Cardiovascular Medicine

Abstracts | Published 01 June 2019 | doi:10.4414/cvm.2019.02038
Cite this as: Cardiovasc Med. 2019;22:w02038

Abstract Session: Basic Cardiology

O06–O10

Joint Annual Meeting 2019 of the Swiss Society of Cardiology and the Swiss Society of Cardiac Surgery

O06

Sirtuin 5 regulates arterial thrombosis by modulating endothelial plasminogen activator inhibitor-1

L. Liberale[1,2], A. Akhmedov[1], V. Nageswaran[1], N.R. Bonetti[1], M.X. Miranda[1], F. Montecucco[2], J.H. Beer[1], T.F. Lüscher[1], G.G. Camici[1]

[1]Center for Molecular Cardiology, University of Zurich, Schlieren, Switzerland, [2]Department of Internal Medicine, University of Genoa, Genoa, Italy

Introduction: Arterial thrombosis as a result of plaque rupture or erosion is a crucial event in myocardial infarction and stroke. Sirtuin 5 (SIRT5), a NAD+-dependent protein desuccinylase and demalonylase, is implicated in several pathophysiological conditions. In this study we investigate the putative role of this protein in arterial thrombosis by using an established in vivo mouse model. The translational value of animal findings as well as the molecular mechanism underlying the observed effect will be investigated also in primary human aortic endothelial cells (HAECs).

Methods: SIRT5 KO as well as SIRT5 TG animals were used for in vivo experiments. HAECs treated with SIRT5 silencing RNA (si-SIRT5) were used for in vitro assays.

Results: When compared to WT animals, SIRT5 KO mice display blunted carotid artery thrombus formation as underlined by delayed time to occlusion in a photochemical injury model. Oppositely, in SIRT5 TG mice the formation of an occlusive thrombus is accelerated (Fig. O06-1). Mechanistically, SIRT5 KO and WT animals show no difference in terms of vascular tissue factor (TF) activity, TF concentration in plasma and expression of TF pathway inhibitor in the aorta. In line with the observed reduced thrombogenicity, SIRT5 KO animal express reduced level of the pro-thrombotic plasminogen activator-1 (PAI-1), as assessed by western blot in aorta lysate. Of interest, SIRT5 genetic deletion does not affect platelet aggregation, as assessed by ex-vivo collagen-induced aggregometry (data

Figure: O06-1.

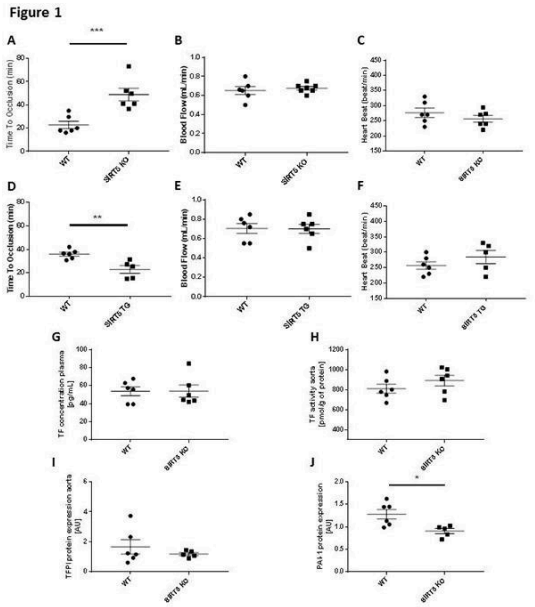


Figure 1. A. SIRT5 KO mice show delayed time to thrombotic occlusion, as compared to WT ones. **B-C.** No difference in terms of initial blood flow and initial heart rate is reported between the two groups. **D.** Accordingly, in SIRT5 overexpressing animals the time to thrombus formation is lower than in WT ones. **E-F.** Again, initial blood flow and initial heart rate are comparable among the groups. **G-I.** SIRT5KO and WT animals show similar levels of tissue factor (TF) activity in aorta lysate, TF concentration in plasma and TF pathway inhibitor (TFPI) in aorta lysate. **J.** SIRT5 KO animals display reduced levels of plasminogen activator-1 (PAI-1) in aorta lysate, as compared with WT mice.

Figure: O06-2.

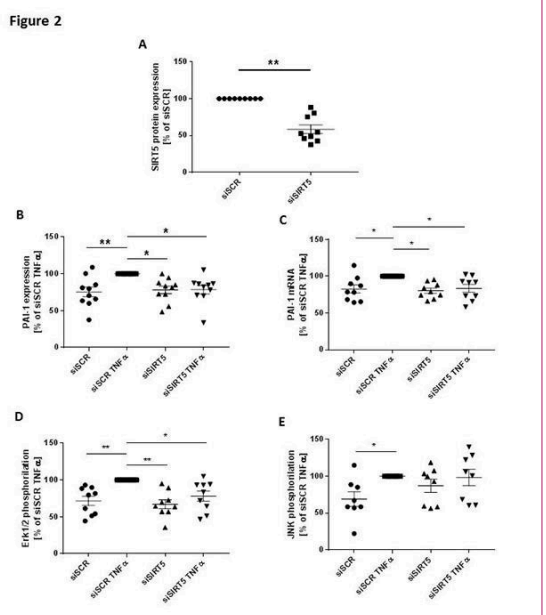


Figure 2. A. Human aortic endothelial cells (HAECs) treated with SIRT5 silencing RNA (siSIRT5) display 50% reduction in SIRT5 protein levels with respect to those treated with scramble RNA (siSCR). **B.** Treatment with TNF α induces PAI-1 protein expression in siSCR-treated HAECs but not in siSIRT5-treated ones. **C.** Treatment with TNF α (10 ng/mL) increases the transcription of PAI-1 in siSCR-treated HAECs but not in siSIRT5-treated ones. **D-E.** siSIRT5-treated cells display reduced activation of the MAP kinase Erk 1/2, but not JNK in response to TNF α (10 ng/mL).

not shown). In HAECs, SIRT5-silencing inhibits PAI-1 expression in response to TNF α. Real-time polymerase chain reaction revealed that inhibition of PAI-1 expression occurs at the mRNA level. This effect is mediated by reduced activation of the MAP kinase Erk 1/2, but not JNK (Fig. O06-2).

Conclusions: SIRT5 mediates arterial thrombosis by increasing endothelial PAI-1 expression. Hence, modulation of SIRT5 may be a therapeutic target in the context of atherothrombosis.

O07

The plant derived omega 3 fatty acid alpha linolenic acid prevents age-dependent arterial stiffness and improves outcome after stroke in mice

N. Bonetti[1], C. Diaz-Canestro[1], L. Pasterk[1], L. Liberale[1], D. Vdovenko[1], S. Gobbato[1], T.F. Lüscher[1,2], J.H. Beer[1,3], G.G. Camici[1]

[1]Center for Molecular Cardiology | Cardiology Research, University of Zurich, Schlieren, Switzerland, [2]Royal Brompton & Harefield Hospital, Imperial College, London, United Kingdom, [3]Internal Medicine, Cantonal Hospital Baden, Baden, Switzerland

Background: A key determinant of cardio- and cerebrovascular diseases is vascular aging, characterized by arterial stiffness. Indeed, arterial stiffness is an independent predictor of adverse cardio- and cerebrovascular events and mortality.

Fish-derived omega-3 fatty acids (n3 FA) have been described to decrease cardiovascular complications in high risk populations.

Little is known on the effects of the plant-derived n3 FA alpha linolenic acid (ALA). More insight is urgently needed, because of ALA´s lower costs and greater global supply.

Thus, we aimed to study the effects of a long-term dietary intervention with ALA on age-dependent arterial stiffness and the magnitude of these effects on a specific vascular endpoint - stroke - in a mouse model of aging.

Methods: C57BL/6 wildtype males were either fed an ALA-rich (high ALA) or a respective control diet for 12 months, starting from 6 months of age.

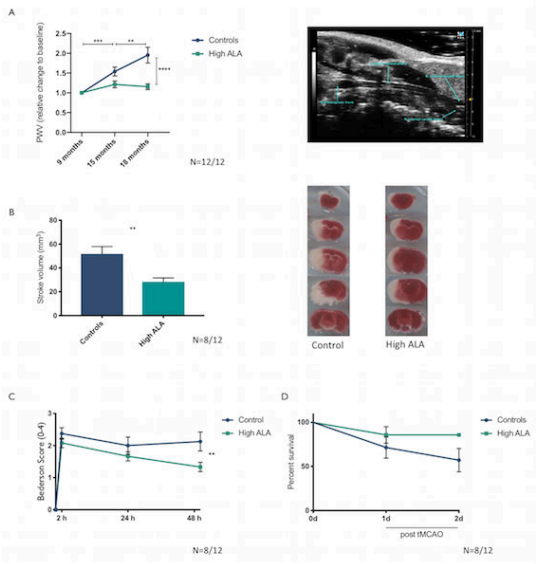
At 9, 15 and 18 months, arterial stiffness was assessed by measuring pulse wave velocity (PWV) in the right common carotid artery using a Vevo 3100 system (VisualSonics, Fig. O07-1A).

At 18 months, ischemic stroke was induced by transient middle cerebral artery occlusion (30 mins/48 h). Stroke size was assessed by triphenyl tetrazolium chloride staining and neurological function by a Bederson based score.

Results: Arterial stiffness steadily and significantly increased in controls over time, while ALA prevented said increase (Fig. O07-1A).

Stroke size at 18 months was significantly decreased in ALA-fed animals compared to controls (Fig. O07-1B). In line with morphological changes, controls performed significantly worse neurologically (Fig. O07-1C). Additionally, post-stroke survival at 48 h was improved in ALA-fed animals compared to controls, with 85% survival compared to 57% (Fig. O07-1D)

Figure: O07-1. Effects of ALA on arterial stiffness and stroke.



Conclusion: We demonstrate that long-term dietary supplementation of ALA fully prevents the development of age-dependent arterial stiffness.

The magnitude of this effect is clearly represented in significantly decreased stroke sizes, improved neurological performance and post-stroke survival observed in ALA-fed animals.

This study not only demonstrates beneficial physiological effects of ALA, but also links them to improved outcome of a specific vascular endpoint. Future analyses will aim at delineating the molecular basis of the observed benefits. This could aid in better understanding ambiguous results from clinical trials and defining a population likely to benefit from ALA.

O08

Chromatin modifications by methyltransferase SETD7 regulate endothelial cell migration and angiogenic response in experimental diabetes

S.A. Mohammed[1], S. Costantino[1], A. Akhemedov[1], S. Ambrosini[1], K. Gergely[2], F.L. Thomas[1,3], F. Paneni[1,4]

[1]Centre for Molecular Cardiology, Universität Zürich, [2]Institute of Clinical Chemistry, Universitätsspital Zürich, Zurich, Switzerland, [3]Royal Brompton and Harefield Hospital Trust, London, United Kingdom, [4]Cardiology, University of Heart Center, Zurich, Switzerland

Introduction: Peripheral artery disease (PAD) is highly prevalent in people with diabetes (DM), and associates with limb ischemia and a poor prognosis. Understanding the mechanisms of impaired blood vessel growth in DM patients is of paramount importance to develop new angiogenic therapies in this setting. Mono-methylation of histone 3 at lysine 4 (H3K4m1) - a specific epigenetic signature induced by the methyltransferase SETD7 - favours a chromatin conformation which enables the transcription of genes involved in endothelial inflammation and oxida-

tive stress. The present study investigates whether SETD7 modulates angiogenesis in DM.

Methods: Human aortic endothelial cells (HAECs) were exposed to normal glucose (NG, 5 mM) or high glucose (HG, 20 mM) for 48 hours. SETD7 protein and H3K4me1 levels were investigated by Western blot and Chromatin immunoprecipitation (ChIP). Knockdown of SETD7 was achieved by small interfering RNA (siRNA). Pharmacological blockade of SETD7 was performed by using the highly selective inhibitor *(R)*-PFI-2, while its inactive enantiomer, *(S)*-PFI-2, was used as a control. Scratch and tube formation assays were performed to investigate the angiogenic properties of HAECs. RNA sequencing (RNA-seq) and Ingenuity Pathway Analysis (IPA) were employed to unveil genes regulated by SETD7. SETD7 expression was also investigated in diabetic (*db/db*) and non-diabetic mice undergoing hindlimb ischemia for 21 days.

Results: HG exposure in HAECs led to a time-dependent increase of both SETD7 gene and protein expression, as compared to NG. Perturbation of SETD7 expression was associated with increased H3K4me1 as well as defective endothelial cell migration and tube formation. Both gene silencing and pharmacological blockade of SETD7 rescued HG-induced impairment of angiogenic properties in HAECs. RNA-seq in HG-treated HAECs with and without SETD7 depletion unveiled an array of differentially expressed genes, which were mainly involved in angiogenesis. We found that SETD7-dependent chromatin changes regulate the transcription of Semaphorin-3G (SEMA-3G), a known repressor of angiogenesis. Finally, SETD7 was also upregulated in *db/db* mice with chronic hindlimb ischemia as compared to non-diabetic animals.

Conclusion: Our study suggests that SETD7 may act as a regulator of angiogenesis in the setting of diabetes. These results may provide insights for novel epigenetic therapies to boost neovascularization in diabetic patients with PAD.

O09

The functional relevance of bile acids in the improvement of HDL-mediated endothelial protection after Roux-en-y gastric bypass

A. Jomard[1,2], A. Taheri[2,3], M. Kraljevic[4], T. Köstler[4], U. Zingg[4], A. Von Eckardstein[2], C. Wolfrum[1], T. Lutz[3], E. Osto[1,2]

[1]IFNH | D-HEST, ETH Zurich, Schwerzenbach, [2]Institute for Clinical Chemistry, Universitätsspital Zürich, Schlieren, [3]Vetsuisse, Universität Zürich, Zurich, [4]Department of General Surgery, Limmattal Hospital, Schlieren, Switzerland

Background: Roux-en-Y gastric bypass (RYGB) reduces cardiovascular mortality. We showed that early after RYGB HDL-mediated vasoprotection is improved. Bile acids (BA) are increasingly recognized as signaling molecules contributing to cardio-metabolic health. HDL carries a portion of circulating BA delivering them directly to endothelial cells.

Purpose: We studied whether and how changes in composition of BA bound to HDL after RYGB may contribute to HDL's endothelial-protective effects.

Methods: HDL functionality was measured in human aortic endothelial cell (HAEC) stimulated in vitro with HDL isolated from 47 morbidly obese patients before and 1 year after RYGB. Furthermore, the BA composition of HDL was quantified by liquid chromatography-mass spectrometry (LC/MS).

Results: Higher concentration (up to 25%) of BA bound to HDL and increased total circulating BA were observed 1 year after RYGB. Further, we found that after RYGB there was a specific remodeling of BA bound to HDL. Specific BA subspecies, which are either agonists for the endothelial nuclear farnesoid X receptor (FXR), e.g. cholic acid (CA) and chenodeoxy-CA (CDCA), or for the membrane TGR5 receptor, e.g. deoxy-CA (DCA) and taurolitho-CA (TLCA), showed a preferential increase of their concentration in the fraction bound to HDL as compared to their presence measured in plasma. One year after RYGB, HDL cholesterol levels were increased and HDL dysfunction induced by obesity were reversed with restored endothelial NO production, HDL anti-apoptotic and cholesterol efflux capacity. The composition-function analysis revealed that among all BA subclasses present on HDL, the specific enrichment of TLCA correlated with enhanced HDL's capacity to induce endothelial NO production. Similarly, higher CA and CDCA bound to HDL correlated with better HDL's anti-apoptotic capacity. Further, exogenous loading of CA onto healthy HDL potentiated anti-apoptotic function, while loading of CA onto obese, pro-apoptotic HDL completely restored its endothelial anti-apoptosis capacity.

Conclusions: RYGB achieves a dual benefit by first increasing the concentration and second improving the function of HDL. One year after RYGB, higher amounts of BA bound to HDL may mediate HDL's improved endothelial-protective effects via enhanced endothelial activation of FXR and TGR5.

O10

Cardiac progenitor cell exosome-associated periostin triggers reentry of cardiomyocytes into cell cycle

C. Balbi[1], S. Bolis[1], L. Barile[1], G. Vassalli[1,2]

[1]Cardiocentro Ticino, [2]Faculty of Biomedicine, Lugano, Switzerland

Introduction: Nanovesicles known as exosomes (Exo) from cardiac-derived progenitor cells (CPCs) are cardioprotective and improve cardiac function after myocardial infarction; however the mechanisms of benefit are incompletely understood, especially with respect to endogenous cardiomyocytes (CM) renewal. Periostin (POSTN), a secreted extracellular matrix protein, is emerging as a matricellular factor that can trigger CM proliferation. We have identified POSTN as a protein secreted by CPC and enriched in their exosomal fraction.

Purpose: We sought to determine whether Exo-CPC can induce proliferation of CM and to explore the role of exosomal POSTN in inducing reentry of CM into the cell cycle.

Methods: Exo were isolated from CPC condioned medium by density gradient ultracentrifugation. Fractions were analyzed by Western blotting for the presence of POSTN as well as specific Exo markers (TSG101, CD9). POSTN-depleted Exo (ExoCPC_SiPOSTN) were obtained by trans-

fecting CPC with specific siRNA. Active DNA synthesis was assessed on primary cell culture of rat neonatal CM by EdU incorporation. H9C2 cardiomyocytic cells were used to assess by real-time RT-PCR the expression of downstream genes Hippo/Yes-associated protein (YAP) signaling pathway.

Results: Western blotting analysis allowed to specifically determining the presence of Exo markers and POSTN in the different fractions of secreted vesicles. Smaller fractions (f1-f3) have the highest amount of TSG101 and CD9 as well as POSTN, thus suggesting that CPC secrete POSTN associated with Exo. The silencing of POSTN in cells resulted in a 60% reduction of Exo-associated POSTN compared to naïve ExoCPC. ExoCPC but not ExoCPC_SiPOSTN, were able to increase phosporylation of AKT and ERK in H9C2 cells. YAP phosporylation and its degradation was decreased resulting in the activation of the downstream gene AurBKinase. By real-time PCR, AurBKinase expression was increased by 2.6 folds with ExoCPC and 1.5 folds with ExoCPC_SiPOSTN compared to cells not exposed to Exo. ExoCPC were able to increase 1.5 fold EdU incorporation in cardiac troponin-positive primary rat CM. ExoCPC_SiPSTN did not affect proliferation.

Conclusion: These results suggest that POSTN may promote cardiomyocyte proliferation through the direct activation of the AKT/ERK/Hippo-Yap pathway. Exosomes released by CPC are an important source of POSTN and may have a potential for promoting cardiac regeneration.