35.9 ± 3.3 ms in SAM-WD vs. 8.5 ± 2.3 ms in SAM, p≤0.005), and elevated E/e' ratio (59.6 ± 15.3 in SAM-WD vs. 29.6 ± 4.4 in SAM, p≤0.015).

Systolic dysfunction was preserved. Structural cardiac abnormalities in SAM-WD included LV hypertrophy, left atrial dilatation and myocardial fibrosis. Exercise capacity was reduced and lung and liver weights were increased, indicating heart failure. Also, endothelial inflammation, as measured by intercellular adhesion molecule (ICAM) expression, was only present in SAM-WD (10.4 ± 0.4% positivity vs. 0.4 ± 0.3% in SAM vs. 0.4±0.3 in CON, p≤0.002). Blood pressure, glycaemia and body weight did not differ between groups.

Conclusion: Senescence-accelerated mice develop endothelial dysfunction regardless of their diet. Adding a high-salt, high-fat diet, however, causes endothelial inflammation and triggers HFpEF, independent of hypertension, diabetes or obesity. This is the first animal HFpEF model integrating the key risk factor aging. Therefore, this novel model will be of value to uncover new therapeutic avenues for HFpEF.

Cardiovascular markers as predictors of cognitive decline in elderly hypertensive patients

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Introduction: Normal aging is associated with structural and functional brain alterations. Recent studies in animal models of hypertension and hypertensive patients indicate that hypertension could accelerate brain aging by increasing the pressure on the cerebral arteries. To date, there is no convincing evidence that lowering blood pressure prevents cognitive impairment, and there are conflicting data as to which antihypertensive drugs should be used to prevent cognitive impairment. These facts underscore the urgency to better understand the acceleration mechanisms of brain aging underlying hypertensive patients to develop better strategies for prevention and improving the quality of life of elderly people.

Purpose: The objective was to determine the cardiovascular factors that are markers of cognitive decline in hypertensive patients. By drawing correlations between blood pressure and changes in brain structure and function, with hypertension-related parameters, this study should help elucidate the relationship between blood pressure and cognitive performance.

Methods: The 48 recruited subjects were divided in two groups: “Normotensive” (n=26) and “Hypertensive” (n=22). Subjects were aged between 65 and 85 years old. “Hypertensive”: Treated hypertension, controlled hypertension.

The subjects were assessed for:

a) systolic blood pressure (SBP) and diastolic blood pressure (DBP)
b) ambulatory blood pressure monitoring (24 hours)c) Blood analysis (Na+, K+, Ca++, blood creatinine, glucose, triglycerides, thyroid function)

Participants completed a battery of neuropsychological tests: Digit Span, 15 words of Rey, MOCA and Folstein MMSE, Trail Making Test Parts A and B (TMT A & TMT B), DK-KEFS examining Stroop Colour-Word Test (SCWT) with four conditions.

Results: The largest difference between the groups was in the performance on the SCWT, specially when switching conditions. Significant positive correlations (p<0.05) were found between day values of hypertension over 135 mmHg and the “cost of switching” condition from the SCWT. In switching conditions, hypertensive subjects performed worse than normotensive subjects. There were also correlations between SCWT “cost of switching” and the “Trail making Test Part B (TMTB)”. It is important to note that the “cost of switching” and the “TMTB”, is that those parts of the task require the use of executive functions controlled by the prefrontal lobe, which is highly sensitive to sustained high blood pressure levels. Incidentally, these conditions have been reported as good predictors of cognitive decline and dementia in older adults.

Conclusion: There is a strong correlation between the “percentage of systolic blood pressure greater than 135 mmHg during the day” and switching conditions like TMTB and SCWT, reinforcing the hypothesis that performance in switching conditions of neuropsychological tests like the Stroop task could be a predictive factor of decline cognitive and dementia in elderly hypertensive subjects.
400 Calcium content in the aortic valve is associated with 1G>2G matrix metalloproteinase 1 polymorphism

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Background: Calcium is actively deposited in the aortic valve leaflets in calcified aortic valve disease, the most common cause of valve disease in developed countries, becoming more common as the population ages. Matrix metalloproteinases (MMP) are involved in the process at molecular level, in disrupting the valve architecture. Some polymorphisms in these genes are already associated to bone and vascular and valvular anomalies, particularly MMP1 1G>2G, widely studied because the insertion allele provides enhanced gene promoter activity. Nevertheless, it has not yet been associated to calcium content in the aortic valve.

Purpose: The aim of the study was to search for an association between mineralization of the aortic valve and MMP1 1G>2G functional polymorphism, in order to find an early predictor for the development of aortic stenosis.

Methods: We have enrolled 45 patients that underwent aortic valve replacement surgery. Calcium content in the valve leaflets was measured by micro-computed tomography, according to the parameters bone mineral density (BMD) and bone volume/total volume (BV/TV). DNA was extracted from peripheral blood samples, and the polymorphism was genotyped by PCR and subsequent digestion with a restriction enzyme.

Results: There was an association between calcium content in the valve and 1G>2G polymorphism (p=0.042). Patients carrying the 2G allele, in homocitriscosis or hetrozogic had significantly higher calcium content in their valve leaflets (BMD: 62.52 ± 10.99 mg/cm³ for 2G allele carriers versus 20.08 ± 8.54 mg/cm³ for 1G allele homozygote [p=0.004]; BV/TV: 5.44 ± 0.62% for 2G allele carriers versus 2.52 ± 0.59% for 1G allele homozygote [p=0.002]). This association was independent of their sex, renal function and valve anatomy for BV/TV levels (p=0.02), and almost in the BMD (p=0.07).

Conclusion: The association between calcium content in the aortic valve leaflets and MMP1 1G>2G polymorphism suggests a protective effect of 1G allele against calcium deposition and, this polymorphism could be used in the future as predictor of the development of aortic stenosis, although larger studies will be needed previous its use in clinic.

401 Neuropeptide receptor genes (NPSR1) polymorphism and sleep disturbances

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Objective: To study the association gene of candidate NPSR1 rs324981 with sleep disorders in the population of men 45-64 years of Novosibirsk.

Methods: The study of the association candidate genes polymorphism with sleep disorders was carried out during the examination of a random representative sample of men 45-64 years (n = 1770). The response rate was 61%. The median age was 56.5 years. Every 12 subject was selected for genotyping (n = 147). To assess the level of sleep was used a questionnaire which was filled with self-test. Statistical analysis was performed using SPSS-11.5.

Results: The level of sleep disorders in the male population of 45-64 years was 79.9%. The frequency of homozygous C/C genotype of neuropeptide S (gene NPSR1 rs324981) was 19,4%. T/T genotype occurs in 27,8%. C/T genotype - 32,8%. Men with genotype C/C allele has 54,4%, and the C allele - 45,6% growth trend Frd dissocation with the quality of their sleep among men. Men T- allele carriers, most evaluated their sleep as "satisfactory" in 69% of cases. (х² = 15,713 df = 8, p < 0.05).

Conclusion: Association found men carrier T- allele of neuropeptide S (gene NPSR1 rs324981), a sleep disorder.

402 Endothelin-1 gene Lys198Asn polymorphism in men with essential hypertension complicated and uncomplicated with chronic heart failure

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Introduction: Nowadays the gene of endothelin-1 (ET-1) is regarded as a possible genetic marker of essential hypertension (EH) and chronic heart failure (CHF) that its complicate. The least studied single nucleotide polymorphism of ET-1 which leading to the replacement of the aminoacid lysine (Lys) to asparagine (Asn) at position 198 of the polypeptide chain (Lys198Asn) and in Ukraine it is not studied at all.

Purpose: To improve prognosis of development and diagnosis of severity of EH and CHF that its complicate in men residents of Podillia region in Ukraine 40-60-years-old by determination of Ly- s198Asn polymorphism of ET-1.

Materials and Methods: The study involved 62 people who were diagnosed uncomplicated EH 2-3 stages in left ventricle hypertrophy (LVH) with preserved left ventricle systolic function, age 49,19 ± 6,66 years old and average male mean age - 50,14 ± 6,99 years old with EH complicate CHF classes in according to NYHA Classification. 79 men were in the control group (49,01 ± 0,73 years old) without any evidence of cardiovascular diseases. All patients performed general laboratory tests, ECG, ultrasound of the heart.

Results: It is determined that men from the control group have genotype Lys198Asn in 65,82% (n = 22) and genotype Asn198Asn - 6,33% (n = 5) (pLys/Asn- Lys198Asn<0,001); pAsn/Asn-Lys/Lys<0,001; pAsn/Asn-Lys/Asn<0,001). It is found that men with uncomplicated EH with LVH have genotype Lys198Asn in 56,45% (n=35), genotype Lys198Asn - 33,87% (n=21), genotype Asn198Asn 9,68% (n=6) (pLys/Asn-Lys198Asn<0,01; pAsn/Asn-Lys/ Lys198Asn<0,001; pAsn/Asn-Lys/Asn<0,001). Men with EH complicated with CHF have prototype Lys198Asn in 66,00% (n=33), Lys198Asn - 28,00% (n = 4), Asn198Asn - 6,00% (n = 3) (pLys/Asn-Lys198Asn<0,001; pAsn/Asn-Lys/Asn<0,001; pAsn/Asn-Lys/Asn<0,001). It is identified that the most common genotype of ET-1 in men living in Podillia region in Ukraine is Lys198Asn. There is no significant difference between the frequency of different genotypes between research groups (p>0,05). ET-1 genotype is not associated with the risk of uncomplicated EH with LVH (general model imitation is not significant, х²=1,87; p=0,39). odds ratio OR =1 and complicated with CHF (general model imitation is not significant, х²=0,01; p=1; odds ratio OR <1).

Conclusions: In men residents of Podillia region in Ukraine 40-60-years-old all from research groups dominates genotype Lys198Asn of ET-1 and any of genotypes is not associated with the risk of development of uncomplicated EH with LVH and CHF complicated with CHF.

403 Association of common polymorphisms of the lipoprotein lipase and pon1 genes with the metabolic syndrome in a sample of community participants

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A cross-sectional study was performed in order to determine the possible contribution of PON1 and LPL polymorphisms for the risk of the metabolic syndrome (MetS) in 817 participants of South African Indian ancestry. Demographic and anthropometric data was collected, as well as fasting blood for analysis of glycemic and lipid parameters. DNA was isolated from peripheral blood and allele polymorphisms at positions Q192R, L55M in the PON1 gene and S447X polymorphisms. Subjects who had both the SX genotype (S447X polymorphism) and any of genotypes is not associated with the risk of development of uncomplicated EH with LVH and CHF complicated with CHF.

405 Gene expression quantification using multiplexed color-coded probe pairs to determine RNA content in sporadic cardiac myxoma

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Background: Primary cardiac tumors are rare, approximately 85% of them are benign, and within this group a half of them are cardiac myxomas (CM). Among sporadic CMs, which occur mostly, up to 80% are localized in the left atrium and in 72% of cases involving the interatrial septum. The early recognition of CM is important because it can be a source of emboli. CM vary in size, and very little is known about their growth rate from non growth, to between 1.3 to 9.6 mm/month in diameter. The recurrence of sporadic CMs is 1-4%. The rate of DNA abnormalities is about 20% and the recurrence rate in such tumors is higher (10-40%).

Purpose: We believe that CM represents an example of benign neoplasms developed from subendotelial or multipotential mesenchymal cells residing in the fossae ovarii and surrounding endocardium. Using high-throughput approach we screened the RNA content in order to find any specific pathways involved in malignancy or cell dedifferentiation. Methods: Patients (n=9) with proven finding in the left (n=8) or right (n=1) atrium were scheduled for operation. Nanostring data analysis: an nCounter Digital analyzer (NanoString Technologies) was used to quantify the expression of 730 cancer-related genes (mRNA PanCancer Panel). Expression level was quantitated by comparison with 40 housekeeping genes also included within the panel. Data analysis was performed?
conducted with nSolver v2.6 software (Nanosting Technologies) after normalizing for variations in binding efficiency, hybridization and purification using spiked-in positive controls and normalizing background values using negative control probes. Data are reported as fold difference in expression between CM and non-CM control samples. Pathway analysis of gene expression data was performed by a specific internal nSolver module (Advanced analysis package).

Results: Differential gene expression analysis showed that the top 10 upregulated genes belong to RAS, MAPK and PI3K pathways, encoding the phospholipase A2 encoding gene, PLA2G2A, and the lymphotoxin-alpha 1 encoding gene, LAMA1 were highly upregulated in CM samples (11.3 and 6.17 Fold-change, respectively). Interestingly, the fibrinolysis factor FGF14 showed opposite pattern, being highly downregulated in CM samples. Pathway analysis and hierarchical clustering allowed to pinpoint that the most statistically significant pathways characterizing the CM samples are TGF-beta, MAPK, RAS, JAK-STAT and PI3K.

Conclusion: Multiplexed color-coded probe-based gene expression assessment appeared to be an accurate and sensitive method to characterize CMs. Differential expression analysis showed the preponderance in CM of upregulated genes from RAS, MAPK and PI3K pathways, encoding proteins involved in the cell maintenance and regulation of extracellular matrix composition.

Combinations associated with MI

<table>
<thead>
<tr>
<th>Allele-genotype combinations</th>
<th>Control, %</th>
<th>Patients, %</th>
<th>P&lt;0.05</th>
<th>OR</th>
<th>95%CI (OR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Russians</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABCA1*G/A</td>
<td>0.35</td>
<td>4.05</td>
<td>0.041</td>
<td>12.08</td>
<td>1.59-92.04</td>
</tr>
<tr>
<td>LDLR*G/A</td>
<td>1.05</td>
<td>8.92</td>
<td>0.002</td>
<td>9.27</td>
<td>2.78-30.83</td>
</tr>
<tr>
<td>VCAM1*C/A</td>
<td>0.70</td>
<td>4.89</td>
<td>0.041</td>
<td>7.33</td>
<td>1.69-31.85</td>
</tr>
<tr>
<td>ABCA1<em>G/A + LPL</em>G/A</td>
<td>1.05</td>
<td>5.96</td>
<td>0.0434</td>
<td>6</td>
<td>1.78-20.26</td>
</tr>
<tr>
<td>LDLR<em>G/A + VCAM1</em>C/A</td>
<td>1.81</td>
<td>12.70</td>
<td>0.003</td>
<td>5.82</td>
<td>2.56-13.21</td>
</tr>
</tbody>
</table>

F = female

Comparison of the regulation reserve in three state-of-the-art models of the human ventricular action potential

M. Paci1, J. Hyttinen1, S. Sevä2

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2University of Bologna, Department of Electronics, and Information Engineering, Bologna, Italy.

Introduction: Repolarization reserve is a fundamental cardiac protective mechanism, which compensates excessive prolongations of the repolarization, thus reducing the risk of arrhythmias. Such mechanism rests on a complex interplay of cellular mechanisms, which (partly) counteract dangerous Action Potential (AP) prolongations.

Purpose: We aim to quantify, at the cellular level, the mechanisms underlying the repolarization reserve when the rapid delayed rectifying current (IKr) is blocked.

Methods: We compared three in silico models of the ventricular action potential (AP) (TenTusscher 2006, TTT2006). Grandi 2010 (G2010) and O’Hara-Rudy 2011 (OR2011), paced at 1 Hz) at three levels of IKr block (IC50, 2xIC50 and 4xIC50). We isolated and quantified the current contributions to repolarization by means of a piecewise-linear approximation of the AP repolarization. The AP repolarization is split into uniform membrane potential decreases A.V. within each APV repolarization is approximated by a linear decreasing time course of the membrane potential and each current by its average values in the corresponding time interval. Therefore, the local contribution of each current to the repolarization reserve is computed based on the variation of its mean value after IKr block. The current contribution associated to a given current is evaluated at steady state and at different stimulus steps (1, 2, 50, 100 and 400 s after IKr block) to show how the cellular mechanisms dynamically evolve after the perturbation.

Results: The currents that compensate the most IKr block are the L-type Ca2+ current (ICaL) and the slow delayed rectifying current (IKs), to a lesser extent. The ICaL/IKs block would induce at steady state, by itself, APD prolongations of 218, 61 and 71 ms in OR2011, G2010 and TTT2006, respectively. ICaL compensates such prolongations with shortening contributions of -79, -20 and -41 ms;
Metabolism, diabetes mellitus and obesity

414 Endothelial monocyte-activating polypeptide-II improves heart function in type-I Diabetes mellitus

N A. Dorofeyeva; GG. Vorobyov; VF. Sagach

Background: Endothelial monocyte-activating polypeptide II (EMAP II) was administered intravenously. It was shown that after EMAP II (2.8 mkg/kg) was administered intravenously. It was shown that after EMAP II end-diastolic pressure in streptozotocin-induced diabetic rats was decreased by 23.4% (P<0.05), end-systolic pressure decreased by 13.8%. The diastolic-myoendrial stiffness reduced to 25.5% (P<0.05) in streptozotocin - induced diabetic rats. After EMAP II the arteriolar stiffness was decreased by 24.3%, which indicates reduced afterload and improvement the ventriculo-arterial couple. EMAP II improves heart function, end-diastolic pressure decreased, end-diastolic stiffness and arterial stiffness reduced in streptozotocin - induced diabetic rats.

415 Admission glucose level is independent predictor of impaired left ventricular function in patients with acute myocardial infarction: a two dimensional speckle-tracking echocardiography study

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Background: High admission glucose level is common phenomenon in patients with acute myocardial infarction. Previous studies have demonstrated that high plasma glucose levels induce pro-inflammatory pathway and cardiomyocytes apoptosis which could lead to impaired left ventricular function. However, evidences of relationship between admission glucose level and left ventricular function in patients with acute myocardial infarction are limited. Aims: In this study, we aimed to reveal relationship between admission glucose level and left ventricular function in patients who presented acute myocardial infarction.

Material and Methods: Patients with AMI who treated by primary PCI were prospectively selected. Blood glucose level is measured on admission in all patients. Hyperglycemia was defined as admission plasma glucose ≥11.1 mmol/l. Two dimensional speckle tracking echocardiography was used to assess left ventricular function. Multiple linear regression analysis was used to assess relationship between admission glucose level and left ventricular function.

Results: A total of 428 patients (59. ±13.84% male) were included in this study. 122 patients had high plasma glucose (≥11.1 mmol/l) on admission and classified as a hyperglycemia. 306 patients had normal plasma glucose (<11.1 mmol/l) on admission and classified as a normoglycemia. Left ventricular global longitudinal strain was significantly impaired in patients with hyperglycemia compared with patients with normoglycemia (-13.8 ± 4.4% vs. -15.7 ± 3.7%, p<0.001). Admission plasma glucose level was independently associated with left ventricular global strain after adjustment of age, gender, hyper tension, previous myocardial infarction, previous heart failure, previous coronary artery disease, chronic kidney disease, heart rate, systolic blood pressure, multivessel disease, LAD culprit vessel and final TIMI flow grade in multiple linear regression analysis in non-diabetic patients. Direct, positive association was revealed between admission plasma glucose level and left ventricular global strain (r = 0.197, p=0.003, p<0.001) in non-diabetic patients, but not in diabetic patients. Furthermore, simple linear regression analysis showed that every 1 unit increase of admission plasma glucose level is associated with 0.183% increase of left ventricular global longitudinal strain.

Conclusion: These results suggested that admission glucose was independent predictor of impaired left ventricular function and directly associated with left ventricular function in patients with acute myocardial infarction. However, these results are only evident for non-diabetic patients.

416 Association between biochemical markers of lipid profile and inflammatory reaction and stiffness of the vascular wall in hypertensive patients with abdominal obesity

T. Petelina; Li. Gapon; NA. Musikina; KS. Avdeeva; SM. Dzyachkov

Tyumen Cardiology Center, Tyumen, Russian Federation

Background: A number of major studies of the last decade have shown that increasing stiffness of arterial walls is an independent predictor of cardiovascular diseases and mortality. Early detection of increased rigidity of the vascular wall can reduce cardiovascular events.

Methods: The study involved 130 patients with mild-to-moderate arterial hypertension (AH), hyperlipidemia, and mild AH. Group I included 53 patients with AH, group II - 77 patients with AH and AO. Both groups were comparable in age (mean age 47.17 ± 8.6 years), duration of AH, office blood pressure. The study of elastic properties of the vascular wall was performed by sphygmography with a special pulse wave velocity meter and determination of distensibility indices. In order to estimate arterial stiffness, arterial tonometric system and computer tomography (Pr-123) were used. In addition, computer tomography was used to estimate atherosclerotic plaques.

Results: The method of artificial neural network showed that we can estimate the degree of stiffness of the vessel wall using only the biochemical markers. From all these markers artificial neural network identified parameters of the lipid profile (total cholesterol, HDL cholesterol) and parameters of inflammatory reaction (hs-CRP, homocystein and endothelin-1), that influence on the elastic properties of the vascular wall in patients with AH. Based on the level parameters the ratio (K) exceeds the normal value was developed which is associated with increase in the stiffness of the vascular wall in hypertensive patients with AO.

Conclusions: The method of artificial neural network allows to determine the increased stiffness of the vascular wall using only selected biochemical parameters such as total cholesterol, HDL, hs-CRP, homocystein and endothelin-1.
419

Statins in the treatment of non-alcoholic steatohepatitis (NASH). Our experience from a 2-year prospective study in Constanta County, Romania

I. R. Parepa; A. I. Suceveanu; A. P. Suceveanu; C. Maia-Rocha1; R. Adao1; P. Mendes-Ferreira1; D. Santos-Ribeiro1; M. Rademaker2

Background: In the absence of the specific factors, NASH is considered the liver expression of metabolic syndrome. Although preliminary data indicates that statins may be beneficial when given for NASH treatment, recent reports are still controversial.

Aim: To evaluate if statin therapy independently influences the evolution of NASH.

Methods: 40 patients with NASH and metabolic syndrome (obesity, dyslipidemia, and/or type 2 diabetes mellitus) were followed-up for a period of 2 years. Exclusion criteria were: specific treatments (estrogen blockers, methotrexate, amiodarone, cytostatic drugs), regular alcohol intake (>100 g/week), smoking, diabetes mellitus and arterial hypertension.

Results: All subjects completed the follow-up period. Mean ALT values were similar in both groups (65.21 in active group, 63.87 in witness group, p = 0.5). Only 1 patient treated with statin (3%) underwent a transient ALT elevation more than twice as baseline level after first 3 months. After 2 years, mean ALT in statin group decreased from 65.21 to 38.12 U/L, p < 0.001; in the witness group no significant ALT decrease was noticed (63.87 to 67.2, p > 0.5). There was also an improvement in ultrasound examination. Patients were randomized in two groups: active group (n=20) receiving low-dose hydrophilic statin (rosuvastatin 5mg/day) and witness group (n=20) receiving placebo. Patients underwent liver enzymes testing every 3 months and at closing. At the end of follow-up period subjects accomplished a second ultrasound examination.

Conclusion: In our study, statins proved to be safe and efficient for the treatment of NASH, but larger clinical studies are needed to further refine this beneficial effect.

420

Epidermal adipose tissue as a predictor of cardiovascular outcome in patients with ACS undergoing PCI

M. Teschke1; M. Rohla1; C. Hauser1; K. Huber1; H. Wögler2; T. Weis3

Aims: We sought to investigate the association between epidermal adipose tissue (EAT) and cardiovascular events in patients with acute coronary syndrome (ACS) undergoing percutaneous coronary intervention (PCI).

Methods: Out of 1198 patients undergoing PCI, 438 had a transthoracic echocardiography during index hospitalisation. EAT thickness was measured in the parasternal long-axis view, perpendicular to the free wall of the right ventricle at end-systole in 3 independent cardiac cycles. Patients were stratified by the median of EAT thickness. The primary endpoint was cardiovascular mortality, the secondary endpoint was all-cause mortality.

Results: Patients were included between 2004-2012, 33.1% were female. Median EAT was 2.65 mm (IQR 1.89-4.51). Patients suffering from diabetes (p=0.049) and patients with previous myocardial infarction (MI) (p=0.017) had significantly higher EAT thickness. Moreover, EAT was correlated with BMI (R=0.69 vs 1.0; p=0.001), mean age (R=0.68 vs 0.6; p=0.001), and weight (R=0.67 vs 0.6; p=0.001). There was also an improvement in ultrasound examination. Patients were randomized in two groups: active group (n=20) receiving low-dose hydrophilic statin (rosuvastatin 5mg/day) and witness group (n=20) receiving placebo. Patients underwent liver enzymes testing every 3 months and at closing. At the end of follow-up period subjects accomplished a second ultrasound examination.

Conclusion: In our study, statins proved to be safe and efficient for the treatment of NASH, but larger clinical studies are needed to further refine this beneficial effect.

424

Molecular mechanisms underlying the beneficial effects of Uro cortin 2 in pulmonary arterial hypertension

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Pulmonary arterial hypertension (PAH) leads to right ventricular (RV) failure and death. Urocortin (UCN)-2 is a peptide highly expressed in the cardiovascular system. It was previously shown, in an experimental model of MCT-induced PAH, that UCN-2 treatment is able to restore PAH-induced right ventricular hypertrophy and pulmonary artery pressure. We investigated the underlying molecular mechanisms to the beneficial effects of UCN-2 in myoccardial function, in the same experimental model.

Male Wistar rats randomly received monocrotaline (MCT, 60mg/Kg) or vehicle. After 14 days, animals were randomly assigned to receive UCN-2 (5ug/Kg/day) or vehicle. The study resulted in 4 groups: CTRL (n=9); CTRL+UCN-2 (n=9); MCT (n=7) and MCT+UCN-2 (n=10). RV sample collection for RT-PCR and western blot was performed 24-25 days after MCT administration. Significant results (mean ± SEM, p<0.05) are given.

Molecular analysis showed that expression of UCN-2 and its receptor CRHR2 are altered in MCT animals. In the RV, the UCN-2 levels are increased in MCT animals (MCT vs. CTRL: 2.5 ± 0.9 vs. 1.0 ± 0.3AU), while CRHR2 levels are decreased (0.5 ± 0.1 vs. 1.0 ± 0.1AU). These values are

Arterial and pulmonary hypertension

423

Dependence between heart rhythm disorders and ID polymorphism of ACE gene in hypertensive patients

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The uncontrolled hypertension is serious problem of the modern cardiology and strongly associated with stroke and myocardial infarction. Arrhythmias development determines by structural alterations of the heart, electrolyte disorders and renin-angiotensin system activity.

The purpose of the study was to evaluate the role of ID polymorphism of ACE gene in patients with uncontrolled hypertension.

We examined 75 patients (the mean age 55 (9) years) with uncontrolled hypertension and 50 patients with controlled hypertension. Among these were 53 (10) years. Patients with acute myocardial infarction in history, diabetes mellitus and autoimmune diseases were excluded from the study. In all patients performed ECG in 12 standart leads, Holter ECG monitoring. EchoCG (AS/EASE recommendations 2012) and was evaluated ID genotype polymorphism of ACE by the polymerase chain reaction method. Statistical significance was defined at the level of methods for p<0.05.

Distribution of different types of arrhythmias in dependence of ID polymorphism of ACE gene is shown in the Table 1. We didn’t found statistically significant differences in arrhythmias development in dependence of ID polymorphism in patients with controlled hypertension. But in patients with uncontrolled hypertension supraventricular arrhythmias were observed significantly frequently in persons with DD genotype in comparison with persons with II genotype (92% (25 persons) vs 66.7% (8 persons). Chi2 =5.82; p=0.016). Also ventricular extrasystoles were observed frequently in patients with DD genotype in comparison with patients with II genotype (78% (21 persons) vs 58.3% (7 persons); Chi2=4.2; p=0.04).

Thus, the results of the study show that significant increase of frequency of arrhythmias occurrence is observed in patients with uncontrolled hypertension with DD genotype.

Arthromas and ACE gene polymorphism

<table>
<thead>
<tr>
<th>Type of arrhythmias</th>
<th>Genotypes of ACE (n=123)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controlled hypertension (n=50)</td>
<td>Uncontrolled hypertension (n=73)</td>
</tr>
<tr>
<td>II (n=17)</td>
<td>ID (n=23)</td>
</tr>
<tr>
<td>SVVP</td>
<td>10 (58.8%)</td>
</tr>
<tr>
<td>Frequent SVVP</td>
<td>2 (11.8%)</td>
</tr>
<tr>
<td>Ventricular extrasystoles</td>
<td>8 (47%)</td>
</tr>
<tr>
<td>Frequent ventricular extrasystoles</td>
<td>1 (5.8%)</td>
</tr>
<tr>
<td>SVVP and paroxysmal AF</td>
<td>1 (-)</td>
</tr>
</tbody>
</table>

Results are shown as n (%). * - p < 0.05 in comparison with patients with II genotype and uncontrolled hypertension. SVVP - supraventricular premature beat; SVT - supraventricular tachycardia; AF - atrial fibrillation.
reversed with UCN-2 treatment (0.5 ± 0.1 and 0.9 ± 0.1AU, respectively). Pathology markers are increased in MCT animals, namely BNP (15.3 ± 2 x 10 ± 0.1AU), ET-1 (3.4 ± 0.4 x 10 ± 0.2AU) and HIF-α (1.6 ± 0.3 x 10 ± 0.2AU) and are attenuated or reversed with UCN-2 treatment (6.9 ± 2.1, 18 ± 0.6, 10 ± 0.1AU, respectively). At the metabolic level, the MCT animals showed higher expression of GLUT1 (3.8 ± 0.8 x 10 ± 0.1AU) and lower expression of GLUT4 (0.5 ± 0.1 x 10 ± 0.1AU) when compared to control animals. UCN-2 treated animals treated GLUT1 expression is reversed (1.5 ± 0.4 x 10 ± 0.1AU) and GLUT4 expression lowered (0.6 ± 0.1 x 10 ± 0.1AU). Caspases 3 and 8, two apoptotic markers are elevated in the MCT animals (3.9 ± 0.6 vs 10 ± 0.1AU and 2.8 ± 0.31 x 10 ± 0.2AU, respectively) and are attenuated by UCN-2 treatment (2.0 ± 0.4 and 1.3 ± 0.2AU, respectively). We have found decreased expression levels of ERK and AKT protein phosphorylation in MCT animals (0.6 ± 0.1, 0.5 ± 0.1 and 0.5 ± 0.04AU, values which were also reversed with UCN-2 treatment (1.0 ± 0.1, 0.9 ± 0.1, 1.1 ± 0.2AU).

In conclusion, we show that UCN-2 treatment is able to restore the changes in expression of markers of cell turnover, hypertrophy and hypoxia induced by PH. The beneficial effects of UCN-2 treatment appear to be modulated with the association of different signaling pathways, namely apoptotic, metabolic, and survival/ proliferation pathways.

425 Inhibition of TGFβα axis and action of renin-angiotensin system in human ascending aorta aneurysms

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Aneurysms of the ascending aorta are usually noninflammatory and characterized by dilatation of the artery, with loss of extracellular components and smooth muscle cell (SMC) apoptosis as a result of the action of metalloproteinases. We recently demonstrated that the TGFβ-SMAD-2 pathway, that modulates the processes of tissue synthesis and repair, is inhibited. There are evidences indicating that the linking of caveolin-1 with TGFβ receptor is involved such inhibition. Another point to take into account is that most patients have systemic arterial hypertension—thus, it is expected that the renin-angiotensin system (RAS) may contribute to the lesion by actions on the SMC. The RAS shares intracellular signaling ligands with elements of the TGFβ axis, and may trigger intracellular events via the angiotensin II receptor (AT1). The action of RAS associated to the inhibition of the TGFβ pathway may be pivotal events in the pathogenesis of the aneurysms. The objective of the present study is to evaluate the presence of caveolin-1, angiotensin converting enzyme (ACE), and AT1 in the media layer of human ascending aorta aneurysms. Samples of ascending aortas from hypertensive patients with aneurysms submitted to surgical correction (n=10) and, as controls, from 10 patients with aneurysms submitted to coronary artery bypass surgery (n=10) underwent standard histological preparation and immunohistochemical reactions to caveolin-1, ACE, and AT1. Medial positive immunostaining was quantified by using a image analysis system coupled to a light microscope. Groups were compared by 1 or Mann-Whitney tests, with p<0.05 being taken as significant. All three components were increased in patients with aneurysms: means of 14.17%, 3.76%, and 7.77% (medians 13.54%, 3.60%, and 7.29%) respectively for caveolin-1, ACE, and AT1 in aneurysms, and 5.47%, 17.7%, and 2.61% (medians 5.24%, 13.7%, and 2.55%) in controls, with p<0.01 for all analyses. The expressive increase of caveolin-1 in the media layer in cases of aneurysms reinforces the hypothesis of the inhibition of the TGFβ pathway in these cases, suggesting that TGFβ receptor can bind this molecule, thus impairing the processes of synthesis and tissue repair. The increases in ACE and AT1 indicate the presence of angiotensin and its probable participation in the SMAD-2 signaling cascade throughout a TGFβ-independent, which would result in an (hyper-) contractile phenotype. Long-term telemetric tracing identified significantly reduced basal systolic and diastolic blood pressure in Tie-2ΔSMC mice. DOCA salt-induced hypertension experiments revealed a significant decrease in the systolic and diastolic blood pressure in Tie-2ΔSMC mice, validating in an unbiased genetic model an important role of VSMC-expressed Tie-2 in regulating blood pressure. To obtain further mechanistic insight into the role of Tie-2 in VSMC, we performed microarray gene expression profiling experiments on isolated aortic VSMC from control and Tie-2ΔSMC mice. Contractile smooth muscle cell markers were upregulated in VSMC from Tie-2ΔSMC mice. Moreover, the proliferation of VSMC from Tie-2ΔSMC mice was significantly reduced as compared to control mice, further supporting a role of VSMC expressed Tie-2 in regulating the contractile VSMC phenotype.

Conclusion: Taken together, this study demonstrates for the first time a functional role of VSMC-expressed Tie-2 in the regulation of blood pressure, vascular phenotype and vascular tone during hypertension.

428 Cardiac and vascular remodelling in the development of chronic thrombo-embolic pulmonary hypertension in a novel swine model

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Background: Pulmonary hypertension (PH) complicates several highly prevalent chronic diseases. It is a chronic disease of the pulmonary vasculature which almost invariably leads to right heart failure and death. One form of PH is chronic thrombo-embolic pulmonary hypertension (CTEPH) which develops in about 4% of patients after a pulmonary embolism.

Purpose: We aim to develop a chronic CTEPH swine model to facilitate early recognition of vascular and cardiac remodelling during development of PH.

Methods: Swine (n=14) were chronically instrumented with catheters in the right venous (RV), pulmonary artery and aorta, a flow probe around the ascending aorta, for blood sampling, measurement of blood pressures and cardiac output. CTEPH was induced in 6 of these swine by repeated infusion of microspheres (070µm, total ~60000) in the pulmonary circulation. During long-term follow-up (7-9weeks) pulmonary hemodynamics at rest and during exercise were measured and RV- dimensions were obtained using echocardiography.

Results: Repeated embolization of the pulmonary vasculature resulted in an increase in pulmonary artery pressure (PAP) and pulmonary vascular resistance (PVR) (Table 1). PVR did not change during exercise in either the control or CTEPH swine. The elevation in PAP was sustained during exercise, despite an attenuated increase in cardiac index (CI). RV dilation and hypertrophy occurred as evidenced by an increase in RV end-diastolic area of 27.4 ± 3.37 ± 2mm2/kg and RV/body- weight ratio from 0.76 ± 0.06 to 1.67 ± 0.11 in control and CTEPH resp. After 7weeks of CTEPH, the elevation of PAP and PVR were sustained while CI was still reduced at rest. However, CI did increase slightly during exercise at 7 weeks, suggesting that the RV hypertrophy had occurred as a result of the sustained increase in afterload. Indeed RV hypertrophy was closely related to the increase in PVR (r=0.51).

Conclusions: Repeated embolization procedures result in sustained PH. In early PH, the RV dilates and RV dysfunction is present as evidenced by the attenuated increase in CI during exercise. When PH is sustained, RV hypertrophy occurs, which, together with an increase in heart rate is able to support an increased CI during exercise.
**Biomarkers**

### 431 Arrhythmogenic cardiomyopathy: a new, non-invasive biomarker


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**Introduction:** The diagnosis of Arrhythmogenic Cardiomyopathy (ACM) is challenging and often delayed in its onset. It has a scoring system of major and minor criteria, requiring several clinical, instrumental and genetic tests. Diagnosis confirmation is often obtained by invasive procedures. To address the lack of non-invasive biomarkers for ACM we hypothesized that microRNAs (miRNAs), which have been proposed as circulating biomarkers for many cardiac diseases (e.g. heart failure, myocardial infarction, atrial fibrillation) may be used as novel potential diagnostic tools in ACM.

**Purposes:** We aimed at identifying plasma miRNAs presenting differential expression in ACM patients vs healthy controls (HC) and patients with ventricular arrhythmias of different aetiology.

**Methods:** Plasma expression of 367 miRNAs was evaluated in ACM patients (n=33), as defined by current guidelines, vs age and sex-matched HC (n=33) using TaqMan Arrays. Validation was conducted by qRT-PCR in ACM patients (n=34) and in non-ACM controls (n=34) and analysed using MAGMA software.

**Results:** miR-320a showed a statistically significant lower expression in ACM when compared to HC (p<0.043, p=0.014 vs IVT; 0.345+0.043 vs 0.001 vs IVT; 0.435+0.034 vs 0.001 vs HC). No significant difference was found in pairwise comparisons between IVT, HC and IC groups. The differentiating potential of miR-320a expression in ACM vs HC and IVT was substantiated by ROC analysis (area under the curve 0.703, p=0.001 vs HC, 0.695, p=0.017 vs IVT). No different miR expression was found between subjects (n=12 ACM, n=17HC).

**Conclusions:** This is the first study that evaluated the diagnostic potential of circulating miRNAs in ACM. Plasma levels of miR-320a are consistently lower in ACM patients with respect to HC, IVT, and IC subjects. Further, miR-320a shows a fairly good accuracy in discriminating ACM vs HC or IVT patients. Plasma levels of mir-320a are consistently lower in ACM patients with respect to HC, IVT, and IC subjects. Further, miR-320a shows a fairly good accuracy in discriminating ACM vs HC or IVT patients.

### 432 Can circulating miRNAs distinguish type 1 and type 2 myocardial infarction?

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1Medical University of Vienna, Department of Internal Medicine II, Division of Cardiology, Vienna, Austria; 2Department of Internal Medicine, Cardiovascular Medicine, Vienna, Austria

**Background:** A type 1 myocardial infarction (MI) is usually the result of atherothrombotic coronary artery disease, whereas myocardial necrosis in type 2 MI occurs because of an increase in myocardial oxygen demand or a decrease in myocardial blood flow. Clinical discrimination between type 1 and type 2 MI is often difficult. MicroRNAs (miRNAs) are short, non-coding RNA with remarkable stability. miRNAs have emerged as a new group of potential biomarkers in several diseases, including prognosis in acute MI.

**Purpose:** Here we aimed to investigate the value of circulating miRNAs as possible biomarkers to distinguish type 1 and type 2 MI.

**Methods and Results:** Expression profiling of 372 circulating miRNAs was performed in plasma samples of patients from 2 independent cohorts. First, 20 patients presenting with type 2 MI were randomly selected from cohort of 621 acute coronary syndrome patients. 20 age- and gender-matched type 1 MI patients were selected from the same cohort. Patients with unstable angina and ST-elevation MI were excluded. Samples were pooled and miRNA arrays were performed in duplicates. miRNA expression was normalized to C. elegans glyceraldehyde 3-phosphate dehydrogenase (C. elegans GAPDH). We have observed 28 differently expressed miRNAs between the 2 groups of patients. In order to reduce the number of candidate miRNAs, we have repeated the screening in 14 consecutive patients presenting with type 1 (n=8) and type 2 (n=6) MI. Again, patients with ST-elevation MI were excluded. We have observed 18 differently expressed miRNAs between the 2 groups. Among these, miR-1183 and -1207 demonstrated the highest differences between the two groups of patients with a fold upregulation (p<0.001) in patients suffering type 2 MI in both study cohorts.

**Conclusion:** Circulating miRNAs represent potential novel biomarkers to distinguish type 1 and type 2 MI. However, further analysis in a larger, prospective cohort is necessary to elucidate the diagnostic value of specific miRNA candidates in patients with type 1 and type 2 MI.

#### 433 Design of a high-throughput multiplex proteomics assay to identify left ventricular diastolic dysfunction in diabetes

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**Introduction:** Heart failure (HF) has reached epidemic proportions in Europe, affecting approximately 2% of the population. These figures will be exacerbated by the increasing prevalence of diabetes mellitus (DM) which is a significant driver of cardiac fibrosis, cardiac dysfunction, and subsequent HF. Cardiovascular death is the leading cause of death among diabetes patients. It is important to focus care on this population with the aim to prevent the development of diabetic cardiomyopathy and the progression to HF. Early cardiac dysfunction is detected using resource-intensive Doppler-echocardiography. The aim of this ongoing study is to identify serum protein biomarkers that can be used to identify diabetic patients that have asymptomatic left ventricular diastolic dysfunction (LVDD) using multiple reaction monitoring (MRM) mass spectrometry. Asymptomatic LVDD is a precursor to HF that occurs due to cardiac fibrosis and reduced compliance of the LV.

**Methods:** The study population consists of 1346 patients from within the STOP-HF cohort in Ireland. A subset of 200 samples was used for the discovery phase of this project, consisting of 48 patients and gender matched groups of 50 patients: DM, LVDD, DM + noLVDD, noDM + LVDD, noDM + noLVDD. LVDD was defined as left atrial volume index equal to or greater than 34ml/m2 and an E<10 cm/s. Samples were enriched via immune-depletion and digested using trypsin before being run on a Q-Exactive mass spectrometer (Thermo Scientific).

**Results:** Statistical analysis using Perseus and MRM software packages revealed 261 unique proteins from the discovery phase. Of these 261 proteins, 68 were identified as potential biomarkers for the identification of LVDD in diabetic patients. GO Term enrichment analysis of these 68 proteins revealed a trend that points to the involvement of a large number of inflammatory processes. Terms such as “humoral immune response”, “complement activation”, “acute inflammatory response” and “regulation of complement activation” were all significantly overexpressed (q-values: 1.00E-30, 1.00E-30, 1.00E-30, respectively).

**Conclusions:** 50 of the shortlisted 68 proteins are to be verified using quantitative MRM mass spectrometry. Proteotypic surrogate peptides are used to selectively target the individual proteins which will be subsequently validated in a larger independent cohort. The process of determining the potential validity of a peptide is aided by Skyline software which visually displays any interference and the optimum transitions of the peptides. The GO Term inflammatory trends have revealed a known key pathological process. Both heart failure and diabetes have significant inflammatory components, which may be a key contributor to the development of diabetic cardiomyopathy. Verification and validation of protein biomarkers and their subsequent functional analysis, will lead to the identification of key contributors to pathological processes, inflammation-related or otherwise.

### 434 Monocyte-derived and P-selectin-carrying microparticles are differently modified by a low fat diet in patients with cardiovascular risk factors who will and who will not develop a cardiovascular event

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**Background:** Circulating microparticles (cMPs) are small size phospholipid-rich blebs shed by cells that will not suffer a CVE throughout the 5 years study follow-up (from now, no-CVE). Blood samples were taken at baseline and after one year of dietary intervention. Annexin V positive cMPs from plaques were identified using flow cytometry. A t-test was performed to analyse: a) changes after one year for no- and CVE subgroups; and b) differences of changes in cMPs after one year between no- and CVE patients.

**Methods:** Subjects were randomly allocated to a low fat diet in patients with cardiovascular risk factors who will and who will not develop a cardiovascular event.

**Results:** Statistical analysis using Perseus and MRP software packages revealed 261 unique proteins from the discovery phase. Of these 261 proteins, 68 were identified as potential biomarkers for the identification of LVDD in diabetic patients. GO Term enrichment analysis of these 68 proteins revealed a trend that points to the involvement of a large number of inflammatory processes. Terms such as “humoral immune response”, “complement activation”, “acute inflammatory response” and “regulation of complement activation” were all significantly overexpressed (q-values: 1.00E-30, 1.00E-30, 1.00E-30, respectively).

**Conclusions:** 50 of the shortlisted 68 proteins are to be verified using quantitative MRM mass spectrometry. Proteotypic surrogate peptides are used to selectively target the individual proteins which will be subsequently validated in a larger independent cohort. The process of determining the potential validity of a peptide is aided by Skyline software which visually displays any interference and the optimum transitions of the peptides. The GO Term inflammatory trends have revealed a known key pathological process. Both heart failure and diabetes have significant inflammatory components, which may be a key contributor to the development of diabetic cardiomyopathy. Verification and validation of protein biomarkers and their subsequent functional analysis, will lead to the identification of key contributors to pathological processes, inflammation-related or otherwise.
Conclusions: Monocytes and platelets are activated in patients who will suffer a future CVE demonstrated by their increased MP shedding. This activation is not passivated by the standard pharmacological treatment or a nutritionally controlled low fat diet. Therefore, monocyte-MP and platelet-MP shedding associates to atherosclerosis and presentation of a future cardiovascular events in a high CV risk cohort of patients.

435 Red blood cell distribution width assessment by polychromat interferences microscopy of thin films in chronic heart failure

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Background: Red blood cells (RBC) distribution width (RDW) is a promising hematology parameter with broad applications in cardiology. RDW has been shown to be associated with increased risk of heart failure (HF) in general population. Clinical usefulness of RDW significantly limited by high variability of this parameter measured by different hematology analyzers.

The purpose of the study was to assess thickness-profile measurement of thin blood smears by white-light scanning interferometry as a novel tool to evaluate red blood cells morphology and functional parameters in chronic heart failure patients.

Methods: Object of the study was thin blood smears prepared on polished silicon plate. Patients with chronic HF were included in the study (total involvement, n = 1 321). Blood tests were performed by automatic hematology analyzers ABS Micros and Datacell-16. Acute hyperglycemia test was used to verify variability of the thickness-profile measurements. Using created dependence between colors vs. optical thickness the optical thickness (OT) in chosen point was measured in our study. RDW was calculated as following: RDW = (ΔOT/M)*100, where ΔOT is standard deviation of measured thicknesses of RBC and M is mean value of RBC thickness in studied blood samples. Additionally, aggregation ability of RBC was estimated as aggregated cells count.

Results: If OT of the object is less than half of length of temporal coherence of incident light interference of waves reflected by object boundaries appears. The OT of the film determines conditions of interference max and min observation. In case of illumination using white light the locations of interference max and min are not coincided for permanent object OT. As a result, color mixing occurs in different proportions, and an image of thin film acquires a particular color. Usage of high reflectance polished silicon plate significantly increases reflection of lower boundary and get bright interference colors in the images of red blood cells (Fig. 1, a). Developed method of OT measurement bases on comparison of interference color of investigated image with color of simulated interference pattern.

The stage of simulating is process of creating color image presenting the dependence of interference colors vs. object OT. RBC OT in HF patients was 877.6 ± 114.2 nm, variance =1303.69 (Fig. 1 c,d). RBC OT reveal strong positive correlation with MCV, RDW (R = 0.84, 0.79 p < 0.05), BNP morphology significantly changes during acute hyperglycemia test (0–10–61 min: Chi Sqr. (n = 220, df = 2) = 8.12 p = 0.017). Variability of studied method was 0.2 compare to 0.5 and 0.4 (automatic hematology analyzers ABS Micros and Datacell-16).

Conclusion: Received data elucidate interference microscopy as a promising method for RDW assessment in routine clinical practice. This method is particularly high sensitive promising tool to assess morphological and functional RBC properties as a prognostic biomarkers in HF patients.

436 Invasive and noninvasive evaluation of quality of radiofrequency-induced cardiac denervation in patients with atrial fibrillation

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Aim: The aim of the study was to develop the methods for evaluation of the efficacy of radiofrequency-induced cardiac denervation.

Materials and Methods: A total of 42 patients aged 60 ± 9.4 years with long-standing atrial fibrillation (AF) and heart failure NYHA class III–IV were included in the study. Patients were assigned to two groups: group 1 comprised 39 patients who received radiofrequency ablation (RFA) for AF together with ablation of paragangial nerve plexi according to the scheme proposed by C. Pappone (2004); group 2 (control) comprised 15 patients with sinus rhythm (no denervation was performed).

Patients were operated using cardiopulmonary bypass. Both groups received scintigraphy-based evaluation of sympathetic tone with metaiodobenzylguanidine (MIBG) with assessment of washout and accumulation defect (50%) before operation and after surgery at a time of discharge from hospital. Levels of norepinephrine and metanephrine were assessed before cardiopulmonary bypass and 10 min after release of aortic clamp.

Results: MIBG washout rates were 30.4 ± 16.7% before and 37.83 ± 14.2% after surgery (p < 0.011). The values of early-H/M were 1.69 ± 0.18 before and 1.47 ± 0.17 after operation (p=0.025). The values of delay-H/M were 1.62 ± 0.249 and 1.39 ± 0.17 after surgery (p=0.014). Accumulation defects were 10.35 ± 3.52% before and 23.67 ± 6.12% after surgery (p=0.012). The levels of norepinephrine and metanephrine were significantly higher in group 1 in blood samples from ascending aorta before surgery. After main step of surgery, a significant increase in the content of norepinephrine (p<0.0003) was detected in the coronary sinus in patients after AF RFA suggesting a decrease in myocardial norepinephrine uptake.

Conclusions: Total sympathetic tone in patients with AF was initially higher. In patients who received destruction of paragangial nerve plexi ablation, myocardial norepinephrine uptake was affected suggesting the efficacy of cardiac denervation.

437 The effect of therapeutic hypothermia on the level of brain derived neurotrophic factor (BDNF) in sera following cardiopulmonary resuscitation

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Introduction: Neuroprotective effect of therapeutic hypothermia after cardiopulmonary resuscitation (CPR) has been claimed to be beneficial. However, there are no studies which investigated BDNF levels in patients treated with mild hypothermia.

Materials and Methods: 46 patients were evaluated as a prospective way between 2009 and 2012. Acute myocardial infarction was proved by coronaryography after resuscitation in 37 patients. All patients were cooled for 24 h. Blood samples were collected before hypothermia (0 h) and after 6 and 24 h later. The levels of BDNF in sera were measured by ELISA. Neurological outcome was determined with Cerebral Performance Categories (CPC) scale at discharge.

Results: At the time of admission all patients were discharged with good neurological outcome (CPC-I-II) and 17 patients with poor neurological outcome (CPC-III-IV). 10 patients died in the intensive care unit. During the 30-day follow-up another 12 patients died, 24 patients survived. BDNF values measured at 0, 6 and 24 hours showed a rising tendency, but the trend was not significant (9.4 ng/mL (2,145) vs. 10.8 ng/mL (3,16-25) vs. 12.5 ng/mL (8,4-16,2)). No significant difference was found between patients with good and poor neurological outcome or according to mortality. When BDNF values in AMI patients after 24-h hypothermia were compared to STEMI patients similar age and gender, results were significantly higher in the STEMI group: 15.7 ng/mL (14,4-22,5) vs. 12.1 ng/mL (7,15), p<0.0001.

Conclusion: Cardiac arrest provoked by infarction decreases the BDNF level compared with STEMI patients, and this effect can be reversed with mild, 24-h therapeutic hypothermia. However, no correlation was found between BDNF results and neurological outcome in our patients, thus it is presumably can’t be used for prediction of neuroprotection.

438 Novel biomarkers to predict outcome in patients with heart failure and severe aortic stenosis

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Introduction: Severe Aortic stenosis (AS) and non-valvular heart failure (HF) appear to have different pathological processes, and therefore cardiac remodelling is likely to involve different pathways.

Novel biomarkers have been developed to assess prognosis, response to treatment, and also to understand the underlying mechanism of cardiac remodelling. We identified from the literature the biomarkers that have shown to demonstrate promise in assessing aortic stenosis and heart failure. We compared the differences in levels between the two groups and to see how they predict outcome (all-cause mortality). We identified biomarkers of fibrosis (ST2, galectin-3), matrix remodelling (osteopontin, PIINP, TIMP1), stretch (NT-pro BNP, cardioprotein).

Methods: We studied a total of 140 patients. 48 patients were recruited with chronic heart failure and EF ≤ 40% from the HF clinics in Hospital, on optimal doses of HF medication and device therapy according to European Society of Cardiology Guidelines. In addition, we also examined 92 patients who were awaiting TAVI for severe aortic stenosis. Prior consent was sought for analysis of NT-pro NP, serum ST2, Galectin 3, osteopontin, TIMP1, cardioprotein and PIINP. These patients were followed up as part of routine care for the time of the study. No patients were lost to follow-up. Statistical analysis was performed on SPSS, and median biomarker values were analysed non-parametrically. We chose 2 year follow up because of many landmark studies investigating the prognosis of severe aortic stenosis and the impact of TAVI are around 2 years.

Results: Out of a total of 140 patients, 24 patients were registered dead at one year and 81 at the end of 3 year follow up. Baseline ejection fraction remains the best predictor of prognosis of all causes mortality at one year in keeping with previous studies. However, the area under the curve for ST2 at baseline appeared to be the most promising of all the biomarkers (0.612) compared with NT-pro BNP (0.610), TIMP1 (0.610), Cardioprotein (0.602) osteopontin (0.601), PIINP (0.353), Galectin 3 (0.370). When we repeated the ROC analysis with 2 year mortality, NT-pro BNP still had the highest AUC (0.685), followed by TIMP 1 (0.639), Osteopontin (0.631), Cardioprotein (0.628), TIMP2 (0.555), ejection fraction (0.528), Galectin 3 (0.458) and PIINP (0.322). Combining biomarkers in a multi-marker panel improved the AUJC even further.

Conclusions: Novel biomarkers appear to give important prognostic information as good as ejection fraction and traditional biomarkers like NT-pro BNP in patients with severe aortic stenosis and optimally managed heart failure. Novel biomarkers which may provide similar prognostic information individually or as a multi-marker panel may be an alternative worthy of a larger trial in future.
439 Biological factors linking depression and anxiety to cardiovascular disease

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Introduction: Depression and anxiety has been related to a higher risk of developing coronary heart disease, but the mechanism that accounts for this association is unclear. Recent advances in biological psychiatry have included the discovery of numerous neurochemical, neuroendocrine, and neuroanatomical alterations in depression and anxiety disorder. The aim of our study was to investigate the association between depressive episode and anxiety and presence of high cholesterol as well as C-reactive protein (Crp) level in patients with aorto-coronary bypass graft surgery and coronary angioplasty. Methods: The research was performed in n=70 patients. Research group was divided into two groups. The first was angioplasty group (n=35 patients) and the second was aorto-coronary bypass graft surgery group (n=35 patients). Investigation was made in pre- and postoperative periods of coronary angioplasty and aorto-coronary bypass graft surgery. To evaluate depression we used Beck depression scale. Anxiety was assessed by the Spielberg State-trait anxiety scale.

Results: Our study demonstrated strong association between depression, state anxiety and increased total cholesterol level in pre and post-operative periods (after 6 months) of coronary angioplasty (n=0.6498; p<0.001; r=0.4867, p<0.001). It was also revealed correlation between depression, state and trait anxiety and increased total cholesterol level in pre and post-operative periods (up to 1 year) of aorto-coronary bypass graft surgery group (n=0.66522 p<0.001; r=0.56865 p=0.003; r=0.4767, p<0.001). All these patients in postoperative periods received anti-ischemic treatment (β-blockers, statins, antiplatelet drugs). Results show that increased level of C-reactive protein (Crp) were registered in aorto-coronary bypass graft surgery group (n=38, 70%), in angioplasty group C-reactive protein was elevated in n=12 (30%), p=0.012. In angioplasty group patients had increased level of Crp had high degree of depression p=0.010. In these group was revealed high degree of trait anxiety too p=0.001. In aorto-coronary bypass surgery group elevated level of Crp was associated with high degree of depression p=0.00.

Conclusions: In this study of patients with documented coronary artery disease it was revealed strong association between moderate and high level of depression and anxiety and total cholesterol level in both groups. Our study also demonstrated association between depression, anxiety and increased C-reactive protein level. Inflammation, the key regulator of Crp synthesis, plays a pivotal role in some cardiovascular disease. These results may have important implications in explaining the diverse physiological mechanisms linking depression and anxiety to cardiovascular disease. Greater attention to depression and anxiety in patients may allow physicians to provide more appropriate and cost-effective care for them and avoid complications.

440 Troponins and myoglobin dynamic at coronary arteries grafting

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Coronary arteries bypass grafting (CABG) is the most effective method of treatment of ischemic heart disease. CABG is naturally accompanied by cardiomyocytes damage. Troponins (Tns) and myoglobin (Myo) are indicators of development of myocardial damage, ischemia and necrosis, and the subsequent adverse reverse.

The aim of the study was to investigate features of Tns and Myo concentrations changes in an estimation of myocardial ischemic damage at various kind of CABG.

Materials and Methods: Depending on a kind of CABG patients were divided into groups: group 1—off-pump CABG; group 2—beating heart-on-pump CABG; group 3—on-pump CABG. Before, in the end, in 6 and 12 hours after surgery measured concentration Tns (mkg/l) and Myo (mkg/l).

Results: In the end of operation the increase in Tns at all groups maximum in group 3 (p=0.065 vs groups 1-2) was marked. In 6 hours per group 1 Tns does not change, whereas in groups 2 and 3 it was decreased (p=0.009). In 6 and 12 hours after surgery Measured concentration Myo (mkg/l) and Tns (mkg/l) in the end of operation at all patients sharp increase of Myo level was observed, is authentic.

Increases of Trs concentration after CABG is not necessarily connected with myocardial ischemic damage and caused by duration CPB and aorta cross clamping, and also a surgical trauma myocardium: occurrence local microcnicuss owing to manipulations on heart. Considerable increase of Myo in early terms after CABG reflects damage of skeletal muscles during surgical intervention.

Invasive, non-invasive and molecular imaging

443 Diet composition effects on the genetic typing of the mouse ob mutation: a micro-ultrasound characterization of cardiac function, macro and micro circulation and liver steatosis

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Obesity is an independent risk factor for cardiovascular disease. The obese (ob) gene mutation plays a central role in the development of this pathology, but less is known about the interaction between the genetic typing and diet composition. Aim of this study was to investigate the effects of genodietetic combination in mice in terms of cardiac function, macro/micro circulation and hepatic fat content. 7 Ob/+ mice on standard diet (Ob/+_SD, 25wo), 6 Ob/+ mice on high-fat diet (Ob/+_HF, 25 wo) and 7 Ob/Ob mice on standard diet (Ob/Ob/SD, 25 wo) were examined using micro-ultrasound (Veo2100, VisioSonics). The high-fat diet (45% fat) group was fed for 18 weeks using US scans.

Cardiac output (CO), ejection fraction (EF) and E/A ratio were calculated. Mean diameter (Dm_abd,Dm_car), relative distention (rdi_abd,rdi_car) and pulse wave velocity (PWV_abd,PWV_car) were assessed for abdominal aorta and carotid artery. Renal resistivity and pulsatility index (RI,PI) and coronary flow reserve (CFR) were evaluated. Hepatic fat content was estimated calculating the hepatic/renal ratio (HR_ratio).

No significant differences were found for cardiac and large arteries parameters (Table 1). RI and PI were significantly higher in Ob/+_HF (RI 0.73±0.034; PI 1.19±0.10) and Ob/Ob/SD (RI 0.70±0.046; PI 1.13±0.11) than in Ob/+_SD (RI 0.64±0.039; PI 0.99±0.084). CFR values were significantly lower for Ob/Ob/SD (1.57±0.86) than for Ob/+_HF (2.12±0.92) and Ob/+_SD (2.97±0.73). HR_ratio was significantly higher for Ob/Ob/SD (1.43±0.28) than for both Ob/+_HF (0.92±0.21) and Ob/+_SD (0.75±0.17).

High-fat diet influences renal microcirculation but not coronary one and does not condition liver fat content. Therefore, the Ob/+_HF model is comparable to the Ob/Ob/SD one in terms of renal microcirculation, but is closer to the Ob/+_SD for the coronary one and liver steatosis grading.

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Cardiovascular Research Supplements

444 Characterization of pig coronary and rabbit aortic lesions using IV-OCT quantitative analysis: correlations with histology

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Background: Optical coherence tomography (OCT) is the highest-resolution imaging technique available for studying the structure and composition of atherosclerotic plaques. Currently, interpreting the OCT images is mainly based on the experience of the trained professionals.

Purpose of the Study: To investigate possible application of OCT analysis software developed at our university in atherosclerotic plaque imaging.

Methods: Lesions were induced in 6 pigs, 3 New Zealand white (NZW) rabbits and 2 Watanabe heritable hyperlipidemic (WHHL) rabbits using high cholesterol diet (excluding WHHL rabbits) and causing endothelial over dilatation injury with a balloon catheter via femoral artery. After 42 days the injured arteries were imaged in vivo with IV-OCT, the animals were sacrificed and their arteries harvested. Lesions were identified and classified based on their histological structure to inflamed, fibrous, smooth muscle cell (SMC), -rich and calcific lesions. Some hematomas following injuries were also classified as their own group. Same lesions were then analyzed from OCT images using five different parameters: ORC, YSR, OBS, alpha and gamma. Statistical analyses between parameters and histology were conducted using SPSS software.

Results: Seventy-two lesions were classified and analyzed. Inflamed plaques had to be excluded from final analysis due their small n. In addition, we could find clear statistical differences (P<0.009) between other lesion types and hematomas using 2 of our parameters: Optical backscatter (OBS) and optical reflectance coefficient (OCF).

Conclusions: We demonstrated good correlation between certain OCT parameters and histological features of the lesions proving that quantitative OCT analysis seems a promising tool in the characterization of atherosclerotic plaques in vivo.
Enhancing the survival and angiogenic potential of mouse atrial mesenchymal cells
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Endogenous cardiac progenitor cells (CPCs) have the potential to regenerate damaged tissue following myocardial infarction. Various CPC populations have been proposed but doubt remains as to which is the optimal population for regenerative therapy. In order to induce regeneration, progenitor cells expanded in vitro need to be robust enough to survive after transplantation into the peri-infarct region of the heart. We have isolated a mouse atrial cell population by collagenase and trypsin digestion. Here we have compared these cells (known as CT cells) to cardiac-sphere-derived cells (CDCs) and established the ability of both cell types to survive under serum-starvation. MicroRNA210 has been shown to have anti-apoptotic and pro-angiogenic effects in HE1 cardiomyocytes, so here we have investigated whether transfection with miR210 increased the therapeutic potential of CT cells. Cells from murine cardiac atria were expanded as CDCs by explanting atrial tissue on fibronectin and culturing explant derived cells as cardiospheres in hanging drops, followed by monolayer culture on fibronectin; or as CT cells after digestion using 0.1% collagenase and trypsin for 1 hour, followed by slow adhesion and monolayer culture on fibronectin. Gene expression was assessed using qRT-PCR. Cell survival was determined after culture in serum-free medium for 72 hours and 10 days. VEGF release over 24 hours was measured by ELISA. CT cells were transfected with miR210 using DharmaFECT.

Atrial CDCs and CT were isolated from mouse atria (n = 6) and cultured to passage 4 over 2 months or 28 days, respectively. Both cell types had a mesenchymal phenotype with comparable expression of the progenitor and early cardiac genes Oct3/4, c-kit, TERT, Nkx2.5, GATA4, MEF2c. However expression of Scx1 was higher in CT cells than CDCs whilst CDCs had 10-fold higher expression of the epicardial marker WT1 than CT cells. Culture in serum-free medium increased cell death over 72 hours in both cell types (n = 3) but was more pronounced in CDCs than CTs after 72 hours. CDC cell number further decreased from 3 to 10 days, whereas CT cells continued to proliferate, albeit at a much slower rate than in normal medium containing 20% serum. VEGF release per cell was comparable from CDCs and CT cells after 72 hours in serum-free medium. Transfection of CT cells (n = 3) with miR210 resulted in a 1.5 fold increase in the number of surviving cells after 10 days in serum-free medium and a 25% increase in VEGF release per cell. The CT protocol generated a mesenchymal cell population that could be expanded rapidly from atrial tissue and which survived long-term culture in serum-free medium. Transfection with miR210 enhanced cell survival and release of VEGF to further increase the therapeutic potential of the cells.

Gene therapy and cell therapy

Enhancing the survival and angiogenic potential of mouse atrial mesenchymal cells

VCAM-1 expression in experimental myocardial infarction and its relation to bone marrow-derived mononuclear cell retention
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Background: Therapeutic efficacy of intraocularly infused bone marrow-derived mononuclear cells (BMMNCs) for acute myocardial infarction (AMI) is limited, possibly because of the number of cells retained within the affected area is consistently very low. Thus, optimizing retention rates could improve therapeutic outcome. Previously we found that BMMNCs are retained in infarcted, but not in remote, myocardium. We therefore hypothesize that adhesion molecules on activated endothelium including vascular adhesion molecule 1 (VCAM-1) are essential for BMMNC retention. Consequently, we studied the relation of VCAM-1 expression and BMMNC retention in AMI and compared these to remote myocardium. Methods: We studied the role of VCAM-1 in BMMNC retention in swine with reperfused AMI produced by 120 min ischemia followed by 24 h reperfusion. We studied 6 pigs per time point (1, 3, 7, 14, and 35 days post-AMI). VCAM-1 expression was quantified with immunohistochemistry at 1, 3, 7, 14 and 35 days post-AMI (Phase I, n=6 per group). In a parallel study, composition of isolated BMMNCs (flow cytometry) was correlated to retention within each time point (Phase IIb, n=5 per group). In a parallel study, composition of isolated BMMNCs (flow cytometry) was correlated to retention within each time point (Phase IIb, n=5 per group). Data are mean ± SEM.

Conclusion(s): VCAM-1 expression influences, but is not the sole determinant of, BMMNC retention in reperfused infarcted myocardium.
Neonatal rat ventricular myocytes (NRVMs) were cultured on glass, 4.5% and 9% weight/volume bovine serum albumin (BSA) hydrogels for 3 days prior to assessment. NRVM viability was maintained on the hydrogels, with clear sarcomeric striations identified by immunofluorescence staining for alpha actinin. Expression of genes encoding cardiac proteins were quantified using RT-qPCR and demonstrated an upregulation in most of the genes of cells cultured on the hydrogels compared to glass (for example the relative expression (log2-ΔΔCt) of ryanodine receptor 2: glass = -2.3 ± 0.5, 4.5% BSA = -0.3 ± 0.1, 9% BSA = -0.2 ± 0.2, p < 0.01; cardiac alpha actinin: glass = -1.7 ± 0.5, 4.5% BSA = -0.2 ± 0.1, 9% BSA = -0.5 ± 0.2, p < 0.01; connexin 43: glass = -1.7 ± 0.5, 4.5% BSA = 0.3 ± 0.1, 9% BSA = 0.1 ± 0.2, p < 0.01, n=4-6). NRVMs were field stimulated at 1Hz and optically mapped using Fluo-4AM to produce calcium transients. We found that, compared to glass, the cells on the 4.5% and 9% BSA hydrogels have an increased time to peak (tp glass = 38 ± 3 ms, tp 4.5% BSA = 54 ± 2 ms, tp 9% BSA = 52 ± 4 ms p < 0.01, n=4-6). Compared to glass the 4.5% BSA hydrogels also have a reduced time 50% decay (t50 glass = 108 ± 13 ms, t50 4.5% BSA = 78 ± 6 ms, p < 0.05, n=4-6) and 80% decay (t80 glass = 217 ± 19 ms, t80 4.5% BSA = 152 ± 10 ms, p < 0.05, n=4-6).

The BSA hydrogels are compatible with NRVMs and the changes observed in cardiac gene expression suggest they may be used to prevent cardiomyocyte de-differentiation in culture. Further study is required to determine the mechanisms involved in calcium handling alterations and then translate this model to human serum albumin hydrogels with iPSC-CMs suitable for a tissue engineered patch.

454

A novel paintbrush technique for transfer of low viscosity ultraviolet light curable cyan methacrylate on saline immersed in-vitro sheep heart

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Aim: To develop a novel technique for the transfer of cyan methacrylate on the inner and outer surfaces of the heart.

Methods: Low viscosity ultraviolet curable cyan methacrylate was mixed with commercially available dye to demonstrate the ability of transfer on the outer and inner surfaces of the in-vitro heart model. 0.5 ml of cyan methacrylate was mixed with 0.2 ml of dye, and the material was injected over the surface of the heart in dry air; and it was allowed to fix for 2-3 seconds. Subsequently, the whole preparation was immersed in saline, and it was vigorously shaken to remove unbound compound. The similar experiment was performed without cyan methacrylate. By visual assessment, a significant quantity of the compound attaches to the surfaces of the heart compared to the dye alone, which was visualized after washing. After that, various techniques were investigated for efficient transfer of the compound to the inner and the outer surfaces of the heart after the tissue was soaked in saline. Of the various techniques paintbrush technique for the targeted transfer of the compound was effective. With this technique, it was very effective to transfer the compound on the epicardial and endocardial surfaces. Also targeted areas like left atrial appendage, left ventricular inner surfaces at the origin of papillary muscle and left ventricular apex were some of the areas investigated successfully.

Light emission diode based source of ultraviolet light was selected and tested for the ultraviolet activation properties of the compound. The ultraviolet (UV) light source was a pen shaped device, which was immersed in normal saline and activation of the compound was tested after UV screening for 5-10 seconds at a distance of 2-3 mm from the tissue. It was observed after the ultraviolet treatment to certain extent precipitation of the compound was observed. The experiment was repeated in 3 different samples to observe the techniques.

Conclusion: There is potential for transfer of low viscosity, ultraviolet curable cyan methacrylate for the study on the inner and outer surfaces of the heart. This could be potentially of use for transfer of growth factors or protein molecules on the surface of the heart.