Hemopexin counteracts systolic dysfunction induced by heme overload

Can-Martin Sag1; Nikola Rev1; Stefan Wagner1; Lucia De Franceschi1; Francesca Vechi1; James Cimino1; Sara Petrelli1; Lorenzo Stiengo1; Fiorenza Atruda1; Lars S. Maier2; Emilie Hinrich1; Alessandra Ghigo1; Emanuela Tolosano1

1Department of Cardiology, Universitàslokklinikum Regensburg, Germany; 2Department of Cardiology, Universitàslokklinikum Regensburg, Germany; 3Department of Cardiology, Universitàslokklinikum Regensburg, Germany; 4Department of Pathology, Universitàslokklinikum Regensburg, Germany; 5Department of Cardiology, University of Turin Medical Center, Germany; 6Department of Biotechnology and Health sciences, University of Turin, Italy; 7Department of Biotechnology and Health sciences, University of Turin, Italy; 8Department of Biotechnology and Health sciences, University of Turin, Italy; 9Department of Biotechnology and Health sciences, University of Turin, Italy; 10Department of Cardiology, University of Florence, Italy; 11Department of Biotechnology and Health sciences, University of Turin, Italy; 12Department of Cardiology, Universitàslokklinikum Regensburg, Germany; 13Department of Biotechnology and Health sciences, University of Turin, Italy

Purpose: Hemopexin is the plasma protein with the highest binding affinity for heme. Recently, it was associated with increased oxidative stress. These data were confirmed on neonatal cardiomyocytes. Heme loading in the heart of Hx+/− mice led to oxidation of Calcium/calmodulin-dependent protein kinase II (CaMKII). We also found modifications in the oxidation and phosphorylation of Ryanodine receptor 2 (RYR2) at the serine 2814 and in Phospholamban (PLB) at threonine 17, suggesting that alteration of redox status due to heme accumulation might alter Ca2+-homeostasis within the cell. In agreement with this conclusion, functional analysis of the heart demonstrated reduced fractional shortening not associated to cardiac remodeling in Hx−/− mice. Moving on pathological models, we found that β-thalassemic mice had heart heme overload and serum hemopexin consumption. As a result of the heart heme accumulation, β-thalassemic mice showed reduced fractional shortening.

Results:

We found that the heart of Hx−/− mice accumulated heme. Heme accumulation resulted in the up-regulation of Heme Oxygenase (HO)-1 and Ferroportin expression in the down-regulation of Transferrin Receptor (TfR1) and Divalent Metal Transporter 1 (DMT1), and it was associated with increased oxidative stress. These data were confirmed on neonatal cardiomyocytes. Heme loading in the heart of Hx−/− mice led to oxidation of Calcium/calmodulin-dependent protein kinase II (CaMKII). We also found modifications in the oxidation and phosphorylation of Ryanodine receptor 2 (RYR2) at the serine 2814 and in Phospholamban (PLB) at threonine 17, suggesting that alteration of redox status due to heme accumulation might alter Ca2+-homeostasis within the cell. In agreement with this conclusion, functional analysis of the heart demonstrated reduced fractional shortening not associated to cardiac remodeling in Hx−/− mice. Moving on pathological models, we found that β-thalassemic mice had heart heme overload and serum hemopexin consumption. As a result of the heart heme accumulation, β-thalassemic mice showed reduced fractional shortening.

Determination of serum hemopexin consumption in β-thalassemic mice. Serum hemopexin consumption was measured in wild-type (WT) and β-thalassemic (β-Thal) mice prior to shear stress. If treatments were used, cells were pre-treated for one minute with IKK inhibitor, BAY11-7085 (10 µM), or DMSO control. The exposure of HUVECs to acute shear stress for 30 and 90 minutes significantly increased nuclear translocation of NF-κB (p65) compared to static controls (n = 3, 3.06 ± 0.24 and 1.79 ± 0.008 fold change ± SEM respectively, p < 0.05). Nuclear translocation at 30 and 90 minutes was associated with significant iNOS degradation (n = 3, 0.43 ± 0.08 and 0.35 ± 0.1 respectively, p < 0.05). Activation of p65 following exposure to shear stress at 30 minutes (n = 3, 1.55 ± 0.03, p < 0.05) was also confirmed using a p65 ELISA-based assay. The NF-κB inhibitor BAY11-7085 significantly reduced the induction of mRNA for the pro-inflammatory genes MCP-1, ICAM-1, IL-8 and IL-6 at 240 minutes (n = 3, −85.95% reduction, p < 0.05).

Our data suggest that inhibition of the activation of the canonical NF-κB pathway appears to dampen the pro-inflammatory response in endothelial cells to acute high shear stress and may provide a novel pre-treatment option for vein graft failure.
Conclusion: This work demonstrates that defibrillation energy can be reduced by applying tailored stimulation patterns, opening translational perspectives for improving current anti-arrhythmic therapies.

505 Tachypacing-induced heart failure: a metabolomic investigation
Amy Watkins1; Stephanie Church2; Paul Begley3; David Esiner4; Andrew Trafford4; Garth Cooper4
1University of Manchester, UK; 2Institute of Human Development, Faculty of Medical and Human Sciences, University of Manchester, Manchester, UK; 3Centre for Advanced Discovery and Experimental Therapeutics (CADET), Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Sciences Centre, Manchester, UK; 4Institute of Human Development, Faculty of Medical and Human Sciences, University of Manchester, Manchester, UK; 5Centre for Advanced Discovery and Experimental Therapeutics (CADET), Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Sciences Centre, Manchester, UK

Background: Heart failure (HF) refers to a complex disease state whereby cardiac function has deteriorated to such a degree that the heart is unable to cope with the demands of the body. Despite the economic and social burden of HF little is understood about the metabolic profile underlying the disease.

Purpose: We applied untargeted GC-MS and LC-MS to investigate the metabolic profile of cardiac left ventricle (LV) tissue during the induction and reversal of HF using an ovine tachypacing model.

Methods: HF was induced in female sheep by right ventricular tachypacing at 210 bpm until symptomatic end-stage HF. Another cohort of animals was allowed to recover at the point of end-stage HF. LV free wall was harvested from control (n = 7) and their wild type (WT) littermates mice (n = 7) were used. LV function was evaluated by inserting the catheter tip retrograde into LV. mRNA levels of cytokines and chemokines (IL-6, IL-1β, MCP-1, MIP2 and KC), NRGI, Erbb1 and Erbb4 in LV were determined by RTPCR.

Results: HFN mice showed severe arthritis such as paw swelling in association with smaller body and heart weight in comparison to WT littermates (P < 0.01). LV systolic pressure and the rate of LV pressure rise was decreased in HFN mice (P < 0.01). In comparison with WT littermates mRNA expression of MCP-1 and KC were increased in HFN mice (P < 0.01).

NRGI expression gradually increased in HFN mice compared to WT littermates. Female mice showed attenuation of Erbb2 expression, irrespective of RA.

Conclusion: HFN mice have showed impaired LV systolic function in association with the upregulation of MCP-1 and KC. NRGI expression increased in both gender which might be a compensatory mechanism downregulation of Erbb2 was observed in female mice, irrespective of RA. These results may represent a potential novel therapeutic point to improve LV function and reduce risk of cardiovascular disease in RA.

507 Circadian rhythm in heart rate is due to an intrinsic circadian clock in the sinus node
Yanwen Wang1; Alicia D’Souza2; Anne Bert Johnson3; Servé Olieslagers4; Annalisa Buchi5; Paula Da Costa Martins6; Dario DiFrancesco7; Halina Dobrzynski8; Hugh D. Piggins9; Mark R. Boyett10
1Department of Internal Medicine III, Medical University of Vienna, Austria; 2Medical University of Vienna, Department for Biomedical Research, Austria; 3Department for Biomedical Research, Austria; 3Department of Internal Medicine III, Medical University of Vienna, Austria; 4Maastricht University, Maastricht; 5The University of Milan, Italy; 6Maastricht University, Maastricht; 7The University of Milan, Italy; 8The University of Manchester, UK; 9The University of Manchester, UK; 10The University of Manchester, UK

Introduction & purpose: There is a circadian rhythm in heart rate (HR), which slows at night. We hypothesised that it is the result of an intrinsic circadian clock in the pacemaker, the sinus node.

Methods: In conscious C57BL/6J mice maintained during 12 h light-12 h dark cycle, there was a circadian rhythm in (i) in vivo HR measured using telemetry and (ii) intrinsic HR measured using denervated isolated Langendorff-perfused hearts - in vivo HR was 61 beats/min slower and intrinsic HR was 49% lower (P < 0.05). HCN4 mRNA was 89% higher at ZT0 compared to ZT12, whereas HCN4 protein was 49% lower (P < 0.05).

Conclusion: It is concluded that the well-known circadian rhythm in HR is the result of a circadian rhythm in transcription of the HCN4 gene driven by an intrinsic circadian clock in the sinus node.