XXI Sympozjum Sekcji Kardiologii Eksperymentalnej Polskiego Towarzystwa Kardiologicznego oraz Komitetu Nauk Fizjologicznych i Farmakologicznych Polskiej Akademii Nauk

Rynia

13–15 października 2016 roku

PROGRAM I STRESZCZENIA

„Nauka jest jak niezmierne morze. Im więcej jej pijesz, tym bardziej jesteś spragniony."
Stefan Żeromski

Szanowni Państwo,

Już po raz XXI spotykamy się na Konferencji organizowanej przez Sekcję Kardiologii Eksperymentalnej Polskiego Towarzystwa Kardiologicznego we współpracy z Komitetem Nauk Fizjologicznych i Farmakologicznych Polskiej Akademii Nauk.

Tegoroczne spotkanie stanowi podsumowanie kolejnego roku prac naukowców zainteresowanych kardiologią eksperymentalną, którzy pragną podzielić się swoimi wynikami i przemyśleniami.

Dzięki zaangażowaniu Redaktora Naczelnego „Kardiologii Polskiej" — prof. dr. hab. n. med. Krzysztofa J. Filipiaka, ukoronowaniem efektów badań jest ich publikacja w postaci doniesień zjazdowych w czasopiśmie propagującym nowości z zakresu kardiologii, w tym eksperymentalnej, o istotnym wskaźniku oddziaływania.

Chcielibyśmy serdecznie podziękować wszystkim osobom zainteresowanym rozwojem kardiologii eksperymentalnej, w tym Władzom Polskiego Towarzystwa Kardiologicznego, Polskiej Akademii Nauk oraz Warszawskiego Uniwersytetu Medycznego, bez których zaangażowania organizacja konferencji, a także ta publikacja byłyby niemożliwe.

Mamy nadzieję, że wygłoszone i opublikowane streszczenia staną się dla wszystkich zainteresowanych tym wiecznym, niezaspokojonym pragnieniem, wspomnianym przez Stefana Żeromskiego.

Dr hab. n. med. Agnieszka Cudnoch-Jędrzejewska
Prof. dr hab. n. med. Maciej Kurpisz

Szanowni Państwo,

Z przyjemnością publikujemy na łamach „Kardiologii Polskiej" doniesienia naukowe z dziedziny kardiologii eksperymentalnej, wierząc, że powstaną z nich pełnotekstowe prace oryginalne, które chętnie poddamy recenzji i zamieścimy na łamach naszego pisma.

Kolegium Redakcyjne i Rada Naukowa „Kardiologii Polskiej" mają głębokie przekonanie, że o ile kardiologia kliniczna jest bogato i z sukcesami cytowalności reprezentowana na łamach naszego narodowego pisma, o tyle nadal odczuwamy deficyt prac z zakresu kardiologii eksperymentalnej czy nauk podstawowych.

Tym bardziej dziękujemy, że mogliśmy stać się partnerem Państwa sympozjum naukowego, organizowanego z takim powodzeniem przez dr hab. n. med. Agnieszkę Cudnoch-Jędrzejewską i prof. dr. hab. n. med. Macieja Kurpisza.

Życzymy miłych obrad i prosimy pamiętać o naszym piśmie — chętnie zaprezentujemy Państwa wyniki w „Kardiologii Polskiej".

Prof. dr hab. n. med. Krzysztof J. Filipiak, FESC
Redaktor Naczelny „Kardiologii Polskiej"

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Dr Tymoteusz Żera
Warszawski Uniwersytet Medyczny

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16.00–18.00 Rejestracja Uczestników

18.00–18.20 **Expanding the functional role of miRNAs in the establishment of permanent atrial fibrillation**
— Francisco J. Enguita, B.Pharm., Ph.D.

18.20–18.50 **Pre-clinical studies and clinical trials with application of myogenic stem cells**
— Prof. dr hab. Maciej Kurpisz

19.00–20.00 **Spotkanie Zarządu Sekcji**

20.00–22.00 Kolacja

PIĄTEK, 14.10.2016 r.

8.45–9.00 **OTWARCIE SYMPOZJUM**

Dr hab. Agnieszka Cudnoch-Jędrzejewska

Prof. dr hab. Maciej Kurpisz

9.00–11.00 **SESJA I. ŚRÓDBŁONEK, NACZYNIA, CZĘŚĆ I**

Przewodniczący: Prof. dr hab. Maciej Kurpisz; Prof. dr hab. Ewa Szczepańska-Sadowska

9.00–9.20 **Local vascular delivery of atherogenic human lipoproteins causes pathological neointimal thickening. Insights into development of porcine coronary model of atherosclerosis** — Piotr P. Buszman, Bartłomiej Orlik, Krzysztof P. Milewski, Tomasz Roleder, Wojciech Wojakowski, Michał Jelonek, Magdalena Michalak, Adam Janas, Filip Polczyk, Frank D. Kolodgie, Renu Virmani, Paweł E. Buszman

9.20–9.40 **Sensitivity of optical coherence tomography for detection of neointimal microvessels in porcine model of coronary artery injury** — Maciej Pruski

9.40–10.00 **Characterization of restenotic tissue depending on the prevalence of neovascularization evaluated by optical coherence tomography in porcine artery model** — Aleksandra Błachut, Maciej Pruski, Adam Janas, Magdalena Michalak, Krzysztof Milewski, Piotr Buszman

10.00–10.20 **The influence of early magnetic resonance on safety after stent implantation in the porcine coronary and peripheral arterial model** — Łukasz Konarski, Karolina Misztal

10.20–10.40 **Comparison between sirolimus eluting stents coated with biodegradable polymers with short and long degradation kinetics** — Adam Janas

10.40–11.00 **Role of P2Y1 and P2Y12 receptors in release of platelet-derived extracellular vesicle** — Aleksandra Gąsecka, Anita Bóing, Najat Hajji, Edwin van der Poi, Auguste Sturk, Pia Siljander, Paul Harrison, Krzysztof J. Filipiak, Grzegorz Opolski, Rienk Nieuwland

11.00–11.20 Przerwa na kawę

11.20–13.30 **SESJA II. NIEWYDOLNOŚĆ I CHOROBA NIEDOKRWIENNA SERCA**

Przewodniczący: Prof. dr hab. Grzegorz Opolski; Prof. dr hab. Paweł Włodarski

11.20–11.40 **Wykład Animalab — Najnowsze rozwiązania do tradycyjnych badań izolowanych naczyń krwionośnych i serca. Kompaktowy system Langendorffa i zautomatyzowany miograf do małych naczyń — funkcjonalność i analiza danych** — mgr Anna Sarzyńska

11.40–12.00 **Profibrotic role of angiotensin receptor type 1 (AT1) signaling in inflammatory monocytes in mouse model of experimental autoimmune myocarditis (EAM)** — Marcin Czepiel

12.00–12.20 **Potential use of superparamagnetic iron oxide nanoparticles for bioimaging of myoblasts in post-infarction heart stem cells therapy** — Kamil R. Wierzbiński, Tomasz Szymański, Natalia Rozwadowska, Jakub D. Rybka, Agnieszka Zimna, Karolina Nowicka-Bauer, Agnieszka Malcher, Magdalena Przybył, Michael Giersig, Maciej Kurpisz

12.20–12.40 **Myocardial hypoxia effect on expression of selected proangiogenic genes in postinfarcted heart as well as on therapeutic properties of human myoblasts** — Agnieszka Zimna, Bartosz Wiernicki, Tomasz J. Kolanowski, Agnieszka Malcher, Natalia Rozwadowska, Maciej Kurpisz

12.40–13.00 **Cytoprotection role of antioxidants towards human myogenic cells of tissue reservoir** — Magdalena Przybył, Agnieszka Malcher, Agnieszka Zimna, Kamil Wierzbiński, Karolina Nowicka-Bauer, Wojciech Łabędź, Łukasz Kubaszewski, Jacek Kaczmarczyk, Natalia Rozwadowska, Maciej Kurpisz

13.00–13.20 **The relative expression of hsa-miR-21-5p in serum in patients with myocardial infarction comparing to patients with stable coronary disease and healthy volunteers** — Michał Kowara, Wiktor Paskal, Agata Gondek, Renata Główczyńska, Katarzyna Czarzasta, Grzegorz Opolski, Paweł Włodarski, Agnieszka Cudnoch-Jędrzejewska

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14.30–15.30 **SESJA PLAKATOWA**

Moderatorzy: Dr hab. Ewa Koźniewska-Kołodziejska; Dr Tymoteusz Żera

1. **The role of angiotensin II receptor I in the pathophysiology of post-inflammatory myocardial fibrosis in mouse model of experimental autoimmune myocarditis** — Edyta Grzyb
2. **Effect of carvedilol on platelet activity in laser-induced thrombosis** — Natalia Marcińczyk, Dominika Jarmoc, Agnieszka Leszczyńska, Karol Kramkowski, Anna Gromotowicz-Popławska, Ewa Chabielska, Stefan Chłopicki
3. **The effect of antiplatelet $P2Y_{12}$ receptor blockers on vascular endothelial cells *in vitro*** — Katarzyna Korybalska, Rafał Rutkowski, Konrad Karpiński, Natalia Czepulis, Janusz Witowski
4. **Atithrombotic potential of Potentilla erecta extract in rat** — Natalia Marcińczyk, Dominika Jarmoc, Agnieszka Leszczyńska, Agnieszka Zakrzeska, Karol Kramkowski, Jakub Strawa, Anna Gromotowicz-Popławska, Ewa Chabielska, Michał Tomczyk
5. **Brain TNF in blood pressure regulation in spontaneously hypertensive and normotensive Wistar-Kyoto rats** — Agnieszka Segiet, Paweł Smykiewicz, Tymoteusz Żera
6. **Brain interleukin 10 in blood pressure regulation in spontaneously hypertensive and normotensive Wistar-Kyoto rats** — Paweł Smykiewicz, Agnieszka Segiet, Tymoteusz Żera
7. **Genetic causes of resistance to vitamin K antagonists in real-life Polish patients: a novel mutation p.Ile123Met in VKORC1 gene** — Joanna Wzorek, Ewa Wypasek, Magdalena Awsiuk, Daniel P. Potaczek, Anetta Undas
8. **Effects of dabigatran on prothrombin, factor VII and tissue factor expression in human aortic valve interstitial cells** — Ewa Wypasek, Joanna Natorska, Przemysław Kapusta, Piotr Mazur, Anetta Undas
9. **Human aortic valve interstitial cells express coagulation factors: the impact of inflammatory stimulation** — Ewa Wypasek, Joanna Natorska, Przemysław Kapusta, Piotr Mazur, Anetta Undas

15.30–17.30 **SESJA III. ŚRÓDBŁONEK, NACZYNIA, CZĘŚĆ II**

Przewodniczący: Prof. dr hab. Ewa Chabielska, Dr hab. Urszula Mackiewicz

15.30–15.50 **Wykład Bionicum — Japońska odpowiedź na pilną potrzebę mierzenia efektów leczenia DOAC** — dr inż. Janusz Pińkowski

15.50–16.10 **Does perivascular tissue of human radial artery release factor with anticontractile/vasorelaxing properties?** — Karolina Kociszewska, Marek Andrzej Deja

16.10–16.30 **Zmiany regulacji napięcia środkowej tętnicy mózgu i tętnicy zaopatrującej mięsień szkieletowy szczura w warunkach zwiększonej podaży sodu i sodo-zależnego nadciśnienia tętniczego. Wpływ leczenia enalaprilem** — Aneta Uszyńska, Krzysztof H. Olszyński, Ewa Koźniewska

16.30–16.50 **Elevated cellular fibronectin is a modifier of clot properties in type 2 diabetes: association with cardiovascular disease** — Małgorzata Konieczyńska, Agata Hanna Bryk, Krzysztof Malinowski, Katarzyna Draga, Anetta Undas

16.50–17.10 **New concepts of the mechanism of vascular oxidative stress. Protective and harmful roles of NADPH oxidase** — Anna Gajos-Draus, Andrzej Beręsewicz

17.10–17.30 **Assessment of the impact of heart rate on hemodynamic properties of left ventricle** — Monika Petelczyc, Maja Jędrzejczak, Urszula Mackiewicz, Michał Mączewski

17.30–17.50 Przerwa na kawę

17.50–19.30 **SESJA IV. KARDIOMIOPATIE. CHOROBA NIEDOKRWIENNA SERCA**

Przewodniczący: Dr hab. Agnieszka Cudnoch-Jędrzejewska; Dr hab. Ewa Łucja Stępień; Dr hab. Tomasz Wierzba

17.50–18.10 **Toll-like receptor 4 expression and apoptosis in the hearts of female rats with Takotsubo cardiomyopathy induced by isoprenaline** — Agnieszka Kołodzińska, Katarzyna Czarzasta, Benedykt Szczepankiewicz, Monika Budnik, Renata Główczyńska, Tomasz Ilczuk, Anna Fojt, Miłosz Folta, Agnieszka Cudnoch-Jędrzejewska, Barbara Górnicka, Grzegorz Opolski

18.10–18.30 **Hemodynamic effects of heart rate reduction in heart failure** — Przemysław Leszek, Aleksandra Paterek, Marta Kępska, Joanna Kołodziejczyk, Urszula Mackiewicz, Michał Mączewski

18.30–18.50 **The effect of age and sex on the susceptibility to ventricular arrhythmias in the model of induced arrhythmia in the perfused rat heart** — Marta Kępska, Joanna Kołodziejczyk, Aleksandra Paterek, Michał Mączewski, Urszula Mackiewicz

18.50–19.10 **Ferric carboxymaltose reduces mortality in a rat model of myocardial ischemia and reperfusion injury** — Aleksandra Paterek, Marta Kępska, Joanna Kołodziejczyk, Urszula Mackiewicz, Przemysław Leszek, Ewa Jankowska, Piotr Ponikowski, Michał Mączewski

19.10–19.30 **Hartowanie na odległość ludzkiej mięśniówki serca — protokół randomizowanego badania klinicznego z podwójnie ślepą próbą** — Magda Piekarska

20.00–00.00 Uroczysta kolacja

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9.30–13.00 **SESJA V. NADCIŚNIENIE TĘTNICZE. REGULACJA ODRUCHOWA**

Przewodniczący: Prof. dr hab. Andrzej Beręsewicz, Prof. dr hab. Barbara Malinowska

9.30–10.00 **Współczesne poglądy na proces hemostazy** — Prof. dr hab. Ewa Chabielska

10.00–10.20 **Influence of primary hypertension on the function of presynaptic cannabinoid CB1 receptors modulating neurogenic vasopressor response** — Marek Toczek, Barbara Malinowska

10.20–10.40 **Suppression of central control of heart rhythm after inhibition of superoxide dismutase in rats** — Stanisław Zajączkowski, Piotr Badtke, Wiesław Ziółkowski, Damian Flis, Tomasz H. Wierzba

10.40–11.00 **Vasopressin V1a receptors mediate respiratory depression induced by vasopressin and are present in the carotid body's chemoreceptor cells** — Tymoteusz Żera, Jacek Przybylski, Tomasz Grygorowicz, Kaja Kasarełło, Dagmara Mirowska-Guzel, Agnieszka Cudnoch-Jędrzejewska

11.00–11.20 **Prominent role of heart rate in handgrip induced arterial pressure rise and essential inter-individual differences in mechanisms of this rise as revealed by novel method of analysis of circulatory response** — Anna Strasz, Małgorzata Skupińska, Wiktor Niewiadomski, Gerard Cybulski, Anna Gąsiorowska

11.20-11.40 **Na2S, a fast-releasing H2S donor, given in suppositories exerts a prolonged hypotensive effect in rats** — Adrian Drapała, Lenka Tomasova, Marcin Ufnal

11.40–12.00 **Intracolonic indole and hydrogen sulfide, gut-bacteria metabolites, lower arterial blood pressure in hypertensive rats** — Marcin Ufnal, Adrian Drapała, Lenka Tomasova, Piotr Konopelski, Kinga Pham

12.00–12.00 **Increased expression of cardiac and medullar neuropeptide Y Y1 receptor in the rat model of multiple sclerosis** — Sonia Borodzicz, Kaja Kasarełło, Katarzyna Czarzasta, Agnieszka Cudnoch-Jędrzejewska, Dagmara Mirowska-Guzel

13.00–14.00 Obiad

14.00 **ZAKOŃCZENIE SYMPOZJUM**

Dr hab. Agnieszka Cudnoch-Jędrzejewska

Prof. dr hab. Maciej Kurpisz

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Background: Neovascularization in restenotic tissue may play a key role in neointimal proliferation and progression of neoatherosclerosis. There are still insufficient in vivo data regarding in-stent neovascularization.

Methods: We evaluated 48 stent segments (2 per stent) using optical coherence tomography (OCT) from 90 days follow-up after stent implantation in porcine artery model. Ali measurements and qualitative analysis were performed by two independent investigators. Microvessels were defined as well delineated Iow backscattering structures with diameter less than 200 microns that show a trajectory within the vessel. There were two groups: with (1) or without (2) occurrence of neovascularization. OCT findings were compared between both groups.

Results: There were no statistically significant differences between the two groups, but lesions with microvessels had a larger average stent area (8.83 ± 1.39 vs. 8.27 ± 1.36, p = NS) and larger average neointimal area (2.62 ± 1.46 vs. l.95 ± 0.87, p = NS) than those without microvessels. Minimal lumen cross-sectional area (CSA) were almost identical in both groups (2.56 ± 0.47 vs. 2.57 ± 0.33, p = NS).

Conclusions: Occurrence of neovascularization might be associated with the extent of neointimal area, but further studies are required to estimate factors associated with strut neovascularization.

Increased expression of cardiac and medullar neuropeptide Y Y1 receptor in the rat model of multiple sclerosis

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Background: Experimental allergic encephalomyelitis (EAE) is the animal model of multiple sclerosis (MS), human chronic and progressive autoimmunological disease that leads to neurodegeneration in the central nervous system (CNS). MS may be associated with cardiovascular dysfunction, although precise pathophysiological mechanisms responsible for the observed abnormalities remain to be determined. Recently, the involvement of autonomic dysfunction in the cardiovascular disorders associated with MS has been suggested.

Aim: The aim of this study was to assess the expression of the neuropeptide Y (NPY) Y1 receptor mRNA and beta-1 adrenergic receptor mRNA in the myocardium and the medulla of the rat model of MS.

Methods: EAE was induced in female Lewis rats (180––200 g) by injection of Guinea pig spinal cord homogenate prepared in Freund Adjuvant with Mycobacterium tuberculosis (n = 9); intact animals (n = 6) served as controls. All animals were weighed, and clinical symptoms were assessed throughout the experiment. At 21st day after EAE induction, animals were sacrificed, hearts and medullas were collected, and NPY Y1 receptor mRNA and beta-1 adrenergic receptor mRNA expression were analyzed with real time PCR.

Results: The expression of NPY Y1 receptor mRNA in medulla and myocardium of EAE rats were significantly increased in comparison to the control group. The expression of beta-1 adrenergic receptor mRNA was not significantly altered in both medulla and myocardium of EAE rats when compared to the control group.

Conclusions: These results demonstrate, that the expressions of NPY Y1 mRNA, but not the beta-1 adrenergic receptors mRNA are significantly altered in the rat model of MS. Altered signaling of NPY Y1 in the heart may be one of the pathophysiological mechanisms involved in the cardiovascular dysfunction observed in MS.

Local vascular delivery of atherogenic human lipoproteins causes pathological neointimal thickening. Insights into development of porcine coronary model of atherosclerosis

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Background: The preclinical studies of vascular response are limited due to lack of underlying disease. The available diet and genetic atherosclerotic models are not satisfactory due to long breeding, unpredictable lesion formation and low plaque burden and degree of stenosis.

Aim: We aimed to evaluate the vascular response to local, intramural delivery of highly atherogenic lipids in the healthy domestic swine (DS) coronary arteries.

Methods: A total of 24 coronary artery segments of 8 DS were enrolled. Following triple, 130% balloon overstretch, segments were randomly assigned to local delivery of 2 mL of oxydated human LDL from apheresis (400 mg/dL, n = 9), 0.9% NaCl (control, n = 7) or to balloon injury alone (PBA). The solutions were infused with the transcatheter, circumferential micro-needle system (Peregrine, ASI) into the vessel wall. Following 28 days, optical coherence tomography (OCT), virtual histology IVUS (VH-IVUS) and NIRS spectroscopy was performed. The vessel segments were harvested for independent pathological analysis.

Results: The balloon injuries expressed as balloon to artery ratios were comparable among groups and the delivery of solutions was feasible in all cases. At 28 days the plaque burden in IVUS was not different between LDL, control and POBA groups respectively (23.4 ± 12% vs. 16.7 ± 9.7%, 16.7 ± 9%; p = 0.45) and the % Area Stenosis in OCT was highest in the LDL group (23.6 ± 13 vs. 10.8 ± 7 vs. 8.1 ± 7%; p = 0.02). The presence of necrotic core (LDL: 55.5%, Control: 37.5% and POBA: 42.8%; p = 0.77) and dense calcium (LDL: 33.3%, Control: 28.5%, POBA: 37.5%; p = 0.94) in VH-IVUS were comparable between groups. The Lipid Core Burden index in NIRS was negative in all cases. In histopathology, the injury was comparable between groups (LDL: 1.6 ± 0.4, Control: 1.7 ± 0.8, POBA: 1.7; p = 0.8). In pathology the specimens showed no signs of necrotic core, cholesterol or calcium, however foamy macrophages were noted and the tissue consisted of fibrointimal hyperplasia and proteoglycan-rich matrix.

Conclusions: Local delivery of saturated human LDL into the coronary wall was feasible, resulted into higher degree of stenosis caused by neointimal thickening and hyperplasia, with no atheromatosus lesions. The discrepancy between histopathological findings and VH-IVUS was also noted.

Expanding the functional role of miRNAs in the establishment of permanent atrial fibrillation

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Atrial fibrillation (AF) is the most common cardiac arrhythmia and is characterized by the loss of coordinated electrical activity in the atria. The pathophysiology of AF involves initial depolarization trigger events that subsequently evolve to the establishment of a chronic condition. At the cellular level, persistent AF showed a specific gene expression fingerprint characterized by a decreased expression of genes related to ion channel function and transcription factors involved in inflammation and cellular stress responses. The role of other genetic and epigenetic players in the onset and establishment of AF is starting to be unveiled. Among epigenetic factors, non-coding RNAs (ncRNAs) are key regulatory players in the control of gene expression. Some ncRNAs such as micro-RNAs (miRNAs) have been recently associated with the pathophysiology of AF. Despite the presence of a characteristic miRNA expression profile in AF, to our knowledge no studies reported the possible role of these negative post-transcriptional regulators in the phenotypic transition from paroxysmal to permanent AF and posterior establishment of a chronic condition. To better define this putative regulatory role, we performed an unbiased transcriptional study in left atrial tissue samples collected during surgical valvuloplasty to simultaneously characterize the miRNA and mRNA expression levels from a cohort of 14 patients: 3 showing permanent AF, 5 with paroxysmal AF and 6 controls in sinus rhythm. We showed the presence of redundant regulatory activity mediated by miRNAs concomitant to the transcript down-regulation in permanent AF, suggesting

that the contribution of miRNA-mediated regulatory networks is an important factor that collaborates to the establishment and stabilization of the chronic stage of the disease.

Profibrotic role of angiotensin receptor type 1 (AT1) signaling in inflammatory monocytes in mouse model of experimental autoimmune myocarditis (EAM)

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Heart-specific inflammation called myocarditis is a common cause of pathological fibrogenesis, which leads to tissue stiffening resulting in impaired heart function. Using a mouse model of experimental autoimmune myocarditis (EAM) it has been established that bone marrow-derived CD133+ monocytes infiltrate the heart during myocarditis and represent the major cellular source of fibrosis and TGFβ signaling plays critical role in cardiac fibrogenesis in this model. Likewise, angiotensin II has been identified as another profibrotic factor in cardiovascular disorders.

In this study we investigated the role of angiotensin receptor type 1 (AT1 — the main angiotensin II receptor) signaling in TGFβ-mediated transformation of CD133+ inflammatory monocytes into pathogenic myofibroblasts. We showed that CD133+ monocytes isolated from myocarditis-affected hearts of AT1 knock out (AT1-/-) mice fail turning into pathogenic myofibroblasts after TGFβ stimulation. Moreover, myofibroblasts differentiation of wild-type CD133+ monocytes could be abolished by AT1 inhibitor — telmisartan. We showed that TGFβ-induced myofibrobalst differentiation process is associated with Wnt signaling activation. In contrast to wild-type cells, AT1-/- monocytes fail to upregulate gene expression and secrete Wnt proteins. TGFβ-mediated Wnt signaling secretion was accompanied by the downstream activation of canonical Wnt pathway (nuclear translocation of β-catenin), which was defected in AT1-/- cells. Moreover, myofibroblasts differentiation of wild-type CD133+ cells was completely inhibited by Wnt pathway antagonist sFRP2 underlining the pivotal role of the Wnt pathway in cardiac fibrosis formation. Furthermore, AT1 is also required to transduce TGFβ signaling via small GTPase RhoA, as demonstrated by reduced formation of Rho-GTP complex in AT1-/- cells. We identified A-kinase anchoring protein-Lbc (AKAP-Lbc) as a critical factor involved in AT1-dependent signal transduction of TGFβ-induced RhoA activation. In conclusion, our data underpin the critical role of AT1 signaling in TGFβ-mediated cardiac fibrogenesis. Targeting TGFβ, AT1 and Wnt signaling might represent promising clinical strategies against inflammatory-driven heart failures in the future.

Na2S, a fast-releasing H2S donor, given in suppositories exerts a prolonged hypotensive effect in rats

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Background: A number of studies provide evidence that hydrogen sulfide (H2S) plays a key role in the regulation of arterial blood pressure and pathogenesis of hypertension. It has been shown that H2S donors lower arterial blood pressure and, therefore, H2S donors have attracted attention as potential antihypertensive drugs. Sodium sulfide (Na2S) is a fast-releasing H2S donor which exerts powerful but brief effect on arterial blood pressure. One of the methods to maintain longer an effective dose of a compound characterized by a short half-life is to administer it in slow-release formulation such as suppository.

Aim: The aim of the study was to evaluate the hemodynamic effects of per rectal administration of Na2S, a fast-releasing H2S donor, in suppositories in normotensive and hypertensive rats.

Methods The study was performed on male, 18–20 weeks-old, normotensive Wistar Kyoto (WKY) rats and Spontaneously hypertensive rats (SHR). Arterial blood pressure and heart rate (HR) were recorded under general anesthesia at baseline and after per rectal administration of cacao butter suppositories containing either vehicle or Na2S at a dose of 0,2, 0.7 or 2.1 mmol/kg BW.

Results: Administration of vehicle suppositories did not result in significant changes in mean arterial pressure (MABP) in WKY and SHR. In contrast, rats treated with Na2S suppositories responded with a dose-dependent hypotensive effect that lasted ~60 min in WKY and ~80 min in SHR. There were no significant differences between WKY and SHR in MABP changes after the treatment with Na2S suppositories. Rats treated with vehicle suppositories showed a moderate, but significant increase in HR in WKY and SHR. Administration of Na2S suppositories led to a decrease in HR in WKY and an increase in HR in SHR.

Conclusions: This study provides further evidence for a significant hypotensive effect of H2S donors. Furthermore,

we found that fast-releasing H2S donors given in suppository formulation may be an attractive option for basic cardiovascular research as well as may have a therapeutic potential.

Role of P2Y1 and P2Y12 receptors in release of platelet-derived extracellular vesicles

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Background: Human platelets have two ADP receptors, the P2Y1 and P2Y12 receptors. Antagonists against the P2Y12 receptor, which are widely used in secondary prevention of acute coronary syndromes, have unexplained anti-inflammatory effects. Because activated platelets release proinflammatory and procoagulant platelet-derived vesicles (PEV), we hypothesized that suppression of the inflammatory response by P2Y12 receptor antagonists might also be explained by inhibition of PEV release.

Aim: To investigate the role of the P2Y1 and P2Y12 receptors in the release of PEV.

Methods: Citrate-anticoagulated blood was collected from three healthy donors with informed consent. Platelet-rich plasma was pre-incubated for 30 minutes in room temperature with saline, P2Y1 receptor antagonist MRS2179, P2Y12 receptor antagonist ticagrelor, and a combination of both antagonists (final concentrations 100 and 1 |iM, respectively). Subsequently, platelets were recalcified (2.5 mM CaCb) and activated by ADP (10 |iM) under stirring conditions and the reactivity was assessed by Multiplate impedance aggregometry. Concentrations of PEV exposing glycoprotein Ula (CD61), P-selectin (CD62p) and phosphatidylserine (PS) were determined by flow cytometry (Apogee A60 Micro).

Results: ADP-induced aggregation (52 Area Under Curve [AUC]) was inhibited by MRS2179 (13 AUC, 75% decrease), by ticagrelor (5 AUC, -90% decrease), and by a combination of both (6 AUC, -90% decrease). The release of PEV exposing CD61 was observed already after 30 minutes (mean concentration 3.0 ± 1.0 x 108 events/ml), and this release was not inhibited by MRS2179 (p = 0.17, 34% decrease) or by ticagrelor (p = 0.11, 37% decrease), but was abolished when both ADP receptors were blocked (p = 0.02, 66% decrease). On the contrary, the release of PEV exposing CD61/P-selectin/PS was observed only after 1 hour (mean concentration 5.0 ± 3.8 x 107 events/ml, 17% of total CD61 + PEV), and this release was not inhibited by MRS2179 (p = 0.92, 38% decrease), but was decreased by ticagrelor (p = 0.05, 68% decrease) and by a combination of both antagonists (p = 0.05, 76% decrease).

Conclusions: ADP-activated platelets released two distinct subpopulations of PEV: a fast-formed population of PEV exposing CD61, but not P-selectin or PS, and a slow-formed population exposing CD61, P-selectin and PS. The release of these subpopulations differ in their sensitivity to inhibition of different ADP receptors. Whereas the release of CD61+ PEV requires inhibition of both ADP receptors, the release of CD61+/CD62P+/PS+ PEV is sensitive to inhibition of the P2Y12 receptor alone. Because CD62P+/PS+ PEV are considered to disseminate inflammation and thrombosis, the anti-inflammatory effects of the P2Y12 receptor antagonists may in part be due to inhibition of this PEV subpopulation.

The role of angiotensin II receptor I in the pathophysiology of post-inflammatory myocardial fibrosis in mouse model of experimental autoimmune myocarditis

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Heart-specific inflammation — myocarditis associated with the autoimmune response against the heart results in formation of myocardial fibrosis causing heart failure. Pathological cardiac tissue remodeling involves cellular substrates such as cardiac fibroblasts and inflammatory CD133+ bone marrow-derived progenitors and molecular regulators including angiotensin II, Rho kinases, and TGF-β signaling. The use of animal models allows for investigation of cellular and molecular mechanisms of post-inflammatory fibrogenesis.

Animal models of experimental autoimmune myocarditis (EAM) offer an attractive option to study the critical process of transition from active inflammation to the myocardial fibrosis phenotype. Heart inflammation in the EAM model is induced in susceptible mouse strains by immunization with alpha

myosin heavy chain (αMyHC) peptide together with Complete Freund's Adjuvant (CFA). EAM is mediated by cardiac-specific CD4+ T helper cells that invade into the myocardium and induce acute myocarditis between days 17–21 after immunization. Myocarditis progresses to a common final pathway of heart fibrosis (day 40).

In this project we studied wild-type BALB/c and mice lacking angiotensin II receptor I ($AT1^{-/-}$). To characterize cell populations involved in inflammatory and fibrotic phase of the disease we perform the flow cytometry and histological analyses. We demonstrated that $AT1^{-/-}$ mice showed similar extent of inflammatory cells ($CD45^+$, $CD3^+$ and $F4/80^+$) infiltration in the heart during myocarditis, but significantly reduced fibrotic tissue area compared to wild-type animals. Furthermore, our data revealed reduced collagen, fibronectin and tissue inhibitors of metalloproteinase (timp-2, timp-3) expression in hearts of $AT1^{-/-}$ compared to wild-type mice. Additionally, we found that in contrast to wild-type mice, $AT1^{-/-}$ animals fail to activate Wnt signaling pathway during heart inflammation. Using lethally irradiated mice reconstituted with bone marrow cells we showed that only chimeric mice reconstituted with wild-type bone marrow cells developed myocardial fibrosis after EAM induction. Both, wild-type and $AT1^{-/-}$ mice reconstituted with $AT1^{-/-}$ bone marrow cells failed to develop myocardial fibrosis at the post-inflammatory stage of EAM.

Insight from animal models is required to better understand mechanisms of fatal myocardial fibrogenesis. Our results suggest that targeting AT1 should be considered as an attractive option in the development of novel treatment strategies in inflammatory cardiomyopathy.

Comparison between sirolimus eluting stents coated with biodegradable polymers with short and long degradation kinetics

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Background: Biodegradable polymers as the drug reservoirs on stents surface may be safer in a clinical use than those with durable polymers. Nevertheless, the period of optimal polymer degradation has never been evaluated. Therefore, we sought to compare safety and efficacy of sirolimus eluting stents with short polymer degradation kinetics of 2 months with thin (70 m BIOSS A Balton) and thick struts (120 m BIOSS K Balton) versus sirolimus eluting stents with long polymer degradation kinetics of 12 months and with thin struts (Orsiro, Biotronic) as a control group.

Methods: In 6 domestic pigs 6 BIOSS K, 6 BIOSS A and 6 Orsiro stents were implanted with 20% overstretch. After 28 days optical coherence tomography (OCT) was performed in areas of interest. Afterwards, the samples with the stents were harvested and sent to the independent pathology laboratory for evaluation.

Results: The OCT analysis showed there were no differences in % diameter stenosis, % area stenosis and neointimal area. Moreover, there was no difference in the number of embedded as well as protruded and covered struts in the BIOSS K, BIOSS A and Orsiro, which was 93% and 6.8%, 91% and 5.7%, 92% and 7.8%, respectively. There were no differences in the number of protruding and uncovered struts in all groups. Histology evaluation showed no differences between groups.

Conclusions: Due to the fact that all groups achieved similar results both in OCT and histology evaluation, it may be of important clinical meaning to apply polymers with faster degradation time.

The effect of age and sex on the susceptibility to ventricular arrhythmias in the model of induced arrhythmia in the perfused rat heart

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Background: The life-threatening ventricular arrhythmias are predominantly of reentrant nature and are initiated by a premature ventricular beats (trigger) coexisting with pro-arrhythmic electrophysiological and/or morphological substrate, such as dispersion of repolarization, slowing of intercellular conduction and extensive fibrosis. Premature ventricular betas are triggered by early afterdepolarizations (EAD), appearing during the action potential and late afterdepolarizations (DAD), occurring after action potential termination. The major source of DAD are disturbances of the intracellular Ca2+ handling, while EAD appear when the action potential is prolonged due to reduced function of potassium channels or increased function of sodium and calcium channels. Studies show that both Ca2+ handling and the action potential duration are age- and sex-dependent, which suggests the different propensity to formation of EAD and DAD in males and females in the ageing process. Arrhythmic substrate, including the extent of cardiac fibrosis is also different in both sexes and changes with age. That is why the effectiveness of EAD and DAD in initiating of the arrhythmia may be different.

Aim: The aim of the study is to verify the hypothesis that susceptibility to the ventricular arrhythmias is age- and sex-dependent.

Methods: Hearts (n = 6 in each group) were collected from the rats of both sexes in three age groups (2 months, 18 months and 26–28 months) and Langendorff-perfused with Krebs solution with addition of isoproterenole (0.1 μM for 20 minutes) and hydrogen peroxide (0.2 mM for 10 minutes and 0.5 mM for 10 minutes) to induce DAD and EAD, respectively. Electrodes implanted in the heart provided continuous ECG recording that was analyzed to verify the effectiveness of induction of ventricular arrhythmias: ventricular tachycardia (defined as at least 3 consecutive premature ventricular beats with a frequency of at least 600 bpm) and/or ventricular fibrillation (defined as ventricular beats of variable frequency and morphology, or the ECG signal, where you cannot distinguish individual QRS complexes).

Results: Hydrogen peroxide led to induction of arrhythmias in 17% of young animals, regardless of sex. However, in older animals arrhythmia occurred more frequently in males than in females (67% vs. 33%). Isoproterenole did not cause arrhythmia in hearts of the females regardless of age, and caused arrhythmias in males in all age groups (in 17% of young males, 50% of middle-aged and 33% in the oldest age). Preliminary analysis of the recordings shows that the EAD is a stronger pro-arrhythmic factor and far more likely to induce arrhythmias than DAD. In young animals there was no difference in the incidence of arrhythmias induced by EAD while in older animals probability of arrhythmia was twice as high in males as in females. DAD induced arrhythmias only in the hearts of males, mostly in the middle age. Females in this model seem to be not susceptible to induction of arrhythmias by DAD.

Does perivascular tissue of human radial artery release factor with anticontractile/ /vasorelaxing properties?

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Background: We previously described anticontractile properties of human internal thoracic artery (ITA) associated with adipose tissue or adipocyte-derived relaxing factor (ADRF). The present study was performed to assess, if perivascular tissue (PVT) of human radial artery (RA) also exhibits such anticontractile/vasorelaxant properties. It could be especially relevant in preventing radial artery spasm.

Methods: The study was performed on isolated segments of human pedicled RA discarded after the conduit had been trimmed to the length necessary for coronary bypass grafting. The discarded RA fragments were next placed in the Krebs--Henseleit solution and then skeletonized free of the surrounding PVT. Subsequently, they were suspended on stainless steel wire hooks. In the first part of the experiment the arteries were gradually contracted with serotonin (from 10 -9M and rising in negative logarithm half molar cumulative steps up to 10 -4,5M) to establish the concentration-effect relationship in the presence/absence of PVT. In the second part skeletonized RA segments were precontracted with a single dose of 10-6 serotonin (EC80). The 5 ml PVT aliquots were next transferred to the RA tissue bath resulting in its relaxation.

Results: The radial artery without PVT contracted stronger to serotonin in comparison to RA with concomitant PVT. PVT relaxed precontracted with serotonin radial artery rings.

Conclusions: Perivascular tissue of human radial artery exhibits anticontractile/vasorelaxant properties.

Toll-like receptor 4 expression and apoptosis in the hearts of female rats with Takotsubo cardiomyopathy induced by isoprenaline

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Background: Takotsubo cardiomyopathy (TTC) is a stress induced, acute, reversible heart failure that affects in 90% women. A few animal models of TTC were performed. Catecholamines may induce different TTC pattern in an afterload dependent manner. ISO induce hypotensive reaction with apical akinesia. Toll like receptors (TLRs) serve as pattern recognition conservative motifs on pathogens and may also recognize molecular patterns of endogenous host material that is released during cellular injury-damage-associated molecular patterns. TLRs play important role in the ischemia/reperfusion injury in acute coronary syndromes, and in heart failure. Apoptosis is an important mechanism of both acute and chronic loss of cardiomyocytes in both myocardial infarction and heart failure.

Aim: The aim of the study was to characterize expression pattern of TLR4 and apoptosis.

Methods: 61 SPRD female rats were treated with isoprenaline (ISO), 150 mg/kg of body weight injected intraperitoneally to induce TTC. 24 hours post ISO transthoracic echocardiography was performed to confirm TTC. The next examination was done 48 h, 72 h and 7 days post ISO. Animals were sacrificed 24, 48, 72 h and 7 days post ISO. Hearts were stained in paraffin wax for immunohistochemistry and immunofluorescence analysis (TLR4 identification, anti-caspase-3 antibody to identify caspase-3 dependent programmed cell death [PCD]) or were frozen for real time polymerase chain reaction (TLR4).

Results: In histochemistry staining inflammatory foci were distinguishable in ISO treated animals. In control rats sporadic, rare caspase-3 activity was observed in cardiomyocytes. 24 hours post ISO high-intensity apoptosis was observed in cardiomyocytes both inside and outside of the foci and also concerned infiltrating heart muscle inflammatory cells, rarely was visible in the vessels. 48 and 72 hours post ISO PCD was observed mainly in the foci and concerned infiltrating cells. The intensity of the process was stronger than in control animals and much weaker than 24 hours post ISO. 7 days post ISO apoptosis was of higher intensity then 2 and 3 days post ISO, and then in control animals, concerned mainly vessels and infiltrating inflammatory cells both inside and outside of the foci. TLR-4 protein expression was not observed in heart muscle of control animals. TLR4 was expressed both in cardiomyocytes and inflammatory cells inside as well as outside the inflammatory foci. In ISO treated rats cardiomyocytes of inflammatory foci presented TLR4 expression in 50–66.6% of rats 24 h post ISO, in 83.3–100% 48 h post ISO, 33% 72 h post ISO, 0–28.5% 7 days post ISO. While in cardiomyocytes outside of the foci TLR4 expression presented 50–66.6% of animals 24 h post ISO, 100% 48 h post ISO, 50% 72 h post ISO, 28.5% 7 days post ISO. Inflammatory cells were TLR4 positive both inside and outside the foci. TLR4 mRNA was up-regulated 48 h and 72 h post ISO in comparison to control animals, while 7 days post ISO was down-regulated.

Conclusions: Apoptosis presents as significant process in the heart remodeling in TTC. TLR4 may play an important role in reversible heart failure in TTC. Future studies are needed.

The influence of early magnetic resonance on safety after stent implantation in the porcine coronary and peripheral arterial model

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Background: Magnetic resonance imaging (MRI) has emerged as a reliable and clinically important imaging technique. Nevertheless this method may contribute to metallic device dislodgement and thermic injury of tissues. Currently, a variety of coronary and peripheral stents are available, consisting of different alloys, however the effect of early MRI imaging post stent implantation in the in vivo setting has not been assessed.

Aim: The aim of the study was to assess safety of early MRI immediately after angioplasty with a variety of stents in the porcine arterial model.

Methods: This is a pivotal, observatory study in which 2 domestic swine were included. Each animal underwent coronary, carotid and peripheral stent implantation. In total 8 drug eluting stents with cobalt-chromium or stainless steal platforms were implanted in the coronary arteries. Additionally, 8 nitinol stents were implanted in carotid and femoral arteries. One animal had magnetic resonance imaging with 1.5 Tesla magnet (GE) immediately after stent implantation. Angiographic control with optical coherence tomography (OCT) was performed immediately at 28 days post MRI imaging.

Results: In quantitative coronary angiography (QCA) all stents preserved their implantation position. Minimal lumen diameter (MLD) and reference diameter (RD) did not differ before and after MRI. There were no cases of stents dislodgement. Artifacts connected with metallic alloy were not observed during MR imaging. The 28 days vascular effects assessed by OCT revealed good vascular response.

Conclusions: MR imaging performed shortly after stent implantation appears to be safe in the pre-clinical, in-vivo model at short term observation. Presence of stents did not influence on quality of MR.

Elevated cellular fibronectin is a modifier of clot properties in type 2 diabetes: association with cardiovascular disease

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Type 2 diabetes (T2DM) is associated with faster formation of poorly lysable fibrin clots. The association with cellular fibronectin (cFn), the marker of vascular damage elevated in T2DM, is unclear. We aimed to investigate the contribution of cFn to clot properties in T2DM with and without documented cardiovascular disease (CVD). In a single-center cross-sectional study, 200 consecutive patients with T2DM (median disease duration 5 years; median HbA1c 48.6 mmol/mol) aged ≥ 18 years were assessed in terms of cFn concentration and plasma clot formation and degradation using high throughput turbidimetric assays and clot permeation. The median cFn was 3.985 (2.865–4.810) μg/ml. Patients with CVD had increased cFn (4.53 [3.68–4.95] μg/ml), permeation coefficient (Ks: 7.0 [6.2–7.5]), clot density (MaxAbsC: 0.340 [0.331–0.362] au) and decreased clot lysis time (LagL: 262 [231–344] s) compared to remaining subjects (cFn: 3.09 [2.21–3.89] μg/ml, $p < 0.001$; Ks: 7.3 [6.9–7.5], $p < 0.02$; MaxAbsC: 0.33 [0.32–0.34] au, $p < 0.001$; LagL: 306 [237–370] s, $p = 0.024$). cFn correlated positively with MaxAbsC ($r = 0.412$, $p < 0.001$), MaxAbsL and AUC ($r = 0.169$, $p = 0.017$ and $r = 0.265$, $p < 0.001$, respectively); negatively with Ks ($r = –0.147$, $p = 0.038$) and LagL ($r = –0.150$, $p = 0.034$). In CVD positive patients, cFn positively correlated with MaxAbsC and AUC ($r = 0.44$, $p < 0.001$ and $r = 0.26$, $p = 0.002$, respectively). After adjustment for age, creatinine, glucose and fibrinogen, cFn accounted for 18.2% in variance of MaxAbsc, 10.2% of Ks and 6.6% of MaxAbsL. This study is first to show that cFn ufavorably modifies clot properties in T2DM.

The effect of antiplatelet $P2Y_{12}$ receptor blockers on vascular endothelial cells *in vitro*

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Background: Normal vascular endothelial cells constitute a barometer for circulatory function, regulating a complicated intravascular homeostasis. Main objective of cardiac pharmacotherapies is the reduction of cardiovascular complications, ending with death and the enhancement of endothelial function. New-generation anti-platelet drugs prevent unfavourable complications during and after angioplasty. It is emphasised that apart from its antiplatelet effect, they have beneficial impact on a blood vessel through improvement of endothelial function (FMD improvement — *flow mediated vasodilatation*). Nucleotide receptor — $P2Y_{12}$, through which they act, was also identified at the surface of vascular endothelial cells. Most of the findings documenting the beneficial effect of drugs from this group, come from clinical trials. We know much less about its direct impact on endothelium in isolated conditions, such as *in vitro* farm.

Aim: Objective of our project is to assess the impact of $P2Y_{12}$ platelet receptor inhibitors on endothelial cells in the conditions of *in vitro* cultivation.

Methods: HUVEC vascular endothelial cells line EA.hy926 were exposed for 24 h to: anti-platelet drugs, in concentrations detected in the blood during standard therapy, with loading doses (L) and maintenance doses (M) (ticagrelor — L: 2 μM, M: 1 μM, prasugrel — L: 2 μM, M: 0.3 μM, clopidogrel — L: 0.4 μM, M: 0.04 μM). After exposure the percentage of healthy, apoptotic and necrotic cells was assessed (FACSARIA III flow cytometer) along with generation of oxidative stress (measurement of DCFDA — 2',7'-dichlorodihydrofluorescein diacetate, staining fluorescence after oxidation under the influence of free radicals).

Results: Findings are presented in the Table 1.

Conclusions: Studied $P2Y_{12}$ platelet receptor inhibitors in maintenance doses are safe for vascular endothelial cells. They do not change their life span, they do not cause apoptosis, necrosis and they do not generate oxidative stress.

Table 1

	Control	Ticagrelor	Prasugrel	Clopidogrel
Live cells (%)				
Loading dose	98 ± 0.8	98 ± 0.5	98 ± 0.5	98 ± 1.5
Maintenance dose	98 ± 0.8	98 ± 1.8	97 ± 1.0	98 ± 1.4
Apoptotic cells (%)				
Loading dose	0.5 ± 0.5	0.6 ± 0.6	0.5 ± 0.3	0.6 ± 0.9
Maintenance dose	0.5 ± 0.5	0.7 ± 0.6	1.4 ± 1.1	0.7 ± 0.6
Necrotic cells (%)				
Loading dose	1.5 ± 0.6	1.4 ± 0.4	1.5 ± 0.3	1.4 ± 0.4
Maintenance dose	1.5 ± 0.6	1.3 ± 1.1	1.6 ± 0.4	1.3 ± 0.9
Oxidative stress, RFU/μg protein				
Loading dose	42.7 ± 20.2	43.9 ± 21.3	46.2 ± 22.3	35.3 ± 17.8*
Maintenance dose	42.7 ± 20.2	40.7 ± 19.3	45.1 ± 21.1	37.7 ± 19.3

*relevance vs. control $p < 0.05$

Flow-cytometry in nanoworld

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Flow cytometry (FC) is a one of powerful method, which is widely used for high-throughput quantitative and qualitative analysis of small particles in the cells. We specifically refer to Apogee A50 Micro plus which is one of the series flow cytometer of unique technical parameters. Is very specific in a class of its own for sub-micron biological analyses of extracellular vesicles exosomes, microplates, bacteria, viruses, fluorescent proteins and their polydispersity, and lower refractive index, with detection limit of < 70 nm, resolution < 10 nm and flexibility to 100 μm.

The traditional of conventional FC has the minimal detection limit for light scattering in a range of 200–500 nm.

Apogee flow cytometer is specially designed for small particles, which can be used without adjustments prior to fast data acquisition based on the latest technology Altera; up to 12 fluorescence detectors, 3 light scatter detectors, 3 spatially separated lasers and high flow rates (> 100 k events/sec). It has an innovative calibration module detecting scattered light and volumetric sample injection, adjustable sample aspiration volume from 100–400 μl. Selectable, precise sample flow rate from 1 to 150 μl/min. Sample concentrations up to 109/ml. The Micro Flow Cytometer's high sensitivity is well suited to platelet applications including: platelet reactivity, platelet aggregates, circulating activated, platelets, platelet-derived, microparticles, calcium flux, bacterial contamination.

The relative expression of hsa-miR-21-5p in serum in patients with myocardial infarction comparing to patients with stable coronary disease and healthy volunteers

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MicroRNA particles may be promising biomarkers of vulnerable atherosclerotic plaque — an important element in the pathogenesis of myocardial infarction. A potential candidate is hsa-miR21-5p, which inhibits RECK expression and, in turn, causes metalloproteinases activation. In this work we determine relative expression of hsa-miR21-5p in serum of patients with myocardial infarction (n = 10), of patients with the stable coronary artery disease patients (n = 10) and in serum of healthy volunteers (n = 5). microRNA levels were assessed by qPCR (TaqMan®) with exogenic "spike-in" control. We found that hsa-miR21-5p level in patients admitted to hospital for myocardial infarction was similar to the one in patients with stable coronary disease (median = 3.4033 vs. 3.1321, p = NS). The hsa-miR21-5p expression in serum of myocardial infarction patients was highly dispersed (median

3.4033, CI = 1.8330 to 9.2529). Nevertheless, hsa-miR21-5p relative expression level in serum was higher in stable coronary disease patients than in healthy volunteers, but the results haven't reached statistical significance (median = 3.1321 vs. 1.8622; p = 0.1416).

Our data show, that hsa-miR21-5p relative expression is increased in patients with stable atherosclerotic plaque compared to healthy volunteers. Unfortunately, we were unable to show that this mircoRNA distinguishes patients with stable and vulnerable atherosclerotic plaque (maybe due to high dissemination of the results in myocardial infarction patients). However, since number of patients participating in this study was relatively low, ultimate conclusions should be drawn with caution.

Hemodynamic effects of heart rate reduction in heart failure

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Background: From the perspective of hemodynamic efficiency the optimal heart rate (HR) in heart failure (HF) is unknown.

Aim: To determine optimal HR in the HF (post-myocardial infarction — MI rat HF model).

Methods: Wistar-Kyoto rats were followed-up for in acute HF 3, 7 days and chronc HF 4 or 8 weeks after MI induction (ligation of left descending artery) or sham operation (ShO). At each time point they underwent basic echocardiographic assessment and left ventricle pressure-volume (PV) loops recording. Then the same assessment was performed at various HR — basic 400 bpm (corresponding roughly with human 75 bpm), reduced by Ivabradine — 320 bpm (human 58 bpm), increased by atrial pacing — 480 bpm (human 90 bpm), at baseline and after preload increase (iv 5 ml NaCl).

Results: ShO group presented: at the baseline no changes in ejection fraction (EF 55%) or end-diastolic pressure (EDP 4 mm Hg). The preload increase resulted in cardiac output (CO) increase at each HR 320, 400, 480 bpm, (55%, 67%, 84% respectively, $p < 0.05$). HF group presented: at 4 and 8 weeks reduced EF (18%), elevated EDP (17 mm Hg). In acute HF the preload increase resulted in CO increase however in much lower extend compared to ShO. In chronic HF the preload increase resulted in CO increase only at HR 320 bpm, without increase at HR 400 and 480 bpm.

Conclusions: In HF — a rat model — HR changes bring different hemodynamic effects (acute vs. chronic HF). Our results suggest that HR-reducing interventions could be beneficial for chronic HF not only by reduction of progression of heart failure, but also offer immediate hemodynamic benefits.

Atithrombotic potential of Potentilla erecta extract in rat

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The genus Potentilla LINNAEUS (L.) (Rosaceae family) consist of about 700 species of annual, biennial plants and small shrubs. Potentilla species that have been investigated so far, display pharmacological activity mainly due to presence of polyphenols [Tomczyk, Latté, J Ethnopharmacol, 2009; 122: 184–204]. Recently it was shown that polyphenol-rich extract from rhizome of Potentilla erecta (PER, tormentil extract) affects metabolism of arachidonic acid (AA) and in vitro and in vivo exterts both anti-inflammatory and anti-oxidant activities, suggesting possible effect on thrombosis. Accordingly, the aim of the study was to evaluate the effect of PER on haemostasis in rat model of thrombosis.

Lyophilized water-methanol extract from Potentilla erecta rhizome was administrated per os for 14 days in doses of 100, 200 or 400 mg/kg in volume of 2 mL/kg in 5% water solution of gummi arabici (VEH). In in vivo experiment an electrically induced carotid artery thrombosis model with blood flow monitoring has been used [Schummacher et al., 1993]. Collected blood samples were analyzed ex vivo functionally and biochemically for changes in haemostatis.

PER (400 mg/kg) significantly decreased thrombus weight (0.68 ± 0.05 mg vs. VEH 0.98 ± 0.07 mg, $p < 0.01$, $n = 11–19$) and prolonged time to occlusion (16.67 ± 0.19 min vs. VEH 12.05 ± 0.71 min, $p < 0.01$, $n = 4–5$). In ex vivo experiment, based on the vigorous blood stirring, the decrease in thromboxane B2 production has been observed (56.88 ± 11.17 pg/mL vs. VEH 97.65 ± 7.76 pg/ml, $p < 0.01$, $n = 9–12$). Additionally PER tended to inhibit collagen-induced

Table 1.

VEH	Carve 3 mg/kg 30 min	Carve 3 mg/kg 40 min	Carve 3 mg/kg 50 min	Carve 10 mg/kg 30 min	Carve 10 mg/kg 40 min	Carve 10 mg/kg 50 min	Carve 10 mg/kg +KETO	KETO 50 mg/kg
8488 ± ± 1081	531 ± ± 117**	2372 ± ± 1155*	3642 ± ± 1731	459 ± ± 178**	1691 ± ± 521**	2332 ± ± 392**	5530 ± ± 1400	7997 ± ± 1630

*p < 0.05; ** p < 0.01 vs. VEH

platelet aggregation in whole blood (7.22 ± 0.94 Ω vs. VEH 8.91 ± 0.49 Ω, p = 0.09, n = 11–19). Suprisingly, PER (400 mg/kg) significantly decreased t-PA activity (0.34 ± 0.03 ng/mL vs. VEH 0.48 ± 0.04 ng/mL, p < 0.05, n = 11–19) while t-PA concentration, as well as PAI-1 concentration and PAI-1 activity, prothrombin time (PT), activated partial thromboplastin time (APTT), QUICK index and fibrinogen level remained unchanged.

Taking together, for the first time in vivo we have shown, that PER inhibits arterial thrombosis in platelet- and t-PA-dependent mechanism, without disturbing the thrombin generation. Further studies, both under detailed mechanism of PER action on thrombosis, as well as under other polyphenol extracts should be done.

Effect of carvedilol on platelet activity in laser-induced thrombosis

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Carvedilol is a third generation non-selective β-blocker and a competitive antagonist of α_1-adrenoreceptor. Kozlovski et al. demonstrated that carvedilol exerts antithrombotic activity *in vivo*, in rat model of extracorporeal circulation superfusing collagen strips, probably due to its metabolite, which acts as a β_2 adrenoreceptor agonist [Pharmacol Rep, 2015].

The aim of the present work was to evaluate antithrombotic properties of carvedilol (10 mg/kg, 3 mg/kg, *per os*) using the method of intravital real time laser-induced thrombosis. Varying to Kozlovsky's method, we assessed the effect of carvedilol on platelet cummulation in the area of endothelium laser injury, for the first time inside of the mesenteric vein. Moreover, the model considers all the haemostatic interactions, including platelet initiating role in thrombosis [Furie et al. Thromb Res, 2012]. The maximal temporary area of the platelet thrombus was assessed during the 8 minutes after the injury.

To assess possible involvement of carvedilol metabolites, as well as involvement of β-receptors in antithrombotic effect of carvedilol, CYP3A4 was inhibited with ketoconazole (50 mg/kg, *per os*).

Carvedilol, in dose- and time-dependent manner decrease thrombus area (Table 1) and this effect is reversed by ketoconazole (Table 1). Ketoconazole does not exert direct anitplatelect effects in our hands.

Concluding, CYP3A4-dependent metabolites of carvedilol show antiplatelet effects.

Ferric carboxymaltose reduces mortality in a rat model of myocardial ischemia and reperfusion injury

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Anemia and iron deficiency are associated with a worse prognosis in acute coronary syndromes. Studies indicate that intravenous administration of the iron in patients with heart failure and iron deficiency improves patient outcomes. On the other hand administration of iron chelators after ischemia, at the beginning of reperfusion has been shown to reduce reperfusion cardiac injury.

The aim of the study was to assess the effect of intravenous iron administration in rats subjected to ischemia and reperfusion on mortality, post-myocardial infarction (MI) remodeling, left ventricular function and toxicity.

Wistar rats, male (n = 90) were implanted with ECG telemetry transmitters for continuous ECG recorded for 24 hours. Then 30 minutes ischemia was induced by left coronary artery clamping (IR). Clamp was removed to result in a reperfusion for additional 30 minutes. Rats in the control group (sham) were subjected to the same operation as above, excepted for the left coronary artery occlusion. After 30 minutes of reperfusion echocardiography imaging was performed in each rat. Half of the subjects from each group received ferric carboxymaltose (CFe, 1 IV, 12.5 mg/kg b.w.), whereas the other half received saline. Echocardiography imaging was performed 24 hours, 4 and 8 weeks after surgery, and after 8 weeks pressure-volume (PV) loops were obtained additionally in order to measure left ventricular function and diameters. The animals were sacrificed 8 weeks after induction of IR/sham surgery.

Lower post-MI mortality was observed in rats that received CFe compared to rats that received saline (36% vs. 63%). Iron supplementation did not cause arrhythmias within 24 hours after myocardial infarction. At the time of randomization (30 minute of reperfusion) the average infarct size assessed by echocardiography did not differ between the groups, while after 8 weeks it was greater in the IR + CFe group (39.8% vs. 33.6%), probably due to increased number of deaths in rats with large myocardial infarction in IR group. Despite the larger infarct size, dilation of the left ventricle, ejection fraction, systolic and end-diastolic left ventricular pressures were not different between the groups.

Ferric carboxymaltose administered intravenously 30 minutes after myocardial ischemia and reperfusion in rats reduces post-MI mortality, has no proarrhythmic effects, exhibits beneficial effects on hemodynamic parameters and remodeling of the left ventricle. This iron therapy is a potentially promising treatment in acute coronary syndromes that warrants further studies.

Assessment of the impact of heart rate on hemodynamic properties of left ventricle

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Presented study focuses on the assessment and comparison of hemodynamics of left ventricle in two groups of animal models. In experiment, Wistar-Kyoto rats were selected (weight: 350 g, age: 3 months) for both cases. Nine healthy rats were in first group. Six rats after the induced infarction were in second group. In experimental protocol, animals response to intravenous load of physiological saline (4 ml) was studied. Data collection was performed by catheter, which provides measurements for complete pressure-volume loop analysis. In the paper, we present the assessment of common variation of heart rate and hemodynamic parameters by cross-correlation method. The increase of heart rate after physiological saline load was smaller in healthy than in post-infarction group. We observed the effect of the load on the relation between heart rate and hemodynamic parameters in both animal models. Healthy rats were characterized by high cross-correlation parameter (above 0.7) between heart rate and: end-systolic, end-diastolic pressure as well between heart rate and: end-systolic, end-diastolic volume. In post-infarction rats the largest cross-correlation was observed between heart rate and: end-systolic, end-diastolic volume.

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Hartowanie na odległość ludzkiej mięśniówki serca — protokół randomizowanego badania klinicznego z podwójnie ślepą próbą

Magda Piekarska

Wstęp: Hartowanie jest podstawowym zjawiskiem fizjologicznym i jego potencjalnie ochronne działanie zostało wykazane w eksperymentach u różnych gatunków zwierząt i w doświadczeniach na izolowanych modelach narządów. Hartowanie na odległość jest zjawiskiem umożliwiającym

wywołanie odporności na uraz niedokrwienny i reperfuzyjny wskutek przejściowego niedokrwienia odległego narządu. W serii eksperymentów zwierzęcych wykazano, że krótkotrwałe niedokrwienie szeregu tkanek wywołuje efekt ogólnoustrojowy chroniący przed następowym urazem niedokrwienno-reperfuzyjnym. W piśmiennictwie brak jest jednak doświadczeń in vitro dowodzących, że po zastosowaniu protokołu hartowania na odległość ludzka mięśniówka serca wykazuje większą oporność na szkodę niedokrwienno-reperfuzyjną. Do dnia dzisiejszego powstało kilka prac badających wpływ zjawiska hartowania na odległość na funkcję narządów u ludzi; ich wyniki pozostają ze sobą w sprzeczności.

Cel: Celem niniejszej pracy jest określenie, czy ludzka mięśniówka serca może być hartowana na odległość.

Metody: Do badania włączonych zostanie 120 pacjentów przyjętych do planowej operacji pomostowania aortalno-wieńcowego w krążeniu pozaustrojowym (CABG), którzy wymagają co najmniej 3 pomostów aortalno-wieńcowych. Pacjenci metodą randomizacji przydzieleni zostaną do grupy badanej lub grupy kontrolnej. W dniu operacji, po indukcji znieczulenia, przed nacięciem skóry, przeprowadzany będzie protokół hartowania, który polegać będzie na 3-krotnym napełnieniu mankietu do ciśnienia umieszczonego na prawym ramieniu do 200 mm Hg na okres 5 minut z 5-minutową przerwą. W grupie kontrolnej na kończynę górną zakładany będzie mankiet do pomiaru ciśnienia, ale nie będzie on napełniany. W trakcie zabiegu przy kaniulacji prawego przedsionka pobrany będzie fragment uszka prawego przedsionka oraz zostanie wykonana biopsja mięśnia lewej komory serca. W badaniu oceniane będą: (1) oporność na szkodę niedokrwienno-reperfuzyjną mięśniówki serca *in-vitro*, oceniana w modelu czynnościowym izolowanej beleczki uszka przedsionka prawego; (2) indukcja apoptozy i stan mitochondriów w mięśniówce uszka przedsionka prawego poddanego protokołowi symulowanego niedokrwienia i reperfuzji *in-vitro* — oceniany przy użyciu metody Western Blot, immunohistochemicznej, TUNEL i w mikroskopii elektronowej; (3) wielkość martwicy mięśnia sercowego *in-vivo* w okresie pooperacyjnym, oceniana za pomocą profilu stężenia troponiny T oraz CK-MB w surowicy krwi; (4) czynność skurczowa mięśnia sercowego w okresie pooperacyjnym, oceniana za pomocą metody termodilucji oraz na podstawie zapotrzebowania na leki inotropowe i wazokonstrykcyjne, (5) indukcja apoptozy i stan mitochondriów w mięśniu sercowym w następstwie operacji kardiochirurgicznej, oceniana w bioptatach mięśnia lewej komory serca — przy użyciu metody Western Blot, immunohistochemicznej, TUNEL i w mikroskopii elektronowej. Po zakończeniu doświadczenia będziemy korelować ze sobą wyniki uzyskane od każdego pacjenta: z badań klinicznych, z analizy materiału biopsyjnego mięśnia sercowego oraz analizy beleczek uszka prawego przedsionka.

Wyniki: Przeprowadzone badanie pozwoli na jednoznaczne określenie, czy ludzkie kardiomiocyty poddawane procedurze hartowania na odległość w warunkach *in vitro* są oporne na szkodę niedokrwienno-reperfuzyjną. Możliwe będzie określenie, czy hartowanie na odległość wpływa na występowanie apoptozy w mięśniu sercowym w warunkach *in vivo* i określenie, czy hartowanie na odległość wpływa na przebieg pooperacyjny pacjentów poddawanych operacjom kardiochirurgicznym. Równocześnie ocenimy, czy hartowanie na odległość modyfikuje indukcję apoptozy i stan mitochondriów w odpowiedzi na okres niedokrwienia i reperfuzji. Potwierdzenie możliwości hartowania na odległość ludzkiej mięśniówki będzie stanowić punkt odniesienia dla wszystkich dalszych prób klinicznego zastosowania tego zjawiska.

A new method of testing hemostasis using microchip technology

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The Japanese firm Fujimori Kogyo has developed T-TAS (Total Thrombus Analysis System) system for the analysis of the hemostatic system using microchip technology and enables the analysis of the creation of thrombus in the semi-physiological conditions in the flow of blood through to the artificial blood vessels in two types of microchips coated with biological material; (i) AR (coated with collagen and tissue thromboplastin) to observation and analysis of mixed white thrombus formation (WTF) mainly composed of activated platelets and fibrin (ii) PL (coated with collagen) to the observation and analysis of platelet plug formation (PTF) composed mainly of activated platelets. The system was designed to test the effectiveness of single or synergies of various anticoagulants, antiplatelet and thrombolytic agents which are commonly used for the prevention of thromboembolic disorders. The system analyzes the formation of a blood clot in real time in the arteries or veins depending on the desired shear force with corresponding video analysis. T-TAS allows comprehensive analysis of hemostatic or activated platelets from the plasma coagulation system and fibrinolysis. The innovative monitoring system T-TAS, allows selection of most favorable treatment for patients requiring antiplatelet, antithrombotic or thrombolytic therapy.

New possibilities of expansion of the adherent stem cells in the cell therapy

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The Scinus Cell Expansion system is a temperature controlled cabinet with an integrated controller to monitor the expansion process, based on the controller technology of Applikon Biotechnology. As a result of automation and a tight control of temperature, dissolved oxygen and pH, the bioreactor provides an environment for superior cell growth and optimal product quality. The system is equipped with a Carboxy-Oxygenator which provides the gas exchange to the medium to control the dissolved oxygen (accuracy of ± 5%) and the pH (accuracy of ± 0.03).

The system use single bioreactor bag contains a dissolved oxygen and pH sensor, which enables the control of the culture inside the bioreactor bag. The volume increase is linked to a biomass sensor. These controls give users the freedom to optimize cell growth using settings that are tailored to the needs of different cells.

The Scinus Cell Expansion system uses a microcarrier-based process that can be customized to accommodate a host of adherent cell types. These cell types include mesenchymal stem cells, embryonic stem cells, fibroblasts, chondrocytes or pancreatic duct cells. Cells can be cultured from any tissue source that contains an adherent cell population. Potential sources include bone marrow, adipose tissue, umbilical cord, pancreas and kidney.

Cells are cultured on microcarriers, which allows high surface/volume ratios. The maximum culture surface inside the bioreactor bag depends on the type of microcarrier and process. Ratios of at least 4 m^2 (43 ft2) per L are possible. This is equivalent to 250 standard (T-175) culture flasks and allows expansion up to 1 billion cells.

Expansion requires a control of volume and contact surface during the expansion process: the volume of the bioreactor bag can be adjusted (of increased) from 150 mL to 1 L. the surface for cells is increased by feeding additional microcarriers into the bioreactor bag. The flexibility of the system makes it possible to combine both expansion factors in an optimal process. Hawing a the automatic volume and surface expansion, the system allows a one step process from a patient cell aspirate to clinically relevant amounts of cells, while maintaining optimal cell/medium volume ratio.

The Scinus system is developed to replace expensive and labour-intensive 2D culture procedures with a cheaper and easy-to-operate 3D alternative. The system is especially suited for the (decentralized) production of GMP-grade cell therapy products. In addition, the system can be used to standardize and simplify cell culture for experimentation and screening activities for academia and R&D-focussed companies.

Sensitivity of optical coherence tomography for detection of neointimal microvessels in porcine model of coronary artery injury

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Background: Studies suggest that the presence of microvessels on coronary optical coherence tomography (OCT) is associated with larger neointimal area and in-stent restenosis. This study assessed the specifity and sensitivity of OCT for detection of microvessels in comparison with histopathology.

Methods: A total of 48 stent segments from 24 stents implanted in a swine model of coronary artery injury were studied. OCT was performed in-vivo at 90 days post implantation and histopathology analysis of the stents was performed by an independent core laboratory. Subsequently OCT frames matching histopathology were identified by the following criteria: distance from stent edge, vessel shape, position of side branches, position and coverage of stent struts, and microvessels. Microvessels were defined as low backscattering structures < 200 μm in diameter on OCT.

Results: Microvessels were identified in 25 stent segments by histopathology and in 10 segments by OCT. The sensitivity of OCT for detection of microvessels as compared to matched histopathology frames is 40%. The specifity was 100% (no false positive results).

Conclusions: OCT has low sensitivity for detection of microvessels in the neointima compared to histopathology.

Cytoprotection role of antioxidants towards human myogenic cells of tissue reservoir

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Reactive oxygen species (ROS) play a crucial role in human metabolism. During "oxidative stress" the balance between the ROS formation and capacity of antioxidant system is impaired. Excess of free radicals and other pro-oxidants causes an oxidation of lipids, proteins and DNA, thereby increasing the likelihood of tissue injury. Overexpression of superoxide dismutase (SOD3) and/or incorporation of a N-tert-butyl-alpha-phenylnitrone (PBN) to the culture medium reduced the amount of free radicals, potentially affecting myoblasts in vitro. The SOD3 exerted a positive effect on viability and proliferation potential of myoblasts, increasing the abundance of young cells in both normoxic and hypoxic conditions. Genetically modified cells maintained myogenic character by CD56 expression and MyOD at higher levels than in initial population. The SOD3 overexpression leads to increased of lifespan and expression of antiapoptotic genes: SOD1, BCL2,SIRT1,FOXO1 and SOD2, CAT (around 2-fold upregulation; $p < 0.05$).

On the other hand PBN in the culture medium led to increased viability of myoblasts and expression of some antioxidant genes like CAT, SOD1, SOD2 and myogenic genes: MyOD and MyOG (at least 2-fold regulation; $p < 0.05$).

Implementation of SOD3 overexpression and PBN has shown the cytoprotective effect on myogenic cells, thus a potential of myoblasts modified by antioxidansts could be feasible for regenerative medicine applications.

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Brain TNF in blood pressure regulation in spontaneously hypertensive and normotensive Wistar-Kyoto rats

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Background: Neuroinflammation is considered as an important contributing factor in the pathogenesis of arterial hypertension. An archetypal proinflammatory cytokine, tumour necrosis factor (TNF), exerts its proinflammatory effects via TNF type 1 receptors (TNFR1). A growing body of evidence indicates that TNF is involved in the central control of arterial blood pressure.

Aim: In the present study, we checked how TNF administered into the cerebral ventricles affects baroreflex in spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto (WKY) rats and whether these rats differ in the expression of TNF and TNFR1 in the key cardiovascular brain centres.

Methods: Under anaesthesia with urethane, we recorded arterial blood pressure during intracerebroventricular (ICV) infusions of TNF (200 ng/10 μl, bolus) or sterile saline (10 μl, bolus) in adult male SHR and WKY rats and evaluated gain of the baroreflex with intravenous infusion phenylephrine. We also measured systolic blood pressure in conscious adult male SHR and WKY rats with tail-cuff method and collected blood and brains for further analysis. From coronal sections of the brain, we isolated the hypothalamus (HTH), the rostral ventrolateral medulla (RVLM) and the nucleus of the solitary tract (NTS), which were homogenized and centrifuged. Using enzyme-linked immunosorbent assays we determine concentration of TNF in the supernatants of HTH, RVLM and NTS and concentration of TNFR1 in the respective precipitates. We also measured norepinephrine (NE), TNF and TNFR1 in serum.

Results: ICV infusion of TNF decreased gain of the baroreflex in both SHR and WKY rats. Conscious SHR rats had significantly higher systolic blood pressure than WKY rats. Expression of TNF in RVLM and NTS of SHR rats was significantly increased in comparison to WKY rats. Expression of TNFR1 was also significantly higher in NTS of SHR than in WKY rats. Serum NE was significantly higher in SHR than in WKY rats.

Conclusions: Our results indicate that TNF participates in the central control of blood pressure and that hypertensive SHR rats have higher expression of both TNF and its receptor TNFR1 in the brainstem than normotensive WKY rats. These findings suggest that proinflammatory conditions in the central nervous system contribute to pathogenesis of hypertension.

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Brain interleukin 10 in blood pressure regulation in spontaneously hypertensive and normotensive Wistar-Kyoto rats

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Background: A growing body of evidence points to the role of neuroinflammation in the development of hypertension. Interleukin 10 (IL-10) is a major anti-inflammatory cytokine, which limits inflammatory response.

Aim: In the present study, we aimed at finding how intracerebroventricular (ICV) administration of IL-10 affects baroreflex in spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto (WKY) rats whether these rats differ in the expression of IL-10 and and its receptor (IL-10R) in the key cardiovascular brain centres.

Methods: Under anaesthesia with urethane, we recorded arterial blood pressure, ICV infused IL-10 (200 ng/10 μl, bolus) or sterile 0.9% NaCl solution (10 μl, bolus) and evaluated the gain of baroreflex with intravenous phenylephrine in adult male SHR and WKY rats. We also recorded systolic blood pressure in conscious adult male SHR and WKY rats with volumetric tail-cuff technique and collected blood and brains for further evaluation. We isolated the hypothalamus (HTH), the rostral ventrolateral medulla (RVLM) and the nucleus of the solitary tract (NTS) from coronal sections of the brain. The tissues were homogenized and centrifuged. Using enzyme-linked immunosorbent assays we determine concentration of IL-10 in the supernatants of HTH, RVLM and NTS and concentration of IL-10R in the precipitates.

Results: ICV administration of IL-10 resulted in decrease in arterial blood pressure in SHR and WKY rats without significant changes in the gain of baroreflex. SHR rats had significantly higher systolic blood pressure and serum NE than WKY rats. Protein expression of IL-10 in RVLM and NTS of SHR rats was significantly higher than in WKY rats, whereas IL-10R was significantly lower in NTS of SHR than WKY rats and lower in RVLM of SHR than WKY rats, however without reaching significant level ($p = 0.056$, Student's t-test).

Conclusions: Our results suggest that IL-10 and its receptor in the central nervous system are involved in the pathogenesis of hypertension. The upregulation of IL-10 in hypertensive rats' brain might be a compensatory mechanism for decreased expression of IL-10 receptor.

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Prominent role of heart rate in handgrip induced arterial pressure rise and essential inter-individual differences in mechanisms of this rise as revealed by novel method of analysis of circulatory response

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Background: Measurement of pressor response to handgrip is used as standard test to assess reactivity of sympathetic system. Such interpretation of handgrip test is based on premise, that changes of blood pressure are mediated predominantly by sympathetic system. Thus, the possible role of parasympathetic system, acting via change in heart rate is overlooked.

Aim: The aim of this study was to assess the relative role heart rate change in pressor response to handgrip.

Methods: In the study participated 34 young, healthy subjects: 16 men and 18 women, resting in supine position. Heart rate and arterial blood pressure were recorded continuously 2 min before and during 2 min lasting handgrip, performed at 30% of maximal voluntary contraction. Based on ratios: MAPH/MAPB and HRH/HRB, where MAP — mean arterial pressure, HR — heart rate, index H denotes value during handgrip, index B denotes value before handgrip, we conceived novel measure: contribution index Hm, which has the following properties: Hm = 0 means no role of HR in MAP increase, Hm = 1 exclusive (i.e. 100%) role of HR in MAP increase.

Results: Heart rate and MAP were elevated during handgrip. Resting HR was similar in men and women and rose more in men during handgrip. Resting MAP was higher in men and rose more in men during HG. Average value of contribution index Hm was 0.96 ± 0.63, what means that almost whole rise of MAP was attributable to the increase in HR. Hm was greater in women (1.10 ± 0.75) than in men (0.83 ± 0.48), however between-sex difference was statistically insignificant. This lack of significance was probably due to the wide range of individual values of Hm: (min Hm = = 0.05, max Hm = 3.12). Such wide range of Hm values means, that the role of HR in MAP rise is individually very different: from partial to complete.

Conclusions: We demonstrated, that in average the rise of mean arterial blood pressure in response to handgrip should be attributed to increase in heart rate. This in turn points toward at least partial role of parasympathetic system in pressor response to handgrip. Moreover, it is necessary to analyze individual contribution of HR to pressor response, as it may considerable vary.

Influence of primary hypertension on the function of presynaptic cannabinoid CB1 receptors modulating neurogenic vasopressor response

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Background: Stimulation of presynaptic cannabinoid CB1 receptors localized on sympathetic nerves inhibits noradrenalin release i.e. neurogenic vasopressor response [Malinowska et al., Naunyn Schmiedeberg's Arch Pharmacol, 1997; 356:197–202]. In DOCA-salt hypertension we observed enhanced function of CB1 receptors inhibiting neurogenic pressor response. It might serve as a protective response in hypertension [Toczek et al., Life Sciences, 2015; 138: 78–85].

Aim: Examination of the influence of primary hypertension on the function of presynaptic cannabinoid CB1 receptors modulating neurogenic vasopressor response in rats.

Methods: We used 9–11 weeks old spontaneously hypertensive rats (SHR) and appropriate normotensive control animals — Wistar Kyoto rats (WKY). Experiments were performed on vagotomised and pithed animals. Before experiments systolic blood pressure (SBP) and heart rate (HR) were measured in conscious animals using the tail-cuff method.

Results: SBP in SHR was higher ($p < 0.001$) than in control group and amount to 192 ± 7 mm Hg (n = 65) and 106 ± 4 mm Hg (n = 69), respectively. Similarly in anaesthetised, vagotomised and pithed animals basal diastolic blood pressure (DBP) was higher ($p < 0.01$) in SHR (48 ± 1 mm Hg, n = 33) than in WKY (43 ± 1 mm Hg, n = 37). The antagonist of cannabinoid CB1 receptors — AM251 (3 μmol/kg) and inhibitors of enzymes — (1) monoacylglycerol lipase (MAGL) — MJN110 (3 μmol/kg) and (2) fatty acid amide hydrolase (FAAH) URB597 (9 μmol/kg) degrading endocannabinoids, 2-arachidonylglycerol and anandamide, respectively, did not modified DBP in any group. The electrical stimulation of preganglionic symphatetic nerves innervating resistant vessels increased DBP comparatively in SHR and WKY (by 28 ± 1 mm Hg, n = 25 and 32 ± 2 mm Hg, n = 24, respectively). AM251 i MJN110 did not affect the electrically stimulated increases in DBP, while URB597 decreased ($p < 0.05$) this parameter both in SHR and WKY. The chemical stimulation of postsynaptic α1-adrenergic receptors by intravenous injection of phenylephrine (0.01 μmol/kg) increased DBP comparatively in SHR and WKY, by 32 ± 4 mm Hg (n = 13) and 30 ± 2 mm Hg (n = 13), respectively. The agonist of cannabinoid receptors — CP55940 (0.01; 0.1; 1 μmol/kg) dose-dependently inhibited electrically, but not chemically, stimulated increases in DBP maximally by about 20–30% in both groups, and this effect was abolished by AM251. The inhibitor of endocannabinoid cellular uptake AM404 (3 μmol/kg) inhibited the electrically stimulated increase in DBP by about 15%, but only in SHR. MJN110 and URB597 in hypertensive rats enhanced or tended to enhance the inhibitory influence of CP55940 on neurogenic pressor response, otherwise in normotensive animals they diminished this effect.

Conclusions: 1. The inhibition of neurogenic vasopressor response via cannabinoid CB1 receptors is not modified in primary hypertension. 2. The degree of inhibition of the neurogenic vasopressor response via presynaptic cannabinoid CB1 receptors depends on experimental model of hypertension. 3. Modulation of neurogenic vasopressor response by endogenously formed cannabinoids in primary hypertension might play a protective role in hypertension.

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Intracolonic indole and hydrogen sulfide, gut-bacteria metabolites, lower arterial blood pressure in hypertensive rats

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Background: Increasing evidence suggests that hypertension is associated with gut microbiota dysbiosis; however, the involvement of the gut microbiota in the control of arterial blood pressure is not determined. Hydrogen sulfide (H2S) and indole are abundant metabolites of gut bacteria in mammals. The goal of the study was to evaluate the effects of intracolonic indole and H2S on arterial blood pressure, heart rate and electrical activity of the heart.

Methods: Arterial blood pressure and ECG were recorded in anesthetized, male, 16-week old, normotensive Wistar Kyoto rats at baseline and after intracolonic injection of either saline (controls) or indole or Na2S.

Results: Both, indole and the H2S donor produced a significant, dose-dependent decrease in mean arterial blood pressure. We found no apparent cardiotoxic effects of the gut bacteria-derived compounds.

Conclusions: Our study shows that intracolonic H2S and indole may contribute to the control of arterial blood pressure and etiology of hypertension.

Zmiany regulacji napięcia środkowej tętnicy mózgu i tętnicy zaopatrującej mięsień szkieletowy szczura w warunkach zwiększonej podaży sodu i sodo-zależnego nadciśnienia tętniczego. Wpływ leczenia enalaprilem

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Nadmierne spożycie sodu jest czynnikiem ryzyka rozwoju chorób sercowo-naczyniowych, w tym nadciśnienia tętniczego. Liczne doniesienia wskazują na to, że nawet w przypadku braku wzrostu ciśnienia tętniczego, nadmiar sodu w diecie wpływa niekorzystnie na naczynia krwionośne i narządy. Wiadomo również, że wysokie spożycie soli jest czynnikiem ryzyka udaru mózgu, niezależnie od nadciśnienia tętniczego. Celem podjętych przez nas badań jest porównanie wpływu diety wysokosodowej i sodo-zależnego nadciśnienia tętniczego na regulację napięcia tętnicy zaopatrującej mięsień smukły (GMA) i tętnicy środkowej mózgu (MCA) szczura. Oba naczynia mają charakter oporowy, przy czym GMA bierze udział w regulacji całkowitego oporu obwodowego, a MCA reguluje opór naczyniowy w krążeniu mózgowym. Zmiany drożności MCA są najczęstszą przyczyną udaru niedokrwiennego u ludzi.

Badania prowadzone były na szczurach szczepu Sprague Dawley (SD) w następujących grupach: 1. SD z pozorowaną nefrektomią na diecie standardowej zawierającej 0,25% Na (SHAMNS), 2. SD z jednostronną nefrektomią na diecie wysokosodowej zawierającej 4% Na (UNXHS), 3. SD z pozorowaną nefrektomią na diecie wysokosodowej zawierającej 4% Na (SHAMHS), 4. UNXHS leczone enalaprilem (UNXHS + Enal), 5. SHAMHS leczone enalaprilem (SHAMHS + Enal). W okresie trwania diety (28 dni) co tydzień w sposób nieinwazyjny mierzono ciśnienie tętnicze i oznaczano stężenie jonów sodu w próbkach osocza krwi. Po 28 dniach diety pobierano oba naczynia, umieszczano je w komorze arteriografu i po uzyskaniu odpowiedniego napięcia badano ich reaktywność na endotelinę (ET-1: 10–10, 5 x 10–10, 10–9, 5 x 10–9, 10–8 M), agonistę receptora AT1 angiotensyny (AT1agon: 5 x 10–10, 10–9, 5 x 10–9, 10–8 M) oraz substancje NO- i śródbłonko-zależne: acetylocholinę (ACh: 10–9, 10–8, 5 x 10–8, 10–7, 5 x 10–7 M) w przypadku GMA i adenozyno-5-trifosforan (ATP: 10–8, 5 x 10–8, 10–7, 5 x 10–7, 10–6 M) w przypadku MCA. Badano również udział NO w utrzymaniu napięcia podstawowego naczyń, podając inhibitor syntazy tlenku azotu (L-NAME, 10–5 M). U zwierząt serii 4 i 5 po 2 tygodniach doświadczenia włączono na kolejne dwa tygodnie enalapril (10 mg/kg *per os*).

Dieta wysokosodowa nie wywarła *per se* wpływu na ciśnienie tętnicze, które w grupie SHAMHS utrzymywało się na poziomie podobnym do ciśnienia u szczurów SHAMNS. Natomiast w grupie UNXHS ciśnienie tętnicze wzrastało począwszy od 7 dnia diety i osiągało wartość maksymalną po 21 dniach. Ciśnienie skurczowe było średnio o 30 mm Hg wyższe a ciśnienie rozkurczowe średnio o 20 mm Hg wyższe niż ciśnienie wyjściowe. Podawanie enalaprilu przez 2 tygodnie prowadziło do statystycznie istotnego ($p < 0,05$) obniżenia ciśnienia skurczowego o 10 mm Hg i do normalizacji ciśnienia rozkurczowego w grupie UNXHS + Enal, nie wywołując wpływu w grupie SHAMHS + Enal. Stężenie Na+ w osoczu szczurów przez cały czas trwania doświadczenia utrzymywało się w granicach prawidłowych wartości od 135 do 142 mM.

W badaniach reaktywności na substancje naczyniokurczące stwierdzono, że tętnica GMA odpowiada silniejszym skurczem na podanie zarówno ET-1, jak i AT1agon w obu grupach na diecie wysokosodowej — SHAMHS i UNXHS w porównaniu z GMA izolowanych ze zwierząt na diecie standardowej. Natomiast odpowiedź MCA na podanie ET-1 i agonisty receptora AT1 w obu grupach na diecie wysokosodowej nie różniła się od odpowiedzi obserwowanych w grupie kontrolnej SHAMNS.

Badania odpowiedzi na substancje śródbłonko-zależne wykazały istotne różnice we wpływie diety wysokosodowej i sodo-zależnego nadciśnienia tętniczego na GMA i MCA. Dieta wysokosodowa nie wywołała zmian w śródbłonko- i NO -zależnym rozszerzeniu zarówno GMA jak i MCA. Natomiast w sodo-zależnym nadciśnieniu tętniczym w grupie UNXHS obserwowano istotne upośledzenie odpowiedzi MCA na ATP i brak zmiany w odpowiedzi GMA na ACh w porównaniu z grupami SHAMNS i SHAMHS. Zablokowanie syntezy NO spowodowało mniejszy skurcz w przypadku GMA jak i MCA w grupach SHAMHS i UNXHS w porównaniu z grupą kontrolą SHAMNS. Leczenie enalaprilem nie przywróciło prawidłowej odpowiedzi GMA na podanie agonisty receptora AT1, ale znormalizowało odpowiedź MCA na ATP co prawdopodobnie ma związek z normalizacją ciśnienia krwi.

Uzyskane wyniki wskazują, że: 1) dieta wysokosodowa prowadzi do zwiększenia odpowiedzi naczyń regulujących całkowity opór obwodowy na substancje naczyniozwężające, nie wywierając takiego wpływu na duże naczynia oporowe krążenia mózgowego, 2) dieta wysokosodowa zmniejsza udział NO w utrzymaniu napięcia podstawowego w na-

czyniach oporowych obwodowych i mózgowych, 3) upośledzenie funkcji śródbłonka naczyń krwionośnych mózgu w sodo-zależnym nadciśnieniu tętniczym pojawia się we wczesnym etapie nadciśnienia.

Potential use of superparamagnetic iron oxide nanoparticles for bioimaging of myoblasts in post-infarction heart stem cells therapy

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Myocardial infarction (MI) is one of the most frequent causes of death in industrial countries. Stem cells therapy seems to be a very promising field of regenerative medicine, including recovery of cardiac muscle after myocardial infarction.

Skeletal myoblasts transplantation into the border area of postinfarction scar has been shown as prospective stem cell therapy for the failing heart. However, this procedure has also some limitations, among other, low cell retention and survival of transplanted stem cells. This brings a need to improve methods of in vivo cell imaging.

To achieve this goal we investigate superparagmetic iron oxide nanoparticles (SPIONs) as an agent for direct cell labelling, which can be used for stem cells therapy imagining. Human skeletal myoblasts were treated with DMSA-coated iron nanoparticles and tested in vitro, considering the influence of SPIONs on investigated cell populations, including their functional properties and gene expression.

The presence of iron nanoparticles within myoblasts was confirmed by Prussian Blue staining. Influence of SPIONs on tested cells was evaluated by investigating their proliferation, ageing, differentiation potential and production of reactive oxygen species. Cytotoxicity of iron nanoparticles and apoptosis of myoblasts were also tested, as well as iron-related and coating-related gene expression. We examined the impact of SPIONs on overexpression of two pro-angiogenic factors introduced via an efficient myoblast electroporation method. Functionality of overexpressed proteins at capillary formation was evaluated.

Obtained results demonstrated slight influence of SPIONs on treated skeletal myoblasts, which should, however not exert negative effects on basic cell functions.

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Effects of dabigatran on prothrombin, factor VII and tissue factor expression in human aortic valve interstitial cells

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Background: Recently, it has been suggested that thrombin inhibition may play a key role in the development and stability of atherosclerotic lesions. It has been shown that dabigatran attenuated the mean plaque growth in the aortic arch of apolipoprotein-E deficient mice. It may be speculated that the role of coagulation proteins in the processes leading to calcification of the aortic valves during aortic stenosis (AS) is equally important and thrombin inhibition may abolish the effects of coagulation protein expression within aortic stenotic valves.

Aim: The objective of this study was to evaluate the effects of dabigatran on factor (F) II, FVII and tissue factor (TF) expression in human valve interstitial cells (VICs) from patients with severe AS.

Methods: Primary cultures of VICs derived from stenotic aortic valves were digested with collagenase for 30 min at 37°C and grown in Dulbecco's Modified Eagle's Medium supplemented with 10% heat-inactivated fetal bovine serum and antibiotics at 37°C and 5% CO2. For immunofluorescence analysis, cells were seeded on a coverslip in 6-well plates and stimulated with TNF-α (50 ng/mL) for 4 h and 24 h to induce inflammatory response. Stimulated VICs were pre-treated with dabigatran (50 and 500 nM). The expression of FII, FVII and TF was analysed by immunofluorescence using corresponding monoclonal antibodies and visualized with the secondary antibodies conjugated with fluorochromes. Evaluation of positive cells was performed as: a number of cells presenting antigen expression/total cell number.

Results: VICs control cultures revealed no expression of FII. Exposure of VICs to TNF-α caused the expression of

FII up to 29.5 ± 15.3% in comparison to the control group (p = 0.02) while treatment of VICs with dabigatran for 4 h decreased FII expression about 70% (29.5 ± 15.3% vs. 9.7 ± 5.7%, p = 0.001). After 24 h of treatment the percentage of FII positive cells was decreased by 50% (29.5 ± 15.3% vs. 14.9 ± 4.8%, p = 0.008). 500 nM dabigatran produced similar effects. Interestingly, a decrease in TF and FVII expression was observed in response to dabigatran on stimulated VICs (83.2 ± 12.1% vs. 52.5 ± 25.1%, p = 0.02 and 74.3 ± 10.3% vs. 36.2 ± 12.6, p = 0.001, respectively).

Conclusion: It can be speculated that the dabigatran may inhibit the effects of thrombin at levels encountered in vivo within aortic stenotic valves and lead to slower aortic valve fibro-calcification in humans.

Human aortic valve interstitial cells express coagulation factors: the impact of inflammatory stimulation

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Background: Aortic stenosis (AS) is currently the most common acquired valvular heart disease. Despite similarities to atherosclerosis the involvement of coagulation proteins in the pathophysiology of AS is not clear.

Aim: To evaluate if human interstitial cells (VICs) from the valves of patients with severe AS have the ability to express: factor (F) II, FVII, FX , tissue factor (TF) and protease-activated receptors (PAR1 and PAR2).

Methods: Primary cultures of VICs derived from stenotic aortic valves were digested with collagenase for 30 min at 37°C and grown in Dulbecco's Modified Eagle's Medium supplemented with 10% heat-inactivated fetal bovine serum and antibiotics at 37°C and 5% CO2. For immunofluorescence analysis, cells were seeded on a coverslip in 6-well plates and stimulated with TNF-α (50 ng/mL) for 4 h to induce inflammatory response. Unstimulated cultures were used as controls. The expression of FII, FVII, FX, TF and PARs was analysed by immunofluorescence using corresponding monoclonal antibodies and visualized with the secondary antibodies conjugated with fluorochromes. Evaluation of positive cells was performed as: a number of cells presenting antigen expression/total cell number.

Results: VICs control cultures revealed the presence of TF (12.2 ± 1.3%), FX (76.5 ± 14.5%), PAR1 (0.8 ± 0.2%) and PAR2 (1.6 ± 0.8%) antigens. No expression of FII and FVII was observed. Exposure of VICs to TNF-α caused the up-regulated expression of TF (83.2 ± 12.1% vs. 12.2 ± 1.3%, p < 0.001). Moreover, under inflammatory stimulation, 74.3 ± 10.3% of VICs revealed the expression of FVII and 29.5 ± 15.3% the expression of FII (p < 0.001 and p = 0.02, respectively, in comparison with the control group). The expression of FX, PAR1 and PAR2 antigens remained unaltered upon stimulation.

Conclusions: We showed for the first time that VICs express constantly FX, PAR1 and PAR2 and are able to express FII and FVII under inflammatory stimulation. The presence of PARs suggests a possible action of thrombin and FXa directly on the VICs. We conclude that in the diseased aortic valves, local expression of coagulation proteins derived in part from VICs might contribute to disease progression.

Genetic causes of resistance to vitamin K antagonists in real-life Polish patients: a novel mutation p.Ile123Met in VKORC1 gene

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Background: Vitamin K antagonists (VKAs) including acenocoumarol and warfarin are highly effective in the prevention and treatment of thrombotic episodes. These drugs exert their anticoagulant effects by inhibition of subunit 1 of vitamin K epoxide reductase complex (VKORC1), thereby limiting the regeneration of reduced vitamin K from vitamin K epoxide. *VKORC1* and cytochrome P450 (*CYP*) *2C9* genetic variants contribute largely to inter-individual variations in the VKA requirements. Several missense mutations have been reported to cause a partial or complete resistance to VKAs. To our knowledge there has been no study on VKA resistance in Polish patients.

Aim: The aim of this study was to investigate the genetic background of VKAs resistant Polish patients.

Methods: We analyzed the *VKORC1* gene sequence and *CYP2C9* known polymorphisms in 25 selected warfarin — (> 10 mg/d) and acenocoumarol — resistant (> 8 mg/d) patients by Sanger sequencing and real-time PCR technique. Dietary or medication-related causes of VKA resistance were excluded.

Results: 21 and 24 patients possess the wild-type alleles of *VKORC1* and *CYP2C9* genes, respectively. Moreover, 12 of VKAs-resistant were homozygous for *VKORC1*3* and 9 were heterozygous for *VKORC1*3* and *VKORC1*4* haplotypes. We identified a novel mutation in a 70 year old male patient receiving 16 mg acenocoumarol daily (missense substitution, p.Ile123Met (c.368 C > G) in *VKORC1*gene that is probably associated with oral anticoagulant resistance. In silico study confirmed that this variant is damaging and can affects VKORC1 function, presumably by a perturbation of the spatial protein structure of residues required for stabilizing oral anticoagulants binding. No known VKORC1 mutations were observed in our group.

Conclusions: We show here that screening of *VKORC1* gene is needed to identify genetic causes of VKAs resistance. However most patients do not have rare mutations in VKORC1 which could be responsible for this state. A role of environmental causes of VKA resistance is important in everyday clinical care.

Suppression of central control of heart rhythm after inhibition of superoxide dismutase in rats

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Background: Reactive oxygen species, including superoxide anion, have been suggested to play a role in cardiovascular regulation as chemoreflex triggers.

Aim: The study aimed to test the effect of inhibition of superoxide dismutase (SOD) on heart rate variability (HRV) in rats.

Methods: Diethylthiocarbamate (DETC; 250 mg/kg i.p, was used as a nonspecific SOD inhibitor. ECG was continuously recorded at sampling rate 4 kHz (PowerLab, AdInstruments, Australia) from previously instrumented unrestrained male Wistar rats (n = 14). SOD activity was measured by using Cayman Assay Kit (no: 706002; Cayman Chemical, MI, USA) in erythrocyte lysate. HRV was analyzed (Kubios HRV Pro software; Kuopio, Finland) in time- and frequency domains (FFT method) from time series of 1024-RR-intervals (RRi). Frequency ranges: 0.27 to 0.75 Hz (LF) and 0.75 to 2.5 Hz (HF) were selected for spectral powers. Nonlinear dynamics of HRV was analyzed through recurrence plots, sample and approximate entropy (SampEn, ApEn) and detrended fluctuation analysis (DFA).

Results: Intraperitoneal injection of DETC resulted in SOD inhibition by 30%. DETC evoked a significant decrease in RRi (from 190 ± 21 to 141 ± 15 ms; p = 0.003), SDNN (from 3.70 ± 0.98 to 1.50 ± 1.08 ms; p = 0.002), LF (from 2.3 ± 1.2 to 0.13 ± 0.08 ms^2; p = 0.001) and LF/HF ratio (from 0.85 ± 0.34 to 0.12 ± 0.03; p = 0.002). SOD inhibition did not change rMSSD (from 3.1 ± 0.97 to 1.7 ± 0.95 ms; p = 0.08) and HF (from 2.6 ± 1.3 to 1.3 ± 1.1 ms^2; p = 0,17). In contrast to unchanged SampEn and ApEn, DETC resulted in a significant reduction of the following nonlinear parameters: recurrency (%REC; from 46 ± 4.4 to 33 ± 10; p = 0.02), determinism (%DET from 99.4 ± 0.27 to 97.9 ± ± 1.3; p = 0.04), DFA $\alpha 1$ (from 1.08 ± 0.15 to 0.53 ± 0.17; p = 0.001) and DFA and $\alpha 2$ (from 1.25 ± 0.12 to 1.01 ± ± 0.27; p = 0.03).

Conclusions: REDOX unbalance induced by SOD inhibition resulted in suppression of autonomic control upon the heart with an increased sympathetic drive. Decrease in %DET and %REC suggests lower predictability of the regulatory control and potential higher risk of unexpected severe events. Changes in HRV evoked by DETC reconfirm hypothesis that exogenous modulators of superoxide generation play a role in cardiovascular regulation.

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Myocardial hypoxia effect on expression of selected proangiogenic genes in postinfarcted heart as well as on therapeutic properties of human myoblasts

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Heart infarction leads to local hypoxia which is defined as a state when the the O2 level decreases below physiological levels characteristic for particular tissue. In eukaryotic cells, hypoxia-inducible factor-1 (HIF-1) is a primary transcriptional mediator of the hypoxic response and a master regulator of O2 homeostasis. Its transcriptional activity leads to activation of genes, particularly those associated with angiogenesis. Regenerative medicine indicates genetically modified myoblasts

stem cells as a potential therapeutic tool for heart regeneration. Coupling hypoxia resistant cells with proangiogenic genes may result in combined cell/gene therapy which is able to bring function to postinfarction scar and enhance the microperfusion in the postinfarcted region.

Our first step was to verify how the murine postinfarcted hearts may regulate the proangiogenic genes and their respective receptor expressions, along with the expression of Hif-1α, at different time points after myocardial infarction. We aimed to determine whether there exist any correlations between our genes of interest and hypoxia.

Because in vitro studies with genetically modified myoblasts were scheduled to be implemented next in preclinical studies, we also wanted to establish the most likely in vitro oxygen conditions corresponding to those prevailing in the heart after infarction.

We also evaluated the influence of established hypoxia and myoblasts normoxia (3% O2 and 5% O2) on the biological functions of genetically modified cells to define how these conditions may impact selected gene/protein expressions to induce proangiogenic and myogenic effects.

The last part of the study was dedicated to test the effect of implanted human myoblasts modified with PlGF gene on post infarction heart function in murine model.

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Vasopressin V1a receptors mediate respiratory depression induced by vasopressin and are present in the carotid body's chemoreceptor cells

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Background: Carotid bodies mediate the peripheral chemoreflex, which is triggered by hypoxia, hypercapnia or acidity and results in increase in ventilation and elevation of arterial blood pressure. The activity of carotid bodies is modulated by key mediators involved in the regulation of cardiovascular system, such as angiotensin II, dopamine, nitric oxide, acetylcholine or ATP. Vasopressin is one of the key pressor hormones released in response to hyperosmolarity, hypovolemia and stress. Its cardiovascular effects are mostly dependent on activation of vasopressin type 1a receptors (V1aRs); however, little is known about involvement of AVP and its V1aRs in the control of breathing.

Aim: The aim of our study was to evaluate how AVP affects ventilation and if the effects are mediated by V1aRs.

Methods: In normotensive Sprague-Dawley rats we evaluated hemodynamic and ventilatory resonse to intravenous AVP under control conditions and after blockade of V1aRs. The functional part of the experiment was carried out on rats anesthetized with urethane. Occurrence of V1aRs in carotid body cells was examined by immunofluorescence method and visualized in confocal microscopy.

Results: Intravenous AVP increased arterial blood pressure and decreased respiratory frequency. Systemic blockade of V1aRs prevented these changes. Staining of the bifurcation of the common carotid artery revealed that V1aRs are mostly present in the carotid bodies on glomus cells type 1, which was confirmed by co-expression of tyrosine hydroxylase.

Conclusions: In the present study we show that AVP administered intravenously increases arterial blood pressure and suppresses respiratory function, which is mediated by V1aRs, as systemic blockade if V1aRs abolishes the responses. For the first time we show that glomus cells type 1 in the carotid bodies express vasopressin V1aRs, which may participate in mediating respiratory and cardiovascular effects of AVP.

New concepts of the mechanism of vascular oxidative stress. Protective and harmful roles of NADPH oxidase

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Background: The cardiovascular disease (CVD) remain the leading cause of morbidity and mortality in contemporary societies. Vascular oxidative stress, defined as increased vascular production of reactive oxygen species leading to endothelial dysfunction and cellular damage arising from disturbed ROS-mediated redox-signalling reactions, are likely common underlying mechanisms of CVD. The central role in the mechanism of CVD play (i) an increase vascular production of reactive oxygen species (ROS) such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2); (ii) an inactivation of endothelial nitric oxide (NO) caused by ROS and (iii) a forma-

tion of toxic peroxynitrite. Actually, in healthy vascular system, cellular signalling is dominated by endothelial NO that induces an anti-atherosclerotic phenotype of the endothelium and the vascular wall. CVD risk factors are associated with ROS--mediated decreased NO bioavailability (oxidative stress) and adverse signalling by $ONOO^-$ (nitrosative stress). Nevertheless, using antioxidants to prevent CVD has been demonstrated to be ineffective in clinical trials, which most probably reflects an incomplete understanding of the oxidative stress. A major source of the vascular ROS and a mediator of CVD is the NADPH oxidase family of enzymes which produce ROS as their primary function. Four NADPH homologues, Nox1, Nox2, Nox4, and Nox5, differing in various features and biological functions are expressed in the cardiovascular system. The experimental evidence suggests that Nox1/2/5 and Nox4 are oppositely regulated by agonists and each other. The data support that the mechanism of the vascular oxidative stress and endothelial dysfunction encompasses two interrelated processes: the increase of the harmful Nox1/2/5, probably by the involved activation of the transcription factor NF-κB, and the decrease of the protecting Nox4, what appeared to be secondary to an increased expression of the transcription factor Nrf2. Our previous studies showed that in models with an increased expression of Nox2 observed the reduce the expression of Nox4. This, in turn, implicates that treatment of CVD should involve interventions directed at either selective inhibition of Nox1/2/5 or selective activation of Nox4.

Aim: The aim of the study is further verification of the above hypothesis, including the examination of the impact of physical training and nutritional supplementation of nitrites (two interventions to prevent CVD and increase vascular NO production) on cardiac production of ROS, the expression of isoforms of Nox, the activity of NF-κB and Nrf2, and other markers of oxidative stress rat heart type I diabetes.

Methods: All studies will consist of three consecutive steps: (i) in vivo experiments in rats (diabetes induction, exercise training and feeding animals with nitrite (50 mg/l) by 7 weeks), measurement of nitrite in the blood as a measure of systemic NO production; (ii) in vitro perfusion of hearts isolated from the animals that completed experimental step I, and various measurements in these hearts, including the measurement of cardiac O_2^- production, and (iii) biochemical measurements in cardiac tissue of activity and expression of Nox isoforms and another proteins and activity of NF-κB and Nrf2.

Results and conclusions: Diabetes accompanies with oxidative stress/nitrosative, as evidenced by: (i) an increase the production of superoxide anion, an activity of NADPH and the expression of Nox1 and Nox2 and a decrease of Nox4; (ii) a decrease the level of nitrite in plasma (iii) an increase concentrations of 8-isoprostane in myocardium and plasma and an increase the concentration of 3-nitrotyrosine in the myocardium; (iv) an increase activity of NF-κB and a decrease activity of Nrf2; (v) an increase expression of iNOS and a decrease of eNOS; (vi) a decrease in the expression of antioxidant enzymes SOD-1 and SOD-2. The above effects of diabetes prevented physical training and supplementation of nitrites which resulted an increase of eNOS expression and an increase levels of nitrite. These results confirm the hypothesis and suggest the introduction of NO in involved in the interactions between signalling pathways induced by Nox2 and Nox4.

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