28th & 10th

Kraków Conference on Endothelium

Cardiovascular Research Days

International Cultural Center,
Rynek Główny 25, Kraków

12th -14th December 2022

JCET Jagiellonian Centre for Experimental Therapeutics

Jagiellonian University in Krakow

Center for Biomedical Research Medical University of Vienna

Jagiellonian Medical Research Center

Ludwig Boltzmann Institute Cardiovascular Research

Book of abstracts

Introduction

Dear Colleagues,

We are pleased to welcome you to the joint conference: 28th Kraków Conference on Endothelium and 10th Cardiovascular Research Days held in Krakow from 12th to 14th of December 2022 at the International Cultural Centre (MCK), at Rynek Główny in Krakow.

The series of conferences recently named **"Krakow Conference on Endothelium"** has been organized by Prof. Ryszard Gryglewski and then by Prof. Stefan Chłopicki over many years in Krakow or surroundings and were devoted to endothelial and vascular pharmacology. The **"Cardiovascular Research Days"**, the bi-annual meetings organized by Prof. Bruno Podesser, have also had a long tradition to bring together researchers from basic and clinical sciences in the area of cardiovascular research and were held in Austria, Germany, Italy or Hungary before.

This year we are jointly organizing the highly interdisciplinary and translational meeting combining both traditions of meetings in Krakow and Vienna. The conference will be dealing with a wide scope of aspects within the major topics of the meeting: **Vascular Inflammation in Ageing and Diseases: from bench to bed and back.**

We are happy to host ca 150 participants, and to present a programme with 24 invited lectures, including the keynote and special lectures, and 14 talks selected from abstracts and 78 posters with their abstracts presented in this book. As in our previous conferences, the central point of the programme is the poster session with one-minute long presentations followed by the classical poster session. The latter will be held in the poster area, which is located one minute walking distance away from the venue of the conference.

We would like to thank all of you for joining us. Together with the Scientific Committee from Krakow and Vienna, which has undertaken the difficult task of selecting abstracts for oral presentation among so many good abstracts, and with the Organizing Committee – Anna Glodowicz (JCET-UJ) and Dominika Piszczek (JMRC) - who took the difficult organization tasks onto their shoulders, we hope that all of you will find the scientific contents of the conference stimulating for creative exchange of knowledge, experience and thoughts.

Last but not least, in the past the conferences in Krakow often hosted guests from Ukraine. We stand alongside Ukraine, and very much hope that colleagues from Ukraine, so badly suffering now due to the Russian invasion, will join us in our next conference and the war over the border, far and near in the same time, will come to an end soon.

Prof. Stefan Chlopicki **Prof. Bruno Podesser**

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12th December 2022

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Abstracts of posters

1.1.

Accelerated ageing and coronary microvascular dysfunction in chronic heart failure in Tgαq*44 mice

Piotr Berkowicz[1], Justyna Totoń-Żurańska[2], Grzegorz Kwiatkowski[1], Agnieszka Jasztal[1], Tamás Csípő[3,4], Kamil Kus[1], Urszula Tyrankiewicz[1], Anna Orzyłowska[5], Paweł Wołkow[2], Attila Tóth[3], Stefan Chlopicki[1,6]
[1] Jagiellonian University, Jagiellonian Centre for Experimental Therapeutics (JCET), Krakow, Poland
[2]Centre for Medical Genomics OMICRON, Jagiellonian University Medical College, Krakow, Poland
[3]Division of Clinical Physiology, Department of Cardiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
[4]Department of Public Health, Faculty of Medicine, Semmelweis University, Budapest, Hungary
[5]Department of Neurosurgery and Paediatric Neurosurgery, Medical University of Lublin, Lublin, Poland
[6]Jagiellonian University Medical College, Faculty of Medicine, Chair of Pharmacology, Krakow, Poland

Abstract:

Age represents a major risk factor in heart failure (HF). However, the mechanisms linking ageing and HF are not clear. We aimed to identify the functional, morphological and transcriptomic changes that could be attributed to cardiac ageing in a unique model of slowly progressing HF in Tgαq*44 mice in reference to the cardiac ageing process in FVB mice.

In FVB mice, ageing resulted in the impairment of diastolic cardiac function and in basal coronary flow (CF), perivascular and interstitial fibrosis without changes in the cardiac activity of angiotensin-converting enzyme (ACE) or aldosterone plasma concentration. In Tgαq*44 mice, HF progression was featured by the impairment of systolic and diastolic cardiac function and in basal CF that was associated with a distinct rearrangement of the capillary architecture, pronounced perivascular and interstitial fibrosis, progressive activation of cardiac ACE and systemic angiotensin-aldosterone-dependent pathways. Interestingly, cardiac ageing genes and processes were represented in Tgαq*44 mice not only in late but also in early phases of HF, as evidenced by cardiac transcriptome analysis. 34 genes and 8 biological processes, identified as being ageing related, occurred early and persisted along HF progression in Tgαq*44 mice and were mostly associated with extracellular matrix remodelling and fibrosis compatible with perivascular fibrosis resulting in coronary microvascular dysfunction (CMD) in Tgαq*44 mice.

In conclusion, accelerated and persistent cardiac ageing contributes to the pathophysiology of chronic HF in Tgαq*44 mice. In particular, CMD with prominent perivascular fibrosis represents an accelerated cardiac ageing phenotype that requires targeted treatment in chronic HF.

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1.2.

Ang 1-12/Ang II/TXA2 pathway in endothelial dysfunction the murine model of heart failure

Tasnim Mohaissen
JCET

Abstract:

Introduction
Angiotensin 1-12-dependent Angiotensin II generation plays a role in cardiac pathology, but the involvement of this pathway in peripheral endothelial dysfunction due to heart failure is still not clear.
The aim
To characterize the possible involvement of Angiotensin 1-12-dependent pathway in vascular TXA2 production, and endothelial dysfunction in chronic HF (Tgαq*44 mice).
Methods and Results
Aortic rings isolated from 12-month-old Tgαq*44 mice and incubated 24-hours (ex vivo) displayed impaired endothelium-dependent vasodilation in contrast to 4-month-old Tgαq*44 mice or age-matched FVB mice as assessed in wire myograph system. Interestingly, in this experimental setup incubated with Ang I, Ang II, or Ang-(1-12) did not show major differences in angiotensin profile generation in the aortic rings isolated from 4- and 12- month-old Tgαq*44 mice as measured by LC-MS/MS. However, 24-hours-incubation of aortic rings with Ang-(1-12) -induced increase Ang II production in 12- month-old Tgαq*44 mice as compared to age-matched FVB mice. Interestingly, there was a clear-cut upregulation of TXB2 generation in response to Ang I, Ang II or Ang-(1-12) in aortic rings isolated from 12- month-old Tgαq*44 mice as compared with 4- month-old Tgαq*44 mice or age-matched FVB mice as measured by LC–MS/MS. Deterioration of endothelial function in aortic rings induced by Ang II or Ang-(1-12) was improved in the presence of TP antagonist, SQ-29548 or AT1 antagonist, losartan in 12-month-old- Tgαq*44 mice, but was without of effect in 4-month-old-Tgaq 44 mice. Although, adenosine deaminase (ADA) activity in aorta isolated from 12-month-old Tgαq*44 mice was increased as compared to age-matched FVB mice, the inhibition of ADA did not improve endothelial dysfunction in aortic rings isolated from 12-month-old Tgαq*44 mice.
Conclusion
Our data suggest a possible role of intravascular-Ang 1-12/angiotensin II- thromboxane A2- pathway in peripheral endothelial dysfunction in chronic heart failure in Tgαq*44 mice.

Acknowledgments:

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1.3.

Effect of mitochondrial ROS-inhibition on vasodilation and renal function in a mouse model of endothelial-specific aging

Annika A. Jüttner[1], Ehsan Ataei Ataabadi[1], Keivan Golshiri[1], Rene de Vries[1], A. H. Jan Danser[1], Adrianus C. van der Graaf[3], Robert H. Henning[3 4], Guido Krenning[3 4], Jenny A. Visser[2], and Anton Roks[1]
[1]Division of Vascular Medicine and Pharmacology, Department of Internal Medicine, Erasmus Medical Centre, Rotterdam. [2]Division of Metabolism and Reproduction, Department of Internal Medicine, Erasmus Medical Centre, Rotterdam, [3]SulfateqB.V., Groningen. [4]UniversityMedical Centre Groningen, Groningen

Abstract:

Vascular aging, a major cause of morbidity and mortality, is marked by decreased nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) signaling. This is partly caused by increased reactive oxygen species (ROS) levels in endothelial cells (EC), believed to be produced by mitochondria. Here, we investigated the effect of chronic treatment with the chromanol SUL138, an inhibitor of reverse mitochondrial electron flow which reduces free electron spill-over to ROS, in a model of EC-specific aging. EC-specific deletion of DNA repair endonuclease Ercc1 in mice (EC-KO) led to accelerated vascular aging features, marked by reduced endothelium-dependent NO-cGMP vasodilation at 22 weeks of age. Chronic treatment with SUL138 from 14 to 22 weeks of age restored vasodilator function. EC-KO additionally displayed overt albuminuria, polyuria, polydipsia and increased senescence and inflammatory marker expression which were normalized by SUL138. Therefore, reduction of mitochondrial ROS by maintaining forward mitochondrial flow might constitute an effective treatment of vascular aging and the accompanied kidney dysfunction. oxane A2-pathway in peripheral endothelial dysfunction in chronic heart failure in Tgαq*44 mice.

1.4.

Vitamin K1 deficiency impairs endothelial function in normolipidemic and dyslipidaemic mice

Agnieszka Kij[1], Anna Kieronska-Rudek[1,2], Anna Bar[1], Izabela Czyzynska-Cichon[1], Magdalena Strus[3], Lucja Kozien[3], Grazyna Wiecek[3], Natalia Zeber-Lubecka[4,5], Maria Kulecka[4,5], Grzegorz Kwiatkowski[1], Kamil Przyborowski[1], Tasnim Mohaissen[1], Magdalena Sternak[1], Elzbieta Buczek[1], Agnieszka Zakrzewska[1], Bartosz Proniewski[1], Kamil Kus[1], Magdalena Franczyk-Zarow[6], Renata B. Kostogrys[6], Elsbeth J. Pieterman[7], Hans M.G. Princen[7], Stefan Chlopicki[1,2]

[1]Jagiellonian University, Jagiellonian Centre for Experimental Therapeutics (JCET), Bobrzynskiego [14], 30-348 Krakow, Poland [2]Jagiellonian University Medical College, Chair of Pharmacology, Grzegorzecka [16], 31-531 Krakow, Poland [3]Jagiellonian University Medical College, Department of Bacteriology, Microbial Ecology and Parasitology, Chair of Microbiology, Czysta [18], 31-121 Krakow, Poland [4]Centre of Postgraduate Medical Education Department of Gastroenterology, Hepatology and Clinical Oncology, Roentgena [5], 02-781 Warszawa, Poland [5]Department of Genetics, Maria Sklodowska-Curie National Research Institute of Oncology, Roentgena [5], 02-781 Warszawa, Poland [6]University of Agriculture in Krakow, Faculty of Food Technology, Department of Human Nutrition and Dietetics, Mickiewicza [21], 31-120 Krakow, Poland [7]The Netherlands Organization of Applied Scientific Research (TNO), Metabolic Health Research, Gaubius Laboratory, 2333BE Leiden, The Netherlands

Abstract:

The biological action of vitamin K extends far beyond haemostasis including vasoprotection. It is thought that vitamin K2 rather than K1 participate in regulation of endothelial function. Therefore, this study aimed to explore the involvement of vitamin K1 in regulating vascular function based on the in vivo effects of vitamin K1 deficiency on endothelial function in normolipidaemic C57BL/6J and dyslipidaemic E3L.CETP mice.

The specially designed vitamin K1-deficient diet was given to mice throughout 10 weeks. Endothelial function was evaluated in vivo using magnetic resonance imaging. Concentrations of vitamin K homologs were measured in the aorta, plasma, liver and faeces using liquid chromatography-tandem mass spectrometry. Blood coagulation status was assessed by the thrombin-antithrombin complex measurements and calibrated automated thrombography. Gut microbiota composition was evaluated by culturing and next-generation sequencing methods.

Vitamin K1 deficiency markedly reduced vitamin K1 levels in plasma and liver without affecting blood coagulation. Importantly, vitamin K1-deficient mice featured with significant impairment of endothelium-dependent vasodilation in vivo in normolipidaemic mice, that was fully reversed by vitamin K1 supplementation. Vitamin K1 deficiency-induced endothelial dysfunction resulted in decreased K2(MK-4) content in the aorta, that was restored by vitamin K1 supplementation. Gut microbiota composition and vitamin K2 homolog production remained unchanged irrespective of vitamin K1 content in the diet. In dyslipidaemic E3L.CETP mice vitamin K1 liver level was reduced as compared to normolipidaemic mice, and interestingly vitamin K1 deficiency exacerbated preexisting endothelial dysfunction.

Concluding, vitamin K1 has vasoprotective action and maintains healthy endothelial function under both normolipidemia and dyslipidemia. oxane A2- pathway in peripheral endothelial dysfunction in chronic heart failure in Tgαq*44 mice.

Acknowledgments:

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1.5.

Senolitic activity of vitamin K in endothelial cells and vascular smooth muscle cells

Anna Kieronska-Rudek[1,2], Anna Kurpinska[1], Agnieszka Kij[1], Agnieszka Panek[3], Monika Pietrowska[4], Catherine M. Shanahan[5], Stefan Chlopicki[1]
[1] Jagiellonian University, Jagiellonian Centre for Experimental Therapeutics (JCET), Krakow, Poland;
[2] Jagiellonian University, Department of Pharmacology, Medical College, Krakow, Poland
[3] Institute of Nuclear Physics Polish Academy of Sciences, Krakow, Poland.
[4] Centre for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie National Research Institute of Oncology, Gliwice, Poland.
[5] School of Cardiovascular and Metabolic Medicine and Sciences, James Black Centre, King's College London, London, United Kingdom

Abstract:

Despite the well-described role of vitamin K (VK) in coagulation and calcification, the newest data provide evidence that VK may also improve calcification-independent vascular function1. However, the basis of the vasoprotective role of VK has not been explained up till now.

Considering reports indicating that VK may inhibit ageing and inflammation 2,3. The main goal of the present research was to study the influence of VK on senescence and senescence-related inflammation in the cells of the vessel wall in vitro.

The influence of Vitamin K1(VK1) and K2(VK2) has been determined in the replicative senescence model of Porcine Aortic Endothelial Cells (PAEC) and Primary HumanVascular Smooth Muscle Cells (04/35F/11A). Additionally, senescence in Human Aortic Endothelial Cells (HAEC) and 11A/35F/04 inducted by ionizing radiation (HAEC) and upregulation of PrelaminA (04/35F/11A) The influence of VK1 and VK2 on senescence was analysed by senescence-associated b-Galactosidase activity and expression of senescence- and inflammation-related proteins by proteomic analysis or PCR method. Additionally, to explain the mechanisms of senolitic activity of VK the influence on DNA damage was analysed.

Our results coherently demonstrate that both VK1 and VK2 inhibit senescence and senescence-associated inflammation in endothelial cells and vascular smooth muscle cells. Most likely, by the mechanisms related to the prevention or repair of DNA damage.

Concluding, VK appears to be a promising agent for supporting therapies of ageing-related cardiovascular diseases.

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1.6.

Endothelial cell ageing, but not their function, impacts abdominal aortic aneurysm formation in mice

Aleksandra Kopacz[1], Damian Kloska[1,2], Anna Bar[3], Marta Targosz-Korecka[4], Dominik Cysewski[5], Stefan Chłopicki[3], Alicja Jozkowicz[1], Anna Grochot-Przeczek[1]

[1]Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland
[2]Molecular Mechanisms of Diseases Laboratory, Malopolska Centre of Biotechnology, Jagiellonian University, Krakow, Poland
[3]Jagiellonian Center of Experimental Therapeutics, Krakow, Poland
[4]Department of Physics of Nanostructures and Nanotechnology, Institute of Physics, Jagiellonian University, Krakow, Poland
[5]Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, [02-106] Warsaw, Poland

Abstract:

An abdominal aortic aneurysm (AAA) is a life-threatening dilatation of the abdominal aorta. Its incidence is significantly affected by ageing, however the mechanisms behind are not elucidated. Moreover the involvement of endothelial cells (ECs) in this disease is vague. This research aimed to define the mechanisms underlying the development of AAA, focusing on EC function and ageing. As a model, we used the mice lacking the transcriptional activity of NRF2. It is a central modulator of stress response, and its deficiency leads to premature ageing of the aorta and predisposes to AAA, which is concomitant with the induction of miRNA-34a – a hallmark ageing-inducer.

We efficiently rescued EC NRF2-dependent premature ageing and AAA formation in NRF2 transcriptional knockout mice using the EC-specific knockout of miRNA-34a, which implies the significance of intimal layer senescence in the susceptibility to AAA. Therefore, endothelial cells can be imperative regulators of AAA formation. However, contrary to previously postulated mechanisms, the maintenance of specialised functions of ECs is not the primary determinant of aneurysm formation. We propose instead that EC proliferation protects against AAA and can confer aneurysm stability.

To sum up, we conclude that EC ability to proliferate and tighten the arterial wall can be the primary determinant of the onset of AAA. Therefore, contrary to current opinions, the age-dependent proneness to AAA does not rely on the functional endothelium features but rather stems from the cessation of EC proliferation.

Acknowledgments:

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1.7.

Endothelial dysfunction profiling in young and aging endotoxemic mice; a role of endothelial Nrf2-dependent pathway

Joanna Suraj-Prażmowska[1]*, Magdalena Sternak[1], Anna Kurpińska[1], Anna Bar[1], Izabela Czyzynska-Cichon[1], Łukasz Mateuszuk[1], Elżbieta Buczek[1], Barbara Sitek[1], Aleksandra Kopacz[3], Anna Grochot-Przeczek[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]Department of Medical Biotechnology, Faculty of Biochemistry Biophysics and Biotechnology, Jagiellonian University, Gronostajowa [7]a, [30-387], Krakow, Poland

Abstract:

Sepsis, known as a heterogenous disease caused by infection, is classified as one of the major health problems in developed countries. Interestingly, most laboratory experiments are based on young animal models whereas sepsis is largely a disease of the elderly.

The main aims of the research are **a)** to comprehensive assess the endothelial phenotype and organ injury in LPS-challenged young and aging mice and **b)** to demonstrate that the inactivation of the expression of the Nrf2 transcription factor in the endothelium may recapitulate increased susceptibility to LPS-induced endothelial dysfunction and organ injury in aging mice.

The experiments will be performed in 3 and 18-month-old C57Bl/6 mice and 3-5-month-old genetically modified Nrf2floxxVE-cad-Cre+ mice. In the study endotoxemia will be induced by a single i.p. administration of LPS at a dose of 3 mg/kg b.wt.

The research plans include a comprehensive characterization of the pattern of protein changes observed in plasma of studied mice using the innovative microLC/MS-MRM method, which will constitute prognostic/diagnostic value in sepsis. Moreover, the assessment of endothelial function in vivo using MRI method in thoracic and abdominal aorta will be also executed. The biochemical parameters of organ damage and systemic inflammatory parameters will be also investigated.

We assume that the obtained research results using mainly biomarker-oriented methodology and MRI method will be helpful in understanding the mechanisms underlying the pathophysiological changes of the vessels induced by age and hopefully will enable in the future the development of new therapeutic approaches dedicated to elderly patients.

Acknowledgments:

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1.8.

Vascular function in a rat model of autosomal dominant polycystic kidney disease

Baoer Liu[1], Tobias Mayer[1], Patricia Kraft[1], Jana Heigwer[2], Tilmann Clanget[2], Maik Brune[3], Alex Ali Sayour[1,4], Franz Schaefer[3], Matthias Karck[1], Gábor Szabó[1,5], Sevil Korkmaz-Icöz[1,5]
[1]Department of Cardiac Surgery, Heidelberg University Hospital, [69120] Heidelberg, Germany
[2]Division of Pediatric Nephrology, University Hospital Heidelberg, [69120] Heidelberg, Germany
[3]Department of Medicine I and Clinical Chemistry, University Hospital Heidelberg, [69120] Heidelberg, Germany
[4]Heart and Vascular Center, Semmelweis University, [1122] Budapest, Hungary
[5]Department of Cardiac Surgery, University Hospital Halle (Saale), [06120] Halle, Germany

Abstract:

Objective: Vascular abnormalities are the most important non-cystic complications in polycystic kidney disease (PKD). Endothelial dysfunction and oxidative stress are evident in patients with autosomal dominant PKD (ADPKD). The aim of the study was to evaluate vascular function in rat model of ADPKD.

Methods: Thoracic aortic rings from six month-old PKD (n=10; 470±24g) and age-matched control (n=13; 460±14g) male rats were used 1) to evaluate both contractile/relaxation responses ex vivo using organ bath experiments and 2) to study aortic morphometry assessed by hematoxylin&eosin staining.

Results: Although there was no significant difference in body weight, serum creatinine levels were increased in the PKD rats compared to controls (2.02±0.24mg/dl vs. 0.46±0.01mg/dl, $p<0.05$). Compared to controls, PKD rats showed decreased maximum relaxation to acetylcholine (55±2% vs. 77±1%, $p<0.05$), indicating impaired endothelial function. Additionally, in PKD aortas the concentration-response curve to sodium nitroprusside, an endothelium-independent vasodilator, was shifted to the right compared to controls (pD2-value: -log50% maximum response: 7.7±0.1 vs. 8.0±0.1, $p<0.05$). Contractile responses to phenylephrine (3.0±0.1g vs. 3.4±0.1g, $p<0.05$) and high potassium (3.3±0.1g vs. 3.7±0.1g, $p<0.05$) were reduced in the PKD-group compared to controls. Morphometrical analysis revealed that wall thickness and wall cross-sectional area normalized to body weight, and the wall:lumen area ratio were significantly higher in PKD-aortas compared to controls.

Conclusions: Six month-old ADPKD rats show endothelial dysfunction, impaired smooth muscle contraction and relaxation, and changes in aortic morphometry in a non-renal vascular territory. The rat model of ADPKD can be used to identify novel targets for the treatment of this disease.

1.9.

The impact of long-term social isolation on the anxiety and depression level and the activation of endothelial mechanisms of vascular dysfunction

Anna Kurpińska[1], Joanna Suraj-Prażmowska[1], Ewa Niedzielska-Andres[2], Jakub Jurczyk[3], Bartosz Pomierny[3], Józef Mizera[2], Klaudia Szklarczyk-Smolana[4], Justyna Mojsa-Kaja, Agnieszka Zakrzewska[1], Maria Walczak[2], Stefan Chłopicki[1]

[1]Jagiellonian Centre for Experimental Therapeutics, Jagiellonian University, Krakow, Poland
[2]Chair and Department of Toxicology, Jagiellonian University Medical College, Krakow, Poland
[3]Department of Toxicological Biochemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Krakow, Poland
[4]Department of Neurobiology and Neuropsychology, Institute of Applied Psychology, Jagiellonian University, Krakow, Poland

Abstract:

Social isolation and loneliness have the possible causal role in the development of loneliness-associated cardiovascular diseases. Despite the existing evidence that social isolation affects the endothelial function, the dynamics of the process remains poorly explored. Some studies reported lower levels of nitric oxide, inflammation, and increase in endothelial permeability after social isolation [BMC Medicine 2020:18:305], highlighting the complexity of the problem.

The aim of research is to verify how social isolation, accompanied by increased anxiety and depression, contributes to the activation of mechanisms related to vascular endothelial dysfunction. The animal studies were performed on Wistar male, 6 months-old rats. Experimental rats were kept solitarily for 3, 5, and 8 weeks. Respective control animals were kept in groups. Metabolomic and proteomic analysis were planned in plasma and aorta.

The observed changes suggest that the stress related to isolation early affected endothelium function, however further studies have been undertaken to verify this hypothesis. Metabolites of nitric oxide – nitrates, were impaired both in the aorta and plasma in rats isolated for three weeks. This tendency was also noticed for nitrites in plasma but not in the aorta. Interestingly, at this time-point in the plasma lower levels of SDMA, ADMA and NMMA were noticed and further decreased until the 8th week of isolation. These changes were accompanied by unaltered arginine, ornithine and citrulline levels.

In order to further characterize the time-dependent changes and determine the processes accompanying and responsible for the development of endothelium dysfunction targeted proteomic and metabolomic LC-MS/MS analysis will be performed.

Acknowledgments:

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1.10.

Vascular inflammation induced by IL-1β causes endothelial dysfunction and changes in vascular metabolism in the isolated murine aortic rings ex vivo

Agnieszka Karaś[1], Patrycja Kaczara[1], Anna Kurpińska[1], Janusz Pyka[1], Stefan Chłopicki[1,2]
[1]Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Krakow, Poland;
[2]Chair of Pharmacology, Faculty of Medicine, Jagiellonian University Medical College, Krakow, Poland

Abstract:

Vascular inflammation is one of the key drivers of atherosclerosis and cardiovascular diseases and is associated with endothelial dysfunction and arterial stiffness, phenotypes that predict morbidity and mortality. Yet, it is still ambiguous how inflammatory activation of vascular wall cells affects their bioenergetics in situ, as in most cases metabolism of the vascular wall is studied in isolated endothelial or vascular smooth muscle cells cultured alone. In this study, we aimed to address the question of how acute vascular inflammation influences bioenergetics of vascular wall on a tissue level and how is it linked to endothelial dysfunction. For that reason, we have applied the new approach of analysis of vascular bioenergetics in aortic rings ex vivo using Seahorse XFe96 Extracellular Flux Analyzer. We have examined the influence of acute inflammation induced by ex vivo stimulation with IL-1β on vascular metabolism with Seahorse and endothelial function using a wire myograph. The results have shown that IL-1β increases basal respiration linked to ATP production and glycolysis. Interestingly, metabolic activation seems to precede severe endothelial dysfunction caused by IL-1β. To confirm that impairment of vasorelaxation is NO-dependent, we have applied the EPR technique to measure NO production. Moreover, we have compared the functional and metabolic vascular response to acute inflammation in aged, healthy mice compared to young mice. Altogether, this study has provided new insight into vascular bioenergetic response to proinflammatory stimuli and its connection with vascular function considering the influence of vascular ageing.

Acknowledgments:

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1.11.

SIRT1 regulation of adipocyte calcium channel activity and perivascular adipose tissue modulation of vascular tone

Daniels Konja[1,2], Yu Wang[1,2]
[1]State Key Laboratory of Pharmaceutical Biotechnology and the
[2]Department of Pharmacology and Pharmacy, The University of Hong Kong, Hong Kong SAR, China.

Abstract:

Backgrounds: Adipose tissues play a crucial role in systemic energy homeostasis. Perivascular adipose tissue (PVAT) surround the vasculature, and are critically involved in regulation of vascular tone. Mitochondria play an important buffering role in intracellular calcium homeostasis, especially at the mitochondria-associated ER membrane, by utilizing calcium as a positive effector of the electron transport chain for ATP production. Adipose SIRT1 induces increases in mitochondria ATP output due to increased interaction with ER, but mitigates the increased ROS production via activation of UPRmt to enhance the overall adipose tissue metabolic function. The present study investigated the impact of the SIRT-mediated increased interaction between ER and mitochondria on adipose tissue mitochondria Ca2+ concentration, and how this affects PVAT regulation of vascular tone.

Methods and results: Calcium sensitive probes were used to measure calcium concentration in mitocondria isolated from PVAT of mice with selective overexpression of human SIRT1 in adipoe tissues (Adipo-SIRT1) and their wild type (WT) littermates. The calcium concentration and subsequent ATP output in mitochondria of Adipo-SIRT1 PVAT was significantly higher when compared to that of the WT littermates. Also, gene expression analysis of intracelluar calcium channels showed significantly upregulated SERCA, IP3R, and RyR complexes in PVAT of Adipose-SIRT1 compared to WT. Wire myography showed that, Adipo-SIRT1 PVAT had enhanced anticontractile activity conpared to WT PVAT. However, pharmacological inhihibition of RyR, but not IP3R channel significantly reduced SIRT1-mediated mitochondria calcium concentration, prevented UPRmt, and abolished PVAT anticontractile activity of Adipo-SIRT1 mice. SIRT1 differential regulation of triadin, a component of the RyR complex, was found to mediate the mitochondriaL calcium uptake from the ER and the subsequent PVAT anticontractile activity.

Conclusion: SIRT1 regulates RyR complex to modulate mitochondrial function and enhance PVAT anticontractile activity.

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1.12.

Vascular lipid droplets formed in response to TNF, hypoxia or OA: biochemical composition and prostacyclin generation

Marta Z. Pacia[1], Natalia Chorazy[1], Magdalena Sternak[1], Kamila Wojnar-Lason[1], Stefan Chlopicki[1,2]
[1]Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, 14 Bobrzynskiego Str., 30-348 Krakow, Poland
[2]Chair of Pharmacology, Jagiellonian University, [16] Grzegorzecka Str., [31-531] Krakow, Poland

Abstract:

Biogenesis of lipid droplets (LDs) in various cells plays an important role in various physiological and pathological processes. However, LDs function in endothelial physiology and pathology is not well understood.

In the present work, the formation of LDs and prostacyclin (PGI2) generation was investigated in the vascular tissue of isolated murine aorta activated by pro-inflammatory factors: tumor necrosis factor (TNF), lipopolysaccharides (LPS), angiotensin II (AngII), hypoxic condition or oleic acid (OA).
The abundance, size and biochemical composition of LDs was characterized based on Raman and fluorescence imaging. Blockade the lipolysis by the adipose triglyceride lipase (ATGL) delayed LDs degradation and simultaneously blunted PGI2 generation in aorta treated by all tested pro-inflammatory stimuli. The analysis of Raman spectra of LDs in the isolated vessels stimulated by TNF, LPS, AngII or hypoxia uncovered that they were all rich in highly unsaturated lipids and had a negligible content of phospholipids and cholesterols. Additionally, by comparing the Raman signature of endothelial LDs under hypoxic or OA-overload conditions in the presence or absence of atglistatin, it was shown that atglistatin did not affect the biochemical composition of LDs. Altogether, independently whether LDs was induced by pro-inflammatory stimuli, hypoxia or oleic acid, and whether displayed highly unsaturated or less unsaturated composition, LDs formation was invariably associated with ATGL-dependent PGI2 generation. In conclusion, vascular LDs formation and ATGL-dependent PGI2 generation represent a universal response to vascular pro-inflammatory insult.

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1.13.

Electron and fluorescence microscopies in the study of endothelial glycocalyx in isolated blood vessels

Natalia Chorazy[1], Marta Z. Pacia[1], Stefan Chlopicki[1,2]
[1]Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, 14 Bobrzynskiego Str., 30-348 Krakow, Poland
[2]Chair of Pharmacology, Jagiellonian University, [16] Grzegorzecka Str., [31-531] Krakow, Poland

Abstract:

The endothelial glycocalyx is a negatively charged net of carbohydrates and proteins anchored to the endothelium. This structure fulfills multiple functions such as controlling vascular tone, participating in mechanotransduction, or modulating vascular homeostasis. However, the most significant role of the glycocalyx is acting as a protection of the endothelium, due to its unique position in the vessels [1]. It has been shown that pro-inflammatory factors such as TNF, interleukins, or hypoxia cause disruption to the glycocalyx layer, leading to endothelial dysfunctions[2].

According to the glycocalyx's delicate structure, imaging its construct is difficult. Currently, two main methods are used: transmission electron microscopy and atomic force microscopy, but these methods are not flawless and have few limitations [3]. Here, we are developing
a new comprehensive method for glycocalyx imaging based on scanning electron microscopy and fluorescence microscopy. The aforementioned methods are applied to the endothelial glycocalyx within isolated blood vessels, which partially enable the maintenance of vessels' functionality and metabolism.

In conclusion, the endothelial glycocalyx is an essential structure for general homeostasis, but its methods of imaging in (dys)functional conditions are still challenging. The presented idea of the new approach to glycocalyx visualization may highly contribute to future glycocalyx research.

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1.14.

Human CD73-derived adenosine in pigs endothelial cells – protective role in the xenotransplant rejection

Ada Kawecka[1], Foy N Osborne[2], Ada Yuen[2], Michal R Smolenski[3], Gabriela Harasim[1], Alicja Braczko[1], Zain Khalpey[4], Kiran Parmar[5], Charlotte Lawson[2], Satsuki Fukushima[2], Ken Suzuki[2], Roberto Giovannoni[6], Marialuisa Lavitrano[7], Magdi H Yacoub[2], Barbara Kutryb-Zajac[1], Ryszard T Smolenski[1]

[1] Department of Biochemistry, Medical University of Gdansk, 80-211 Gdansk, Poland [2] Heart Science Centre, Harefield Hospital, Harefield, U.K. [3] Department of Biochemistry, Medical University of Warsaw, Warsaw, Poland [4] Division of Cardiac Surgery, Department of Surgery, Banner University Medical Center, [5] College of Medicine, University of Arizona, Tucson, USA [5] Department of Haematology, Guys and St Thomas' NHS Foundation Trust, London, U.K. [6] Nutrafood Center and Department of Biology, University of Pisa, Pisa, Italy [7] Department of Surgical Sciences, University of Milano-Bicocca, Milano, Italy

Abstract:

Ecto-5'-nucleotidase (E5'N, CD73) is highly abundant on the surface of endothelial cells converting extracellular adenosine monophosphate (AMP) to cytoprotective adenosine. Thus, it regulates thrombosis and immune responses engaged in xenotransplant rejection. This study compares nucleotide metabolism in porcine endothelial cells with or without human E5'N expression and its effect on thrombotic and immune mechanisms of rejection after xenotransplantation. Porcine endothelial cells transfected with human E5'N (E5'N-PIEC) and human endothelial cells (HMEC-1) maintained elevated adenosine concentration after addition of AMP in both static and flow systems in comparison to mock-transfected (MT-PIEC) and non-transfected PIEC cells. Platelet adhesion to E5'N-PIEC was reduced and media conditioned with E5'N-PIEC attenuated platelet aggregation and lymphocyte proliferation. Lymphocyte adhesion to E5'N-PIEC was also diminished in comparison to MT-PIEC. All these effects were dependent on E5'N activity as they were reversed by E5'N inhibition or adenosine receptor antagonists. We have shown that overexpression of human E5'N on porcine endothelial cells induced multiple cytoprotective adenosine-derived mechanisms that can be relevant in xenogeneic environment. These effects included not only the attenuation of natural killer cell toxicity as previously shown but also antiplatelet effects, the prevention against lymphocyte proliferation and adhesion to endothelium as well as the protection from oxidative damage.

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1.15.

Heterogeneity of endocytosis pathways in endothelial cells as a novel approach for targeted vascular therapy

Edyta Kus[1], Anna Kurpinska[1], Agnieszka Pełesz[1], Kamila Wojnar-Lasoń[1], Małgorzata Janik-Hazuka[3], Szczepan Zapotoczny[3], Stefan Chlopicki[1,2]

[1] Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Bobrzynskiego 14, 30-348 Krakow, Poland
[2] Department of Experimental Pharmacology, Jagiellonian University Medical College, Grzegorzecka 16, 31-531 Krakow, Poland
[3] Faculty of Chemistry, Jagiellonian University, Gronostajowa 2, 30-387 Krakow, Poland

Abstract:

Endothelial cells (ECs) are highly heterogeneous, however, it remains unexplored whether ECs heterogeneity determine also the ECs dominant endocytosis pathway, giving the ability to find a new, vascular-targeted strategies in the treatment of macro- and microvascular diseases.

The aim of this study was to define leading endocytosis pathways in organ-specific, macro- and microvascular ECs and determine the organ-specific-delivery and endocytosis mechanism of hyaluronic acid-based nanocapsules (HyC12-NCs) by ECs as a proposal of EC-specific drug delivery system in vascular treatment.

The study was conducted in vitro using primary macrovascular (HAEC) and microvascular (hLMVEC, LSEC) endothelial cells. The ECs endocytosis heterogeneity and mechanism of HyC12-NCs up-take with involving NCs "corona" impact were assessed using proteomic and fluorescence microscopy techniques. The characteristic of HyC12-NCs was assessed using microflow cytometer ApoGee.

Results showed enhanced endocytosis of transferrin (selective ligand for clathrin mediated endocytosis) by LSEC and albumin (ligand for caveolin mediated endocytosis) by hLMVEC, while in HAECs increased up-take of both, albumin and transferrin was observed. Proteomic analysis showed up-regulation of proteins involved in clathrin coated vesicles in TSEC and caveolae in hLMVECs. The HyC12-NCs showed to be preferentially internalized by hLMVEC and in less extend through other ECs. The HyC12-NCs endocytosis occurred via caveolin mediated endocytosis and was strongly depended on FBS presence.

The organ- and vascular-specific ECs show endocytic heterogeneity which can be used as a novel strategy in targeted therapy of endothelial dysfunction. As that HyC12-NCs, being preferentially internalized via caveolin-mediated endocytosis by lung ECs may act as an effective targeted drug delivery system for the treatment of endothelial dysfunction.

1.16.

Investigating the important role of inflammatory factors in the induction of endothelial-mesenchymal transition in vitro.

Ebrahim H Maleki[1], Marta Smeda[1], Anna Gdula[1], Stefan Chlopicki[1,2]
[1] Jagiellonian University, Jagiellonian Centre for Experimental Therapeutics, Krakow, Poland
[2] Jagiellonian University Medical College, Department of Pharmacology, Krakow, Poland

Abstract:

Endothelial-mesenchymal transition (EndMT), a cellular differentiation in which endothelial cells (ECs) lose their properties and differentiate into mesenchymal cells, has been observed in various pathological states in adults, including cancer progression and metastasis. Although TGFβ has been shown to play a central role in the induction of EndMT, its action could be potentiated by other pro-inflammatory factors, In this study, we aimed to investigate the effects of TGFβ itself, and in combination with IL-1β, a potent pro-inflammatory cytokine, in the induction of EndMT in mouse lung vascular endothelial cells (MLVECs) in vitro. We assessed levels of mesenchymal cell-specific proteins by Western blot. As the expression of the mesenchymal markers in the progression of EndMT is accompanied by the progressive reduction and the eventual loss of endothelial cell-specific proteins, we also evaluated selected endothelial markers. Moreover, since matrix metalloproteinases (MMPs) are known as mesenchymal cell-specific markers, and their activity as mediators of ECM degradation is associated with cancer invasion, we measured the general activity of MMP enzymes in the conditioned media by MMP activity assay kit. Our data indicate that the combination of TGFβ1 and IL-1β induces EndMT in mouse microvascular endothelial cells more potently than TGFβ only. Moreover, the MMP activity assay demonstrates explicit differences between the activity of matrix metalloproteinase enzyme in the presence of inflammatory factors and untreated cells. Therefore, it can be concluded that the combination of TGFβ1 and IL-1β can induce EndMT. It can be suggested that targeting these regulators will help us to develop therapeutic strategies for EndMT and its related diseases, such as cancer soon.

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1.17.

ToF-SIMS-based lipid composition research of endothelial cells and isolated EVs under normoglycemic and hyperglycemic conditions

Magdalena E. Marzeca, Carina Rzącaa and Ewa Ł. Stępieńa
[a] Department of Medical Physics, M. Smoluchowski Institute of Physics, Faculty of Physics, Astronomy and Applied Computer Science, Jagiellonian University in Krakow, Poland

Abstract:

Hyperglycemia (HG) is a condition in which an excessive concentration of glucose circulates in the blood plasma. It affects people who have impaired glucose tolerance. It is important to recognise symptoms and treat HG at the first stage to avoid serious health problems i.e. diabetic renal failure, retinopathy or neurological complications. Usually, HG symptoms develop slowly over weeks and complications are diagnosed even after years of HG deterioration.

In our study, we examined the potential importance of lipid signatures of extracellular vesicles (EVs) derived from endothelial cells (TIME) as prognostic markers of metabolic changes due to HG. We did a comparative analysis using secondary ion mass spectrometry with a time-of-flight analyzer (ToF-SIMS). TOF-SIMS is the method of choice that can be used to analyze biomolecules in membranous structures. It enables qualitative testing in a semi-native state without extraction, fixation or labelling. Here, cells were cultured in NG and HG conditions (35 mM of glucose). Low-pressure filtration and ultracentrifugation were used to separate EVs subpopulations: exosomes and ectosomes. For measurements, EVs in PBS solution were applied on cleaned silicon surfaces.

The results of the analysis include the comparison of cell lipid profiles and isolated EVs at high compared to normal glucose concentrations. The group of analyzed lipids includes fatty acids, sterols, and prenols. Differences in the composition of each of the analysed lipid groups were observed. Hyperglycemic culture conditions caused changes in the profile of cholesterol, vitamin E and several fatty acids in cells and EVs from their origin.

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1.18.

Application of PDMS-based microfluidic channels to study endothelium

Stefano Rocchetti[1]; Kasper Marchlewicz[2-3]; Marek Grosicki[1]; Michal Chudy[3]; Stefan Chlopicki[1-4]
[1] Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, 14 Bobrzynskiego Str., 30-348 Krakow, Poland.
[2] Faculty of Chemistry, Warsaw University of Technology, 3 Noakowskiego Str., 00-664 Warsaw, Poland.
[3] Faculty of Chemistry, University of Warsaw, 1 Pasteura Str., 02-093 Warszawa, Poland.
[4] Chair of Pharmacology, Jagiellonian University, 30-348 Krakow, Poland 30-348 Krakow, Poland.

Abstract:

The endothelium is a major organ formed by endothelial cells that line all the vessels. It is the responsible, within others, for regulating the vascular tone (vasodilatory and vasoconstricting effects, using Nitric Oxide - NO, and Endothelin-1), vascular permeability (e.g., adhesion molecules), and adhesion of platelet and leukocytes (e.g., adhesion molecules ICAM1). A dysfunctional endothelium, mostly characterized by impaired NO bioavailability, is involved in almost all cardiovascular diseases.

The endothelium achieves fine-tuned regulations through complex integrations of stimulation-response patterns, which involve cascade of molecules. A key stimulus sensed by endothelial cells is the fluid shear stress generated by the blood flow. Endothelial cells are very sensitive to changes in shear stress levels and patterns. Most of studies performed in vitro use standard cell culture techniques, which can give information on the cell response and behavior, but lack key features like the fluid shear stress that is present in in vivo conditions. For this reason, the results obtained in laboratory not always reflect the ones obtained in vivo.

Microfluidic devices are diffusing in biological research, thanks to their capabilities of controlling features like surface micro-structures and fluid dynamics. Devices that allow to obtain organ-on-a-chip by co-culturing different cell types, or which demonstrate the cellular increased production of NO in response to shear stress are some of the milestones towards bridging the gap between research and clinical applications.

In this study, microfluidic devices are used to expose endothelial cell to shear stress, and unlock cellular responses that until now were hampered by the unnatural in vitro environment in which cells are immersed, helping to achieve responses closer to in vivo behaviors.

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1.19.

Multimodal imaging of endothelial pathology models in vitro and ex vivo

Basseem Radwan[1], Ewelina Matuszyk[2], Stefano Rocchetti[2], Magdalena Sternak[2], Stefan Chlopicki[2,3], Malgorzata Baranska[1,2]

[1] Faculty of Chemistry, Jagiellonian University, 2 Gronostajowa Str., 30-387 Krakow, Poland.
[2] Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, 14 Bobrzynskiego Str., 30-348 Krakow, Poland.
[3] Chair of Pharmacology, Jagiellonian University, 30-348 Krakow, Poland 30-348 Krakow, Poland.

Abstract:

Raman imaging is regarded as a powerful tool to investigate chemical changes in endothelial cells (EC) and identify spectroscopic markers of endothelial dysfunction (ED)[1]. Although label-free Raman imaging allows obtaining comprehensive information on the samples' chemical composition, Raman reporters have been shown to improve sensitivity and selectivity[2].

Changes in lipids distribution and content is a well-known marker for pathological processes[3,4]. We utilized Raman imaging to investigate the changes in EC lipids following inflammation, introducing a new Raman probe for subcellular lipidic structures. Astaxanthin (AXT) displays resonantly enhanced Raman bands at ca. 1520, and 1159 cm^{-1} when excited with a 532nm laser line. We demonstrated that AXT allows the imaging of lipid-rich structures like lipid droplets and nuclear envelope, using relatively low (3mW) laser power, presenting an advantage over label-free imaging of lipids, especially for fragile samples like live cells[5]. This was applied in ECs from various organs, showing that AXT is utilizable as a lipids probe in ECs despite their heterogeneity[6].

5-ethynyl-2'-deoxyuridine (EdU) is a thymidine analogue that incorporates into newly synthesized DNA during replication and is used for proliferation detection after its copper-catalysed cycloaddition reaction "Click Chemistry" with a fluorescent azide[7,8]. Due to the alkyne tag, EdU gives a Raman band around 2122 cm^{-1} in the "silent region" where there are no interferences in the signal from other biological compounds[9]. We studied the effects of cycloheximide (CHX) and doxorubicin (DOX) on EdU-tagged ECs. CHX and DOX pre-treated cells showed a significantly lower EdU signal from their nuclei and a lower number of EdU-positive cells. Fluorescent detection of Alexa Fluor® 488-tagged EdU was used as a reference method, confirming the Raman findings[10]. Moreover, the Raman-based approach omits cell permeabilization step and allows EdU detection in live cells and without the need for additional dyes. Finally, using Raman and fluorescence imaging approaches, EC and SMC regeneration following insult was detected in isolated murine aorta after ex vivo incubation with EdU[10].

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1.20.

Bisphosphonates disturb calcium homeostasis in isolated endothelial mitochondria

Budzinska Adrianna, Wojcicki Krzysztof, Jarmuszkiewicz Wieslawa
Laboratory of Mitochondrial Biochemistry, Department of Bioenergetics, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

Abstract:

Osteoporosis as the most common bone-metabolic disorder in postmenopausal women poses a threat in successfully aging societies. One of the drugs used in anti-resorptive treatment of osteoporosis are bisphosphonates. The mechanism of their action is to block the mevalonate pathway and inhibit osteoclast-mediated bone resorption. However, the other product of this pathway is coenzyme Q, an important antioxidant and mitochondrial respiratory chain electron carrier. Endothelial cells are the first to be exposed to these drugs during intravenous bisphosphonate therapy. Endothelial dysfunction associated with mitochondrial impairment can cause cardiovascular disease. We studied a direct effect of two nitrogen-containing bisphosphonates, alendronate and zoledronate on respiratory function and membrane potential of mitochondria isolated from human umbilical vein endothelial cells (EA.hy926 cell line). We found that both drugs did not adversely affect mitochondrial function (phosphorylating and nonphosphorylating respiration, ADP/O ratio, respiratory control ratio) in vitro at lower concentrations. However, after prolonged (15 minutes) incubation with zoledronate, the mitochondria showed an increase in nonphosphorylating respiration accompanied by a decrease in membrane potential, indicating mitochondrial uncoupling. In addition, the addition of zoledronate or alendronate to isolated endothelial mitochondria resulted in disturbances in calcium ion uptake and release, indicating inhibition of the calcium uniporter. Our results demonstrate a negative effect on calcium homeostasis in isolated endothelial mitochondria.

Acknowledgments:

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1.21.

Statins and bioenergetic function of mitochondria isolated from human endothelial cells and rat brain

Wojcicki Krzysztof, Budzinska Adrianna, Jarmuszkiewicz Wieslawa
Laboratory of Mitochondrial Biochemistry, Department of Bioenergetics, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland

Abstract:

The purpose of this research was to elucidate the direct effects of popular blood cholesterol-lowering drugs used to treat cardiovascular diseases on respiratory function, membrane potential, and reactive oxygen species formation in mitochondria isolated from human umbilical vein endothelial cells (EA.hy926 cell line) (pravastatin and atorvastatin)# and rat brain (atorvastatin and simvastatin). Hydrophilic pravastatin did not significantly affect endothelial mitochondria function. In contrast, in endothelial and brain mitochondria, hydrophobic simvastatin and/or calcium-containing atorvastatin induced a loss of outer mitochondrial membrane integrity, an increase in hydrogen peroxide formation, and reductions in maximal (phosphorylating or uncoupled) respiratory rate, membrane potential and oxidative phosphorylation efficiency (ADP/O ratio and respiratory control ratio).

The action of statins indicates changes in the functioning of studied mitochondria by impaired functioning at the level of the respiratory chain, probably in complexes I and III, and at the level of ATP synthesis. The effect of simvastatin appears to be weaker than that of atorvastatin at a given concentration. The stronger effect of atorvastatin on the brain mitochondria was highly dependent on the calcium and led to the disturbance of mitochondrial calcium homeostasis. The conclusions from this study indicate that hydrophobic statins, widely used as drugs for the treatment of hypercholesterolemia, have a direct negative effect on isolated endothelial and brain mitochondria.

Broniarek I, Jarmuszkiewicz W (2018) Atorvastatin affects negatively respiratory function of isolated endothelial mitochondria, Archives of Biochemistry and Biophysics, 637, 64-72

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1.22.

Attenuation of mitochondrial BK_{Ca} channel activity during stress induced senescence of vascular smooth muscle cells

Barbara Kalenik[1], Agata Głuchowska[2], Antoni Wrzosek[1], Bogusz Kulawiak[1], Piotr Bednarczyk[3], Grażyna Mosieniak[2], Adam Szewczyk[1]

[1)] Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology PAS, 02-093 Warsaw
[2)] Laboratory of Molecular Bases of Aging, Nencki Institute of Experimental Biology PAS, 02-093 Warsaw
[3)] Department of Physics and Biophysics, Institute of Biology, Warsaw University of Life Sciences -SGGW, 02-776 Warsaw

Abstract:

Cellular senescence is stress response that is induced upon different endo- or exogenous factors. Senescent cells remain viable and metabolically active. They accumulate in the tissue of aging organism but also during development of age-related diseases. Importantly, the presence of senescent vascular smooth muscle cells and endothelial cells was recognized in atherosclerotic plaque. Moreover, elimination of senescent cells significantly attenuates development of atherosclerosis progression. One of the important features of senescent cells is changes in mitochondrial functioning that influence development of senescence. Mitochondria were also shown to modulate proinflammatory phenotype of senescent cells, known as senescence associated secretory phenotype (SASP), which support development of age-related diseases. Although numerous studies analyzed mitochondrial organization and metabolism in senescent cells, still little is known about involvement of mitochondrial potassium channels in this process. Thus, the aim of our study was to estimate potential changes in mitochondrial large conductance calcium-activated (BKCa) channel in vascular smooth muscle cells undergoing stress induced cellular senescence. Human aortic smooth muscle cells were treated with single dose of H2O2 at the concentration that is not toxic for the cells but induce senescence within seven days after treatment. Basic senescence markers were confirmed, change in mitochondrial network visualized and transcriptional differences in genes encoding proteins involved in the biogenesis and function of the mitochondria described. Moreover, we report typical activity (measured by patch-clamp) of mitochondrial BKCa in control, but not in senescent cells. Additionally, we observed decreased of potassium channel protein level regardless of invariant specific-mRNA expression.

Acknowledgments:

This research was funded by the National Science Center, grants nos. 2019/34/A/NZ1/00352 and OPUS UMO-2018/31/B/NZ3/02931.

1.23.

High content screening in the detection of antiviral drug's adverse effect on endothelial cells

Marek Grosicki, Kamila Wojnar-Lasoń, Marta Stojak, Sylwester Mosiołek, Łukasz Mateuszuk, Stefan Chłopicki
Jagiellonian Centre for Experimental Therapeutics (JCET UJ)

Abstract:

Since the first antiviral drugs were successfully introduced into clinics, we have a developed variety of potent drugs, directed against the most harmful viral infections (like HIV, HBV, HCV, HSV, and influenza). Unfortunately, as shown during the anti-HIV HAART treatment, these drugs have determinantal effects on endothelial cells. Here, we present how high-content screening can be used to investigate the toxic effects of antiviral drugs on endothelial cells. For the experiments, the following drugs were chosen (remdesivir, zidovudine, efavirenz, abacavir, lopinavir, ritonavir, and PF-008355231). The effect of drugs was investigated against two human endothelial cell lines (hLMVEC and HAEC). The following cellular properties were studied: overall metabolism, number of cells, cell and nuclei morphology, mitochondrial membrane potential, ROS generation, lipid droplet and lysosome content, autophagy and pro-inflammatory markers. The high content methodology allowed to differentiate the activity of studied drugs. We have detected that PF-008355231, zidovudine, and abacavir were not toxic for endothelium, whereas, efavirenz, lopinavir, ritonavir and remdesivir decreased the number of cells, as well as their metabolic activity. Drug toxicity was followed by cell morphological alternation, the decrease of mitochondrial membrane potential, alternation in lipid droplet synthesis, and ROS generation. Some drugs increased cellular lysosomal content and induced autophagic response. Interestingly, the cellular response to drugs was not homogeneous, but rather drug-specific. Moreover, we have detected different drug responses in hLMVEC and HAEC. These findings are promoting new research on the detailed drug toxicity mechanism, that in future can result in more endothelial-friendly drug development.

Acknowledgments:

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1.24.

Activation of mitophagy in endothelial cells by antiviral protease inhibitors

Łukasz Mateuszuk[1], Marek Grosicki[1], Marta Stojak[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:

Introduction: mitophagy is a mitochondrial-specific kind of autophagy, responsible for the elimination of damaged and dysfunctional mitochondria, which allows the maintenance of bioenergetic balance and reduces the production of reactive oxygen species, thus affecting cell survival. Mitochondrial homeostasis is crucial for the proper function of endothelial cells, forming the vascular endothelium, which constructs the lining of the vascular wall. Autophagy and mitophagy may be induced in cells by various groups of pharmaceuticals, including cationic amphiphilic drugs (CADs), antiviral agents, or PI3K/Akt/mTOR pathway modulators.

Materials and methods: mitophagy in HAEC and HLMVEC lines was studied using immunofluorescent staining, mitophagosome-specific fluorescent dyes, and confocal imaging. As mitophagy inducers, two antiviral protease inhibitors (PIs) used in HIV/AIDS and hepatitis C treatment were applied; lopinavir and ritonavir.

Results: short-term and long-term incubation with lopinavir and ritonavir increased the number of endothelial mitophagosomes, which was reflected by increased expression of autophagy marker LC3IIB and mitophagy-specific Parkin. Prolonged exposure to a higher concentration of PIs resulted in the upregulation of pro-inflammatory and pro-thrombotic markers; von Willebrand Factor (vWF) and ICAM-1.

Conclusions: enhanced mitophagy at the early stage of PI treatment may be considered an anti-inflammatory and anti-apoptotic mechanism triggered by the detrimental effect of lopinavir and ritonavir on mitochondrial homeostasis.

1.25.

Adenosine Deaminase Activity and Microvascular Dysfunction in Coronavirus Disease-2019

Alicja Braczko[1], Agata Jedrzejewska[1], Marika Franczak[1], Ada Kawecka[1], Marzena Romanowska-Kocejko[2], Ewa M. Slominska[1], Ryszard T. Smolenski[1], Marcin Hellmann[2], Barbara Kutryb-Zajac[1]
[1] Department of Biochemistry, Medical University of Gdansk, 80-211 Gdansk, Poland
[2] Department of Cardiac Diagnostics, Medical University of Gdansk, 80-211 Gdansk, Poland

Abstract:

Endothelial cells are a preferential target of SARS-CoV-2 infection resulting in widespread endotheliitis. Previously, we have reported that vascular adenosine deaminase (ADA) activity may serve as a biomarker of endothelial activation and vascular inflammation. In addition, ADA play a critical role in the immune system regulation. In this study we investigated circulating activities of ADA isoenzymes in patients after mild COVID infection (postCOVID) and correlated them with the parameters of inflammatory and endothelial dysfunction as well as with microcirculation assessment. Interestingly, total circulating ADA activity was lower in the serum samples of postCOVID patients in comparison to controls. This derived from the reduced activity of ADA2 isoenzyme, while ADA1 activity was slightly higher in postCOVID group. Endothelial and microvascular dysfunction observed 2 months after SARS-CoV-2 infection correlated with circulating ADA isoenzyme profile. Moreover, in vitro studies revealed that human lung microvascular endothelial cells exposed to post-COVID patients' serum change their adenosine metabolism ecto-enzyme pattern as well as their ability to the neutrophils' adhesion. These results underline that endothelial ADA isoenzymes' activities are profoundly deregulated after COVID-19.

Acknowledgments:

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1.26.

In search of COVID-19 severity predictors: activation of NA-MNA pathway in hospitalised patients

Fedak Filip Aleksander[a], Kij Agnieszka[a], Kania Aleksander[b], Lichołai Sabina[c], Polok Kamil[b], Celejewska-Wójcik Natalia[b], Górka Karolina[b], Stachura Tomasz[b], Chłopicki Stefan[a,d]

[a] Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Kraków, Poland;
[b] Department of Pulmonology, 2nd Department of Internal Medicine, Faculty of Medicine, Jagiellonian University Medical College, Kraków, Poland
[c] Department of Internal Medicine, Jagiellonian University Medical College, Kraków, Poland
[d] Department of Pharmacology, Jagiellonian University Medical College, Kraków, Poland

Abstract:

Introduction:
Nicotinamide N-methyltransferase (NNMT) is an enzyme catalyzing transformation of nicotinamide (NA) to 1-methylnicotinamide (MNA). NNMT overexpression is a well-known biomarker in oncology, however it occurs also in states of endothelial dysfunction such as thrombosis, inflammation or pulmonary hypertension (Gao Y et al.; Drug Discovery Today, 2021). Due to its role in mentioned pathomechanisms, NA-MNA pathway was predicted to be a biomarker in course of viral respiratory infection COVID-19.
Aim: The aim of this study was to evaluate the potential of NA-MNA pathway as a severity predictor of COVID-19 course in hospitalized patients.

Materials & Methods:
Blood samples from patients with confirmed COVID-19 infection were collected at two time points: first at the day of admission to pulmonary ward and second after 7 days of hospitalization. Blood samples from control participants were collected at single time point. Concentrations of NA and MNA were determined using LC-MS/MS method. The MS/MS detection was carried out on a triple quadrupole mass spectrometer TSQ Quantum Ultra (Thermo Scientific, USA) applying SRM mode. Obtained results were analyzed in terms of clinical patient data.

Results & Conclusions:
In samples coming from SARS-CoV-2 patients, concentration of MNA was significantly higher than in the control patients. However, no significant changes between first and second time point were observed. Moreover, MNA level was not correlated with the course of disease severity. To sum up, NA-MNA pathway seems to be activated in early stage of COVID-19 infection. Further investigation of that phenomenon is required to better understand the role of NNMT in this viral infection.

Acknowledgments:

Authors acknowledge medical personnel of The University Hospital in Krakow for support in sample collection.

1.27.

Acute betacoronavirus infection results in persistent tissue inflammation and hepatic fibrosis in a mouse model

Gibler P.[1,2], Salzmann M.[3], Kral-Pointner J.[3], Wojta J.[1,3], Plasenzotti R. [2], Hohensinner P. J.[1,3].
[1] Ludwig Boltzmann Institute for Cardiovascular Research, Austria
[2] Core Facility Laboratory Animal Breeding and Husbandry, Medical University of Vienna, Austria
[3] Internal Medicine II, Medical University of Vienna, Austria
[4] Center for Biomedical Research, Medical University of Vienna, Austria

Abstract:

Introduction:
Controlled thrombosis contains and limits virus spreading but needs to be fibrinolytically resolved to prevent tissue damage. However, virus infections may alter the haemostatic and fibrinolytic environment. We analysed the immediate and long-term impact of a murine betacoronavirus (m-CoV) infection on the thrombotic and fibrinolytic system in the liver.

Methods:
C57Bl/6 mice were infected intranasally with m-CoV. Liver, lung and plasma samples were collected 2, 4, and 10 days after infection. Liver organ cultures were stimulated with TNFα, IL-4, or IFNγ. Thrombosis, inflammation, and fibrinolysis were analysed via histology, ELISA, and qPCR.

Results:
Liver virus titres were increased at day 4 and low at day 2 and 10. Plasminogen mRNA was elevated on day 2 with uPA and PAI-1 mRNA peaking at day 4. Hepatic thrombi were lowest on day 4. Inflammatory proteins were increased in plasma consistently at day 4 with no systemic inflammation at day 10. Organ culture experiments indicated that especially uPA and PAI-1 were induced by a proinflammatory milieu. In contrast, inflammatory markers within the liver including ICAM-1, VCAM-1 and P-selectin remained elevated at day 10. In addition, prothrombin increased at day 10. This inflammation was accompanied by significantly increased liver fibrosis.

Conclusion:
Virus infection caused systemic inflammation with high levels of, IFNγ and TNFα already at day 2 and reached the liver at day 4. Activation of plasminogen happened within the first 4 days with little thrombi observed in the liver. In vitro, IFNγ and TNFα were able to induce uPA and PAI in liver tissue, thereby explaining the fibrinolytic change in vivo. Moreover, we detected persistent tissue inflammation and massive fibrosis, although virus was already cleared in both lung and liver.

1.28.

Pharmacologic masking of the glycocalyx reduces binding of SARS-CoV-2 spike protein.

Marka F. T.[1], Salzmann M.[2], Haider P.[1], Wojta J.[1,2], Hohensinner P. J.1, [3].
[1] Ludwig Boltzmann Institute for Cardiovascular Research, Austria
[2] Internal Medicine II, Medical University of Vienna, Austria
[3] Center for Biomedical Research, Medical University of Vienna, Austria

Abstract:

Introduction:
Corona viruses are able to bind to ACE2 receptors, which are highly expressed in endothelial lung cells, arterial and vein endothelial cells and smooth arterial muscle cells. The binding is mediated via the corona spike protein. Besides direct binding to the receptor, different glycosaminoglycans of the glycocalyx play an important role during the attachment of the virus to the host. Calcium dobesilate (commercial name Doxium), was hypothesized to inhibit or minimize the interaction potential of the corona spike protein with the cellular glycocalyx due to its interaction with the charged chains.

Methods:
To determine the effect of calcium dobesilate on this general adhesion mechanism, we used human pulmonary microvascular endothelial cells (HPMEC), human umbilical vein endothelial cells (HUVEC), human umbilical vein smooth muscle cells (HUVSMC) and human tracheal epithelial cells (HTEpiC), stimulated with different concentrations of corona spike protein and calcium dobesilate. Binding was quantified by confocal microscopy (LSM700). To identify if the binding process of the spike protein is altered by the lack of certain glycosaminoglycans, different enzymes, (chondroitinase, heparinase and hyaluronidase) were used for enzymatic digestion of specific residues.

Results:
The microscopy showed that the corona spike protein was internalized by the cells and is located in/around the nucleus. When cells were incubated with hyaluronidase there was a significant reduction of attached spike protein. This could be also seen when cells were treated with chrondroitinase but not with heparinase. In the microscopy data, we observed a significantly lower fluorescence signal of the spike protein of cells which were treated with calcium dobesilate.

Conclusion:
The corona spike protein is able to bind to a cells surface via glycocalyx. This ability can be minimized by enzymatic digestion of the glycocalyx or by the use of calcium dobesilate which we hypothesize leads to a sterical hindrance of spike protein binding. Therefore, glycosaminoglycans play an important role during the attachment and internalization of the virus. By observing a significant reduction of attached spike protein to the cells, we showed that calcium dobesilate can minimize the ability of the spike protein to enter the cell via the glycocalyx.

1.29.

Gene expression alterations of different SARS-CoV-2 host factors in heart failure patients

Viktória E. Tóth[1,2,3], Tamás G. Gergely[1,2,3], Anikó Görbe[1], Zsófia Onódi[1,2,3], Ágota Apáti[4], Przemyslaw Leszek[5], Tamás Radovits[6], Béla Merkely[6*], Péter Ferdinandy[1,7,8*], Zoltán V. Varga[1,2,3 #*]

[1] Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary [2] HCEMM-SU Cardiometabolic Immunology Research Group, Budapest, Hungary [3] MTA-SE Momentum Cardio-Oncology and Cardioimmunology Research Group, Budapest, Hungary [4] Research Centre for Natural Sciences, Institute of Enzymology, Budapest, Hungary [5] Department of Heart Failure and Transplantology, Cardinal Stefan Wyszyński National Institute of Cardiology, Warszawa, Poland [6] Heart and Vascular Center, Semmelweis University, Budapest, Hungary [7] Pharmahungary Group, Szeged, Hungary [8] MTA-SE System Pharmacology Research Group, Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary

[*] These authors contributed equally

[#] Corresponding author: varga.zoltan@med.semmelweis-univ.hu

Abstract:

Objective:
COVID-19 was the most significant health crisis of the 21st century. ACE2 is the main entry receptor of SARS-CoV-2, but several other host factors have been identified. Heart failure (HF) is a major risk factor for mortality in COVID-19 patients, especially in the elderly. In this study, we aimed to investigate the expression of several SARS-CoV-2 host factors in the cardiovascular system and heart failure, to clarify potential mechanisms for the worse outcomes in HF patients.

Methods:
The mRNA expression levels of 16 host factor targets were analyzed by digital PCR in HUVEC cells, human iPSC-CMs and human AC16 cardiomyocytes (n=3).
Moreover, human hearts samples were investigated. 46 interventricular septum samples were used in the following groups: healthy controls (n = 14), ischemic cardiomyopathy (ICM, n = 16) and dilated cardiomyopathy (DCM, n = 16).

Results:
IFITM3, which is responsible for viral restriction, showed the highest expression on all human cardiovascular cell lines at normal culture conditions, while the key entry receptor, ACE2 showed negligible expressions.
In human heart failure samples, IFITM3 levels were significantly reduced in both DCM and ICM groups. Furthermore, the mRNA expression level of ACE2 was significantly increased and this elevated level correlated with the age of DCM patients.

Conclusions:
Elevated levels of ACE2 and reduced levels of IFITM3 may contribute to the higher mortality in COVID patients with heart failure. The expression of ACE2 in heart failure correlates with age, suggesting a potential mechanism for worse outcomes in elderly HF patients with SARS-CoV2 infection.

Acknowledgments:

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1.30.

Effect of edible insects (tenebrio molitor and gryllus assimilis) on the development of atherosclerotic lesions in apoe/ldlr-/- mice

Franczyk-Żarów M.[1], Hassen H.A.[1], Czosnowska B.[1], Strojewska A.[1], Škvorová P.[2], Pokhrel K.[2], Kouřimská L.[2], Kostogrys R.B.[1]
[1] Department of Human Nutrition and Dietetics, Faculty of Food Technology, University of Agriculture in Krakow; Poland
[2] Department of Microbiology, Nutrition and Dietetics, Czech University of Life Sciences Prague, Czech Republic

Abstract:

Edible insects are promoted as a source of protein with high nutritional value. Studies have shown that edible insects may have various health-related properties, e.g. anticancer, anti-obesity, antimicrobial, antioxidant, antihypertensive, cardio- and neuroprotective.

The aim of the study was to evaluate the effect of edible insects (Tenebrio molitor and Gryllus assimilis) on the development of atherosclerotic lesions in ApoE/LDLR-/- mice.

Animals were divided into 3 groups (n=10) and fed ad libitum for 10 weeks with following diets: AIN-93G (control), AIN-93G with 10% of Tenebrio molitor (TM) and AIN-93G with 10% of Gryllus assimilis (GA).

ApoE/LDLR-/- mice fed supplemented diets showed no significant differences in final body weight, heart or kidney weight compared to control. Liver weight (g/100 g b.w.) was significantly higher in the GA group compared to the TM. A significant decrease in ALT activity was found in the groups receiving insects. There were no significant differences in the lipid profile, glucose concentration, AST activity, nor the level of atherosclerotic lesions. There was a trend to the reduction in the number of atherosclerotic lesions in mice fed the diet supplemented with GA cricket. Tenebrio molitor larvae and Gryllus assimilis cricket significantly affected the proportion of individual fatty acids both in adipose tissue and in the liver.

The addition of edible insects (TM and GA) to the diet has no significant effect on the development of atherosclerotic lesions in ApoE/LDLR-/- mice, but may affect the liver profile. ALT activity and liver weight were significantly different in the GA group. esting a potential mechanism for worse outcomes in elderly HF patients with SARS-CoV2 infection.

1.31.

Effect of D-Allulose on atherosclerosis in ApoE/LDLR-/- mice

Kostogrys R.B.[1], Łanoszka K.[1], Ranasinghe N.[1], Franczyk-Żarów M.[1], Filipiak-Florkiewicz A.[2]
[1]Department of Human Nutrition and Dietetics, University of Agriculture in Krakow
[2]Department of Plant Products Technology and Food Hygiene, University of Agriculture in Krakow

Abstract:

D-Allulose, an epimer of D-fructose at the C-3 position is a monosaccharide naturally found in products such as raisins, figs, molasses, maple syrup. It has gained tremendous interest in the recent years for its significant health promoting potential e.g. antiobesity, anti-inflammation. It may be assumed that D-Allulose may also influence the atherogenesis.

The aim of the study was to evaluate the effect of D-Allulose on the lipid profile and the atherosclerotic index in ApoE/LDLR-/- mice.

2-month-old female ApoE/LDLR-/- mice were divided into 3 groups (n=6) and fed ad libitum for 10 weeks. Two levels of D-Allulose (3% and 5%) in the diet have been be used. AIN-93G diet was used as a control. The body weight of the animals was monitored once a week. At the end of the experiment animals were euthanized. Blood was collected. Lipid profile was analyzed using the ABX Pentra 400 analyzer (Horiba Medical). The results were statistically analyzed using the ANOVA test.

It was shown that body weight was significant decreased in mice fed 3% and 5% of D-Allulose compared to control (21.0 g and 19.9 g, vs. 24.7 g, respectively). The levels of LDL cholesterol as well as triacylglycerols were significantly decreased after feeding diet with 3% and 5% of D-Allulose. Additionally, atherogenic index was significantly decreased in mice fed 3% and 5% D-Allulose.

D-Allulose is considered as a potential anti-atherosclerotic component.

1.32.

Ecto-adenosine deaminase inhibition rescued protective effects of ticagrelor in experimental models of endothelial cell dysfunction and atherosclerosis

Ada Kawecka[1], Alicja Braczko[1], Gabriela Harasim[1], Marika Franczak[1], Agata Jedrzejewska[1], Paulina Mierzejewska[1], Krzysztof Urbanowicz[1], Marta Tomczyk[1], Marcin Hellmann[2], Ewa M. Slominska[1], Roberto Giovannoni[3], Ryszard T. Smolenski[1], Barbara Kutryb-Zajac[1]
1 Department of Biochemistry, Medical University of Gdansk, Debinki 1 St., 80-211 Gdansk, Poland;
2 Noninvasive Cardiac Diagnostics Department, Medical University of Gdansk, Debinki 7 St., 80-211 Gdansk, Poland;
3 Department of Biology, University of Pisa, Via Derna 1, Pisa, Tuscany 56126, Italy;

Abstract:

Background:
Ticagrelor (TIC) is a P2Y12 antagonist with antiplatelet actions that expresses pleiotropic effects, including a beneficial impact on endothelial function. Part of them may be dependent on adenosine (Ado) signaling and related to the inhibition of ENT1 nucleoside transporter by TIC, which protects adenosine from intracellular metabolism. However, little is known about the adverse effects and cytotoxicity of TIC on endothelial cells (EC).

Objectives:
This work aimed to analyze the effects of TIC on EC function and intracellular nucleotide status under hypoxia. We have also investigated how the modulation of the extracellular Ado catabolism will affect the effects of TIC on EC.

Results:
TIC at clinically relevant doses depleted intracellular ATP and NAD concentration in human (HMEC-1) and mouse (H5V) EC and despite its positive effects on increased nitric oxide (NO) production, further diminished nucleotide levels during hypoxia and promoted blood cells adhesion to endothelium. The inhibition of adenosine deaminase (ADA) by
2'-deoxycoformycin (dCF) enhanced the effect of TIC on NO production affecting extracellular Ado signaling via receptor-dependent mechanisms. Moreover, TIC impaired the effectiveness of dCF in suppressing intracellular ADA (iADA), not affecting the dCF effect on ecto-ADA activity, which was related to reduced dCF uptake via ENT1. This may be of particular importance in preventing the adverse immunosuppressive effects of dCF on iADA inhibition. The experiments with porcine EC (PIEC) transfected with human ecto-enzymes engaged in Ado production (hCD73, hCD39) confirmed that the use of dCF significantly improved the effect of TIC on EC function.

Conclusions:
For the first time we have demonstrated that except for its beneficial properties, TIC disrupts the intracellular nucleotide status in EC, which may have negative long-term effects. We have proposed that TIC/dCF therapy has a synergistic effect on improving endothelial function during hypoxic conditions, enhancing the effect of TIC on extracellular adenosine concentration.

Acknowledgments:

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1.33.

Th2-driven pulmonary vascular hyperresponsiveness and remodelling in the Fra-2 transgenic mouse model of systemic sclerosis associated pulmonary arterial hypertension

Anna Birnhuber[1,2], Valentina Biasin[1,2], Pritesh Jain[1], Ekaterina Boiarina[3], Chandran Nagaraj[1], Andrea Olschewski[1,4], Martin Witzenrath[3,5,6], Leigh M Marsh[1], Christoph Tabeling[3,5,7], Grazyna Kwapiszewska[1,2]

[1] Ludwig Boltzmann Institute for Lung Vascular Research Graz, Graz, Austria. [2] Otto Loewi Research Center, Department of Physiology, Medical University of Graz, Graz, Austria. [3] Division of Pulmonary Inflammation, Charité - Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany. [4] Department of Anaesthesiology and Intensive Care Medicine, Medical University of Graz, Graz, Austria. [5] Department of Infectious Diseases and Respiratory Medicine, Charité - Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany. [6] German Center for Lung Research (DZL), Partner Site Charité, Berlin, Germany [7] Berlin Institute of Health at Charité - Universitätsmedizin Berlin, Berlin, Germany

Abstract:

Pulmonary arterial vasoconstriction and remodelling are features of pulmonary arterial hypertension (PAH). Th2 inflammation in the lung induces pulmonary vascular hyperresponsiveness to vasoconstrictive stimuli. In Fra2 overexpressing mice, a model of systemic sclerosis associated PAH, a strong Th2 signature and pulmonary vascular remodelling were reported. We hypothesised that here Th2 inflammation lead to pulmonary vascular hyperresponsiveness and remodelling.

In Fra2 overexpressing (transgenic [TG]) and wild-type (WT) mice, pulmonary vascular responsiveness was investigated ex vivo using isolated perfused and ventilated lungs (IPL) and wire myography. Pulmonary vascular remodelling was analysed in vivo following treatment with IL-13 blocking antibodies or glucocorticoids to suppress Th2 inflammation.

In IPL, mean pulmonary arterial pressure under basal conditions was significantly elevated in TG compared to WT lungs. Hypoxic pulmonary vasoconstriction was largely augmented in TG. Pulmonary vascular hyperresponsiveness to serotonin was observed in TG lungs. Isolated pulmonary arteries of TG mice showed increased vasoconstriction in response to diverse stimuli such as potassium chloride or the thromboxane A2 analogue U46619, indicating stimulus-independent hyperresponiseveness in pulmonary arteries of Fra2 TG mice.

In vivo, increased TG mice had increased vessel wall thickness and muscularisation of pulmonary vessels compared to WT mice. Vascular remodelling was ameliorated by anti-inflammatory treatment using either IL-13 blocking or general immunosuppression using glucocorticoids.

Our data argue for the concept that Th2 driven inflammation induces pulmonary vascular remodeling and pulmonary vascular hyperresponsiveness. We could show that targeting this axis leads to better outcome and is a relevant therapeutic avenue in inflammation-induced vascular pathologies. ng hypoxic conditions, enhancing the effect of TIC on extracellular adenosine concentration.

1.34.

Comprehensive analysis of vascular surface and activation markers in pulmonary fibrosis

Elisabeth Fließer[1], Anna Birnhuber[1,2], Horst Olschewski[3], Michael Kreuter[4], Malgorzata Wygrecka[5], Panja M. Böhm[6], Konrad Hötzenecker[6], Leigh M. Marsh[1], Grazyna Kwapiszewska[1,2,7]

[1] Ludwig Boltzmann Institute for Lung Vascular Research, Graz, Austria. [2] Otto Loewi Research Center, Medical University of Graz, Graz, Austria
[3] Division of Pulmonology, Medical University of Graz, Graz, Austria [4] Center for Interstitial and Rare Lung Diseases Pneumology and Respiratory Critical Care Medicine, Thoraxklinik University of Heidelberg, Translational Lung Research Center Heidelberg (TLRC), Heidelberg, Germany.
[5] Center for Infection and Genomics of the Lung, Universities of Giessen and Marburg Lung Center, Giessen, Germany. Member of the German Center for Lung Research. [6] Division of Thoracic Surgery, Department of Surgery, Medical University of Vienna, Vienna, Austria.
[7] Institute for Lung Health, Giessen, Germany.

Abstract:

Introduction:
Pulmonary fibrosis (PF) is a progressive lung scarring disorder associated with high morbidity and mortality. So far, maladaptive cellular and molecular alterations were assumed mainly epithelial-driven, but evidence is growing, that endothelial cells (EC) significantly contribute to the disease pathogenesis as well. Here, we provide a thorough analysis of vascular markers implicated in EC identity, integrity and activation both locally in the lung and in the systemic circulation of PF patients.

Methods:
Gene expression levels of EC-related markers CD31/PECAM1, VE-Cadherin/CDH5, thrombomodulin/THBD, VEGFR-2/KDR, von Willebrand Factor/VWF, intercellular adhesion molecule 1-3/ICAM1-ICAM3, vascular cell adhesion molecule 1/VCAM1, P-selectin/SELP and interleukin(IL)-8/IL8 were analyzed in lung tissue samples of PF patients (n=17) and donors (n=21) by qRT-PCR. Corresponding protein levels were determined in plasma samples of PF patients at the time of diagnosis (n=18) and in endstage disease samples vs. healthy control (n=19 and n=28, respectively).

Results:
CDH5, ICAM2 and ICAM3 gene expression levels were decreased in PF lung tissue compared to donors ($p=0.0275$, $p=0.0001$ and $p=0.0023$, respectively) and the changes were further confirmed in publicly available microarray datasets. In the systemic circulation, VE-Cadherin was decreased during disease initiation ($p=0.003$) and at disease endstage ($p=0.0043$), while Thrombomodulin, VEGFR-2 and ICAM-3 were only decreased at the endstage ($p=0.0238$, $p=0.0001$ and $p=0.0110$, respectively). Circulating levels of vWF and IL-8 were increased ($p\leq0.0001$) at disease endstage.

Conclusion:
These results support the presence of a dysregulated vascular compartment in the fibrotic lung, which is reflected by changes in soluble levels of EC markers in the systemic circulation.

1.35.

Endocan as contributor in pathogenic remodelling of pulmonary vasculature

Thomas Lins[1], Elisabeth Fliesser[1], Anna Birnhuber[1,2], Katharina Jandl[1,3] and Grazyna Kwapiszewska[1,2,4]
[1]Ludwig Boltzmann Institute for Lung Vascular Research Graz- Graz, Austria;
[2]Otto Loewi Research Center, Division of Physiology, Medical University of Graz - Graz, Austria;
[3]Otto Loewi Research Center, Division of Pharmacology, Medical University of Graz - Graz, Austria;
[4]Institute for Lung Health (ILH), Justus Liebig University - Giessen, Germany.

Abstract:

Systemic Sclerosis (SSc) is a severe chronic disease with a 50% chance of direct lung involvement; this can manifest as either pulmonary fibrosis, pulmonary arterial hypertension, or both. The Fos-related antigen 2 transgenic (Fra2 TG) mouse line has been established as functional model for SSc associated pulmonary fibrosis, recapitulating important features of SSc. Furthermore, this model suggests direct involvement of the pulmonary endothelium.

We have investigated changes in lungs of Fra2 TG mice at two different time-points (8 and 16 weeks) representing early-onset and severe disease progression, using: lung function and hemodynamic measurements, immunofluorescence and electron microscopy and RNA bulk sequencing of sorted endothelial cells. Further, in vitro silencing experiments utilizing human microvascular endothelial cells and GSE datasets from human patients suffering from lung fibrosis are being investigated.

Lung function measurements showed hampered respiratory capabilities and increased pulmonary arterial pressure in Fra2 TG mice. Gene expression analyses of lung homogenates suggested an imbalance of endothelial cell homeostasis. Electron microscopy visualized swelling of the endothelium in pulmonary arteries and capillaries increasing with age in TG subjects. Using RNAseq, we found Endocan as one of the most downregulated genes in the pulmonary endothelium of young Fra2 TG mice. Endocan is involved in extracellular matrix organization, cellular migration and proliferation, processes all linked to fibrosis. Similarly, endocan expression is decreased in human pulmonary fibrosis.

We conclude that endothelial cells have prominent contribution in early onset and development of the disease. Further studies are needed to elucidate pathomechanisms and investigate the role of Endocan.

1.36.

Reactivity of HNO towards alkyl sulfinic acids

Monika Rola[1], Angelika Artelska[1], Jakub Pięta[1], Marlena Pięta[1], Radosław Michalski[1] and Adam B. Sikora[1]
[1]Institute of Applied Radiation Chemistry, Lodz University of Technology, Lodz, Poland;

Abstract:

Azanone (HNO) is a triatomic electrophilic molecule that is one of the most elusive reactive forms of nitrogen. In the last few years, it has finally attracted considerable attention due to its unique chemical and biological properties. Given the rapid dimerization of azanone, chemical and biological researches require the use of compounds that release HNO in a controlled manner (HNO donors). These molecules may exhibit a novel class of vasodilators for the treatment of heart failure. Here we present the use of the competition kinetic method on the reactivity of sulfinic acids towards HNO, which demonstrates the ability of this group of compounds to scavenge azanone under physiological conditions (pH 7.4, 25°C). The products formed in this reaction are presumably, in turn, azanone donors. The applied kinetic method, based on the use of a novel boronate probe FlBA, allowed determining the second-rate constants of the HNO reaction rate with three sulfinic acids - ethanesulfinic acid, hypotaurine and 3-sulfinoalanine.

Acknowledgments:

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1.37.

Characterization of novel, fluorescent HNO donor – N-hydroxydansylamide

Renata Smulik-Izydorczyk[1], Jakub Pięta[1], Radosław Podsiadły[2], Radosław Michalski[1] and Adam B. Sikora[1]
[1]Institute of Applied Radiation Chemistry, Lodz University of Technology, Lodz, Poland;
[2]Institute of Polymer and Dye Technology, Lodz University of Technology, Lodz, Poland.

Abstract:

Azanone (HNO), otherwise known as a nitroxyl, is protonated product of the one-electron reduction of nitric oxide (NO) and similarly to its precursor exhibit a therapeutic potential in the treatment of heart failure diseases. In aqueous solutions HNO spontaneously dimerizes with the rate constant of approximately 8×10^{6} $M^{-1}s^{-1}$ to yield hyponitrous acid, which than dehydrates to give nitrous oxide and water. Propensity of HNO to undergo this reaction causes a necessity to use donor molecules, which decomposition product is a HNO molecule.

Here, we present the spectroscopic and kinetic characterizaion of novel, fluorescent HNO donor N-hydroxydansylamide (DAN-HNO) donor. The decomposition of DAN-HNO yield HNO molecule and fluorescent dansyl-based sulfinic acid.

Acknowledgments:

This study was supported by Polish National Science Center within the OPUS program (Grant no. 2021/43/B/NZ7/01903).

2.1.

Probing microvascular function with Magnetic Resonance Imaging and Doppler Blood Flow Velocity Mapping: proof-of-principle in the heart and lungs

Grzegorz Kwiatkowski[1], Urszula Tyrankiewicz[1], Stefan Chłopicki[1,2]
[1]Jagiellonian University, Jagiellonian Centre for Experimental Therapeutics (JCET), Bobrzynskiego 14, 30-348, Krakow, Poland
[2]Jagiellonian University Medical College, Faculty of Medicine, Chair of Pharmacology, Grzegorzecka 16, 31-531, Krakow, Poland

Abstract:

Coronary vascular dysfunction is considered a hallmark of cardiovascular disease and it has a diagnostic as well as a therapeutic significance. Nevertheless, it is almost exclusively studied in the vasculature of large, conduit vessels. In contrast, very little is known about the mechanisms of coronary microvascular dysfunction, despite the fact that the coronary microvasculature functional status determines patient outcomes in heart failure (HF) and represents an independent prognostic factor. The details on mechanisms of coronary microcirculation impairment in both humans and animal models, that could allow to better understand the mechanisms in which microvascular failure contribute to the development of heart failure, are still limited. Current methods to study microvasculature abruption are restricted and mostly based on an indirect probing of vascular status in the large vessels. Only recently, advances in magnetic resonance-based imaging (MRI) and Doppler Blood Flow Velocity Mapping and data analysis allowed for deriving novel imaging protocols that are specific towards coronary microvascular bed. Hence, as it is increasingly apparent that a reliable, quantitative measurement of microcirculation function is possible, and if applied, could greatly advance preclinical studies on the role of coronary microcirculation in pathophysiology and pharmacology of heart failure.

In this communication recent advances in non-invasive, in-vivo imaging of microvascular function in murine models of heart failure will be discussed.

Acknowledgments:

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2.2.

Alterations in coronary flow pattern in regards to progressing cardiovascular pathology.

Urszula Tyrankiewicz, Grzegorz Kwiatkowski, Stefan Chlopicki;
Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Cracow, Poland;
Chair of Pharmacology, Faculty of Medicine, Jagiellonian University Medical College, Cracow, Poland

Abstract:

The alterations in coronary flow (CF) pattern that precede impaired CF reserve are not well understood and need clarification for better understanding the mechanisms regulating developing cardiac pathology. The goal of the study was to analyze the alterations of CF pattern in regards to: dilated cardiomyopathy (Tgαq44), atherosclerosis (ApoE/LDLR-/-) and endothelial-dependent deletion of Nrf2 protein. Tgαq44 mice (at 5,8 and 12 months of age), ApoE/LDLR-/- mice at the age of 6 months and NRF2-/- mice at the age of 3 months were used. Coronary flow pattern (slope and time of increasing and decreasing coronary blood flow) and CF reserve as well as cardiac systolic and diastolic flow characteristics were studied using Doppler Flow Velocity System. Coronary flow was registered under basal conditions (with 1-1,25% of isoflurane) and under isoflurane or dobutamine-induced CF hyperemia (2,5% and 2mg/kg, i.p., respectively). CF reserve was preserved in all measured groups (except of 12c month-old Tgαq44 mice). However, the analysis of CF pattern uncovered lower dynamic of the increasing CF velocity under dobutamine injection in 5 and 8 month-old Tgαq44 mice, that were followed by decreased dynamic of decreasing CF velocity at in 8month-old Tgαq44 mice. These changes were accompanied with increased cardiac inflow velocities and decreased outflow velocities. The ApoE/LDLR-/- mice had decreased slope of increasing as well as of decreasing coronary blood flow under dobutamine stimulation (accompanied with impaired systolic and diastolic LV flows). In contrast, NRF2-deficient mice had enhanced slope of the increasing CF, that was followed by decreased LV systolic peak acceleration. In conclusion, the in vivo obtained data of coronary flow pattern (at the basal and stress-induced conditions) allowed for the detection of early CF functional changes, that precede impaired CF reserve. Decreased CF dynamic under stress conditions for 6 month-old ApoE/LDLR-/- and for 8 month-old Tgαq44 mice may indicate similar coronary functional impairment for different pathologies, while changes in NRF2-deficient mice rather present coronary adaptation to subtle impaired LV flows.

Acknowledgments:

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2.3.

Evaluation of the cardiac function in mice with abnormal endothelial SIRT1 activities

Sandeep Singh, Daniels Konja, Bowen Fu, Kulsuma M. Begum, Beijia Zhang, Aimin Xu, Yu Wang.
The State Key Laboratory of Pharmaceutical Biotechnology and Department of Pharmacology and Pharmacy, The University of Hong Kong, Hong Kong SAR, China.

Abstract:

Introduction:
SIRT1 is an NAD-dependent deacetylase that is involved in energy homeostasis and longevity regulation. Mutation of the H363Y residue leads to negative dominant SIRT1 and loss of deacetylase function. SIRT1 is regulated by cyclin dependent kinase 5 (CDK5)/ P25 (regulatory subunit of CDK5) pathway. Hyperactivation of the CDK5)/ P25 signaling in endothelial cells leads to augmented SIRT1 activity and increased SIRT1 fragmentation. This study is designed to investigate the cardiac function of mice with endothelium-specific overexpression of H363Y (EC-H363Y) or P25 (EC-P25) mice.

Methods:
Transgenic mice with overexpression of a deacetylase mutant of SIRT1 (EC-H363Y) or P25 (EC-P25), in the endothelium were used as experimental groups. Littermate control mice were used as the control groups. All mice were kept in a 12/12 light-dark cycle with adequate standard chow diet and normal drinking water. Echocardiography was used to evaluate the systolic and diastolic functions including B mode, M mode, Tissue Doppler, Color Doppler and analysis was performed using the Vevo Lab 1.7.0 version software.

Results:
Isovolumic Contraction time (IVCT) was found to be shorter in mice with loss of endothelial SIRT1 function (EC-H363Y) compared with controls, but longer in EC-P25 compared to their controls. However, Isovolumic relaxation time (IVRT) was shorter in EC-P25 mice compared with their controls. Longitudinal strain was higher in the EC-H363Y while Radial strain SR was decreased compared to the controls. Ratio of Peak velocity of early to late diastolic mitral annular motion (E'/A'), Mitral valve ratio of E to E' (MV E/E') were both found to be significantly decreased in EC-H363Y compared to their controls. On the other hand, A'/E' and MV E/E' were found to be significantly increased for EC-P25 mice compared to their controls.

Conclusion:
Abnormal endothelial SIRT1 activities lead to cardiac diastolic dysfunction which is evident by changes in IVCT,IVRT, MV E/E', E',A', E'/A' ratio.

Acknowledgments:

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2.4.

Characterization of mechanical stretch-induced responses of lymphatics in left ventricular hypertrophy

Zsombor Ocskay[1*], Simge Baydar[3*], Gülsüm Yilmaz Lauber[3], Lukas Weber[3], Zsuzsanna Bakó[1], Sabrina Rohringer[3], Peter Pokreisz[3], Karin Zins[2], Dietmar Abraham[2], Bruno K. Podesser[3], Attila Kiss[3*], Zoltan Jakus[1*]

[1] Department of Physiology, Semmelweis University School of Medicine, Budapest, Hungary
[2] Center for Anatomy and Cell Biology, Division of Cell and Developmental Biology, Medical University of Vienna, Vienna, Austria
[3] Ludwig Boltzmann Institute for Cardiovascular Research at the Center for Biomedical Research, Medical University of Vienna, Vienna, Austria,
[*] These authors contributed equally to this work.

Abstract:

The lymphatics regulate interstitial fluid balance, immune cell trafficking. Importantly, lymphatics and lymphangiogenic signaling are involved in cardiac growth and regeneration. However, the possible involvement of lymphatics in the pathogenesis of heart in left ventricular hypertrophy due to pressure overload remains largely unclear.

Left ventricular hypertrophy was induced by transverse aortic constriction (TAC) in adult male mice. Mice were allocated into the following groups Sham, 1 week and 6-8 weeks follow-up after TAC operation. Heart samples were processed for paraffin-based histology followed by immunostainings against lymphatic markers. Alteration of cardiac lymphatic vessels was quantified in perivascular area and myocardium. Lymphatic endothelial cells (LEC) were exposed to two stretch conditions including low elongation and high elongation. The expression of NF-κB p65 subunit as well as markers of inflammation and apoptosis were assessed.

Morphological and functional results indicated time-dependent disease progression in our TAC-operated animals. Importantly, we detected specific region-dependent alterations in lymphatic growth in left ventricular hypertrophy. E.g. one week after the TAC surgery a dramatic regression of lymphatic structures was observed in the perivascular compartments, while regrowth of new lymphatic vessels in the reverse TAC model was detected. In addition, Phospho-NF-κB p65 expression shows a tendency towards to increase in LEC after stretch condition as well as markers of pro-inflammatory and apoptosis were markedly increased.

Our results revealed that mechanical forces induce dynamic spatiotemporal changes of lymphatic morphology in left ventricular hypertrophy suggesting that efficient modulation of lymphangiogenesis might be a new possible way of the intervention of the disease. olic dysfunction which is evident by changes in IVCT,IVRT, MV E/E', E',A', E'/A' ratio.

2.5.

Novel GPCRtargets forheart failure with reduced ejection fraction identified by droplet digital PCR

Nabil V. Sayour[1,2], Viktória E. Tóth[1,2], Regina N. Nagy[1], Noémi Nagy[3], Csaba Bödör[3], Barnabás Váradi[1], Bence Ágg[1], Mihály Ruppert[4], Tamás Radovits[4], Anikó Görbe[1], Péter Ferdianady[1,5],Zoltán V. Varga[1,2]
[1] Department of Pharmacology and Pharmacotherapy, Semmelweis University, Faculty of Medicine, Budapest, Hungary
[2] HCEMM-SU Cardiometabolic Immunology Research Group, Budapest, Hungary
[3] 1st Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary
[4] Experimental Research Laboratory, Heart and Vascular Center, Semmelweis University, Budapest, Hungary
[5] Pharmahungary Group, Szeged

Abstract:

Despite the currently available comprehensive therapies of heart failure with reduced ejection fraction (HFrEF), identification of novel drug targets is still needed to improve outcomes for patients' benefit. G-protein coupled receptors (GPCRs) represent the largest family of targets for already approved drugs providing a great opportunity for drug repurposing.Here,we aimed to identifynovel GPCRtargets forHFrEF in a rat model,andan in vitromodel of cardiomyocyte hypertrophy.8-10 weeks old, male Wistar rats were subjected to transverse aortic constriction (TAC) or sham (SHAM) surgery. 15-18 weeks after surgery, cardiac echocardiography was performed, andcardiac samples were collected for bulk RNA sequencing byNGS, followed byddPCRon288 GPCR genes. Differential expression of GPCRs of TAC and SHAM hearts identifiedby both techniques was collected, and a potential GPCR candidate wastested in vitroon hypertrophic cardiomyocyte cells, induced by 1 uM angiotensin-II (Ang2). TAC animals showed significantlydecreased ejection fraction. Out of 288 GPCRs, NGS identified 69, anddPCRidentified 27 genes to be significantly differently expressed in TAC vs. SHAM animals, 14 of which were identified by both methods.Blockade of the prostaglandine-F2α-receptor(Ptgfr)by 10 uM of AL-8810reverted Ang2-induced in vitrocardiomyocyte hypertrophy.This is the first use ofddPCR as a screening method for identifying differentially expressed genes in a disease. We identified 14 differentially expressedGPCRsby NGS and ddPCR in failing rat hearts, of which Ptgfrwas shown to be potentially targetable in vitro.

Acknowledgments:

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2.6.

Interactions between the RAAS Signaling and Tenascin C in Post-Infarct Cardiac Remodeling

Anna Stampfer, Lukas Weber, Gülsüm Yilmaz Lauber, Eylem Acar, Maximilian De Zordo, Petra Fichtinger, Karin Zins, Dietmar Abraham, Bruno K Podesser, Attila Kiss, Peter Pokreisz
Medical University of Vienna, Vienna, Austria
Center for Biomedical Research, (AS, LW, GYL, EA, MDZ, BKP, AK, PP)
Center for Anatomy and Cell Biology (PF, KZ, DA)

Abstract:

Introduction:
Cardiac remodeling rapidly alters the expression of extracellular matrix proteins, including tenascin-C (TnC) and activates the renin-angiotensin-aldosterone-system (RAAS) after myocardial infarction (MI). We investigated the interactions between TnC and RAAS in a murine model of MI.

Methods:
MI was induced via permanent ligation of the left anterior descending coronary artery in mice lacking TnC (TnC-KO) and their wild-type littermates (A/J). After six weeks, cardiac function and morphology of A/J (n=8) and TnC-KO (n=5) was compared by echocardiography (Visualsonics Vevo3100). Cardiac tissue was analyzed postmortem using (immune)-histochemical techniques and quantitative reverse transcription PCR (qRT-PCR), while activities of circulating and tissue angiotensin convertase enzyme 1 and 2 (ACE1/ACE2) were measured using fluorescent assays. To mimic the post-MI inflammatory, oxidative, and activated RAAS conditions, human ventricular cardiac fibroblasts and cardiac microvascular endothelial cells were exposed to transforming growth factor beta1 (TGFb1), hydrogen peroxide, and angiotensin II, respectively.

Results:
After six weeks, TnC-KO and A/J animals had subnormal, but comparable left ventricular (LV) ejection fraction (53±2% vs 47±13%), accompanied by chamber dilatation to similar extent measured by end-diastolic internal diameter. RT-qPCR analyses of LVs displayed increased expression of alpha-smooth muscle actin (aSMA), angiotensin 1 and toll-like receptor 4 in TnC-KO. Oxidative stress and TGFb1 had no significant effect on ACE1/2 activity in fibroblasts, but TGFb1 substantially increased aSMA expression.

Conclusion:
The absence of TnC may positively influence murine cardiac structural changes, especially by augmenting aSMA expression and transition from fibroblasts to myofibroblasts, as well as represents a potential target.

Acknowledgments:

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2.7.

Reverse remodeling in STZ induced diabetic Tenascin C knock-out mice

Ibrahim Aykac[1], Zsuzsanna Arnold[1], Eylem Acar[1], Gyorgy Nadasy[2], Bruno Podesser[1], Attila Kiss[1]
[1]Ludwig Boltzmann Institute for Cardiovascular Research at the Center for Biomedical Research, Medical University of Vienna, Austria;
[2]Department of Physiology, Medical Faculty, Semmelweis University Budapest, Budapest, Hungary;

Abstract:

Background:
Upregulation of Tenascin C (TNC) is associated with cardiac fibrosis in mice with pressure or volume overload-induced heart failure as well as worse clinical outcome in patients with diabetes mellitus. However, the exact role of TNC in diabetes are largely unknown.

Aim:
1) address the impact of lacking TNC in the progression of cardiac dysfunction and

2) to discover the potential signaling using RNA-sequencing.

Methods:
Diabetes was induced by streptozotocin injections (in total n=24 animals) in Wild type (A/J) and TNC KO mice, and 16 weeks later heart tissue was collected for RNA-Sequencing.

Results:
TNC deficiency was accompanied by preserved ejection fraction and less cardiac fibrosis in TNC-KO diabetic animals than in the AJ diabetic group. Genes associated with ER-stress (Eif2a), mitochondrial stress (Mrps28, Tsfm, Sdhd,) were significantly downregulated in TNC KO diabetic mice vs. Wt diabetic mice, indicating decreased cell-stress pathways. Diabetic TNC KO mice also showed altered insulin signaling by upregulation of phenylalanine hydroxylase; trough downregulation of Pdk 4, increased fat metabolism and less glucose utilization, and a decreased cholesterol metabolism by downregulation of Srebf1 was observed. In addition, the decrease of expression of cellular communication network factor 1 and 2 was found in diabetic TNC KO mice, indicating reduced cell chemotaxis of fibroblasts.

Conclusion:
TNC induces fibrosis, mitochondrial dysfunction and altered energy metabolism in diabetes which leads to cardiomyocyte dysfunction. Thus, TNC seems to be a critical mediator in progression of cardiac dysfunction but at the same time, it represents as potential target for therapy.

Acknowledgments:

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2.8.

Dapagliflozin ameliorates cardiac dysfunction in a mouse model of chronic pressure overload

Lukas Weber[1,2], Patrick Pilz[1], Niklas Baumgartner[1], Petra Lujza Szabo[1], Zsuzsanna Arnold[1,3], Simge Baydar[1,4], Christopher Dostal[1], Attila Kiss[1,4], Bruno Podesser[1,4]
[1] Center for Biomedical Research, Medical University of Vienna, Austria
[2] AIT Austrian Institute of Technology GmbH, Tulln, Austria
[3] Wiener Gesundheitsverbund, Klinik Floridsdorf, Vienna, Austria
[4] Ludwig Boltzmann Institute for Cardiovascular Research, Vienna, Austria udapest, Budapest, Hungary;

Abstract:

Objective:
This study aimed to investigate whether the SGLT2 inhibitor dapagliflozin (DAPA) could ameliorate left ventricular hypertrophy (LVH) and cardiac dysfunction in a mouse model of pressure overload induced LVH.

Methods:
LVH was induced in adult male C57BL/6J mice by transverse aortic constriction (TAC). The animals were divided in four groups: 1) TAC for eight weeks; 2) TAC and DAPA treatment for eight weeks; 3) TAC for eight weeks and DAPA treatment for only two weeks before sacrification and 4) served as a sham control group (no TAC). Cardiac function was assessed using transthoracic echocardiography and invasive hemodynamic measurements by applying a microtip catheter into LV. ACE and ACE 2 activity measurements were performed in serum, lung and kidney tissue. In addition, isolated adult mice cardiomyocytes were used to test the anti-hypertonic effect of DAPA after mechanical stretch conditions.

Results:
TAC resulted in a significant reduction in LV ejection fraction (LVEF) and LV hypertrophy compared to sham ($p<0.001$). Furthermore, TAC mice showed a significant increase of LV systolic pressure and end-diastolic pressure compared to sham ($p<0.01$). Both the LVEF and LV functional parameters were markedly improved in mice treated with DAPA for eight weeks ($p<0.05$). Interestingly, DAPA treatment for only two weeks already slightly improved LVEF and alleviated LVH in comparison with untreated TAC mice ($p<0.05$). ACE and ACE 2 activity in serum, lung and kidney did not change upon DAPA treatment.

Conclusions:
DAPA treatment improved LV contractile function and adverse remodelling following TAC in a diabetes independent setup. ACE and ACE 2 activity are not affected by long-term or short-term DAPA treatment.

Acknowledgments:

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2.9.

Sex-dependent deterioration of cardiac function and molecular alterations in age- and disease-associated RAGE overexpression

Patrick R. Winterhalter [a], Mandy Wirkner [a,b], Babett Bartling [a,c], Kristin Wächter [a], Arina Urazova [a], Anne Großkopf [a], Claudius Diez [a,d], Gábor Szabó [a], Andreas Simm [a]

[a] Martin-Luther-University Halle-Wittenberg, University Hospital and Polyclinic for Cardiac Surgery, Ernst-Grube-Straße 40, D-06120 Halle (Saale), Germany
[b] Gesundheitszentrum Bitterfeld/Wolfen gGmbH, Clinic for Anesthesiology and Intensive Care, Friedrich-Ludwig-Jahn-Straße 2, D-06749 Bitterfeld-Wolfen, Germany
[c] Martin-Luther-University Halle-Wittenberg, Institute of Agricultural and Nutritional Sciences, Animal Health Management, Theodor-Lieser-Str. 11, D-06120 Halle (Saale), Germany
[d] Acute Care Therapies - Getinge Group, Maquet GmbH, Kehler Straße 31, D-76437 Rastatt, Germany

Abstract:

Elevated expression of the receptor for advanced-glycation endproducts (RAGE) in cardiac tissue is well-known in the elderly, in diabetes mellitus, and after acute cardiac infarction or ischemia/reperfusion injuries. RAGE and its binding partners affect the clinical outcome of heart failure and may play an essential role in accelerating the functional decline in cardiovascular aging. Therefore, hearts of wild-type (WT) C57black6/N and cardiac-specific RAGE-overexpressing transgenic (TR) mice were analyzed for their function by ultrasound at young (4-5 months) and old (22-23 months) ages. Transgenic mice exhibit significantly increased systolic (LVD-sy) and diastolic (LVD-di) diameters of their left ventricles. The left ventricular ejection fraction (EF) was significantly reduced in young male TR mice. Omics of the heart did not reveal direct activation of cytokine-induced inflammation. Instead, energy metabolism-associated genes were enriched in downregulated transcripts and proteins of TR animals, causing decreased ATP production. In a sex-specific manner, there was a reduced expression of the four-and-a-half LIM-domains protein 2 (FHL2). In conclusion, transgene-induced RAGE overexpression, as a model for age- and disease-associated RAGE alteration, leads to a sex-dependent EF decline, in which FHL2 and energy depletion might play crucial roles.

2.10.

The impact of St.Thomas‘ Hospital polarizing cardioplegia on hemodynamic recovery in a chronically infarcted rat model.

L. Wolner[1], PL. Szabo[1], M. Inci[1], L. Weber[1], A. Kiss[1], BK. Podesser[1]
[1] Ludwig Boltzmann Institute for Cardiovascular Research at the Center for Biomedical Research, Medical University of Vienna, Austria

Abstract:

Aim:
We investigated isolated rat hearts after myocardial infarction in a cardiac arrest, ischemia/reperfusion model with the aim to compare the efficiency of the novel cold St.Thomas' Hospital polarizing cardioplegic solution (STH-Pol) and standard St.Thomas' Hospital cardioplegia (STH2).

Methods:
Myocardial infarction (MI) was induced by permanent ligation of the left anterior descending artery in male adult Sprague Dawley rats. Six weeks post-MI, the animals were sacrificed, the hearts were isolated and perfused via an erythrocyte-perfused system. Cold ischemia (4oC) for 60 min was induced by one of the cardioplegic solution and was applied every 20 min, followed by 30 min of reperfusion. The hearts were allocated to STH2 (n=9) and STH-Pol group (n=10). Hemodynamic variables were continuously recorded. Finally, pump function assessment was performed and tissue samples were taken for analysis of troponin-T and high-energy phosphates.

Results:
STH-Pol shows similar hemodynamic recovery (in % in comparison to baseline values ± SEM) as STH2 cardioplegia in infarcted rat hearts. Accordingly, the left atrial flow (LAF: 33.59 ± 6.33 vs. 43.67 ± 6.17), coronary flow (CF: 58.02 ± 8.92 vs. 66.6 ± 6.71) and heart rate (HR: 87.18 ± 2.95 vs. 95.7 ± 3.3), as well as the left ventricular pressure (LVSP: 83.53 ± 2.03 vs. 80 ± 4.07) did not show a statistically significant difference between the groups at 30 minutes of reperfusion.

Conclusion:
Polarizing cardioplegic arrest does not show inferior effects on hemodynamic recovery after cold ischemia and reperfusion in infarcted rat hearts in comparison to depolarizing cardioplegic arrest.

Acknowledgments:

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2.11.

In vitro characterization of human placenta extracellular matrix (hpECM) hydrogels aiming for cardiac tissue engineering

Marvin Dötzlhofer [a,b,c], Sabrina Rohringer [a,b,c], Bruno Podesser [a,b,c], Helga Bergmeister [a,b,c], Karl H. Schneider [a,b,c]

[a] Medical University of Vienna, Center for Biomedical Research, Währinger Gürtel 18-20, 1090, Vienna, Austria
[b] Ludwig Boltzmann Institute for Cardiovascular Research, Währinger Gürtel 18-20, 1090, Vienna, Austria
[c] Austrian Cluster for Tissue Regeneration, Vienna, Austria

Abstract:

Extracellular matrix derived from decellularized human placenta (hpECM) is a promising biomaterial for cardiovascular tissue engineering. With its excellent biocompatibility paired with a composition of important extracellular matrix proteins the hpECM can be used in various applications to generate artificial soft tissue.

In this preliminary work placental chorionic tissue was decellularized, followed by enzymatic digestion to produce hpcECM hydrogels. The material was evaluated for biochemical composition, gelation kinetics by rheological measurements and surface structure by scanning electron microscopy. To investigate cell-matrix interactions and the ability to form 3D constructs, human fibroblasts were seeded to hpcECM hydrogels with different protein concentrations ranging from 2 to 4% (w/v). Biochemical characterization confirmed high levels of collagen and glycosaminoglycans preservation as well as complete decellularization shown by a neglectable DNA content below 50 ng/mg dry weight. Independent of protein concentration cells were evenly spread throughout the 3D matrix and the hpcECM hydrogels depicted high cytocompatibility. Above 3% protein concentration only moderate hydrogel contraction was observed over time and cells were larger in size compared to cells loaded to gels with lower protein concentrations.

This pilot study confirmed great potential of the hpcECM hydrogels to support natural cell behaviour and tissue growth. Future experiments will address enhancing the mechanical properties of the hpcECM hydrogels and the use of cardiac muscle cells to mimic cardiac tissue. In order to produce more complex shapes, the hpcECM hydrogel will be further tested as a 3D printable cell-laden bioink.

Keywords: artificial tissue design, human placenta hydrogel, extracellular matrix, 3D cell culture

2.12.

S-nitroso human serum albumin in drug-eluting small diameter vascular grafts

Marjan Enayati [a,b,c], Karl H. Schneider [a,b,c], Ciarra Almeria [a], Christian Grasl [b,d], Christoph Kaune, Barbara Messner [f], Sabrina Rohringer [a,b], Ingrid Walterg, Johann Wojta [b,e], Lubos Budinsky [h,j], Beat H. Walpoth [k], Heinrich Schima [b,d], Gerd Kageri, Seth Hallströmi, Bruno K. Podesser [b,c], Helga Bergmeister [b,c]

[a] Center for Biomedical Research, Medical University Vienna, Austria [b] Ludwig Boltzmann Institute for Cardiovascular Research, Vienna, Austria [c] Austrian Cluster for Tissue Regeneration, Vienna, Austria [d] Center for Medical Physics and Biomedical Engineering, Medical University Vienna, Austria [e] Department of Internal Medicine II, Medical University of Vienna, Vienna, Austria [f] Cardiac Surgery Research Laboratory, Department of Cardiac Surgery, Medical University Vienna, Austria [g] Department of Pathobiology, University of Veterinary Medicine, Vienna, Austria [h] Preclinical Imaging Laboratory, Division of Molecular and Gender Imaging, Department of Biomedical Imaging and Image-guided Therapy, Medical University of Vienna, Vienna, Austria [i] Division of Physiological Chemistry, Otto Loewi Research Center, Medical University of Graz, Graz, Austria. [j] Faculty of Chemical and Food Technology, Central Laboratories, Slovak University of Technology, Bratislava, Slovakia. [k] Emeritus, Cardiovascular Surgery and Research, University Hospital & University of Geneva, Geneva, Switzerland

Abstract:

Introduction:
Despite of the intensive research in vascular graft development, there is still no appropriate substitute to autografts yet. Numerous obstacles are thrombogenicity and intimal hyperplasia. One of the most substantial signaling molecules that are involved in homeostasis of the vascular system is nitric oxide. Therefore, biofunctionalized grafts releasing nitric oxide (NO) in situ may overcome these limitations. In this study, a drug-eluting vascular graft was designed by blending polycaprolactone (PCL) with S-nitroso-human-serum-albumin (S-NO-HSA), a nitric oxide donor with prolonged half-life.

Methods:
PCL-S-NO-HSA grafts were fabricated via electrospinning. Various processing parameters were optimized. Morphology, drug release profile, biomechanics, inflammatory effects, cell proliferation, and expression of adhesion molecules of the optimized grafts were fully characterized in-vitro. Grafts were additionally evaluated in a small rodent model as aortic implants up to 12 weeks. Grafts were assessed by magnetic resonance imaging angiography (MRI) in vivo and after retrieval by histology.

Results:
Grafts composed of 8% PCL showed the highest NO encapsulation with superior morphological, mechanical characteristics. NO attenuated the expression of ICAM-1, VCAM-1 and TF. Furthermore, it up-regulated the anti-inflammatory cytokines (IL10) and M2 macrophage marker (CD163). All grafts remained 100 % patent with no signs of thrombosis or calcification. 8%PCL-S-NO-HSA vascular grafts supported rapid endothelialization, whereas SMCs proliferation was hampered in earlier phases.

Conclusion:
This study indicates that 8%PCL-S-NO-HSA grafts effectively support long-term in situ release of bioactive NO. The beneficial effects observed can be promising features for long-term success of small diameter vascular grafts. cold ischemia and reperfusion in infarcted rat hearts in comparison to depolarizing cardioplegic arrest.

Acknowledgments:

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2.13.

The senomorphic agent Ruxolitinib protects vascular grafts of mice from endothelial ischemia/reperfusion injury

Paula Naujoks[1], Lotta Hartrumpf[1], Sabine Pohl[1], Andreas Simm[1], Gábor Szabó[1], Lars Saemann[1]
[1] Department of Cardiac Surgery, University Hospital Halle, Halle (Saale), 06120 Halle, Germany

Abstract:

Objectives
The storage of the vascular graft in saline solution followed by reperfusion after anastomozation during coronary artery bypass surgery leads to an ischemia/reperfusion (I/R) situation. This causes an endothelial dysfunction in the bypass graft partially mediated through the release of proinflammatory cytokines. Senomorphics inhibit the release of specific cytokines and could provide a protective effect on the long-term patency of the graft. However, the effect of the senomorphic agent Ruxolitinib on the endothelial function of vascular grafts has not been investigated yet.

Method
Aorta segments of young male and female C57BL/6J mice (N=12/group), were divided into three groups: Control, ischemia/reperfusion (I/R), and I/R with Ruxolitinib (I/R+Ruxo). The I/R segments were stored in deaired saline solution for 24 hours with or without Ruxolitinib. Afterwards they were mounted in organ bath chambers. The reperfusion injury was induced by 200 µM sodiumhyperchlorid. We invested different vascular reactions of the graft with potassium chloride (KCl), phenylephrine (PE), acetylcholine (ACh) and sodium nitroprusside (SNP).

Results
Maximal endothelial-dependent vasorelaxation to ACh (Rmax) was highly significantly stronger in the I/R+Ruxo compared to the I/R group. (Rmax ACh: 74.4±2,6 vs. 48.6±3.4; $p<0.05$). Rmax to ACh was also significantly higher in the control group compared to the I/R group (Rmax Ach: 83.6±2.4 vs. 48.6±3.4; $p< 0.05$). The maximal vasoconstriction to PE was significantly stronger in both I/R groups compared to the control group. (PEmax: I/R: 88.7±5.2; I/R+Ruxo: 86,8±3.5 vs. Control: 62.6±4.3). A sex-related analysis revealed no significant differences between female and male mice regarding PEmax, Rmax Ach, Rmax SNP.

Conclusion
Ruxolitinib during storage of vascular grafts from mice protects the endothelium from I/R-mediated damage.

2.14.

3D printing an auxetic scaffold with anisotropic mechanics for cardiac tissue engineering

Luis Pichelkastner, Marjan Enayati, Karl H. Schneider, Martin Stoiber, Heinrich Schima, Bruno K. Podesser, Helga Bergmeister
Medical University of Vienna

Abstract:

Introduction:
Cardiac patches are functioning cardiac tissue constructs, grown in-vitro to support damaged tissue after myocardial infarction. Auxetic microstructures can add mechanical properties to a patch's scaffold, which are favorable for a demanding, anisotropic implantation site like the myocardium. Auxetics have a Poisson's ratio $\nu < 0$, which means that they expand, when stretched, perpendicular to the applied force. The objective of this study was to 3D print an auxetic polycaprolactone (PCL) scaffold.

Methods:
Micropatterned PCL scaffolds were fabricated on a 3D bioprinter. The printing process was divided into two steps to gain precise control over the printhead's movement path. Printing parameters were optimized to further improve printing quality. Morphological characterization was done via Scanning Electron Microscopy (SEM). Mechanical properties were studied in two orthogonal directions (x, y) in unidirectional, quasistatic tensile tests. Biocompatibility of HUVECs seeded on the scaffolds was studied via XTT assay and SEM images (timepoints 24, 48, and 72 hours).

Results:
Fabrication process optimizations resulted in uniform scaffolds with small pore sizes (~0.2 mm2) and good structural integrity. Tensile tests showed a clear anisotropic stiffness-ratio Ex/Ey = 2.48. Ultimate strain was 7-20%; close to the range of physiological deformations of the myocardium (15-22%).
ν was well below -0.3 for all samples, proving a clear auxetic behavior. Good biocompatibility was shown in XTT assay and SEM images for all timepoints.

Conclusion:
High-resolution 3D printing of auxetic and highly porous PCL scaffolds was achieved. Conducted tests showed promising results for the use of this mechanically tunable biomaterial in cardiac tissue engineering. schemia and reperfusion in infarcted rat hearts in comparison to depolarizing cardioplegic arrest.

Acknowledgments:

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2.15.

Identification of mechanisms involved in the early healing phase of different vascular graft materials

Daniel Prem [1,2,3], Sabrina Rohringer [1,2,3], Pia Hager [1,2,3], Sophie Johanna Specht [1,2,3], Marvin Dötzlhofer [1,2,3], Roman Lieber [1], Christian Grasl [2,4], Barbara Kapeller [1], Katharina Ehrmann [5], Stefan Baudis [5], Robert Liska [5], Heinrich Schima [2,], Bruno K. Podesser [1,2,3], Helga Bergmeister [1,2,3]

[1] Center for Biomedical Research, Medical University of Vienna, Vienna, Austria
[2] Ludwig Boltzmann Institute for Cardiovascular Research, Vienna, Austria
[3] Austrian Cluster for Tissue Regeneration, Vienna, Austria
[4] Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Vienna, Austria
[5] Institute of Applied Synthetic Chemistry, Technical University of Vienna, Vienna, Austria

Abstract:

Due to the high number of deaths caused by cardiovascular disease (CVD), coronary bypass grafting became one of the most important cardiovascular surgical interventions. Preferably autologous vessels are used because of higher patency rates compared to synthetic small-diameter (<6 mm) vascular grafts. This study aims to elucidate the mechanisms involved in early graft healing to identify potential reasons for graft failure.

In an infrarenal aorta replacement model in rats, expanded polytetrafluorethylene (ePTFE), biodegradable thermoplastic polyether urethane-urea (TPUU) grafts and autologous vessels (abdominal aorta) were implanted. Grafts were retrieved after 24 hours and 7 days and analysed by immunohistochemistry, scanning electron microscopy and cytokine array. Based on the cytokine arrays, an intense analysis of the involved pathways was conducted. Gene expression analyses were performed to elucidate the profile of downstream activated cells and the related cytokines.

Significantly more pro-inflammatory and proliferation-inducing cytokine expression was observed in the synthetic grafts than in autologous conduits whereby TPUU showed the highest response.

In conclusion, different graft materials led to varying responses in host tissue during the early vascular graft healing period. Additionally, our in-depth analyses of cytokine expression profiles and related signalling pathways suggest a direct involvement of adaptive immune cells. These findings may help to develop synthetic grafts with improved performance in the future. ring. schemia and reperfusion in infarcted rat hearts in comparison to depolarizing cardioplegic arrest.

Acknowledgments:

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2.16.

Biocompatibility and performance evaluation of biodegradable, self-strengthening small-diameter vascular grafts

Sabrina Rohringer[1,2,7], Pia Hager[1,2,7], Clemens Hahn[1,2], Sophie J. Specht[1,2,7], Katharina Ehrmann[3,7], Christian Grasl[2,4,7], Ingrid Walter[5], Barbara Kapeller[1], Karl H. Schneider[1,2,7], Lydia Zopf[6,7], Stefan Baudis[3,7], Robert Liska[3,7], Heinrich Schima[2,4,7], Bruno K. Podesser[1,2,7], Helga Bergmeister[1,2,7,*]

[1] Center for Biomedical Research, Medical University of Vienna, Vienna, Austria [2] Ludwig Boltzmann Institute for Cardiovascular Research, Vienna, Austria [3] Institute of Applied Synthetic Chemistry, Technical University of Vienna, Vienna, Austria [4] Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Vienna, Austria [5] Department of Pathobiology, University of Veterinary Medicine, Vienna, Austria [6] Ludwig Boltzmann Institute for Traumatology, Vienna, Austria [7] Austrian Cluster for Tissue Regeneration, Vienna, Austria

Abstract:

Clinically available small diameter polymeric vascular grafts (SDVGs) have significantly lower patency rates compared to autologous implants due to impaired graft healing. Despite the promising features of biodegradable implants, their poor biomechanical properties during degradation limit their clinical use. A self-strengthening modification could overcome this problem. Therefore, our aim was to fabricate a new biodegradable, self-reinforcing biomaterial for safe and long-term application as SDVGs.

Electrospun thermoplastic polyether urethane-urea (TPUU) grafts were subjected to burst pressure, tensile strength, and compliance measurements. The biocompatibility was assessed by cell seeding experiments and hemocompatibility assays in vitro. The grafts were further implanted into the abdominal aorta of rats for up to 6 months. The in vivo performance and graft/host interactions were evaluated via scanning electron microscopy, µCT, histology and gene expression analyses.

In vitro evaluations confirmed the excellent biocompatibility of TPUU grafts. The grafts remained patent for up to 6 months and the biomechanical properties remained high despite constant wall thinning. Gene expression analyses showed the absence of chronic inflammation. Moreover, no aneurysms, intimal hyperplasia or thrombogenicity of the grafts were found. Further evaluations of graft healing showed a decrease of inflammation and active remodeling markers over time.

In the current study, biodegradable, self-reinforcing electrospun TPUU SDVGs were shown to remain biomechanically stable during the degradation process and exhibited similar gene expression profiles to autologous implants. Therefore, our TPUU SDVGs could be promising candidates for clinical use in the future.

Acknowledgments:

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2.17.

Development of a perivascular adipose tissue mimicking hydrogel to study vascular graft healing in-vitro

Anna-Maria Schmitt[1,2,3], Sabrina Rohringer[1,2,3], Marvin Dötzlhofer[1,2,3], Pia Hager[1,2,3], Sophie J. Specht[1,2,3], Karl H. Schneider[1,2,3], Herbert Kiss[4], Bruno K. Podesser[1,2,3], Helga Bergmeister[1,2,3]

[1] Center for Biomedical Research, Medical University of Vienna, Vienna, Austria
[2] Ludwig Boltzmann Institute for Cardiovascular Research, Vienna, Austria
[3] Austrian Cluster for Tissue Regeneration, Vienna, Austria
[4] Department of Obstetrics and Gynaecology, Division of Obstetrics and Feto-Maternal Medicine, Medical University of Vienna, Vienna, Austria

Abstract:

Perivascular adipose tissue (PVAT) is an important determinant of vascular homeostasis and contractility and has therefore attracted increasing interest from researchers over the past decade. Although the paracrine effects of PVAT on the vasculature have not been fully elucidated, it is hypothesized that it may influence vascular remodeling and improve vascular graft healing. The aim of this study was to develop a 3D cell culture matrix that mimics PVAT in-vitro to investigate the effect of PVAT on the healing process of small diameter vascular grafts.

A 3D collagenous matrix consisting of gelatine and extracellular matrix (ECM) derived from decellularized human placenta was seeded with endothelial cells, smooth muscle cells, fibroblasts as well as mesenchymal stem cells and macrophages. Immunofluorescence staining, histological assessments, viability assays, and gene expression analyses were performed.

All cells remained viable in the constructs for up to one week and showed cell-specific gene and protein expression profiles. In addition, histology confirmed uniform distribution of cells in the gel.

The cell-populated gel shows potential to mimic the interaction of perivascular adipose tissue with small vessel grafts in-vitro.

Acknowledgments:

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2.18.

Distinct chemical changes in perivascular adipose tissue due to cardiovascular pathologies studied by Raman spectroscopy

Krzysztof Czamara[a], Zuzanna Majka[a], Stefan Chlopicki[a,b], Agnieszka Kaczor[a,c]
[a]Jagiellonian Centre of Experimental Therapeutics (JCET), Jagiellonian University, 14 Bobrzynskiego Str., 30-348 Krakow, Poland.
[b]Chair of Pharmacology, Jagiellonian University, 16 Grzegorzecka Str., 31-531 Krakow, Poland.
[c]Faculty of Chemistry, Jagiellonian University, 2 Gronostajowa Str., 30-387 Krakow, Poland.

Abstract:

The last decades have shown that the perivascular adipose tissue (PVAT) surrounding the aorta contributes to cardiovascular and lifestyle diseases. PVAT adipocytes secrete pro- and anti-inflammatory vasoactive substances that after diffusion into the vessel wall affect the endothelial layer leading to its dysfunction. Moreover, excess lipid accumulation that causes impaired PVAT function directly induces vascular inflammation that intensifies the development of cardiovascular diseases. Thus, PVAT is recognized as a novel factor involved in the progression of vascular diseases, i.e. atherosclerosis and obesity.

Raman spectroscopy, as a sensitive tool for the study of biological tissues, especially those that contain a high level of lipids, was applied for estimating PVAT chemical composition. Based on Raman spectra relevant markers can be established including the lipid unsaturation degree. We investigated the PVAT of three main arteries aorta, femoral and mesentery upon development of atherosclerosis compared to common white and brown adipose tissue depots. The results show local-dependent variations in lipid unsaturation indicating inter alia the earlier changes in the abdominal aorta, visible for 16-week-old animals, than in the thoracic aorta. Additionally, our results show that the lipid composition of the thoracic PVAT along the aorta is homogeneous in contrast to the significantly mixed composition of the abdominal PVAT. We demonstrated the heterogeneity in the chemical composition of the PVAT based on lipid unsaturation that enables to shed more light on the biochemistry of PVAT.

Acknowledgments:

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2.19.

Pathway-dependent anti-obesity effect of short-chain fatty acids via modulation of the gut microbiome and adipose tissue

Zuzanna Majka[a,b], Barbara Zapala[c], Agnieszka Krawczyk[d], Krzysztof Czamara[a], Joanna Mazurkiewicz[b], Ewa Stanek[a], Dominika Salamon[d], Tomasz Gosiewski[d] , Agnieszka Kaczor[a,b]
[a]Jagiellonian Centre of Experimental Therapeutics (JCET), Jagiellonian University, 14 Bobrzynskiego Str., 30-348 Krakow, Poland.
[b]Faculty of Chemistry, Jagiellonian University, 2 Gronostajowa Str., 30-387 Krakow, Poland.
[c]Department of Clinical Biochemistry, Jagiellonian University Medical College, 8 Skawinska Str.,31-066 Krakow, Poland.
[d]Department of Molecular Medical Microbiology, Faculty of Medicine, Jagiellonian University Medical College, 18 Czysta Str., 31-121 Krakow, Poland.

Abstract:

Currently, the gut microbiome-adipose tissue axis is recognized as one of the key factors controlling systemic metabolism via short-chain fatty acids (SCFAs). SCFAs are produced in the colon by fermentation of non-digestible carbohydrates (e.g. fiber, resistant starch). Adipose tissue (AT) is highly heterogenous and therefore it can store lipids (the white adipose tissue; WAT) or control the whole-body energy expenditure (the brown adipose tissue; BAT). Also, the response of AT to disease factors such as a high-fat diet (HFD) is phenotype- and local-dependent.

To evaluate the direct impact of SCFAs on the AT vs the influence of the SCFAs due to fiber fermentation in the gut, we undertook here an investigation of the effect of an oral butyrate supplementation and SCFAs released via β-glucan consumption on AT and gut microbiota composition in the murine model of HFD-induced obesity. Changes in various AT depots were studied using Raman-based methods (ex vivo and in vivo), while gut microbiome evaluation was performed using Next Generation Sequencing. We confirmed that both supplements show a similar anti-obesity effect on young mice on HFD, however via different pathways. Butyrate reduced epididymal WAT (eWAT, the main WAT deposit) mass, decreased lipid saturation in WAT-like AT: eWAT and abdominal aortic perivascular AT (AA PVAT), and the lipid content in single PVAT adipocytes. Whereas, β-glucan significantly changed the composition and diversity of the gut microbiome, with no effect on eWAT.

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2.20.

In vitro models of adipocytes – cell stimulation and compound delivery studied by Raman microscopy

Ewa Stanek[1], Krzysztof Czamara[1], Joanna Mazurkiewicz[2], Aleksandra Orlef[2], Agnieszka Kaczor[1,2]
[1] Jagiellonian Centre of Experimental Therapeutics (JCET), Jagiellonian University, 14 Bobrzynskiego Str., 30-348 Krakow, Poland.
[2] Faculty of Chemistry, Jagiellonian University, 2 Gronostajowa Str., 30-387 Krakow, Poland.

Abstract:

Adipose tissue (AT) is a key regulator of systemic metabolism and the main target for anti-obesity treatment. Due to its heterogeneous composition, AT possesses an abundant pool of various cell-type populations including, among others, primary adipocytes and stromal vascular fraction (SVF) with adipose-derived stem cells. Excessive accumulation of lipids in AT and the rise of associated disorders, are one of the most prominent causes of death worldwide. Therefore, in the context of therapeutic strategies, it leads to the development of appropriate cellular models and technical approaches.

This work aimed to evaluate the phenotype of primary and SVF-derived adipocytes originated from epididymal (eWAT) and interscapular (iBAT) adipose tissues of C57Bl/6 mice and determine their response to antioxidative compounds such as carotenoids. The results show that adipocytes vastly differ in the chemical composition of lipids, whether they are derived from cell culture or have been freshly isolated from tissue. Raman spectra revealed major changes manifested by, among other, levels of lipid unsaturation. We also tested two methods of transport into cells of potent AT active carotenoid, i.e. astaxanthin, encapsulated in nanocarriers or complexed with protein. Both ways provide effective release of carotenoids to primary murine adipocytes in a time-dependent manner. Our work demonstrates the applicability of Raman microscopy in verifying different models of adipocytes that may serve as a future methodology for delivering i.e. fat-burning drugs.

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2.21.

Progression of Cardiovascular Dysfunction In Tumour-bearing Mice

Simge Baydar[1], Christopher Dostal[1], Lujza P. Szabo[1], Casian-Simon Aioanei[2], Peter Pokreisz[1], Dietmar Abraham[3], Karin Zins[3], Henry M. Gong[4], Jonathan Kirk[4], Latifa Bakiri[2], Erwin F. Wagner[2], Bruno K. Podesser[1], Attila Kiss[1]

[1] Center for Biomedical Research and Translational Surgery, Medical University of Vienna;
[2] Genes and Disease Group, Medical University of Vienna;
[3] Center for Anatomy and Cell Biology, Medical University of Vienna,
[4] Department of Cell and Molecular Physiology, Loyola University Stritch School of Medicine, Maywood, IL

Abstract:

Background:
Cancer is independently associated with altered cardiac function prior to cardiotoxic chemotherapy exposure. Similar to cancer-associated-cachexia, the elevation of IL-6 in plasma was associated with reduced cardiac function in heart failure. Cancer cells manipulate BCL-2–associated-athanogene-3(BAG3)-HSP70-regulated pathways, same proteins regulate sarcomere assembly in cardiomyocytes. Here, we aimed to characterize the progression of cardiovascular dysfunction in tumour-bearing mice.

Methods:
Colon-26-adenocarcinoma-cells(C26;n=22) with/without shIL-6 (C26-shIL-6;n=22) were injected subcutaneously in adult male BALB/c mice. Control mice were injected with PBS (n=13). Echocardiography and invasive hemodynamic measurements in-vivo and ex-vivo isolated working heart experiments were performed 10 (early-stage) and 20 (late-stage) days post injection. The expression of BAG3 and HSP70 in cardiac tissue was determined by Western blot and vascular function was assessed using wire myography.

Results:
Tumour-size was comparable between the cancer groups. However, only C26-group showed significantly reduced subcutaneous fat and skeletal muscle($p<0.05$), confirming cancer-cachexia. Echocardiography shows tendency towards a decline of left ventricular (LV) ejection fraction at the early-phase, which turned significantly lower at late-stage($p<0.05$) in tumour-bearing mice. Invasive hemodynamic and isolated working heart measurements confirmed LV systolic and diastolic dysfunction (late-stage, $p<0.05$). Interestingly, heart rate and aortic flow were declined in cachectic animals ($p<0.05$). These alterations were associated with a reduced expression of BAG3 and Hsp70 as well as with a reduction in Ca2+-sensitivities of force production of LV permeabilized cardiomyocytes.

Discussion:
Tumour and cancer-cachexia resulted in significant cardiac contractile dysfunction in mice. Additionally, our data suggest that targeting BAG3-Hsp70-complex in cardiomyocytes may provide a novel strategy to improve cancer-associated-cardiovascular-dysfunction.

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2.22.

A comparative evaluation of tyrosine kinase inhibitors effects on endothelial function in mice in vivo.

Brygida Marczyk[1,2], Anna Bar[1], Agnieszka Kij[1], Iwaine Benbouziane[1], Joanna Suraj-Prażmowska[1], Grzegorz Kwiatkowski[1], Stefan Chłopicki[1,2]

[1] Jagiellonian University, Jagiellonian Centre for Experimental Therapeutics (JCET), Bobrzyńskiego 14, 30-348, Krakow, Poland
[2] Jagiellonian University Medical College, Faculty of Medicine, Chair of Pharmacology, Grzegórzecka 16, 31-531, Krakow, Poland

Abstract:

Tyrosine kinase inhibitors (TKIs) are widely used in the treatment of various cancers. However, they exert undesirable vascular effects, that may be associated with TKIs-induced effects on endothelial function. Here, we comprehensively compared the effects of various TKIs on endothelial function in mice in vivo.

MRI-based assessment of endothelial function in vivo was performed in 10–12-week-old C57BL/6 male mice treated with nilotinib or imatinib (10-360 mg/kg/day) or ponatinib (1-10 mg/kg/day) for 4-8 weeks, in comparison to untreated mice .

TKIs induced a dose-dependent impairment of endothelium-dependent vasodilation in response to acetylcholine in the thoracic and abdominal aorta as well as impairment of flow-mediated vasodilation in the femoral artery, while sodium nitroprusside-induced vasodilatation remained unaltered. Importantly, the potency of TKIs to induce endothelial dysfunction was different and achieved after 4-week-treatment with imatinib, nilotinib and ponatinib at doses equal or higher than 120 mg/kg, 30 mg/kg, and 1 mg/kg, respectively. Analysis of 18 plasma endothelial biomarkers of endothelial function by LC/MS-based targeted proteomic show moderate changes.

In conclusion, the potency to induce the impairment of endothelium-dependent vasodilation in vivo in mice by tested TKIs was compatible with their known risks for vascular toxicity reported for these compounds in clinical settings and their plasma concentration measured by UPLC-MS/MS technique show plasma concentrations relevant to clinical setting of CML patients treated with TKIs.Altogether, in vivo functional endothelial profiling by MRI may be useful to predict vascular toxicity in preclinical studies and pave the way towards “endothelial safe” TKIs. ytes may provide a novel strategy to improve cancer-associated-cardiovascular-dysfunction.

Acknowledgments:

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2.23.

Larval zebrafish as a model of vascular toxicity induced by a chemotherapeutic drug - ponatinib

Emilia Seta [1], Stefan Chłopicki [2], Tomasz Prajsnar [1]
[1] Department of Evolutionary Immunology, Faculty of Biology, Jagiellonian University, Krakow, Poland
[2] Jagiellonian Centre for Experimental Therapeutics, Jagiellonian University, Krakow, Poland

Abstract:

Despite growing rate of success in cancer treatment, the increasing number of severe side effects on cardiovascular system have been observed among patients. The mechanisms of such vascular toxicity of many chemotherapeutic compounds are largely unknown.

A chemotherapeutic agent called ponatinib is a BCR-ABL tyrosine kinase inhibitor and a third generation drug used in therapy against chronic myeloid leukaemia and Philadelphia chromosome- positive acute lymphoblastic leukaemia. It has been shown in the phase I and II trials that treatment with ponatinib contributed to adverse vascular events in 48 % or 24 % of patients, respectively.

In this study, we use the zebrafish model that is currently gaining popularity in biomedical research as it combines the low cost, ease of use or amenability to high throughput screening of in vitro systems with complexity or genetic and functional similarity of mammalian systems. Our experiments involved microinjections of fluorescent (FITC) dextran to the bloodstream of transgenic zebrafish larvae with fluorescently tagged (mTurquoise) endothelium at 2 days post fertilization. Subsequently, the injected larvae were treated with ponatinib at 10 μM by immersion for 12 hours and finally imaged by confocal microscopy. In order to quantify the extravasation of dextran, the fluorescence intensity within 6 areas inside and outside a blood vessel (dorsal aorta) was measured in each individual.

We observed reduced vessel diameter and the enhanced vascular leakage in ponatinib-treated larvae in comparison to vehicle controls. Therefore, we believe zebrafish is a valuable model for studying endothelial toxicity in vivo.

2.24.

Pharmacokinetic study of tyrosine kinase inhibitors to understand their detrimental effects on endothelial function

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

Abstract:

The vascular endothelium maintains the health of the cardiovascular system and its alteration in response to applied oncotherapy can lead to the development of cardiovascular diseases. Inhibitors of tyrosine kinase (TKIs) induce endothelial dysfunction (B.Marczyk et al., unpublished), and are known to produce serious vascular side effects, but it is not known whether pharmacokinetics (PK) of TKIs is of importance in this respect. The aim of this study was to perform pharmacokinetic study and to build a suitable PBPK model.

In vivo pharmacokinetic experiments were performed in C57BL/6 male mice. Animals were injected intraperitoneally (IP) with imatinib, nilotinib, ponatinib and asciminib at doses: 12 mg/kg, 3 mg/kg, 0.3 mg/kg and 0.3 mg/kg, respectively. Plasma and selected tissues (brain, heart, lung, spleen, kidney, fat, intestine, aorta, liver) were collected at the following time points: 0, 5, 15, 30, 45, 60, 120, 180, 240 and 480 min. The concentration of TKIs in plasma, aorta and liver samples was determined by LC-MS/MS technique, and the PK parameters were calculated using R Studio. Results showed the differences in PK parameters for studies TKIs (for example, NIL 3 mg/kg, IP injection, AUC = 16 902 ng*h/mL and NIL 10 mg/kg, IV injection, AUC = 76 252 ng*h/mL), that might be linked to vascular toxicity but further studies are needed. In particular the pharmacokinetic experimental data in mice will be used with an attempt to build a predictive PBPK model to relate PK profile with the risk of the endothelial dysfunction induced by these compounds.

Acknowledgments:

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2.25.

Effect of BRAF and MEK inhibitors on the endothelial function in murine aorta ex vivo and in vivo.

Elżbieta Buczek[1], Marta Smęda[1], Anna Bar[1], Agnieszka Karaś[1], Brygida Marczyk[1], Janusz Pyka[1], Ebrahim H. Maleki[1], Anna Gdula[1], Stefan Chłopicki[1]
[1] Jagiellonian Centre for Experimental Therapeutics, Jagiellonian University, Cracow, Poland

Abstract:

Persistent hyperactivation of the RAS–RAF–MEK–ERK pathway commonly contributes to carcinogenesis. Many of the recently developed cancer therapies target this pathway, like BRAF and MEK inhibitors, used in combination to treat metastatic melanoma, non-small cell lung cancer and colon cancer. Unfortunately, cardiovascular adverse events associated with BRAF and MEK inhibitors, such as hypertension, left ventricular dysfunction, venous thromboembolism have been reported.

The mechanisms of BRAF and MEK inhibitors cardiotoxicity are incompletely understood. The occurrence of arterial hypertension could be triggered by the reduced bioavailability of nitric oxide, because of an impaired vascular endothelial growth factor pathway, mediated through the MAPK pathway.

To assess the effect of BRAF and MEK inhibitors ex vivo in the isolated mice aortas incubated for 24 hours with selected drug, endothelial dependent vasorelaxation was assessed, NO production was measured by EPR spin trapping with DETC and chosen endothelial markers were analyzed using Western Blot. In vivo endothelial function was assessed by MRI in mice treated i.p. with chosen inhibitors.

In the ex vivo model impaired endothelial function in an aorta incubated with BRAF inhibitor (dabrafenib), but not MEK inhibitor (trametinib), was found. Interestingly, toxic effect of dabrafenib was attenuated by the simultaneous incubation with vitamin K1.

Moreover, the decreased expression of claudin-5 in aortas treated with dabrafenib may indicate that BRAF inhibitors affect also the endothelial permeability.

Our findings may suggests that in the vascular endothelium, inhibition of the BRAF-AKT but not MEK signaling, is crucial for the occurrence of adverse effects of combined therapy.

2.26.

PDI A3-based pharmacology regulates cross-talk between the endothelium and cancer cells.

Marta Stojak[1], Joanna Suraj-Prazmowska[1], Anna Kurpinska[1], Kamila Wojnar-Lason[1] Magdalena Milczarek[2], Martyna Stachowicz-Suhs[2], Joanna Rossowska[2], Ivars Kalviņš[4], Joanna Wietrzyk[2], Stefan Chlopicki[1,3]
[1] Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Krakow, Poland
[2] Department of Experimental Oncology, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland
[3] Department of Pharmacology, Jagiellonian University Medical College, Krakow, Poland
[4] Laboratory of Carbofunctional Compounds,Latvian Institute of Organic Synthesis, Riga, Latvia

Abstract:

Dynamic interaction between cancer cells and the vascular endothelium plays the crucial role during metastasis formation. Recent studies demonstrate that protein disulfide isomerases (PDIs) can modulate cell-cell or cell-extracellular matrix interactions.

Our recent study shows that extracellular PDIA1 regulates the adhesion and transendothelial migration of breast cancer cells via disulphide re-arrangement of ecto-sulfhydryls. However, it is not known whether, apart from PDIA1, other PDI isoforms are involved in regulation of cancer cells interactions with microenvironment components. The aim of the present work was to identify the relative importance of PDIA3 in regulating interactions of breast cancer cells with lung microvascular endothelial cells. A particular attention was dedicated to mechanistically explain whether direct adhesion between breast cancer cells and pulmonary endothelial cells or indirect interactions of these cells via extracellular matrix proteins were regulated by PDIA3. Experiments were performed in vitro on human lung microvascular endothelial cells and two breast cancer cell lines (MCF-7 and MDA-MB-231). To assess the biological role of PDIA3, we used two approaches: 1/ inhibition of PDIA3 using novel PDIs inhibitor, C-3399 and 2/ silencing of PDIA3 in breast cancer cells. We found that using of non-selective PDI inhibitors with preference toward PDIA3 (C-3399) is the most effective way to inhibit breast cancer cells adhesion to various substrates (endothelial cells, collagen I, fibronectin). Moreover, the inhibition of PDIA1 affords weak anti-adhesive effect, while inhibition of PDIA3 seems to be the most effective way to modulate interactions between cancer and the endothelium.

Acknowledgments:

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2.27.

Protein disulfide isomerase A1 – a new player in the regulation of fenestrations of hepatic endothelium

Izabela Czyzynska-Cichon[1], Magdalena Giergiel[2], Anna Kurpinska[1], Kamila Wojnar-Lason[1,5], Patrycja Kaczara[1], Marek Szymonski[2], Malgorzata Lekka[3], Ivars Kalviņš[4] and Bartlomiej Zapotoczny[2,3*], Stefan Chlopicki[1,5*]

[1] Jagiellonian University, Jagiellonian Centre for Experimental Therapeutics (JCET), Bobrzynskiego 14, 30-348 Krakow, Poland [2] Jagiellonian University, Centre for Nanometer-Scale Science and Advanced Materials, NANOSAM, Faculty of Physics, Astronomy, and Applied Computer Science, Lojasiewicza 11, 30-348 Krakow, Poland [3] Department of Biophysical Microstructures, Institute of Nuclear Physics, Polish Academy of Sciences, Radzikowskiego 152, 31-342 Krakow, Poland [4] Laboratory of Carbofunctional Compounds, Latvian Institute of Organic Synthesis, LV-1006 Riga, Latvia [5] Jagiellonian University Medical College, Faculty of Medicine, Department of Pharmacology, Grzegorzecka 16, 31-531, Krakow, Poland [*] corresponding authors

Abstract:

Protein disulfide isomerases (PDIs) are involved in many processes including adhesion and cytoskeleton reorganization, but their contribution to the fenestration formation in liver sinusoidal endothelial cells (LSECs) remains unknown. Therefore, this study aimed to investigate the repertoire of PDIs in primary mouse LSECs and the role of major isoforms in the regulation of fenestration dynamics.

A repertoire of PDIs and integrins was analyzed using a proteomic approach, while the number of fenestrations after PDIs inhibition was investigated taking advantage of the AFM measurements. LSECs function and cytoskeleton organization were comprehensively studied using cytotoxicity and adhesion assays, bioenergetic measurements (Seahorse FX), Western blot analysis, and confocal microscopy.

PDIA1 and PDIA3 were the most expressed isoforms in primary mouse LSECs, while the most abundant integrins were β-1 and α-1. Inhibition of PDIA1 significantly reduced the number of fenestrae in the LSECs, while inhibition of PDIA3 had no effect. Blocking of free thiols by the cell-penetrating reagent N-ethylmaleimide, but not by the non-cell-penetrating 4-chloromercuribenzenesulfonate (pCMBS), induced LSEC defenestration. Neither the PDIA1 inhibitors nor the pCMBS affected cellular adhesion. The effects of PDIA1 inhibition on fenestrations were not linked to changes in LSECs viability or bioenergetics nor with a clear-cut rearrangement of cytoskeleton. However, blocking of PDIA1 did not prevent actin depolymerization by cytochalasin B, a known fenestration stimulator, when added after PDIA1 blockade resulted in a complete reversal of LSECs defenestration.

These results demonstrate that PDIA1, but not PDIA3, plays a regulatory role in the fenestration dynamics in LSECs through intracellular disulfide exchange.

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2.28.

Increased sex hormone-binding globulin concentration is accompanied by endothelial dysfunction in older men

Marcin Grandys[1], Joanna Majerczak[1], Stefan Chlopicki[2,3], Jerzy A. Zoladz[1]
[1]Chair of Exercise Physiology and Muscle Bioenergetics, Faculty of Health Sciences, Jagiellonian University Medical College, Krakow, Poland
[2]Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Krakow, Poland
[3]Department of Experimental Pharmacology, Chair of Pharmacology, Jagiellonian University Medical College, Krakow, Poland,

Abstract:

It has been demonstrated that sex hormone-binding globulin (SHBG) level may be related to cardiovascular risk, but there is still controversy about its role in the health of vascular system. Therefore the aim of this study was to determine the age-dependent alterations in basal serum SHBG in relation to endothelial function. In the group of young (22.0 ± 2.3 yrs, n=17) and older, physically non-active subjects (60.9 ± 6.9 yrs, n=16) SHBG concentration, endothelial markers (hyaluronic acid – HA, syndecan-1 – SDC-1, sum of nitrite and nitrate – NO_x, 6-keto-prostaglandin $F_{1\alpha}$ – 6-keto-$PGF_{1\alpha}$ and 1-methylnicotinamide – MNA concentrations) and inflammatory markers were analyzed. We have found that basal serum SHBG concentration was significantly higher ($p<0.001$) in older men in comparison to young men in the absence of differences in testosterone concentration. At the same time, older men presented with almost 2-fold higher basal serum HA and significantly lower plasma 6-keto-$PGF_{1\alpha}$ concentrations (both $p<0.01$) as well as with significantly worse blood lipid (higher concentrations of triglycerides and total and LDL cholesterol) and inflammatory profiles (higher concentrations of tumor necrosis factor-α, c-reactive proteins and ferritin). Although there were no significant differences in other endothelial markers, older group had a tendency to lower plasma NO_x and MNA concentrations ($p=0.09$ and $p=0.11$, respectively). Interestingly, SHBG was significantly positively correlated with HA and TNF-α concentrations and negatively with 6-keto-$PGF_{1\alpha}$ and MNA concentrations in all studied individuals (n=33). We have concluded that age-related increase in basal serum SHBG concentration is accompanied by a significant worsening of endothelial function.

Acknowledgments:

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2.29.

Aging-related decline of working memory performance is associated with impaired neurovascular coupling responses in healthy adults

Tamas Csipo[1,2], Agnes Lipecz[1,2], Peter Mukli[2], Stefano Tarantini[1,2], Adam Nyul-Toth[2], Anna Csiszar[2], Zoltan Ungvari[1,2], Andriy Yabluchanskiy[2]
[1] Department of Public Health, Faculty of Medicine, Semmelweis University, Budapest, Hungary
[2] Vascular Cognitive Impairment and Neurodegeneration Program, Oklahoma Center for Geroscience and Healthy Brain Aging, Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

Abstract:

Introduction
Normal brain function requires continuous adjustment of cerebral blood flow (CBF), through the process also known as neurovascular coupling (NVC). Therefore, impairment of the resting CBF or impairment of NVC in the brain may be responsible for the development of vascular cognitive deficits in aging. In this study, we test whether cognitive function and the cognitive challenge-evoked NVC is different in two separate age groups.

Methods
We enrolled 37 healthy young (y: <40 y. o.) and 66 healthy aged (a: >65 y. o.) adults. Participants were asked to perform the n-back cognitive test, that consisted of tasks of gradually increasing difficulty levels (i. e. 1-back, 2-back). During cognitive testing, either Transcranial Doppler sonography (TCD) was used to assess change of CBF velocity (y: n=27, a: n=25) or functional Near Infrared Spectroscopy (fNIRS) was used to assess changes in cortical hemoglobin concentration (y: n=12, a: n=41).

Results
Cognitive n-back test revealed an age-associated decrease in working memory performance (2-back accuracy: y: 3.8 [3.4 to 3.8], a: 2.8 [2.4 to 3.1] $p<0.001$, 2-back reaction time: y: 584±176 ms, a: 693±189 ms, $p=0.03$). fNIRS measurements revealed increased NVC in the prefrontal cortex during the 2-back task, when compared to the easier 1-back task only in the young group.

Conclusion
Our results suggest that aging is associated with the decline of working memory performance. An NVC pattern was also observed in the aged group that is indicative of the exhaustion of resources, which may contribute to the impairment of cognitive function.

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2.30.

Anti-inflammatory pharmacotherapy as a preventive strategy for vascular stiffness: A bridge to the clinic

Soroush Mohammadi Jouabadi[1,2], Paula K. Bautista Nino[3], Keivan Golshiri[1], Annika Jüttner[1], Ehsan Ataei Ataabadi[1], René de Vries[1], A.H. Jan Danser[1], Anton Roks[1*]

[1] Vascular disease and Pharmacology, Department of Internal Medicine, Erasmus Medical Center, Rotterdam, the Netherlands.
[2] Department of Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands.
[3] Research Center Floridablanca, Colombian Cardiovascular Foundation, FL, Colombia.
[*] Corresponding Author.

Abstract:

Vasculature aging is an important determinant of cardio/cerebrovascular diseases. Vascular aging is associated with chronic low-grade inflammation, which might contribute to disease. Vascular stiffness in one of the main features associated to aging and Pulse Wave Velocity (PWV) is a well-established technique for estimating arterial stiffness. We therefore hypothesized that long-term therapy with anti-inflammatory agents inhibits persistent vascular inflammation, enhances endothelium function and hence decelerate vascular aging. To address this, we first performed experiments in age-accelerated mouse models with smooth muscle-selective Ercc1 DNA repair gene excision (SMC-Ercc1$^{d/-}$), and then translated the results into the clinic through a meta-analysis of clinical trials. At the age of 22 weeks, higher PWV levels in SMC-Ercc$^{1/d}$-(KO) than in Wild-Type littermates (WT) confirmed the effect of aging on vascular stiffness. Colchicine therapy for 12 weeks resulted to decreased PWV levels compared to the vehicle group (P-value = 0.006) and normalized them to WT levels. SMC-KO mice showed diminished reactive hyperemia response measured by Laser Doppler device compared to WT while no effect of the treatment was observed. In line with our findings in animal setting, the meta-analysis of clinical trials comparing the effectiveness of anti-Rheumatoid Arthritis drugs (as the representative of long-term anti-inflammatory treatments) to placebo, trended towards PWV reduction (Random effect-Pooled SMD= -0.10, 95%CI [-0.43; 0.23], prediction interval [-0.95; 0.76]). Future post-hoc analysis of this study aims to identify whether changes in endothelium function could explain the effectiveness of colchicine in prevention of vascular stiffness. icative of the exhaustion of resources, which may contribute to the impairment of cognitive function.

2.31.

Macrophage-secreted enzymes as biomarkers of inflammatory diseases

Attila Ádám Szabó, Enikő Edit Enyedi, Tamás Pintér, István Tibor Altorjay, Péter Hajnal, Zoltán Papp, Attila Tóth, Miklós Fagyas
Division of Clinical Physiology, Department of Cardiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Abstract:

Objective:
Angiotensin-converting enzyme (ACE) plays an important role in the pathogenesis of cardiovascular diseases and proved to be an excellent target for treating these illnesses. In macrophage-mediated inflammatory diseases (sarcoidosis, idiopathic bowel disease etc.), activated macrophages can produce high amounts of ACE and other enzymes such as chitotriosidase (CTO). The aim of our study was to investigate the role of serum ACE and CTO activities as biomarkers in inflammatory diseases.

Methods:
Patients with sarcoidosis and idiopathic bowel disease were included in the study and their serum ACE and CTO activities were determined by fluorescence kinetic assays at several time points. Patients' clinical data and medications are recorded and evaluated.

Results:
ACE inhibitor drugs significantly reduced serum ACE activity in sarcoidosis population (median [interquartile range], treated with ACE inhibitor: 4.42 [2.93-6.75] U/L, n=302; vs. untreated with ACE inhibitor: 11.32 [8.79-13.92] U/L, n= 1521; $p<0.01$), which should be taken into account when using ACE activity as a biomarker. Serum ACE and CTO activities were significantly higher in patients with sarcoidosis (11.84 [10.1-13.5] U/L; 2882 [1497-4166] mU/L, n= 80, respectively) than in controls (9.19±2.1 U/L; 539 [316-884] mU/L, n= 133, respectively). A similar result was observed in idiopathic bowel disease. Significant reductions in ACE and CTO activity of up to 50% were also detected after initiation of anti-inflammatory treatment. In the follow-up of some patients, we observed that serum ACE and CTO activities increased with disease progression or relapse.

Conclusions:
Serum ACE and CTO activities can be good biomarkers of macrophage-mediated inflammatory diseases. In addition to disease activity, their level may be a good indicator of response to drug therapy and may can predict relapse. icative of the exhaustion of resources, which may contribute to the impairment of cognitive function.

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2.32.

Impact of endurance training on the circulating branched-chain amino acids concentration in rats

Lucyna Widacha[1], Agnieszka Kij[2], Hanna Drzymala-Celichowska[3,4], Jan Celichowski[3], Kamil Kuś[2], Stefan Chłopicki[2,5], Jerzy A. Zoladz[1], Joanna Majerczak[1]

[1] Chair of Exercise Physiology and Muscle Bioenergetics, Faculty of Health Sciences, Jagiellonian University Medical College
[2] Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University
[3] Department of Neurobiology, Faculty of Health Sciences, Poznan University of Physical Education
[4] Department of Physiology and Biochemistry, Faculty of Health Sciences, Poznan University of Physical Education
[5] Chair of Pharmacology, Faculty of Medicine, Jagiellonian University Medical College

Abstract:

BACKROUND

Elevated levels of circulating branched-chain amino acids (BCAAs; leucine, isoleucine and valine) constitute a hallmark of the metabolic disorders and chronic heart failure. BCAAs exert also pro-arrhythmic effects. The main aim of the present study was to establish the impact of 8-week of endurance training on the plasma BCAAs concentration.

METHODS

Twenty-four adult male rats were randomly allocated to a sedentary control (n=12) or a training group (n=12). The rats from training group performed 8-weeks of strenuous endurance training on a treadmill in which the total distance covered by them amounted to 36.8±12.8 km. The effect of endurance training on circulating branched-chain amino acid concentrations was analyzed for the sum of three branched-chain amino acids (∑BCAAs) as well as separately, for leucine, isoleucine and valine.

RESULTS

We found that intense endurance training in rats reduced circulating concentration of ∑BCAAs ($p<10^{-4}$; ~19%▼). Moreover, when analysis were performed separately for three BCAAs, we noticed that intense endurance training decreased plasma concentration of leucine ($p<10^{-4}$; ~24%▼) and isoleucine ($p<10^{-4}$; ~27%▼), whereas no significant changes were observed for valine (p=0.16; ~6%▼).

CONCLUSIONS

The main finding of the present study was that the strenuous endurance training decreases BCAAs in plasma. Since, elevated plasma BCAAs level is a hallmark for metabolic and cardiovascular disorders, training-induced reduction of BCAAs levels in plasma, seems to be a potential promising strategy in enhancing cardiovascular health. te to the impairment of cognitive function.

Acknowledgments:

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2.33.

Circulating and myocardial BCAAs level is elevated in the heart failure Tgαq*44 mice

Joanna Szramel[1], Lucyna Widacha[1], Agnieszka Kij[2], Stefan Chlopicki[2,3], Jerzy A. Zoladz[1], Joanna Majerczak[1]

[1] Chair of Exercise Physiology and Muscle Bioenergetics, Faculty of Health Science, Jagiellonian University Medical College
[2] Jagiellonian Centre of Experimental Therapeutics (JCET), Jagiellonian University
[3] Chair of Pharmacology, Jagiellonian University Medical College

Abstract:

BACKROUND
Failing heart is characterized by an impaired branched-chain amino acids (BCAAs) oxidation and higher BCAAs concentration ([BCAAs]) in myocardium, which is linked to the decrease in cardiac contractility.

The aim of the study was to determine the impact of spontaneous physical activity (wheel running, WR) on the [BCAAs] in plasma, heart (HE) and gastrocnemius muscle (GAS) in relation to the mitochondrial oxidative capacity (based on CIV-complex) in heart failure (HF) mice model (homozygous Tgαq*44).

METHODS
Adult female wild-type (WT) and Tgαq*44 (Tg) mice were randomly assigned to either sedentary (Sed) or trained group (Tre) which were subjected to the 8-weeks of WR.

RESULTS
Tg mice revealed significantly higher [BCAAs] in plasma and heart compared to WT mice, whereas [BCAAs] in gastrocnemius was not significantly different between WT and Tg mice. No significant difference between WT and Tg mice in the CIV-complex content was observed, both in heart and in the gastrocnemius muscle.

Exercise physical capacity (e.g. total distance of running) of Tg mice was significantly reduced when compared to WT mice.

No effect of spontaneous physical activity on the mitochondrial CIV-complex content in the heart of both WT and Tg mice was observed, although, in the gastrocnemius muscle CIV-complex content increased to a similar extent in both groups after the WR.

CONCLUSIONS
We found that circulating and myocardial BCAAs level is higher in the heart failure mice. This might contribute to the poorer exercise tolerance of the heart failure mice. of the exhaustion of resources, which may contribute to the impairment of cognitive function.

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2.34.

The studies of platelets biochemistry and biomechanics by Quartz Crystal Microbalance with Dissipation Monitoring system.

Katarzyna Derszniak[1,2], Michał Pacia[2], Szczepan Zapotoczny[2], Maria Nowakowska[2], Stefan Chłopicki[1,3]
[1] Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University
[2] Faculty of Chemistry, Jagiellonian University
[3] Chair of Pharmacology, Jagiellonian University

Abstract:

Besides the widely studied platelets biochemistry, platelets biomechanics has become a new complementary study's target influencing platelets adhesive properties and thrombus/ platelets clot stability. Under physiological conditions, both functions could be regulated by blood rheology and endothelial mediators such as prostacyclin (PGI2), nitric oxide (NO), and carbon monoxide (CO). One of the promising systems for studying platelets biochemistry and biomechanics is Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D) – the flow acoustic-gravimetric sensors-based system.

Human washed convulxin-stimulated (Cvx), Cvx–eptifibatide-treated, non-stimulated, non-stimulated–eptifibatide-treated, and non-stimulated cPGI2–, PAPA-NO– and CORM-A1–treated platelets were studied by QCM-D. Fibrinogen-coated/gold-polystyrene sensors were used. The frequency (Δf) and dissipation (ΔD) shifts were registered for platelets-sensors interactions (100µl/min, 37°C, 30 min, 3rd – 13th overtone numbers). Platelets were imaged at 30 min by SEM.

The registered Δf, ΔD shifts supported by SEM showed that αIIbβ3-dependent Cvx platelets-sensor interactions run more dynamically than for non-activated platelets, both led to rigidifying the biointerface under the elastic regime due to fully spread platelets. The ΔD/Δf ratio supported by SEM revealed that: PGI2–treated platelets developed focal adhesion points that preceded the progressive platelets spreading; NO held platelets adhesion and spreading at the early stages, and cytoskeleton remodeling was running then; CO strongly reduced the platelets-sensor adhesion but did not significantly affect the platelets morphology.

The inhibition of platelets cAMP, cGMP pathways, and platelets bioenergetics differently affected platelets morphology which was translated into varied biointerface viscoelasticity sensed by the QCM-D which could be a useful tool in the bio-mechano-medicine field. esources, which may contribute to the impairment of cognitive function.

Acknowledgments:

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2.35.

Assessment of the effects of selected drugs or compounds with antiplatelet activity on the profile of molecules released from platelet granules

Anna Kurpińska[1], Agnieszka Kij[1], Kamil Przyborowski[1], Anna Bar[1], Agata Malinowska[2], Ewa Niedzielska-Andres[3], Renata Budzyńska[1], Stefan Chłopicki[1]

[1] Jagiellonian Centre for Experimental Therapeutics, Jagiellonian University, Krakow, Poland
[2] Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland.
[3] Chair and Department of Toxicology, Jagiellonian University Medical College, Krakow, Poland

Abstract:

The content of platelet granules and microvessicles is selectively released regulating e.g. coagulation processes, immune response or angiogenesis. Inadequate modulation of the platelet response can lead to failure or side effects of sub-optimal therapy and, despite having beneficial effects on anticoagulant mechanisms, induce e.g. a pro-inflammatory response.

To answer the question of how the platelet secretion is modulated, whether and what favorable or unfavorable mechanisms are activated or inhibited by selected antiplatelet drugs/compounds (ASA, cangrelor, bepristat, C-3389, C-3399), a series of molecular studies including proteomic, lipidomic and transcriptomic analyzes were performed. Additionally, the platelet releasate proteomic and lipidomic analyzes were conducted in the aged ApoE -/- mice compared with age matched C57Bl/6 mice.

In isolated mice platelets incubated with selected compounds, bepristat resulted in greater response when compared to C-3399. Both compounds downregulated coagulation proteins and affected cytoskeleton remodeling. Bepristat also highly downregulated proteins related to lipid and pyruvate metabolism, adhesion, immunological/inflammatory response and angiogenesis. C-3399 downregulated and bepristat upregulated the proteins related to protein folding, protein disulphide isomerase activity, translation and junction formation.

In the animal model of atherosclerosis lipidomic studies revealed pro-inflammatory profile of the platelet releasates in ApoE mice. Proteomic studies additionally highlighted altered coagulation proteins, actin cytoskeleton remodeling, upregulation of the proteins related to VEGF signaling pathway, transendothelial migration, peroxiredoxin and chaperone activity.

The analysis of the composition of the platelet releasate allows for monitoring of the effects of antiplatelet therapy, as well as to determine the effect on the surrounding tissues and organs, and may potentially form a diagnostic platform for the assessment of the pathological condition.

Acknowledgments:

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2.36.

How do the combinations of antiplatelet mediators with metabolic inhibitors affect the activity of platelets?

Marta Lech[1], Kamil Przyborowski[1], Stefan Chłopicki[1,2], Patrycja Kaczara[1]
[1]Jagiellonian Centre for Experimental Therapeutics, Jagiellonian University, Krakow, Poland
[2]Chair of Pharmacology, Faculty of Medicine, Jagiellonian University Medical College, Krakow, Poland

Abstract:

Thrombosis, which is associated with various cardiovascular diseases, is one of the leading clinical concerns associated with high morbidity and mortality. Despite antithrombotic treatment, the risk of cardiovascular events related to thrombosis is not eliminated, and, in addition, unwanted side effects, such as bleeding, can occur. Platelets are important players in vascular homeostasis and thrombosis; therefore, antiplatelet drugs are used to reduce thrombosis. However, some patients with metabolic diseases show increased platelet reactivity despite antiplatelet treatment. It suggests that platelet hyperreactivity is intrinsically linked to altered energy metabolism and that current antiplatelet strategies are not optimal for regulating platelet activity. The hypothesis of our project is that partial inhibition of metabolic pathways enhances the antiplatelet action of selected drugs and compounds acting via cAMP- and cGMP-dependent mechanisms.

We exposed platelets isolated from healthy volunteers to carbaprostacycline (cPGI2), PAPA NONOate (nitric oxide donor) and 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO; metabolic inhibitor), followed by analysis of platelet aggregation (96-well light transmission aggregometry), glycolysis and mitochondrial function (Seahorse XFe96 analyzer).

cPGI2 and PAPA NONOate at concentrations that substantially inhibited platelet aggregation, only slightly reduced oxidative phosphorylation, and activated glycolysis. In turn, 3PO insensibly reduced platelet aggregation, but inhibited both oxidative phosphorylation and glycolysis. The combination of compounds tested at concentrations, at which each of them individually only slightly affected platelet aggregation, allowed platelet aggregation to be substantially reduced. It suggests that the tested compounds act synergistically. In conclusion, pharmacologic regulation of platelet energy metabolism can improve the antiplatelet effect of drugs while reducing the concentrations used.

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2.37.

C-3399 inhibitor of PDI isoforms with an attractive profile of antiplatelet action

Kamil Przyborowski[1,2], Agnieszka Pelesz[1], Filip Fedak[1], Ivars Kalvins[3], Kerstin Jurk[2] and Stefan Chlopicki[1,4]
[1.] Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Krakow, Poland
[2.] Center for Thrombosis and Hemostasis (CTH), University Medical Center of the Johannes Gutenberg University, Mainz, Germany.
[3.] Laboratory of Carbocyclic Compounds, Latvian Institute of Organic Synthesis, Riga, Latvia
[4.] Chair of Pharmacology, Jagiellonian University Medical College, Krakow, Poland

Abstract:

As protein disulfide isomerases (PDI/PDIA1, 3, 4, 5, 6) significantly regulate platelet activity, their inhibition is becoming an attractive approach for the development of new antiplatelet drugs.

We are investigating a newly in-house synthesized inhibitor, C-3399, blocking PDI isoforms PDIA1, PDIA3 and PDIA6.

To show antiplatelet effects of C-3399 compared to PDIA1 selective inhibitor, bepristat 2a, and reference PDIA1/3/6 inhibitor, PACMA-31, platelet aggregation and platelet surface activation markers in response to the GPVI receptor agonist convulxin versus TXA2-mimetic, U46619, were evaluated using light-transmission aggregometry and flow cytometry, respectively.

C-3399 significantly inhibited in a concentration-dependent manner the activation of platelet αIIbβ3 integrin induced by convulxin, while it partially affected CD62P or CD63 surface expression in diluted platelet-rich plasma (PRP). The effects of PACMA-31 were similar to those observed for C-3399, whereas bepristat displayed the strongest inhibitory effect on αIIbβ3 integrin activation and nearly completely reduced surface expression of CD62P or CD63 compared to C-3399 or PACMA-31.

C-3399 and bepristat partially reduced platelet aggregation in PRP induced by high convulxin concentration, while PACMA-31 had no effect.

Interestingly, the blockade of PDIA1, PDIA3 and PDIA6 by C-3399 or PACMA-31 nearly completely inhibited U46619-induced activation of αIIbβ3 integrin, CD63 surface expression and platelet aggregation, while the selective blockade of PDIA1 by bepristat resulted in significantly weaker inhibitory effects on these platelet responses.

These results suggests that C-3399 blocks distinct targets in convulxin- and TXA2-mediated platelet activation, which shows agonist-dependent similarities and differences to bepristat and PACMA-31. However, further studies are required to explore mechanisms behind.

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2.38.

Pharmacological modulation of platelet-derived extracellular vesicle release; focus on PDI inhibition

Agnieszka Pelesz [1], Kamil Przyborowski [1], Stefan Chlopicki [1,2]
[1] Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Krakow, Poland
[2] Chair of Pharmacology, Jagiellonian University Medical College, Krakow, Poland

Abstract:

The knowledge about the effects of antiplatelet drugs on platelet-derived extracellular vesicle (pEVs) release playing a crucial role in thrombosis, hemostasis and non-hemostatic effects of platelets is limited. Various pharmacological modulators of platelet activity may affect vesiculation mechanisms differently and subsequently lead to distinct effects on crosstalk between platelets and other cells. The study aims to comprehensively characterize changes in pEVs formation in murine platelets after inhibition of protein disulfide isomerase 1 (PDIA1), as well as other platelet mechanisms by reference antiplatelet drugs using nanoflow cytometry protocol.

The preliminary results indicate that activation of the platelet GPVI receptor by convulxin and activation of calcium–*calpain* dependent pathway by ionophore A23187 caused excessive pEVs formation. PEVs formation via GPVI stimulation was dependent on platelet aggregation and was reduced by αIIbβ3 integrin antagonist. Furthermore, PEVs release involved ADP-dependent feedback mechanism of platelet activation because PEVs formation was blocked by P2Y12 receptor antagonist, cangrelor, however this process was independent of TXA2 synthesis as evidenced by lack of the effects of aspirin on PEVs. In contrast,the calcium–calpain dependent pathway, was αIIbβ3 integrin independent. Interestingly, selective PDIA1 blockage by bepristat 2a inhibited pEVs formation induced by either by convulxin or by ionophore A23187.These results emphasize that both aggregation-dependent and agreggation-independent pEVs formation can be modulated by the PDIA1 inhibition, but further studies are need to confirm this conclusion and to define the role of PDIA3 in pEVs formation.

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2.39.

Age-dependent effects of platelet releasates on lung microvascular endothelial barrier

Marta Smeda[1], Marta Stojak[1], Anna Gdula[1], Ebrahim H Maleki[1], Stefan Chlopicki[1,2]
[1] Jagiellonian University, Jagiellonian Centre for Experimental Therapeutics, Krakow, Poland
[2] Jagiellonian University Medical College, Department of Pharmacology, , Krakow, Poland

Abstract:

Ageing is associated with impairment of endothelial function. Platelets support endothelial integrity in health and alongside inflammatory diseases and are indispensable to maintain endothelial integrity of the lungs. However, platelets are a rich source of TGFβ that disrupts endothelial barrier as well as initiates mesenchymal transformation of endothelial cells (EndMT).

In this study, we investigated effects of thrombin-induced platelet releasates obtained from young 10-week-old and middle-aged 40-week-old female BALB/c mice on the integrity of endothelial barrier in vitro formed by mouse lung vascular endothelial cells (MLVECs). Changes in lung microvascular endothelial barrier were measured in real time with ECIS system upon addition of platelet releasates with and without TGFβ activation.We also assessed levels of proteins building adherens and tight junctions between endothelial cells by Western blot upon treatment with platelet releasates with and without TGFβ activation.

Our data indicate that thrombin-induced platelet releasates, once TGFβ is not activated, do not increase the overall permeability of lung microvascular endothelial barrier in vitro and do not affect the level of proteins warranting intergrity of adherens and tight junctions between endothelial cells. However, upon activation of TGFβ, thrombin-induced platelet releasates from 40-week-old mice increased permeability of lung microvascular endothelial barrier in vitro while this effect was not detected in case of platelet releasates obtained from younger mice. Western blot analysis revealed that it could be associated with lover levels of claudin-5, an endothelium-specific tight junction protein, in MLVECs cultures treated with thrombin-induced platelet releasates obtained from 40-week-old mice.

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