0.0951±0.291; p<0.0001), indicating a more heterogeneous trapping of bigger than smaller particles in necrotic central zones. Moreover, the larger PFC particles were more concentrated in central zones of necrosis, whereas the smaller peptide particles more evenly permeated the entire area at risk, signifying heterogeneous severities of vascular damage, extravasation, and microvascular obstruction throughout the area at risk. These patterns persisted at all time periods after reperfusion indicating barrier disruption well beyond reperfusion.
Conclusion: Vascular barrier damage is rapid and persistent in cardiac IRI and extends throughout the area at risk. Microvascular damage is less severe in regions adjacent to well perfused tissue regions (minimal extravasation of PFC NP, but homogeneous extravasation of peptide NP), and more severe in central necrotic regions (maximal extravasation of PFC NP with skewed distributions). Thus, the heterogeneity of vascular damage is likely a key factor governing the potential for salvage of myocytes after IRI.
Funding Acknowledgements: HL073646-08

P945
PCSK9 deficiency is not associated with impaired cardiac repair capacity early after myocardial infarction

J. Winter[1], M.L. Barcena De Arrellano[2], J. Jansen[2], E. Na[1], S. Knueppel[1], V. Regitz-Zagrosek[2], U. Landmesser[1]. [1]*Charite - Campus Benjamin Franklin, Department of Cardiology, Berlin, Germany;* [2]*Charite - Campus Mitte (CCM), Institute of Gender in Medicine, Berlin, Germany*

Background: Proprotein convertase PCSK9 plays a crucial role in LDL cholesterol metabolism. PCSK9 inhibitory antibodies target circulating PCSK9, thereby preventing LDLR degradation in liver cells, ultimately decreasing serum LDL levels. Recently, they have been introduced as a potential novel treatment option in patients after a myocardial infarction. However, knockout (KO) of PCSK9 was accompanied by impaired liver regeneration after hepatectomy in mice. Here we therefore investigate the impact of PCSK9 deficiency on the cardiac repair response early after myocardial infarction (MI). It is of pivotal importance to understand the safety of PCSK9-Inhibition early after an acute MI.
Purpose: The purpose of our study was to evaluate potential adverse effects of PCSK9 absence in acute MI, with a focus on cardiac tissue remodelling and cardiac function post MI.
Methods: We analysed PCSK9 KO as well as wildtype (WT) mouse hearts by echocardiography before and after permanent ligation of the left anterior descending coronary artery (myocardial infarction, MI). Sirius red staining of tissue sections was performed to analyse fibrosis in the remote area and to determine thickness of remaining left ventricular wall of the infarcted hearts.
Results: Our study revealed, that PCSK9 KO itself did not cause significant changes in cardiac output (CO), left ventricular end systolic or diastolic volume (LVESV, LVEDV), stroke volume (SV) or ejection fraction (EF) compared to WT mice. Four weeks after MI, no significant differences in cardiac function (CO, EF, SV, LVESV, LVEDV) could be observed between WT and PCSK9 KO mice, either. These results indicate, that reduction of PCSK9 in patients with MI is not a risk factor. Additionally we observed, that the remaining left ventricular wall in the infarct area tends to be thicker in mice lacking PCSK9 (p=0,054). This was accompanied by reduced fibrosis in the remote area of infarcted hearts, even if infarct size determined by echocardiography did not show any differences between KO and WT mice. These data suggest less maladaptive remodelling in PCSK9 KO. The remaining wall in PCSK9 KO mice seems to be more stable, which could point to a lower rate of ventricular rupture and improved outcome in patients with big infarct sizes when treated with PCSK9 inhibitors.
Conclusion: Taken together, our study shows for the first time, that knockout of PCSK9 is not associated with impaired repair capacity of the heart after tissue damage during MI. Less maladaptive remodeling was observed after MI, supporting the concept that PCSK9 inhibition is safe for application early after MI.
Funding Acknowledgements: This study is funded by the DZHK (Deutsches Zentrum für Herz-Kreislauf-Forschung e.V.).

81645

ABSTRACT WITHDRAWN

ABSTRACT WITHDRAWN

P946
Stamp2 diminishes structural remodeling and systolic dysfunction in post-ischemic hearts by its anti-inflammatory properties

S. Bokredenghel, M. Mollenhauer, S. Bablok, T. Ruther, C. Proksch, E.M. Berghausen, M. Vantler, S. Rosenkranz, S. Baldus, V. Rudolph, H. Ten Freyhaus. *Cologne University Hospital - Heart Center, Cardiology, Cologne, Germany*

Background and hypothesis: The six-transmembrane protein of prostate (Stamp)-2 acts as an anti-inflammatory protein in human and murine macrophages by preventing NF-κB-dependent inflammatory signaling. Stamp2 deficiency promotes atherosclerosis in mice, but its role in other cardiovascular diseases remains unclear. We hypothesized that Stamp2 might preserve systolic and diastolic left ventricular function in myocardial ischemia-reperfusion injury.
Methods and results: Stamp2 expression was significantly reduced in isolated murine leukocytes upon lipopolysaccharide (LPS) treatment (100 ng/ml, 2 h) as compared to controls (100±6.3 vs. 68.5±23.3%, p<0.05). Next, myocardial ischemia-reperfusion injury (I/R) was induced by a temporary (40 minutes) ligation of the left anterior descending artery (LAD) followed by 7 days of reperfusion. Following I/R, Stamp2-/–mice showed significantly worse left ventricular (LV) systolic function as compared to WT as indicated by echocardiography (LV cardiac output 12.61±0.57 ml/min vs. 21.82±1.3 ml/min, p<0.05; LV fractional shortening 5.2±0.47% vs. 8.6±0.47%, p<0.05). In line with this observation, Stamp2-/– mice exhibited aggravated structural cardiac remodeling as compared to WT as shown by increased left ventricular fibrosis (picrosirius red staining, 42.2±8% vs. 25.1±11%, p<0.05).
As inflammatory processes may drive enhanced susceptibility of Stamp2 deficiency to I/R, the role of neutrophils was explored. We found significantly elevated plasma levels of the inflammatory enzyme myeloperoxidase (MPO) under baseline conditions in Stamp2-/–mice (ELISA, 145±42.5 vs. 75.9±10.5 ng/ml, p<0.05 vs. WT). Consistently, immunohistochemistry revealed increased neutrophil infiltration in the infarct region of Stamp2-/–mice as compared to WT 3 days following I/R (6±1.5 vs. 3.8±2.2 neutrophils/100.000 um^2). As p38 MAPK phosphorylation may link actions in neutrophils and structural cardiac remodeling, we examined protein phosphorylation of p38 MAPK (p-p38) in primary isolated murine cardiac fibroblasts, the major effector cells of myocardial fibrosis after infarction. These analyses revealed a significant increase of p38 phosphorylation (1.43±0.25 vs. 1±0.1 AU, p<0.05) in fibroblasts derived from Stamp2-/–mice as compared to WT. Moreover, upon stimulation with MPO elevated smooth muscle actin protein expression was observed in primary isolated cardiac fibroblast from Stamp2-/–mice as compared to WT, suggesting an increased myofibroblast transdifferentiation.
Conclusion: Our data reveal that Stamp2 deficiency profoundly enhances the susceptibility towards myocardial ischemia/reperfusion injury. Cross-talk between neutrophils and cardiac fibroblasts, possibly via p38 MAPK, arises as a potential mechanism.

Downloaded from https://academic.oup.com/eurheartj/article/39/suppl_1/ehy564.P946/5081016 by guest on 23 April 2024

P947

The RNA-binding protein SRSF10 is necessary to avoid cardiac rupture after myocardial infarction

J. Larrasa-Alonso, M. Villalba, C. Marti-Gomez, F. Sanchez-Cabo, E. Lara-Pezzi. *National Centre for Cardiovascular Research (CNIC), Madrid, Spain*

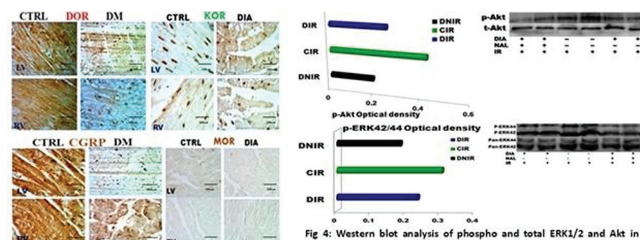
Introduction and aim: Incomplete knowledge about the molecular mechanisms that drive cardiovascular diseases, which are the first cause of death worldwide, precludes the development of effective therapies. In particular, little is known about the role and regulation of alternative splicing (AS) in heart disease. Our previous AS analysis of mouse infarcted hearts and human heart failure samples showed an enrichment in RNA binding motif protein 5 (RBM5) and serine/arginine-rich splicing factor 10 (SRSF10) binding motifs in alternatively spliced exons in both species. The aim of this study was to investigate the role of these RNA binding proteins (RBPs) in the heart, specifically after myocardial infarction.
Methods and results: We generated three adeno-associated viruses (AAV9) containing the Luciferase reporter gene followed by one hundred repeats in tandem of the binding motif for SRSF10, RBM5 or both together under the control of the cardiac-specific troponin T promoter. With this strategy, the targeted RBPs are sequestered by the viral RNA, precluding their access to the endogenous mRNAs. Echocardiography showed that, in the absence of injury, none of the viruses had any effect on cardiac function, although inhibition of SRSF10 resulted in elevated levels of cardiac stress markers Acta1 and BNP. Following myocardial infarction, a significant number of mice infected with the SRSF10 or the RBM5-SRSF10 loss of function viruses died due to cardiac rupture, which was confirmed by histological analysis. Surviving mice showed a thinner left ventricular anterior wall, compared to animals infected with the control virus or with the RBM5 loss of function virus, which showed no effect. To identify the molecular mechanism of action of SRSF10, we carried out an RNA-Seq analysis comparing the gene expression patterns between control and SRSF10-depleted mouse hearts. The results showed a significant decrease in elastin and several extracellular matrix proteins, which was confirmed by qRT-PCR.
Conclusions: We show that loss of function of SRSF10 in cardiomyocytes after myocardial infarction results in cardiac rupture. Our results suggest that SRSF10 controls, either directly or indirectly, the expression of extracellular proteins necessary to maintain the structural integrity of the scar, such as elastin. Increasing our understanding of the molecular mechanism of action of SRSF10 in cardiomyocytes will allows us to fully understand its role in the response to myocardial infarction.
Funding Acknowledgements: ProCNIC Foundation. Ministry of Economy, Industry and Competitiveness of Spain

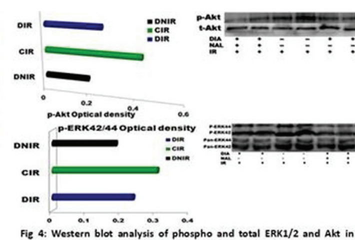
P948

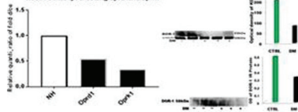
Characterization of classical opioid receptors, and their effects on ischemia-reperfusion injury in hearts of diabetic rats

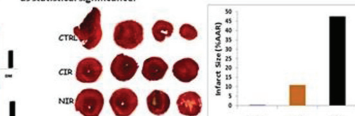
M.A. Gebrie[1], A. Rungatscher[1], D. Linardi[1], A. Andrioli[2], N. Ahmed[1], S. Ahmed[1], M. Ismail[1], M. Tessari[1], A. Soave[1], G. Innamorati[1], G.B. Luciani[1], G. Faggian[1]. [1]*University of Verona Medical School, Department of Surgery, Division of Cardiac Surgery, Verona, Italy;* [2]*University of Verona, Neuroscience, Biomedicine and Movement Science, Verona, Italy*

Despite several functions of opioid receptors (ORs) in cardiovascular physiology and neurotransmission, their expression and cardioprotective roles in hearts of diabetic rats after ischemia-reperfusion injury have not been addressed. The aims of this study were i) to investigate whether the myocardial opioidergic system in the heart of diabetic rat was altered, ii) to study the activity of Akt and ERK1/2 upon ORs blockade, and iii) to measure levels of infarct size, in the presence of non-selective antagonist.
This study engaged a total of 56 Sprague-Dawley rats (340–380g). For characterization study, induction of ischemia-reperfusion, 2, 3, 5-triphenyl tetrazolium chloride/TTC staining n=21, n=20, and n=15 rats were used, respectively. Diabetic rat heart tissues before and after ischemia-reperfusion injury were collected and preserved in formalin and liquid nitrogen and processed for RNA isolation, protein extraction, and immunohistochemistry. Some (Akt and ERK1/2) signaling pathways and 2, 3, 5-triphenyl tetrazolium chloride/TTC staining was also applied. Immunohistochemistry analysis demonstrated the expression and dual labeling co-localization of δ- & κ- ORs in the heart with a significant reduction in immunoreactivity in Streptozotocin-induced diabetic rats' heart. However, MOR-1 wasn't reactive in the tissues tested. The reduction of δ- & κ- ORs was correlated with the decline in immunoreactivity of the neuronal marker (CGRP-1). Down-regulation of messenger RNA transcript encoding the Oprd1 and Oprk1 was identified in hearts of diabetic rats. Nevertheless, the Oprm1 was not identified in the hearts tested. Undetected Oprm1 RT-qPCR products also were verified by agarose gel electrophoresis. Western blot analysis revealed that δ- & κ- ORs reduced in diabetic rat heart. Blockade of ORs by naloxone reduced the phosphorylation of pro-survival kinases (AKT, ERK1/2) in the diabetic and ischemia-reperfusion-induced heart in the rat. Moreover, TTC-stained ventricular slices showed a higher percentage of infarct size after ORs blockade.
The opioid receptors (δ & κ) possess a protective role in the heart against ischemia-reperfusion-injury; however, the down-regulation of ORs may compromise the effectiveness of pharmacological activities of opioids in the diabetic heart that could reduce the potential role of ORs in the regulation of cardiac tissue. The ORs might promote cell survival by mediating the action of Akt and ERK1/2.









Immunohistochemistry, Western blot, TTC

Funding Acknowledgements: University of Verona

P949

Effect of dipeptidyl peptidase 4 inhibitor Sitagliptin against ischemia reperfusion injury in normolipidemic and hyperlipidemic rat models

A. Al-Awar[1], N. Almasi[1], R. Szabo[1], Z. Murlasits[2], S. Torok[1], A. Posa[1], K. Kupai[1], C. Varga[1]. [1]*University of Szeged, Physiology, Anatomy and Neuroscience, Szeged, Hungary;* [2]*Qatar University, Doha, Qatar*. On behalf of Endocrinology group

Introduction: Hyperlipidemia has been associated with increased risk of myocardial infarction (MI). Inhibitors of dipeptidyl peptidase 4 (DPP-4) such as sitagliptin (Sitg) approved as a class of oral anti-diabetic drugs with pleiotropic secondary effects on cardiovascular parameters. However, this cardioprotective mechanism remains unknown in normal and hyperlipidemic conditions.
Purpose: To investigate the cardioprotective effect of Sitg pre-treatment against heart ischemia- reperfusion (IR) injury originated from normal and hyperlipidaemic rats.
Methods: Male wistar rats were fed with normal (N) rat chow or mixed with fats (High fat= HF) for 12 weeks to induce hyperlipidemia. At the end of last two weeks of feeding, animals were treated orally with Sitg of different doses (25mg, 50mg, 100mg, and 150 mg/kg/day), or its saline as a vehicle (control). Hearts were isolated to test infarct size (IS) and clarify the biochemical pathway of Sitg. Heart tissues were assigned to two different IR- injury protocols: 10 min perfusion, 45 min regional ischemia, and 120 min reperfusion for IS measurement or: 10 min reperfusion, 45 min regional ischemia and 10 min reperfusion, for biochemical analysis in both N and HF animals.
Following parameters were measured: 1) DPP-4 activity, 2) NOS activity, 3) TRPV-1 level by ELISA, and 4) TRPC-1 expressions by western blot. To clarify the potent role of NOS proteins in Sitg- induced cardioprotection, in a separate experiment NOS inhibitor (L-NAME, 25 mg/kg/day, i.p) was injected in parallel with Sitg treatment in N and HF animals to test the IS.
Results: Infarct size decreased significantly in hearts isolated from both N (23±3.34 vs. 37.45±2.73%) and HF (22.39±3.25 vs. 39±3.29%) animals. DPP-4 activity significantly decreased in N (552.32±100.02 vs. 1005.92±190.96 μU/ml) animals. NOS activity significantly increased in N (210.6±58.57 vs. 77.48±15.67 pmol/min/mg protein) and HF (96.51±13.75 vs. 52.38±11.56 pmol/min/mg protein) animals. The abovementioned results are expressed as Sitg (50mg) group compared to its control (Saline) group (Sitg vs. Control), in both N and HF diet animals. In the presence NOS- inhibitor, cardioprotective effect of Sitg (50mg) was lost, and IS increased in N (36.99±3.82 vs. 23±3.34%) and HF (59.17±6.67 vs. 23.42±3.26%) groups, comparing Sitg (50mg)+L-NAME group to Sitg (50mg) group. Comparing Sitg (50mg) groups to their Controls (Saline), a significant increase in TRPV-1 level (458.49±27.62 vs. 351.04±17.40 ng/mg protein) and TRPC-1 expression (408.12±83.93 vs. 129.38±38.58 mm^2) were displayed in N animals, but not in HF diet ones.
Conclusions: Sitg was effective not only in lowering the infarct size (IS), but also in increasing NOS activity, TRPV-1 and TRPC-1 levels. Sitg appears to contribute in cardioprotective actions due to the significant change in the latter parameters in normal animals, with less extent in hyperlipidemic condition.
Funding Acknowledgements: This work was supported by GINOP 232-15-2016-00035

Downloaded from https://academic.oup.com/eurheartj/article/39/suppl_1/ehy564.P946/5081016 by guest on 23 April 2024