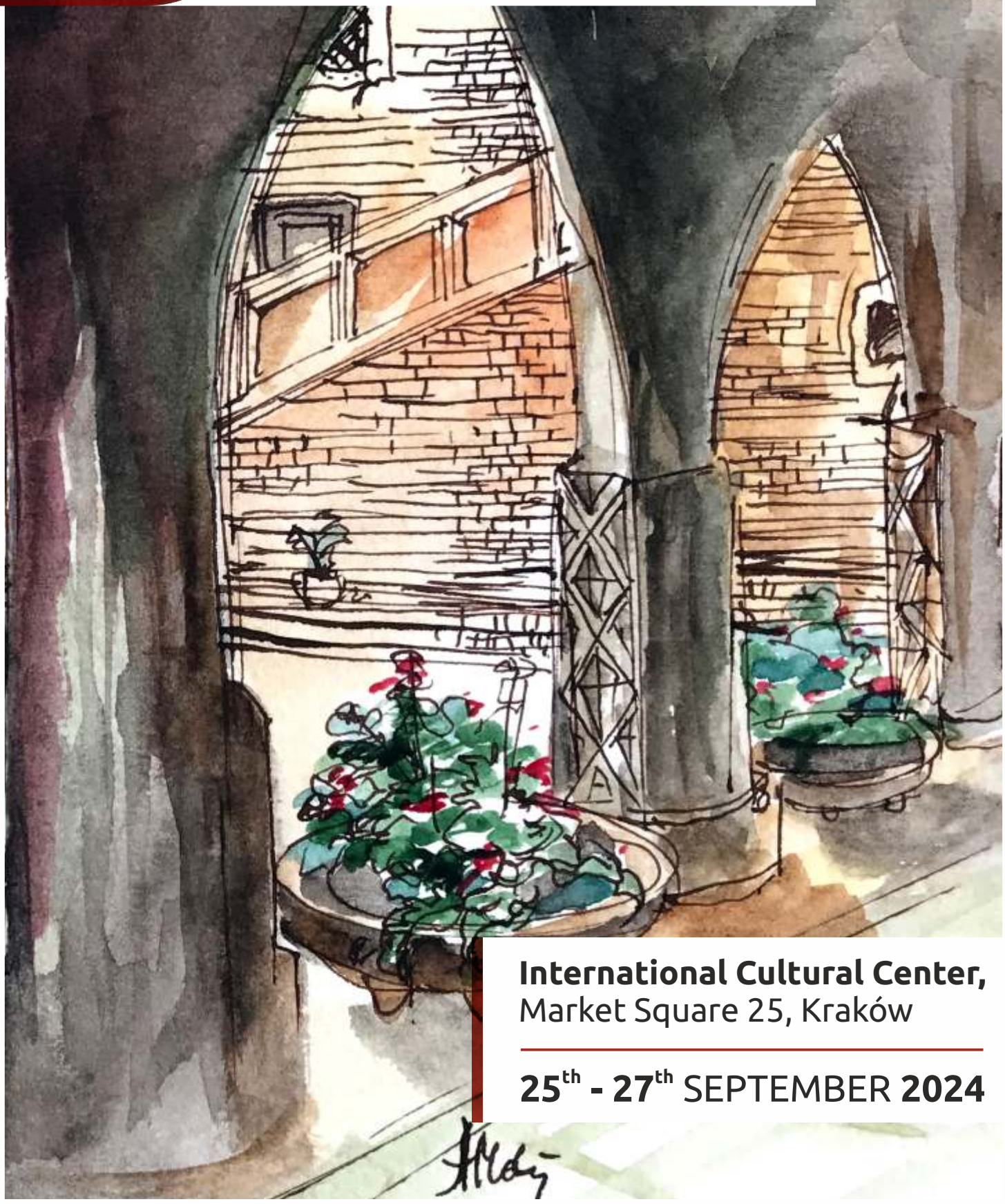
29th Kraków Conference on Endothelium



International Cultural Center,
Market Square 25, Kraków

25th - 27th SEPTEMBER **2024**

JCET Jagiellonian Centre for Experimental Therapeutics

JAGIELLONIAN UNIVERSITY IN KRAKÓW

Jagiellonian Medical Research Center

Ministry of Science and Higher Education
Republic of Poland

Introduction

Dear Colleagues,

We are pleased to welcome you to the 29th Krakow Conference on Endothelium. Enclosed you will find the final programme, followed by the submitted abstracts, separated into Short Oral Presentations and Poster Presentations. This year's conference programme includes 4 keynote lectures (30 minutes plus 10 minute discussion), nearly 50 presentations, almost half of which will be short presentations (20 minutes plus 5 minute discussion, or 10 minutes plus 5 minute discussion). As usual, the conference will include one-minute presentations of all posters (nearly 60 in total), divided into two sessions and the classical poster sessions.

This year's conference, just as our previous conferences in this series, will be dealing with a wide range of aspects of Vascular Biomedicine, but has a very special feature as we organize it jointly with three co-chairs from internationally renowned vascular biology research centres from Augusta, Madrid and Maastricht: Gabor Csanyi (VBC – Augusta University, USA), Tilman Hackeng (CARIM, Maastricht, The Netherlands), and Vicente Andrés (CNIC, Madrid, Spain).

The idea of this conference is to share the knowledge and research experience, establish scientific collaborations as well as give the opportunity to young researchers from our Centers as well as from other Institutions in Poland and abroad to present their results.

We hope that the conference will stimulate you to creatively exchange of knowledge, experience and thoughts.

Stefan Chłopicki (JCET, Krakow, Poland),
Gabor Csanyi (VBC, Augusta, USA)
Tilman Hackeng (CARIM, Maastricht, The Netherlands)
Vicente Andrés (CNIC, Madrid, Spain)

Programme

25th September 2024

13:30 – 13:45 **Welcome addresses and opening remarks:**
Maciej Malecki, *Vice-Rector Rector of the Jagiellonian University Medical College*
Stefan Chlopicki, *Jagiellonian Centre for Experimental Therapeutics - **JCET**, Jagiellonian University, Krakow, Poland,*
Gabor Csanyi, *Vascular Biology Center-**VBC**, Augusta, USA,*
Tilman Hackeng, *Cardiovascular Research Institute Maastricht - **CARIM**, Maastricht, the Netherlands*
Vicente Andrés, *Spanish National Centre for Cardiovascular Research Carlos III-**CNIC,** Madrid, Spain*

Keynote lecture

13:45 – 14:25 **Jose J. Fuster, *CNIC, Madrid, Spain***
Clonal hematopoiesis and atherosclerotic disease: from humans to mice and back

Session I **Vascular Ageing (part 1)**
Chairs: Stefan Chlopicki (Poland), Tilman M. Hackeng (The Netherlands)

14:25 – 14:50 **Vicente Andrés, *CNIC, Madrid, Spain***
Endothelial cell and vascular smooth muscle cell dysfuntion in Hutchinson-Gilford progeria syndrome

14:50 – 15:05 **Iñigo Ruiz-Polo de Lara, *CNIC, Madrid, Spain***
Role of endothelial cell A-type lamin expression in age-related cardiovascular disease

15:05 – 15:30 **Catherine Shanahan, *King's College London, London, UK***
Senescence and the extracellular matrix in vascular ageing

15:30 – 15:55 **Pauls Shiels, *University of Glasgow, Glasgow, Scotland***
The exposome and vascular ageing

15:55 – 16:25 **Coffee break**

Session II **Vascular Ageing (part 2)**
Chairs: Gabor Csanyi (USA), Vicente Andrés (Spain)

16:25 – 16:50 **Yu Wang, *The University of Hong Kong, Hong Kong, China***
Cardiovascular protective function of SIRT1 - an update

16:50 – 17.05 **Magda R. Hamczyk, *Aarhus Institute of Advanced Studies (AIAS), Denmark***
Endothelial-to-mesenchymal transition in progeria-associated atherosclerosis

17:05 – 17:20 **Anna Grochot-Przeczek, *Jagiellonian University, Krakow, Poland***
Insights into the function of NRF2 / KEAP1 in endothelial biology

17:20 – 17:35 **Anna Bar, *JCET, Krakow, Poland***
Accelerated ageing in endothelial-specific NRF2 knockout mice is linked to increased endothelial permeability, detected by MRI in vivo

17:35 – 17:50 **Soroush Mohammadi Jouabadi, *Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands***
Metformin enhances lifespan and endothelial health-span in an ERCC-1 accelerated mouse model of aging

17:50 – 18:05 **Grzegorz Kwiatkowski, *JCET, Krakow, Poland***
Sex-specific phenotype of heart failure in ageing APOE*3Leiden. CETP dyslipidemic mice

18.05 – 18:20 **Rafal Gulej, *University of Oklahoma Health Science Center, USA***
Young blood rejuvenates cerebromicrovascular endothelial function in aged mice: Lessons from heterochronic parabiosis experiments

18:30 – 20:00 **Welcome Reception** (Collegium Maius Courtyard)

26th September 2024

08:00 – 08:40	**Keynote lecture** **Joseph M. Miano, *Vascular Biology Center (VBC), Augusta, USA*** A new genetic model of accelerated coronary atherosclerosis
Session III	**Vascular Inflammation (part 1)** ***Chairs: Cherry Wainwright (UK), Jerzy Zoladz (Poland)***
08:40 – 09:05	**Gabor Csanyi, *VBC, Augusta, USA*** Mechanisms of foamy monocyte formation in atherosclerosis
09:05 – 09:30	**David Stepp, *VBC, Augusta, USA*** Impact of skeletal muscle metabolism on vascular function in obesity
09:30 – 09:55	**Xiaochun Long, *VBC, Augusta, USA*** New Mechanisms for vascular smooth muscle degeneration in vascular disease
09:55 – 10:20	**Jiliang Zhou, *Pharmacology & Toxicology, Augusta, USA*** Regulation of smooth muscle phenotype by long non-coding RNAs
10:20 – 10:45	**Tohru Fukai, *VBC, Augusta, USA*** Interplay between ROS and Copper transport protein in Vascular Disease
10:45 – 11:15	**Coffee break**
Session IV	**Vascular Inflammation (part 2)** ***Chairs: Ryszard T. Smolenski (Poland), Anton Roks (The Netherlands)***
11:15 – 11:40	**Eric Belin de Chantemele, *VBC, Augusta, USA*** CD4+ T cells expressing HIV proteins induced endothelial dysfunction and hypertension via IL-1a-mediated, Nox1-derived ROS production
11:40 – 12:05	**Philippe Vangrieken, *CARIM, Maastricht, The Netherlands*** Methylglyoxal as a driver of vasculopathy in diabetes and prediabetes
12:05 – 12:30	**Masuko Ushio-Fukai, *VBC, Augusta, USA*** Integrating redox signaling, mitochondrial dynamics, and endothelial metabolism: driving angiogenesis
12:30 – 12:55	**Jaime Millán, *Centro de Biología Molecular Severo Ochoa, Spanish National Research Council (CSIC), Madrid, Spain*** Harnessing Rho GTPases to preserve human endothelial barrier function during inflammation
12:55 – 13:10	**Marta Smeda, *JCET, Krakow, Poland*** Impact of platelet-derived TGFβ1 on integrity of murine lung microvascular endothelial barrier: age-related disruption and effect of platelet releasate milieu
13:10 – 13:25	**Andrew Guilfoyle-Speese, *VBC, Augusta, USA*** Prevention of Sarcopenic Obesity Maintains Angiogenic Capacity in PAD
13:25 – 13:40	**Lukasz Mateuszuk, *JCET, Krakow, Poland*** Effect of spermidine on vascular function and endothelial phenotype
13.40 – 14:10	**Invitation to Poster Session 1** (rapid fire presentations) **Chairs: Magdalena Sternak, Marek Grosicki**
14.10 – 15.55	**Lunch and Poster Viewing – Poster Session (part 1)**
Session V	**Platelets and Thrombosis** ***Chairs: Gabor Csanyi (USA), Tilman M. Hackeng (The Netherlands)***
15:55 – 16:20	**Judith Cosemans, *CARIM, Maastricht, The Netherlands*** Platelet-endothelial interaction in health and disease
16:20 – 16:45	**Paola van der Meijden, *CARIM, Maastricht, The Netherlands*** Monitoring antiplatelet therapy and associated bleeding risk in high-risk patients
16:45 – 17:00	**Patrycja Kaczara, *JCET, Krakow, Poland*** Pharmacological inhibition of platelet energy metabolism as a way to potentiate antiplatelet drug action
17:00 – 17:15	**Kamil Przyborowski, *JCET, Krakow, Poland*** PDIA1 and PDIA3 differentially regulate platelet function
17:15 – 17:45	**Coffee break**
Session VI	**Tissue Factor Pathway Inhibitor (TFPI) and Vascular Wall** ***Chairs: Stefan Chlopicki (Poland), Vicente Andrés (Spain)***
17:45 – 18:10	**Tilman M. Hackeng, *CARIM, Maastricht, The Netherlands*** Tissue factor pathway inhibitor (TFPI) and the attenuation of venous thrombosis
18:10 – 18:35	**Rory Koenen, *CARIM, Maastricht, The Netherlands*** Regulation of TFPI by neutrophil extracellular traps (NETs)
18:35 – 18:50	**Vanessa Bröker, *CARIM, Maastricht, The Netherlands*** The effects of tissue factor pathway inhibitor on the proliferation and phenotype of vascular cells
18:50 – 19:15	**Leon Schurgers, *CARIM, Maastricht, The Netherlands*** Using the young to study the old: iPS cells to study vascular ageing and cardiovascular disease
19:30	**Get Together - Party, Venue: Pałac pod Baranami, Market Square 27**

27th September 2024

	Keynote lecture
08:00 – 08:40	**Csaba Szabo,** ***University of Fribourg, Switzerland*** Hydrogen cyanide, the next mammalian gaseous
Session VII	**Inflammation Resolution** ***Chairs: Magnus Bäck (Sweden), Zoltán Ungvári (USA)***
08:40 – 09:05	**Jesmond Dalli,** ***Queen Mary University of London, London, UK*** Unveiling common pathways: Tackling joint and vascular inflammation to resolve multimorbidities
09:05 – 09:30	**Dianne Cooper,** ***The William Harvey Research Institute, Queen Mary University of London, London, UK*** Resolution pharmacology to control comorbidities in arthritis
09:30 – 09:55	**Ana Briones Alonso,** ***Universidad Autónoma de Madrid, Madrid, Spain*** Role of Resolvin D2 in vascular dysfunction and remodeling in hypertension
09:55 – 10:10	**Agnieszka Kij,** ***JCET, Krakow, Poland*** Systemic and local lipid mediator status reflecting vascular health
10:10 – 10:40	**Coffee break**
Session VIII	**Cardio-vasco-oncology** ***Chairs: Rafał Olszanecki (Poland), Tomasz Rutkowski (Poland)***
10:40 – 11:05	**Laura Cádiz Barrera,** ***CNIC, Madrid, Spain*** Anthracycline cardiotoxicity: Pathophysiological mechanisms and novel therapeutic targets
11:05 – 11:30	**Pieter-Jan Guns,** ***University of Antwerp, Antwerp, Belgium*** SERPINA3, a novel marker of endothelial cell dysfunction in chemotherapy-related cardiovascular dysfunction?
11:30 – 11:55	**Jeanette Woolard,** ***University of Nottingham, Nottingham, UK*** Role of Endothelial Receptors in the Hypertension Induced by VEGFR-2 Inhibitors
11:55 – 12:20	**Zoltán V. Varga,** ***Semmelweis University, Budapest, Hungary*** Immune checkpoint signaling and heart failure
12:20 – 12:45	**Marijke J E Kuijpers,** ***CARIM, Maastricht, The Netherlands*** Tyrosine kinase inhibitors for treatment of solid tumors: effects on platelet function and signalling
12:45 – 13:00	**Elzbieta Buczek,** ***JCET, Krakow, Poland*** BRAF and MEK inhibitors: investigating the mechanisms of vascular toxicity
13.00 – 13.30	**Invitation to Poster Session 2** (rapid fire presentations) **Chairs: Marta Stojak, Marta Pacia**
13.30 – 15:15	**Lunch and Poster Viewing – Poster Session (part 2)**
Session IX	**Heterogeneity of Vascular Beds** ***Chairs: Zoltan Benyó (Hungary), Yu Wang (Hong Kong)***
15:15 – 15:40	**Aernout Luttun,** ***Centre for Molecular and Vascular Biology, Catholic University of Leuven, Leuven, Belgium*** Transcriptional regulation of endothelial heterogeneity in health and disease
15.40 – 16:05	**David Fulton,** ***VBC, Augusta, USA*** The role of PBK in pulmonary vascular remodeling
16:05 – 16:20	**Ignacio Benedicto Español,** ***Centro de Investigaciones Biológicas Margarita Salas (CIB Margarita Salas), Madrid, Spain*** Role of endothelial cells in age-related defective liver regeneration
16:20 – 16:35	**Izabela Czyzynska-Cichon,** ***JCET, Krakow, Poland*** Liver sinusoidal endothelial cells (LSECs) and hepatocyte metabolism in chronic heart failure
16:35 – 16:50	**Katarzyna Mleczko-Sanecka,** ***International Institute of Molecular and Cell Biology in Warsaw, Poland*** Liver sinusoidal endothelial cells constitute a major route for hemoglobin clearance
17:50 – 17:05	**Mateusz Gawrysiak,** ***Medical University of Lodz, Poland*** The involvement of the lung vascular endothelium in antiviral immunity during respiratory viral infections
17:05 – 17:20	**Elisabeth Fließer,** ***Medical University of Graz, Austria*** Unveiling the role of Lung Endothelial Cell Dynamics in the progression of pulmonary fibrosis
	Closing lecture
17:20 – 18:00	**Rhian M Touyz,** ***Institute of the McGill University Health Centre (RI-MUHC), Montreal, Canada*** Aging and the vasculature in health and disease
18.00 – 18.15	**Conference closing and poster awards ceremony**
18:30 – 20:00	**Wawel Castle Visit** (special tour after Wawel is closed)

SHORT ORAL PRESENTATIONS

O1 **Ruiz-Polo I.** “Lamin A/C downregulation as a new mediator of age-related endothelial dysfunction and cardiovascular disease”

O2 **Hamczyk M.** “Endothelial-to-mesenchymal transition in progeria-associated atherosclerosis”

O3 **Grochot-Przęczek A.** "Insights into NRF2/KEAP1 function in endothelial biology”

O4 **Bar A.** “Accelerated ageing in endothelial-specific NRF2 knockout mice is linked to increased endothelial permeability, detected by MRI in vivo”

O5 **Mohammadi Jouabadi S.** “Metformin enhances lifespan and endothelial health-span in an ERCC-1 accelerated mouse model of aging”

O6 **Kwiatkowski G.** “Sex-specific phenotype of heart failure in aging APOE*3Leiden. CETP dyslipidemic mice”

O7 **Gulej R.** "Young blood rejuvenates cerebromicrovascular endothelial function in aged mice: Lessons from heterochronic parabiosis experiments"

O8 **Smęda M.** “Impact of platelet-derived TGFβ1 on integrity of murine lung microvascular endothelial barrier: age-related disruption”

O9 **Mateuszuk Ł.** “Effect of spermidine on vascular function and endothelial phenotype”

O10 **Guilfoyle-Speese A.** "Prevention of Sarcopenic Obesity Maintains Angiogenic Capacity in PAD”

O11 **Kaczara P.** “Pharmacological inhibition of platelet energy metabolism as a way to potentiate antiplatelet drug action”

O12 **Przyborowski K.** “PDIA1 and PDIA3 differentially regulate platelet function”

O13 **Bröker V.** “The effects of tissue factor pathway inhibitor on the proliferation and phenotype of vascular cells”

O14 **Kij A.** “Systemic and local lipid mediator status reflecting vascular health”

O15 **Buczek E.** “BRAF and MEK inhibitors: investigating the mechanisms of vascular toxicity”

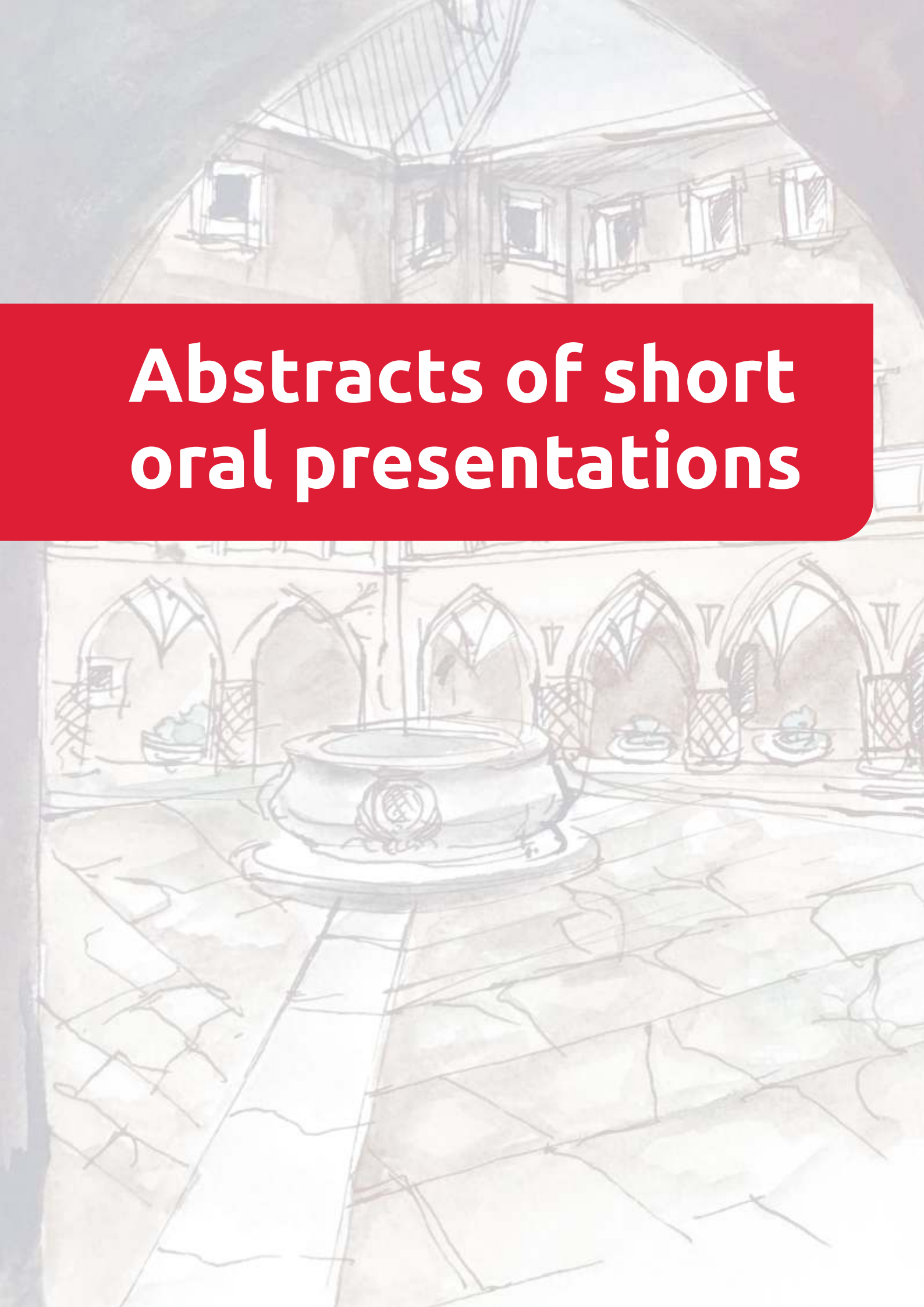
O16 **Benedicto I.** “Role of endothelial cells in age-related defective liver regeneration”

O17 **Czyżyńska-Cichoń I.** “Liver sinusoidal endothelial cell (LSEC) dysfunction and alterations in hepatocyte metabolism in chronic heart failure”

O18 **Mleczko-Sanecka K.** “Liver sinusoidal endothelial cells constitute a major route for hemoglobin clearance”

O19 **Gawrysiak M.** "The involvement of the lung vascular endothelium in antiviral immunity during respiratory viral infections”

O20 **Fließer E.** "Unveiling the role of Lung Endothelial Cell Dynamics in the progression of pulmonary fibrosis"



Abstracts of short oral presentations

O1: Lamin A/C downregulation as a new mediator of age-related endothelial dysfunction and cardiovascular disease

Iñigo Ruiz-Polo de Lara[1], Alberto del Monte[1,2], María González-Amor[1,2], Pilar Gonzalo[1,2], María J. Andrés-Manzano[1,2], Marta Amorós-Pérez[1], Cristina Rodríguez[2,3], José Martínez- González[2,4], Ignacio Benedicto[1,5], Vicente Andrés[1,2]

[1] Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain
[2] Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV), Spain
[3] Institut de Recerca Sant Pau (IR SANT PAU), Barcelona, Spain
[4] Instituto de Investigaciones Biomédicas de Barcelona (IIBB-CSIC), Barcelona, Spain
[5] Centro de Investigaciones Biológicas Margarita Salas (CIB-CSIC), Madrid, Spain

Abstract:

Nuclear lamin A/C are essential proteins for the maintenance of nuclear integrity and proper regulation of various cellular processes, including DNA replication, transcription, and cell division. Aging is the main risk factor for hypertension, vessel stiffness, and atherosclerosis and its complications, including myocardial infarction, heart failure and stroke. Here, we investigated the regulation of lamin A/C expression in the vasculature during ageing and its potential role in age-related cardiovascular disease.

Western blot analysis revealed age-dependent downregulation of lamin A/C expression in human coronary arteries (≤30 years, ≥58 years) and mouse aortas (3-week-old, 65-week-old, 109-week-old). Flow cytometry analysis of mouse aorta revealed that lamin A/C downregulation occurs in both endothelial cells (ECs) and vascular smooth muscle cells, but not in adventitial cells. Mice with EC-specific lamin A/C ablation (Ldlr-/-Lmnaflox/floxCdh5-CreERT2) exhibited defective post-natal growth, elevated arterial systolic blood pressure, and reduced lifespan. Moreover, aortic rings from these mice showed impaired endothelium-dependent vasorelaxation, which was also observed in wild-type 114-week-old mice compared with 12-week-old controls. Echocardiography studies revealed diastolic dysfunction in both young and old Ldlr-/-Lmnaflox/floxCdh5-CreERT2 mice, which was associated with increased collagen deposition in the heart, and elevated serum NT-proBNP levels. High-throughput omics revealed several altered biological processes in Ldlr-/-Lmnaflox/floxCdh5-CreERT2 mice, indicating endothelial dysfunction, including reduced Nos3 expression, and disrupted nitric oxide signaling pathways.

Based on these findings, we propose that lamin A/C downregulation in ageing ECs plays a crucial role in age-associated endothelial dysfunction, hypertension, vascular tone dysregulation, and diastolic dysfunction. Our ongoing studies are addressing the underlying mechanisms.

Acknowledgments:

Work in V.A.'s laboratory is supported by grants from the Spanish Ministerio de Ciencia, Innovación y Universidades (MICIU)/Agencia Estatal de Investigación (AEI) (Grant PID2022-141211OB-I00 funded by MICIU/AEI/10.13039/501100011033 and by ERDF/EU) and Instituto de Salud Carlos III (ISCIII) (Grant AC22/00020, ERANET E-RARE 2022, with co-funding from the European Social Fund ("The ESF invests in your future") and by the "European Union NextGenerationEU/PRTR"), Ramón Areces Foundation (project code CIVP21A7006), Fundació la Marató TV3 (grant 202033-31) and Fundación Inocente-Inocente through a donation from Asociación Progeria Alexandra Peraut. IR-P is supported by a predoctoral FPI contract from MICIN/AEI10.13039/ 501100011033. I.B. was supported by RYC2021-033805-I (MICIU/AEI/10.13039/501100011033, European Union NextGenerationEU/PRTR). The Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC) is supported by the MICIU, the ISCIII, the Pro-CNIC Foundation, and is a Severo Ochoa Center of Excellence (grant CEX2020-001041-S funded by MICIU/AEI/10.13039/501100011033).

O2: Endothelial-to-mesenchymal transition in progeria-associated atherosclerosis

Magda Rita Hamczyk[1,2,3,*], Rosa María Nevado[3,4], Pilar Gonzalo[3,4], María Jesús Andrés-Manzano[3,4], Paula Nogales[4], Jacob Fog Bentzon[4,5], Carlos López-Otín[2] & Vicente Andrés[3,4]
*presenting author
[1] Aarhus Institute of Advanced Studies (AIAS), Denmark
[2] Departamento de Bioquímica y Biología Molecular, Instituto Universitario de Oncología (IUOPA), Universidad de Oviedo, Spain,
[3] Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV), Spain
[4] Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain
[5] Department of Clinical Medicine, Aarhus University, Denmark

Abstract:

Atherosclerosis is the main medical problem in Hutchinson-Gilford progeria syndrome (HGPS), a rare premature aging disorder caused by the mutant lamin-A protein progerin. Recently, we generated the first atheroprone mouse model of HGPS that fully recapitulates disease symptoms. We also found that limiting progerin expression to vascular smooth muscle cells (VSMCs) is sufficient to hasten atherosclerosis and death in Apoe-deficient mice. However, the impact of progerin-driven VSMC defects on endothelial cells (ECs) remained unclear.

Here, we show that both ubiquitous and VSMC-specific progerin expression in Apoe-null mice provoke EC alterations in the aorta, including increased permeability to low-density lipoprotein and leukocyte recruitment. Moreover, atherosclerotic lesions in both progeroid mouse models contain abundant cells combining endothelial and mesenchymal features, indicating extensive endothelial-to-mesenchymal transition (EndMT). Likewise, the intima of both models at the onset of atherosclerosis present increased expression of EndMT-linked genes, especially those specific to fibroblasts and extracellular matrix. These EC defects are dependent on progerin-driven VSMC alterations, as the mouse model expressing progerin solely in ECs does not show EC alterations, accelerated atherosclerosis, or shortened survival. Furthermore, aorta and atheromata in the ubiquitous and VSMC-specific progeroid models show activation of the TGFβ1/SMAD3 pathway, a major trigger of EndMT. Accordingly, treatment of VSMC-specific progeroid mice with a specific SMAD3 inhibitor alleviates the aortic phenotype.

Our results demonstrate that progerin-induced VSMC alterations promote EC dysfunction and EndMT via TGFβ1/SMAD3, identifying this pathway as a candidate target for HGPS treatment. These findings also provide insights into the complex role of EndMT during atherogenesis.

Acknowledgments:

V.A.'s lab is supported by grant PID2022-141211OB-I00 funded by MICIU/AEI/10.13039/501100011033 and ERDF/EU, and a donation from Asociación Progeria Alexandra Peraut. The CNIC is supported by the Instituto de Salud Carlos III (ISCIII), the Ministerio de Ciencia, Innovación y Universidades (MICIU), and the Pro-CNIC Foundation and is a Severo Ochoa Center of Excellence (grant CEX2020-001041-S funded by MCIN/AEI/10.13039/501100011033). Microscopy was conducted at the CNIC Microscopy & Dynamic Imaging ICTS (Unique Science and Technology Infrastructure)-ReDib funded by MCIN/AEI/10.13039/501100011033 and ERDF-A way to make Europe. R.M.N was supported by the Ministerio de Educación, Cultura y Deporte (predoctoral contract FPU16/05027). M.R.H. is supported by the AIAS-AUFF fellowship.

O3: Insights into the function of NRF2 / KEAP1 in endothelial biology

Aleksandra Kopacz[1], Damian Kloska[1], Aleksandra Piechota-Polanczyk[1], Anna Bar[2], Marta Targosz-Korecka[3], Dominik Cysewski[4], Bartosz Proniewski[2], Stefan Chlopicki[2], Alicja Jozkowicz[1], Anna Grochot-Przeczek[1]

[1]Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland
[2]Jagiellonian Centre for Experimental Therapeutics, Jagiellonian University, Krakow, Poland
[3]Department of Physics of Nanostructures and Nanotechnology, Institute of Physics, Jagiellonian University, Krakow, Poland
[4]Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Abstract:

NRF2 is a stress-responsive cytoprotective transcription factor repressed by KEAP1. Its protein level and activity decline with age. NRF2 plays a crucial, multimodal role in endothelial cell biology. Our results show that NRF2 regulates angiogenesis by tethering KEAP1 and preventing podosome disassembly. This effect is independent of NRF2 transcriptional activity. Knockdown of NRF2 in endothelial cells induces premature senescence, which is associated with a potent protein S-nitrosation (SNO) and lack of oxidative damage. Mechanically, in the absence of NRF2, KEAP1 binds to GAPDH and NOS, forming an enzymatic SNO complex. SNO modification of NOX4 prevents oxidative damage and endothelial cell death. Genetic ablation of NRF2 activity in vivo favours the formation of abdominal aortic aneurysms (AAA), which can be counteracted by endothelial specific knockout of miR-34a. The deletion of miR-34a enhances MTA2-dependent proliferation of EC induced by angiotensin II, which may confer the protection of miR-34aΔEC mice from AAA. These data show the critical role of the NRF2/KEAP1 axis in vascular biology and underline their non-classical activities, reaching beyond gene transactivation and NRF2 repression.

Acknowledgments:

Funding: National Science Centre grants 2016/22/E/NZ3/00405 and 2021/43/B/NZ4/02130

O4: Accelerated ageing in endothelial-specific NRF2 knockout mice is linked to increased endothelial permeability, detected by MRI in vivo

Anna Bar[1*], Anna Gdula[1], Agnieszka Karaś[1], Patrycja Kaczara[1], Zuzanna Kuryłowicz[1], Agnieszka Jasztal[1], Zeinep Berkimbayeva[1], Anna Grochot-Przęczek[2], Stefan Chlopicki[1,3]
[1]Jagiellonian University, Jagiellonian Centre for Experimental Therapeutics (JCET), Krakow, Poland,
[2]Jagiellonian University, Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Kraków Poland,
[3]Jagiellonian University Medical College, Faculty of Medicine, Chair of Pharmacology, Krakow, Poland.

Abstract:

Early detection of endothelial dysfunction (ED) features is important for endothelium-targeted treatment, but sequence of events occurring during early ED development, in clinically relevant animal models of ED, has not been well defined as yet.
In 6-month-old endothelial-specific NRF2 knockout mice (EC-NRF2KO), representing an accelerated, age-dependent vascular oxidative stress model, impaired acetylcholine-induced response in the aorta, with preserved endothelium-independent response to sodium nitroprusside and impaired flow-mediated response in the femoral artery were detected by MRI in vivo. While aortic stiffness measured by USG-Doppler in vivo was unchanged, decreased NO production and impaired vascular mitochondrial metabolism in aorta ex vivo were also detected. Importantly, increased endothelial permeability (ECp), assessed by MRI in vivo, was detected in the femoral artery, brachiocephalic artery and aorta, already in 3-month-old EC-NRF2KO mice, what was associated with lower expression of tight-junction (ZO-1, CLDN5, OCLN) but not with adherent junction (CDH5) proteins and with increased pro-inflammatory (ICAM-1, IL-6, IL-1β) and senescence markers (p21, p16).
In conclusion, in EC-NRF2KO mice increased ECp preceded impaired NO production and endothelium-dependent vasodilation, regarded as a classical functional readout of ED, as well as impaired vascular metabolism, and increased vascular stiffness. These results indicate that altered ECp, linked to impaired tight-junction function may be detected in vivo by MRI, and represents the sensitive parameter for ED development, in the setting of oxidative stress. Altogether, the results of this study may open up new perspectives for early ED detection, to monitor the early effects of endothelium-targeted pharmacotherapy in murine models in vivo.

Acknowledgments:

This work was supported by the Polish National Science Centre (NCN) grant: OPUS No 2020/39/B/NZ5/02305.

O5: METFORMIN ENHANCES LIFESPAN AND ENDOTHELIAL HEALTH-SPAN IN AN ERCC-1 ACCELERATED MOUSE MODEL OF AGING

Soroush Mohammadi Jouabadi[1], Sabrina Ribeiro Gonzales[1], Philippe Vangrieken[2], Annika Juttner[1], René de Vries[1], Richard van Veghel[1], A.H. Jan Danser[1], Casper G. Schalkwijk[2], Anton J.M. Roks[1]
[1] Department of Internal Medicine, Erasmus MC, Rotterdam, the Netherlands.
[2] Department of Internal Medicine, Maastricht university medical centre, Maastricht, the Netherlands.

Abstract:

Background:
Vascular aging is a critical contributor to cardio/cerebrovascular diseases, with chronic low-grade inflammation and senescence implicated as key factors. However, the effectiveness of anti-inflammatory therapies in preventing cardiovascular aging remains underexplored. Here, we hypothesized that long-term metformin therapy, a safe biguanide with anti-inflammatory properties used in type 2 diabetes treatment, could prevent age-associated pathological changes.
Methods:
Using vascular age-accelerated mouse models with endothelium-selective Ercc1 DNA repair gene excision (EC-KO), we administered metformin (150mg/kg/day) or vehicle from 10 to 20 weeks of age. In-vivo and in-vitro assessments of kidney, vascular, cardiac, and endothelial function were performed.
Results:
EC-KO mice exhibited a significantly lower survival rate compared to littermates (LM) (70.5% vs 100%) by the study conclusion (week 20). Notable, Metformin treatment increased the survival rate of EC-KO mice to 88.8%. EC-KO mice also displayed increased water intake and urine volume compared to LM (P=0.001), which was completely restored by metformin treatment (P=0.002). Furthermore, endothelium-dependent vasodilation was decreased in EC-KO mice ($p<0.01$), but chronic metformin treatment restored this function. We have identified enhanced nitric oxide-cGMP signalling as a potential mechanism underlying the effects of metformin.
Conclusion:
Our study highlights endothelial cell aging as a significant contributor to increased mortality and impaired nitric oxide-mediated endothelium-dependent vasodilation. Metformin therapy emerges as an effective intervention for slowing vascular aging and preventing endothelial dysfunction. Further investigations are warranted to elucidate the underlying mechanisms driving its longevity benefits.

Acknowledgments:

NA

O6: Sex-specific phenotype of heart failure in aging APOE*3Leiden.CETP dyslipidemic mice

Grzegorz Kwiatkowski[1], Anna Bar[1], Urszula Tyrankiewicz[1], Hans M.G. Princen[2], Stefan Chłopicki[1,3]
[1]Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Krakow, Poland
[2]The Netherlands Organization of Applied Scientific Research (TNO), Metabolic Health Research, Leiden, The Netherlands
[3]Faculty of Medicine, Chair of Pharmacology, Jagiellonian University Medical College, Krakow, Poland

Abstract:

Dyslipidemia, characterized by abnormal lipid levels in the blood, presents differently in men and women and therefore might lead to a distinctly different pattern of heart failure (HF) development, influencing disease progression and outcomes. We have utilized a unique mode model of APOE*3Leiden.humanCholesteryl Ester Transfer Protein (E3L.CETP) mice that displayed a human-like lipid profile to track age- and sex-specific changes in cardiac function between 2- and 18 months of age. A comprehensive MRI and Doppler imaging protocol was run to assess subtle changes in left ventricle function and coronary blood flow pattern. We have found that while aging female mice developed signs of HFpEF (fully preserved ejection fraction, reduced peak longitudinal strain, reduced E/A ratio), on the contrary, male aging mice were characterized with symptoms of HFrEF (reduced ejection fraction, reduced peak circumferential strain). Both male and female mice were present with reduced basal coronary blood flow velocity as compared to age- and sex-matched control animals, suggesting early signs of coronary microvascular dysfunction. Moreover, a sex-specific response to dapagliflozin treatment (10 mg/kg/day, 2 months) was assessed showing a significantly better LV function salvage in females.

Acknowledgments:

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O7: Young blood rejuvenates cerebromicrovascular endothelial function in aged mice: Lessons from heterochronic parabiosis experiments

Rafal Gulej[1,2], Boglarka Csik[1,2], Ádám Nyúl-Tóth[1,2,3], Siva Sai Chandragiri[1,2], Santny Shangumarama[1,2], Stefano Tarantini[1,2,4], Anna Csiszar[1,2,5], Zoltan Ungvari[1,2,3]
[1]Neurosurgery Department, College of Medicine, University of Oklahoma Health Science Center, Oklahoma City, OK, USA;
[2]Vascular Cognitive Impairment and Neurodegeneration Program, Reynolds Oklahoma Center on Aging/Oklahoma Center of Geroscience, University of Oklahoma Health Science Center, Oklahoma City, OK, USA;
[3]International Training Program in Geroscience, Doctoral School of Basic and Translation Medicine/Department of Public Health, Semmelweis University, Budapest, Hungary;
[4]Peggy and Charles Stephenson Cancer Center, Oklahoma City, OK, USA;
[5]International Training Program in Geroscience/Vascular Cognitive Impairment and Neurodegeneration Program, Department of Translation Medicine, Semmelweis University, Budapest, Hungary.

Abstract:

Introduction: Maintaining healthy cognitive function requires moment-to-moment adjustment of cerebral blood flow to neuronal activity through neurovascular coupling (NVC) and an intact blood-brain barrier (BBB), both of which are disrupted in aging due to cerebromicrovascular endothelial dysfunction. Recent research demonstrates that cell-autonomous mechanisms alone are inadequate to explain all aspects of vascular aging. In this study, we investigated the contribution of cell non-autonomous mechanisms to cerebrovascular aging.
Methods: To determine the cerebrovascular effects of circulating factors enriched in young and aged blood, we intravitally measured cerebromicrovascular outcomes, such as NVC responses, BBB integrity, and brain capillarization in heterochronic (Young-Aged pairs consisting of 6- and 20-month-old C57BL/6 mice, respectively) and isochronic (Young-Young and Aged-Aged) parabionts. Furthermore, to mechanistically test the contribution of insulin-like growth factor 1 receptor (IGF-1R) signaling to young blood-mediated cerebrovascular rejuvenation, we assessed these cerebromicrovascular outcomes in endothelial IGF-1R-deficient aged parabionts exposed to young blood (AgedVE-Cadherin-IGF1R-KD–(YoungWT)).
Results: We found that exposure to young blood improved NVC responses, BBB integrity, and brain capillarization in aged parabionts. Importantly, in endothelial IGF1R-deficient parabionts, exposure to young blood led to a significantly decreased rescue of NVC responses and a loss of young blood-mediated rejuvenation of BBB integrity and brain capillarization.
In conclusion, relatively short-term exposure to young blood can rescue vascular aging phenotypes, demonstrating a significant role of cell non-autonomous mechanisms, such as IGF1/IGF-1R signaling, in the regulation of cerebromicrovascular aging processes.

Acknowledgments:

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O8: Impact of platelet-derived TGFβ1 on integrity of murine lung microvascular endothelial barrier: age-related disruption

Marta Smeda[1], Mohammadebrahim Hosseinzadehmaleki[1,2], Marta Stojak[1], Anna Kurpinska[1], Anna Gdula[1], Agnieszka Kij[1], Marek Grosicki[1], Kamila Wojnar-Lason[1], Stefan Chlopicki[1,3]
[1]Jagiellonian University, Jagiellonian Centre for Experimental Therapeutics (JCET), Krakow, Poland
[2]Jagiellonian University, Doctoral School of Exact and Natural Sciences, Krakow, Poland
[3] Jagiellonian University Medical College, Department of Pharmacology, Krakow, Poland

Abstract:

Ageing leads to the progressive decline of endothelial function, alters the phonotype of circulating platelets, and exacerbates the course of lung diseases. Platelets protect the endothelial barrier integrity of the lungs in health and alongside inflammatory diseases. On the other hand, platelets are the major source of transforming growth factor β (TGFβ1) in the body. This study examines the effects of platelet-derived TGFβ1 present in a mixture of factors released from platelets isolated from healthy 10-week-old mice and from 40-week-old mice with age-related endothelial dysfunction on the barrier integrity formed by primary lung microvascular endothelial cells (mLMVECs s) in an ex vivo setting. We found that platelet-derived TGFβ1 increased the permeability of the barrier formed by mLMVECs s when embedded in the platelet releasate milieu of older mice. By analyzing the proteins released by platelets, we identified Serpina3k (S3K) as a protein that could modulate the barrier-disruptive function of platelet-derived TGFβ1. S3K addition to mLMVECs culture at concentrations found in platelet releasates of 10-week-old and 40-week-old mice protected against barrier-disruptive action of TGFβ as well as prevented TGFβ1-driven tight junction protein claudin-5 decrease. The barrier-protective effect of S3K was linked to its role as a serine protease inhibitor. These findings highlight the potential significance of platelet-derived S3K as a crucial regulator of lung endothelial barrier integrity during the aging process and a potential target for therapeutic intervention.

Acknowledgments:

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O9: Effect of spermidine on vascular function and endothelial phenotype.

Łukasz Mateuszuk[1], Sylwester Mosiołek[1], Stefan Chłopicki[1,2]
[1] Jagiellonian Centre for Experimental Therapeutics (JCET), Bobrzynskiego [14], [30-348] Krakow, Poland
[2] Department of Pharmacology, Chair of Pharmacology, Jagiellonian University, Medical College, Grzegórzecka 16, 31-531 Krakow, Poland

Abstract:

Spermidine, among other polyamines, is an essential metabolite regulating important cellular functions, including RNA-to-protein translation, cellular growth and proliferation, autophagy, immune responses, and RNA/DNA stability. Polyamine pathway is interconnected with arginine, nitric oxide, nicotinamide and methionine metabolism, which are important factors for maintaining proper vascular and endothelial function. Most beneficial cellular effects regulated by spermidine are mechanistically linked to mitophagy, an autophagy subtype specific for the lysosomal degradation and recycling of dysfunctional and obsolete mitochondria. Spermidine is a substrate for the posttranslational hypusination of eukaryotic initiation factor 5A (eIF5A), a translation factor involved in elongation and termination, stimulating autophagy and mitophagy. Spermidine is considered a geroprotective agent, and since eIF5A hypusination declines with aging in vivo, it can be restored by spermidine supplementation, which improves autophagic pathways. The aim of this study, using in vitro, ex vivo and in vivo models of endothelial dysfunction, was to examine whether exogenous spermidine may act as a vasoprotectant in a mitophagy-dependent manner. Materials and methods: the effect of spermidine was tested on HAEC line treated with TNFα or IL1β, mouse aortic vessels incubated with IL1β, or taken from atherosclerotic mice with endothelial dysfunction, or aortic vessels isolated from mice treated with LPS administered intraperitoneally. Preliminary results: spermidine triggered mitophagy and lysosome formulation in HAEC cells. Nitric oxide-dependent vasodilation of aortic vessels was improved after spermidine treatment. More studies on inflammatory, mitochondrial and mitophagic markers will be conducted to confirm the link between vasoprotective action of spermidine and mitophagy.

Acknowledgments:

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O10: Prevention of Sarcopenic Obesity Maintains Angiogenic Capacity in PAD

Andrew C. Guilfoyle-Speese, B.S.[1], Cody L. Rosewater, M.S.[1], Caleb A. Padgett Ph.D.[1], Hunter Sellars B.S.[1], James D. Mintz M.B.A.[1], David J. Fulton Ph.D.[1,2], and David W. Stepp Ph.D.[1,3]
[1]Vascular Biology Center,
[2]Department of Pharmacology and Toxicology, and
[3]Department of Physiology, Medical College of Georgia, Augusta University, Augusta, GA USA

Abstract:

Objective: To test the hypothesis that prevention of sarcopenia achieved through MSTN KO restores angiogenic capacity in ischemic limbs of obese mice through the resolution of GAL3-NOX1-mediated endothelial dysfunction.

Results: Myostatin deletion (MSTN -) results in significantly increased skeletal muscle (SKM) mass and restored insulin sensitivity without altering whole-body mass, fat percentage, or activity in obese leptin receptor mutant (db/db) mice. Previous studies in our lab revealed that MSTN deletion was sufficient to restore endothelial function in db/db mice. Additionally, our lab has shown that galectin 3 or NADPH oxidase 1 KO (GAL3 - and NOX1 - respectively) in db/db mice phenocopied the vascular improvements seen in db/db_MSTN - mice without restoring SKM size or quality. RNA-Seq and RT-qPCR analysis of SKM and endothelial cells (ECs) revealed significant upregulation of GAL3 and NOX1 expression in db/db_MSTN + mice but not in db/db_MSTN - and db/db_GAL3 - mice. We observed that obesity inhibits and MSTN, GAL3, or NOX1 KO restores limb perfusion and angiogenesis in both hind limb ischemia (HLI) and aortic ring assay models respectively.

Conclusion: In summary, restoration of muscle mass in obese individuals provides potent protection of vascular and metabolic health. A correlate of this improvement is the attenuation of a novel MSTN-GAL3-NOX1 axis, which we have shown to mediate vascular inflammation/redox signaling in obesity. These data suggest that pharmacological targeting of upstream MSTN, downstream GAL3 and/or NOX1 could be effective in treating cardiovascular disease in the context of obesity and metabolic syndrome.

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O11: Pharmacological inhibition of platelet energy metabolism as a way to potentiate antiplatelet drug action

Patrycja Kaczara, Olena Lytvynenko, Anna Kurpińska, Agnieszka Kij, Katarzyna Rafa-Zabłocka, Piotr Berkowicz, Kamil Przyborowski, Stefan Chłopicki
Jagiellonian University in Krakow, Jagiellonian Centre for Experimental Therapeutics (JCET), Kraków, Poland

Abstract:

A major strategy to reduce cardiovascular events related to thrombosis is antiplatelet therapy, but some patients with metabolic diseases show platelet hyperreactivity despite treatment. Pharmacological regulation of platelet energy metabolism can help reduce effective doses of antiplatelet drugs and their unwanted side effects. We investigated the effects of partial inhibition of platelet energy metabolism on the antiaggregatory action of cangrelor, an antiplatelet drug that acts by inhibiting the purinergic P2Y12 receptor. Washed platelets, isolated from blood donated by healthy volunteers, were exposed to the carbon monoxide releasing molecule A1 (CORM-A1; inhibits platelet aggregation by inhibiting mitochondrial respiration and glycolysis) or a combination of 2-deoxy-D-glucose (2DG; an inhibitor of hexokinase) with oligomycin (an inhibitor of ATP synthase), and cangrelor, individually and in combinations. Platelet aggregation was measured by light transmission aggregometry on a multiwall plate, mitochondrial respiration and glycolysis rates were measured by the Seahorse technique, while intraplatelet metabolites and eicosanoid levels were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Cangrelor inhibited platelet aggregation and did not affect platelet energy metabolism. Treatment of platelets with CORM-A1 or a combination of oligomycin with 2DG led to simultaneous partial inhibition of both ATP-producing pathways and synergistically potentiated the antiaggregatory effect of cangrelor. Furthermore, the approach of combined platelet treatment with energy metabolism inhibitors and an antiplatelet drug allowed for the reduction of effective concentrations of all applied reagents. These results provide new evidence for targeting metabolic regulatory mechanisms as a way of improving an antithrombotic strategy.

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O12: PDIA1 and PDIA3 differentially regulate platelet function

Kamil Przyborowski[1,4], Agnieszka Pelesz[1,2], Filip Fedak[1], Natalia Marcinczyk[3], Saskia von Ungern-Sternberg[4], Ivars Kalvins[5], Ewa Chabielska[3], Kerstin Jurk[4] and Stefan Chlopicki[1,6]

[1] Jagiellonian University, Jagiellonian Centre for Experimental Therapeutics (JCET), Krakow, Poland
[2] Jagiellonian University, Doctoral School of Exact and Natural Science, Krakow, Poland
[3] Medical University of Bialystok, Department of Biopharmacy and Radiopharmacy, Bialystok, Poland
[4] University Medical Center of the Johannes Gutenberg University, Center for Thrombosis and Hemostasis (CTH), Mainz, Germany
[5] Latvian Institute of Organic Synthesis, Laboratory of Carbocyclic Compounds, Riga, Latvia
[6] Jagiellonian University Medical College, Chair of Pharmacology, Krakow, Poland

Abstract:

Background
Platelet disulfide isomerases (PDIA) regulate activation of platelet surface αIIbβ3 integrin and platelet aggregation.
Aims
We investigated a newly in-house synthesized inhibitor, C-3399, blocking PDI isoforms PDIA1, PDIA3 and PDIA6.
Methods
To show antiplatelet effects of C-3399 compared to PDIA1 selective inhibitor, bepristat 2a, platelet aggregation, expression of platelet surface activation markers, TXA2 production and phosphorylation of signaling proteins were evaluated in response to GPVI or TXA2 receptor-mediated platelet activation.
Results
C-3399 significantly inhibited in a concentration-dependent manner the activation of platelet αIIbβ3 integrin induced by convulxin, and partially diminished CD62P or CD63 expression in platelet-rich plasma (PRP). Bepristat displayed the strongest inhibitory effect on αIIbβ3 integrin activation and nearly completely abolished expression of CD62P or CD63. C-3399 and bepristat partially reduced platelet aggregation induced by convulxin in PRP. C-3399 and bepristat reduced convulxin-induced ROS generation and downregulated p-Akt (S473) and p-ERK1/2 (T202/Y204) with no effect on p-38 (T180/Y182). Interestingly, the blockade of PDIA1, PDIA3 and PDIA6 by C-3399 nearly completely inhibited U46619-induced activation of αIIbβ3 integrin, CD63 expression and platelet aggregation, while the selective blockade of PDIA1 by bepristat resulted in significantly weaker inhibitory effects.
Conclusions
C-3399 shows differential inhibitory effects on GPVI-mediated platelet activation compared to bepristat. In contrast to bepristat, C-3399 blocks not only GPVI-mediated TXA2 synthesis but also TXA2-mediated activation of human platelets. Our results suggest that PDIA1 plays a major role in GPVI-mediated activation of platelet αIIbβ3 integrin activation and granule exocytosis while PDIA3 and/or PDIA6 predominantly regulate these platelet responses induced by TXA2.

Acknowledgments:

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O13: The effects of tissue factor pathway inhibitor on the proliferation and phenotype of vascular cells

Vanessa Bröker, Jarno JJ Koetsier, Stella Thomassen, Elisabetta Castoldi, Kanin Wichapong, Philip Wenzel, Leon J Schurgers, Tilman M Hackeng
Department of Biochemistry, CARIM School for Cardiovascular Diseases, Maastricht University, Maastricht, The Netherlands

Abstract:

Tissue factor pathway inhibitor (TFPI) is a constitutive inhibitor of the extrinsic coagulation pathway by inhibiting of the TF/FVIIa/FXa complex. Recent studies highlight TFPI's non-canonical functions, such as protection against vascular remodeling, but underlying molecular mechanisms remain unclear.
This study aims to elucidate the impact of TFPI on phenotypic switching of vascular smooth muscle cells (VSMC) and endothelial cells (EC) using a genetically modified variant of TFPI.
Pluripotent cells were genetically modified by CRISPR/Cas9 editing to express a partially functional TFPI variant lacking Kunitz-domain 2, and were differentiated into vascular cells. In silico modeling revealed binding impairments of this modified TFPI to FXa. TFPI expression and secretion were quantified via Western blot and ELISA. Cellular proliferation was assessed with EdU assays. Extracellular vesicle secretion was measured using a particle tracer device, and VSMC were analyzed for phenotypic switching and vascular calcification. TFPI functional assays determined remaining inhibitory functions.
Modified VSMC exhibited decreased TFPI expression and secretion. Increased cellular proliferation was mitigated by genetic repair of the mutation and rivaroxaban treatment. Synthetic mutant VSMC had higher vesicle secretion and showed increased vascular calcification under hypercalcemic conditions. EC phenotypes were altered, with changes in CD144 and CD31 expression. The mutant TFPI variant lost its ability to inhibit FXa but retained some functionality in FVIIa and prothrombinase inhibition assays.
The partially functional TFPI variant impacts proliferation, vesicle secretion, and vascular calcification, indicating a pivotal role in vascular cell phenotype maintenance. Further investigation into TFPI's molecular mechanisms is essential to fully understand its non-canonical functions.

O14: Systemic and local lipid mediator status reflecting vascular health

Agnieszka Kij[1], Anna Bar[1], Grzegorz Kwiatkowski[1], Anna Kieronska-Rudek[1], Agnieszka Karas[1], Barbara Sitek[1], Hans M.G. Princen[2], Ana Chopo Pizarro[3], Matthew Dooley[3], Jesmond Dalli[3], Stefan Chlopicki[1]

[1]Jagiellonian University, Jagiellonian Centre for Experimental Therapeutics (JCET), Krakow, Poland
[2]The Netherlands Organization of Applied Scientific Research (TNO), Metabolic Health Research, Leiden, The Netherlands
[3]William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, John Vane Science Centre, Queen Mary University of London, London, United Kingdom

Abstract:

Endothelial dysfunction (ED) is governed by various complex mechanisms, and it can be considered a state of chronic vascular inflammation. Importantly, the inflammatory response is mediated by potent signalling molecules such as lipid mediators including pro-inflammatory eicosanoids generated from arachidonic acid (AA) as well as specialized pro-resolving mediators (SPMs) involved in the resolution of inflammation biosynthesized from eicosapentaenoic (EPA)-, docosahexaenoic (DHA)- and docosapentaenoic (DPA) acids. The constant need for novel diagnostic and therapeutic tools for the early prevention of ED-induced cardiovascular diseases prompted us to verify whether the changes in lipid mediator biosynthesis could mirror the ED in murine models of dyslipidaemia (E3L.CETP mice) and acute inflammation (LPS-injected mice).

In dyslipidaemic E3L.CETP mice, the most affected pathways included down-regulation of EPA and DHA metabolome in both plasma and aorta which was age- and gender-dependent rather than determined by dyslipidaemia and dyslipidaemia-induced effect on ED. In LPS-induced inflammation, transient alternations in the plasma lipid mediator profile including mainly AA and EPA pathways were observed. In our studies, we measured both free and total fraction of lipid mediators revealing that esterified fraction could also reflect the state of endothelium under different pathological states. Altogether, our preliminary studies indicate that the most significant changes in lipid mediators were related to ageing rather than dyslipidaemia in E3L.CETP mice, and the plasma pattern of inflammation-resolution metabolome linked to EPA, DHA and DPA did not reflect the severity of endothelial dysfunction in both models. On the other hand, acute EPA or DHA treatment improved endothelial function in E3L.CETP mice, but this promising direction for endothelial function regulation requires further studies.

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O15: BRAF and MEK inhibitors: investigating the mechanisms of vascular toxicity.

Elżbieta Buczek[1,2], Marta Smęda[1], Anna Bar[1], Brygida Marczyk[1], Ebrahim H. Maleki[1,2], Janusz Pyka[1], Anna Gdula[1], Agnieszka Karaś[1,2], Kenchana Pandian[3], Stefano Rochetti[1], Stefan Chlopicki[1]
[1] Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Krakow, Poland
[2] Jagiellonian University, Doctoral School of Exact and Natural Sciences, Krakow, Poland
[3] Leiden Academic Centre for Drug Research, Leiden, Netherlands

Abstract:

Combination of BRAF and MEK inhibitors (BRAFi, MEKi), used as targeted therapy of BRAF-mutated cancers (e.g. Melanoma), significantly improved survival, but increased the cardiovascular complications in patients, by unknown mechanisms. It is not clear whether BRAF and/or MEK inhibitors are responsible for cardiovascular toxicity of their combination.
We aimed to verify, whether BRAFi and MEKi have detrimental effects on endothelial function in vivo and ex vivo and to identify underlying molecular mechanisms. For that purpose, we applied wide range of techniques such as wire myography, EPR, MRI, Western Blot, LC-MS, RT-PCR and PamGene kinase activity profiling.
In the in vivo study, acute (6h) and short-term (7days) treatment with Dabrafenib (BRAFi) but not Trametinib (MEKi) induced an impairment of endothelium-dependent response of mice aorta to acetylcholine, without effect on response to sodium nitroprusside. Similarly, ex vivo, in murine aorta incubated 24 hours with Dabrafenib but not Trametinib, impaired vasorelaxation of aorta to acetylcholine was evidenced as well as decreased NO production measured by EPR and impaired NOS activity measured by LC/MS and tracer-based metabolomics. Kinome profiling identified number of kinases that were downregulated (e.g. RSK4) or upregulated (e.g ROCK2, CAMK2 and PKA) in response to Trametinib and Dabrafenib, respectively, underscoring distinct profile of kinase response for these drugs.
In conclusion, cardiovascular adverse effects of BRAF/MEK inhibitors therapy in cancer patients may be linked to endothelial dysfunction induced by BRAFi rather than MEKi. The mechanism of Dabrafenib - induced endothelial dysfunction may involve up-regulation of number of kinases including CAMK and Rho/ROCK signaling.

Acknowledgments:

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O16: Role of endothelial cells in age-related defective liver regeneration

Ignacio Benedicto
Centro de Investigaciones Biológicas Margarita Salas (CIB-CSIC), Madrid, Spain

Abstract:

Liver sinusoidal endothelial cells (LSECs) regulate liver regeneration after partial hepatectomy by providing angiocrine factors that induce hepatocyte proliferation. After partial resection of the liver, LSECs become stretched due to the elevation in blood pressure that occurs in the non-resected remaining organ fraction. Such mechanical stress promotes the expression of pro-regenerative angiocrine factors by LSECs but can also induce inflammatory responses that compromise liver homeostasis. A fine-tuned response of LSECs to cell stretching seems therefore essential for productive organ regeneration. Liver regeneration is drastically reduced in aged patients and animal models, but the potential causal role of LSECs in the impairment of aged liver regeneration remains largely unexplored. I will present bulk and single-cell RNAseq analyses of young and aged mouse LSECs in basal conditions and after partial hepatectomy. I will also discuss preliminary data from in vitro stretching experiments designed to explore a potential pharmacological approach to improve the pro-regenerative response of endothelial cells upon mechanical stress.

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O17: Liver sinusoidal endothelial cell (LSEC) dysfunction and alterations in hepatocyte metabolism in chronic heart failure

Izabela Czyzynska-Cichon[1], Kamila Wojnar-Lason[1,2], Grzegorz Kwiatkowski[1], Anna Kurpinska[1], Agnieszka Kij[1], Urszula Tyrankiewicz[1], Marta Stojak[1], Patrycja Kaczara[1], Magda Sternak[1], Marta Pacia[1], Stefan Chlopicki[1,2]

[1] Jagiellonian University, Jagiellonian Centre for Experimental Therapeutics (JCET), Krakow, Poland
[2] Jagiellonian University Medical College, Department of Pharmacology, Krakow, Poland

Abstract:

The heart-liver axis represents a complex bidirectional relationship, which becomes crucial in the effective care of patients with heart failure (HF), but mechanisms involved in cardio-hepatic interactions still remain unclear. In particular, the liver may support the function of the heart by providing bioenergetic substrates. Thus, liver dysfunction, accompanying the pathophysiology of HF and associated with increased mortality of HF patients, may be related to altered hepatocyte metabolism.

In our recently published paper by Wojnar-Lason et al.[1], taking advantage of Tgαq*44 mice, a unique murine model of slowly developing chronic HF reflecting the human pathophysiology, we demonstrated that LSEC dysfunction with decreased porosity represents an early event in chronic HF progression, occurring earlier than systemic endothelial dysfunction. LSEC defenestration was linked to impaired hepatic microvascular perfusion, as determined by MRI-based assessment of hepatocyte-specific contrast agent uptake. Importantly, LSEC dysfunction was also accompanied by prolonged postprandial lipoprotein clearance and augmented β-hydroxybutyrate production. Moreover, the hepatocyte proteome exhibited alterations suggesting metabolic reprogramming of the liver including Acat1, Got2, Mcat, Sdhc, Cbr4, Aldh7a1. To validate proteomic data the pro-ketogenic phenotype was also studied in primary hepatocytes isolated from Tgαq*44 by measurement of β-hydroxybutyrate production and Seahorse functional analysis. Finally, effects of pharmacological inhibition of ACE (perindopril, 2 mg/kg) or SGLT-2 (empagliflozin, 300 mg/kg), or of spontaneous exercise on pro-ketogenic phenotype of hepatocytes in Tgαq*44 mice were also studied. Interestingly, endothelial mediators such as NO or PGI2, can modulate hepatocyte metabolism as revealed by our studies in the isolated perfused liver.

Taken together, our data suggest that chronic HF results in changes in lipid and ketone body metabolism in the hepatocytes that accompany LSEC dysfunction, and this bioenergetic alterations may impact cardiac function. Accordingly, LSEC dysfunction and hepatocyte metabolism may represent an novel therapeutic target to regulate cardio-hepatic interactions in HF treatment.

[1] Wojnar-Lason et al. Chronic heart failure induces early defenestration of liver sinusoidal endothelial cells (LSECs) in mice. Acta Physiol (Oxf). 2024. doi: 10.1111/apha.14114.

Acknowledgments:

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O18: Liver sinusoidal endothelial cells constitute a major route for hemoglobin clearance

Gabriela Zurawska[1,*], Zuzanna Sas[2,*], Aneta Jończy[1], Raghunandan Mahadeva[1], Patryk Slusarczyk[1], Marta Chwałek[1], Maria Kulecka[3], Izabela Rumieńczyk[3], Morgane Moulin[4], Kamil Jastrzębski[1], Michal Mikula[3], Anders Etzerodt[4], Remigiusz Serwa[5], Marta Miączyńska[1], Tomasz P. Rygiel[2,6,#] and Katarzyna Mleczko-Sanecka[1,#]

[1] International Institute of Molecular and Cell Biology in Warsaw, Warsaw, Poland
[2] Medical University of Warsaw, Warsaw, Poland
[3] Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Poland
[4] Department of Biomedicine, Aarhus University, Aarhus, Denmark
[5] ReMedy International Research Agenda Unit, IMol Polish Academy of Sciences, Warsaw, Poland
[6] Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
* # equal contribution

Abstract:

The heart-liver axis represents a complex bidirectional relationship, which becomes crucial in the effective care of patients with heart failure (HF), but mechanisms involved in cardio-hepatic interactions still remain unclear. In particular, the liver may support the function of the heart by providing bioenergetic substrates. Thus, liver dysfunction, accompanying the pathophysiology of HF and associated with increased mortality of HF patients, may be related to altered hepatocyte metabolism.

In our recently published paper by Wojnar-Lason et al.[1], taking advantage of Tgαq*44 mice, a unique murine model of slowly developing chronic HF reflecting the human pathophysiology, we demonstrated that LSEC dysfunction with decreased porosity represents an early event in chronic HF progression, occurring earlier than systemic endothelial dysfunction. LSEC defenestration was linked to impaired hepatic microvascular perfusion, as determined by MRI-based assessment of hepatocyte-specific contrast agent uptake. Importantly, LSEC dysfunction was also accompanied by prolonged postprandial lipoprotein clearance and augmented β-hydroxybutyrate production. Moreover, the hepatocyte proteome exhibited alterations suggesting metabolic reprogramming of the liver including Acat1, Got2, Mcat, Sdhc, Cbr4, Aldh7a1. To validate proteomic data the pro-ketogenic phenotype was also studied in primary hepatocytes isolated from Tgαq*44 by measurement of β-hydroxybutyrate production and Seahorse functional analysis. Finally, effects of pharmacological inhibition of ACE (perindopril, 2 mg/kg) or SGLT-2 (empagliflozin, 300 mg/kg), or of spontaneous exercise on pro-ketogenic phenotype of hepatocytes in Tgαq*44 mice were also studied. Interestingly, endothelial mediators such as NO or PGI2, can modulate hepatocyte metabolism as revealed by our studies in the isolated perfused liver.

Taken together, our data suggest that chronic HF results in changes in lipid and ketone body metabolism in the hepatocytes that accompany LSEC dysfunction, and this bioenergetic alterations may impact cardiac function. Accordingly, LSEC dysfunction and hepatocyte metabolism may represent an novel therapeutic target to regulate cardio-hepatic interactions in HF treatment.

[1] Wojnar-Lason et al. Chronic heart failure induces early defenestration of liver sinusoidal endothelial cells (LSECs) in mice. Acta Physiol (Oxf). 2024. doi: 10.1111/apha.14114.

Acknowledgments:

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O19: The involvement of the lung vascular endothelium in antiviral immunity during respiratory viral infections

Mateusz Gawrysiak[1], Robert Szewczyk[1], Marta Chuncia[1], Jonatan Rataj[1], Adrian Gajewski[1], Aleksandra Likońska[1], Izabela Gulbas[1], Maciej Chałubiński[1]
[1] Department of Immunology and Allergy, Medical University of Lodz, Poland, Pomorska str. 251, 92-213 Lodz

Abstract:

Introduction. Human rhinovirus 16 (HRV16) and coronavirus 229E (HCoV229E) may infect the human lung vascular endothelium (ECs) in vitro. HRV16 stimulates rapid IFN-β production and activates mechanisms of antiviral immunity. The relevance of antiviral immune responses orchestrated by virus-infected ECs is unknown.
Purpose of the work. To determine the role of IFN-β and IFN-dependent mechanisms in limiting lung endothelium respiratory virus infection.
Materials and methods. Human lung microvascular endothelial cells (HMVEC-L) were infected with HRV16 (MOI 3.0) and HCoV229E (MOI 1.0) in vitro. mRNA expression and viral RNA were assessed by real-time PCR. Protein levels were measured in FACS and ELISA assay. IFN-β blocking was performed after HRV16 infection using anti-IFN-β antibodies.
Results. In HMVEC-L, the virus copy number peaked at 5h upon incubation with HRV16 and then decreased. The decrease in viral load was accompanied by up-regulation of IFN-β, OAS-1, PKR mRNA expression, and an increase at the protein level. IFN-β blocking caused the reduction of expression of OAS-1 and PKR accompanied by the rise of viral RNA. After successfully silencing OAS-1 or PKR, higher viral copy numbers were observed. Interestingly, the number of HCoV229E copies was significantly lower in endothelium primarily infected with HRV16.
Conclusions. Interferon response is essential for sustaining the first line of antiviral defense. We demonstrated that IFN-β and OAS-1, PKR, as proteins responsible for the inhibition of HRV16 replication, are associated with antiviral immunity of endothelium.

Acknowledgments:

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O20: Unveiling the role of lung endothelial cell (EC) dynamics in the progression of pulmonary fibrosis

Elisabeth Fließer[1], Katharina Jandl[2], Thomas Lins[1], Konrad Hoetzenecker[3], Grazyna Kwapiszewska[1,4]
[1]Lung Group, Otto Loewi Research Center, Medical University of Graz, Graz, Austria
[2]Division of Pharmacology, Otto Loewi Research Center, Medical University of Graz, Graz, Austria
[3]Department of Thoracic Surgery, Medical University of Vienna, Vienna, Austria
[4]Institute for Lung Health, Cardiopulmonary Institute, Member of the German Lung Center (DZL), Giessen, Germany

Abstract:

Progressive pulmonary fibrosis is a rapidly advancing, deadly disease triggered by uncontrolled tissue scarring due to chronic lung injury. The severe remodeling of the lung parenchyma is accompanied by pronounced alterations of the vascular compartment, manifested as heterogeneous vascularization, vascular inflammation and vessel remodeling. Whether lung fibrosis is associated with local endothelial shifts or an actual loss of vasculature remains unsolved. Here, we propose that a disrupted ability of the endothelium to proliferate and thus to regenerate exacerbates pulmonary fibrosis.
Markers of ECs, proliferation, and apoptosis have been measured by flow cytometry in single-cell suspensions from human end-stage disease lungs, donor lungs, and murine lungs 3 or 14 days after bleomycin administration.
The portion of CD31+ ECs was significantly decreased in murine lungs 3d after bleomycin administration and normalized again after 14d as compared to saline treated mice. Additionally, the relative proportion of EC marker Thrombomodulin and von-Willebrand-Factor increased 14d after bleomycin administration. This was accompanied by a significant increase of Ki67+ ECs. In human transplant lungs, significantly lower percentages of CD31+ ECs were Ki67+ as compared to donor lungs. Generally, the proportion of CD31+ was lower in transplant lungs, however, ECs showed significantly higher Thrombomodulin and von-Willebrand-Factor positivity.
Our preliminary data suggest that EC proliferation might contribute to fibrosis resolution in the bleomycin mouse model. On the contrary, an impaired proliferative capacity in the human disease may foster the disease progression. Pharmacological interference with endothelial proliferation in-vivo will unravel whether dysfunctional vascular growth affects the degree of parenchymal distortion.

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26th SEPTEMBER 2024

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P3 **Benbouziane I.** "Pharmacokinetic Analysis of Ponatinib: Tissue Distribution, Accumulation in Perivascular Fat (PVAT); A Possible Implication for PVAT-Targeted Vascular Toxicity of Ponatinib"

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P7 **Cichoń M.** "Can age-related protein aggregation affect endothelial cell function?"

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P9 **De Moudt S.** "Pro-resolving receptor GPR18 regulates vascular physiology in a vascular bed-specific manner"

P10 **Domagała A.** "Recognition of peptide and protein structural alterations using ROA-CPL spectroscopy and Eu(III) probes"

P11 **Dziaczkowski F.** "The role of PKD2 in the hyperlipidemia and atherosclerosis development"

P12 **Franczyk-Żarów M.** "Evaluation of atherosclerotic lesions in ApoE/LDLR-/- mice fed diets supplemented with fermented products as a source of vitamin K"

P13 **Gergely T.** "Prior cardiac ischemic injury exacerbates immune checkpoint inhibitor-induced cardiotoxicity"

P14 **Habibi A.** "Rivaroxaban reverses the adverse effects of warfarin on plaque composition and vessel wall remodeling"

P15 **Harasim G.** "NAD+-boosting therapy by nicotinamide riboside alters extracellular adenine nucleotides metabolism in human endothelial cells"

P16 **Hegedűs Z.** "Expression of key immune checkpoints in end-stage heart failure"

P17 **Jaminon A.** "Role of medial vascular smooth muscle cells in atherogenesis and features of a vulnerable plaque"

P18 **Jaźwa-Kusior A.** "Endothelial Nrf2 deficiency enhances atherosclerotic lesion formation and alters vascular inflammatory crosstalk: insights from scRNA-seq"

P19 **Jüttner A.** "The modified 6-chromanol SUL-138 protects from accelerated vascular aging and associated end organ dysfunction"

P20 **Karaś A.** "The effects of ageing and inflammation on vascular metabolism in the isolated murine aorta"

P21 **Kawecka A.** "Do all experimental models of inflammation stimulate ecto-adenosine deaminase?"

P22 **Kierońska-Rudek A.** "Cytoprotective role of increased hydrogen sulfide (H2S) turnover and polysulfide production in replicative cell senescence"

P23 **Kocsis M.** "Thymic modulation of Immune Checkpoint Inhibitor-induced cardiotoxicity: New perspectives in Immunotherapy"

P24 **Kolczyńska-Matysiak K.** "Role of platelet-derived lipid mediators in glucose homeostasis"

P25 **Kostogrys RB.** "A negative effect of caloric restriction on atherosclerosis in young ApoE/LDLR-/- mice"

P26 **Kulawiak B.** "The relationship between the mitochondrial BKCa channel and the cellular redox state"

P27 **Kurpińska A.** "Anti-platelet inhibitors of protein disulfide isomerases (PDI) afford vasoprotection mediated by platelet cargo"

P28 **Kuś E.** "Hyaluronan-based nanocapsules – mechanisms of targeted drug delivery in endothelial dysfunction"

P29 **Kutryb-Zając B.** "Nucleotide precursor supplementation as a strategy to improve cardiac bioenergetics and function in hypertrophic cardiomyopathy"

P30 **Maleki E.** "Age-Dependent Induction of Endothelial-Mesenchymal Transition in Murine Lung Microvascular Endothelial Cells by Platelet-Derived TGFβ1"

P31 **Mansi S.** "Investigating the influence of shear stress and substrate stiffness on human endothelial cells from different vascular beds"

P32 **Marczyk B.** "Acute effects of selected kinase inhibitors and doxorubicin on vascular endothelial function in vivo; vasoprotective effects of methylnicotinamide (MNA) and vitamin K"

P33 **Pieczara A.** "Single-cell drug response analysis using spectroscopic profiling – searching for adrenaline mechanism of action"

P34 **Radziwon-Balicka A.** "Platelets in Vaccine-induced Immune Thrombotic Thrombocytopenia (VITT)"

P35 **Rafa-Zabłocka K.** "The effects of CORM-A1 on platelet aggregation in mice – in vitro and in vivo studies"

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P39 **Sellers H.** "Expanding the Toolkit for Obesity Research: Murine AAV Models of Obesity"

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P41 **Seta E.** "A novel in vivo model for multiparameter assessment of tyrosine kinase inhibitor-associated cardiovascular toxicity"

P42 **Silva Sousa L.** "Development of a murine model of arteriovenous malformations in Hereditary Hemorrhagic Telangiectasia"

P43 **Sitek B.** "Impaired resolution of inflammation-induced endothelial dysfunction in a murine model of endotoxemia"

P44 **Skoczek D.** "Endothelial actin cytoskeleton alterations and glycocalyx disruption associated with HNF1A-MODY"

P45 **Stanek E.** "Acute phenotype switch of stem cell-derived preadipocytes under proinflammatory action of palmitic acid and TNF"

P46 **Stawarska K.** "Modulators of adenosine metabolism and signaling prevent against cardiac hypertrophy in mouse experimental models"

P47 **Suraj-Prażmowska J.** "Characterization of profiles of endothelial biomarkers in young and old endotoxemic mice"

P48 **Tóth A.** "Circulating ACE and ACE2 as Endothelial Biomarkers: Insights into Cardiovascular Disease"

P49 **Tóth V.** "Tirzepatide, a GIP/GLP1-receptor co-agonist preserves cardiac function and improves survival in mice with angiotensin II-induced heart failure"

P50 **Tyrankiewicz U.** "The influence of spontaneous physical activity on coronary adaptations to heart failure in Tgaq44 mice in course of early cardiac pathology"

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P52 **Wrzosek A.** "Light modulation of mitochondrial potassium channels activity isolated from Guinea Pig cardiomyocytes"

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Abstracts of posters

P1: Effects of the combination of ambrisentan and tadalafil on vasoresponsiveness in pulmonary hypertension in rats

Marta Baranowska-Kuczko[1,2], Monika Kloza[1], Anna Krzyżewska[1], Patryk Remiszewski[1], Piotr Ryszkiewicz[1], Krzysztof Mińczuk[1], Anna Pędzińska-Betiuk[1], Hanna Kozłowska[1], Barbara Malinowska[1]
[1] Department of Experimental Physiology and Pathophysiology, Medical University of Białystok, Białystok - Poland
[2] Department of Clinical Pharmacy, Medical University of Białystok, Białystok - Poland

Abstract:

Combination therapy is the standard of care in pulmonary arterial hypertension (PAH). Endothelin receptor antagonists (ambrisentan, AMB) and phosphodiesterase-5 inhibitors (tadalafil, TAD) are used as an upfront treatment for PAH. The aim of the study was to test the effect of AMB+TAD on vascular responses in pulmonary hypertension (PH) induced by monocrotaline (MCT) or Sugen5416 and hypoxia (SuHx) in rats. AMB+TAD (each at 10 mg/kg) or their vehicles were administered once daily per os for 3 weeks starting at day 7 in MCT and in day 14 in SuHx post 3 weeks of 10% hypoxia. Then, right ventricular systolic pressure (RVSP) and vascular responses in isolated intralobar pulmonary arteries (PAs) and aortas were measured.
RVSP was greater in PH rats. PAs and aortas of PH rats showed the reduced potency of endothelium-dependent acetylcholine-and endothelium-independent sodium nitroprusside (SNP)-mediated relaxation. The efficacy of thromboxane analogue U46619-induced contraction in PAs and aortas were enhanced in SuHx, but not in MCT. Treatment with AMB+TAD reduced RVSP and improved potency of the acetylcholine-mediated vasorelaxation in PAs and aortas in MCT and SuHx. It enhanced SNP-induced vasorelaxation in PAs of MCT and aortas of SuHx and diminished U-46619-mediated vasoconstriction in aortas of SuHx only.
In summary, AMB+TAD exert potent vasoprotective activity in endothelium-dependent vasorelaxation in both PH models. However, caution should be taken when choosing PH models, considering the model- and artery type-dependent specific variations in the effectiveness of the vascular therapy. So, efforts should be done to reveal the underlying mechanisms of this phenomenon.

Acknowledgments:

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P2: Hb-NO adducts in the red blood cells uncovered by resonance Raman spectroscopy

A. Bartkowiak, E. Szczesny-Malysiak, J. Dybas
Jagiellonian Centre for Experimental Therapeutics, Jagiellonian University, Krakow, Poland

Abstract:

Nitric oxide (NO) is a key mediator in the cardiovascular system, playing a crucial role in regulating vascular tone, smooth muscle cell proliferation, and the expression of endothelial adhesion molecules. The primary source of NO in the vasculature is endothelial cells (ECs), which produce NO via endothelial nitric oxide synthase (eNOS). Hemoglobin (Hb) within red blood cells (RBCs) closely controls and regulates the physiological pool of available NO. The interaction between NO and Hb varies depending on the oxidation state of the heme iron ion and environmental factors, leading to the formation of different nitrosyl Hb adducts. For instance, the reaction with deoxyHb forms a stable low-spin HbIINO adduct, while binding of NO to metHb can result in the formation of HbIIINO, considered a labile form of NO. Thus, the balance between ECs and RBCs is essential for maintaining NO bioavailability, and understanding this interplay is crucial for elucidating cardiovascular pathologies.
In this study, resonance Raman (rR) spectroscopy with a 405 nm excitation wavelength was employed to detect and characterize Hb adducts formed within RBCs after interacting with ECs under static and dynamic flow conditions. Human aortic endothelial cells (HAECs) were stimulated to produce NO using calcium ionophore A23187. Isolated RBCs were then allowed to interact with HAECs for 1 hour under either static or dynamic conditions before being subjected to rR measurements with a 5 μW laser to prevent NO dissociation.
This work confirmed the formation of different nitrosyl Hb adducts, including HbIINO and HbIIINO. Interestingly, the amount of formed nitrosyl Hb adducts was higher under static conditions. Additionally, reductive nitrosylation was observed over the course of EC-RBC interaction, indicating that HbIIINO could serve as a labile NO reservoir.

Acknowledgments:

This work was supported by the Polish National Science Centre (UMO-2021/41/B/NZ3/04146).

P3: Pharmacokinetic Analysis of Ponatinib: Tissue Distribution, Accumulation in Perivascular Fat (PVAT); A Possible Implication for PVAT-Targeted Vascular Toxicity of Ponatinib

Iwaine Benbouziane, Agnieszka Kij, Krzysztof Czamara, Stefan Chlopicki
Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Krakow, Poland

Abstract:

Tyrosine kinase inhibitors (TKIs) are associated with severe vascular side effects, but the mechanisms involved are not clear. This study aimed to investigate the in vivo pharmacokinetics of imatinib, nilotinib and ponatinib in C57BL/6 mice, to identify potential differences in PK profiles, and assess tissue distribution and accumulation over an extended period that could help to understand the role of pharmacokinetics (PK) in vascular toxicity of ponatinib. Ponatinib was administered via oral (10 mg/kg), intravenous (3 mg/kg), or intraperitoneal (0.3 mg/kg) routes. Initial PK experiments were conducted over 8 hours, followed by a 4-week treatment regimen. Ponatinib concentrations were measured in plasma, liver, aorta (with and without peripheral fat), and adipose tissues using LC-MS/MS. PK parameters were calculated using R Studio with PKNCA and NonCompart packages. Distinct PK profiles were observed across administration routes. After oral administration, prolonged half-lives were seen in the liver (5.33 h) and aorta with PVAT (7.29 h) compared to plasma (5.73 h), but ponatinib was not detected in aorta without PVAT. The 4-week treatment revealed significant accumulation in lipid-rich environments, with high concentrations in aorta with PVAT (10447.17 ng/g of tissue) and interscapular brown adipose tissue (3286.54 ng/g of tissue). Plasma levels remained elevated (80.63 ng/mL) after 4 weeks, confirming sustained systemic exposure. Intravenous administration resulted in rapid distribution to vascular tissues with levels detected in aorta with PVAT but not without PVAT. Adipose-derived mesenchymal stem cells treated with ponatinib revealed a concentration-dependent inhibition of adipogenesis by TKIs. Specifically, ponatinib (1 μM) prominently inhibited adipocyte differentiation and reduced the number and size of formed lipid droplets, in comparison to nilotinib and imatinib suggesting toxic effects of ponatinib on PVAT - known to regulate vascular homeostasis. Taken together, these findings suggest accumulation of ponatinib in fat, including PVAT and a possible involvement of perivascular adipose tissue (PVAT) in vascular toxicity of ponatinib. Further studies are needed to confirm this hypothesis.

Acknowledgments:

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P4: Assessment of the anti-inflammatory therapy with RS-102895 on cardiac inflammation in Tgαq*44 mice

Piotr Berkowicz[1], Filip Rolski[2,3], Justyna Totoń-Żurańska[4], Grzegorz Kwiatkowski[1], Agnieszka Jasztal[1], Urszula Tyrankiewicz[1], Paweł Wołkow[4], Przemysław Błyszczuk[2], Stefan Chlopicki[1]

[1] Jagiellonian Centre for Experimental Therapeutics, Jagiellonian University, Cracow, Poland;
[2] Department of Clinical Immunology, Jagiellonian University Medical College, Cracow, Poland;
[3] Centre of Postgraduate Medical Education, Department of Clinical Physiology, Warsaw;
[4] Centre for Medical Genomics OMICRON, Jagiellonian University, Cracow, Poland

Abstract:

Background: The major treatment strategy for heart failure (HF) involves neurohormonal blockade but targeting cardiac inflammation in chronic HF brought conflicting results. Better understanding of molecular background of pathomechanisms of cardiac inflammation in chronic HF is needed. The aim of this study was to evaluate the effect of anti-inflammatory therapy with RS-102895 (Ccr2 receptor antagonist) on cardiac function, cardiac inflammatory and cardiac remodeling processes in early-phase of HF development in Tgαq*44 mice.

Methods: Tgαq*44 mice were treated with RS-102895 (3 mg/kg of body weight/day in a diet) for 2 months. Cardiac transcriptomic analyses were performed by next generation sequencing, cardiac function by magnetic resonance imaging, infiltration of inflammatory cells into the cardiac tissue by flow cytometry, cardiac ultrastructure by transmission electron microscopy in chronic HF in Tgαq*44 mice.

Results: Cardiac transcriptomic analyses revealed activation of inflammation at early-phase of HF in Tgαq*44 mice. Infiltration of cardiac macrophages, extracellular matrix accumulation and perivascular fibrosis were increased in early-phase of HF in Tgαq*44 mice. RS-102895 therapy slightly improved heart function in Tg α q*44 mice.

Conclusions: In the model of chronic HF in Tgαq*44 mice activation of inflammation is evident in early stage of disease and is accompanied by abundant perivascular fibrosis. Ccl2-Ccr2 pathway seem to be promising anti-inflammatory target to alleviate HF progression.

Acknowledgments:

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P5: Ticagrelor vs. clopidogrel: different effects on microvascular function and endothelial cell bioenergetics

Alicja Braczko[1*], Barbara Kutryb-Zając[1*], Ada Kawecka[1], Iga Walczak[1], Krzysztof Urbanowicz[1], Ryszard T. Smoleński[1], Marcin Hellmann[2]
[1]Department of Biochemistry, Medical University of Gdańsk, Poland
[2]Department of Cardiac Diagnostics, Medical University of Gdańsk, Poland

Abstract:

Background: Ticagrelor (TIC) and clopidogrel (CLO) are the most widely used P2Y12 receptor antagonists. Antiplatelet effects of TIC are reversible and involve direct binding to the receptor. In contrast, CLO binds irreversibly to P2Y12, is converted to an active metabolite through liver metabolism, and inhibits ADP signaling. This study aimed to analyze the effects of antiplatelet drugs on microvascular function and endothelial cell bioenergetics.
Methods: Microvascular function was monitored in coronary artery disease (CAD) patients treated w/ and w/o P2Y12 antagonists using flow-mediated skin fluorescence technique (FMSF). Endothelial cell bioenergetics was analyzed in human (HMEC-1) and mouse (H5V) microvascular endothelial cells in vitro by ultra-high-performance liquid chromatography (UHPLC) and extracellular flux analyzer (Seahorse®). Nitric oxide (NO) production was measured using a special kit. Cells were treated for 24h with CLOP (0; 0.1; 1; 10 M), which was previously activated with microsomal fraction (MF) obtained from the rat liver and TIC (0; 0.1; 1; 5 M).
Results: TIC, but not CLO, improved microvascular function in CAD patients, especially the parameters of ischemic and reactive hyperemic response. Both, TIC and MF-activated CLO effectively inhibited ADP-induced platelet aggregation in vitro. However, only TIC augmented NO production, affected bioenergetics and adenosine triphosphate concentration in endothelial cells.
Conclusions: This study shows a superior effect of ticagrelor over clopidogrel on the microvascular function in humans and experimental cell culture models. This may be related to the influence of TIC on endothelial cell bioenergetics and nitric oxide production.

Acknowledgments:

Study supported by National Science Centre of Poland (projects 2023/51/B/NZ4/03017 and 2019/35/D/NZ3/03512).

P6: The effect of sodium glucose co-transporter 2 inhibitors-based pharmacology on endothelial glycocalyx disruption in endothelial dysfunction within isolated murine aorta

Natalia Chorazy[1,2], Kamila Wojnar-Lason[1,3], Stefan Chlopicki[1,3], Marta Z. Pacia[1]
[1]Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Krakow, Poland
[2]Doctoral School of Exact and Natural Sciences, Jagiellonian University, Krakow, Poland
[3]Chair of Pharmacology, Jagiellonian University, Krakow, Poland

Abstract:

Sodium glucose co-transporter 2 inhibitors (SGLT2-I) are novel attractive therapeutics, initially developed as a new generation of anti-diabetic drugs. Recently, SGLT2-I have displayed a vast array of beneficial therapeutic effects independent of anti-diabetic action, including cardioprotective and anti-inflammatory effects that may involve endothelial effects. Here we hypothesize that SGLT2-I have a positive influence on endothelial glycocalyx (GLX) disruption associated with endothelial inflammation.
The effect of SGLT2-I on coverage of GLX and expression of inflammation-associated proteins was investigated in the tumor necrosis factor (TNF, 10 ng/ml)-stimulated isolated murine aorta using fluorescence microscopy. TNF-induced vascular inflammation was associated with the disruption of GLX observed after 1 and 4 hs, but not after 24 hours, suggesting a transient effect or self-recovery of GLX. In the presence of the SGLT2-I: empagliflozin (Empa) dapagliflozin or ertugliflozin (10 μM concentration for each), not only TNF-induced overexpression of intercellular adhesion molecule I and von Willebrand factor was blunted but also endothelial GLX was preserved (4 hs incubation with TNF). The preservation of TNF-induced GLX disruption was also afforded by cariporide (Cari), sodium-hydrogen exchanger inhibitor (NHE-I), to a similar extent as Empa.
In conclusion, SGLT2-I prevent inflammation-associated endothelial GLX injury in the isolated murine aorta. As Cari protected GLX injury by TNF similarly to Empa, SGLT2-I may act on GLX via NHE, not via SGLT2, yet the mechanisms' details require further studies.

Acknowledgments:

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P7: Can age-related protein aggregation affect endothelial cell function?

Milena Cichoń, Jakub Bartuś, Aleksandra Kopacz, Damian Klóska, Alicja Józkowicz, Anna Grochot-Przęczek
Jagiellonian University, Faculty of Biochemistry, Biophysics and Biotechnology, Department of Medical Biotechnology

Abstract:

Cardiovascular diseases (CVDs) are the leading cause of death globally. Endothelial cells (ECs) that line the inner surface of blood and lymph vessels pay critical role in maintaining healthy phenotype of vasculature. Aging dysregulates function of blood vessels which contributes to a higher risk of CVDs in the elderly. Our data suggest that proteins NRF2 and KEAP1 can be involved in this process.
KEAP1 (Kelch-like ECH-associated protein 1) mainly regulates the activity of NRF2 (NFE2L2 – nuclear factor (erythroid-derived 2)-like 2) – a transcription factor that mediates protective response against oxidative stress. But, as we recently proposed, KEAP1 can act independently from NRF2 and along with nitric oxide synthase (NOS) and transnitrosating protein GAPDH be involved in the formation of S-nitrosothiols, leading to changes in protein function. In NRF2 deficient ECs this leads to premature senescence due to impaired autophagy, to the loss of proteostasis and accumulation of protein aggregates, and further to the loss of function.
Our data show that with age NRF2 level decreases in ECs, which suggests that the same dysfunctional phenotype of ECs can be occurring in physiological aging. Therefore, we study the relation between protein aggregation and cell function in young and aged human-derived primary endothelial cells with the possible involvement of S-nitrosation mediated by KEAP1/NOS/GAPDH complex. Our results indicate that a higher level of protein aggregates appears with age along with the impairment of function. In aging we also observed an increase in the mRNA expression level of proteins forming S-nitrosation complex.

P8: Effect of high-fat diet reversal on endothelial function and perivascular adipose tissue phenotype

K. Czamara[1], I. Czyzynska-Cichon[1], A. Bar[1], E. Stanek[1,2], M. Wawro[3], Z. Berkimbayeva[1], B. Marczyk[1], M.Z. Pacia[1], E. Bragado-García[4], P. Palma-Guzman[4], M.S. Fernandez-Alfonso[4], S. Chlopicki[1,5]
[1]Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Krakow, Poland
[2]Doctoral School of Exact and Natural Sciences, Jagiellonian University, Krakow, Poland
[3]Department of Cell Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland
[4]Instituto Pluridisciplinar, Faculty of Pharmacy, Universidad Complutense de Madrid, Madrid, Spain
[5]Department of Pharmacology, Jagiellonian University Medical College, Krakow, Poland

Abstract:

Background: Perivascular adipose tissue (PVAT) plays a crucial role in the regulation of vascular function. Structural and functional alterations of PVAT in, e.g., high-fat diet (HFD)-induced obesity, are associated with impaired anticontractile function and perivascular inflammation. Prolonged (8 weeks) HFD feeding causes endothelial dysfunction and alters PVAT, but these changes can be restored by HFD replacement with a chow diet.

Methods and results: A multi-faceted approach was involved to evaluate the effects of diet reversal after eight weeks of HFD on the aorta and PVAT. After one week of HFD reversal, significant improvements in glucose tolerance were observed. Using a magnetic resonance imaging (MRI)-based technique to characterize endothelial function in vivo in the presence of PVAT, it was found that the endothelial dysfunction in the thoracic aorta (TA) was partially reversed after one week, with full recovery requiring at least six weeks. Conversely, Raman spectroscopy revealed that the lipid unsaturation degree in the abdominal aorta (AA) PVAT fully recovered early, which is reflected in Scd1 gene expression assessed by qPCR, while recovery in the TA PVAT was only partial.

Conclusion: We demonstrated that 8-week HFD feeding results in the impairment of the endothelial function in the entire aorta (TA and AA) and alterations in PVAT lipid composition. However, the response to HFD reversal is location-dependent, with the TA showing quicker restoration, associated with brown-like PVAT. In the AA, endothelial dysfunction recovery does not appear to be insulin-dependent, nor is it related to lipid unsaturation in the AA PVAT.

Acknowledgments:

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P9: Pro-resolving receptor GPR18 regulates vascular physiology in a vascular bed-specific manner

Sofie De Moudt, Ameziane Herzine, Frances T. Yen, Nathalie Mercier, Magnus Back
INSERM U1116 Défaillance Cardiovasculaire Aiguë et Chronique, Université de Lorraine, Vandoeuvre-lès-Nancy, France

Abstract:

GPR18 is the specific receptor for pro-resolving-lipid-mediator resolvinD2 involved in atheroprotection through immunomodulation and transducing vascular functions. GPR18's role in site-specific vascular physiology was investigated by assessing vascular reactivity of thoracic aortas and femoral arteries of 3-month old GPR18+/+ and GPR18-/- mice using ex vivo pin or wire myography.

Contractile tension was measured using high K+ (5.6-50 mM) or phenylephrine (PE, 3E-9 to E-5 M). Relaxations to acetylcholine (ACh, 3E-9 to E-5 M) in the absence/presence of 10 µM indomethacin, NO-donor DEANO (3E-10 to E-5 M), or voltage-gated-calcium-channel (VGCC) blocker diltiazem (E-8 to E-4 M) were measured after 10 µM PE-precontraction. Intracellular vascular smooth muscle cell (VSMC) calcium handling was assessed in a calcium-free environment.

ACh-induced relaxations were impaired in the GPR18-/- femoral artery (IC50, log(M): 7.1±0.12; GPR18+/+ 7.4±0.16) (p=0.006). Although the passive stress-strain relation was heightened (p=0.001), high K+ elicited lower contractile tension in GPR18-/- mice (Emax, mN/mm: 3.56±0.28, GPR18+/+ 4.2±0.22) (p=0.007).

In contrast, ACh-induced relaxations were enhanced in the GPR18-/- thoracic aorta (IC50, log(M): -7.3±0.09; GPR18+/+ -7.0±0.06) (p=0.003). Passive stress-strain relation was attenuated (p<0.001), whereas K+-induced contractility was increased (Emax, mN/mm: 2.91±0.37; GPR18+/+ 2.44±0.13) (p<0.001). Furthermore, observed heightened activation of VGCC (p=0.004) coincided with an elevated VSMC cytoplasmic calcium load and altered IP3-dependent contractions.

Exogenous NO-dependent relaxations and PE-induced contractions were unaltered between genotypes (both vessel types).

In summary, GPR18 transduces differential and vascular-bed specific effects at both the endothelial and medial layers (i.e., passive arterial stiffness, endothelial function, contractility), demonstrating the importance of GPR18 in vascular function.

Acknowledgments:

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P10: Recognition of peptide and protein structural alterations using ROA-CPL spectroscopy and Eu(III) probes

Agnieszka Domagała[1,2], Małgorzata Barańska[1,3], Grzegorz Zając[1*]
[1]Jagiellonian University, Jagiellonian Centre for Experimental Therapeutics (JCET), Bobrzyńskiego 14, 30-348 Krakow, Poland
[2]Jagiellonian University, Doctoral School of Exact and Natural Sciences, prof. S. Łojasiewicza 11, 30-348 Krakow, Poland
[3]Jagiellonian University, Faculty of Chemistry, Gronostajowa 2, 30-387 Krakow, Poland

Abstract:

Protein alterations, such as post-translational modifications (PTMs) and aggregation, often contribute to common lifestyle diseases. For instance, PTMs like phosphorylation, acetylation, methylation, and glycosylation are linked to cancers, cardiovascular diseases, and neurodegenerative disorders. Additionally, the aggregation of amyloid fibrils in brain tissue is associated with conditions such as Alzheimer's disease (tau protein) and Parkinson's disease (α-synuclein). Therefore, studying modified proteins is essential for a deeper understanding of pathogenic processes and could enhance diagnostic and treatment strategies.

Raman Optical Activity (ROA) and Circularly Polarized Luminescence (CPL) are chiroptical spectroscopy techniques sensitive to the chirality of molecules and complex systems. They provide unique information about biologically important compounds' structure and optical activity in both model and biological systems. Both techniques measure the difference in interaction between chiral matter and right- and left-circularly polarized light, using Raman scattering (ROA) or luminescence (CPL). The combination of these techniques, ROA-CPL spectroscopy, has several documented applications in biomolecular studies. This approach allows for simultaneous recording of ROA and CPL spectra using an ROA spectrometer and sensitive europium(III) molecular probes. Induced CPL signals of Eu(III) probes are observed in the ROA spectrum, further enhanced by the strong laser radiation used in the spectrometer.

In this study, we demonstrate the first application of ROA-CPL spectroscopy with various Eu(III) probes for the spectral recognition of the glutathione peptide (GSH) and its derivatives as model PTMs. Additionally, we confirm the potential of this approach for monitoring the tau protein fibrillization pathway at very low protein concentrations.

Acknowledgments:

This work was supported by the National Science Centre in Poland (Grant No. 2019/35/B/ST4/04161 to GZ)

P11: The role of PKD2 in the hyperlipidemia and atherosclerosis development

Filip Dziaczkowski[1],Małgorzata Stelmach[1], Izabela Hawro[1,†], Magdalena Wit[1], Bozhena Luniv[1], Katarzyna Kolczyńska-Matysiak[1] and Grzegorz Sumara[1]
[1]Dioscuri center for metabolic diseases, Nencki
Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
[†]:current affiliation: Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, University of Illinois at Chicago, Chicago, IL, US

Abstract:

Cardiovascular diseases are the leading cause of death globally, with atherosclerosis being a major contributor. Elevated blood cholesterol and triglyceride levels are significant risk factors for atherosclerosis development, while low-density lipoproteins (LDL) contain a cholesterol-enriched lipid core and are crucial for atheroma formation. The PKD family consists of three members: PKD1, PKD2, and PKD3, which integrate hormonal and nutritional stimuli as effectors of Protein Kinase C (PKC) and Diacylglycerol. Our previous findings suggest that deletion or inactivation of Protein Kinase D 2 (PKD2) in the intestine could be a promising strategy to lower the absorption of dietary lipids and thereby the levels of triglycerides in circulation, which overall improves the metabolic profile of the mice. However, the impact of PKD2 on dietary cholesterol uptake in the intestine and its potential contribution to atherosclerosis development has not been defined. Our data show that global inactivation of PKD2 ameliorates hypercholesterolemia and protects from atherosclerosis. Our results indicate that CRT0066101 inhibits intestinal PKD2, reduces cholesterol and triglyceride levels, and reduces atherosclerotic plaque area in the aorta and aortic roots. To further explain mechanistically the impact of PKD2 on cholesterol transport in the intestine and thus its role in the development of atherosclerosis, we aim to use LDLR-deficient mice depleted from PKD2 specifically in the intestine as well as intestinal organoids.

Acknowledgments:

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P12: Evaluation of atherosclerotic lesions in ApoE/LDLR-/- mice fed diets supplemented with fermented products as a source of vitamin K

Hayat Hassen, Renata B. Kostogrys, Magdalena Franczyk-Żarów
Department of Human Nutrition and Dietetics, Faculty of Food Technology, University of Agriculture in Krakow, Poland

Abstract:

Vitamin K is a group of several lipophilic compounds among which vitamin K1 (phylloquinones) and vitamin K2 (menaquinones) can be distinguished. The Western diet mainly provides vitamin K1, which is 90% of the total vitamin K supply. As vitamin K2 (with the exception of the MK-4 derivative) is formed by bacterial synthesis, the products that are the richest source of these compounds may be the soft rennet cheeses or pickles. While the richest source of vitamin K2 MK-7 is fermented soyabean – natto, one of the most popular Japan dish. Fermented products have many health benefits, including antihypertensive, anticancer, antioxidant, anti-inflammatory and positive effects on the functioning of the digestive system. Fermented foods can therefore improve antioxidant and anti-inflammatory status. Therefore, the objective of the project was to verify the hypothesis about the effectiveness of dietary vitamin K2 as an anti-inflammatory agent, in the prevention of atherosclerosis.

The aim of the study was to assess the effect of dietary fermented foods on atherosclerosis. Two-month-old female ApoE/LDLR−/− mice were fed AIN-93G modified diets containing vitamin K-rich products: natto, cheese, sauerkraut and synthetic vitamin K2 MK-7 (100 μg/kg b.w./day) for 8 weeks. At the end of the experiment the animals were sacrificed, and blood, aortas and hearts were dissected. Measurements of inflammatory markers (IL-6 and TNFα), adipocytokins were made by immunoenzymatic method by ELISA. Analysis of atherosclerotic lesions in the aortic roots using cross-section method was done. Total atherosclerotic area did not show significant differences between experimental groups and control. Notably, in mice fed natto a significant decrease in atherosclerotic plaque area was observed in cross-section of the aortic root at the level of 800 μm ($p<0.05$). Our study suggests that natto as fermented foods rich in Vitamin K2 may protect against atherosclerosis in ApoE/LDLR−/− mice.

Acknowledgments:

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P13: Prior cardiac ischemic injury exacerbates immune checkpoint inhibitor-induced cardiotoxicity

[1,2,3]Tamás G. Gergely, [4,6]Zsófia D. Drobni, [1,2,3]Tamás Kovács, [1,2,3]Nabil V. Sayour, [1,2,3]Viktória É. Tóth, [1,2,3]Dániel Kucsera, [1,2,3]Zsófia Onódi, [1,2,3]Márton Kocsis, [5]Nóra Fekete, [5]Éva Pállinger, [5]Edit I. Buzás, [6]Tomas G. Neilan, [1,7]Péter Ferdinandy, [1,2,3]Zoltán V. Varga

[1]Center for Pharmacology and Drug Research & Development, Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest
[2]HCEMM-SU Cardiometabolic Immunology Research Group, Budapest
[3]MTA-SE Momentum Cardio-oncology and Cardio-immunology Research Group, Budapest
[4]Heart and Vascular Center, Semmelweis University, Budapest, Hungary
[5]Department of Genetics and Cell- and Immunbiology, Semmelweis University, Budapest, Hungary
[6]Cardiovascular Imaging Research Center (CIRC), Department of Radiology and Division of Cardiology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
[7]Pharmahungary Group, Szeged, Hungary

Abstract:

Introduction: Immune checkpoint inhibitors (ICI), such as antibodies targeting programmed death ligand-1 (PD-1), revolutionized cancer treatment. However, they can lead to several cardiovascular adverse effects, including heart failure and myocarditis. The mechanisms and risk factors behind ICI-induced cardiotoxicity are not entirely understood currently. We hypothesized that a prior cardiac ischemic injury can exacerbate the cardiotoxicity caused by anti-PD-1 therapy. Furthermore, we investigated whether abatacept, a T-cell co-stimulation blocker, can ameliorate ICI-induced cardiac effects.

Methods: First, we treated C57BL/6J mice with isoprenaline (ISOP group) or with its solvent (CON group), to induce reversible cardiac ischemia. After this, the animals underwent 16 weeks of recovery period, followed by echocardiography to confirm cardiac functional recovery. Here, mice from both groups were randomized to three further treatment groups: isotype control, anti-PD-1 alone, or anti-PD-1 combined with abatacept and were treated for two weeks, with three intraperitoneal injections per week. Echocardiography, qRT-PCR and histology was performed to evaluate cardiac function and inflammation.

Results: Mice with prior ischemic injury and anti-PD-1 treatment (ISOP + anti-PD-1 alone) showed significant cardiac dysfunction, while animals with abatacept treatment (ISOP+anti-PD-1+abatacept) showed normal cardiac function. Increased infiltration of T-cells and macrophages was seen in the myocardium of the ISOP+anti-PD-1 treated group compared to CON animals, with increased expression of pro-inflammatory cytokines, which was alleviated by abatacept co-treatment.

Conclusions: Prior cardiac ischemic injury exacerbates cardiac inflammation and cardiotoxicity induced by anti-PD-1 ICI therapy. Patients with pre-existing ischemic heart disease may be at greater risk for developing ICI-induced severe cardiac adverse events.

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P14: Rivaroxaban reverses the adverse effects of warfarin on plaque composition and vessel wall remodeling

Anxhela Habibi, Armand Jaminon, Jenny Surholt, Juliana Boukhaled, Magdi Nagy, Peter Leenders, Leon Schurgers
Department of Biochemistry, CARIM, Cardiovascular Research Institute Maastricht, Maastricht University, Maastricht, The Netherlands

Abstract:

Atherosclerosis is a major cause of cardiovascular disease (CVD) deaths globally, characterized by vascular calcification, increasing rupture risk. Patients with atherosclerosis often undergo lifelong warfarin therapy, raising concerns due to its association with increased calcification and plaque progression. Rivaroxaban offers an alternative anticoagulant, but its effects on warfarin-induced vascular damage remain unclear. This study investigated whether switching from warfarin to rivaroxaban benefits plaque progression and calcification.

ApoE-/- mice were fed a Western-type diet (WTD) for 18 weeks, with either warfarin or rivaroxaban supplementation (both at full anticoagulation dose). A reversing group received warfarin for 6 weeks, then rivaroxaban for 12 weeks. Plaque size was comparable in both the continuous rivaroxaban group and the reversing group and was decreased compared to the continuous warfarin and WTD groups. Notably, calcification decreased in both rivaroxaban groups, with a more significant decrease observed in the reversing group compared to continuous warfarin treatment. Additionally, the reversing group showed a more stable plaque phenotype with increased aSMA-positive vascular smooth muscle cells, reduced protease-activated receptor 2 and matrix-metalloprotease 9 expression, and fewer elastic breaks.

Our results suggest rivaroxaban reverses the adverse effects of warfarin, offering significant benefits on atherosclerotic burden and plaque stability. Switching from warfarin to rivaroxaban may mitigate or reverse warfarin's adverse effects. This anticoagulant strategy could significantly influence clinical practice for patients on anticoagulation with underlying cardiovascular disease.

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P15: NAD+-boosting therapy by nicotinamide riboside alters extracellular adenine nucleotides metabolism in human endothelial cells

Gabriela Harasim[1], Ada Kawecka[1], Marika A. Frańczak[1,2], Agata Jędrzejewska[1], Oliwia Król[1], Barbara Kutryb-Zając[1], Ryszard T. Smoleński[1], Ewa M. Słomińska[1]
[1]Department of Biochemistry, Medical University of Gdansk, 80-210 Gdańsk, Poland
[2]Department of Medical Oncology, Amsterdam University Medical Centers, Location VUmc, Cancer Center Amsterdam, 1081 HV Amsterdam, The Netherlands

Abstract:

Objectives:
Nicotinamide riboside (NR) is a potent NAD+ booster that may be beneficial for endothelial cells. This study aimed to test the effects of NR on intracellular NAD+ concentration, cell energy status and activities of cell-surface ecto-enzymes engaged in ATP and AMP hydrolysis and adenosine deamination in human endothelial cells.
Materials and methods:
Human microvascular endothelial cells (HMEC-1) were treated with 500 μM NR for 24h and 48h. The intracellular adenine nucleotides (ATP, ADP and AMP) and NAD+ concentrations were measured using RP-HPLC. The activity of ecto-enzymes was assessed after 24h incubation with NR by adding substrates (50 μM adenosine, ATP or AMP) and measuring catabolites in incubation buffer using RP-HPLC. The activity of ecto-adenosine deaminase (eADA), ATP and AMP hydrolysis were expressed as μmol/min/L.
Results:
24h and 48h incubation of HMEC-1 cells with NR resulted in increasing intracellular NAD+ by 27.7% and 35.6%, respectively, compared to the control. There were no differences in adenine nucleotides' pool between control and NR-treated cells after 24h nor 48h incubation. After treatment with NR for 24h, extracellular AMP hydrolysis rate were higher than control (1.37±0.09 vs. 0.61±0.04). ATP hydrolysis were also increased (5.35±0.4 vs. 3.90±0.37). Moreover, treatment with NR decreased total eADA1 activity by 26% with no significant differences in activity of eADA2.
Conclusions:
In addition to improving intracellular NAD+ in human endothelial cells, NR supplementation significantly affects the extracellular adenine nucleotides metabolism that promotes formation of vasoprotective adenosine. However, the specific action of NR through adenosine receptor pathways requires further studies.

Acknowledgments:

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P16: Expression of key immune checkpoints in end-stage heart failure

[1,2]Zsombor I. Hegedűs, [1,2,3]Tamás G. Gergely, [1,2,3]Tamás Kovács, [1,2,3]Zsófia Onódi, [4]Bálint Barta, [4]Alex Ali Sayour, [4]Tamás Radovits, [6]Przemyslaw Leszek, [4]Béla Merkely, [1,5]Péter Ferdinandy, [1,2,3]Zoltán V. Varga

[1]Center for Pharmacology and Drug Research & Development, Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest
[2]HCEMM-SU Cardiometabolic Immunology Research Group, Budapest
[3]MTA-SE Momentum Cardio-oncology and Cardio-immunology Research Group, Budapest
[4]Heart and Vascular Center, Semmelweis University, Budapest, Hungary
[5]Pharmahungary Group, Szeged, Hungary
[6]Department of Heart Failure and Transplantology, Cardinal Stefan Wyszyński National Institute of Cardiology, Warszawa, Poland

Abstract:

The key regulatory molecules of the adaptive immune response are known as immune checkpoints (ICs). These ICs are the basis of novel immunotherapies for several cancer types. ICs-targeting therapies often have severe side effects related to the activation of the immune system. Certain IC inhibitors have cardiotoxic adverse effects, and cause heart failure (HF), suggesting that ICs are essential for preserving cardiac homeostasis. There is a lack of information about the expression of ICs in the healthy and failing human heart.

We aimed to characterize the expression of co-inhibitory ICs in patients with end-stage HF. During heart transplantation, samples of myocardial tissue were obtained from patients suffering from advanced heart failure of non-ischemic (DCM, n=7) and ischemic origin (ISC, n=7). Our healthy controls (n=6) were organ donors whose hearts were not transplanted due to technical issues. The expression level of different co-inhibitory IC proteins (PD-L1, PD-1, CTLA-4, LAG3, and B7-H3) in the heart was characterized with Western blot analysis. There were no differences in PD-1, CTLA-4, and B7-H3 expression between the groups. Meanwhile, in the ISC group, LAG3 significantly increased. Furthermore, PD-L1 showed a significant increase in HF groups compared to the control. Thus, we validated the results of PD-L1 in a larger cohort (n=80). Myocardial PD-L1 expression showed a significant negative correlation with left ventricular ejection fraction. There was a negative correlation between cardiac function and the upregulation of myocardial PD-L1 in failing hearts. In HF, PD-L1 is a promising diagnostic and perhaps therapeutic target, however, further investigation is required to understand its role in cardiac homeostasis.

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P17: Role of medial vascular smooth muscle cells in atherogenesis and features of a vulnerable plaque

Armand Jaminon[1], Brecht Willems[1], Rick van Gorp[1], Paul Schiffers[2], Marc van Zandvoort[3], Chris Reutelingsperger[1], Murray Clarke[4], Martin Bennett[4], Leon Schurgers[1].
[1]Department of Biochemistry, CARIM, Maastricht University, Maastricht, The Netherlands
[2]Department of Pharmacology, CARIM, Maastricht University, Maastricht, The Netherlands
[3]Department of Molecular Cell Biology, CARIM, Maastricht University, Maastricht, The Netherlands
[4]Department of Medicine, University of Cambridge, Cambridge, United Kingdom

Abstract:

Objective: Vascular smooth muscle cells (VSMC) are essential in maintaining vessel wall structure and function. Vascular medial remodeling contributes to the development of atherosclerosis. To investigate the role of VSMC in the vascular media on the initiation and progression of atherosclerosis and atherosclerotic plaques, we used SM22α-hDTR+ ApoE-/- mice.
Methods: SM22a-hDTR+/- ApoE-/- mice were injected intraperitoneally with Diphtheria Toxin (DT) 3 times a week for 21 days. After one recovery week, mice were fed a Western Type diet (WTD) for either 6 or 18 weeks. Mice were sacrificed and vessels were isolated. First-order mesenteric and carotid artery were used in myograph and two-photon experiments. Aortic arch was used for immunohistochemistry.
Results: After 4 weeks, hDTR+ mice showed a 50% decrease in VSMC aortic vessel wall cellularity compared to hDTR- mice ($p<0.001$), which was confirmed by two-photon imaging in mesenteric and carotid arteries. hDTR+ mice developed more and larger atherosclerotic plaques after both 6 ($p<0.05$) and 18 ($p<0.01$) weeks of WTD. hDTR+ mice showed increased macrophage infiltration ($p<0.05$) and necrotic core size ($p<0.01$) in atherosclerotic plaques after 18 weeks WTD. Myograph experiments revealed that maximal contraction of mesenteric arteries in hDTR+ mice was retained compared to hDTR- mice, while endothelial mediated mesenteric vessel wall relaxation was decreased (>50% relative to hDTR-).
Conclusions: Medial VSMC ablation causes a significant decrease in vessel wall relaxation that is endothelial cell dependent. Furthermore, hDTR+ mice demonstrated accelerated atherogenesis and plaque progression with features of a vulnerable plaque.

Acknowledgments:

n/a

P18: Endothelial Nrf2 deficiency enhances atherosclerotic lesion formation and alters vascular inflammatory crosstalk: insights from scRNA-seq

Katarzyna Sarad[1,2], Monika Stefańska[3], Izabela Kraszewska[1], Gabriela Burda[1,2], Krzysztof Szade[4], Przemysław Błyszczuk[3,5], Józef Dulak[1], Agnieszka Jaźwa-Kusior[1]

[1]Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland;
[2]Jagiellonian University, Doctoral School of Exact and Natural Sciences, Kraków, Poland;
[3]Department of Clinical Immunology, Jagiellonian University Medical College, Kraków, Poland;
[4]Laboratory of Stem Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland;
[5]Department of Rheumatology, University Hospital Zurich, University of Zurich, Zurich, Switzerland

Abstract:

Dysfunctional endothelium contributes to the initiation and progression of atherosclerosis. Impairment in endothelial function may be caused by various factors, such as oxidative stress, chronic inflammation, and the natural aging process. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcriptional regulator of antioxidant gene expression across all cell types, but its decreased activity (e.g., associated with aging) may have cell type-dependent effects on phenotype and function. The aim of this study was to analyze, at the single-cell level, the molecular and cellular changes in atherosclerotic mice with endothelial Nrf2 deficiency. Atherosclerosis was induced in mice with transcriptionally inactive Nrf2 in Cdh5-expressing cells ($Nrf2^{Cdh5tKO}$) and appropriate control $Nrf2^{flox/flox}$ mice via adeno-associated viral vector (AAV)-mediated overexpression of murine proprotein convertase subtilisin/kexin type 9 (Pcsk9) in the liver and high-fat diet feeding. We observed that atherosclerotic $Nrf2^{Cdh5tKO}$ mice have larger lesions in comparison to their control littermates. Single-cell RNA sequencing (scRNA-seq) analysis performed on the aortas of these mice revealed a specific transcriptomic profile of endothelial cells lacking Nrf2 activity, including altered expression of genes regulating shear stress, inflammation, vascular permeability, and secretion of pro-atherogenic factors. Cellular crosstalk analysis revealed several alterations in the atherosclerotic aortas of $Nrf2^{Cdh5tKO}$ mice compared to diseased $Nrf2^{flox/flox}$ controls. In particular, the dysregulated communication with macrophages may promote a pro-inflammatory environment that could accelerate atherosclerosis progression in $Nrf2^{Cdh5tKO}$ mice. Our findings provide new insights into the role of decreased Nrf2 activity in contributing to endothelial dysfunction in atherosclerosis.

Acknowledgments:

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P19: The modified 6-chromanol SUL-138 protects from accelerated vascular aging and associated end organ dysfunction

Annika A. Jüttner[1], Soroush Mohammadi Jouabadi[1], Sabrina Ribeiro Gonsalez[1], Rene de Vries[1], Adrianus C. van der Graaf[2], Daniël H. Swart[2, 3], A.H. Jan Danser[1], Robert Henning[3], Jenny A. Visser[4], Guido Krenning[2, 3], Anton J.M. Roks[1]

[1]Division of Vascular Medicine and Pharmacology, Department of Internal Medicine, Erasmus MC, University Medical Center, Rotterdam, The Netherlands
[2]Sulfateq B.V., Groningen, The Netherlands
[3]Department of Clinical Pharmacy and Pharmacology, University Medical Centre Groningen, University of Groningen, The Netherlands
[4]Division of Endocrinology, Department of Internal Medicine, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

Abstract:

Vascular aging is marked by decreased vasodilation due to lower nitric oxide bioavailability caused by dysfunctional mitochondria. Nitric oxide loss can partially be compensated by endothelium-derived hyperpolarization (EDH) during aging. Another postulated adaptation to vascular aging is an alteration in mitochondrial calcium-signalling leading to increased EDH in aged vessels. Modulation of mitochondrial function is therefore a potential treatment target to alleviate age-related vascular dysfunctions. In this study, we investigated the effect of chronic treatment with the modified 6-chromanol SUL-138, a reverse electron flux inhibitor, in vascular aging mouse models. Accelerated aging was induced by KO of DNA repair endonuclease ERCC1, leading to DNA damage accumulation in the target cells. We investigated the effect of SUL-138 (30 mg/kg/day) in the accelerated aged endothelial (EC-KO) and vascular smooth muscle cell (SMC-KO) mouse models. We hypothesize that SUL-138 might be able to prevent vascular dysfunction and accompanied end organ dysfunctions.

The chronic treatment with SUL-138 prevented vascular dysfunction in the EC-KO mice and increased vasodilation in SMC-KO mice by enhancing EDH-contribution in both models. Finally, an accompanied detrimental end organ effect, namely salt-wasting tubulopathy in EC-KO mice, was attenuated by SUL-138.

To conclude, the treatment with SUL-138 prevented the development of age-associated vascular dysfunctions including arterial stiffness, decreased vasodilation and end organ dysfunction. Vasodilation was modulated by enhanced EDH. This seems to be an additional mechanism of SUL-138 and according to our knowledge the only drug with this capacity which opens new fields of application.

Acknowledgments:

N.A.

P20: The effects of ageing and inflammation on vascular metabolism in the isolated murine aorta

Agnieszka Karaś[1,2]; Anni I. Niemnen[3]; Anna Bar[1]; Janusz Pyka[1]; Barbara Kutryb-Zając[4], Klaudia Stawarska[4]; Elżbieta Buczek[1,2]; Patrycja Kaczara[1]; Stefan Chłopicki[1,5]

[1]Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Bobrzynskiego 14, 30-348, Krakow, Poland
[2]Doctoral School of Exact and Natural Sciences, Jagiellonian University, Lojasiewicza 11, 30-348, Krakow, Poland
[3]Metabolomics Unit, Institute for Molecular Medicine Finland, University of Helsinki, Tukholmankatu 8, 00290, Helsinki, Finland
[4]Department of Biochemistry, Medical University of Gdansk, Debniki 1, 80-211 Gdansk, Poland
[5]Chair of Pharmacology, Faculty of Medicine, Jagiellonian University Medical College, Grzegorzecka 16, 31-531, Krakow, Poland

Abstract:

Endothelial dysfunction and arterial stiffness represent the major phenotypes od aged vessels that pose the risk of cardiovascular diseases, but the role of with metabolic alterations in the age-dependent deterioration of vascular phenotype is yet to be established. The aim of this study was to assess the influence of ageing on vascular metabolism and phenotype using aged C57BL/6 mice, and to investigate the contribution of vascular inflammation, a pivotal factor in vascular aging. To induce acute inflammation, isolated aorta was ex vivo stimulated with IL-1β. Aged vessels displayed increased stiffness in vivo and vascular wall remodelling including collagen deposition. Furthermore, ageing was associated with functional impairment of glycolytic and respiratory capacity and a fall in vascular NAD pool. The acute IL-1β-induced vascular inflammatory response impaired endothelium-dependent vasodilation and caused functional activation of mitochondrial respiration and glycolysis, as well as ATP production, in young but not in old mice. Moreover, targeted fluxomics revealed an increase in glycolysis and a shift to the pentose phosphate pathway (PPP) and purine synthesis caused by IL-1β. Even under basal conditions, old mice appeared to have an activated PPP compared to young mice. Additionally, we found that the inhibition of mitochondrial ATP production impairs NO release and NO-dependent functions of the vessel wall, thereby suggesting a direct link between vascular metabolism and the regulation of vascular function. These findings highlight the importance of further research into the vascular metabolism as a potential therapeutic target for mitigating age- and inflammation-related endothelial dysfunction and its impact on cardiovascular health.

Acknowledgments:

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P21: Do all experimental models of inflammation stimulate vascular ecto-adenosine deaminase 1?

Ada Kawecka[1], Paulina Mierzejewska[1], Klaudia Stawarska[1], Alicja Braczko[1], Ryszard T. Smoleński[1], Stefan Chłopicki[2], Barbara Kutryb-Zając[1]
[1]Department of Biochemistry, Medical University of Gdańsk, Poland
[2]Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Poland

Abstract:

Background: Cell surface ecto-adenosine deaminase (eADA1) is critical in modulating the inflammatory response triggered by cytokines or lipopolysaccharides (LPS). Ameliorating eADA activity can provide therapeutic effects due to the elevated adenosine signaling, dampening the inflammatory processes activated by cytokines or LPS. This study aims to identify the pro-inflammatory factors that most significantly activate eADA1 in vitro, ex vivo, and in vivo.

Methods: Human lung microvascular endothelial cells (HULEC) were treated with 10 ng/mL TNFα, with or without an ADA1-CD26 binding inhibitor gp120, for 24 hours in EBM-2 medium supplemented with 10% FBS and 1% P/S, at 37°C 5% CO2. Fragments of descending aortas harvested from 12-week-old male C57BL/6J mice were incubated for 2 or 6 hours in MEM medium with 0.1% FBS and 1% P/S, at 37°C 5% CO2, in the presence of 10 g/ml LPS, 10 g/ml IL1β, or 10 g/ml TNF-α / 50 ng/ml IL17. Aortas were also collected from 12-week-old BALB/c mice, 24h after intraperitoneal injection of 10 mg/kg body weight of LPS. E-ADA1 activity assay was conducted on HULEC and aortic surface, followed by THP-1 monocyte/macrophage, HL-60 neutrophils or Jurkat lymphocytes adhesion test.

Results: LPS-induced inflammation in ex vivo and in vivo models increased eADA1 activity and enhanced THP-1 adhesion. Pro-inflammatory cytokines in vitro modulated both inflammatory cell adhesion and eADA1 activity, while their ex vivo effects were not equivalent.

Conclusions: In summary, not all pro-inflammatory experimental conditions affect eADA1 activity, even if they induce inflammation, as evidenced by increased immune to endothelial cell adhesion.

Acknowledgments:

Study supported by National Science Centre of Poland (project no. 2019/35/D/NZ3/03512).

P22: Cytoprotective role of increased hydrogen sulfide (H2S) turnover and polysulfide production in replicative cell senescence

Anna Kieronska-Rudek[1,2], Kelly Ascencao[1], Stefan Chlopicki[2], and Csaba Szabo[1]
[1] Section of Pharmacology, Department of Science and Medicine, University of Fribourg, Fribourg, Switzerland
[2] Jagiellonian University, Jagiellonian Centre for Experimental Therapeutics (JCET), Cracow, Poland

Abstract:

The endogenous mammalian gasotransmitter hydrogen sulfide (H2S) is generated by cystathionine b-synthase (CBS), cystathionine gamma-lyase (CSE), 3-mercaptopyruvate sulfurtransferase (3-MST) and mitochondrial cysteinyl-tRNA synthetase (CARS2). H2S exerts cytoprotective effects in various cells and tissues under physiological conditions. However, recent data suggest that H2S may also play an anti-aging role.
In the current project we investigated the alteration and the potential role of endogenous H2S and polysulfide production in replicative senescence in murine macrophages RAW 264.7. H2S and polysulfide were measured using the fluorescent probes 7-azido-4-methylcoumarin (AzMC) and Sulfane Sulfur Probe 4 (SSP4), respectively. The expression of CBS, CSE, 3-MST and CARS2, which are responsible for H2S synthesis, as well as the H2S degradation enzymes: rhodanese, ethylmalonic encephalopathy 1 protein (ETHE1), sulfide quinone reductase-like (SQRDL), superoxide dismutase 1 (SOD1) and sulfite oxidase (SUOX) were measured by Western blotting. CBS, CSE and 3-MST were inhibited using aminooxyacetic acid (AOAA, 1 mM), propargylglycine (PAG, 1 mM) or HMPSNE (300 μM), respectively. Senescence-associated beta-galactosidase (SA-β-Gal) activity was measured using a chromogenic substrate (X-gal). Cell proliferation was assessed by the BRDU method and immunocytochemical staining for p21 expression (cell cycle inhibitor).
Senescence was associated with a trend towards upregulation of CBS (16±7% increase), a marked (over 30-fold) upregulation of CSE and a 44±7% upregulation of 3-MST. The H2S degradation enzymes rhodanese, SQRDL and SUOX were also significantly upregulated in senescent cells. Inhibition of CBS, CSE or 3-MST all reduced cellular H2S levels as expected, but interestingly increased cellular polysulfide signals. The H2S biosynthesis inhibitors reduced cell proliferation and increased SA-β-Gal and p21 levels. When we compared the expression of various H2S producing and degrading enzymes in the spleen of young vs. old mice, downregulation of CBS and ETHE1, but upregulation of rhodanese and SUOX were found.
Thus, simultaneous upregulation of multiple H2S biosynthesis and degradation pathways induces increased reactive sulfur turnover in senescent macrophages. Endogenous H2S/polysulfides support cell proliferation and protect against the development of cellular senescence.

Acknowledgments:

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P23: Thymic modulation of Immune Checkpoint Inhibitor-induced cardiotoxicity: New perspectives in Immunotherapy

Márton Kocsis, Lilla Szabó, Sayour Viktor Nabil, Viktória Tóth, Luca Varga, Tamás Gergely, Anna Kulin, Barnabás Váradi, Tamás Kovács, Zoltán Varga
Semmelweis University, Department of Pharmacology and Pharmacotherapeutics, Budapest, Hungary
HCEMM-SU Cardiometabolic-Immunology Research Group, Budapest, Hungary

Abstract:

Immune checkpoint inhibitors (ICI) are monoclonal antibodies that enhance the anti-tumor activity of the immune system. However, this therapy could lead to immune overactivation, resulting in severe side effects associated with high mortality rates. Previously, our group demonstrated that ICI increases the expression of inflammatory genes in the thymus, and furthermore in clinical studies, ICI therapy for thymoma treatment caused severe immune related adverse events, including fatal cardiotoxicity. Based on these findings, we hypothesized that thymic activity could play an important role in ICI-induced autoimmune side effects.
The main aim of our study was to investigate the correlation between thymic activity and the pathophysiological processes of immune-mediated cardiotoxic and systemic side effects during ICI therapy using a preclinical mouse model. Since the thymus gradually atrophies and thymic activity decreases with age, we examined the side effects of ICIs in two differently aged groups, plus artificially degraded the thymus with pharmacological treatment in young animals.
Based on our results, thymic immune response plays a central role in ICI-induced cardiotoxicity in mice. In aged animals undergoing ICI treatment and combined immunotherapy with parallel pharmacologically induced involution of the thymus significantly reduced side effects, indicating that the immune response following ICI treatment varies depending on thymic activity.
Thus, modulation of thymus activity may serve as a therapeutic target in reducing immune-mediated side effects and monitoring the morphology and activity may be an important factor in predicting the severity of side effects and identifying high-risk patients.

Acknowledgments:

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P24: Role of platelet-derived lipid mediators in glucose homeostasis

Katarzyna Kolczyńska-Matysiak[1], Till Karwen[2], David Stegner[2], and Grzegorz Sumara[1]
[1]Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur Street, 02-093 Warszawa, Poland
[2]Rudolf-Virchow-Zentrum. Center for Integrative and Translational Bioimaging, University of Würzburg, 97080 Würzburg, Germany.

Abstract:

Platelets are small anucleate blood cells originating from megakaryocytes responsible for the maintenance of vascular integrity. Although platelets were originally described for their role in blood coagulation, now are also recognized as key players in modulating inflammation, tissue regeneration, angiogenesis, and carcinogenesis. The multifaceted functions of platelets are mediated by the factors stored in their alpha and dense granules. Interestingly, platelets store and release several bioactive molecules that have been reported to potentiate insulin secretion by pancreatic beta cells. This raises the possibility that platelet may affect insulin secretion and be a regulator of glucose homeostasis.
Our studies showed that genetic or pharmacological ablation of platelet functionality causes impaired glucose tolerance and decreased blood insulin level. We observed that elevated glucose level and factor(s) derived from beta cells stimulate platelet activity and that platelets selectively localize to the vascular endothelium of pancreatic islets. We found platelet-derived lipid-based molecules to promote insulin secretion upon platelet activation and identified 20-hydroxyeicosatetraenoic acid (20-HETE) as one of the factors promoting beta cells function. Furthermore, our study showed that dense granules, but not alpha granules, mediate platelets-dependent insulin secretion from pancreatic beta cells. At last, we observed that the impact of platelets declines with age, which is related to decreased level of platelet-derived 20-HETE in older individuals. Altogether, obtained data show new, unexpected role of platelets in the regulation of glucose homeostasis and insulin secretion.

Acknowledgments:

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P25: A negative effect of caloric restriction on atherosclerosis in young ApoE/LDLR-/- mice

Renata B. Kostogrys[a*], Magdalena Franczyk-Żarów[a], Artur Gurgul[b], Igor Jasielczuk[b], Anna Zaczek[c], Magdalena Hubalewska-Mazgaj[d], Sabina Lichołai[e] Angelika Manterys[a] and Iwona Wybrańska[f]
a Department of Human Nutrition and Dietetics, Faculty of Food Technology, University of Agriculture in Krakow, ul. Balicka [122], [30-149] Kraków, Poland
b Center for Experimental and Innovative Medicine, University of Agriculture in Krakow, Rędzina [1]c, [30-248], Kraków, Poland
c Department of Microbiology, Institute of Medical Sciences, Medical College of Rzeszow University, al. W. Kopisto [2]a, [35-959] Rzeszów, Poland
d Department of Drug Addiction Pharmacology, The Institute of Pharmacology of the Polish Academy of Sciences (IF PAS) Department of Drug Addiction Pharmacology, Smetna [12], [31-343] Kraków, Poland
e Division of Molecular Biology and Clinical Genetics, Faculty of Medicine, Jagiellonian University Medical College, [8] Skawinska Street, [31-066] Krakow, Poland
f Department of Clinical Biochemistry, Faculty of Medicine, Jagiellonian University Collegium Medicum, ul. Kopernika [15], [31-501] Kraków, Poland

Abstract:

Caloric restriction (CR) has proved to be the most effective dietary intervention which leads to the reduction of CVD associated with obesity. Depending on the age of the mice the effect of caloric restriction was diversed. Therefore, the effect of CR on the development of atherosclerosis in young and adult ApoE/LDLr-/- mice has been evaluated.
8-week-old and 20-week-old male mice received a control diet. Young mice were fed for 8 weeks whereas adult one for 5 weeks. To assess if keeping the animals individually influences the tested parameters, the control animals were kept in colony cages (AL) or individually (stressAL; sAL) and fed ad libitum. Individually kept caloric restriction (CR) mice received a 30% less diet in comparison to AL group.
The body weight of CR mice has been significantly lower vs. AL and sAL groups. TCh and LDL levels were significantly increased in young CR mice. No differences in adult animals were observed. TAG levels significantly decreased in both young and adult CR mice. CR-induced atherosclerosis in young mice. FMO3 gene has been upregulated in young animals. Microbiota has been changed. At the genus level, compared to the control, CR group resulted in a higher relative abundance of the Enterococcus, Clostridium_sensu_stricto_1, Rikenella and a lower relative abundance of the CAG_352 ($P < 0.05$). Caloric restriction showed a negative effect on atherosclerosis in young ApoE/LDLr-/- mice.
260/250 words – need to be shortened

P26: The relationship between the mitochondrial BK_{Ca} channel and the cellular redox state

Bogusz Kulawiak[1], Shur Gałecka[1], Karolina Pytlak[1], Kamila Maliszewska-Olejniczak[2], Piotr Bednarczyk[2], Monika Żochowska[1], Antoni Wrzosek[1], Adam Szewczyk[1]
[1] Laboratory of Intracellular Ion channels, Nencki Institute of Experimental Biology PAS, Warszawa, Poland
[2] Department of Physics and Biophysics, Institute of Biology, Warsaw University of Life Sciences -SGGW, Warszawa, Poland

Abstract:

Mitochondrial potassium (mitoK) channels play an important role in mitochondrial physiology. Activation of mitoK channels protects brain and cardiac tissue against injury induced by ischemia-reperfusion. In cancer cells, it has been observed that some mitoK channels can be overexpressed, and their inhibition may induce the death of cancer cells.
In our work, we focus on the mitochondrial large conductance calcium-activated potassium (mitoBKCa) channel. This channel has been found in various tissues, including cardiac tissue, the brain, and cancer cells. The basic properties of the mitoBKCa channel are similar to those of other BKCa channels, including those found in the plasma membrane or endoplasmic reticulum. Previously, we suggested a functional and perhaps structural interaction between respiratory chain complexes and the mitoBKCa channel in glioma cells. Our recent studies have shown that the loss of this channel leads to deregulation of redox homeostasis in mitochondria. We also observed that the removal of this channel results in changes in the mitochondrial proteome, particularly related to mitochondrial metabolism. On the other hand, the overexpression of this channel in HEK293 cells leads to changes in mitochondrial function, particularly in the rate of mitochondrial respiration, which is likely a consequence of increased respiratory chain capacity. Changes in the capacity and activity of the respiratory chain directly affect the synthesis of reactive oxygen species in mitochondria.
Our data indicate a strong correlation between the presence and activity of the mitoBKCa channel and mitochondrial function, metabolism, and redox homeostasis.

Acknowledgments:

This work was supported by the National Science Center grant no. 2019/35/B/NZ1/02546 and 2019/34/A/ NZ1/00352.

P27: Inhibitors of protein disulfide isomerases (PDI) afford antiplatelet effects and improve endothelial function; a possible involvement of platelet cargo

Anna Kurpińska[1], Anna Bar[1], Kamil Przyborowski[1], Agnieszka Kij[1], Grzegorz Kwiatkowski[1], Joanna Suraj-Prażmowska[1], Agata Malinowska[2], Stefan Chłopicki[1]
[1]Jagiellonian University. Jagiellonian Centre for Experimental Therapeutics (JCET), Krakow, Poland
[2]Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland

Abstract:

Interactions of hyperactivated platelets with endothelium contribute to the progression of atherosclerosis and occurrence of cardiovascular events. Modulation of platelet release reaction by antiplatelet agents can affect the vessel function.
The aim of the studies was to (1) characterize endothelial dysfunction and platelet cargo released by activated platelets in atherosclerosis (2) assess the platelet ability to afford protective anti-inflammatory role on the endothelial cells and vessel wall in the course of atherosclerosis (3) verify the vasoprotective properties of the newly developed anti-platelet compounds, inhibitors of PDIs.
The studies were performed in animal model of atherosclerosis (ApoE/LDLR-/- mice) and age-matched C57BL/6 mice. Cargo released by activated platelets was defined by proteomic analysis, eicosanomics and metabolomics. Endothelial function was characterized using MRI and selected protein markers. Novel potential anti-platelet drugs, inhibitors of PDIA1 and PDIA3 - sulphonamides of aziridine-2-carboxylic acid derivatives (C-3399, C-3389), reference PDIA1 inhibitor - bepristat, and COX-1 inhibitor - aspirin as a reference were tested in vivo against their ability to provide the vasoprotective action.
In atherosclerosis we confirmed endothelium dysfunction by MRI analysis. Glycocalyx disruption, imbalance in hemostasis were noted. Platelet releasates were characterized by pro-inflammatory profile of eicosanoids, changes in the proteins related to e.g. VEGF signaling, trans-endothelial migration, chaperone activity, also e.g. higher levels of TCA and glycolysis metabolites and glutamate were observed. Platelet cargo eicosanoids were the most potently reduced by aspirin and bepristat. Aspirin also decreased e.g. proteins related to the immune response, bepristat and C-3389 related to angiogenesis, inflammation, C-3389 and C-3399 increased the proteins of oxidative stress. MRI analysis proved that the tested inhibitors showed a positive effect on the vessel function.
Conclusion: Inhibitors of PDI display a distinct effects on platelet cargo release and exhibit vasoprotective properties.

Acknowledgments:

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P28: Hyaluronan-based nanocapsules and vascular drug delivery – novel insight into mechanisms involved

Edyta Kus[1], Agnieszka Kij[1], Justyna Bednorz[3,4,5], Małgorzata Janik-Hazuka[3], Izabela Czyzynska-Cichon[1], Jakub Dybaś[1], Filip Aleksander Fedak[1], Anna Kieronska-Rudek[1], Kamila Wojnar-Lason[1], Aleksandra Paterek[6], Filip Rolski[6], Michal Maczewski[6], Szczepan Zapotoczny[3], Stefan Chlopicki[1,2]

[1] Jagiellonian University, Jagiellonian Centre for Experimental Therapeutics (JCET), Michala Bobrzynskiego 14, 30-348 Krakow, Poland
[2] Jagiellonian University Medical College, Chair of Pharmacology, Faculty of Medicine, Grzegorzecka 16, 33-332 Krakow, Poland
[3] Jagiellonian University Department of Physical Chemistry and Electrochemistry, Faculty of Chemistry, Gronostajowa 2, 30-387 Krakow, Poland
[4] CHDE Polska S.A., Biesiadna 7, 35-304 Rzeszow, Poland
[5] Jagiellonian University, Doctoral School of Exact and Natural Sciences, Prof. St. Lojasiewicza 11, 30-348 Krakow, Poland
[6] Centre of Postgraduate Medical Education, Department of Clinical Physiology, Marymoncka 99/103, 01-813 Warsaw, Poland

Abstract:

Objective: The utilization of hyaluronan-based nanocapsules has been already presented as a promising and innovative approach in the realm of targeted drug delivery of lipophilic compounds to vascular wall. However the intricate mechanisms by which these nanocapsules significantly improve lipophilic agents delivery specifically to dysfunctional endothelial cells remain poorly known.

Methods: The stability of nanocapsules in the gastrointestinal tract was evaluated using infrared spectroscopy in simulated gastric juice and LC-MS/MS analysis in fecal samples. The pharmacokinetic profile of nanocapsules was assessed in mice following a single oral administration of vitamin K1 either in oil or encapsulated in nanocapsules, employing LC-MS/MS. The intestinal absorption mechanism and systemic transport of nanocapsules were visualized in mice using intravital confocal microscopy.

Results: The obtained results showed that nanocapsules were highly stable in digestive tract and protected encapsulated vitamin K1 against its degradation in the gastric juices. Moreover, encapsulated vitamin K1 showed significantly improved bioavailability and prolonged absorption as compared to nonencapsulated form. Importantly, encapsulated vitamin K1 was transported via the villi of the small intestine to lymphatic vessels and was abundantly located in the liver.

Conclusion: Hyaluronan-based nanocapsules offer a stable and effective delivery system for lipophilic compounds, protecting them from degradation, prolonging its absorption and enhancing its bioavailability. This targeted delivery system has the potential to significantly improve therapeutic outcomes and warrants significant improvement in patient comfort and compliance.

P29: Nucleotide precursor supplementation as a strategy to improve cardiac bioenergetics and function in hypertrophic cardiomyopathy

Barbara Kutryb-Zając[1,2], Remco Hoogervorst[2], Marcin Lipiński[3], Ryszard T. Smoleński[1], Jolanda van der Velden[2]
[1]Department of Biochemistry, Medical University of Gdańsk, Poland
[2]Department of Physiology, Amsterdam University Medical Center, Netherlands
[3]Department of Pharmaceutical Biochemistry, Medical University of Gdańsk, Poland

Abstract:

Background: The main scientific goal of the project was to conduct a comprehensive analysis of the nicotinamide adenine dinucleotide (NAD+) metabolome and the metabolic mechanisms of nucleotide precursor supplementation in the experimental models of hypertrophic cardiomyopathy (HCM).

Methods: Intracellular nucleotides and derivates were measured by ultra-high-performance liquid chromatography (UHPLC) in human induced pluripotent stem cell-derived cardiomyocytes harboring an HCM mutation (MYH7-R403Q) and isogenic control cells (R403Qic). Extracellular NAD+, nicotinamide mononucleotide (NMN) and nicotinamide (NAM) interconversions were also analyzed on the surface of both cell types. In addition, the effects of nicotinamide riboside (0; 0.2; 1; 5 mM NR) supplementation on the contractile phenotype and intracellular bioenergetics were investigated using CytoCypher MultiCell High Throughput System and UHPLC.

Results: MYH7-R403Q demonstrated decreased ratios of intracellular ATP/ADP and ATP/NAD+ and higher AMP and IMP concentration than R403Qic. No changes were observed in ATP, ADP, AMP, NAD+, NADH, NADP and NADPH concentrations nor ecto-enzymes' activities engaged in extracellular NAD+ metabolism. 24h NR treatment prolonged the relaxation time in both cell types in dose-dependent mode. Moreover, NR increased intracellular NR and NAD+ concentration and decreased ATP/NAD+ ratio. In the incubation medium, NR treatment led to the accumulation of ADPR and inosine.

Conclusions: In vitro HCM model used in this study showed subtle changes in cell bioenergetics, without affecting the NAD+ pool. NR supplementation demonstrated an additional increase in intracellular NAD+ concentration correlated with a significant negative chronotropic effect. Further studies are needed to investigate the mechanisms of NR on cardiac bioenergetics and function in HCM.

Acknowledgments:

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P30: Age-Dependent Induction of Endothelial-Mesenchymal Transition in Murine Lung Microvascular Endothelial Cells by Platelet-Derived TGFβ1

Ebrahim H. Maleki [1,2], Marta Stojak[1], Anna Gdula[1], StefanChlopicki[1,3], Marta Smeda [1]
[1] Jagiellonian University, Jagiellonian Centre for Experimental Therapeutics (JCET), Krakow, Poland
[2] Jagiellonian University, Doctoral School of Exact and Natural Sciences, Krakow, Poland
[3] Jagiellonian University Medical College, Department of Pharmacology, Krakow, Poland

Abstract:

Endothelial-mesenchymal transition (EndMT) plays a critical role in pulmonary vascular remodeling. Platelets serve as the body's primary reservoir of Transforming Growth Factor β1 (TGFβ1), releasing TGFβ1 close to endothelial cells. TGFβ1 is a key inducer of EndMT, initiated by a sustained decrease in endothelial barrier integrity, followed by reduced endothelial markers and increased mesenchymal markers. This study investigates whether a 5-day treatment with platelet releasates from young healthy 10-week-old and older 40-week-old mice, which exhibit endothelial dysfunction and contain active endogenous platelet-derived TGFβ1 (~2 ng/ml), can induce EndMT in murine lung microvascular endothelial cells (mLMVECs).
The treatment resulted in a permanent decrease in barrier integrity only in mLMVECs treated with platelet releasates from 40-week-old mice, similar to the effects of exogenous TGFβ1 at 1 ng/ml and 10 ng/ml, but without downregulation of endothelial junction proteins. Moreover, only cells treated with platelet releasates from 40-week-old mice showed elevated levels of both early mesenchymal marker transgelin-like actin modulating protein Stg1 (SM22) and the myofibroblast marker α-smooth muscle actin (α-SMA), along with increased TGFβ1 concentration in the conditioned medium. Interestingly, treatment with exogenous TGFβ1 at 10 ng/ml did not increase α-SMA protein levels.
In conclusion, (1) TGFβ1 present in platelet releasates from both 10-week-old and 40-week-old mice promoted more robust EndMT progression in older mice, and (2) the EndMT phenotype induced by platelet-derived TGFβ1 was distinct from that induced by exogenous TGFβ1.

Acknowledgments:

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P31: Investigating the influence of shear stress and substrate stiffness on human endothelial cells from different vascular beds.

Salma Mansi, Sophie Bauer, Diana Rojas-Gonzalez, Petra Mela
Technical University of Munich, Germany; TUM School of Engineering and Design, Department of Mechanical Engineering, Chair of Medical Materials and Implants; Munich Institute of Biomedical Engineering; Munich Institute of Integrated Materials, Energy and Process Engineering.
Boltzmannstraße 15, 85748 Garching, Germany

Abstract:

Endothelialization is a critical process that significantly influences the success of cardiovascular implants. A properly endothelialized surface reduces the risk of thrombosis, inflammation, and infection, and regulates vascular permeability. Therefore, understanding and promoting endothelialization of implants remains an important research focus. Here, we show that the source of endothelial cells does not influence their response to flow with shear stresses up to 30 dyne/cm2 when cultivated on stiff substrates. Human arterial, venous, and microvascular endothelial cells showed similar gene expression of flow-induced marker KLF2, upregulation of anti-thrombotic (TM and TPA) and antioxidant markers (NQO1 and HO1) and downregulation of pro-inflammatory markers (SELE, IL8 and VCAM1). In this study we designed a flow chamber to investigate the combined influence of shear stress and substrate stiffness on the endothelialization process and endothelial morphology, phenotype, and function. The flow chamber allows for the preparation of flat hydrogel layers of different stiffnesses and defined heights, controlling the shear stress, and live tracking of endothelial cell proliferation, migration and alignment under flow. This study addresses the need for a comprehensive approach to studying the mechanical environment of endothelial cells. With this platform we aim to give insight into the key requirements for the design of implant materials that deliver the proper biomechanical cues for restoring endothelial health and vascular function.

P32: Acute effects of selected kinase inhibitors and doxorubicin on vascular endothelial function in vivo; vasoprotective effects of methylnicotinamide (MNA) and vitamin K

Brygida Marczyk, Anna Bar, Zeinep Berkimbayeva, Stefan Chłopicki
Jagiellonian Centre for Experimental Therapeutics (JCET)

Abstract:

The use of kinase inhibitors has significantly improved the efficacy of cancer treatment; however, their long-term use is associated with adverse cardiovascular effects that could be linked to detrimental effects of these drugs on endothelial function. Previously we showed that 4-weeks therapy with imatinib, nilotinib, ponatinib, or asciminib induced dose-dependent endothelial dysfunction in mice in vivo with the strongest effect on endothelial function by ponatinib and asciminib (1-10 mg/kg b.w./day), weaker by nilotinib (30-360 mg/kg b.w./day), followed by imatinib (120-360 mg/kg b.w./day), compatible with the cardiovascular risk of these compounds reported in clinical trials.
Here, we investigated whether selected anti-cancer drugs could induce endothelial dysfunction after a singlpe drug administration. Then, we tested the efficacy of various vasoprotective interventions to protect against anti-cancer drug-induced acute endothelial dysfunction. Endothelial effects of studies compounds (ponatinib, asciminib, doxorubicin, axitinib) were studied in vivo, based on MRI-based methodology to assess endothelium-dependent responses induced by acetylcholine in the aorta, or by an increase in blood flow in the femoral artery.
All of the tested anti-cancer drugs including ponatinib, asciminib (10 mg/kg b.w./day), doxorubicin (10 mg/kg b.w./day) and axitinib (30 mg/kg b.w./day) induced endothelial dysfunction after a single administration within 6 hours after drug administration.
Vasoprotective therapy was based on 1-methylnicotinamide-MNA (100 mg/kg b.w./day), vitamin K2(MK-7) (0.5 mg/kg b.w./day) or endothelin- antagonist (bosentan, 100 mg/kg b.w./day). Endothelial dysfunction induced by ponatinib, was prevented by MNA or K2(MK-7) when given prophylactically for 2 weeks prior ponatinib administration or when given acutely together with ponatinib. MNA also prevented doxorubicin-induced endothelial dysfunction.
In summary, based on in vivo studies using MRI-based methodology, we showed that various anti-cancer drugs impaired vascular endothelial function even after single drug administration. Importantly, MNA or vitamin K2(MK-7) can attenuate endothelial dysfunction induced by some anti-cancer drugs.

Acknowledgments:

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P33: Single-cell spectral profiling for the mechanism of action of adrenaline

Anna Pieczara[1,2], Jakub Machowski[2], Małgorzata Barańska[1,2]
[1]Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Bobrzynskiego 14, 30-348, Krakow
[2] Faculty of Chemistry, Jagiellonian University, Gronostajowa 2, 30-387 Krakow

Abstract:

Cellular responses to various chemicals involve the modifications that occur in cells as a result of exposure to such compounds, as changes in metabolism, protein levels, or organelles functions. Capturing these changes is very important to understand the cellular response as well as the action of the substances of interest for example hormones. Hormones work by triggering reactions in specific organs or tissues, influencing many aspects of the body's functioning like increasing the rate of protein synthesis, influencing the cellular metabolism, and promoting the release of stored fats. Due to such a wide range of effects, the mechanism of action (MoA) of hormones is quite complicated to clearly define. Here comes vibrational techniques in combination with biological tests that show great potential in evaluating the effects of different compounds on individual cells by offering a detailed analysis of the cellular structure and function through a biochemical signature.

This study focused on determining adrenaline's MoA on fatty acid metabolism in single live endothelial cells. For this purpose, a new methodology based on labeled Raman microscopy and biological tests was employed. Five substances influencing lipid metabolism and mitochondria activity with known MoA (forskolin, niclosamide, oligomycin, CCCP) and adrenaline were examined to define their spectroscopic signature. By employing a semi-quantitative approach based on Raman band ratios it was possible to compare their biochemical signature and conclude that adrenaline has a very similar pattern to forskolin and niclosamide. Raman`s results were also correlated with biological tests (MTT), which confirmed the results obtained from Raman.

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P34: Platelets in Vaccine-induced Immune Thrombotic Thrombocytopenia (VITT)

Aneta Radziwon-Balicka, Tølbøll Sørensen AL, Paul Jurasz
JCET

Abstract:

Bacground: Platelet microparticles (PMPs) shed to the circulation are (0.1-1μ) fragments of their plasma membranes. They have procoagulant activity, mainly due to phosphatidylserine exposure and tissue factor expression (1); therefore, they play a significant role in blood hemostasis and thrombosis (2). PMPs are present in the blood under physiological conditions; however, their elevated levels are associated with many diseases such as heparin-induced thrombocytopenia (3), thrombotic thrombocytopenia, idiopathic thrombocytopenic purpura (4) and arterial thrombosis (5) Microparticles are formed following activation of platelets with strong physiological platelet agonists such as thrombin and collagen. Moreover, platelet microparticles have also been demonstrated to form by stored platelets exposed to shear (6) and following exposure to complement proteins C5b-9 (7). Also, PMPs promote adhesion of platelets and leukocytes under flow conditions (8).
Methods: We describe a 30-year-old woman who developed thrombocytopenia and multiple thromboses after she received the ChAdOx1 nCoV-19 vaccine (9). Platelets were isolated from VITT's patient and healthy volunteer and prostacyclin-washed platelets were prepared. The platelet aggregation-mediating activated integrin αIIβ3, P-selectin and PMPs were measured in fixed platelets using flow-cytometry. Platelet functionality was assessed using Multiplate and Platelet Mapping analysis. The whole blood TEG® Platelet Mapping™ assay measures clot strength as maximal amplitude (MA) and enables for quantification of platelet function, including the contribution of the ADP and thromboxane A2 (TxA2) receptors to clot formation.
Results: Utilizing flow cytometry, we identified a potentially elevated level of platelet microparticles (10.1% of CD42b positive events) in the patient's blood sample after detecting a thrombus in the patient's liver. For comparison, flow cytometry measurements demonstrated a much smaller fraction of PMPs (2.9% of CD42b positive events) in the blood of a healthy volunteer. Moreover, a slightly higher number of PMPs were positive for activated integrin αIIβ3 (0.6%) in the patient's blood sample. Additionally, flow cytometry measurement of platelet activation markers surface P-selectin (4.7% vs. 2.9% of platelets; patient vs. healthy control) and activated integrin αIIβ3 (1.9% vs 3.4% of platelets; patient vs. healthy control) demonstrated that the patient's overall platelet activation was comparable to that observed for a healthy control. Interestingly, stimulation of the patient's blood sample with high concentrations of adenosine diphosphate (ADP) and thrombin receptor-activating peptide (TRAP) demonstrated a lower percentage of platelets with a surface exposure of P-selectin and activated integrin αIIβ3 compared to that of the healthy control. Similar to the flow cytometry data, Multiplate and Platelet Mapping analysis revealed lower platelets respond to stimuli.
Conclusions: Together, these analyses suggest a lowering of the patient's platelet responses to different agonists and may indicate that the platelets were refractory to stimulation due to prior activation. This potential desensitization of platelets to the ADP might be an adaptive response in an attempt to prevent further major thrombotic events when they are exposed to continuous stimulation.

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P35: The effects of CORM-A1 on platelet aggregation in mice – in vitro and in vivo studies

Katarzyna Rafa-Zabłocka, Jakub Dybaś, Renata Budzyńska, Patrycja Kaczara
Jagiellonian Center for Experimental Therapeutics, Jagiellonian University

Abstract:

Platelet hyperreactivity, increasing the risk of thrombotic events, is associated with diabetes mellitus, atherosclerosis, or ageing. Currently, several antiplatelet strategies are being used, mainly targeting platelet surface receptors. However, the side effects and inefficiency of drugs observed in some patients require the search for improved antithrombotic strategies. Since platelet aggregation is an energy–demanding process, agents that modulate platelet energy metabolism are supposed to reduce their reactivity. We studied the effects of the carbon monoxide releasing molecule (CORM-A1; inhibits mitochondrial respiration and glycolysis) on platelet aggregation (activated by convulxin (CVX) or protease activated receptor 4 (PAR4) and energy metabolism in in vitro and in vivo models using light transmission aggregometry and the Seahorse XFe96 technique. Carboxyhemoglobin levels were measured using UV-VIS spectroscopy. We showed that CORM-A1, applied to isolated murine platelets in vitro, clearly inhibited platelet energy metabolism and aggregation. Furthermore, CORM-A1 synergistically enhanced the antiaggregatory action of cangrelor (an antiplatelet drug that acts by inhibiting the purinergic P2Y12 receptor). In turn, CORM-A1, administered to male C57Bl/6J mice (10 mg/kg, every other day for 2 weeks) in vivo, increased carboxyhemoglobin levels from 1.2 to 5.2% and slightly impaired mitochondrial function (reduced maximal respiration and spare respiratory capacity). Surprisingly, CORM-A1 also slightly increased platelet aggregation in response to CVX and PAR4. We did not demonstrate the antiplatelet effect of CORM-A1 administered to mice in vivo. However, since the results obtained in the in vitro model are promising, we will continue experiments under conditions that result in higher levels of carboxyhemoglobin.

Acknowledgments:

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P36: Endothelial pharmacology under flow conditions

Stefano Rocchetti[1]; Sylwester Mosiołek[1]; Marek Grosicki[1]; Stefan Chłopicki[1,2]
[1] Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, 14 Bobrzynskiego Str., 30-348 Krakow, Poland.
[2] Chair of Pharmacology, Jagiellonian University, 30-348 Krakow, Poland 30-348 Krakow, Poland.

Abstract:

The endothelium is a major organ in the body, formed by endothelial cells, with different endocrine, paracrine, and autocrine functions. The endothelial cells, are constantly exposed to different patterns of shear stress, produced by the blood that flows on their surface. Has been proved that the shear stress act as one of several stimuli that can activate and change the behaviour of the endothelial cells. In the pharmacology of endothelium, the effects of drugs are tested mostly in culture ware that allow for high throughput and scalability, but limit the exposure on cells that are cultured without the shear stress stimulus. The exposure to shear stress can help in bridging the bench to bedside gap, generating results that get closer to the ones obtained in in vivo studies, and possibly improve the translation

P37: Anti-rheumatic treatment as a preventive strategy for vascular aging: A network meta-analysis

Sahar Sahafi[1], Soroush Mohammadi Jouabadi[2], Paula Katherine Bautista-Nino[3], Anton Roks[2]
[1]Student Research Committee, Isfahan University of Medical Sciences, Isfahan, Iran
[2]Division of Vascular Medicine and Pharmacology, Department of Internal Medicine, Erasmus MC Medical Centre, Rotterdam, the Netherlands
[3]Fundación Cardiovascular de Colombia, Floridablanca, Colombia.

Abstract:

Introduction
Vascular aging significantly contributes to cardiovascular and cerebrovascular diseases, with chronic low-grade inflammation identified as a major factor. The potential of anti-inflammatory therapies to prevent cardiovascular aging is still not well understood. In this study, we systematically evaluated and indirectly compared evidence to assess and rank the effectiveness of anti-rheumatic agents as potential therapeutic options for vascular aging.

Method
We conducted a systematic search of PubMed, Embase, and Cochrane databases for randomized controlled trials (RCTs) evaluating the effects of anti-rheumatic agents compared to either placebo or another drug within the ATC-M01 class on vascular aging. Vascular aging was defined by changes in Pulse Wave Velocity (PWV) and carotid Intima-Media Thickness (cIMT). Data screening and extraction were performed independently by two reviewers. We utilized a random-effects Bayesian network meta-analysis (NMA) to assess and rank the effectiveness of these anti-rheumatic agents.

Results:
Seven RCTs were included for the PWV outcome and eight RCTs for the cIMT outcome. The NMA revealed that, compared to placebo, Sulfasalazine was the most effective in preventing the progression of cIMT (SMD= -10.57 [-12.75;-8.39]), while Tocilizumab was the most effective in reducing vascular stiffness, as indicated by PWV (p-score=0.85).

Conclusion:
These findings indicate that certain anti-inflammatory agents, specifically Sulfasalazine and Tocilizumab, may be beneficial in preventing vascular aging in patients with rheumatoid arthritis. Future research and clinical practice may consider these agents for managing vascular dysfunction in this population.

Acknowledgments:

NA

P38: Mitochondrial decay of iPSC-derived VSMCs as a sign of vascular ageing in Marfan Syndrome

Pepijn J.M.H. Saraber[1,2,*], Rudolf Bittner[1], Rogier J.A. Veltrop[1], Armand M.G. Jaminon[1], Elham Bidar[3], Koen D. Reesink[2], Leon J. Schurgers[1]

[1]Department of Biochemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, the Netherlands
[2]Department of Biomedical Engineering, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, the Netherlands
[3]Department of Cardiothoracic Surgery, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University Medical Centre (MUMC+), Maastricht, the Netherlands

Abstract:

Background.
Marfan Syndrome (MFS) involves accelerated tunica media decay and VSMC dysfunction, often leading to severe aortic dissection. Mitochondrial function in vascular smooth muscle cells (VSMCs) regulates vascular remodelling during ageing. MFS shares vascular remodelling traits with ageing, suggesting decreased metabolic function could be an important player in MFS pathogenesis.
Aim. Examining traits of vascular ageing in Marfan syndrome to gain insight in shared underlaying metabolic mechanisms.

Methods.
Non-aneurysmal control and MFS iPSCs were differentiated into VSMCs (iVSMCs). From the same MFS donor, subsequent primary VSMCs (pVSMCs) were isolated and cultured. VSMCs were characterized by VSMC marker expression, calcification potential, cellular stiffness via nanoindentation, and mitochondrial function via mitochondrial membrane potential assay.

Results.
MFS patient-derived iVSMCs showed a reduction in contractile markers, increased calcification, decreased mitochondrial membrane potential, lower total mitochondrial area, and reduced cellular stiffness compared to non-aneurysmal control iVSMCs. pVSMCs showed an increase in contractile markers, higher number of mitochondria and mitochondrial membrane potential when compared to same patient iVSMCs. However, no difference in cellular stiffness and a further increase in calcification potential between Marfan pVSMCs and iVSMCs was observed.

Conclusion.
iVSMCs from Marfan patients show increased age-related hallmarks, characterized by loss of contractile function and altered metabolic activity. Changes in VSMC phenotype and mitochondrial function coherently promote vascular calcification, which is also noticed in Marfan iVSMCs. pVSMCs from the same Marfan patient showed a milder phenotype with partial rescue of these markers, but increased calcification potential persisted.

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P39: Expanding the Toolkit for Obesity Research: Murine AAV Models of Obesity

Hunter G. Sellers, Zachary L. Brown, Stephen B. Haigh, Mitchell A. Shivers, Tej V. Patel, Caleb A. Padgett, David W. Stepp, David J.R. Fulton
Vascular Biology Center, Medical College of Georgia at Augusta University, Augusta GA, USA

Abstract:

Obesity persists as one of the foremost threats to global health; serving as a contributor to many chronic diseases such as atherosclerosis, neurodegenerative disorders, and cancer. Because of this, it is imperative that basic scientists have robust and well-described models to better understand the pathophysiology of obesity. Current models of obesity such as the db/db, ob/ob, and HFD models present some obvious strengths, such as morbid obesity and progression to metabolic disease and hypertension, these models have key weaknesses including loss of leptin signaling, early onset severe metabolic syndrome, lack of temporal control, and infertility. To address these limitations and attempt to maintain the strengths of the aforementioned models, we hypothesized that utilizing a brain penetrating AAV (PHP.eB) to overexpress orexigenic peptides in the brain would lead to hyperphagia, obesity, and would recapitulate the metabolic deficits seen in other models of obesity. Treated mice gained weight to a final mass of 63.1±2.1g. Male mice were implanted with telemeters and a non-dipping circadian phenotype was observed with no increase in mean arterial pressure. Male mice exhibited an increase in HbA1c and blood glucose with hyperinsulinemia and no impairment in glucose disposal. AgRP-AAV mice have impaired lipid metabolism measured by increased plasma leptin, cholesterol, triglycerides, and NEFA. As early as 5 weeks post injection, male but not female AgRP-AAV.PHP.eB treated mice display profound impairment in endothelial function. Overexpression of orexigenic peptides in the brain using AAV-PHP.eB is a convenient, temporally controllable, and effective method to induce obesity in the C57BL/6J mouse

Acknowledgments:

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P40: Long-Term Effects of Ketogenic Diet on Endothelial Cell Bioenergetics: An In Vitro Study

Anna Selmi[1,2], Patrycja Kaczara[3], Lidia Ostrowska-Nawarycz[1], Stefan Chłopicki[3], Aneta Balcerczyk[2]
[1] Medical University of Lodz, Department of Experimental and Clinical Physiology, Department of Biophysics
[2] University of Lodz, Department of Oncobiology and Epigenetics
[3] Jagiellonian University, JCET

Abstract:

In the last decade, interest in the ketogenic diet as a solution for obesity has surged. The complications associated with overweight and obesity drive researchers to explore new solutions, such as inducing a state of ketosis in the body. Ketone bodies, primarily produced in the liver from fatty acid precursors, become the main energy source during carbohydrate deficiency. Paradoxically, a high-energy ketogenic diet aids in mobilizing adipose tissue. The endothelium is crucial for maintaining homeostasis, not only within blood vessels, and its dysfunction marks many diseases.

We examined the impact of high ketone body levels on endothelial cells, focusing on the molecular mechanisms in vitro under ketosis and variable glucose conditions. Endothelial cells exposed to ketone bodies (beta-hydroxybutyrate [BHB] and acetoacetate [AcAc]) under different glycemic conditions showed changes in genes involved in ketone body metabolism. Increased expression of these genes in low-glucose conditions, alongside increased oxygen consumption as assessed by Seahorse technology, indicates ketone body mobilization and ketolysis activation. This suggests that appropriate BHB concentrations or increased in vivo production, coupled with glucose reduction, can enhance the metabolic rate of endothelial cells. Cells in high-glucose conditions with ketone body treatment did not exhibit these changes. Therefore, to shift cellular metabolism towards increased activity, a ketogenic diet, minimizing carbohydrates and inducing ketosis, appears necessary.

Acknowledgments:

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P41: A novel in vivo model for multiparameter assessment of tyrosine kinase inhibitor-associated cardiovascular toxicity

Emilia Seta[1,2,3], Bartosz Michno[2,3], Stefan Chłopicki[1],Tomasz Prajsnar[2]
[1]Jagiellonian Centre for Experimental Therapeutics, Jagiellonian University, Bobrzynskiego 14, Krakow, Poland,
[2]Department of Evolutionary Immunology, Faculty of Biology, Jagiellonian University, Gronostajowa 9, Krakow, Poland,
[3]Doctoral School of Exact and Natural Sciences, Jagiellonian University, Lojasiewicza 11, Krakow, Poland

Abstract:

Tyrosine kinase inhibitors (TKIs) of BCR-ABL contributed to a major breakthrough in therapy against chronic myeloid leukaemia (CML) and other malignancies. Unfortunately, treatment with TKIs contributes to adverse cardiovascular events whose underlying mechanisms are largely unknown.

In this study, we employed the larval zebrafish model to determine the effect of TKI from every generation (imatinib, nilotinib, ponatinib and asciminib) on cardiac and vascular dysfunction.

Firstly, we explored the cardiotoxicity of TKIs by treating wild-type zebrafish larvae via immersion at 2 days post fertilization (dpf) and measuring the area of pericardium 24, 48 and 72 hours post treatment. Subsequently, to assess TKI vascular toxicity, transgenic zebrafish larvae 2 dpf with labelled endothelium were injected with FITC-dextran, treated with TKIs for 12 or 40 hours and then imaged using a confocal microscope. To quantify the vessel permeability, mean dextran fluorescence intensity was determined inside and outside the dorsal aorta. Moreover, measurements of blood vessel diameters were taken. Finally, the uptake of TKIs into zebrafish larvae was assessed with ultra pressure liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS).

We observed a pericardial oedema in nilotinib- and ponatinib-treated larvae after 48 or 24 hours, respectively. Only ponatinib induced vascular hyperpermeability and constriction after 12 hours. Nevertheless, 40 hours long treatment with nilotinib or ponatinib resulted in similar vasculotoxicity in both groups. We found the absorption of imatinib and asciminib by zebrafish larvae was very low, contrary to nilotinib and ponatinib. Currently, we are investigating the role of immune cells in TKIs cardiovascular toxicity.

Acknowledgments:

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P42: Development of a murine model of arteriovenous malformations in Hereditary Hemorrhagic Telangiectasia

Silva-Sousa, L. MSc; Alarcón-Torrecillas, C. MSc; Ayuso-Íñigo, B. MSc; Solano-SC, I. MSc; Rodríguez-Barbero, A. PhD; Pericacho, M. PhD.
Dpto. de Fisiología y Farmacología, Universidad de Salamanca. CARD-$_{05}$. Grupo de Fisiopatología del Endotelio Vascular (ENDOVAS). Instituto de Investigación Biomédica de Salamanca (IBSAL), Salamanca, España

Medical University of Vienna, Center for Biomedical Research and Translational Surgery,
Vienna, Austria

Abstract:

Objectives: To investigate therapies that can prevent the development of vascular malformations, a reproducible and measurable animal model is essential. This project aims to establish a model of arteriovenous malformations (AVMs) using a murine model of Hereditary Hemorrhagic Telangiectasia (HHT).

Methods: The study was conducted using iKO-Eng mice, an endoglin tamoxifen-induced knockout model that mimics HHT1. A skin wound was created on the back of each mouse to stimulate blood vessel formation around the wound, which was expected to develop AVMs. Ten days after the wound was made, blue latex was perfused into the left ventricle to visualize the vasculature. The tissues were then processed, photographed, and analyzed.

Results: The iKO-Eng mice developed arteriovenous malformations with varying degrees of severity. The area occupied by blood vessels and the ratio of perimeter to area were significantly greater compared to control mice. Additionally, the blood vessels around the wound in endoglin-deficient mice were more tortuous and dilated, characterized by larger diameters, more branches, and increased ramification.

Conclusions: We successfully developed a murine model of HHT1 that replicates the formation of skin-wound induced arteriovenous malformations. This model simulates the potential development of new skin or nasal malformations in HHT1 patients following injury. It could serve as a valuable tool for evaluating current and future therapies aimed at preventing the formation of arteriovenous malformations.

Acknowledgments:

Dpto. de Fisiología y Farmacología, Universidad de Salamanca. CARD-05. Grupo de Fisiopatología del Endotelio Vascular (ENDOVAS). Instituto de Investigación Biomédica de Salamanca (IBSAL), Salamanca, España and Medical University of Vienna, Center for Biomedical Research and Translational Surgery,
Vienna, Austria

P43: Impaired resolution of inflammation-induced endothelial dysfunction in a murine model of endotoxemia

Barbara Sitek[1,2], Anna Bar[1], Elżbieta Buczek[1], Łukasz Mateuszuk[1], Tasnim Mohaissen[1], Katarzyna Kieć-Kononowicz[3], Nathalie Mercier[4], Magnus Back[5,6], Stefan Chłopicki[1]
[1]Jagiellonian Centre for Experimental Therapeutics (JCET), Bobrzyńskiego 14, 30-348 Kraków, Poland
[2]Jagiellonian University, ul. Gołębia 24, 31-007 Kraków
[3]Department of Technology and Biotechnology of Drugs, Collegium Medicum of Jagiellonian University, ul. św. Anny 12, 31-008 Kraków, Poland
[4]Université de Lorraine, Inserm, Défaillance Cardiovasculaire Aiguë et Chronique (DCAC), Nancy, France
[5]Division of Cardiovascular Medicine, Center for Molecular Medicine, Department of Medicine Solna, Karolinska Institute
[6]Theme Heart and Vessels, Division of Valvular and Coronary Disease, Karolinska University Hospital, Stockholm, Sweden

Abstract:

The aim of this study was to investigate the mechanisms responsible for endothelial dysfunction and recovery of normal function in a mice model of endotoxemia in order to find a pharmacological path to accelerate this process.

LPS (3 mg/kg) response was characterized in young and aged C57BL/6 mice and in young WT and KO mice lacking the GPR18. Endothelial function was examined by MRI, organ injury - based on biochemical parameters, histology and lung myeloperoxidase. Various GPR18 agonists and other compounds were tested in vitro and then in vivo in the LPS model.

In young WT mice endothelial function was significantly impaired, but then returned to the normal state 24 h after LPS injection. In aged WT mice and in young GPR18 KO mice LPS induced endothelial dysfunction was not followed by endothelial recovery. GPR18 agonists had a vasodilator effect in the aorta previously treated with phenylephrine, an effect abolished in the presence of GPR18 antagonist. In the treated animals the most promising results were observed in the group treated with RTP-022 or amygdalin.

Our results suggest that an impaired ability of endothelial dysfunction recovery may contribute to the worse course of endotoxemia and higher organ injury in older mice. Pharmacotherapy that accelerate the resolution of inflammation can effectively reverse endothelial dysfunction occurring in endotoxemia but the mechanism of action of the investigated compounds requires further research.

Acknowledgments:

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P44: Endothelial actin cytoskeleton alterations and glycocalyx disruption associated with HNF1A-MODY

Dawid Skoczek[1,2], Damian Klóska[1], Marta Targosz-Korecka[3], Neli Kachamakova-Trojanowska[1]
[1]Malopolska Centre of Biotechnology, Jagiellonian University, Poland
[2]Doctoral School of Exact and Natural Sciences, Jagiellonian University, Poland
[3]Faculty of Physics, Astronomy, and Applied Computer Science, Jagiellonian University, Poland

Abstract:

Maturity Onset Diabetes of the Young (MODY) is a monogenic form of diabetes, with a common subtype caused by mutations in the hepatocyte nuclear factor 1-alpha (HNF1A) gene. Although the disease mechanism is poorly understood, HNF1A-MODY patients frequently develop endothelial dysfunction (ED), like retinopathy. This study aims to reveal the molecular basis of ED in HNF1A-MODY.

To model the ED, two HNF1A-MODY disease sets of human induced pluripotent stem cells (hiPSCs), patient and isogenic, were used. The patient set included two control and two HNF1A-MODY patient-specific lines, while the isogenic set comprised a control line and two CRISPR-Cas9-generated lines with monoallelic (MAC) or biallelic (BAC) HNF1A truncation. All hiPSC lines were differentiated into endothelial cells (hiPSC-ECs) and cultured under normoglycemic conditions. Additionally, key results were validated with primary endothelial cells (ECs) where HNF1A expression was silenced by siRNA.

Global RNA sequencing was conducted with the isogenic HNF1A-MODY set, revealing decreased expression of glycosylation-related genes and alterations in actin cytoskeleton pathways in HNF1A-mutated lines. Phenotypically, glycosylation changes resulted in a diminished glycocalyx identified in both HNF1A-MODY disease sets. Actin cytoskeleton alterations in the HNF1A-mutated isogenic lines were subsequently confirmed by mass spectrometry. These cytoskeletal disturbances, linked to reduced HNF1A expression, led to increased cell migration accompanied by decreased presence of actin stress fibers. A similar phenotype was observed after silencing HNF1A expression in HNF1A-MODY patient-specific lines and primary ECs.

To summarize, ECs with HNF1A mutations show diminished glycocalyx layer and alterations in the actin cytoskeleton. These changes may contribute to non-hyperglycemia-related ED.

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P45: Acute cellular response of stem cell-derived preadipocytes under proinflammatory action of palmitic acid and TNF

Ewa Stanek[1,2], Krzysztof Czamara[2], Agnieszka Kaczor[3]
[1]Jagiellonian University, Doctoral School of Exact and Natural Sciences, 11 Lojasiewicza Str., 30-348 Krakow, Poland
[2]Jagiellonian Centre for Experimental Therapeutics, Jagiellonian University, 14 Bobrzynskiego Str, Krakow, Poland
[3]Faculty of Chemistry, Jagiellonian University, 2 Gronostajowa Str., 30-387 Krakow, Poland

Abstract:

Background: Adipocyte development is highly influenced by lifestyle and diet. Excessive intake of high-fat foods, especially rich in saturated lipids like palmitic acid (PA), is linked to white adipose tissue dysfunction. Its expansion disrupts physiological functions, causing abnormal adipokine secretion and TNF-induced inflammation, which are associated with obesity and related disorders. While PA's effects on adipose tissue are known, the potential for PA uptake and resulting lipid changes at different stages of adipogenesis are not well understood.

Methods: The study was conducted on stem cell-derived preadipocytes, mature adipocytes and primary ones isolated from the epididymal white adipose tissue of C57Bl/6J male mice at the age of 10 and 20 weeks. Studied cells were exposed to deuterium-labeled palmitic acid-d31 (d-PA) and TNF for short (24 h) or prolonged incubation time (8 days) and measured using the confocal Raman WITec system equipped with a 532 nm laser. Additionally, to verify the inflammation process, immunostaining of ICAM-1 was applied.

Results: d-PA significantly changed the lipid composition of stem cell-derived adipocytes, especially in preadipocytes. TNF caused less inflammation and harmful effects than d-PA, while primary adipocytes were unaffected by either. Moreover, the potential for the uptake of the saturated fatty acids correlated directly with the inflammation reflected as the ICAM-1 expression. These findings suggest that the effects of PA and TNF differ based on adipocyte maturation and highlight the critical role of diet in the early stages of adipogenesis, with preadipocytes being the most vulnerable to dysfunction.

Conclusions: PA has a significant impact on cells in the early stage of adipocyte development. Interestingly, the less mature the adipocytes, the chemical composition is more changed.

Acknowledgments:

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P46: Modulators of adenosine metabolism and signaling prevent against isoproterenol-induced cardiac hypertrophy in mouse experimental models

Klaudia Stawarska[1], Ada Kawecka[1], Magdalena A. Zabielska-Kaczorowska[2], Elizabetta Caiazzo[3], Paulina Mierzejewska[1], Barbara Kutryb-Zając[1], Ryszard T. Smoleński[1]
[1]Department of Biochemistry, Medical University of Gdansk, Gdansk, Poland
[2]Department of Physiology, Medical University of Gdansk, Gdansk, Poland
[3]Department of Pharmacy, School of Medicine, University of Naples Federico II, Naples, Italy

Abstract:

Background: Pathological cardiac hypertrophy, due to increased mechanical overload, leads to cardiomyocyte dysfunction and heart failure. Adenosine has been proposed to exert anti hypertrophic effects. This study explored the protective role of adenosine in isoproterenol-induced cardiac hypertrophy in mice.

Methods: Experiments involved 4- and 8-week-old ecto-5'-nucleotidase (CD73) knock-out mice and wild-type C57Bl/6J mice (WT), treated with -agonist isoproterenol (ISO) with/without adenosine deaminase (ADA) inhibitor (deoxycoformycin, dCF). Mice underwent an echocardiography and blood sampling, while left ventricle fragments were preserved to measure enzyme activities with high-performance liquid chromatography (HPLC) or embedded in O.C.T. compound for immunofluorescence assays. Alternatively, mice cardiomyocyte cell line (HL-1) was used to determine ISO's impact on cell size, calcification and CD73, tissue nonspecific alkaline phosphatase (TNAP) and ADA ecto-enzymes' activities.

Results: Mice treated with ISO and dCF exhibited a higher survival rate and increased systolic volume than solely ISO-treated WT and CD73 knock-out mice. Adenosine metabolism enzyme assays in heart homogenates confirmed the effective inhibition of ADA by dCF and the lack of ecto-5'-nucleotidase activity in CD73-/- mice. Interestingly, ADA inhibition increased CD73 activity in left ventricular homogenate (488,332,42 v.s 1256192,4 mol/min/g wt), while CD73-/- mice decreased ADA activity (440,187,91 vs. 323,223,98 mol/min/g wt). ISO significantly enhanced HL-1 cross-sectional and calcification area, while adenosine receptor modulators interrupted these processes.

Conclusions: This study indicates that adenosine deaminase inhibition improves the survival rate and cardiac function in mice experimental models of -agonist-induced hypertrophy. Specific mechanisms need to be further investigated by adenosine receptor signaling seems to be critical in these beneficial anti-hypertrophic outcomes.

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P47: Characterization of profiles of endothelial biomarkers in young and old endotoxemic mice.

Joanna Suraj-Prażmowska[1*], Magdalena Sternak[1], Anna Kurpińska[1], Anna Bar[1], Izabela Czyzynska-Cichon[1], Łukasz Mateuszuk[1], Marta Pacia[1], Elżbieta Buczek[1], Krzysztof Czamara[1], Kelly Ascencao[3], Csaba Szabo[3], Stefan Chlopicki[1,2]

[1]Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Bobrzynskiego 14, 30-348, Krakow, Poland
[2]Chair of Pharmacology, Jagiellonian University Medical College, Grzegorzecka 16, 31-531, Krakow, Poland
[3]Chair of Pharmacology, Section of Medicine, University of Fribourg, Fribourg, Switzerland

Abstract:

Age has a significant influence on both the course and increased intensity of inflammatory processes and a degree of organ damage during the development of sepsis. However it has not been defined as yet, whether endothelial dysfunction in aged mice is more severe and has the same pathophysiological profile or is rather characterized by a distinct profile of endothelial response.

The aim of the study was therefore to comprehensively assess early and late endothelial response to LPS in young and old mice on functional (MRI) and biochemical (LC-MS/MS-based endothelial profiling) levels. Endothelial response to LPS was analyzed in the relation to LPS-induced organ injury assessed by classical biomarkers.

In young mice, the systemic inflammatory response appeared more rapid, whereas in old mice developed slower but was more intense. Similarly, LPS-induced increase in several cytokines in plasma, was more pronounced in old mice (e.g. IL-1β). Impairment of endothelial-dependent vasodilation in vivo assessed by MRI was more severe in old mice. Organ damage was confirmed in both young and old mice at the late stage of endotoxemia development with more pronounced increase of urea in old mice. Interestingly, among multiple biomarkers characterizing endothelial response, endothelial inflammation was not different between old and young mice, however indicators of glycocalyx disruption (ESM-1), and hemostatic markers, in particular sTM, displayed a clearly distinct pattern of response.

In conclusion, in old mice systemic inflammatory response to LPS was more intense, resulting in more severe impairment of NO-dependent function and robust glycocalyx injury and impaired thrombomodulin response.

Acknowledgments:

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P48: Circulating ACE and ACE2 as Endothelial Biomarkers: Insights into Cardiovascular Disease

Attila Tóth, Miklós Fagyas, Szilárd Nagy, Ottó Tatai, Tamás Pintér, Zoltán Papp
Division of Clinical Physiology, Department of Cardiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Abstract:

The endothelial system critically influences the circulating levels and activity of angiotensin-converting enzyme (ACE) and its homolog ACE2, with implications for cardiovascular health and disease.

Circulating ACE2 levels are notably elevated in patients with hypertension and heart failure, especially those with reduced ejection fraction (HFrEF). Conversely, ACE2 activity does not change significantly in heart failure with preserved ejection fraction (HFpEF), suggesting differing endothelial pathophysiological mechanisms.

Circulating ACE activity is influenced by endogenous inhibitors such as human serum albumin (HSA) while endothelial ACE is resistant to inhibition by HSA. ACE shedding and endothelial regulation defines its role in cardiovascular outcomes.

Our findings integrate with existing knowledge to emphasize the significance of circulating ACE and ACE2 as biomarkers of endothelial function and cardiovascular disease. Their levels not only reflect endothelial dysfunction but also offer valuable biomarkers for assessing disease severity and therapeutic outcomes. Understanding these biomarkers can guide the development of targeted therapies aimed at improving endothelial function and overall cardiovascular health.

Acknowledgments:

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P49: Tirzepatide, a GIP/GLP1-receptor co-agonist preserves cardiac function and improves survival in mice with angiotensin II-induced heart failure

Viktória E. Tóth[1,2*], Zsombor I. Hegedűs[1,2*], Nabil V. Sayour[1,2], Márk Jakab[1,2], Tamás G. Gergely[1,2], Ayham Haddad[1,2], Péter Ferdinandy[1,2,3,4], Varga V. Zoltán[1,2]
[1]Center for Pharmacology and Drug Research & Development, Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary
[2]HCEMM-SU Cardiometabolic Immunology Research Group, Budapest, Hungary
[3]Pharmahungary Group, Szeged, Hungary
[4]MTA-SE System Pharmacology Research Group, Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary
*These authors contributed equally
Corresponding author: toth.viktoria@semmelweis.hu

Abstract:

Introduction
Incretin analogs used for the treatment of type 2 diabetes mellitus and obesity, such as GLP1-receptor agonists have been shown to reduce major adverse cardiac events in recent clinical trials, while dual GIP/GLP1-R agonists, such as tirzepatide (TZP) are under investigation.

Aim
We performed a comparative study in a mouse model of HFrEF induced by continuous angiotensin II (AngII) infusion to investigate the effects of Lira and TZP on mortality and cardiac structural and functional changes.

Methods
Osmotic minipumps were inserted for subcutaneous (s.c.) administration of AngII (1.5mg/kg/day) in 5-month-old male Balb/c mice or sham surgery was performed. After this, animals were treated with vehicle (Veh), Lira (300 µg/day i.p.) or TZP (10 nmol/day s.c.) for 14 days. Cardiac structural, functional and molecular characteristics were assessed by echocardiography, ECG, immunohistochemistry, and qRT-PCR.

Results
Mortality was significantly higher in AngII/Veh animals compared to controls, while AngII/TZP mice showed significantly reduced mortality after 14 days of treatment. Both Lira and TZP caused significant weight loss. Treatment with both compounds preserved cardiac systolic and diastolic function compared with AngII/Veh animals, as shown by normal ejection fraction and E/e', respectively. Both Lira and TZP decreased the AngII-induced elevation of cardiac fibrosis and hypertrophy markers, including Ctgf, Col1a1, Col3a1, and Nppa, while TZP also reduced the elevated Nppb level.

Conclusion
Lira and TZP preserved cardiac function and decreased markers of hypertrophy and fibrosis in mice with AngII-induced heart failure, whereas TZP also significantly decreased mortality. The use of incretin analogs may have beneficial effects in the treatment of HFrEF.

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P50: The influence of spontaneous physical activity on coronary circulation adaptations in the early phase of heart failure in Tgaq44 mice

Urszula Tyrankiewicz[1], Grzegorz Kwiatkowski[1], Magdalena Sternak[1], Magdalena Stojak[1], Marta Pacia[1], Izabela Czyżynska-Cichon[1], Stefan Chlopicki[1];
Jagiellonian Centre for Experimental Therapeutics, (JCET)

Abstract:

The changes in coronary circulation correspond to alterations related to the progression of heart failure. Accurate profiling of coronary flow can help understand early changes related to the course of cardiac pathology. The goal of the study was to: (1) delineate coronary flow pattern in course of heart failure in regards to maximal velocities and flow dynamics and to (2) characterize the effects of spontaneous physical activity on coronary circulation in Tgaq44 mice at the very early stages of disease. For that purpose murine model of heart failure was used (Tgaq44 mice) and their control FVB strain at the age of 2 months. Of note, Tgaq44 mice develop early HF at the age of 4 months, but end-stage HF at the age of 12 months. Mice were divided on trained (Tgaq44 with free access to running wheel) and sedentary group (Tgaq44 and FVB mice). After 8 weeks of experiment in vivo cardiac function was measured by magnetic resonance imaging and coronary flow profile was assessed by Doppler Flow Velocity System. The effects of spontaneous physical activity on congestive hepatopathy biomarkers and altered lipid metabolism previously reported in this model [1] was also analyzed. Spontaneous activity improved cardiac performance as evidenced by improved LV contractility and inhibited diastolic deterioration. There were also changes detected in coronary circulation (related to peak velocities and flow dynamics) in sedentary Tgaq44 mice, that were not observed in control FVB mice neither in Tgaq44 mice with applied physical therapy. To conclude: (1) changes in coronary flow pattern were clearly observed already at the very early stage of HF in Tgaq44 mice; (2) they precede LV functional deterioration and (3) physical activity applied even at very early stage of HF (in 2 month-old mice) was able to improved cardiac and coronary performance. These results underscore the pronounced beneficial effects of early exercise intervention on coronary circulation and cardiac performance that might be lost at the later phases of HF as previously demonstrated in the same model [2].

[1]Wojnar-Lason K et al., Chronic heart failure induces early defenestration of liver sinusoidal endothelial cells (LSECs) in mice. Acta Physiol (Oxf). 2024 May;240(5):e14114
[2]Tyrankiewicz U et al. Physical Activity and Inhibition of ACE Additively Modulate ACE/ACE-2 Balance in Heart Failure in Mice. Front Pharmacol. 2021 Jun 7;12:68243

P51: Dapagliflozin improves cardiac endothelial cell function and energy metabolism

Iga Walczak[a], Alicja Braczko[a], Aleksandra Paterek[b], Filip Rolski[b], Krzysztof Urbanowicz[a], Ryszard T. Smoleński[a], Marcin Hellmann[c], Michał Mączewski[b], Barbara Kutryb-Zajac[a]
[a]Department of Biochemistry, Medical University of Gdańsk, Poland
[b]Department of Physiology, The Centre of Postgraduate Medical Education, Warsaw, Poland
[c]Department of Cardiac Diagnostics, Medical University of Gdańsk, Poland

Abstract:

Background: Flozins (sodium-glucose cotransporter 2 inhibitors, SGLT2i) are a new class of antidiabetic drugs that reduce cardiovascular mortality and hospitalization rates in heart failure patients, regardless of type 2 diabetes status. Besides lowering glycemia by inhibiting renal glucose reabsorption, SGLT2 inhibitors may exert sodium-dependent hemodynamic effects and improve cardiomyocyte energy metabolism, substrate preference, and mitochondrial function. However, their impact on the metabolism and function of endothelial cells remains largely unknown.

Methods: Mouse cardiac microvascular endothelial cells (H5V) were pre-treated with dapagliflozin (DAPA) for 30 min followed by a 24-hour co-treatment with hypoxia-mimicking agent $CoCl_2$. The concentration of intracellular nucleotides was measured using high-performance liquid chromatography. Mitochondrial function and nitric oxide (NO) production were assessed in live cells by SeahorseXFp and fluorescence DAF-FM staining, respectively. The impact of DAPA on coronary artery flow-mediated dilatation (FMD) and real-time NO production in C57Bl/6J mice was also examined.

Results: In a murine model, DAPA improved FMD and enhanced coronary artery NO synthesis. In a cell culture model, DAPA affected intracellular ATP/ADP ratio and improved NO production that correlated with enhanced mitochondrial respiration parameters.

Conclusions: This study highlights the beneficial effects of flozins on cardiac endothelial cell function and metabolism via mitochondrial mechanisms and NO-dependent effects. Regulation of cardiac endothelial cell energy metabolism may be an undervalued mechanism of flozins to delay the development of HF and support cardiac regeneration. These studies may accelerate endothelial-targeted strategies to support heart failure treatment.

Acknowledgments:

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P52: Light modulation of mitochondrial potassium channels activity isolated from Guinea Pig cardiomyocytes

Antoni Wrzosek[1], Joanna Lewandowska[1], Barbara Kalenik[1], Piotr Bednarczyk[2], Adam Szewczyk[1]
[1] Laboratory of Intracellular Ion channels, Nencki Institute of Experimental Biology PAS, Warszawa, Poland
[2] Department of Physics and Biophysics, Institute of Biology, Warsaw University of Life Sciences-SGGW, Warszawa, Poland

Abstract:

It is well documented that mitochondrial potassium channels (mitoK) present in the inner mitochondrial membrane (IMM) have cytoprotective effects against damage induced by an ischemia-reperfusion event. The oxidative nature of ATP synthesis in the heart muscle, its sensitivity to hypoxia is very high and compounds that can protect cardiomyocytes from damage are extremely desirable. The currently available method for regulating the activity of mitoK channels remains pharmacological modulation. However, modulators often evince "off-targeting", so methods are being sought that do not have this undesirable feature. Western Blot and RT-PCR analysis confirmed the presence of high conductance Ca2+ activated mitochondrial potassium channels (mitoBKCa) in the IMM of Guinea Pig cardiomyocytes. Electrophysiological data showed that the mitoBKCa channel had a conductance of 130 pS, was Ca2+ sensitive, and was inhibited by the canonical blocker paxilline. Studies with patch-clamp technique showed down-regulation of the mitoBKCa channels under oxidizing conditions, which was abolished by infrared light with a wavelength of 820 nm.
Previous studies have shown that activation of the mitochondrial respiratory chain affects the activity of the mitoBKCa channels and the putative interaction between the mitoBKCa channel and cytochrome c oxidase (COX). The COX has metal centers capable of absorbing infrared light, including one (CuA) which in the oxidized state absorbs a wavelength of 820 nm. The obtained results indicate possible activation of the mitoBKCa channel from the IMM of cardiomyocytes through changes in COX activity.

Acknowledgments:

Study was supported by the Polish National Science Centre (grant MAESTRO No. 2019/34/A/NZ1/00352 to AS).

P53: Towards untargeted metabolomics in zebrafish larvae using liquid chromatography coupled with Orbitrap Exploris 120 mass spectrometer (LC-HRMS).

Agnieszka Zakrzewska[1], Renata Wawrzyniak[2], Emila Seta[1,3], Tomasz K. Prajsnar[3], Michał Markuszewski[2], Stefan Chłopicki[1,4]

[1] Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Krakow, Poland.
[2] Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdansk, Gdansk, Poland.
[3] Department of Evolutionary Immunology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Poland.
[4] Department of Experimental Pharmacology, Chair of Pharmacology, Jagiellonian University Medical College, Krakow, Poland.

Abstract:

Metabolomics is an emerging and powerful tool, that gives scientists a snapshot of the cellular metabolic state at a given time. In the present study, we utilized liquid chromatography coupled with Orbitrap Exploris 120 mass spectrometer (LC-HRMS), applied to zebrafish (Danio rerio) larvae to analyze changes in metabolic profiles associated with ponatinib (tyrosine kinase inhibitor) exposure. Zebrafish embryos were obtained by the natural spawning of adult zebrafish (line AB/TL). In this work HILIC chromatography column (BEH Z-HILIC, 2.1×100 mm, 1.7 µm, Waters) was used. The mass spectrometer was operated in positive and negative ionization modes. Data were acquired in full and MS2 scan modes. Full scan data acquisition was conducted as follows: resolution of 120,000 and a scan range of 60–900 m/z. Raw data were processed by Compound Discoverer 3.3 SP3 software (CD). Principle component analysis (PCA) was applied to get an overview of the data. Afterwards, orthogonal projection to latent structure discriminant analysis (OPLS-DA) was utilized to discriminate between the studied group. The number of annotated metabolites by CD (level 3 - annotations are based on library searches using the accurate mass and elemental composition alone) in zebrafish larvae was 312 and 120 for positive and negative mode, respectively. These results suggest significant alterations in zebrafish larvae exposed to ponatinib, known for its vasotoxic effects. Whether this pattern of metabolomics changes reflects plasma profile of metabolites in mice and humans after ponatinib treatment and could be linked to vasotoxicity of this compound, requires further studies.

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P54: Cardioprotective function of iso-branched chain fatty acids in mice with double knockout of KO ApoE/LDLR -/-

Agata Zwara[1], Agata Jędrzejewska[2], Szymon Formella[3], Marta Toczek[2], Tomasz Śledzinski[3], Adriana Mika[1,3]

[1] Department of Environmental Analytics, Faculty of Chemistry, University of Gdansk, Wita Stwosza 63, 80-308 Gdansk, Poland
[2] Department of Biochemistry, Faculty of Medicine, Medical University of Gdansk, Debinki 1, 80-211 Gdansk, Poland
[3] Department of Pharmaceutical Biochemistry, Faculty of Pharmacy, Medical University of Gdansk, Debinki 1, 80-211 Gdansk, Poland

Abstract:

Cardiovascular diseases (CVD) are classified as diseases of civilization. The basis of the CVD is atherosclerosis, which is characterized by the abnormal accumulation of lipids leading to dyslipidemia and chronic inflammation [1]. Available data indicate that iso branched-chain fatty acids (iso BCFA) have anti-inflammatory properties [2]. The aim of the study was to investigate the effect of supplementation with iso BCFA on atherosclerosis.

Three- and nine-month old mice with double knockout of apolipoprotein E and LDL receptor (KO ApoE/LDLR-/-) were divided into groups: (1) fed with standard chow (SD group), (2) fed with chow enriched with 13-methyl-14:0, 14-methyl-15:0 and 15-methyl-16:0 (BCFA group) for three months. Hearts and livers were sampled for gas chromatography-mass spectrometry and real time PCR analysis.

In both age groups, the content of supplemented iso BCFA was higher in the BCFA group compared to the SD group in both tissues. The expression of the branched-chain ketoacid dehydrogenase E1 (Bckdh) gene showed a downward trend in the liver of BCFA group compared to the SD group in both age groups. A decreasing trend of Bckdh expression was observed in the older hearts of the BCFA group compared to the hearts of the SD group.

In conclusion, iso BCFA supplementation was successful. Also, iso BCFA supplementation had an effect on the expressionof Bckdh, which is involved in the catabolism of branched chain amino acids. [1] Minelli S et al. J Multidiscip Healthc (2020) 13:621–633 [2] Ran-Ressler RR et al. PLoS One (2011) 6:e29032

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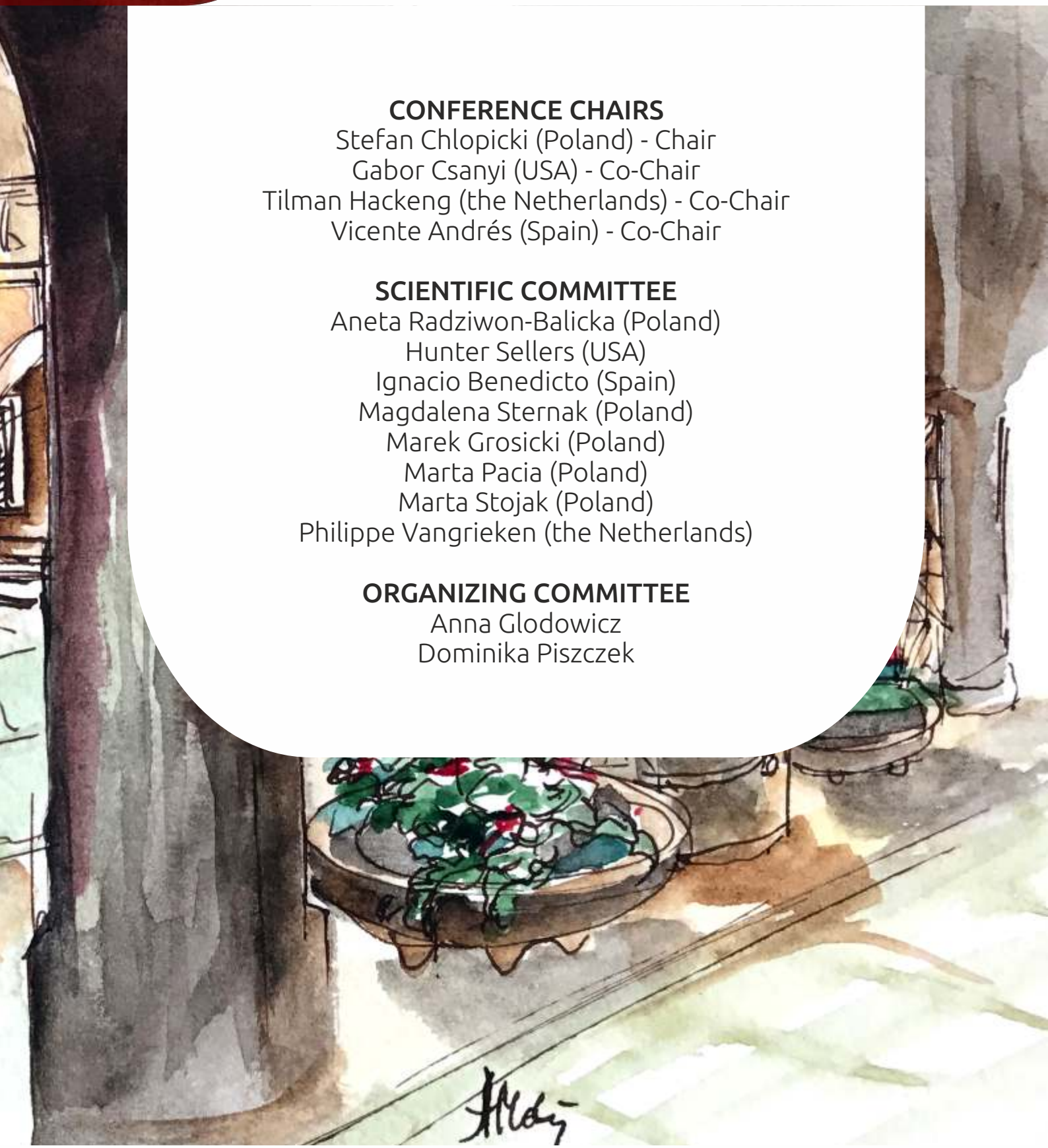

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