„VICTOR BABEȘ“ UNIVERSITY
OF MEDICINE AND PHARMACY
FROM TIMISOARA

9th European Section Meeting of the International Academy of Cardiovascular Sciences

october
4 | 7
2023

PROGRAMME & ABSTRACT BOOK

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Indicativ CNCSIS: 324

ISBN 978-606-786-341-3

„VICTOR BABEȘ" UNIVERSITY
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9th European Section Meeting of the International Academy of Cardiovascular Sciences

October 4th – 7th, 2023

Timișoara, Romania

PROGRAMME & ABSTRACT BOOK

Editors: Danina M. Muntean, Maria D. Dănilă, Adrian Sturza

Dear Colleagues,

It is our great pleasure to welcome you to the **9th European Section Meeting of the International Academy of Cardiovascular Sciences (IACS-ES)**, in Timișoara, Romania.

The meeting will be held between October 4th – 7th, at "Victor Babeș" University of Medicine and Pharmacy from Timișoara, the largest medical university with international accreditation from Western Romania.

We kindly invite you to participate in this fascinating scientific meeting focusing on the following scientific topics:

Pathophysiology and Therapeutics of Heart Failure
Basic and Translational Approaches in Cardiac Arrhythmias
Myocardial Ischemia-Reperfusion Injury: Mechanisms and Cardioprotective Strategies
Cardiovascular Dysfunction and Adaptation to Stress
Pathomechanisms and Therapeutic Targets in Cardiometabolic Diseases
Cardiovascular Dysfunction Due to Toxicity
Natural Compounds, Ageing and Cardiovascular Risk
Comorbidities and Novel Therapeutic Strategies in Cardiovascular Diseases
Cardiac Regeneration and Response to Infection
Novel Targets and Therapies in Cardiovascular Diseases: What Animal Models Have Taught Us?
Novel Biomarkers, Therapeutic Targets and Unusual Cases: What Humans Have Taught Us?

The meeting will feature both basic science and clinical sessions, including lectures of invited speakers and free oral communications selected from submitted abstracts. It will serve as a platform for the exchange of new ideas and concepts and will offer a great educational opportunity for advancing career development of early and mid-career trainees, personal interactions and networking for all the participants.

We would like to provide the opportunity for several young investigators to present their latest research results and compete in both oral and poster sessions; we also strongly encourage the attendance of undergraduate students.

In addition to the thought-provoking conference, the organizers wish to inform you that Timișoara is one of three nominated European Capitals of Culture 2023, having as slogan a vivid message: "Shine Your Light!" and promise you lasting impressions besides the productive exchanges.

We cordially invite you to join us, shine your light at this meeting, renew old friendships, and make new ones!

With best regards,

Prof. Danina M. Muntean, MD, PhD
Chair of the Meeting

Honorary Chair of the Meeting: Prof. Naranjan S. Dhalla, PhD, MD (Hon), DSc (Hon)

President of the Meeting: Prof. Octavian M. Crețu, MD, PhD, Rector of the University

Main organizers, Vice Rectors of the University: **Prof. Claudia Borza, MD, PhD**
Prof. Daniel Lighezan, MD, PhD
Prof. Cristian Oancea, MD, PhD

If we let our own light shine, we unconsciously give other people permission to do the same. (Nelson Mandela)

Meeting Venue:

"Victor Babeș" University of Medicine and Pharmacy from Timișoara
E. Murgu Sq., nr. 2, 300041 Timișoara, Romania

Organizing secretariat:

Dr. Adrian Sturza, MD, PhD - Director of the Local Organizing Committee
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Organizing Committees

International Organizing Committee

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Vladimir Jakovljevic, President IACS-European Section (Kragujevac, Serbia)

Grant Pierce, President IACS (Winnipeg, Canada)

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(Members and Collaborators of the Center for Translational Research and Systems Medicine, University Advanced Research Center)

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Ioana M. Mozoș

Adrian Sturza

Sorin Ursoniu

Overview of Conference Programme

DAY 1 (October 4, 2023) *Aula Magna Hall*	
12:00 -	**Registration – Central Lobby of the University**
15:00 - 15:30	**OPENING CEREMONY / WELCOME MESSAGES** **Danina Muntean (Romania, Chair of the Meeting)** **Vladimir Jakovljevic (Serbia, President of IACS-ES)** **Naranjan Dhalla (Canada, Honorary Life President of IACS)** **Grant Pierce (Canada, President of IACS)** **András Varró (Hungary, President elect of IACS)**
15:30 - 16:00	**IACS RECOGNITIONS:** **Presentation by Profs. Grant Pierce, Vladimir Jakovljevic, and András Varró** **1. Lifetime Achievement Award:** **Prof. Octavian Crețu** , Rector of "*Victor Babeș*" University of Medicine and Pharmacy from Timișoara, Romania **2. Distinguished Leadership Awards:** **Prof. Daniel Lighezan**, Vice-Rector for Education, "*Victor Babeș*" University of Medicine and Pharmacy from Timișoara, Romania **Prof. Claudia Borza**, Vice-Rector for International Affairs, "*Victor Babeș*" University of Medicine and Pharmacy of Timisoara, Romania **3. Distinguished Service Award:** **Assoc. Prof. Adrian Sturza**, Director of the Organizing Committee, "*Victor Babeș*" University of Medicine and Pharmacy of Timișoara, Romania
16:00 - 16:30	**UNIVERSITY RECOGNITIONS:** **Presentation by Profs. Danina Muntean, Daniel Lighezan and Claudia Borza** **1. Lifetime Leadership in Cardiovascular Sciences Award:** **Prof. Naranjan Dhalla**, University of Manitoba, Canada **2. Excellence in Cardiovascular Research Awards:** **Prof. Roberto Bolli**, University of Louisville, USA **Prof. Zoltán Papp**, University of Debrecen, Hungary **3. Visiting Professor Title:** **Prof. Vladimir Jakovljevic**, University of Kragujevac, Serbia
16:30-19:00	**PLENARY LECTURES**
Chairs:	**Vladimir Jakovljevic (Serbia), Danina Muntean (Romania)**
16:30-17:00 17:00-17:05	**Naranjan Dhalla Honorary Lecture** **Gerd Heusch (Essen, Germany)** *Remote Ischemic Conditioning and Its Translation* Presentation of Naranjan Dhalla Honorary Lecture Award
17:05-17:35	**Rodolphe Fischmeister (Paris, France)** *Enhancing Cardiac Phosphodiesterase Activity: A Therapeutic Strategy in Heart Failure?*
17:35-18:00	**Coffee Break**
Chairs:	**István Baczkó (Hungary), Andrei Motoc (Romania)**

18:00-18:30	**Michael Czubryt (Winnipeg, Canada)** *Scleraxis: A Master Regulator of Wound Healing and Scarring*
18:30-19:00	**Devendra Agrawal (Pomona, USA)** *Novel Therapeutic Targets to Prevent Atherosclerotic Plaque Burden and Rupture*
19:00 -	**Welcome Reception (University building - sponsored by VOLVO)**
DAY 2 (October 5, 2023) ***Parallel sessions in Aula Magna Hall, Senate Hall and Iagnov Hall***	
08:30-10:25	**Session 1 – Pathophysiology and Therapeutics of Heart Failure I** ***Aula Magna Hall***
Session chairs:	**Zoltán Papp (Hungary), Lucian Petrescu (Romania)**
08:30-08:55	**David Eisner (Manchester, UK)** *Calcium Entry Into the Ventricular Myocytes: Beyond the L-Type Calcium Current*
08:55-09:20	**Zoltán Papp (Debrecen, Hungary)** *From Myosin Activation to Myosin Inhibition for the Treatment of Cardiac Diseases*
09:20-09:45	**Coert Zuurbier (Amsterdam, The Netherlands)** *Vascular and Myocardial Effects of SGLT2 Inhibitors*
09:45-10:10	**Dan Gaiță (Timișoara, Romania)** *Is SGLT2 Inhibition the Perfect Pathway to Heart Failure Prevention?*
10:10-10:25	**Dan-Alexandru Cozac (Târgu Mureș, Romania)** *Effects Of Chronic Beta-Blockers Therapy On In-Hospital Outcomes In Patients With ST-Segment Elevation Myocardial Infarction Treated With Primary Percutaneous Coronary Intervention*
08:30-10:25	**Session 2 – Basic and Translational Approaches in Cardiac Arrhythmias** ***Senate Hall***
Session chairs:	**José Jalife (Spain), Torsten Christ (Germany)**
08:30-08:55	**Torsten Christ (Hamburg, Germany)** *Engineered Heart Tissue Based on Human Induced Pluripotent Stem Cell Derived Atrial Cardiomyocytes: A Useful Model To Study Human Atrial Electrophysiology?*
08:55-09:20	**José Jalife (Madrid, Spain)** *Dysfunction of the $Na_v1.5$-Kir2.1 Channelosome and the Mechanism of Arrhythmias in Inherited Channelopathies*
09:20-09:45	**István Baczkó (Szeged, Hungary)** *Transgenic Rabbit Models of Congenital LQT Syndromes*
09:45-10:10	**Norbert Nagy (Szeged, Hungary)** *Assessment of Cardiac Alternans in a Canine Model of Elite Exercise*
10:10-10:25	**Băraru-Bojan Iris (Iași, Romania)** *The Genetic Characteristics Associated With The Prothrombotic Condition In Individuals Diagnosed With Type 2 Diabetes*
10:25-11:00	**Coffee Break**
11:00-12:55	**Session 3 – Myocardial Ischemia-Reperfusion Injury: Mechanisms and Cardioprotective Strategies**

	Aula Magna Hall
Session chairs:	**Peter Ferdinandy (Hungary), Ranko Škrbić (Bosnia & Herzegovina)**
11:00-11:25	**Naranjan S. Dhalla (Winnipeg, Canada)** *Mechanism Defects in Adrenergic Signal Transduction System in Ischemic Heart Disease*
11:25-11:50	**Peter Ferdinandy (Budapest, Hungary)** *Development of miRNA Therapeutics for Cardioprotection*
11:50-12:15	**Rakesh Kukreja (Virginia, USA)** *cGMP Signaling in Cardioprotection and Beyond*
12:15-12:40	**Ranko Škrbić (Banja Luka, Bosnia & Herzegovina)** *Liraglutide, A GLP-1 Receptor Agonist, Attenuates Isoprenaline-Induced Acute Myocardial Injury via Inhibition of the Wnt/B-Catenin Signaling Pathway*
12:40-12:55	**Nevena Mihailovic-Stanojevic (Belgrade, Serbia)** *Caffeic Acid Improves Antioxidant Defense And Attenuates Oxidative Stress In Heart Of Spontaneously Hypertensive Rat*
11:00-12:55	**Session 4 – Cardiovascular Dysfunction and Adaptation to Stress** ***Senate Hall***
Session chairs:	**Petr Ostadal (Czech Republic), Inna-Rabinovich-Nikitin (Canada)**
11:00-11:25	**Petr Ostadal (Prague, Czech Republic)** *Extracorporeal Membrane Oxygenation in the Therapy of Cardiogenic Shock*
11:25-11:50	**Inna Rabinovich-Nikitin (Winnipeg, Canada)** *Retinoic Acid-Related Orphan Receptors Regulate Autophagy and Cell Survival in Cardiac Myocytes During Hypoxic Stress*
11:50-12:15	**Saadeh Suleiman (Bristol, UK)** *Cardio-resilience Due to Chronic (Atherosclerosis) or Brief (Conditioning) Ischemia*
12:15-12:40	**Péter Bencsik (Szeged, Hungary)** *Immuno-Cardiovascular Diseasome: A Novel Approach to Reveal Confounding Factors Between Myocardial Infarction and Immunological Diseases*
12:40-12:55	**Jovana Joksimovic-Jovic (Kragujevac, Serbia)** *Relationship Between Hypertensive-Like Traits in a Rat Model of PCOS and Oxidative Stress*
11:00-12:55	**Symposium: *Cardiometabolic Alterations and Regulation of the Associated Pathways in Mammalian Heart Function and Regeneration I* – Iagnov Hall**
Session chairs	**Belma Turan (Turkey), Suresh Tyagi (USA)**
11:00-11:20	**Belma Turan (Ankara, Turkey)** *Comparisons of Pleiotropic Effects of SGLT2 Inhibition and GLP-1 Receptor Agonism on Cardiac Glucose Intolerance*
11:20-11:40	**Erkan Tuncay (Ankara, Turkey)** *The Relationship Between Cytosolic/Mitochondrial Labile Zinc Ratio and Mitochondrial Dynamics in Cardiomyocytes: Role of Znt6*
11:40-12:00	**Yusuf Olgar (Ankara, Turkey)** *Acute Action of Incretin-Based Therapeutics on Ageing Myocardium*
12:00-12:20	**Ceylan Verda Bitirim (Ankara, Turkey)**

	Improving Stem Cell-Based Therapy in Cardiac Failure: The Effect of Estrogen on Regenerative Capacity of Cardiac Progenitor Cells
12:20-12:40	**Aysegul Durak (Ankara, Turkey)** *GLP-1 Receptor Agonist Attenuates Ageing-Associated Cardiac Insufficiencies Through Recovery in Mitochondria*
12:40-12:55	**Anica Petrovic (Kragujevac, Serbia)** *From Forest o Healing: Development and Evaluation of Pinus Sibirica Essential Oil Based-Topical Gel for Wound Healing in Diabetic Rats*
13:00-13:10	**GROUP PHOTO in Front of the University**
13:10-14:00	**Lunch Break** *(lunch boxes provided)* **Work Lunch for IACS Officials in Petru Drăgan Hall**
14:00-15:55	**Session 5 – Pathomechanisms and Therapeutic Targets in Cardiometabolic Diseases** ***Aula Magna Hall***
Session chairs:	**Denis Angoulvant (France), Vesna Vucic (Serbia)**
14:00-14:25	**Denis Angoulvant (Tours, France)** *Targeting Inflammation in Cardiovascular Diseases*
14:25-14:50	**Vladimir Jakovljević (Kragujevac, Serbia)** *Sacubitril/Valsartan Promotes White Adipose Tissue Browning in Rats with Metabolic Syndrome*
14:50-15:15	**Ramaroson Andriantsitohaina (Montpellier, France)** *NLRP3-Inflammasome Carried by Extracellular Vesicles Validates the Inflammatory Hypothesis of Atherosclerosis in Metabolic Syndrome Patients*
15:15-15:40	**Vesna Vucic (Belgrade, Serbia)** *Osteosarcopenic Adiposity - Implications for Cardiometabolic Disorder*
15:40-15:55	**Avram Vlad-Florian (Timișoara, Romania)** *Improving Mitochondrial Respiration of Human Platelets With Cell-Permeable Succinate in Metabolic Disease*
15:55-16:15	**Coffee break**
14:00-15:55	**Session 6 – Natural Compounds, Ageing and Cardiovascular Risk** ***Senate Hall***
	Monika Bartekova (Slovakia), Adrian Sturza (Romania)
14:00-14:25	**Monika Bartekova (Bratislava, Slovak Republic)** *Role of Ageing and Metabolic Comorbidities in Beneficial Effects of Quercetin in Cardiovascular System*
14:25-14:50	**Isidora Milosavljevic (Kragujevac, Serbia)** *Melissa Officinalis as Cardioprotective Tool in Experimental Autoimmune Myocarditis*
14:50-15:15	**Ioana Mozoș (Timișoara, Romania)** *Arterial Stiffness and Cardiovascular Risk*
15:15-15:40	**Simona Drăgan (Timișoara, Romania)** *Cardioprotective Effects of Polyphenols and Future Perspectives for Functional Foods*
15:40-15:55	**Minodora Andor (Timișoara, Romania)** *Evaluation of Black Chokeberry Bio Juice Effect on Blood Pressure Levels and Endothelial Damage in Patients Under Chronic Antihypertensive Treatment (Preliminary data)*
15:55-16:15	**Coffee Break**

16:15-17:30	**Session 7 – Comorbidities and Novel Therapeutic Strategies in Cardiovascular Diseases** ***Aula Magna Hall***
Session chairs:	**Ferenc Gallyas (Hungary), Patrycja Kaczara (Krakow, Poland)**
16:15-16:40	**Ferenc Gallyas (Pécs, Hungary)** *Protective Effect of Olaparib in Experimental Crohn's Disease*
16:40-17:05	**Patrycja Kaczara (Krakow, Poland)** *Pharmacology of Energy Metabolism to Regulate Platelet Activation*
17:05-17:30	**Ștefan Mihăicuță (Timișoara, Romania)** *Obstructive Sleep Apnea and Cardiovascular Comorbidities Might Tailor Different Pathogenetic Treatment Strategies*
17:15-18:30	**POSTER SESSION I (Sport Hall)** **Chairs: Thomas Jespersen (Denmark), Péter Bencsik (Hungary), Miloš Stojiljković (Bosnia & Herzegovina)**
14:30-17:00	***Meeting of the MCB Editors (Ms. Aicha Hanna) – Petru Drăgan Hall***
DAY 3 (October 6, 2023) ***Parallel sessions in Aula Magna Hall, Senate Hall and Iagnov Hall***	
08:30-10:25	**Session 8 – Identifying the Right Targets and Therapeutic Approaches: Uncharted Paths** ***Aula Magna Hall***
Session chairs:	**Martin Morad (USA), András Varró (Hungary)**
08:30-08:55	**Martin Morad (Charleston, USA)** *What Are We Learning From Gene Editing of Human Ryr2 About CPVT1 Arrhythmia and Cardiac EC-Coupling?*
08:55-09:20	**Antonio Zaza (Milano, Italy)** *SERCA2a Loss vs. Gain of Function: Which Is Worse?*
09:20-09:45	**András Varró (Szeged, Hungary)** *The Electrophysiological Effect of Orange Alkaloid Hesperetin in Dog Cardiac Ventricular Preparations*
09:45-10:10	**Hector Martinez-Navarro (Oxford, UK)** *In Silico Trials for Ischemic Heart Disease: The Future of Diagnosis and Treatment*
10:10-10:25	**Muhammad Naveed (Szeged, Hungary)** *Arrhythmias Triggered By Medication: The Proarrhythmic Risk Associated With Class 1 Antiarrhythmics And Cannabinoids*
10:25-11:00	**Coffee Break**
08:30-10:25	**Session 9 – Pathophysiology and Therapeutics of Heart Failure II** ***Senate Hall***
Session chairs:	**Katharine Dibb (UK), Dragoș Cozma (Romania)**
08:30-08:55	**Katharine Dibb (Manchester, UK)** *Atrial Fibrillation in Heart Failure and Ageing: Understanding the Mechanisms*
08:55-09:20	**Suresh Tyagi (Louisville, USA)** *Interoceptive Inhibition of ADAMTS1 and Angiogenic Strategy for Prevention of HFrEF*
09:20-09:45	**Ruxandra Christodorescu (Timișoara, Romania)**

	New Trends in the Management of Advanced Heart Failure
09:45-10:10	**Dragoș Cozma (Timișoara, Romania)** *From Left Atrial Stretch to Heart Failure*
10:10-10:25	**Niskala Alisha Annikki (Copenhagen, Denmark)** *The Role of Colchicine on Cardiac Fibrosis in a Porcine Model of Atrial Fibrillation*
10:25-11:00	**Coffee break**
11:00-12:55	**Session 10 – Cardiac Regeneration and Response to Infection** ***Aula Magna Hall***
Session chairs:	**Roberto Bolli (USA), Grant Pierce (Canada)**
11:00-11:25	**Roberto Bolli (Louisville, USA)** *Reparative/Regenerative Cardiology: Hype vs. Reality*
11:25-11:50	**Grant Pierce (Winnipeg, Canada)** *Bacterial Infection and Cardiovascular Disease*
11:50-12:15	**Thomas Jespersen (Copenhagen, Denmark)** *In Vitro Reprogramming of Fibroblasts From Human Cadavers to Generate iPSC-Cardiomyocytes*
12:15-12:40	**Sanjiv Dhingra (Winnipeg, Canada)** *Immuno-Engineering Approaches for Cardiac Regenerative Medicine*
12:40-12:55	**Abhay Srivastava (Winnipeg, Canada)** *Development of iPSC-Based Clinical Trial Selection Platform for Patients with Inherited Metabolic and Cardiovascular Disorders*
11:00-12:40	**Session 11 – Cardiometabolic Diseases – From Pathophysiology to Treatment** ***Senate Hall***
Session chairs:	**Olga Pechanova (Slovakia), Luca Constantin (Romania)**
11:00-11:25	**Olga Pechanova (Bratislava, Slovakia)** *The Role of Nitric Oxide in Metabolic Syndrome: An Experimental Study*
11:25-11:50	**Paramjit Tappia (Winnipeg, Canada)** *High Fructose Diet Induces Cardiovascular Dysfunction*
11:50-12:15	**Florinela Cătoi (Cluj-Napoca, Romania)** *Obesity, Metabolic/Bariatric Surgery and Cardiovascular Disease*
12:15-12:40	**Cristian Mornoș (Timișoara, Romania)** *Epicardial Adipose Tissue Thickness as Independent Predictor in Coronary Artery Disease: Assessment by Echocardiography*
11:00-13:20	**Young Investigator Award Competition – Iagnov Hall**
Session chairs	**Antonio Zaza (Italy), Suleiman Saadeh (UK), Michael Czubryt (Canada)**
11:00-11:20	**Jovana Bradic (Kragujevac, Serbia)** *Gallium Verum Extract As A Novel Cardioprotective Agent Against Doxorubicin-Induced Cardiotoxicity In Rats*
11:20-11:40	**Bogdan Halațiu (Târgu-Mureș, Romania)** *Interleukin-8 Inhibition – A Possible Pathway to Reduce Hemodynamic Complications in Post-Coronary Artery Bypass Grafting Surgery Patients*
11:40-12:00	**Leto Luana Riebel (Oxford, UK)** *Human In Silico Trials to Evaluate the Safety of Regenerative Cell Therapy*
12:00-12:20	**Arnela Saljic (Copenhagen, Denmark)**

	Atrial Fat Infiltration Post Myocardial Infarction in a Göttingen Minipig Model of Obesity
12:20- 12:40	**Mélodie Schneider (Copenhagen, Denmark)** *Metformin Reduces Atrial Fibrillation Inducibility in Horses*
12:40- 13:00	**Leila Topal (Szeged, Hungary)** *Increased Arrhythmia Susceptibility Associated With Cardiac Remodelling Following Vigorous Endurance Training in a Canine Athlete's Heart Model*
13:00- 13:20	**Noemi Toth (Charleston, USA)** *Calcium Signalling Consequences of Calcium Release Deficiency Associated Ryr2-S4938F and Ryr2-I4855M Mutations Expressed in HiPSC-CMs*
13:00-14:00	**Lunch Break** *(lunch boxes provided)* **Work Lunch for IACS Officials in Petru Drăgan Hall**
14:00-15:30	**Session 12 – Cardiovascular Dysfunction Due to Toxicity** ***Aula Magna Hall***
Session chairs:	**Lorrie Kirshenbaum (Canada), Theodora Angoulvant (France)**
14:00-14:25	**Lorrie Kirshenbaum (Winnipeg, Canada)** *TRAF2-NF-kB Signaling in Doxorubicin Cardiomyopathy*
14:25-14:50	**Dinender K. Singla (Orlando, USA)** *Exosomes Ameliorates Doxorubicin-Induced Cardiomyopathy*
14:50-15:15	**Theodora Angoulvant (Tours, France)** *Immune Checkpoint Inhibitors-Related Myocarditis*
15:15-15:30	**Stojanovic Aleksandra (Kragujevac, Serbia)** *The Increased Expression of Extracellular Vesicles In Patients With Rheumatoid Arthritis: Novel Potential Biomarker For Disease Monitoring?*
	Symposium: *Cardiometabolic Alterations and Regulation of the Associated Pathways in Mammalian Heart Function and Regeneration II* – Iagnov Hall
Session chairs:	**Belma Turan (Turkey), Dinender Singla (USA)**
14:00-14:20	**Ebru Arioglu-Inan (Ankara, Turkey)** *Diabetic Heart And The Effects of Antidiabetic Drugs On Cardiac Function*
14:20-14:40	**Gizem Kayki-Mutlu (Ankara, Turkey)** *The Metabolic Roles of GRK2 in Cardiac Pathologies*
14:40-15:00	**Asena Gökçay Canpolat (Ankara, Turkey)** *Glucose Lowering Therapies for Cardiovascular Risk Reduction in Diabetes Mellitus*
15:00-15:20	**Firat Akat (Ankara, Turkey)** *The Roles of Sirtuins and K-Acetylation in Metabolic Syndrome Associated Heart Remodeling*
15:30-16:00	**Coffee Break**
16:00-17:15	**Session 13 – Translational Approaches in Cardiac Arrhythmias** ***Aula Magna Hall***
Session chairs:	**Norbert Jost (Hungary), Viviana Ivan (Romania)**
16:00-16:25	**Belma Turan (Ankara, Turkey)** *The Electrotonic Modulation in Mixed-Mode Electrical Conduction Can Be a Novel Approach for SQT-Characterized Cardiac Remodelling*

16:25-16:50	**Norbert Jost (Szeged, Hungary)** *Comparative Study of the Rapid (IKr) and Slow (IKs) Delayed Rectifier Potassium Currents in Undiseased Human, Dog, Rabbit and Guinea Pig Cardiac Ventricular Preparations*
16:50-17:15	**Viviana Ivan (Timișoara, Romania)** *Antiarrhythmic Effect of Metabolic Therapy: The Role of Late Potentials In Risk Assessment*
16:00-17:15	**Session 14 – Natural Compounds in Cardiovascular Protection** ***Senate Hall***
Session chairs:	**Jerzy Beltowski (Poland), Alina Pârvu (Romania)**
16:00-16:25	**Miloš Stojiljković (Banja Luka, Bosnia & Herzegovina)** *Antioxidative Capacity of Pomegranate Peel Extracts: Results of In Vitro and In Vivo Studies*
16:25-16:50	**Jerzy Beltowski (Lublin, Poland)** *Green Tea Extract Improves Vasodilating Effect of Insulin on Resistance Arteries in Rats with Metabolic Syndrome By Converting H_2S to Polysulfides*
16:50-17:15	**Alina Pârvu (Cluj-Napoca, Romania)** *Cardioprotective Effects of Mahonia Aquifolium Extracts*
17:15-18:30	**POSTER SESSION II (Sport Hall)** **Chairs: Devendra Agrawal (USA), Zoltán Papp (Hungary), Vladimir Zivkovic (Serbia)**
20:00-24:00 GALA DINNER & AWARDS CEREMONIES (bus transfer provided)	
DAY 4 (October 7, 2023)	
09:00-10:40	**Session 15 – Novel Targets and Therapies in Cardiovascular Diseases: What Animal Models Have Taught Us?** ***Aula Magna Hall***
Session chairs	**Ivan Srejovic (Serbia), Oana Aburel (Romania)**
09:00-09:25	**Melanie Hezzel (Bristol, UK)** *A One Health Approach to Cardiovascular Research – What Can Dogs Teach Us About Heart Disease?*
09:25-09:50	**Slavica Mutavdzin Krneta (Belgrade, Serbia)** *Effects of Vitamin B6 and Folic Acid on the Cardiovascular System of Rats with Streptozotocin-Induced Diabetes Mellitus*
09:50-10:15	**Vladimir Zivkovic (Kragujevac, Serbia)** *The Effects of Different Exercise Types on a Rat Model of Myocardial Ischemia/Reperfusion Injury*
10:15-10:40	**Ivan Srejovic (Kragujevac, Serbia)** *The Effects of L-Glutamate on Heart Function, Morphology and Redox Balance in Rats*
09:00-10:40	**Session 16 – Novel Biomarkers, Therapeutic Targets and Unusual Cases: What Humans Have Taught Us?** ***Senate Hall***
Session chairs	**Isidora Milosavljevic (Serbia), Caius Streian (Romania)**
09:00-09:25	**Manuela Ciocoiu (Iași, Romania)**

	The Development of Aortic Wall Aneurysms: Insights Into Molecular and Cellular Mechanisms
09:25-09:40	**Adrian Apostol (Timișoara, Romania)** *RAS Inhibition Therapy in Dialized Patients and the Effects on Positive Remodelling*
09:40-09:55	**Caius Streian (Timișoara, Romania)** *An Unusual Form of Left Ventricular Tumor*
09:55-10:10	**Ana Lascu (Timișoara, Romania)** *Concurrent Causes For Acute Severe Right Ventricular Failure In An Apparently Healthy Patient*
10:10-10:25	**Sonia Rațiu (Timișoara, Romania)** *Vitamin D Mitigates Oxidative Stress in Varicose Veins Explants From Obese and Non-Obese Patients*
10:25-10:40	**Răzvan Adrian Bertici (Timișoara, Romania)** *Evolution and New Etiological Trends in Patients With Pulmonary Arterial Hypertension*
10:45-11:00	**Closing Remarks (Aula Magna)**
11:30-	**Social program**

Social Program

Welcome Reception will take place at the meeting venue, "Victor Babeș" University of Medicine and Pharmacy of Timișoara, E.Murgu Sq., nr.2 Timișoara, Romania, on Wednesday, October 4.
Gala Dinner will be organized on Friday, October 6. Bus transportation will be arranged.
Other social events will be available on Saturday, October 7, after the end of the meeting. Further information will be provided on-site.

17:15 - 18:30	POSTER SESSION I (Sport Hall, October 5)
Session chairs	Thomas Jespersen (Denmark), Péter Bencsik (Hungary), Miloš Stojiljković (Bosnia & Herzegovina)

1. **Enache Alexandra (Timișoara, Romania)**
   Molecular Autopsy in Long Qt Syndrome in Sudden Unexplained Death Cases

2. **Bitay Gergő (Szeged, Hungary)**
   Investigation of Action Potential and Ca2+ Transient Alternans in Canine Athlete's Heart Model

3. **Besher Abual'anaz (Winnipeg, Canada)**
   Periostin Knock Out Is Causal to Differential Responses Among Male and Female Mice: Cardiac Extracellular Matrix Proteins and Survival

4. **Haugaard Simon Libak (Copenhagen, Denmark)**
   Metformin Attenuates Human Atrial Fibroblast Activation

5. **Bogdan Carina (Timișoara, Romania)**
   The Heart-Covid Connection: Autonomic Dysfunction - Heart Rate Variability and Atrial Fibrillation

6. **Božidar Pindović (Kragujevac, Serbia)**
   The Effects of Hyperbaric Oxygenation and Insulin Treatment on the Myocardial Function and Oxidative Stress in Diabetic Rats

7. **Demeter-Haludka Vivien (Szeged, Hungary)**
   Molecular Mechanisms Behind the Late Cardioprotective Effects of Inorganic Nitrites

8. **Enyedi Enikő Edit (Debrecen, Hungary)**
   Assessment of Factors Deceptively Lowering ACE Activity

9. **Ionică Loredana (Timișoara, Romania)**
   SGLT2 Inhibitors Reduce Monoamine Oxidase Expression and Oxidative Stress in Human Atrial Tissue: A Novel Off-Target Class Effect

10. **Knudsen Rikke (Copenhagen, Denmark)**
    Fibrosis in the Heart of The Most Commonly Used Animal Models in Atrial Fibrillation Research – A Comparative Study

11. **Déri Szilvia (Szeged, Hungary)**
    A Possible Explanation for the Low Penetrance of Pathogenic Kcne1 Variants in Long QT Syndrome Type 5

12. **Bînă Paul (Timișoara, Romania)**
    Platelet Mitochondrial Respiratory Dysfunction In Sepsis and Covid-19 Is Alleviated by Cell-Permeable Succinate

13. Aiman Mohammed (Szeged, Hungary)
Hesperetin Decreases the Repolarization Reserve and Inhibits the Slow Delayed Rectifier Potassium Current (Iks) in Dog And Rabbit Cardiac Ventricular Muscle Preparations and Isolated Myocytes - A Proarrhythmic Candidate

14. Saljic Arnela (Copenhagen, Denmark)
Atrial Fat Infiltration Post Myocardial Infarction in a Göttingen Minipig Model of Obesity

15. Bețiu Alina (Timișoara, Romania)
Assessment of Drug-Induced Mitochondrial Toxicity in Human Platelets Isolated from Buffy-Coat

16. Sárkány Fruzsina (Debrecen, Hungary)
Investigation of the Effects of the Next-Generation Myosin Inhibitor Aficamten on Canine Left Ventricular Isolated Cardiomyocytes

17. Suhov Loredana (Timișoara, Romania)
Thrombophilia and Venous Thromboembolism in Young Patients

18. Szabó Attila Ádám (Debrecen, Hungary)
Investigation of the Biomarker Role of Angiotensin-Converting and Chitotriosidase Enzymes in Various Inflammatory Diseases

19. Vajic Una-Jovana (Belgrade, Serbia)
Effects of Chronic Supplementation With Urtica Dioica L. Leaf Extract And Chlorogenic Acid on Hemodynamic and Biochemical Parameters in Spontaneously Hypertensive Rats

20. Vlădeanu Maria Cristina (Iași, Romania)
Angiotensin Converting Enzyme Gene D-Allele and Stent Restenosis: A Tangled Story - Case Presentation

21. Sandu Oana (Timișoara, Romania)
Pulmonary Thromboembolism in Adults Under 50 Years Old

22. Karanović Danijela (Belgrade, Serbia)
Natural Versus Synthetic Antioxidant Supplementation of Losartan Treatment in an Experimental Model of Hypertension and Chronic Kidney Disease: Is Together Better?

23. Dăniluc Larissa (Timișoara, Romania)
Chemotherapy - Focus on the Mechanisms of Cardiotoxicity

17:15 - 18:30	POSTER SESSION II (Sport Hall, October 6)
Session chairs	Devendra Agrawal (USA), Zoltán Papp (Hungary), Vladimir Zivkovic (Serbia)

24. Kezia Jerltorp (Copenhagen, Denmark)
Electrophysiological Characterisation of Indomethacin in a Porcine Model of Obstructive Sleep Apnea

25. Soșdean Raluca (Timișoara, Romania)
Role of Monoamine Oxidase in the Pathogenesis of Severe Mitral Regurgitation and The Possible Link With The Patients' Comorbidities

26. Bajic Zorislava (Banja Luka, Bosnia and Herzegovina)
Cardioprotective Effects of Pomegranate Peel Extract in Takotsubo-Like Myocardial Injury in Rats

27. Dannesboe Johs (Copenhagen, Denmark)
Understanding Deadly Intravenous Paracetamol-Induced Hypotension

28. Đorđe Đukanović (Banja Luka, Bosnia and Herzegovina)
The Role of TRPA1 Channels in Regulation of the Vascular Tone - 3D QSAR Modeling with Design of Novel TRPA1 Agonists

29. Mariș Mihaela-Ioana (Timișoara, Romania)
Buerger's Disease With Upper Limbs Ischemia in a Female Patient

30. Nikolic Marina (Kragujevac, Serbia)
mTORC1 Activation Is Essential for Sacubitril/Valsartan Stimulation of Subcutaneous White Adipose Tissue Browning

31. Baba Mirela (Timișoara, Romania)
Crosstalk Between Early Vascular Aging, Nonalcoholic Fatty Liver Disease and Insulin Resistance in Patients with Cardiovascular Risk Factors

32. Canpolat Gökçay Asena (Ankara, Turkey)
The Significance of Body Fat Index and Other Indicators in Identifying Metabolic Subtypes

33. Milivojac Tatjana (Banja Luka, Bosnia and Herzegovina)
Ursodeoxycholic Acid Attenuates Systemic and Liver Inflammation Induced by LPS In Rats

34. Ciubotaru Paul-Gabriel (Timișoara, Romania)
Is a Pacient With Hemophilia Protected Against Ischemia?

35. Todor Ivanka Marian (Timișoara, Romania)
Immunotherapy-Mediated Cardiotoxicity in a Patient With Metastatic Melanoma

36. Moroz Simina (Timisoara, Romania)

The Influence of The Relative Parietal Thickness on The Post-Interventional Hemoglobin Value in Aortic Stenosis After TAVR

37. Maličević Uglješa (Banja Luka, Bosnia & Herzegovina)
Levosimendan Attenuates Lung Injury in Intestinal Ischemia-Reperfusion Model In Rats

38. Goje Iacob Daniel (Timișoara, Romania)
Dynamic Right Ventricular Obstruction Complicating Laparoscopic Cholecystectomy in a Patient With Known Atrial Myxoma

39. Kohistani Mosk (Szeged, Hungary)
The Influence of Ulcerative Colitis on Myocardial Infarct Size in a Co-Morbid Model: The Role of Immuno-Cardiovascular Diseasome Research

40. Nissen Sarah Dalgas (Copenhagen, Denmark)
The Role of M2r-IK,Ach Pathway in the Genesis of Second-Degree Atrioventricular Block in Horses

41. Andjic Marijana (Kragujevac, Serbia)
Wound Healing Effects of an Ointment Containing Helichrysum Italicum Essential Oil

42. Buriman Darius (Timișoara, Romania)
Characterization of Changes in Platelet Mitochondrial Respiration Pre- and Post-Cardiopulmonary Bypass

43. Pintér Jenő Antal (Szeged, Hungary)
Altered Cardiac Repolarization Associated With Cellular Electrophysiological Remodeling Following Chronic Testosterone Administration in a Large Animal Model

44. Başak Neslihan (Ankara, Turkey)
Mitochondrial Dysfunction in iPSC-derived Ventricular Cardiomyocytes in Spinal Muscular Atrophy

45. Kocovic Aleksandar (Kragujevac, Serbia)
Assessing the Cardioprotective Potential of Usnic Acid in Mitigating Doxorubicin-Induced Cardiotoxicity in Rats

46. Brăescu Laurentiu (Timișoara, Romania)
Effects of Statin Therapy on Epicardial Adipose Tissue: Focus on Oxidative Stress

47. Bitay Gergő (Szeged, Hungary)
Investigation of the Action Potential Alternans in Human Heart Failure

48. Gábor Mohácsi (Szeged, Hungary)
Effect of Selective IKur inhibitor XEN-D0103 on the Dog Cardiac Ventricular and Purkinje Fiber Action Potential

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ABSTRACTS OF THE LECTURES

CARDIOPROTECTION BY REMOTE ISCHEMIC CONDITIONING (RIC) AND ITS TRANSLATION

Gerd Heusch

Institute for Pathophysiology, West German Heart and Vascular Center, University Duisburg-Essen/ Germany

There is still a medical need for cardioprotection beyond that by rapid reperfusion, since mortality and morbidity, notably from heart failure, in patients with acute myocardial infarction remain high. In our pig model of reperfused acute myocardial infarction, RIC by cycles of hindlimb occlusion/reperfusion before (preconditioning) or during (perconditioning) coronary occlusion reduces infarct size. The signal transfer of RIC from the periphery to the heart involves humoral and neuronal factors. Humoral factors of RIC can be transferred with plasma preparations from one individual to another, even across species. The neuronal signal transfer involves peripheral sensory afferents, the central nervous system and vagal efferents. The spleen serves as a decisive relay organ of RIC and releases humoral cardioprotective factors upon vagal activation. The protective signal transduction in pig myocardium involves STAT3 activation and improved mitochondrial function. Humoral cardioprotective factors of RIC by arm occlusion/reperfusion in humans can also be transferred to bioassay recipient hearts. In cardiosurgical patients, the myocardial signal tranduction of RIC involves STAT5 activation and improved mitochondrial function. Repeated blood pressure cuff inflation/deflation on the arm or leg has reduced infarct size and improved clinical outcome in patients undergoing coronary bypass surgery when RIC was used in a preconditioning mode and in patients with reperfused acute myocardial infarction when RIC was used in a perconditioning mode. However, larger clinical trials with neutral results have raised disappointment on the translation of RIC. Co-morbidities, co-medications but also a primordial myocardial non-responsiveness can interfere with cardioprotection by RIC. The focus must be on RIC in patients who really need adjunct cardioprotection because they are at high risk and/or have suboptimal reperfusion. Respective trials are underway.

Nat Rev Cardiol 12,2020,773-89; Eur Heart J 44,2023,1687-9

ENHANCING CARDIAC PHOSPHODIESTERASE ACTIVITY: A THERAPEUTIC STRATEGY IN HEART FAILURE?

Rodolphe Fischmeister

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The β-adrenergic stimulation of cardiac function involves the second messenger cAMP which activates the cAMP-dependent protein kinase (PKA) to modulate key proteins of the excitation-contraction coupling (ECC) process. Intracellular cAMP levels produced upon β-stimulation are counterbalanced by the degradation of the cyclic nucleotide by enzymes called phosphodiesterases (PDEs). We have shown in the past that PDEs not only terminate the activation of cAMP effectors but also compartmentalize this second messenger in discrete subcellular domains. Clinically, PDE inhibition has been considered a promising approach to compensate for the catecholamine desensitization that accompanies heart failure (HF). In that respect, PDE3 inhibitors, such as milrinone or enoximone, have been used clinically to improve systolic function and alleviate the symptoms of acute HF. However, their chronic use has proven to be detrimental, increasing adverse remodeling and ventricular arrhythmias. Recently, we proposed to test the opposite strategy, *i.e.* increasing rather than inhibiting PDE activity. We believe that this strategy, which is reminiscent of the counter-intuitive beneficial effect of beta-blockers in HF, could be therapeutically relevant in HF because it would prevent a deleterious accumulation of cAMP during catecholamines spill over. In that line, we found that constitutive overexpression of PDE4B, one of the main PDE4 isoform expressed in the cardiomyocyte to control the β-adrenergic regulation of the ECC, is cardioprotective. We also found that gene therapy with AAV9-PDE4B exerts cardioprotective effects limiting adverse remodeling evoked by catecholamines or increased postcharge. Similarly, we found that PDE2A constitutive overexpression exerts anti-hypertrophic effects and transgenic mice overexpressing PDE2A in the heart have preserved ejection fraction after myocardial infarction and are protected against catecholamine induced ventricular arrhythmia. Also, PDE2 gene therapy limits cardiac adverse left ventricle remodeling and dysfunction induced by sympathomimetic amines as well as ventricular arrhythmias. Altogether, our findings suggest that increasing PDE activity, either with gene therapy or pharmacologically with PDE-specific activators yet to be discovered, could represent an interesting novel therapeutic strategy to treat heart failure.

SCLERAXIS: A MASTER REGULATOR OF WOUND HEALING AND SCARRING

Michael P. Czubryt

Institute of Cardiovascular Sciences, St. Boniface Hospital Albrechtsen Research Centre, University of Manitoba, Winnipeg, Manitoba, Canada

In response to injury or damage, tissues undergo a well-defined wound healing process that includes fibroblast activation to myofibroblasts, which synthesize extracellular matrix to stabilize the wound site. When the injury has healed, myofibroblasts undergo apoptosis and excess matrix is broken down, ideally resulting in scar-less resolution. In the heart, cardiomyocytes fail to reproduce, thus tissue injury is impaired, leading to myofibroblast persistence and fibrosis, which independently increases the risk of heart failure and death. While cardiac fibrosis currently lacks any treatment, attenuating fibroblast activation provides a potential therapeutic mechanism. Our lab has shown that the transcription factor scleraxis is required for the activation of cardiac fibroblasts to myofibroblasts, in part by controlling the expression of a broad program of pro-fibrosis genes. Most recently, using inducible, fibroblast-specific scleraxis knockout mice, we found that pressure overload surgery (thoracic aortic constriction, TAC) resulted in cardiac fibrosis only when scleraxis was present. Scleraxis knockout prevented the generation of myofibroblasts, attenuated the assembly of transcriptional complexes on pro-fibrosis gene promoters, and further improved cardiac systolic function, including ejection fraction. Deleting scleraxis after fibrosis was already established also attenuated fibrosis, arrested functional loss, and resulted in a wave of apoptosis, ostensibly of myofibroblasts. Most significantly, scleraxis deletion prevented death due to heart failure. In preliminary data, we have noted that scleraxis knockout prevented scar formation following myocardial infarction, with an observed increase in cardiac rupture. Similarly, we noted impaired dermal healing of scleraxis knockout mice following full thickness skin injury. Scleraxis thus plays a key role in the balance between wound healing and scarring: attenuating scleraxis function can be beneficial in the setting of fibrosis, but detrimental for normal wound healing. Targeting scleraxis may thus be useful in pathological situations in which excessive extracellular matrix leads to detrimental effects on tissue function.

NOVEL THERAPEUTIC TARGETS TO PREVENT ATHEROSCLEROTIC PLAQUE BURDEN AND RUPTURE

Devendra K. Agrawal

Department of Translational Research, Western University of Health Sciences, Pomona, CA, USA

Chronic inflammation within the atherosclerotic plaque renders a stable plaque vulnerable to rupture ultimately leading to rupture and thrombus formation. We found fewer number of VSMCs due to apoptosis, increased density of CD68+ cells, and increased expression of inflammatory molecules in the carotid endarterectomy tissues from symptomatic than in the asymptomatic patients with carotid stenosis. Atherogenic cytokines inhibited proliferation of VSMCs. Overall, there was an imbalance between the inflammation and reparative process tending to plaque instability. The findings supported the critical role of TLR-4 and TREM-1 in the underlying pathophysiology of plaque instability. Thus, we inhibited these molecules with their selective inhibitors in microswine with carotid artery atherosclerosis. Hypercholesterolemic Yucatan microswine were subjected to intimal injury with balloon angioplasty in the carotid artery followed by ox-LDL The swine were treated with control vehicles and the inhibitors of the inflammatory molecules and sacrificed after 5-6 months. Carotid arteries were evaluated for vessel wall thickness, blood flow, blood volume, and lumen area using color doppler ultrasound, angiography, and optical coherence tomography (OCT) at baseline, 6 weeks after surgery, and before sacrifice at 5-6 months. Radiologic evaluation of carotid arteries revealed decreased neointimal hyperplasia and plaque formation with the treatment with the inhibitors compared to vehicle controls. Histomorphologically, we found neointimal hyperplasia with significantly increased inflammation and elastin degradation in the vehicle groups compared to the inhibitor groups. Real-time PCR revealed significantly decreased carotid artery mRNA expression of TLR-4, MyD88, TREM-1, and collagen I while increased expression of collagen III and MMP-9 in inhibitor-treated swine compared to vehicle controls. Further, the gene and protein expression for the markers of plaque vulnerability (MMP-7, IL-6, IL-12/23, and CD36) were significantly decreased with the treatment with inhibitors compared to individual vehicle. The findings of this study support the therapeutic efficacy of inhibiting either TLR-4 or TREM-1 signaling alone or in combination to attenuate atherosclerotic plaque vulnerability, reduce plaque burden, and thus prevent the occurrence of transient ischemic attack and stroke.

Supported by the research grant R01 HL144125 by the National Institutes of Health, USA

CALCIUM ENTRY INTO THE VENTRICULAR MYOCYTES: BEYOND THE L-TYPE CALCIUM CURRENT

David Eisner, Barbara Niort, Andrew Trafford, David Hutchings

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The cytoplasmic calcium concentration in a ventricular myocyte is about 10,000 times less than that of plasma and increases roughly tenfold during systole. Ca^{2+} ions enter the cell via the L-type Ca current and contribute to both the action potential and the generation of contraction. However, there is also evidence that other mechanisms can contribute to Ca^{2+} influx but their identify and quantitative importance is unknown (PMID: 16563501; PMID: 31999537). The aim of this work was to characterize this "background" Ca^{2+} influx. Experiments were performed on sheep ventricular myocytes. Ca^{2+} entry was increased by elevating external Ca^{2+} concentration. This resulted in the occurrence of waves of Ca^{2+} release from the sarcoplasmic reticulum (SR) indicating an increased SR Ca^{2+} content. The Ca^{2+} waves activate sodium-calcium exchange and the resulting depolarizing current has been shown to produce arrhythmogenic afterdepolarizations. This current also gives a measure of the amount of Ca^{2+} pumped out of the cell, from which Ca^{2+} influx is measured. We found that the influx was inhibited by a TRPC6 inhibitor (BI 749327). This inhibitor also decreased Mn^{2+} influx into the cell, a measure of Ca^{2+} influx, again suggesting a role for TRPC6 in background Ca^{2+} entry (PMID: 35233776). We also found that the background influx is increased in myocytes from sheep with heart failure (rapid pacing). This extra Ca^{2+} influx may therefore contribute to the arrhythmias observed in heart failure. Finally, we find that the phosphodiesterase 5 inhibitor sildenafil decreases the background influx and this is associated with a decrease of arrhythmias (PMID: 34247494). Further work is required to clarify the role of this background Ca^{2+} entry in normal cardiac physiology.

FROM MYOSIN ACTIVATION TO MYOSIN INHIBITION FOR THE TREATMENT OF CARDIAC DISEASE

Zoltán Papp, Fruzsina Sárkány, Arnold Ráduly, Dávid Pásztor, Balázs Máté, István Édes, Attila Tóth, Attila Borbély

Division of Clinical Physiology, Department of Cardiology, Faculty of Medicine, University of Debrecen, Hungary

The function of the myosin molecule is closely related to cardiac systolic and diastolic performances. In the absence of side-effect-free inotropic drugs, the developments of direct myosin activators and direct myosin inhibitors evolve with considerable professional interest. Omecamtiv mecarbil is the most studied representative of direct myosin activators and has recently been shown to be effective in the GALACTIC-HF large randomized clinical trial. However, the accumulation of knowledge regarding less favourable effects of the agent - and the similar behaviour of the second-generation danicamtiv - means that rapid clinical introduction of direct myosin activators is not expected in the near future. As a result of the action of direct myosin activators, systolic duration increases - and consequently diastolic duration decreases - the rates of ventricular contractions and relaxations are slowed, which together raise the possibility of diastolic dysfunction. Direct myosin inhibitors (mavacamten and aficamten) may have a role in the treatment of hypertrophic cardiomyopathy, due to their negative inotropic effects. As far as we know, the reduction of hypercontractility in the latter condition can slow down the hypertrophic transformation of the heart, which may prevent invasive treatments of hypertrophic cardiomyopathy (septal myectomy, septal ablation, heart transplantation). Nevertheless, many questions remain to be answered regarding the development and use of myosin inhibitor drugs. Data so far suggest that myosin inhibition, in a well-chosen patient population, may become part of everyday practice sooner than a positive inotropic treatment based on myosin activation.

VASCULAR AND MYOCARDIAL EFFECTS OF SGLT2 INHIBITORS

Coert Zuurbier

Laboratory of Experimental Intensive Care and Anaesthesiology (L.E.I.C.A.), Academic Medical Centre (AMC), Department of Anaesthesiology, Amsterdam UMC, University of Amsterdam, The Netherlands

SGLT2i's are now known to reduce cardiovascular death and hospitalization for heart failure in various pathologies. The broad protective action suggest a common cellular nodal point being targeted. We postulate that one such important nodal point is intracellular sodium ($[Na^+]_I$). $[Na^+]_I$ is commonly increased in conditions of mechanical, metabolic and/or inflammatory overload. We demonstrated that SGLT2i's directly reduce $[Na^+]_I$ and ($[Ca^{2+}]_I$ in cardiomyocytes and endothelial cells. The lowering of $[Na^+]_I$ and ($[Ca^{2+}]_I$ can explain various effects of SGLT2is: decreased inflammation and oxidative stress, increased NO production, decreased CaMKII activity and a shift in cardiac metabolism away from glucose towards fatty acid metabolism. Possible cellular targets whereby SGLT2i's directly influence $[Na^+]_I$ are the sodium/hydrogen exchanger (NHE), the late Na^+ current, and disease-induced SGLT2 expression. To examine whether protection is through SGLT2 protein inhibition, we have created the SGLT2 KO mouse. In mice subjected to cardiac ischemia-reperfusion injury in vivo, we demonstrated that the SGLT2i empagliflozin was still protective, independent of the SGLT2 protein. Further studies are underway in preclinical heart failure models to examine the role of the SGLT2 protein in SGLT2i's protective effects in chronic heart failure.

ENGINEERED HEART TISSUE BASED ON HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED ATRIAL CARDIOMYOCYTES: A USEFUL MODEL TO STUDY HUMAN ATRIAL ELECTROPHYSIOLOGY?

Torsten Christ

Institute of Experimental Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Background: Atrial specific human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-aCM) allow genetic studies and application of optogenetic tools to study mechanisms in atrial fibrillation. However, the benefit of hiPSC-aCMs as a model will critically depend on how closely properties in hiPSC-aCM resembles the situation in human atrium. Contribution of the ultrarapid delayed (I_{Kur}) and G-protein-gated K^+ current ($I_{K,Ach}$) to repolarization in human atrium has been studied in very detail and it is now clear that both currents contribute to repolarization in hiPSC-aCM. However, effect size varies widely between different cell lines. Data on the contribution of the rapidly activating delayed rectifier currents I_{Kr} to repolarization in human atrium are spare, data on the contribution of the slowly activating delayed rectifier currents I_{Ks} to repolarization still lacking. Thus we compared contribution of 4 individual potassium currents to repolarization in aEHT vs. human atrial tissue.
Method: Right atrial tissues (RA) were obtained from patients with SR and AF undergoing heart surgery. Engineered heart tissue based on human induced pluripotent stem cell derived atrial cardiomyocytes (aEHT) were generated from hiPSC-aCM (cultured in the presence of 1 μM RA). Action potentials were recorded with standard sharp microelectrodes.
Results: As seen in RA block of I_{Kur} by 4-amonipyridine (100 μM) prolonged APD_{20} but shortened APD_{90} in aEHT. In both RA and aEHT block of I_{Kr} by E-4031 (1 μM) prolonged APD_{90}. However, block of I_{Ks} with HMR 1556 (1 μM) did not prolong APD even in the presence of I_{Kur} or I_{Kr} block, neither in RA nor in aEHT.
Conclusion: Our data suggest that aEHT can recapitulate key findings on repolarization in human atrium. The contribution of I_{Kr} resembles closely the situation in human atrium. I_{Ks} has only minor relevance in both human atrium and in aEHT.

DYSFUNCTION OF THE NAV1.5-KIR2.1 CHANNELOSOME AND THE MECHANISM OF ARRHYTHMIAS IN INHERITED CHANNELOPATHIES

José Jalife

Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain

Sudden cardiac death in children and young adults is a relatively rare but tragic event whose pathophysiology is unknown at the molecular level. The objective of this talk is to highlight the emerging role of macromolecular ion channel complexes in the mechanisms of arrhythmias and sudden death in hereditary diseases of young individuals. The recent identification of the interaction at the molecular level of the main sodium channel (NaV1.5) with the strong inward rectifying potassium channel (Kir2.1) in the control of cardiac excitability has led to a new paradigm to establish the molecular bases of arrhythmias and sudden cardiac death in some inheritable cardiac channelopathies. Evidence indicates that these two ion channels physically interact with common partners, including adapter, scaffolding and regulatory proteins, and form "channelosomes" that can traffic together to their eventual membrane microdomains. Most important dysfunction of either or both channels has direct links to hereditary human diseases. For example, certain mutations in the *KCNJ2* gene encoding the Kir2.1 protein impede channel traffic to the membrane and result in Andersen-Tawil syndrome type 1 (also known as long QT syndrome type 7). Similarly, trafficking-deficient mutations in the gene encoding the NaV1.5 protein (*SCN5A*) result in Brugada syndrome. On the other hand, gain-of-function mutations in *KCNJ2* result in short QT syndrome type 3, which is extremely rare but highly arrhythmogenic, and can modify Kir2.1-NaV1.5 interactions in mutation specific ways, further highlighting the relevance of macromolecular protein complexes in ion channel diseases. By expressing mutant proteins that interrupt Kir2.1 or NaV1.5 trafficking from the sarcoplasmic reticulum or the Golgi apparatus, or that modify Kir2.1-NaV1.5 interactions at their common membrane locations of transgenic mouse models and patient-specific iPSC-CMs, investigators are defining for the first time the mechanistic framework of how mutation-induced dysregulation of the NaV1.5-Kir2.1 channelosome affects cardiac excitability resulting in arrhythmias and sudden death in different cardiac diseases.

TRANSGENIC RABBIT MODELS OF CONGENITAL LQT SYNDROMES

István Baczkó

Department of Pharmacology and Pharmacotherapy, Albert Szent-Györgyi School of Medicine, University of Szeged, Szeged, Hungary

Drug-induced proarrhythmia constitutes a potentially lethal side effect of various drugs. This proarrhythmia is often mechanistically linked to the drug's potential to interact with repolarizing cardiac ion channels causing a prolongation of the QT interval in the ECG. Despite sophisticated screening approaches during drug development, reliable prediction of proarrhythmia in novel drug candidates is still a major challenge. Although drug-induced pro-arrhythmia occurs primarily in patients with pre-existing repolarisation disturbances, healthy animals are employed for pro-arrhythmia testing. To improve current safety screening, transgenic long QT (LQTS) rabbit models with impaired repolarisation reserve are useful tools not only to assess I_{Kr}-blocking but also I_{Ks}- and I_{K1}-blocking properties of drugs. In this presentation, the currently available transgenic LQTS rabbit models will be introduced, which carry pathogenic variants in KCNQ1 (LQT1, loss of I_{Ks}), KCNH2 (LQT2, loss of I_{Kr}), KCNE1 (LQT5, reduction of I_{Ks}), or KCNH2+KCNE1 (double-transgenic LQT2-5, loss of I_{Kr} and reduction of I_{Ks}) and the pharmacological proof-of-principle studies that have been performed with these models-highlighting the advantages and disadvantages of LQTS models for proarrhythmia research. In summary, LQTS models represent patients with reduced repolarisation reserve due to different pathomechanisms. Since they demonstrate increased sensitivity to different specific ion channel blockers (I_{Kr} blockade in LQT1 and LQT5 and I_{K1} and I_{Ks} blockade in LQT2 and LQT2-5), their combined use could provide more reliable and more thorough prediction of (multichannel-based) pro-arrhythmic potential of novel drug candidates.

Supported by the Hungarian National Research, Development and Innovation Office (TKP2021-EGA-32).

ASSESSMENT OF CARDIAC ALTERNANS IN A CANINE MODEL OF ELITE EXERCISE

Gergő Bitay[1], Leila Topal[1], Jenő Pintér[3], Noémi Tóth[1], Alexandra Polyák[1], János Prorok[1,2], Zsófia Kohajda[2], Szilvia Déri[1], Vivien Demeter-Haludka[1], Péter Hegyi[4,5], Viktória Venglovecz[1], Zoltán Husti[1], Tibor Hornyik[1], Naveed Muhammad[1], Aiman Saleh A. Mohammed[1], Norbert Jost[1,2,6], László Virág[1,6], István Baczkó[1,6], Attila S. Farkas[3], András Varró[1,2,6], Norbert Nagy[1,2]

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[2]*HU-REN-SZTE Research Group for Cardiovascular Pharmacology*
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[4]*Centre for Translational Medicine and Institute of Pancreatic Diseases, Semmelweis University, Institute for Translational Medicine, Medical School, University of Pécs*
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Background & Aim: Large number of evidences indicate that extreme sport activities - although very rare - can induce various cardiac arrhythmias, such as atrial fibrillation or even sudden cardiac death. A significant number of sudden cardiac deaths among athletes have been attributed to ischemic origin or other deviations revealed by autopsy. However, the underlying cause of the remaining cases remains unclear. Cardiac alternans indicate a periodic, regular oscillation of the action potential (short-long pattern) and parallel intracellular Ca^{2+} release (large-small pattern). Alternans have been clearly associated with the development of ventricular fibrillation and sudden cardiac death. However, the potential role of cardiac alternans in the "athlete heart" is unknown. In this study the potential role of alternans was assessed in arrhythmia development in trained dogs.
Materials & Methods: All measurements were performed on sedentary and trained dogs. Cardiac action potentials were measured by conventional microelectrode technique from right ventricular tissue samples. Ionic currents were recorded by the whole cell configuration of the patch-clamp technique. SR Ca^{2+} content was assessed by 10 mM caffeine. Ca^{2+} transients were monitored by employing fluo-4AM fluorescent dye.
Results: Trained dogs exerted increased susceptibility for ventricular fibrillation as a response of burst pacing. The I_{to} was downregulated and action potentials were prolonged in exercised animals. Action potential alternans and Ca^{2+} transient alternans were increased in trained animals. Parallel alternans of I_{CaL} and Ca^{2+} transient was enhanced in trained dogs. Ca^{2+} transient amplitude was decreased, SR Ca^{2+} content was reduced and Ca^{2+} transient decay was found slower in trained dogs.
Conclusion: Cardiac alternans were found slightly larger in trained animals that could be caused by decreased SERCA function. Alternans could contribute to arrhythmia development in elite athletes.

Key words: exercise, arrhythmia, remodelling, alternans, fibrillation

MECHANISMS FOR DEFECTS IN ADRENERGIC SIGNAL TRANSDUCTION SYSTEM DUE TO ISCHEMIA-REPERFUSION INJURY IN THE HEART

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Background & Aim: Ischemia- reperfusion (I/R) injury to the heart has been reported to cause marked alterations in the β_1- adrenoceptor (β_1- AR) signal transduction pathway; however, the mechanisms for such defects are not fully understood. Since the occurrence of oxidative stress and development of intracellular Ca^{2+}-overload have been shown to be the major mechanisms underlying the I/R induced changes in contractile function and myocardial metabolism, we have carried out extensive studies to examine if these pathologic events are involved in inducing alterations in the β_1- AR signaling system due to I/R injury. **Observations:** Marked attenuation in the positive inotropic effect of catecholamines in isolated hearts subjected to I/R injury was associated with depressions in the β_1-AR density, catecholamine-stimulated adenylyl cyclase activity and cyclic AMP-PKA stimulated phosphorylations of Ca^{2+}-handling proteins in the heart. All these changes were attenuated when I/R was carried out in the presence of oxyradical scavengers or antioxidants. Furthermore, perfusion of hearts with oxyradical generating system or induction of intracellular Ca^{2+} overload by Ca^{2+}-depletion and repletion in the perfusion medium were observed to simulate the I/R induced changes in different components of β_1- AR signaling system. **Conclusions:** It is suggested that attenuation of β_1- AR mediated signal transduction due to I/R injury may be a consequence of the development of oxidative stress and occurrence of intracellular Ca^{2+}-overload in the heart.

Key words: β_1-adrenoceptors/adenylyl cyclase, cyclic AMP/protein kinase, ischemia/reperfusion injury, oxidative stress, intracellular Ca^{2+} overload

ROLE OF CGMP SIGNALING IN CARDIOPROTECTION AND BEYOND

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The c-GMP specific phosphodiesterase 5 (PDE5) inhibitors, including sildenafil (Viagra™), vardenafil (Levitra™), and tadalafil (Cialis™) have been developed for treatment of erectile dysfunction. Preclinical studies have demonstrated that PDE5 inhibitors exert powerful protective effect against myocardial ischemia/reperfusion injury, ischemic and diabetic cardiomyopathy, cardiac hypertrophy and improvement of stem cell efficacy for myocardial repair. Because PDE5 is highly expressed in prostate cancer (PCa), treatment of PCa cells with sildenafil in combination with the potent anti-cancer drugs including doxorubicin (DOX) as well as docetaxal-induced apoptosis, which was mediated by enhanced oxidative stress, nitric oxide generation, up-regulation of caspase-3 and caspase-9 activities, reduced expression of Bcl-xL, and phosphorylation of Bad. Furthermore, co-treatment with sildenafil and DOX or docetaxal in mice bearing PCa xenografts resulted in significant inhibition of tumor growth as compared to individual drug treatment. Co-treatment sildenafil and DOX or docetaxel significantly improved viability of adult cardiomyocytes. Doppler echocardiography showed that sildenafil treatment also ameliorated DOX-induced LV dysfunction. These results provide provocative evidence that sildenafil is both a powerful sensitizer of DOX or docetaxal-induced killing of PCa and a potent cardioprotective small molecule. The results suggest that modulation of cGMP signaling could be a clinically translatable strategy in improving outcome of PCa patients receiving chemotherapy, considering that many PDE5 inhibitors are now approved for treatment of ED.

LIRAGLUTIDE, A GLP-1 RECEPTOR AGONIST, ATTENUATES ISOPRENALINE-INDUCED ACUTE MYOCARDIAL INJURY VIA INHIBITION OF THE Wnt/β-CATENIN SIGNALLING PATHWAY

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Background & Aim: Glucagon-like peptide-1 receptor agonists (GLP-1RAs) are efficiently used for type-2 diabetes mellitus (T2DM) management. GLP-1RAs also have anti-inflammatory and antioxidative effects that can improve cardiac function. The aim of this study was to investigate the cardioprotective effects of liraglutide, a GLP-1RA, on isoprenaline-induced myocardial injury in rats, and to elucidate the possible interplay with Wnt/β-catenin signalling pathway.
Materials & Methods: The study included four groups of animals. They were pretreated with saline for 10 days+ saline on days 9 and 10 (control), saline for 10 days + isoprenaline on days 9 and 10 (isoprenaline group), liraglutide for 10 days+ saline on days 9 and 10 (liraglutide group), and liraglutide for 10 days, and on days 9 and 10 isoprenaline was administered. This study evaluated heart remodelling and heart injury markers, as well as the oxidative stress markers, and pathohistological changes. The severity of tissue inflammatory injury was expressed as cardiac damage scores. The apoptotic cells were detected by TUNEL (Terminal deoxynucleotidyl transferase dUTP Nick End Labelling) and immunohistochemical staining was used for detection of proapoptotic (cleaved caspase-3, Bax), antiapoptotic (Bcl-2) and proinflammatory NF-kB, as well as for the components of Wnt/β-catenin signalling (GSK3β, β-catenin, LEF, AXIN2 and Cyclin-2).
Results: Liraglutide mitigated the isoprenaline-induced cardiac dysfunction recorded by ECG, reduced serum markers of myocardial injury and markers of oxidative stress, and increased activity of antioxidative enzymes and glutathione level. Liraglutide significantly abolished cardiac damage score induced by isoprenaline and significantly decreased apoptotic index, caspase-3 cleavage, Bax and NF-kB, while antiapoptotic Bcl-2 was significantly increased. At the same time liraglutide significantly attenuated β-catenin, LEF, AXIN2 and Cyclin-2 activity and increased the activity of GSK3β.
Conclusion: Taken together, the cardioprotective effects of liraglutide in isoprenaline-induced myocardial injury could be attributed to the inhibition of Wnt/β-catenin signalling pathway.

EXTRACORPOREAL MEMBRANE OXYGENATION IN THE THERAPY OF CARDIOGENIC SHOCK

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Cardiogenic shock is a critical circulatory condition when severely failing cardiac pump is unable to generate sufficient cardiac output leading to tissue hypoperfusion in critical organs. Despite the advances in intensive cardiology care, mortality of cardiogenic shock remains high. During the past years mechanical circulatory support including extracorporeal membrane oxygenation (ECMO) became a standard therapy of cardiogenic shock with the aim to bridge the most critical conditions. Current evidence supporting the use of ECMO (and other mechanical circulatory support systems) in cardiogenic shock remains insufficient. The initiation of ECMO leads to rapid restoration of total circulatory output, blood pressure and tissue perfusion. On the other hand, it can be associated with possible negative effect on already severely damaged left ventricle and with several specific complications. Furthermore, the hemodynamic effect of ECMO depends also on the cause of cardiogenic shock and modulating factors such as the presence of valvular heart disease (e.g. aortic stenosis or mitral regurgitation) or mechanical complications (e.g. ventricular septal defect). The negative effect of ECMO could be at least partially prevented by synchronized pulsatile extracorporeal flow. Recently published randomized clinical trials reported more or less neutral effect of the use of ECMO on clinical outcomes in cardiogenic shock. At the present time numerous clinical questions remain unanswered and another research in this field is fundamentally needed.

CIRCADIAN REGULATED AUTOPHAGY DURING HYPOXIC STRESS

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Background: Virtually, all tissues of the body exhibit a circadian rhythm that follows a 24-hour cycle that coordinates the timing of several key biological and homeostatic processes, including autophagy. Autophagy is a highly conserved evolutionary process that regulates cell quality control through protein degradation, organelle turnover, and recycling of cellular components in response to nutrient cellular stress.
Methods and Results: Herein, we provide new evidence that in contrast to control mice, a marked time dependent decline in *Clock* gene was observed in mice subjected to ischemia reperfusion injury. This coincided with impaired mitochondrial turnover resulting in the accumulation of damaged reactive oxygen species (ROS)-producing mitochondria from loss of *Clock* activity, as well as ultrastructural defects to mitochondria, autophagic dysfunction and impaired cardiac function. The Retinoic Acid-Related Orphan Receptors α (*RORα*) controls expression of *Clock* gene through binding to RORE elements on the *Clock* gene promoter. We therefore show that activation of Clock mediated autophagy can be achieved by treating cardiac myocytes with Nobiletin, a polymethoxy flavonoid, which is a direct target of *RORα.* We further show that treatment with Nobiletin rescued mitochondrial perturbations, and increased cell survival of cardiac myocytes during hypoxia. However, inactivation of autophagy by *Atg7,* or suppression of *RORα* abrogated the cytoprotective effects of Nobiletin.
Conclusions: Collectively, these findings provide the first direct evidence that *Clock* regulates autophagy and cell survival of cardiac myocytes during hypoxic injury, and interdictions that activate autophagy dependent circadian, such as Nobiletin, may prove beneficial in reducing hypoxia- induced cardiac cell death.

CARDIO-RESILIENCE DUE TO CHRONIC (ATHEROSCLEROSIS) OR BRIEF (CONDITIONING) ISCHEMIA

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The myocardial tissue has a built-in innate ability to undergo acute cellular and molecular changes (e.g., conditioning protocols) that can resist a sustained controlled acute injurious insult (e.g., ischemia & reperfusion). However, the progression of the atherosclerotic disease would trigger remodelling and adaptive responses (metabolic, molecular, structural etc) which would determine how the heart responds to an acute sustained insult and the extent of injury. Furthermore, the chronic disease-adaptive responses are likely to alter the signalling pathways associated with conditioning protocols. Understanding coronary artery disease-induced adaptive cardiac remodelling and how this can modify the cardioprotective efficacy of conditioning would help in the design of appropriate cardioprotective interventions. Key in delivering this target is the availability of clinically relevant animal models of atherosclerosis.

IMMUNO-CARDIOVASCULAR DISEASOME: A NOVEL APPROACH TO REVEAL CONFOUNDING FACTORS BETWEEN MYOCARDIAL INFARCTION AND IMMUNOLOGICAL DISEASES

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Background & Aim: In the last 2 decades, cardiovascular comorbidities have extensively been shown to interfere with cardioprotection, and may severely influence the outcome of cardiac diseases e.g. acute myocardial infarction and heart failure. Recently, increased cardiovascular risk has also been linked to immunological diseases. Although their aggravating role is clinically more and more obvious, the availability of such comorbid preclinical models to understand the molecular and cellular backgrounds of their connection, is limited. Bioinformatic network analysis is suitable to screen abundant data based on previous publications. Therefore, our aim was to identify an immunological comorbidity, which may interfere with acute myocardial infarction (AMI) by using bioinformatics, and then to develop and characterize a comorbid animal model.
Methods: Based on available literature data, we used a novel bioinformatics approach, whereby we defined ulcerative colitis (UC) as a comorbidity that may interfere with AMI. UC was induced in male C57Bl/6J mice by oral administration of dextran sulfate sodium (DSS, 2.5%; tap water was administered to controls). Histological and biochemical analyses of colon were assessed to confirm the development of UC. Transthoracic echocardiography was performed to assess cardiac function and morphology 7 days after DDS treatment, then animals were subjected to ischemia/reperfusion (I/R) injury by coronary artery occlusion and reperfusion. Myocardial infarct size was measured by Evans blue and TTC double staining.
Results: Although there was no difference in cardiac functional parameters between UC and control groups, mice with UC showed a significantly increased infarct size and a significantly higher all-cause mortality rate as compared to the control animals.
Conclusion: In summary, we successfully developed an AMI and UC (as immune disease) comorbid disease model in mice, in which an increased severity of major adverse cardiac events has been found as compared to the comorbidity-free controls.

Key words: acute myocardial infarction, ulcerative colitis, myocardial infarct size, diseaseome, immune disease

Funding: This study was supported by Project no. RRF-2.3.1-21-2022-00003 "National Heart Laboratory, Hungary", as well as by Project ID: 2020-1.1.5-GYORSÍTÓSÁV-2021-00011; OTKA-138223; ÚNKP-23-5-SZTE-704; and BO_481_21 – by the Hungarian Academy of Sciences.

TARGETING INFLAMMATION IN CARDIOVASCULAR DISEASES

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In recent years, a growing body of evidence has highlighted the pivotal role of inflammation in the pathogenesis and progression of cardiovascular diseases. Inflammation has emerged as a central player in the initiation and development of atherosclerosis. Chronic inflammation in the vascular endothelium, triggered by risk factors such as hyperlipidemia, smoking, and hypertension, promotes the formation of atherosclerotic plaques. These plaques, when destabilized, can rupture and lead to acute cardiovascular events like myocardial infarction and stroke. Post myocardial infarction inflammatory responses also contributes to adverse cardiac remodeling and heart failure and were also identified as potential contributors of recurrent atherosclerotic events. Recent research has revealed several potential targets for modulating inflammation in cardiovascular diseases. These include cytokines such as interleukin-1β (IL-1β) and interleukin-6 (IL-6), as well as specific cellular pathways like purinergic receptors signaling. Therapeutic interventions aimed at reducing inflammation in cardiovascular diseases range from conventional medications like statins and colchicine to more specialized biologics, such as monoclonal antibodies targeting pro-inflammatory cytokines. Clinical trials investigating anti-inflammatory strategies have shown promise in reducing cardiovascular events, demonstrating the feasibility of this approach. Notably, the Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) targeting IL-1β with canakinumab and the Colchicine Cardiovascular Outcomes Trial (COLCOT) with colchicine significantly reduced the risk of recurrent cardiovascular events in high-risk patients. On the other hand, the Colchicine for Left Ventricular Infarct Size Reduction in Acute Myocardial Infarction study (COVERT-MI) failed to reduce left ventricular remodeling after acute myocardial infarction. understanding and targeting inflammation represents a paradigm shift in the management of cardiovascular diseases. Addressing the pathophysiology of inflammation and the effects of anti-inflammatory therapies in cardiovascular diseases remains a challenge to further improve patient’s prognosis.

NLRP3-INFLAMMASOME CARRIED BY EXTRACELLULAR VESICLES VALIDATES THE INFLAMMATORY HYPOTHESIS OF ATHEROSCLEROSIS IN METABOLIC SYNDROME PATIENTS

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NLRP3 inflammasome pathway inhibition as an anti-inflammatory therapy leads to a significant decreasing rate of recurrent cardiovascular events independent of lipid-level lowering. Inappropriate activation of the NLRP3-inflammasome contributes to atherosclerosis progression and plaque instability in metabolic syndrome (MetS) patients. We studied the role of NLRP3-inflammasome carried by extracellular vesicles (EV) in the inflammatory hypothesis of atherosclerosis in MetS. Circulating large (lEVs) from non-MetS and MetS patients were isolated and characterized. The involvement of NLRP3 in the effects of EVs on permeabilization, proliferation, migration, and cytokine production of human aortic endothelial cells, smooth muscle cells (HASMC), and macrophages were analyzed. Correlations of levels of NLRP3-lEVs with anthropometric parameters of patients were assessed. Pathological relevance was studied in ApoE-/- mice fed a high-fat diet (HFD) and in human atherosclerotic lesions. NLRP3-inflammasome components were overexpressed in both lEVs from MetS patients compared with non-MetS subjects. Circulating levels of NLRP3-lEVs correlated with metabolic risk factors associated with obesity and insulin resistance. lEVs from MetS patients increased endothelial permeability, monocyte transmigration, and secretion of pro-inflammatory molecules from macrophages. In HASMC, MetSlEVs increased migration, proliferation, and IL-1ß and TNF-a secretion. Finally, EVs isolated from HFD mice and from human advanced plaques evidenced an accumulation of EV-associated NLRP3 and their implication in endothelial permeability. Pharmacological inhibition of NLRP3 prevented altered endothelial cellscrosstalk with macrophages and SMC, leading to vascular inflammation and remodeling. This data demonstrates that NLRP3-inflammasome, carried by lEVs, actively participate in vascular inflammation and atherosclerosis development in MetS. We underscore NLRP3 carried by lEVs as potential biomarkers and targets for novel therapeutic strategies of atherosclerosis-related diseases leading to major adverse cardiovascular events.

This work was supported by INSERM, Université d'Angers and CHU Angers. X. Vidal-Gómez wassupported by Fondation pour la Recherche Médicale (SPF201809006985). This work was also supported by the French Federation of Cardiology.

SACUBITRIL/VALSARTAN PROMOTES WHITE ADIPOSE TISSUE BROWNING IN RATS WITH METABOLIC SYNDROME

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Background & Aim: In the middle of an obesity epidemic, the process of white adipose tissue (WAT) browning appeared as promising therapeutic strategy to increase energy expenditure and prevent weight gain. In this study, combined therapy with sacubitril/valsartan was used to promote WAT browning in rats with metabolic syndrome (MetS).
Materials & Methods: The study involved 40 male *Wistar albino* rats (8 weeks old; body weight, bw: 200 ± 20g) divided into four groups: healthy non-treated rats – CTRL; rats treated with sacubitril/valsartan – S/V; rats with MetS – MS and rats with MetS treated with sacubitril/valsartan – MS+S/V. Combination of drugs were applied *per os* in a dose of 68 mg/kg/day during 4 weeks. A day before sacrificing the animals, oral glucose tolerance test (OGTT) was performed. When the protocol was completed, rats were sacrificed and blood samples were used for insulin and lipid status determination. Furthermore, visceral and subcutaneous WAT depots were isolated for hematoxylin/eosin (H/E) staining and immunohistochemical assessment of UCP-1 expression. After homogenization of frozen WAT sections, browning-related genes expression was determined.
Results: Treatment with S/V significantly improved glucose homeostasis in the OGTT test regarding healthy or rats with MetS. Also, applied therapy normalized insulin level and lipid profile compared to untreated rats. H/E staining demonstrated that S/V treatment led to significant morphological changes in lipid droplet size of both visceral and subcutaneous WAT depots of healthy as well as rats with MetS. Moreover, immunohistochemical analysis showed significantly higher UCP-1 expression in WAT of rats from exprerimental groups which is similar with results obtained from relative expression of browning-related genes.
Conclusion: The obtained results showed multifunctional role of S/V against MetS-related dysfunction, which is reflected in the improvement of glucose and lipid profile, but also with a strong potential to promote WAT browning.

Key words: white adipose tissue, browning, rat, sacubitril, valsartan

OSTEOSARCOPENIC ADIPOSITY - IMPLICATIONS FOR CARDIOMETABOLIC DISORDERS

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Body composition impairments, encompassing concurrent deterioration of bone, muscle, and adipose tissue, are defined as osteosarcopenic obesity/adiposity (OSO/OSA). The objective of this scoping review was to evaluate human studies addressing OSA in relation to cardiovascular and metabolic diseases. The search was conducted in PubMed, Scopus, and Web of Science databases to examine relevant articles published until August 2023, using the MeSH terms in the search strategy. Only studies published in English and conducted in adult humans with cardiometabolic disorders (dyslipidemia, hypertension, metabolic syndrome, cardiovascular disease - CVD) were included. A total of $n = 272$ articles were retrieved from all three databases. After removing duplicates and articles unrelated to the topic, only $n = 11$ studies met the inclusion criteria. Six studies were conducted in Asia, one in the USA, one in Brazil and three in the EU. Eight studies were cross-sectional, one was nested case-control and one retrospective study. Additionally, we identified only one interventional study in obese postmenopausal women supplemented with calcium and vitamin D or with dairy products. The studies included 16220 participants (60-4500). In general, a significantly higher prevalence of OSA was found in patients with CVD, hypertension, dyslipidemia, insulin resistance, non-alcoholic fatty liver disease and metabolic syndrome. Higher levels of CRP and other biomarkers of chronic inflammation were also detected in persons with OSA. The only interventional study has shown significant improvement in lipid profile, leptin, adiponectin and hypertension in the intervention groups. In summary, OSA is a frequent comorbidity in patients with cardiovascular and/or metabolic disorders. More studies are warranted to establish a causal relationship between OSA and cardiometabolic diseases.

ANTIARRHYTHMIC EFFECT OF METABOLIC THERAPY - THE ROLE OF LATE POTENTIALS IN RISK ASSESSMENT

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Late potentials represent the expression of the alteration of electrophysiological properties of the ischaemic myocardium, being a noninvasive marker of the arrhythmogenic substrate. The presence of the arrhythmogenic substrate is not equivalent with reentrant arrhythmia, a major role is played by autonomic factors, electrolytes disturbances, ischaemia itself, and the fact that an arrhythmogenic substrate might be protected by an entry or exit block. Signal averaged electrocardiography records parameters of the vector magnitude and help to diagnose patients with impaired left ventricular function or ventricular arrhythmias in which impaired cardiac tissue properties favor the development and sustain life threatening arrhythmias. Late potentials have a powerful negative predictive value and their appearance was determined by: infarct location and evolution of ischemia in acute phase- so patients with residual angina, silent ischaemia or infarct extension had a higher incidence of late potentials. The appearance of late potentials after acute phase was always associated with enzyme elevation and reinfarction. Incidence of late potentials tend to rise in parallel with Killip class, magnitude of myocardial infarction and the hemodynamic response is according to tissue involvement. Our data suggest that metabolic treatment reduces the incidence of late potentials. Trimetazidine (TMZ) has the benefit of metabolic shift, lowering free radicals, modulating cardiac energy metabolism, and alleviating the membrane lesions. All these result in a better performance of the heart without altering the hemodynamic function of the myocardium. Much remains to be understood about the effect of TMZ in protecting against myocardial ischemia, and most of its beneficial effects are revealed in clinical studies showing that TMZ administration may result in less myocardial damage, earlier successful reperfusion, improvement of left ventricular ejection fraction, substantially improved effort tolerance, decreased angina episodes and less cardiac adverse events, such as reduced hospitalization for heart failure and re-infarction. A new class of drugs, recently introduce as a main pillar in treatment of heart failure, SGLT2i, have been also reported to elicit antiarrhythmic effects.

ROLE OF AGEING AND METABOLIC COMORBIDITIES IN BENEFICIAL EFFECTS OF QUERCETIN IN CARDIOVASCULAR SYSTEM

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Despite extensive research in the field of cardioprotection, there are still no effective drugs on the market for prevention/treatment of myocardial ischemia-reperfusion (I/R) injury. Thus, searching for novel cardioprotective compounds is very much needed. Quercetin (QCT), a natural polyphenol enriched in human food, is a promising substance that exerts several beneficial effects in cardiovascular system including preventing cardiac I/R injury. Cardioprotective potential of QCT was largely documented in healthy young animals but only limited data are available regarding cardiac effects of QCT in presence of comorbidities and in ageing subjects. The aim of the current study was to explore potential cardioprotective effects of QCT in ageing individuals suffering from metabolic comorbidity – diabetes type 2 (T2D). For this aim, an animal model of T2D, Zucker diabetic fatty (ZDF) rats of two different ages, 6-months and 1-year, were used. QCT in the dose 20mg/kg/day was administered orally for 6 weeks. Effects of QCT on cardiac morphology and function *in vivo* were monitored using echocardiography. After the end of treatment hearts were isolated and *ex vivo* exposed to I/R (30min global ischemia/2h reperfusion). Recovery of cardiac function and infarct size were assessed as the physiological outputs of the experiments. Molecular signaling pathways involved in QCT action were evaluated using Western blotting method. The results showed that QCT exerts cardioprotective effects on diabetic hearts in vivo by preventing diastolic dysfunction and fibrosis in 1-year-old ZDF rats but is inefficient in preventing I/R injury in T2D rats of both ages. In conclusion, QCT might be potentially cardioprotective in aged diabetic subjects; however, ageing and presence of diabetes may decrease or even abolished its anti-ischemic effects.

Supported by grants: VEGA 2/0104/20, VEGA 1/0775/21, APVV-21-0194 and APVV-20-0242

ARTERIAL STIFFNESS AND CARDIOVASCULAR RISK

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Arterial stiffness, the expression of impaired arterial elasticity, is an early sign of adverse changes of the vessel wall, related to arterio- and atherosclerosis. It predicts cardiovascular events beyond standard cardiovascular risk factors. Arterial stiffness may be assessed using pulse wave velocity (PWV) and augmentation index (AI), a wave reflection parameter, and enables estimation of vascular age. The present paper discusses gender differences for arterial stiffness and arterial age in smokers, revealing a lower exposure to cigarette smoke needed to increase arterial stiffness in female compared to male smokers. There are several serological biomarkers which were significantly associated with arterial stiffness, including high sensitivity C reactive protein, vitamin D level and serum lipids. Electrocardiographic biomarkers, such as QT and Tpeak-Tend intervals, intrinsic heart rate and P wave duration were also significantly associated with variables of pulse wave analysis, especially in hypertensive patients. Cardiovascular risk can be also assessed in younger participants, considering that childhood obesity speeds up the increase of arterial stiffness. Arterial stiffness provides valuable data in several disorders and should be considered a reliable tool in the management of cardiovascular risk, especially in smokers, hypertensive patients and obese children.

Key words: arterial stiffness, pulse wave analysis, early vascular aging, cardiovascular risk

MELISSA OFFICINALIS AS A CARDIOPROTECTIVE TOOL IN EXPERIMENTAL AUTOIMMUNE MYOCARDITIS

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The aim of this study was to investigate the potential of ethanolic Melissa officinalis extract (MOE) in model of experimental autoimmune myocarditis (EAM). For that purpose we used 50 male Dark Agouti rats which were divided into: healthy rats (CTRL); rats with EAM (EAM); rats with EAM treated with 50, 100, or 200 mg/kg of MOE for 3 weeks per os (MOE50, MOE100, and MOE200). EAM was induced using porcine myocardial myosin emulsion. In order to investigate the effects of MOE on EAM we measured in vivo cardiac function via echocardiography, and also we measured the blood pressure and heart rate (HR). At the end of experiment, rats were sacrificed and the hearts were isolated for further histopathological/ immunohistochemical analyses, and PCR analysis. The blood samples were collected to determine the levels of cardiac enzymes and levels of inflammatory markers. The EAM group characteristically showed greater LV wall thickness and lower ejection fraction (50.33 ± 7.94% vs. 84.81 ± 7.74%) and fractional shortening compared to CTRL ($p < 0.05$). MOE significantly improved echocardiographic parameters (EF in MOE200 81.44 ± 5.51%) and also reduced inflammatory infiltrate (by 88.46%; $p < 0.001$) and collagen content (by 76.39%; $p < 0.001$) in the heart tissues, especially in the MOE200 group compared to the EAM group. Additionally, MOE significantly improved HR and decrease the levels of cardiac enzymes (CK-MB and TnT). MOE supplementation significantly decreased the values of pro-inflammatory cytokines (IL-1, 6 and 17 and TNF-α) and increase the level of anti-inflammatory cytokine (IL-10). The present study suggests that ethanolic MOEs, especially in a 200 mg/kg dose, improve cardiac function and myocardial architecture, possibly via decrement of inflammation and apoptosis, thus preventing heart remodeling, development of dilated cardiomyopathy, and subsequent heart failure connected with EAM. MOEs might be considered as a potentially helpful adjuvant therapy in patients with autoimmune myocarditis.

Key words: experimental autoimmune myocarditis, Melissa officinalis, inflammation, apoptosis, rats

CARDIOPROTECTIVE EFFECTS OF POLYPHENOLS AND FUTURE PERSPECTIVES FOR FUNCTIONAL FOODS

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Dietary polyphenols have been shown to exert multiple cardioprotective effects on plasma lipids, blood pressure, platelet and vascular function. They act as antioxidants and protect against development of atherosclerosis by influencing several biomarkers associated with increased cardiovascular risk. Grape and olive oil polyphenols and tea catequins affect hepatic lipoprotein metabolism and decrease inflammation. There continue to persist contradictory opinions on the effects of polyphenols on LDL oxidation, although altering of hepatic cholesterol absorption and triglyceride secretion have been demonstrated, Polyphenols from dark chocolate and flavonoids from cocoa and berries such as cranberries and blueberries improve endothelial function by increasing flow mediated dilation through NO production and inhibit ACE and renin lowering blood pressure. Most of the studies focused to research in this area were in vitro based on endothelial and aortic cells, proving polyphenols have an inhibitory effect on acetylcholinesterase and decrease production of reactive oxygen species. Due to a constantly increasing number of dietary supplements and functional foods containing polyphenolic extracts currently available on the market and to the positive effects of polyphenols resulting from in vitro studies, we consider that more long-term in vivo studies are necessary to demonstrate mechanisms of action and efficacy for proper health claims.

PROTECTIVE EFFECT OF OLAPARIB IN EXPERIMENTAL CROHN'S DISEASE

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The nuclear protein, poly(ADP-ribose) polymerase-1 (PARP-1) is an essential element of the DNA repair system, therefore, inhibitors of the enzyme ($PARP_i$) became increasingly favoured in tumour therapy. Recently, well-considered recommendations were published regarding the re-positioning of $PARP_i$s for non-oncological diseases. Crohn's disease (CD) is an inflammatory disorder of the intestines characterized by epithelial barrier dysfunction and mucosal damage. Focusing on the epithelial barrier integrity and bioenergetics of epithelial cells, we investigated potential of the oral $PARP_i$ olaparib in the 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced mouse colitis model. We determined inflammatory scoring, cytokine levels, colon histology, hematological analysis, and intestinal permeability. Caco-2 monolayer culture was utilized as an epithelial barrier model, on which we used qPCR and light microscopy imaging, and measured impedance-based barrier integrity, FITC-dextran permeability, apoptosis, mitochondrial oxygen consumption rate, and extracellular acidification rate. Olaparib reduced the inflammation score, the concentration of IL-1β and IL-6, enhanced the level of IL-10, and decreased the intestinal permeability in TNBS-colitis. Blood cell ratios, such as lymphocyte to monocyte ratio, platelet to lymphocyte ratio, and neutrophil to lymphocyte ratio were improved. In H_2O_2-treated Caco-2 monolayer, olaparib decreased morphological changes, barrier permeability, and preserved barrier integrity. In oxidative stress, olaparib enhanced glycolysis (extracellular acidification rate), and it improved mitochondrial function (mitochondrial coupling efficiency, maximal respiration, and spare respiratory capacity) in epithelial cells. Based on these results, Olaparib can be repositioned for the therapy of CD.

PHARMACOLOGY OF ENERGY METABOLISM TO REGULATE PLATELET ACTIVATION

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Platelets represent important players in vascular thrombosis, which is a serious clinical concern associated with cardiovascular diseases. Because of this, antiplatelet drugs are widely used to reduce thrombosis. However, some patients with metabolic diseases show platelet hyperreactivity despite the use of antiplatelet drugs, suggesting that platelet reactivity is intrinsically related to altered energy metabolism, an effect that might not be inhibited by current antiplatelet strategies. Therefore, new strategies are needed to limit platelet hyperreactivity, without interference with their basic function. We demonstrated in washed human platelets that carbon monoxide releasing molecule, CORM-A1, inhibits platelet aggregation by inhibiting both mitochondrial respiration, at the level of cytochrome c oxidase, and glycolysis, at the level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), attributed to cytosolic NAD^+ depletion (Kaczara et al. ATVB, 2020;40:2376-2390). Similar effects, but through different molecular mechanisms, can be obtained by 3PO (3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one), a 6-Phosphofructo-2-Kinase/Fructose-2,6-Biphosphatase 3 (PFKFB3) inhibitor, which in platelets inhibits not only glycolysis, but also mitochondrial respiration. Our recent results indicate that the antiplatelet effects of known antiplatelet mediators, such as nitric oxide (NO; delivered by PAPA NONOate; acting *via* the cGMP-dependent mechanism) and carbaprostacyclin ($cPGI_2$; acting *via* the cAMP-dependent mechanism), as well as cangrelor (antiplatelet drug acting *via* the inhibition of platelet P2Y12 receptor), at the concentrations that inhibit platelet aggregation, do not affect platelet energy metabolism. The combinations of CORM-A1 with cangrelor, or 3PO with PAPA NONOate and $cPGI_2$, at concentrations, at which each of them individually only slightly affected platelet aggregation, allowed platelet aggregation to be substantially reduced. Thus, pharmacological regulation of platelet energy metabolism can potentiate antiplatelet effects of drugs.

Acknowledgments: The National Science Centre Poland [OPUS 2021/41/B/NZ7/01426]

OBSTRUCTIVE SLEEP APNEA AND CARDIOVASCULAR COMORBIDITIES MIGHT TAILOR DIFFERENT PATHOGENETIC TREATMENT STRATEGIES

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Obstructive sleep apnea (OSA) is defined as more than 5 stops of breathing during sleep, with an estimated number of 1 billion people being affected worldwide, thus the disease is reported as an epidemic. Also, there is an increasing prevalence of OSA in commercial motor vehicle, estimated t 13–28%. Cardiovascular comorbidities are the most common, such as hypertension, stroke, atrial fibrillation, ischemic heart disease, sudden death. The association beween OSA and obesity leads to a higher risk for the development of diabetes mellitus, and even cancer. Several pathomechanims are triggered by intermittent hypoxia, such as sympathetic activation, cellular and systemic inflammation, oxidative stress, abnormal airway neuromechanical control, increased leptin secretion in obesity, all being responsible for the occurrence and/or progression of cardiovascular co-morbidities in OSA. The extreme risk is linked to the so-called phenotype of very severe sleep apnea (i.e. Apnea Hypopnea Index/AHI ≥ 60). The early diagnosis of these severe cases is paramount. There is an urgent need for the definition of phenotypes, based on polysomnography, clinical and outcome parameters: AHI, excessive daytime somnolence, cognitive impairment, as well as an individualized tratement, the use of CPAP being critical. Accordingly, adequate specialty training and expertise are required with certification in sleep medicine for sleep practitioners. Nowadays, Big Data-driven OSA phenomapping and patient ecosystem pave the road towards a clearer picture of the disease, which will reshape the OSA integrated - "doing better for less" with Big Data analysis using AI and new statistical models. P4 Medicine definition - Predictive, Preventive, Personalized, Participatory, is based on Systems Biology and Systems Medicine, Consumer-Driven Healthcare and Social Networks, Digital Revolution and will result in better diagnoses, targeted therapies, and active participation of patients, including the ones with OSA.

WHAT ARE WE LEARNING FROM GENE EDITING OF HUMAN RyR2 ABOUT CPVT1 ARRHYTHMIA AND CARDIAC EC-COUPLING?

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Cardiac EC-coupling is regulated by calcium entry through L-type Ca^{2+} channels that triggers Ca^{2+} release from the ryanodine receptor (RyR2). While this process is well understood little is known of the role of different RyR2 domains and residues except that mis-sense mutations in over 170 RyR2 residues are associated with arrhythmias. Using CRISPR/Cas9 gene editing in human induced pluripotent stem cells we determined the consequences of mutating several residues in different domains of RyR2 associated with Ca^{2+} binding pocket, FKBP binding domain, carboxylic pore region and have quantified their effects on CICR, the SR Ca^{2+}-leak, and Ca^{2+} signaling. CPVT1-associated mutations in N-terminal R420Q, the mid-section F2483I and carboxylic end Q4201R and S4938F produced aberrant Ca^{2+} releases, more frequent and longer wandering Ca^{2+} sparks, and larger Ca^{2+} leak. Mutation in FKBP binding site (N771D) unexpectedly produced lower Ca^{2+} leak but higher levels of aberrant calcium releases. Mutations in RyR2 Ca^{2+} binding pocket (E3848A and Q3925E) suppressed I_{Ca}- and caffeine-triggered Ca^{2+} releases and CICR. Nevertheless, these cells continued to beat producing arrhythmic calcium transients that triggered no SR calcium release signals and were blocked variably by TRP channels and IP_3 receptors blockers, suggesting EC-coupling remodeling. Mutations in caffeine binding site (W4645R, A4607P) eliminated caffeine-triggered Ca^{2+} release without significantly affecting Ca^{2+} release by 4-CmC, another RyR agonist. We failed to identify mutation-dependent pharmacological specificity in the suppressive effects of dantrolene, JTV compounds, flecanide, even though JTV compounds were most effective in suppressing aberrant Ca^{2+} releases. Mutations in RyR2 Ca^{2+} binding residues reveal remodeling of cardiac calcium signaling pathways when the fundamental pathway of cardiac Ca^{2+} signaling (CICR) is eliminated. It is likely that the heart of patients carrying such mutations may similarly remodel to maintain cardiac contractility and cellular function.

SERCA2a LOSS vs. GAIN OF FUNCTION, WHICH IS WORSE?

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Downregulation of SERCA2a expression/function is an early manifestation of myocardial remodelling, central to the progression of contractile failure and arrhythmogenesis in heart failure. Small-molecule agents selectively increasing SERCA2a function have been recently developed, with the expectation that they may counter HF progression and its consequences. Nonetheless, SERCA2a is physiologically stimulated by cAMP-PKA signalling, which notoriously contributes to remodelling and arrhythmogenesis. This raises the concern that SERCA2a upregulation may be detrimental or, at least, a "double-edged sword". The talk will discuss how and why the effects of selective SERCA2a upregulation diverge from those of cAMP-PKA signalling, their impact on contractile function, electrical stability and cell biology. In "translational" terms, the presentation aims to provide a framework to position SERCA2a activators, an entirely novel class of agents, in the cardiovascular therapy toolkit.

THE ELECTROPHYSIOLOGICAL EFFECT OF ORANGE ALKALOID HESPERETIN IN DOG AND RABBIT CARDIAC PREPARATIONS

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The beneficial anti-inflammatory, antioxidant, anticarcinogen effects of citrus alkaloids are known. However, sporadical reports indicated that citrus flavonoids have inhibitory effects on HERG potassium current. Therefore this raises the possibility that they may cause proarrhythmia in certain situation in certain patients.. Based on these observations the cardiac electrophysiological effects of citrus alkaloids should be investigated. There are reports which showed that flavonoid alkaloids like naringenin and hesperetin are present (in the micromolar range) in the human blood after ingestion of 0,5 or 1 liter/day orange or grapefruit juice. In Xenophus oocytes both naringenin and hesperetin inhibited the HERG potassium current. In addition QTc lengthening was also observed after consumption of orange and grapefruit juice. Therefore in our laboratory we measured the effect of hesperetin on the cardiac ventricular action potential and several transmembrane ionic currents such as the inward rectifier (IK1), transient outward (Ito) and slow and rapid delayed rectifyer (IKs and IKr) transmembrane potassium and late sodium (INaL) and L-type calcium (ICa) inward currents in dog papillary muscle and rabbit and dog ventricular myocytes by the conventional microelectrode and patch clamp techniques. These results showed that hesperetin delayed repolarization in dog papillary muscle and also inhibited these investigated ionic currents particularly or most importantly IKs. Based on the available data in the literature and on our own experimental results it can be suggested that - although the cardiac electrophysiological effects of citrus alkaloids are modest and does not represent significantly enhanced proarrhythmic risk in normal situation, - in certain patients having impaired repolarization reserve ingestion of large quantity of orange or grapefruit should be handled by some caution in order to avoid possible proarrhythmic complication.

IN SILICO TRIALS IN ISCHEMIC HEART DISEASE: THE FUTURE OF DIAGNOSIS AND TREATMENT

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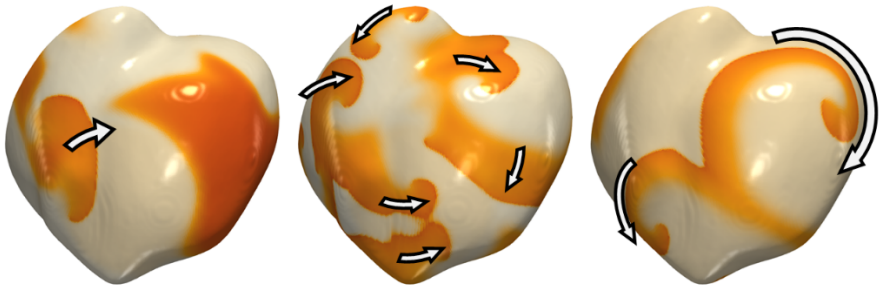
Ischemic heart disease is a major cause of myocardial ischemia, which may lead to ventricular arrhythmias and cardiac arrest. Studies adopting traditional methodological approaches are often limited by ethical, economic, and technical reasons. For instance, human-based research in myocardial ischemia is limited by the prioritisation of the patient's safety. However, modelling and simulation is an attractive alternative and can be applied to conduct human-based *in silico* trials exploring new opportunities in the diagnosis and treatment of ischemic heart disease. For this, we construct and exploit a modelling and simulation framework of human cardiac electrophysiology based on extensive experimental and clinical data, from ion channel kinetics and tissue electrical properties to biventricular function and electrocardiogram. Validation of the models is key for building the credibility of the methods used, ensuring that the results reproduce experimental and clinical findings. The models can be calibrated to reproduce both physiological conditions and pathophysiological mechanisms in cardiac disease, as well as digital twins and populations of models reproducing phenotypes of subpopulations of patients. The use of this technology is very computationally expensive; hence our models are solved in MonoAlg3D, a high-performance computing software deployed on machines equipped with graphics processing units. We provide mechanistic evidence on how variability in size, transmural extent or location of acute myocardial ischemia may lead to marked differences in pro-arrhythmic mechanisms and ECG abnormalities. The results highlight the notable arrhythmic risk arising from silent ischemia, despite a lack of abnormal ECG markers. In addition, our simulations provide evidence of the cardiotoxic side effects caused by sodium blockers in ischemic patients, reproducing results from clinical studies. Once arrhythmia is established, we have assessed the potential of the ECG in the setting of ventricular fibrillation to assess the stability of the arrhythmia for optimising intervention.



Simulated ventricular fibrillation.

ATRIAL FIBRILLATION IN HEART FAILURE AND AGING: UNDERSTANDING THE MECHANISM

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Heart failure (HF) and ageing are key risk factors associated with the development of atrial fibrillation (AF). However, whether HF and ageing share common mechanisms for the development of AF remains to be resolved. In this study, we have used a translationally, relevant large animal model (sheep) of ageing and rapid pacing induced heart failure. Experiments were performed using both in-vivo and cellular electrophysiological approaches. In vivo, we found in both HF and ageing that the onset of AF was often preceded by a period of alternans of the monophasic action potential suggesting alternans can facilitate AF. We investigated the ease by which alternans can be generated in both HF and ageing and found the frequency of stimulation at which alternans first occurred (the alternans threshold) was decreased in both HF and aging. Patch clamp studies showed a similar decrease in the alternans threshold in atrial myocytes from HF and aged sheep. We next sought to determine the mechanism by which alternans occurs in atrial myocytes. In HF sheep atrial myocytes our data suggests that increased levels of calcium in the sarcoplasmic reticulum (SR) coupled with decreased L-type calcium current can promote variation in calcium release. In aged atrial myocytes similar changes to the SR calcium content and L-type calcium current occur suggesting a similar mechanism could facilitate alternans. However, in addition, and unique to ageing, we note an increase in cytosolic calcium buffering which decreases the rate of decay of the calcium transient and we suggest promotes alternans in aged atrial myocytes. In conclusion, we suggest in HF and in ageing an increased susceptibility to alternans can facilitate AF. Some factors promoting alternans are common to both HF and ageing however increased calcium buffering is an additional factor, promoting the development of alternatives in ageing.

INTEROCEPTIVE INHIBITION OF ADAMTS1 AND ANGIOGENIC STRATEGY FOR PREVENTION OF HFrEF

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Previous studies from our laboratory has demonstrated contribution by interoception i.e., by other organs (skeletal muscle and renal) to heart failure with preserved ejection fraction (HFpEF). Here we determined that exercise also preserved EF by releasing myokines that help preserving EF by releasing exosome and interoception. To test this, we created HFpEF by AVF w/o exercises. The blood exosome was analyzed for myokines. The results suggested that the cardiac angiogenic HIF1, VEGF, FGF, TGFb, TMPRSS2 and signaling PAR1, FAK, PKB, MMP-2/TIMP-2, and TIMP-4 levels are preserved during HFpEF and reduced during HFrEF. Instead, anti-angiogenic ADAMTS1, MMP9, myostatin, parstatin, endostatin and angiostatin levels are increased during HFrEF. The exercise and/or the musclin induces the angiogenic HIF1, VEGF, FGF, TGFb, TMPRSS2 and signaling PAR1, FAK, PKB, MMP-2/TIMP-2, and TIMP-4 levels during HFpEF. Instead, anti-angiogenic ADAMTS1, MMP9, myostatin, parstatin, endostatin and angiostatin levels are decreased during HFrEF. These results suggested the interoceptive inhibition of ADAMTS1 and angiogenic strategy for prevention of HFrEF.

NEW TRENDS IN THE MANAGEMENT OF ADVANCED HEART FAILURE

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Advanced heart failure (HF) represents a distinct entity at the end-spectrum of HF. Overall, 5-10% of HF patients progress to advanced HF, characterised by persistent symptoms despite maximal therapy. The prevalence of advanced HF is increasing due to the growing number of patients with HF, an aging population, and better treatment for and survival of HF. Prognosis remains poor, with 1-year mortality ranging from 25% to 75%. The current presentation is centered on the management of advanced HF based on the criteria developed by Heart Failure Association - European Society of Cardiology and on the importance of early and appropriate referrals to advanced HF centers, where durable therapies such as left ventricular assist devices (LVAD) or heart transplantation (HT), can be provided.

FROM LEFT ATRIAL STRETCH TO HEART FAILURE

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Left atrium (LA) is a muscular chamber with a essential role in passage and contractile function involved in left ventricular (LV) filling. Reservoir function of LA during LV systole plays an important role in pathogenesis of atrial fibrillation (AF). During LV filling, LA is directly "exposed" to LV diastolic pressure (during mitral valve opening). The LA dilatation is a consequence of elevated LV end diastolic pressures and is in direct relation with AF, the most common arrhythmia. AF is not a benign condition and has a significant role of ischemic stroke, heart failure and cardiovascular mortality. Overload in pressure or volume induces changes in length in entire segments of atrial myocardium, thus altering the local action potential and electrical properties. Changes in pressure overtime may lead to stretch and geometrical restructure. LA dilatation is mainly not equal, non-symmetrical and the thickness of the LA wall is neither constant nor at the same density concerning fibrosis. Complete characterization of LA remodeling should include shape definition. LA volume offers additional information concerning differentiation between normal and pseudonormal mitral inflow. Increased LA size is associated with increased wall tension due to increased filling pressure and relates to adverse cardiovascular outcomes. Increased filling volumes can lead to an increase in LA size, but the adverse outcomes associated with increased dimension and volume are more strongly associated with increased filling pressure. Increased left atrial size is related to the incidence of atrial fibrillation and stroke. LA enlargement is a marker of diastolic dysfunction and magnitude of LA pressure, and LV end diastolic pressure elevation.

REPARATIVE/REGENERATIVE CARDIOLOGY: HYPE VS. REALITY

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Two decades and >200 trials have shown that adult stem/progenitor cells are safe, and several Phase I/II studies suggest that they are efficacious in heart failure and refractory angina, although large Phase III trials are needed to establish efficacy conclusively. In contrast, embryonic stem cells have never been tested in a controlled trial in cardiovascular medicine and are plagued by major problems, including ethical concerns, arrhythmias, tumorigenicity, rejection requiring immunosuppression, genomic instability, prohibitive dose requirements, phenotypic heterogeneity, and lack of long-term engraftment. The most reasonable interpretation of current data is that embryonic stem cell-based therapies are not likely to have clinical application for heart disease. At the preclinical level, there is overwhelming evidence that various stem/progenitor cells can improve cardiac function after myocardial infarction. The mechanism remains unclear but it does not involve regeneration of new myocytes because all stem/progenitor cells fail to engraft in the heart and disappear quickly after transplantation. Therefore, transplanted cells must work via secretion of factors that act in a paracrine fashion. The use of repeated dosing is particularly important because mounting evidence shows that the efficacy of cell therapy is underestimated if only one dose is used. In the clinical arena, it seems unlikely that cell therapy will prove beneficial in patients with STEMI; instead, heart failure and refractory angina appear to be the main target populations. The use of the intravenous route for cell delivery may fundamentally transform the field of cell therapy. We are at a pivotal stage in the field in which information from many relatively small clinical trials must guide carefully executed efficacy trials. This lecture critically examines the current state of clinical research on cell-based therapies for cardiovascular disease, highlighting the controversies in the field, improvements in clinical trial design, and the application of exciting new cell products.

DIETARY FLAXSEED MODULATES NON-ALCOHOLIC FATTY LIVER DISEASE

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The most prevalent chronic liver disease globally is non-alcoholic fatty liver disease (NAFLD). Obesity, insulin resistance and metabolic syndrome are the most commonly recognized risk factors for NAFLD. With increasing levels of obesity and diabetes expected to increase in the years to come, the incidence of NAFLD is expected to rise. The purpose of the study was to investigate the potential for dietary flaxseed to alleviate symptoms of non-alcoholic fatty liver disease in an animal model, the JCR:LA-corpulent rat. The JCR:LA corpulent rat exhibits almost all of the same metabolic indicators as NAFLD and could be an ideal animal model for NAFLD. Both male and female rats were studied in their homozygous obese form and with their lean genetic counterparts after 12 weeks of ingestion of a control diet, or a diet supplemented with flaxseed, or high fat/high sucrose (HFHS), or HFHS plus flaxseed. Cholesterol, triglyceride, saturated fatty acid levels were elevated in the obese rats and were even higher in rats on a HFHS diet. The HFHS diet also induced a significant two fold elevation in the plasma levels of both aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the obese male and female rats. This is indicative of liver injury consistent with NAFLD. Including flaxseed in the HFHS diet significantly depressed the plasma levels of both AST and ALT in the obese male rats and also reduced hepatic cholesterol and triglyceride content as well as improving the fatty acid profile. In summary, including flaxseed in the diet of male and female obese rats led to an improved lipid composition in the liver and significantly reduced biomarkers of tissue injury despite consuming a HFHS chow.

Supported by CIHR, SaskFlax, ARDI, the Western Grain Research Foundation and St Boniface Hospital Foundation.

IN VITRO REPROGRAMMING OF FIBROBLASTS FROM HUMAN CADAVERS FOR iPSCs GENERATION AND iPSC-CARDIOMYOCYTES DERIVATION

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Sudden cardiac death (SCD) represents a significant proportion of deaths in the young. Postmortem pathological, toxicological, and forensic examination is the existing gold standard procedure to gain any insight into the cause of SCD. Unfortunately, these evaluations lack any functional readout from cadaver samples, which otherwise could be used to better understand the underlying cause of SCD. We sought to establish a methodology for harvesting viable cells from cadaver biopsies, even 3-5 days post-mortem, and to reprogram them into induced pluripotent stem cells (iPSCs) with further differentiation into cardiomyocytes. For this purpose, we harvested Achilles tendons from 7 human cadavers aged below 40 and successfully outgrew viable fibroblasts. The fibroblasts were reprogrammed into cadaver-derived iPSCs (CdiPSCs) and successfully differentiated into cardiomyocytes (CdiPSC-CMs). The applied approach to reprogram fibroblasts from human cadavers, even days after death, was highly efficient (6 out of 7) producing patient-specific live pluripotent stem cells available post-mortem. Hence, this study proves that an electrophysiology and biochemical foot-print of cardiac myocytes from deceased person can be obtained, thereby providing new valuable auxilium to molecular autopsy for the investigation of the mechanisms underlying SCD.

IMMUNO-ENGINEERING APPROACHES FOR CARDIAC REGENERATIVE MEDICINE

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Donor derived allogeneic stem cells including mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSCs) are being tested in animal studies and clinical trials for cardiac regeneration and repair. The outcome of initial studies was very encouraging and transplanted cells were safe in the recipient heart. However, poor survival of transplanted stem cells in the infarcted heart has impaired the clinical translation of stem cells-based therapies. We have performed investigations to understand the mechanisms of poor survival of implanted stem cells in the heart. We found that allogeneic stem cells after transplantation in the ischemic heart turned immunogenic and were subsequently rejected by host immune system. In our ongoing studies we are focusing on understanding the mechanisms of increase in immunogenicity of allogeneic stem cells. We are also developing biomaterials-based strategies to prevent rejection of transplanted cells in the heart. We synthesized and characterized MXene quantum dots (MQDs). MQDs possess intrinsic immunomodulatory properties and selectively reduce activation of $CD4^{+}IFN\text{-}\gamma^{+}$ T-lymphocytes and promote expansion of immunosuppressive $CD4^{+}CD25^{+}FoxP3^{+}$ regulatory T-cells in an activated lymphocyte population. We also incorporated MQDs into a chitosan-based hydrogel to create a 3D platform for stem cell delivery to the heart. This composite immunomodulatory hydrogel-based platform improved survival of stem cells and mitigated allo-immune responses. We also found that MQDs have potential to mitigate allograft vasculopathy and prevent rejection of transplanted organs. These studies highlight the potential of MXene based next generation biomaterials for cardiac regenerative medicine.

THE ROLE OF NITRIC OXIDE IN METABOLIC SYNDROME: EFFECTS OF POLYPHENOLIC SUBSTANCES

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Nitric oxide (NO) plays a crucial role in the pathogeneses of metabolic syndrome components and is involved in blood pressure control, insulin production, or lipid profile regulation. We aimed to determine NO synthase (NOS) activity under conditions of different lipid profiles. In our studies, normotensive Wistar Kyoto rats (WKY), spontaneously hypertensive rats (SHR), obese SHR (SHR/cp), lean and obese Zucker rats have been used. In 12-week-old male rats, the lipid profile in plasma and NOS activity in the left ventricle and aorta have been determined. Simultaneously, we studied the effects of different polyphenolic substances.
We demonstrated that WKY and SHR have the same level of total cholesterol, triglycerides, high density lipoprotein (HDL) and low density lipoprotein (LDL). In SHR/cp, however, the level of total cholesterol, triglycerides, LDL, but also HDL increased significantly. Lean Zucker rats have a similar lipid profile to WKY. However, obese Zucker rats have all investigated lipid parameters significantly higher than SHR/cp. NOS activity was significantly higher in the left ventricle and aorta of SHR compared to WKY. In SHR/cp, however, NOS activity was comparable to that in WKY. This indicates that obesity reduces NOS activity in spontaneous hypertension. Lean and obese Zucker rats had comparable NOS activity in the heart, but it was significantly reduced in the aorta of obese Zucker rats. In accordance with these findings, polyphenol rich natural compounds like *Lonicera caerulea L.* and cornelian cherry varieties, Koralovij Marka and Wilde Type, increased NOS activity in the aorta, while not affecting the activity in the left ventricle. Likewise, the polyphenol rich wine extract had a moderate effect on NOS depending on the level of NOS activity in the studied models of metabolic syndrome. In conclusion, deteriorated lipid profile may reduce NOS activity, while natural polyphenolic substances can moderate it through different signaling pathways.

This study was supported by the national grant agency VEGA 2/0025/23 and 1/0048/23.

HIGH FRUCTOSE DIET INDUCES CARDIOVASCULAR DYSFUNCTION

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Background. Obesity is a global epidemic and a major health hazard. It is well-recognized to contribute to an increase in the risk of cardiovascular disease. Unhealthy dietary practices affect human health and excessive fructose intake have been linked to obesity as well as associated cardiac dysfunction.
Hypothesis. Long-term consumption of a high fructose (HF) diet induces cardiac abnormalities.
Methods/Results. Preclinical investigations, conducted in male SD rats fed a HF (60% w/w) diet plus a moderate level of saturated fat (10% w/w lard) for up to 20 weeks, revealed that systolic blood pressure, and serum levels of insulin, triglycerides and total cholesterol were significantly increased in the HF fed group. In addition, an increase in visceral fat mass and reduced levels of the anti-inflammatory, and anti-atherosclerotic cytokine, IL-10 were observed. Others have demonstrated an increase in cardiomyocytes size, LV wall thickness, and reduced ejection fraction and fractional shortening subsequent to high-fructose feeding of mice for 12 weeks.
Conclusion. Long-term consumption of HF can lead to cardiovascular dysfunction. Thus, an effective intervention to reduce HF intake may attenuate the risk for obesity-related morbidities.

OBESITY, METABOLIC/BARIATRIC SURGERY AND CARDIOVASCULAR DISEASE

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Obesity is a chronic relapsing and progressive disease and one of the most important concerns worldwide due to the associated morbidity and mortality. It is directly linked to cardiovascular risk factors such as type 2 diabetes, atherogenic dyslipidemia hypertension and sleep disorders. Moreover, obesity leads to the development of cardiovascular disease i.e. myocardial infarction, stroke, and heart failure that is a responsible for reduced quality of life and death, therefore making early detection of cardiovascular risk mandatory. Weight loss has been shown to have positive effects on cardiovascular risk factors such as type 2 diabetes, dyslipidemia and hypertension. Post-hoc analyses of the Look AHEAD trial showed that a 10% weight loss through lifestyle interventions induced a 20% reduction of cardiovascular disease risk in patients with type 2 diabetes and high body mass index. However, to maintain a 5-10% decrease of body weight can be a real challenge in many cases. In order to obtain/maintain weight loss, in some patients, pharmacological or metabolic/bariatric surgery treatment is recommended. A very recent study showed that subcutaneous once-weekly semaglutide was associated with 20% reduction in major adverse cardiovascular events in overweight and obese patients with cardiovascular disease without diabetes compared with placebo. Metabolic/bariatric surgery has been shown to induce significant weight loss and a lower cardiovascular risk. Interestingly, one study has demonstrated the minimum needed weight loss in order to attain reduced risk of major adverse cardiovascular events in patients with obesity and diabetes to be 10% for the metabolic surgery group and 20% for the nonsurgical group. The relationship between obesity, type 2 diabetes, cardiovascular disease and metabolic surgery remains entangled as patients with established heart disease and diabetes that undergo metabolic surgery seem to benefit more in terms of major adverse cardiovascular events risk and mortality reduction as compared to patients without type 2 diabetes.

EPICARDIAL ADIPOSE TISSUE THICKNESS AS INDEPENDENT PREDICTOR IN CORONARY ARTERY DISEASE: ASSESSMENT BY ECHOCARDIOGRAPHY

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Background & Aim: Epicardial adipose tissue (EAT), the visceral fat depot of the heart, may play an unfavorable activity through production and secretion of proinflammatory and proatherogenic mediators, and is associated with atherosclerotic diseases, such as coronary artery disease (CAD). The present study aimed to investigate the relationship of EAT thickness measured by echocardiography with the epicardial tissue oxidative stress level in CAD patients.
Materials & Methods: This study included a total of 25 patients referred for cardiac surgery (n = 14 in the CAD group and n =11 in the control group, which included valvular patients without documented CAD. At the time of enrolment, biochemical blood analyses and echocardiographic examinations were performed. EAT was measured perpendicularly on the free wall of the right ventricle at the end of systole in three cardiac cycles using a parasternal long or parasternal short axis view. Epicardial fat was sampled from patients subjected to open-heart surgery once cardiopulmonary bypass was established. Level of oxidative stress in the EAT was assessed by confocal microscopy (dihydroethidium staining) and spectrophotometry (ferrous oxidation xylenol orange method).
Results: There was no statistically significant difference between the two groups *in* terms of age, sex, body mass index, renal function, cardiovascular risk factors (diabetes, LDL level, hypertension, smoking, heredity), early mitral filling velocity/early diastolic mitral annular velocity ratio, left ventricular ejection fraction, global longitudinal strain. EAT was higher in the CAD group compared to the control group (8.15±2.09 mm vs. 5.12±1.8 mm, p=0.001). The epicardial ROS level was higher in the CAD group compared to the control group (21.4±2.47 vs. 15.7±1.55, p<0.001).
Conclusion: EAT may cause an increased risk of cardiovascular diseases by leading to increased oxidative stress in patients with CAD.

CYTOPROTECTIVE ROLE OF TRAF2 IN TNFα MEDIATED DOXORUBICIN-CARDIOMYOPATHY

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Background & Aim: Cytokines such as TNFα have been implicated in cardiac dysfunction and toxicity associated with doxorubicin (DOX). While TNFα can elicit different cellular responses including survival or death, the mechanisms underlying these divergent outcomes in the heart remains cryptic. The E3 ubiquitin ligase TRAF2 provides a critical signaling platform for K63 - linked polyubiquitination of RIPK1, crucial for NF-κB activation by TNFα and survival. Herein, we investigate TRAF2 signaling in the pathogenesis of DOX cardiotoxicity.
Materials & Methods: Using a combination of *in vivo* (4 weekly injections of DOX (5mg/kg/week) in cardiac-myocyte restricted expression of AAV9-GFP and AAV9-TRAF2 mice (C57/BL6J), and in vitro approaches, we monitored TNFα levels, LDH, cardiac ultrastructure and function, mitochondrial bioenergetics and cardiac cell viability.
Results: In contrast to vehicle treated mice, ultrastructural defects including cytoplasmic swelling, mitochondrial perturbations, and elevated TNFα levels were observed in the hearts of mice treated with DOX. While investigating the involvement of TNFα in DOX cardiotoxicity, we discovered that in the absence of DOX, NF-κB was readily activated by TNFα. However, TNFα-mediated NF-κB activation was impaired in cardiac myocytes treated with DOX. This coincided with loss of K63- linked poly-ubiquitination of RIPK1, attributed to the proteasomal degradation of TRAF2. Further, TRAF2 protein abundance was markedly reduced in hearts of cancer patients treated with DOX. Impaired TRAF2 signaling resulted in mitochondrial perturbations, including disrupted bioenergetics, loss of membrane potential and permeability transition pore opening. We further established that the reciprocal actions of the ubiquitinating and de-ubiquitinating enzymes c-IAP1 and USP19 regulated the proteasomal degradation of TRAF2. An E3 ligase mutant of c-IAP1(c-IAP1 H588A) or gain of function of USP19, prevented proteasomal degradation of TRAF2 and DOX -induced cell death. Further, wild type TRAF2 but not a RING finger mutant defective for K63 linked polyubiquitination of RIPK1, restored NF-κB signaling and suppressed DOX-induced cardiac cell death. Finally, cardiomyocyte-restricted expression of TRAF2 (AAV9-TRAF2) *in vivo* protected against mitochondrial defects and cardiac dysfunction induced by DOX**.**
Conclusions: Our findings reveal a novel signaling axis that functionally connects the cardiotoxic effects of DOX to proteasomal degradation of TRAF2. Disruption of the critical TRAF2 survival pathway by DOX, sensitizes cardiac myocytes to TNFα mediated necrotic cell death.

IMMUNE CHECKPOINT INHIBITORS-RELATED MYOCARDITIS

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Immune Checkpoint Inhibitors (ICI) have revolutionized solid cancer treatment in the last 10 years since FDA and EMA approval of ipilimumab, a monoclonal antibody (mAb) that targets Cytotoxic T-lymphocyte antigen 4 (CTLA-4), and the first two mAbs that target Programmed death 1 (PD-1, pembrolizumab and nivolumab). Six other ICI have since been approved in various types of solid cancers and, more recently, in Hodgkin lymphomas. These progresses were made possible by the understanding of the complex interactions regulating the immune system, notably its effectors cells, T and NK cells. Their activity is modulated by activating and attenuating receptors and ligands. Notably, inhibitory mechanisms involving CTLA-4, PD-1 and its ligand, PD-L1, are crucial in healthy patients to prevent autoimmunity. ICI drugs antagonize these inhibitory mechanisms allowing therefore patient's immune system to engage and combat cancer by enhancing T-cell responses. However, very rapidly, even before market authorization for ipilimumab, immune-related adverse events (IRAE) were reported such as rash, colitis, hepatitis. But the major wake-up call regarding the potential severity of these IRAE was in 2016 the publication of two cases of patients with melanoma who developed fatal fulminant myocarditis. In both cases myocarditis was associated with myositis, with the presence of T-cells and macrophages infiltrates. Since, hundreds of articles tried to describe the features of ICI related myocarditis (very heterogeneous in its presentation), its diagnosis (difficult), its prognostic factors (rarely validated, the most solid one being the combination of ICI) and treatment. Despite several Societies' recommendations, treatment of ICI-related myocarditis is still extrapolated from other non-ICI related toxicities and remains a challenge. In this presentation I will try to give an overview of the current knowledge regarding the ICI related myocarditis.

EXOSOMES AMELIORATES DOXORUBICIN-INDUCED CARDIOMYOPATHY

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Doxorubicin (Dox) is an effective chemotherapeutic drug used to treat various cancers. Unfortunately, use of Dox is limited due to serious side effects including cardiomyopathy. Dox-induced cardiomyopathy (DIC) occurs due to enhanced oxidative stress, inflammation, and excessive apoptosis and necrosis. However, whether DOX induces inflammation induced cardiomyopathy is not established. Moreover, the role of embryonic stem (ES) cells derived exosomes in the inhibition of DIC is not established. We will present data to understand the inflammation associated cellular mechanisms that causes cardiac fibrosis and hypertrophy following DOX treatment. We will also present data on inflammation mediated cell death pyroptosis. Role of inflammatory infiltrated cell types will also be established in DIC. Exosomes data will be presented to understand its protective effects on inflammation, pyroptosis and the fate of infiltrated cell types. Furthermore, effects of exosomes on cardiac remodeling and cardiac function will be discussed.

ANTIOXIDATIVE CAPACITY OF POMEGRANATE PEEL EXTRACTS: RESULTS OF IN VITRO AND IN VIVO STUDIES

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Background & Aim: Since numerous beneficial effects of pomegranate can be explained by its antioxidative effects, the antioxidative potential of pomegranate peel extract (PoPEx) was investigated *in vitro* and also in the isoprenaline (ISO) cardiac injury model *in vivo*.
Materials & Methods: The *in vitro* experiments were caried out in a battery of antioxidative assays, including 2,2-diphenyl-1-picrylhydrazyl assay (DPPH), 2,2'-azino-bis(3-ethylbenzothioazoline-6-sulphonic acid assay (ABTS), iron(III)-2,4,6-tripyridyl-S-triazine complex assay (FRAP), reduction of copper(II) ions assay (CUPRAC), Briggs-Rauscher oscillatory reactions, neutralization of OH radicals and lipid peroxidation assay. For the purposes of the *in vivo* expreriments, PoPEx was dissolved in 0.5 % Na carboxymethyl cellulose (CMC) and administered to Wistar rats by gavage, on 7 consecutive days, in one of three doses: 50, 100 or 200 mg/kg. The ISO myocardial injury *in vivo* was induced by two subcutaneous (sc) injections of isoprenaline 85 mg/kg, 24 h apart. The control group of animals received by gavage CMC over 7 days and saline sc on days 6 and 7, three groups received one of the three doses of PoPEx over 7 days and saline sc on days 6 and 7, one group was treated with CMC orally over 7 days and ISO on days 6 and 7, while the additional three groups received either of the three doses of PoPEx as a pretreatment over 7 days and ISO on days 6 and 7. Animals were anaesthetised and euthanised and their hearts, erythrocytes and plasma used for the determination of prooxidative and antioxidative parameters. **Results:** In all the *in vitro* tests, PoPEx exerted strong antioxidative properties. *In vivo*, increasing doses of PoPEx exerted a dose-dependent decrease in thiobarbituric acid reactive substances (TBARS) and increase in levels of the antioxidative molecules, such as catalase (CAT), superoxide dismutase (SOD and reduced glutathione (GSH). ISO caused a strong increase in the prooxidative parameters, such as TBARS in heart homogenates and TBARS, superoxide anion radical (O_2^-) and nitrite (NO_2^-) in plasma. Pre-treatment with PoPEx resulted in a dose-dependent decrease in TBARS and NO_2^- and increase in CAT, SOD and GSH levels. **Conclusion:** PoPEx exerts clear antioxidative effect *in vitro* and a dose-dependent antioxidative effect in the *in vivo* model of ISO myocardial injury. **Key words**: pomegranate, isoprenaline, myocardial injury, oxygen free radicals, antioxidative capacity

GREEN TEAS EXTRACT IMPROVES VASODILATING EFFECT OF INSULIN ON RESISTANCE ARTERIES IN RATS WITH THE METABOLIC SYNDROME BY CONVERTING H_2S TO POLYSULFIDES

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Insulin induces endothelium-dependent vasorelaxation mediated by nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF), and this effect is impaired in obesity/metabolic syndrome. Endogenous hydrogen sulfide (H_2S) serves as the EDHF in some vascular beds. Oxidized products of H_2S such as polysulfides (H_2S_n) hyperpolarize endothelial cells by activating small and intermediate-conductance Ca^{2+}-activated K^+ channels (SK_{Ca} and IK_{Ca}). Green tea polyphenols have beneficial vascular and metabolic effects but the underlying mechanism is incompletely understood. The aim of this study was to examine the effect of green tea extract (GTE) on vasodilating effect of insulin in rats with fructose-induced metabolic syndrome. Male Wistar rat were fed high-fructose diet for 8 weeks and some animals received GTE (10 mg/kg body weight) for the last 2 weeks. Vascular tome was measured by wire myography in isolated 2nd or 3rd order mesenteric arteries. Fructose induced hypertriglyceridemia, increased HOMA index and induced blood pressure elevation. GTE had no effect on triglyceride level and HOMA but decreased blood pressure in fructose-fed rats. Insulin induced relaxation of mesenteric artery rings and this effect was attenuated by either NO synthase inhibitor, L-NAME, or EDHF inhibitors, apamin and TRAM-34. Both NO- and EDHF-dependent components of insulin-induced vasorelaxation were impaired in fructose-fed rats. The effect of insulin on phosphorylation of insulin receptor beta subunit (IRβ), insulin receptor substrate-1 (IRS-1) and protein kinase Akt were impaired in fructose fed rats. Insulin stimulated NO and H_2S production as well as induced hyperpolarization of endothelial cells and these effects were impaired in fructose-fed rats. GTE had no effect on insulin-induced phosphorylation of IRβ, IRS-1 and Akt however, reduced the amount of H_2S and increased the amount of H_2S_n produced by endothelial cells and improved hyperpolarization. In conclusion, metabolic syndrome is associated with insulin resistance of endothelial cells associated with the impairment of both NO and H_2S pathways. GTE treatment has no effect on insulin sensitivity but improves H_2S-EDHF signaling in endothelial cells by converting hydrogen sulfide to polysulfides. The results shed new light on the mechanism of beneficial vascular effect of green tea polyphenols in the metabolic syndrome.

CARDIOPROTECTIVE EFFECTS OF MAHONIA AQUIFOLIUM EXTRACTS

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Plants provide potential health benefits in cardiovascular diseases. Traditional phytotherapy indicates that many plant products have cardioprotective effects in the primary and secondary prevention of coronary heart disease, but the mechanisms were unknown. Lately the mechanism of cardioprotection in the prevention of cardiovascular disorders have been analyzed in many experimental studies. Because the role of oxidative stress in promoting cardiovascular diseases were widely recognized, they became therapeutic targets. *M. aquifolium* was widely used in Traditional Chinese Medicine as well as in North America for many health and medicinal benefice activities, such as anti-inflammatory, antioxidant, antibacterial and antifungal. We tested the prevention effects of *Mahonia aquifolium* green fruits, ripe fruits, leaves, bark and flowers ethanolic extracts in isoprenaline-induced acute myocardial infarction (AMI) at rats. *M. aquifolium* green fruits, ripe fruits, leaves, bark and flowers extracts are rich in antioxidant compounds, mainly alkaloids and flavonoids. The ethanolic extracts were administered orally for seven days prior to AMI. After AMI induction myocardial injury marker enzymes alanine transaminase, aspartate transaminase, and creatine kinase-MB were evaluated, and an electrocardiogram was registered. The oxidative stress was evaluated by total oxidative status, total antioxidant reactivity, oxidative stress index, nitric oxide, 3-nitrotyrosine, malondialdehyde, gamma-glutamyl transferase and total thiols. The tested ethanolic extracts of *M. aquifolium* have a cardioprotective effect by reducing oxidants release and by increasing the antioxidants after AMI induction. Leaves and bark extracts were the most efficient extracts. Taken together, these findings suggest that the pre-treatment with *M. aquifolium* ethanolic extracts has a cardioprotective effect by reducing oxidative stress during AMI. These results can recommend *M. Aquifolium* as a functional cardioprotective food in all populations who have high prevalence of cardiovascular diseases.

THE ELECTROTONIC MODULATION IN MIXED-MODE ELECTRICAL CONDUCTION CAN BE A NOVEL APPROACH FOR SHORT QT-CHARACTERIZED CARDIAC REMODELING

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Short QT-interval induction causes irregular heart rhythms further leading to cardiac arrest. Considering the contributions of macrophage to myofibroblast transition, together with macrophage coupling with cardiomyocytes via Cx43, by hypothesizing the involvement of non-myocyte-associated passive electrical contributions in cardiac remodeling, we examined whether they can contribute to cardiac remodeling of early-stage MetS rats characterized by a short QT-interval. Action potentials (APs) of isolated papillary muscles, and L-type Ca^{2+}-channel currents (LTCC) and K^+-channel currents (I_K) in ventricular cardiomyocytes were studied in the MetS rat hearts fed with high-sucrose for 14-16 weeks (named as early-stage) compared with age-matched controls. The distributions of $Na_V1.5$ Na^+-channels and K^+-channesl as well as the distributions of non-myocytes such as myofibroblasts and macrophages in the intact hearts were evaluated by using immunohistochemistry and immunofluorescence techniques by light and confocal microscopy. The increased heart rate, shorten QT- and PR-intervals, and significantly high response to sympathetic stimulation were determined in the MetS rats. In this group, there was a slightly dense distribution of collagen I-III in the extracellular matrix and increased numbers of positive cells stained with either α-SMA or CD68 in interfibrillar spaces of the hearts with slightly developed fibrosis. Furthermore, the phosphorylated Cx43 was prominently localized on the longitudinal cell membrane while the Cx43 was on the intercalated discs of the MetS hearts. Moreover, we determined a significantly shortened AP duration with depolarized resting membrane potential in the papillary muscle strips of the MetS rats. These data were further supported by a significantly increased density of $Na_V1.5$ on the membranes and depressed LTCC with no change in total I_K. Considering the electrotonic contribution to intercellular propagation of APs in the heart via increases in the numbers of non-myocytes together with changes in phosphorylation and localization of Cx43, we, for the first time, demonstrated that electrical remodeling in early-stage MetS heart is characterized by a short QT-interval with contributions of electrotonic coupling of excitable and non-excitable cells as part of the causal pathway, leading to activation of ephaptic-coupling by mixed-mode conduction in the heart which seems to be a preconditioning stimulus for the development of long QT-interval in chronic condition.

THE COMPARATIVE STUDY OF THE RAPID (I_{Kr}) AND SLOW (I_{Ks}) DELAYED RECTIFIER POTASSIUM CURRENTS IN UNDISEASED HUMAN, DOG, RABBIT AND GUINEA PIG CARDIAC VENTRICULAR PREPARATIONS

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Background & Aim: The purpose of the study was to investigate and compare the properties of the of the rapid (I_{Kr}) and the slow (I_{Ks}) components of the delayed rectifier potassium currents in order to understand the large inter-species variation in the drug effect on repolarization. **Materials & Methods:** Standard microelectrode and whole-cell configuration of the patch-clamp techniques were applied in myocytes isolated from undiseased human donor (HM), dog (DM), rabbit (RM) and guinea pig (GM) ventricles at 37 °C.
Results: The amplitude of the E-4031 sensitive I_{Kr} tail current measured at -40 mV after, a 1 s long test pulse to 20 mV, was very similar in HM and DM but larger in RM and GM. The L-735,821 sensitive I_{Ks} tail current was considerably larger in GM than in RM and DM. In HM I_{Ks} tail was even smaller than in DM. I_{Kr} activated rapidly and monoexponentially in each studied species. The deactivation of I_{Kr} in HM, DM and RM measured at -40 mV, after a pulse to 30 mV was slow and biexponential, while in GM the I_{Kr} tail current was best fitted triexponentially. I_{Ks} measured at 30 mV, activated slowly and had apparently a monoxponential time course in HM, DM and RM. In contrast, in GM the activation was clearly biexponential. In HM, DM and RM I_{Ks} deactivation measured at -40 mV, was fast and monoexponential, while in GM, in addition to the fast component an another slower component was also revealed.
Conclusion: These results suggest that I_K in HM resembles that measured in DM and RM, and considerably differs from that observed in GM. These findings suggest that the dog and rabbit are more appropriate species than the guinea pig for preclinical evaluation of new potential drugs expected to affect cardiac repolarization.

THE DEVELOPMENT OF AORTIC WALL ANEURYSMS: INSIGHTS INTO MOLECULAR AND CELLULAR MECHANISMS

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Aortic aneurysms are a prevalent medical condition that can affect various segments of the aorta, and they commonly manifest in either the thoracic or abdominal regions, with abdominal cases being more frequent. Despite certain shared characteristics, thoracic and abdominal aortic aneurysms are distinct medical conditions. In this presentation, we have conducted an extensive examination of the diverse mechanisms contributing to the development of aortic aneurysms and have explored various treatment approaches. Aortic wall aneurysms often progress asymptomatically over an extended period. However, this gradual expansion of the aneurysm can culminate in a life-threatening complication: aortic rupture. Given the limited therapeutic options available to delay or prevent the emergence of acute aortic syndromes, surgical intervention remains the predominant method of treatment. While significant advancements in surgical techniques have been made in recent years, leading to less invasive and more refined procedures, the associated morbidity and mortality rates remain elevated. Uncovering the intricate cellular and molecular networks responsible for initiating aneurysm formation holds the promise of identifying novel therapeutic targets. The importance of molecular and cellular mechanisms in the complex pathogenesis of aortic aneurysms is steadily growing. Future investigations should aim to bridge the gap between observations in human tissue and animal models of aortic aneurysms. This comparative approach will enable the derivation of clinically relevant insights into aortic aneurysm formation and the potential pharmacological interventions that could block pathogenic pathways. In summary, aortic aneurysms are a widespread medical issue with distinct thoracic and abdominal forms. While surgical treatments have improved, they still carry substantial risks. Advancing our understanding of the molecular and cellular underpinnings of aneurysm development is critical for the development of more effective therapies. Comparative research between human and animal models will be instrumental in achieving this goal.

A ONE HEALTH APPROACH TO CARDIOVASCULAR RESEARCH – WHAT CAN DOGS TEACH US ABOUT HEART DISEASE?

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It is well known that cardiovascular diseases are the leading global cause of mortality in people. However, the impact of heart disease on canine morbidity and mortality is perhaps less well recognised. Heart disease is common, affecting ~10% of all dogs. As the limitations of studying experimentally induced disease in animals are increasingly recognised, using naturally occurring animal models that more accurately recapitulate human disease is becoming increasingly attractive. Selective breeding has resulted in a huge variety of dog breeds. Although some breeds retain a high degree of genetic diversity, especially working breeds (e.g. Labrador retrievers), in others, selective breeding has resulted in genetic bottlenecks and the inadvertent "trapping" of disease-causing genes. As a result, breed predispositions exist for cardiac diseases. The most extreme example is the Cavalier King Charles spaniel, which has a lifetime prevalence of myxomatous mitral valve disease (MMVD; analogous to Barlow's syndrome in people) of 100%. Another striking example is the lifetime prevalence of dilated cardiomyopathy in Dobermann pinschers of 58%. Although dogs do not develop atherosclerosis (in the absence of severe hypothyroidism), regardless of diet, and therefore do not represent a naturally occurring model of coronary artery disease, there is a naturally occurring canine analogue to most other common congenital and acquired cardiac diseases.
My research focuses on the epidemiology of canine MMVD, specifically on the genetics of the disease and the contribution of inflammation and endothelial dysfunction to disease progression. Surgical and interventional mitral valve repair is performed in dogs but is not widely accessible (due to cost and lack of availability). This allows us to study the disease course in patients in which surgery is not performed, which would be unethical in human patients, allowing new markers of prognosis and disease progression to be identified and evaluated, for translation to human patients.

THE EFFECTS OF DIFFERENT EXERCISE TYPES ON A RAT MODEL OF MYOCARDIAL ISHEMIA/REPERFUSION INJURY

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The aim of present research was to examine the influence of different swimming and running protocols, as forms of physiological preconditioning on an isolated rat heart's ischemia/reperfusion injury. The study was conducted on male Wistar albino rats divided into groups following an aerobic or an anaerobic swimming and running protocol. Aerobic and anaerobic swimming trainings lasted 9 weeks while aerobic and anaerobic running trainings lasted 6 weeks. After the preconditioning protocols were completed, an *ex vivo* estimation of myocardial function was performed using the Langendorff technique. After a 30-minute global ischemia session, a 30-minute reperfusion period started. Different parameters for assessing myocardial function were measured with a sensor placed in the left heart ventricle: maximum and minimum rate of pressure development in the left ventricule (dP/dt max and dP/dt min), systolic and diastolic left ventricle pressure (SLVP and DLVP) and heart rate (HR). Coronary flow (CF) was measured flowmetrically. Our results show that anerobic running training decreased heart rate values while anerobic swimming training reduced coronary flow. These differences can be explained with the different physiological response of the heart to aerobic/anaerobic physical training. All data from this experimental study support many training effects: improved contractility, resting heart rate and increased physical work capacity and exercise tolerance. Anaerobic running physical training induces greater heart preconditioning to reperfusion injury in comparison to anaerobic swimming training. Physiological response to aerobic swimming physical training is considered more valuable for improving the exercise tolerance and coronary circulation.

Key words: preconditioning, ischemia/reperfusion, swimming, running, rats

THE EFFECTS OF L-GLUTAMATE ON HEART FUNCTION, MORPHOLOGY AND REDOX BALANCE IN RATS

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Glutamate is crucial excitatory neurotransmitter in the central nervous system, but glutamate receptors are found in variety of tissues and organs, including cardiomyocytes, vascular smooth muscle cells, and endothelial cells. Our previous results indicated role of N-methyl-D-aspartate receptors in regulation of heart function. The exact role of glutamate in regulation of physiological functions of cardiovascular tissues, as well as development of pathological conditions, is not fully elucidated. The aim of this study was to assess the effects of glutamate administration on heart function, morphological changes of myocardium and redox balance. Twenty male Wistar Albino rats were assigned into 2 groups: 1) control group treated with saline, and 2) glutamate group treated with glutamate. Glutamate was intraperitoneally administered to rats for 5-weeks, and after the experimental protocol was completed animals were sacrificed and hearts were attached to Langendorff apparatus. The blood was collected for assessment of changes in redox balance. Current results showed that prolonged exposure to glutamate significantly affected cardiac contractility, systemic and cardiac redox balance, and myocardial morphology. The heart collagen content was significantly increased in glutamate treated animals. This study confirmed possibility of affection of cardiovascular tissues by glutamate and its prooxidative action. Such effects are probably mediated by changes of activity of glutamate receptors in the heart and consequent disturbances in intracellular calcium level, due to the high permeability of N-methyl-D-aspartate receptors for calcium.

Key words: glutamate; heart function; oxidative stress; collagen; fibrosis.

EFFECTS OF VITAMIN B6 AND FOLIC ACID ON THE CARDIOVASCULAR SYSTEM OF RATS WITH STREPTOZOTOCIN-INDUCED DIABETES MELLITUS

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The aim of this study was investigation of the effects of application of vitamin B6 (pyridoxine) and folic acid on cardiometabolic and oxidative stress parameters, activities of lactate and malate dehydrogenase (LDH and MDH) isoforms, cardiac tissue remodeling [matrix metalloproteinase (MMP) -2 and -9 activities], histomorphometric parameters of the heart, aorta and pancreas, and immunohistochemical changes in heart and pancreatic tissue in rats with streptozotocin (STZ)-induced diabetes mellitus (DM).
The study was conducted for four weeks. Experimental animals were divided into 9 groups and treated intraperitoneally: C1 (1x saline, 1 ml/kg); C2 (28x saline, 1ml/kg,); DM (1x STZ, 100mg/kg); P (28x pyridoxine 7mg/kg), DM+P (1x STZ, 100mg/kg and 28x pyridoxine 7mg/kg); FA (28x folic acid 5mg/kg); DM+FA (1x STZ, 10mg/kg and 28x folic acid 5mg/kg); P+FA (28x pyridoxine 7mg/kg and 28x folic acid 5mg/kg); DM+P+FA (1x STZ, 100mg/kg and 28x pyridoxine 7mg/kg and 28x folic acid 5mg/kg).
DM changed many cardio-metabolic biomarkers. An increased cardiac oxidative stress was reflected by increased superoxide dismutase and catalase activities. Changes in LDH isoform activity indicated switch from aerobic to anaerobic metabolism, while increased peroxisomal MDH activity demonstrated peroxisomes activation in diabetic conditions. Significant changes in MMP activities showed cardiac remodeling. DM reduced the number of insulin-positive islets of Langerhans and proliferating cell nuclear antigen (PCNA)-positive cardiomyocytes. Folic acid administration decreased glucose level, while pyridoxine did not influence it. Single and combined administration of pyridoxine and folic acid in rats with DM has significant positive effects on diabetes-altered cardiometabolic biomarkers, oxidative stress parameters, cardiac tissue remodeling, as well as histomorphometric and immunohistochemical parameters.
Since DM represents a global public health problem characterized by multiple organ damage and complications, investigation of the use of supplements in addition to conventional medical treatment, is of great importance in order to prevent or reduce the complications of DM.

Key words: diabetes mellitus, streptozotocin, pyridoxine, folic acid, biochemical markers, oxidative stress, cardiac tissue remodeling, immunohistochemical markers, rat

Acknowledgement: This work was supported by the Ministry of Education science and technological development of Republic of Serbia grant number 175 043 and 200110/00402.

ABSTRACTS OF THE ORAL COMMUNICATIONS

EFFECTS OF CHRONIC *BETA*-BLOCKERS THERAPY ON IN-HOSPITAL OUTCOMES IN PATIENTS WITH ST-SEGMENT ELEVATION MYOCARDIAL INFARCTION TREATED WITH PRIMARY PERCUTANEOUS CORONARY INTERVENTION

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Background & Aim: *Beta*-blockers are among the pillars of pharmacological treatment in patients with ST-segment elevation myocardial infarction (STEMI). However, the impact of previous treatment with *beta*-blockers in patients with STEMI remains unclear. We aimed to determine the impact of chronic *beta*-blocker treatment on the risk of cardiac arrest and cardiogenic shock (CS) in patients with STEMI treated by primary percutaneous coronary intervention (pPCI).
Materials & Methods: Data were retrospectively collected from 977 patients treated by pPCI for STEMI. Cardiovascular risk factors together with factors related to the acute phase of STEMI were evaluated, according to prior *beta*-blocker therapy status. Associations between chronic *beta*-blocker treatment and the occurrence of STEMI-related cardiac arrest and CS were assessed in multiple regression analyses.
Results: The median age of the population was 62 (IQR 53-71) years. Compared to patients without *beta*-blocker therapy, patients undergoing chronic *beta*-blocker treatment were older, more likely to present hypertension, diabetes, chronic kidney disease, heart failure, and previous myocardial infarction (all $p< 0.05$). Prior *beta*-blocker therapy was independently associated with STEMI-related cardiac arrest in older (>62 years), hypertensive, and diabetic patients (OR 2.1, 95%CI 1.1-4.1, $p< 0.01$; OR 1.9, 95%CI 1.1-3.3, $p = 0.01$; and OR 2.8, 95%CI 1.1-7.2, $p = 0.03$, respectively). Chronic *beta*-blocker therapy was also independently associated with new-onset CS in diabetic patients (OR 2.8, 95%CI 1.0-7.3, $p = 0.03$).
Conclusions: In diabetic patients, prior *beta*-blocker therapy was an independent predictor of STEMI-related CS. More importantly, *beta*-blocker therapy before admission for STEMI was associated with an increased risk of cardiac arrest in older, hypertensive, and diabetic patients, even after correction for potential confounders. Therefore, it might be reasonable to ensure closer in-hospital follow-up for this high-risk population and restrict *beta*-blocker treatment to hemodynamically stable patients, particularly in the setting of diabetes mellitus.

Key words: *beta*-blocker treatment, cardiac arrest, cardiogenic shock, acute myocardial infarction

THE GENETIC CHARACTERISTICS ASSOCIATED WITH THE PROTHROMBOTIC CONDITION IN INDIVIDUALS DIAGNOSED WITH TYPE 2 DIABETES

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Background & Aim: Type 2 diabetes mellitus (T2DM) represents a prevalent and widespread medical condition, distinguished by an increased tendency towards blood clot formation, which frequently culminates in severe complications. This heightened prothrombotic state arises from the sustained activation of the coagulation system, coupled with a concurrent decline in the body's inherent ability to dissolve clots, known as fibrinolysis. The primary objective of this investigation was to assess the role of genetic polymorphisms in hemostatic factors in amplifying the risk of thrombosis within the context of insulin resistance commonly observed in T2DM.
Materials & Methods: To conduct this study, a cohort of 60 patients diagnosed with T2DM was subjected to comprehensive metabolic assessments, involving parameters such as serum glucose, total cholesterol, LDL (low-density lipoprotein), HDL (high-density lipoprotein), triglycerides, glycated hemoglobin, and indicators of renal function. All measurements were obtained following a 12-hour fasting period. The evaluation of hemostatic status encompassed the analysis of fibrinogen levels, platelet count and morphology, plasmatic concentration of antithrombin III, and the identification of various PAI-1 (plasminogen activator inhibitor-1) and coagulation factor XIII genotypes.
Results: The outcomes of this study unveiled a noteworthy association between the PAI-14G/4G genotype and elevated levels of glycated hemoglobin, as well as increased total and LDL cholesterol. Conversely, no such correlations were observed with other PAI-1 genotypes. Additionally, our research unveiled a connection between the mutant WT+MT factor XIII heterozygote and elevated total and LDL cholesterol levels, alongside heightened concentrations of CRP (C-reactive protein).
Conclusions: In summary, this investigation illuminates the intricate interplay between genetic variations in hemostatic factors and the prothrombotic milieu inherent to T2DM. These findings underscore the importance of considering individual genetic profiles when assessing the risk of thrombotic complications in patients with insulin resistance and T2DM, ultimately paving the way for more personalized and effective management strategies.

CAFFEIC ACID IMPROVES ANTIOXIDANT DEFENSE AND ATTENUATES OXIDATIVE STRESS IN HEART OF SPONTANEOUSLY HYPERTENSIVE RAT

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Background & Aim: Hypertension is a serious medical condition associated with a high risk of heart, brain, and kidney damage. Oxidative stress is an imbalance between prooxidants and antioxidants in which reactive oxygen species react with cellular macromolecules such as DNA, RNA, proteins, and lipids, causing cellular damage and cell death. Advanced oxidation protein products (AOPPs) are formed during chronic oxidative stress and may contribute to myocardial damage. Caffeic acid (CA), a phenolic acid with antioxidant and anti-inflammatory activities, can improve blood pressure and cardiac performance in spontaneously hypertensive rats (SHR) by increasing nitric oxide bioavailability. Since hypertension is associated with increased oxidative stress, we investigated whether chronic consumption of CA could affect cardiac oxidative stress and antioxidant defense in SHR.
Materials & Methods: Adult, male SHR were divided in two groups: control SHRC received vehicle, and SHR+CA was given CA (3mg/kg/day, by gavage) during a 4-week-period. Cardiac AOPP and superoxide anion (O_2^-) levels, superoxide dismutase (SOD) and catalase (CAT) activities (spectrophotometry), and SOD and CAT proteins expressions (western blotting) were measured. Correlations between systolic blood pressure (SAP, measured by Cardiomax III in anesthetized rats) and oxidative stress parameters were analyzed.
Results: Chronic consumption of CA increased the activities of SOD ($p<0.05$) and CAT ($p<0.001$), and reduced O_2^- and AOPP levels in the heart of SHR. The protein expression of SOD was reduced ($p<0.05$), while CAT remained unchanged in SHR+CA vs. SHRC. SAP exhibited a significant negative correlation with SOD activity ($r = -0.7384$, $p = 0.006$). SOD and CAT activities were in significant positive correlation ($r = 0.6371$, $p = 0.026$), while CAT activity significantly negatively correlated with AOPP ($r = -0.8036$, $p = 0.002$).
Conclusion: Our results showed that chronic administration of caffeic acid ameliorates cardiac antioxidant defense and protein damage induced by oxidative stress in SHR.

Key words: caffeic acid, hypertension, oxidative stress, AOPP

Funding: This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (No. 451-03-47/2023-01/200015).

RELATIONSHIP BETWEEN HYPERTENSIVE-LIKE TRAITS IN RAT MODEL OF PCOS AND OXIDATIVE STRESS

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Background & Aim: Many data imply that PCOS and hypertension (HT) in reproductive-age women of are interrelated. Even though hyperandrogenemia is associated with higher blood pressure (BP) levels, whether HT influences PCOS development remains elusive. The aim of this study was to investigate the differences in ovarian characteristics, blood pressure levels and OS parameters in normotensive and spontaneously hypertensive rats (SHR) in the PCOS rat model**.**
Materials & Methods: Four groups of rats (n=6 per group) —WK (normotensive rats), WK PCOS, SHR, and SHR PCOS were involved in the investigation. Testosterone-enanthate (1 mg/100 g body weight) was injected subcutaneously every day for 5 weeks to induce PCOS.
Results: Estrus cyclicity cessation and higher testosterone levels were caused by PCOS induction in both strains. In both strains, there were more preantral, atretic, and cystic follicles, which are PCOS-related abnormalities. We registered higher testosterone levels, while all blood pressure measurements were greater in SHR. In addition, the number of preantral, atretic, and cystic follicles were higher in SHR compared to WK. Systolic, mean arterial, and pulse pressure were all raised by PCOS modeling in the WK strain, but only mean arterial and pulse pressure were increased in SHR strain. The significant interaction between mean arterial blood pressure and level of nitrites in plasma were found. Thiobarbituric acid reactive substance and superoxide anion radical values were greater, while superoxide dismutase activity and concentration of reduced glutathione were reduced in both PCOS groups, which reflect the molecular basis related to changes in PCOS-related alterations.
Conclusion: This study revealed new information about potential processes underlying the HT seen in hyperandrogenic PCOS rats as well as a closer relationship between these two disorders in terms of oxidative disturbance.

Key words: hypertension, polycystic ovary syndrome, oxidative stress, testosterone, ovary

COMPARISONS OF PLEIOTROPIC EFFECTS OF SGLT2 INHIBITION AND GLP-1 RECEPTOR AGONISM ON CARDIAC GLUCOSE INTOLERANCE

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Worldwide, among adults, the prevalence of insulin resistance reaches to about 40%. Supporting document from the International Diabetes Federation, the number of people with diabetes in the world is expected to increase from about 500 million in 2019 to 700 million in 2045, with about 85% of cases occurring in low and middle income countries. Interestingly, recent studies discuss the evidence of lesser degrees of hyperglycemia contribution to cardiovascular disease (CVD) than impaired glucose tolerance. Indeed, the biggest risk for CVD seems to shift to glucose intolerance in humans with insulin resistance. Although there is a connection between abnormal insulin signaling and heart dysfunction in diabetics, there is also a relation between cardiac insulin resistance and aging heart failure (HF). Moreover, studies have revealed that HF is associated with generalized insulin resistance. Recent clinical outcomes parallel to the experimental data undertaken with antihyperglycemic drugs have shown their beneficial effects on the cardiovascular system through a direct effect on the myocardium, beyond their ability to lower blood glucose levels and their receptor-associated actions. In this regard, several new-class drugs, such as glucagon-like peptide 1 receptor agonists (GLP-1Ra) and sodium-glucose cotransport 2 inhibitors (SGLT2i), can improve cardiac health beyond their ability to control glycemia. In recent years, great improvements have been made toward the possibility of direct heart-targeting effects including modulation of the expression of specific cardiac genes in vivo for therapeutic purposes. However, many questions remain unanswered, regarding their therapeutic effects on cardiomyocytes in heart failure, although there are various cellular levels studies with these drugs. There are also some important comparative studies on the role of SGLT2i versus GLP-1Ra in patients with and without CVD as well as with or without hyperglycemia. Here, we sought to summarize and interpret the available evidence from clinical studies focusing on the effects of either GLP-1Ra or SGLT-2i or their combinations on cardiac structure and function. Furthermore, we documented data from experimental studies, at systemic, organ, and cellular levels. Overall, one can summarize that both clinical and experimental data support that either SGLT2i or GLP-1R agonists have similar benefits as cardioprotective agents in patients with or without impaired glucose tolerance.

DIABETIC HEART AND THE EFFECTS OF ANTIDIABETIC DRUGS ON CARDIAC FUNCTION

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Background & Aim: Diabetes is a chronic endocrine disorder characterised by hyperglycaemia due to insulin deficiency or insulin resistance in target tissues. Diabetes affects the health of millions of people worldwide and its prevalence is increasing dramatically. Cardiovascular complications are a major cause of diabetes-related morbidity and mortality. Therefore, antidiabetic treatments that also target the cardiovascular complications of the disease are crucial. Thus, we aimed to investigate the effects of antidiabetic drugs on cardiac function.
Materials and methods: High-dose streptozotocin (STZ) induced diabetes and high-fat fed (HFD) and low-dose STZ induced diabetes models were used in the studies. Sitagliptin, linagliptin, dapagliflozin and empagliflozin were used as treatment agents. In vivo and in vitro cardiac function was assessed using PV-loop analysis, echocardiography, Langendorff heart preparation and papillary muscle experiments. Results are presented as mean±SD.
Results: We found that both DPP-IV inhibitors, sitagliptin and linagliptin, failed to improve cardiac function in STZ-diabetic rats. Similarly, the SGLT2 inhibitors empagliflozin and dapagliflozin were also ineffective in correcting cardiac function in the STZ diabetic and HFD/low dose STZ diabetic models, respectively.
Conclusion: Our results show that the investigated antidiabetic drugs were unable to improve cardiac dysfunction in experimental diabetic models. On the other hand, the beneficial cardiac effects of these drugs have been reported by others. Future studies are therefore needed to clarify the role of antidiabetic drugs in cardiac dysfunction.

Key words: heart, diabetes, DPP-IV inhibitor, SGLT2 inhibitor

GLUCOSE LOWERING THERAPIES FOR CARDIOVASCULAR RISK REDUCTION IN DIABETES MELLITUS

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Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood sugar levels due to insufficient insulin production or the body's inability to use insulin effectively. Individuals with diabetes are at an increased risk of cardiovascular diseases (CVD), such as heart attacks and strokes. Therefore, managing cardiovascular risk has become a critical aspect of diabetes care. Over the years, research has focused on identifying glucose-lowering therapies that not only help control blood sugar levels but also have a positive impact on cardiovascular outcomes. Several classes of antidiabetic medications have shown potential for reducing cardiovascular risk in people with diabetes. Numerous studies have investigated the cardiovascular benefits of various glucose-lowering agents in individuals with diabetes. These studies have provided valuable insights into how different classes of medications can impact cardiovascular outcomes. Here are some key studies that have explored the cardiovascular benefits of specific classes of glucose-lowering agents. The only glucose-lowering medication major cardiovascular (CV) outcome trials (CVOTs) in type 2 diabetes were from the University Group Diabetes Program and the UK Prospective Diabetes Study (UKPDS) before the twenty-first century. After the 2008 Food and Drug Administration (FDA) guidance and the CV safety concerns from heart failure (HF) events with pioglitazone and myocardial infarction with rosiglitazone, the FDA had mandated a requirement to achieve a maximum hazard of 1.30 in a CVOT with a composite endpoint of first MI, stroke or CV death event for cardiovascular reliability after licensing. There are several important trials about cardiovascular outcome events such as EMPA-REG OUTCOME, LEADER , SUSTAIN-6, CANVAS Program (Canagliflozin Cardiovascular Assessment Study), and EXSCEL.

Key words: glucose lowering therapies, cardiovascular outcome, myocardial infarction, cardiovascular safety

THE ROLES OF SIRTUINS AND K-ACETYLATION IN METABOLIC SYNDROME ASSOCIATED HEART REMODELING

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Background & Aim: Metabolic syndrome is a cluster of distinct metabolic perturbations which markedly increases the incidence of cardiovascular pathologies. Lysine (K)–Acetylation is a crucial posttranslational modification process that regulates lots of pathways in the cell. Sirtuins (SIRT) are a family of deacetylase proteins that have a key role in the K–Acetylation process. We hypothesized that K – Acetylation and the SIRTs could be the key regulator of metabolic syndrome-associated heart remodeling.
Materials & Methods: Male, 8 weeks old, Balb-C mice were randomly divided into two groups: Control (C) and Metabolic Syndrome (MetS). MetS was induced in mice by adding 32% sucrose to drinking water for 16 weeks. Body weight, caloric intake, and fasting blood glucose (FBG) were monitored. Insulin sensitivity was assessed with OGTT. Cardiomyocytes were isolated from the heart tissue to measure mitochondrial membrane potential (MMP) and Reactive Oxygen Species (ROS) with confocal microscopy. In addition, total K-Acetylation, SIRT1, and SIRT2 levels were evaluated with Western Blot. Cells isolated from MetS mice were incubated with a SIRT1 activator (SRT1720, 1μM for 3h) and repeated the same analyzes.
Results: Body weights did not differ between groups. FBG levels were not different in the first months but we observed higher FBG in the MetS group at 4th month. Insulin resistance was observed at 5th week, and it lasted until the end. MMP were significantly depolarized and ROS levels were significantly increased in MetS animals. Total K-Acetylation was markedly elevated and SIRT1 and SIRT2 levels decreased in the MetS group. SRT1720 incubation was restored to all these adverse alterations.
Conclusion: Our results show that there is a possible link between SIRT induced K-Acetylation process and metabolic syndrome-associated cardiac remodeling. In further studies, modification of the SIRT1 activity in cardiomyocytes would be a protective strategy against cardiac remodeling.

Key words: metabolic syndrome, sirtuins, K-acetylation, cardiac remodelling

IMPROVING MITOCHONDRIAL RESPIRATION OF HUMAN PLATELETS WITH CELL-PERMEABLE SUCCINATE IN METABOLIC DISEASE

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Background & Aim: Mitochondrial dysfunction lies at the heart of the pathophysiology of metabolic disease. In the case of diabetes mellitus, mitochondrial dysfunction is implicated in both etiopathogenesis of the disease and development of long-term complications. Statins are the first line treatment for hypercholesterolemia in current guidelines. Platelets are a readily available substitute for more metabolically active cells in the study of mitochondrial dysfunction. The aim of the current study is to evaluate whether NV118, acell-permeable succinate, may be used to improve mitochondrial respiration in patients with type 2 diabetes mellitus (T2DM).
Materials & Methods: Platelets of patients with T2DM were isolated by means of differential centrifugations and were resuspended in the donor plasma. High-resolution respirometry using the O2k-oxygraph (Oroboros Instr., Innsbruck, Austria) was used to measure oxygen consumption in the presence of a cell-permeable succinate or DMSO (control). Non-phosphorylating oxygen consumption and noncoupled respiration were measured in the presence of oligomycin (ATP-synthase inhibitor) and CCCP (protonophore) respectively. Exclusion of non-mitochondrial oxygen consumption was achieved by measurements after exposure to rotenone (complex I inhibitor) and antimycin A (complex III inhibitor).
Results: Mitochondrial respiration the group exposed to NV118 (cell-permeable succinate) was improved as R-L net ROUTINE capacity increased to 124% ±6.3 and E-L net ET capacity increased to 158%±13.7 of control, with an added E-R reserve capacity of 229%±45.8. To exclude the possibility that these increases in mitochondrial respiration were due to uncoupling E-L coupling efficiency was calculated in the presence of NV118 (0.86±0.01) and in its absence (0.9±0.01), showing that the ETS is running in an efficient manner.
Conclusions: Cell-permeable succinates can be used to treat mitochondrial dysfunction in type 2 diabetes mellitus, possibly opening new horizons in the prevention of complications.

Key words: cell-permeable succinate, mitochondria, type 2 diabetes, platelets

EVALUATION OF THE BLACK CHOKEBERRY BIO JUICE EFFECT ON BLOOD PRESSURE LEVELS AND ENDOTHELIAL DAMAGE IN PATIENTS UNDER CHRONIC ANTIHYPERTENSIVE TREATMENT (PRELIMINARY DATA)

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Background & Aim: The modern pre-pharmacological approach to prevention and management of hypertension should be multitarget, suggesting improvement of body weight, quality of diet and eventually inclusion of some dietary supplements.
Some dietary components and natural products seem to be able to significantly lower BP without inducing serious side effects. Aronia berries are a good source of antioxidants, including anthocyanins, quercetin and kaempferol. These antioxidants have been shown to have beneficial effects on cardiovascular health, including reducing inflammation, improving blood flow, and lowering cholesterol levels. The aim of this study was to evaluate (in vivo) the effect of black chokeberry bio juice (AMJ), obtained from Western part of Romania on blood pressure levels and endothelial damage.
Materials & Methods: A prospective study was conducted over a period of 3 months. In the study, 46 patients under chronic antihypertensive treatment (bitherapy, > 1year) but with uncontrolled blood pressure values were included. The study was conducted at the Cardiology Clinic of Timisoara City Hospital. The patients were randomized 1:1 into 2 groups (one receiving 100ml black chokeberry juice/day, and a control group, both groups being under lifestyle change), groups matched by age, sex and characteristics. No changes were made to the chronic pharmacologic treatment of the patients. Demographic and lifestyle data were collected from the patients, and blood tests were performed, including markers of endothelial injury, asymmetric dimethylarginine (ADMA), as well as imaging investigations to confirm the presence of endothelial dysfunction (FMD and IMT).
Results: Both SBP and DBP values decreased in patients on the arm with AMJ from 140.9mmHg to 131.8mmHg, respectively 86.5mmHg to 80.4mmHg, the differences being extremely significant (p=0.000002, respectively p=0.000009). The markers by which inflammation was evaluated also had a beneficial evolution (ADMA values, from 105,304mcg/l to 89,913mcg/l, p=0.0000029). Likewise, the metabolic profile (glucose 104.65mg/dl to 94.30 mg/dl, p=0.00002; uric acid 5.59 mg/dl to 5.01mg/dl, p=0.0006; as well as cholesterol levels 195.47 mg/dl to 178.00 mg/dl, p=0.012 and triglycerides levels 137.13 mg/dl to 113.17 mg/dl, p=0.0017) showed a beneficial evolution of patients on AMJ. Also, the patients on the arm with therapeutic intervention had a decrease in BMI (29.51kg/m² to 28.67 kg/m², p=0.000002).
Conclusion: Black chokeberry juice can be considered as a promising nutraceutical with increased biological potential. However, like other highly bioactive fruits and natural products, black chokeberry requires extensive human studies to determine its safety, efficacy, and mechanisms of action.

ARRHYTHMIAS TRIGGERED BY MEDICATION: THE PROARRHYTHMIC RISK ASSOCIATED WITH CLASS 1 ANTIARRHYTHMICS AND CANNABINOIDS

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Background & Aim: The concept of proarrhythmia is not new and can be described as the escalation of a persisting arrhythmia or the progression of a new arrhythmia resultant to antiarrhythmic drug. Drug-induced arrhythmia can be linked to either exorbitant conduction slowing (e.g; with class Ia and/or Ic Na^+ channel inhibitor) or extreme prolongation of ventricular APD (e.g; with class III agent), or both. These effects are sustained by several confounding factors such as the existence heart diseases, stimulated adrenergic tone, and hypokalemia etc. This study was designed to investigate the proarrhythmic potential of class 1 antiarrhythmic drugs (flecainide and quinidine) by studying steady-state frequency dependence-, offset and onset kinetics of V^+_{max}. Moreover, the electrophysiological effects of cannabidiol was evaluated on action potential and transmembrane potassium currents.
Materials & Methods: In the current study, conventional microelectrode and whole-cell configuration of the patch-clamp techniques were used to record the action potentials and transmembrane ionic currents.
Results & Conclusion: The results show that flecainide 3 µM and quinidine 10 µM significantly reduced the maximum upstroke velocity (V^+_{max}) in the entire frequency range under steady-state conditions. Similarly, the time constant of recovery of V^+_{max}, determined following a constant 1 Hz stimulation was 14.6 s for flecainide and 7.2 s for quinidine. The onset rate constant was 17.5 AP for 3 µM flecainide and 5.6 AP for 10 µM quinidine. Contrary to other class 1 agents, these slowly dissociate from the Na+ channel and hence remarkably delay ventricular conduction and therefore in the presence of *arrhythmic substrate* could further propagate this arrhythmic event. These findings suggest that the frequency dependent, restitution kinetics and onset kinetics are important electrophysiological determinants which can discriminate Na^+ channel blockers with proarrhythmic and antiarrhythmic potential. Moreover, CBD lengthens APD which are attributed to blockade of transmembrane potassium currents (I_{Kr}, I_{Ks}, and I_{to}). Although the EC50 value of CBD was higher than literary Cmax values after CBD smoking and oral intake, our results raise the possibility that hERG channel and repolarizing potassium currents inhibition might have a role in the possible proarrhythmic adverse effects of cannabinoids in situations where metabolism of CBD impaired and/or the repolarization reserve is weakened.

THE ROLE OF COLCHICINE ON CARDIAC FIBROSIS IN A PORCINE MODEL OF ATRIAL FIBRILLATION

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Background: Affecting 37.5 million people worldwide, atrial fibrillation (AF) is the most common cardiac arrhythmia. An enhanced inflammatory response is commonly observed in patients with AF, linked to activation of the NLRP3 inflammasome and structural remodeling. Colchicine is an anti-inflammatory drug that functions as a NLRP3 inflammasome inhibitor, but its effects on cardiac fibrosis and early activators of inflammation in the pathogenesis of AF is relatively unknown.
Materials & Methods: A porcine model of tachypacing induced AF was set up and 15 pigs were divided into three study groups; colchicine (0.5 mg bidaily, n=6), placebo (calcium tablets bidaily, n=6) and sham (no treatment, n=3). The pigs were euthanized after 6 weeks of treatment and the hearts were excised. Whole tissue lysates from the pig's right atrium were collected and analyzed through Western Blot. Different sections of the left and right atrium were stained for collagen secretion through Picro-Sirius Red staining. Colchicine demonstrates a trend in downregulating early fibroblast activation with cross talk from macrophages as observed through decreased protein levels of fibroblast activators and macrophage markers upon comparison of placebo and colchicine; vimentin ($p=0.0572$), αSMA ($p=0.2420$), periostin ($p=0.1413$), TLR4 ($p=0.0583$), and TGF-β ($p= 0.2384$). The accumulated fibrosis in different sections of the right and left atrium confirmed the porcine model of AF as observed by an upregulation of fibrosis upon comparison of the sham and placebo treated group (left atrial lateral wall,*$p=0.0476$ and right atrial lateral wall, *$p=0.0238$). No downregulation of fibrosis was observed in the colchicine group.
Conclusion: Colchicine demonstrates a trend in downregulation of early activation of fibroblasts with cross talk signaling from macrophages; however, colchicine had no effect on the structural remodeling.

Key words: atrial fibrillation, colchicine, fibrosis, inflammation

DEVELOPMENT OF IPSC-BASED CLINICAL TRIAL SELECTION PLATFORM FOR PATIENTS WITH INHERITED METABOLIC AND CARDIOVASCULAR DISORDERS

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Collectively rare metabolic disorders affect 5% of the global population with complications related to heart and circulatory system. Also, novel and ultrarare mutations make it difficult for patients with inherited metabolic disorders to be considered for clinical trials due to non-availability of mutation specific drug response data. Therefore, a “Leap of Faith” approach is used to develop a treatment protocol, often based on empiric observations from patients with overlapping phenotypes but different or more prevalent mutations. This uncertainty reflects the need for development of personalized clinical trial selection platforms to assess drug efficacy to facilitate decision-making process in enrolling such patients in suitable clinical trials. Leigh syndrome is a multisystemic mitochondrial disorder that results in cardiovascular and neurological complications.

In this study we report an 18-year-old male patient with Leigh-like syndrome (LS) harboring compound heterozygous variants in the *ECHS1* gene. This patient had previously participated in two clinical trials with unfavorable responses. We established an induced pluripotent stem cell (iPSC)-based platform for this patient and the safety and efficacy of a panel of drugs on patient cardiomyocytes was assessed. We further demonstrated validity of this platform by observing improved phenotype, function and mitochondrial dynamics of patient cardiomyocytes in response to drug treatment. Hence, post drug screening we administered the most effective drugs to the patient (Ubiquinol, α-Lipoic acid and Riboflavin) for a period of 3 years, where we observed a significant shift in the metabolic profile of this patient towards that of a healthy control, showing improvement in both energy flux and ROS reduction thereby confirming the validity of iPSC-based drug screening platform.

Therefore, this personalized iPSC-based platform can act as a pre-screening tool to help in decision-making with respect to patient's participation in future clinical trials.

GALIUM VERUM EXTRACT AS A NOVEL CARDIOPROTECTIVE AGENT AGAINST DOXORUBICIN-INDUCED CARDIOTOXICITY IN RATS

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Background & Aim: Although previous data confirmed the strong antioxidant capacity and cardioprotective potential of lady's bedstraw herb extract (*Galium verum* L, GVE), there are no information referring to the role of this plant species in DOX-induced cardiac damage. Since natural antioxidant represent a worthwhile tool for reduction of heart damage associated with DOX therapy, we hypothesized that lady's bedstraw extract would alleviate DOX- induced heart damage. Therefore, this study was conducted to assess the influence of two-week GVE intake on doxorubicin-induced cardiotoxicity in rat model.
Materials & Methods: 24 male *Wistar albino* rats were randomly divided into the following groups: healthy (CTRL), doxorubicin (DOX), and DOX+GVE. GVE was applied per os (50 mg/kg/day for 2 weeks). In order to establish DOX-induced cardiotoxicity, DOX was injected as a single dose of 15 mg/kg. Three days after DOX application, all animals were sacrificed, blood samples were collected and hearts were isolated for performing *ex vivo* measurements. Concentration of pro-oxidants and antioxidants was determined spectrophotometrically in blood samples in order to assess the influence of GVE on systemic redox status. Functional cardiac parameters were monitored during the autoregulation protocol (40-120 mmHg) on the Langendorff apparatus. Cardiac redox status was assessed spectrophotometrically in heart tissue homogenates, while histological examinations in heart tissue samples were performed to verify morphological changes.
Results: Our results demonstrated that GVE consumption effectively suppressed disturbed cardiac response induced by DOX. Moreover, GVE treatment was able to diminish most of the measured pro-oxidant parameters, as well as to increase the activity of the antioxidant enzymes compared to the DOX group. Histological data showed that GVE therapy alleviated the pathological injuries caused by DOX injection.
Conclusion: Two-week GVE intake could relieve DOX-induced cardiotoxicity, via improvement in heart contractility, attenuation of oxidative stress and alleviation of structural heart damage.

Key words: Lady's bedstraw, heart; doxorubicin; cardiotoxicity

INTERLEUKIN-8 INHIBITION – A POSSIBLE PATHWAY TO REDUCE HEMODYNAMIC COMPLICATIONS IN POST-CORONARY ARTERY BYPASS GRAFTING SURGERY PATIENTS

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Background & Aim: The pro-inflammatory effect of coronary artery bypass grafting (CABG) surgery has been incriminated as a contributor to post-CABG unfavourable hemodynamic evolution. We aimed to identify inflammatory markers that link CABG duration with post-surgical hemodynamic impairment.
Materials & Methods: Fifty-seven consecutive patients undergoing elective CABG were enrolled in the present study. Blood samples were collected prior and on post-surgery days 2 and 5. High-sensitivity C-reactive protein (hs-CRP) and interleukins 1b (IL-1b), 2 (IL-2), 6 (IL-6), and 8 (IL-8) levels were determined. For each patient, the duration of extracorporeal circulation (ECC), aortic clamping, and the total duration of the CABG procedure were recorded. Prolonged hemodynamic instability (requiring more than 24 h inotropic and/or vasopressor therapy) was recorded.
Results: The mean age of the study population was 60.1 ± 9.6 years, with a male to female ratio of 1.85. A significant increase in all studied inflammatory marker levels was recorded following CABG (all $p< 0.05$), with peak levels being recorded in postoperative day 2. Peak IL-8 levels significantly correlated with total surgery, ECC, and aortic clamping times (all $p< 0.01$). There was no significant correlation between peak hs-CRP levels and any of the three surgery times (all $p >0.05$). Peak IL-1b, IL-2, IL-6, and IL-8 levels positively correlated with the duration of inotropic and/or vasopressor therapy (all $p< 0.05$). In multiple regression analysis, peak IL-8 levels remained the only independent predictor of prolonged post-CABG hemodynamic impairment ($p = 0.02$).
Conclusions: Peak IL-8 levels correlated with CABG surgery duration and independently predicted prolonged post-CABG hemodynamic impairment. These data indicate the IL-8-related pathway as an important link between CABG duration and hemodynamic impairment and suggest that the use of IL-8 inhibitors could be a new concept for prevention of hemodynamic complications in the period following CABG.

Key words: coronary artery bypass grafting, hemodynamic impairment, interleukin-8

HUMAN *IN SILICO* TRIALS TO EVALUATE THE SAFETY OF REGENERATIVE CELL THERAPY

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Cardiovascular diseases remain the leading cause of death worldwide. The intrinsic regenerative capabilities of the human heart and donor organ availability are limited, making the development of new therapies to regenerate the injured heart a priority. The delivery of stem cells has been shown promising in revitalising the damaged human heart but is accompanied by safety concerns over the stem cells' arrhythmogenic potential. Therapy evaluation is complicated by the critical condition of targeted patients, commonly with a prognosis of heart failure. Modelling and simulation offer a fast and cost-effective alternative overcoming translational and ethical concerns of animal models. In silico trials at cell and organ level have repeatedly demonstrated to accurately capture the response of the human heart in diseased conditions and under drug block. Here we present the development, calibration and validation of a comprehensive multiscale modelling and simulation framework of the diseased human heart with Purkinje allowing to evaluate the safety of cell therapy. Each level of our multiscale framework was constructed based on experimental and clinical knowledge. Verification and validation were carried out across single cell, organ level and the ECG to ensure our framework's credibility. We show how our framework can be personalised to a range of patient-specific features, such as biventricular activation, infarct progression, scar size and Purkinje connectivity. Furthermore, we present the application of our framework to mechanistically investigate the safety of regenerative cell therapy under a range of delivery conditions, such as stem cell phenotype and density, as well as pharmacological treatment.
In summary, we have demonstrated the credibility of our novel human-based framework through verification and validation and shown how it can be applied to determine optimal delivery conditions progressing cell therapy safety evaluations for a range of patient-specific features.

Key words: *in silico,* modelling and simulation, cell therapy, human, myocardial Infarction

Acknowledgements: This project was funded by a BBSRC PhD scholarship in collaboration with AstraZeneca (BB/V509395/1) to LR, an EPSRC doctoral award to JC, a Wellcome Trust Senior Research Fellowship in Basic Biomedical Sciences to BR (214290/Z/18/Z), the CompBioMed 2 Centre of Excellence in Computational Biomedicine (European Commission Horizon 2020 research and innovation programme, grant agreement No. 823712), and by the Brazilian Government via CAPES, CNPq, FAPEMIG, UFSJ and UFJF. The Paci2020 model was provided by Dr. Michelangelo Paci and CBIG at the Tampere University Foundation. Simulations were enabled through PRACE-ICEI grants icp013 and 019, carried out at the Piz Daint Platform of the Swiss National Supercomputing Centre.

ATRIAL FAT INFILTRATION POST MYOCARDIAL INFARCTION IN A GÖTTINGEN MINIPIG MODEL OF OBESITY

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Background & Aim: Myocardial infarction (MI) can contribute to alterations in atrial electrophysiology creating a substrate for atrial fibrillation (AF) post MI, but the mechanisms for these alterations are unexplored. Obesity enhances the accumulation of epicardial adipose tissue (EAT) and is an independent risk factor involved in the pathogenesis of AF. Enhanced EAT accumulation also leads to infiltration of adipocytes deep into the myocardium promoting fibrosis and leading to functional disorganization and the formation of a structural arrhythmogenic substrate. Whether obesity related atrial fat infiltration is further promoted upon MI has not been investigated yet. The aim of this study was to investigate the effects of obesity and MI on the development of the atrial arrhythmogenic substrate post MI.
Materials & Methods: 12 lean and 12 obese Göttingen Minipigs were enrolled in the study. MI was induced by 120 min occlusion of the left anterior descending coronary artery in 12 lean pigs and 9 obese pigs, while 3 obese pigs served as a sham operated controls. Tissue was harvested 8 weeks post MI from the right atrium free wall (RA), right atrial appendage (RAA), left atrium free wall (LA) and left atrial appendage (LAA) and fixed in 10% formalin and cryosectioned at 30µm before they were stained either with Sirius Red (fibrosis) or Oil Red O (adipocytes/fat). Total amount of fibrosis and fat including EAT was quantified using automated image segmentation. Furthermore, atrial myocardial fat infiltration was quantified excluding the EAT.
Results: Obesity tendentiously increases total atrial fat in RA, RAA, LA and LAA. MI further increases the total atrial fat in RA, RAA and LAA ($p<0.05$) in obese pigs with MI compared to lean pigs with MI. Furthermore, atrial myocardial fat infiltration in RA and LA was found to be significantly increased in obese pigs with MI compared to lean pigs with MI ($p<0.05$). The amount of total fibrosis remained unchanged at all sites investigated.
Conclusion: These data indicate that MI enhances atrial fat infiltration in obesity suggesting an additive effect of obesity and MI in the formation of an atrial arrhythmogenic substrate post MI. Furthermore, these data indicate that atrial fat infiltration predisposes atrial fibrosis formation.

METFORMIN REDUCES ATRIAL FIBRILLATION INDUCIBILITY IN HORSES

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Background & Aim: Atrial fibrillation (AF) is perpetuated by electrical remodelling of the atria, characterized by a decrease in atrial effective refractory period (aERP) and an increase in AF inducibility. This study investigates the potential of metformin, an antidiabetic drug, in preventing cardiac remodelling during chronic AF.
Materials & Methods: Twenty retired Standardbred racehorses underwent continuous right atrial tachypacing for two weeks to induce AF. Ten horses were treated with 30mg/kg metformin orally twice daily and ten served as controls. After two weeks of tachypacing, heart rhythm was evaluated and, if needed, additional tachypacing was initiated to reinduce AF. After four months of AF, the aERPs were recorded by incremental S1-S2 epicardial pacing under general anaesthesia.
Results: A significant higher proportion of metformin-treated horses (6/10) required additional tachypacing to develop self-sustained AF compared to control horses (1/10) ($p = 0.02$). Preliminary data indicate that metformin-treated horses ($n = 7$) had longer right atrial ERPs after 4 months of AF compared to controls ($n = 8$) (mean right atrial ERP ±SD 1000ms = 177±26 vs 143±26ms ($p = 0.03$) and mean right atrial ERP 500ms = 226±52 vs 189±32 ms ($p = 0.11$)).
Conclusion: These preliminary findings suggest that metformin treatment reduces AF inducibility and may protect against the AF-induced aERP shortening. This ongoing longitudinal *in vivo* study will provide novel insights into the atrial arrhythmogenic remodelling during AF and may shed light on anti-remodelling mechanisms of metformin.

Key words: atrial fibrillation, cardiac remodelling, metformin, EP study, translational cardiology

This work is supported by a research grant from the Danish Cardiovascular Academy and the Danish Heart Foundation (PhD 2022002-HF)

INCREASED ARRHYTHMIA SUSCEPTIBILITY ASSOCIATED WITH CARDIAC REMODELING FOLLOWING VIGOROUS ENDURANCE TRAINING IN A CANINE ATHLETE'S HEART MODEL

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Background & Aim: The health benefits of regular physical activity are unquestionable. However, there is increasing evidence that chronic high-level exercise in elite athletes can evoke malignant cardiac arrhythmias and even sudden cardiac death. The purpose of our study was to determine the cardiac changes and arrhythmia susceptibility induced by long-term intense training in a large animal model that is electrophysiologically relevant to the human heart.
Materials & Methods: Beagle dogs were randomized into a 'Sedentary' and an 'Exercised' group (n=19-19). The latter group was trained for 4 months with an intensive treadmill-running protocol. To establish the development of the athlete's heart echocardiography and ECG measurements were performed. In open-chest anaesthetized dogs arrhythmia susceptibility was tested with high-frequency burst stimulation. Following heart removal, myocardial fibrotic changes were quantified and transmembrane ionic currents and action potential duration were measured.
Results: The vigorous endurance training resulted in increased left ventricular hypertrophy, greater atrial parameters, and enhanced degree of fibrosis. The remodeling of the sino-atrial node was detected which contributes to the bradycardia in the exercised group. ECG recordings presented prolonged PQ and widened QRS intervals and enhanced repolarization parameters in the exercised group. In open-chest anesthetized dogs, high-frequency burst stimulation induced a higher incidence of ventricular fibrillation, besides that, the occurrence of atrial fibrillation was nearly significant in the exercised group. The action potential duration was significantly lengthened in the left ventricular myocytes isolated from the exercised dogs. Additionally, the amplitude of the transient outward current was smaller in the left ventricular myocytes of exercised dogs.
Conclusion: Similar to the observed structural and electrical changes indicating enhanced arrhythmia susceptibility in our athlete's heart model, these alterations may present in elite athletes. However, further studies are essential to clarify this issue in more detail.

Key words: athlete's heart, fibrosis, echocardiography, electrocardiography, patch clamp

CALCIUM SIGNALING CONSEQUENCES OF CALCIUM RELEASE DEFICIENCY ASSOCIATED RyR2-S4938F AND RyR2-I4855M MUTATIONS EXPRESSED IN hiPSC-CMs

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Ryanodine receptor 2 (RyR2) is the major Ca 2+ release channel of cardiac sarcoplasmic reticulum (SR) that regulates the rhythm and strength of the heartbeat, but its malfunction generates arrhythmia leading to heart failure. Here we report on two Ca 2+ release deficiency syndrome (CRDS) associated RyR2 mutation located either in RyR2 carboxyl-terminal (S4938F) or RyR2 pore forming domain (I4855M) that were expressed in human induced pluripotent stem cells derived cardiomyocytes (hiPSC-CMs) using CRISPR/Cas9 gene-editing. S4938F mutation has been identified to cause premature ventricular contractions with ventricular fibrillation episodes in patients, whereas I4855M mutation causes atypical catecholaminergic polymorphic ventricular tachycardia (CPVT) accompanied with left ventricular non compaction.

Ca2+ signaling and electrophysiological properties of cardiomyocytes carrying S4938F or I4855M mutation were studied using total internal reflection fluorescence microscopy (TIRF) and patch clamp technique. The magnitude of spontaneously occurring SR Ca 2+ transients were significantly suppressed in both mutant cell lines. I Ca densities measured either by depolarizations to zero mV or repolarizations from +100mV to -50mV in mutant hiPSC-CMs were significantly smaller, as well as their accompanying Ca 2+ -transients detected by Fura-2 (cytosolic Ca 2+) or ER-GCaMP6 (SR Ca 2+ release), suggesting I Ca induced Ca 2+ release (CICR) was compromised. Caffeine induced Ca 2+ release and the frequency of spontaneously generated Ca 2+ transients and Ca 2+ sparks of I4855M hiPSC-CMs were greatly reduced, while S4938F hiPSC-CMs exerted enhanced caffeine triggered cytosolic and SR Ca 2+ release, with increased Ca 2+ sparks frequency.

Increased Ca 2+ content and increased Ca 2+ spark frequency is consistent with the arrhythmogenic phenotype of S4938F mutation, however the mechanism by which I4855M-RyR2 mutation causes Ca 2+ signaling aberrancies needs to be further elucidated.

Key words: ryanodine receptor, ryanodine receptor mutations, Ca2+ release deficiency, CICR, sparks

Funding: National Institutes of Health 1R01HL153504-01A1

ASSESSING THE INHIBITION POTENTIAL OF PUNICALIN AND UROLITHIN ON THE SARS-COV-2 SPIKE PROTEIN AND NEUROPILIN-1 RECEPTOR: A COMPARATIVE IN SILICO AND IN VITRO STUDY

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This study explored the therapeutic potential of punicalin and its gut metabolite, urolithin, derived from pomegranates, against SARS-CoV-2. The primary objective was to assess their ability to impede the interaction between the SARS-CoV-2 spike protein and the neuropilin-1 receptor, thereby preventing virus internalization. In vitro experiments were conducted to evaluate punicalin and urolithin's capacity to disrupt the spike protein's binding to the neuropilin-1 receptor across various concentrations. This assessment employed a 96-well plate coated with specific antibodies and a colorimetric reaction, which measured color changes and intensity at 450 nm. This method allowed visualization of the spike protein-neuropilin-1 complex formation, aiding in the identification of potential inhibitors or regulators. Notably, the highest punicalin dose displayed a significant 63.96% inhibition of the spike protein-neuropilin-1 receptor interaction. However, it's crucial to recognize that most punicalin undergoes stomach degradation, with urolithin emerging as the primary molecule effectively inhibiting virus internalization via the neuropilin-1 receptor. At the highest tested dose (1 mg/ml), urolithin demonstrated even more potent inhibition, reducing spike protein-neuropilin-1 receptor interaction by 65%. These findings suggest that both punicalin and urolithin hold promise in disrupting SARS-CoV-2 infection by preventing the spike protein from binding to the neuropilin-1 receptor. This inhibition could potentially hinder the virus's entry into host cells, offering a promising therapeutic approach. Moreover, *in silico* investigations unveiled that this inhibition partly occurs through direct interaction with the neuropilin-1 receptor, a multi-domain receptor with implications in the cardiovascular system. This implies that natural polyphenols and their metabolites may serve as therapeutic agents for cardiovascular diseases involving this receptor. Further research is needed to delve into the distinct mechanisms through which punicalin and urolithin interact with neuropilin-1 receptors.

Key words: punicalin, urolithin, SARS-CoV-2, spike protein, neuropilin-1 receptor

THE RELATIONSHIP BETWEEN CYTOSOLIC/MITOCHONDRIAL LABILE ZINC RATIO AND MITOCHONDRIAL DYNAMICS IN CARDIOMYOCYTES: ROLE OF ZNT6

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Background & Aim: Zinc as a cation (Zn^{2+}) participates in various biochemical pathways in living cells with extremely low concentrations in cardiac cells. Changes in intracellular free Zn^{2+} are coordinated by Zn^{2+}-transporters while changes in their expressions are affecting cellular functions. We aimed to examine the possible role of ZnT6 in the pathogenesis and progression of mitochondrial dysfunction in hyperglycemic cardiomyocytes.
Materials & Methods: We used either hyperglycemic (25mM glucose, 24h) or ZnT6 (ZnT6-OE) overexpressed H9c2 cardiomyoblasts. To determine distributions of subcellular free Zn^{2+} levels, we measured $[Zn^{2+}]_{cyt}$, $[Zn^{2+}]_{Mit}$, and $[Zn^{2+}]_{SER}$ separately by Zn^{2+}-sensitive fluorescent FRET-sensors. The mitochondrial localization and protein level of ZnT6 and mitochondrial proteins were determined by immunofluorescence and Western-blotting techniques. The mitochondrial membrane potential (MMP) and reactive oxygen species (ROS) production levels were determined.
Results: Western-blotting and immunofluorescence experiments revealed that ZnT6 was localized in mitochondria and its protein level was increased HG-cells. The $[Zn^{2+}]_{Mit}$ was found to be increased significantly in both ZnT6-OE and HG-cells. However, The $[Zn^{2+}]_{cyt}$ was significantly low in ZnT6-OE cells while it was significantly high in HG-cells compared to the controls. Increased ROS production and depolarized MMP were determined in HG-cells, while ZnT6-OE cells showed minor changes. DRP-1 migration to the mitochondria from cytosol was observed in ZNT6-OE cells with a significant decrease and increase in apoptotic and autophagy markers, respectively. However, the DRP1 level wasn't changed in HG-cells with a significant increase and decrease in apoptotic and autophagy, respectively.
Conclusion: Our findings suggest for the first time that decreasing the $[Zn^{2+}]_{cyt}/[Zn^{2+}]_{Mit}$ ratio through the aberrant ZnT6 expression induces mobilization of DRP-1 from the cytosol to mitochondria in cardiomyocytes, implying its effect on mitochondrial dynamic imbalance towards the fission process. However, the elevation of the $[Zn^{2+}]_{cyt}/[Zn^{2+}]_{Mit}$ ratio as observed in HG-cells diminishes the mobilization of DRP-1 to mitochondria and increases the fusion (Supported by TUBITAK-SBAG-117S386).

Key words: cardiomyocytes, mitochondria, DRP1, ZnT6, zinc

GLP-1 RECEPTOR AGONIST ATTENUATES AGING-ASSOCIATED CARDIAC INSUFFICIENCIES THROUGH RECOVERY IN MITOCHONDRIA

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Background & Aim: Glucagon-like peptide-1 receptor (GLP-1R) agonists improve cardiovascular dysfunction via the pleiotropic effects behind their receptor action. However, it is unknown whether they have a cardioprotective action in the hearts of the elderly. Therefore, we examined the effects of GLP-1R agonist liraglutide treatment (LG, 4 weeks) on the systemic parameters of aged rats (24-month-old) compared to those of adult rats (6-month-old) such as electrocardiograms (ECGs) and systolic and diastolic blood pressure (SBP and DBP).
Materials & Methods: At the cellular level, the action potential (AP) parameters, ionic currents, and Ca2+ regulation were examined in freshly isolated ventricular cardiomyocytes.
Results: The LG treatment of aged rats significantly ameliorated the prolongation of QRS duration and increased both SBP and DBP together with recovery in plasma oxidant and antioxidant statuses. The prolonged AP durations and membrane potentials of the isolated cardiomyocytes from the aged rats were normalized via recoveries in K^+ channel currents with LG treatment. The alterations in Ca^{2+} regulation including leaky-ryanodine receptors (RyR2) could be also ameliorated via recoveries in Na^+/Ca^{2+} exchanger currents with this treatment. A direct LG treatment of isolated aged rat cardiomyocytes could recover the depolarized mitochondrial membrane potential, the increase in both reactive oxygen and nitrogen species (ROS and RNS), and the cytosolic Na+ level, although the Na+ channel currents were not affected by aging. Interestingly, LG treatment of aged rat cardiomyocytes provided a significant inhibition of activated sodium-glucose co-transporter-2 (SGLT2) and recoveries in the depressed insulin receptor substrate 1 (IRS1) and increased protein kinase G (PKG). The recovery in the ratio of phospho-endothelial nitric oxide (pNOS3) level to NOS3 protein level in LG-treated cardiomyocytes implies the involvement of LG-associated inhibition of oxidative stress-induced injury via IRS1-eNOS-PKG pathway in the aging heart.
Conclusion: Overall, our data, for the first time, provide important information on the direct cardioprotective effects of GLP-1R agonism with LG in the hearts of aged rats through an examination of recoveries in mitochondrial dysfunction, and both levels of ROS and RNS in left ventricular cardiomyocytes.

IMPROVING STEM CELL-BASED THERAPY IN CARDIAC FAILURE: THE EFFECT OF ESTROGEN ON REGENERATIVE CAPACITY OF CARDIAC PROGENITOR CELLS

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Background & Aim: Clinical and preclinical studies reported insufficient cell engraftment and delayed cardiac functional recovery problems in stem cell-based therapy in heart failure. Therefore, pretreatment of stem cells with an exogenous factor may improve regenerative efficiency. Here we aimed to induce the transplantation efficiency and regenerative capacity of cardiac progenitor cells (CPCs) through estrogen administration.
Materials & Methods: CPCs were isolated from a male mouse heart and incubated with estrogen (E2:10^{-7}M, 48hours) in vitro. The heart failure model was generated by intraperitoneally *isoproterenol* treatment (ISO;200mg/kg, 6days) in the female mouse. Either estrogen pretreated-CPC (E2-CPC) or untreated-CPCs were intramyocardially transplanted into the failure heart. Engraftment and retention levels of transplanted cells were visualized by IVIS and SRY gene for expression analysis. Electrophysiological changes were analyzed by echocardiographic analysis (ECG) before and after the ISO application. Cardiac regeneration status following the transplantation was also examined at the molecular level through both qRT-PCR from isolated heart samples and immunofluorescence/histochemical staining on heart tissue sections to address the efficiency of the estrogen pre-treated CPC transplantation.
Results: Our IVIS and PCR results have demonstrated that untreated-CPCs diffuse much more rapidly in the heart compared to E2-CPCs. At the end of the 24hours after transplantation, only E2-CPCs were detected in the heart. ECG recordings showed that PR, QT, and QRS-intervals were markedly prolonged in ISO-treated mice. These intervals significantly shortened in E2-CPC transplanted mouse hearts compared to the untreated-CPC transplanted group. E2-CPC transplantation also remarkably reduced the collagen and lipid accumulation in the heart tissues. Our observations also showed that E2-CPC transfer induced cardiomyocyte proliferation and neo-vasculogenesis in transplanted hearts compared to the untreated-CPC group via increasing the Ki67, CXCR, cTNTI, and vWF protein expression levels.
Conclusion: Here, we demonstrated the effect of estrogen administration on the regenerative capacity of CPCs with in vitro and in vivo experiments.

Key words: cardiac progenitor cells, transplantation, heart failure, estrogen, stem cell-based therapy

ACUTE ACTION OF INCRETIN-BASED THERAPEUTICS ON AGEING MYOCARDIUM

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Background & Aim: Considering the crucial role of incretin action in regulating glucose metabolism, it becomes essential to examine the potential influence of these agents, particularly in the context of aging individuals who have elevated blood sugar levels. While the initial effects of incretin activation are well-established in glucose metabolism, its impact on cardiovascular outcomes remains relatively unexplored. Therefore, our study aimed to delve into the early effects of GLP-1 receptor agonists on age-related cardiac dysfunction.
Material and Methods: This study included in-vivo electrocardiography and cellular-based in vitro electrophysiology experiments from 6 months old (Adult) and 24 months old (Aged) BALB/c mice.
Results: Electrocardiographic measurements taken from the body's surface revealed the presence of irregular atypical fibrillations and a notable extension of QT intervals shortly after administering the acute GLP agonist (liraglutide at 0.3 mg/kg). In contrast to the adult group, GLP agonism (using Liraglutide at 1 μM) resulted in the elongation of the action potential, reduction in K^+-currents, and an increase in the frequency of Ca^{+2} sparks within aged cardiomyocytes. Notably, following GLP activation, we have observed a triggering in a resurgence of Na channels, suggesting an increased susceptibility to arrhythmias. We have also observed a marked RyR destabilization in the presence of GLP activation and altered phosphorylation profile in Ca-removal proteins phospholamban and SERCA2a.
Conclusion: Incretin effects contribute to detrimental processes by disrupting the regulation of sodium-calcium handling, consequently elevating the susceptibility to arrhythmias in an aging heart. The inhibition of CK2 effectively counteracted the impacts of GLP1 agonism, indicating a promising avenue for translation in the treatment of cardiovascular ailments.

Key words: aging, GLP-1 agonism, Na^+-Ca^{++} handling

THE METABOLIC ROLES OF GRK2 IN CARDIAC PATHOLOGIES

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Background & Aim: G protein-coupled receptor kinase 2 (GRK2) is a multifunctional kinase that plays a pivotal role in regulating G protein-coupled receptor (GPCR) signaling. While initially recognized for its role in desensitizing GPCRs, emerging research has highlighted the significant involvement of GRK2 in various cardiac pathologies beyond its canonical signaling functions. One such role that has garnered increasing attention is the impact of GRK2 on cardiac metabolism.
Materials & Methods: Original studies performed by our group and literature data are reviewed.
Results. The influence of GRK2 on cardiac metabolism is multifaceted and extends beyond its traditional role in GPCR desensitization. In pathological conditions such as heart failure, myocardial infarction, and diabetic cardiomyopathy, GRK2 expression and activity are often elevated. Following cardiac injury, GRK2 has been shown to translocate to mitochondria where it mediates regulatory processes involved in cellular energy metabolism, oxidative stress response, and apoptotic signalling pathways. Mitochondrial GRK2 functions as a pro-death kinase, contributing to aggravated calcium signaling, increased opening of the mitochondrial permeability transition pore (mPTP), and impaired mitochondrial function. Therapeutic strategies limiting GRK2 localization to the mitochondria have emerged as potential avenue to mitigate the metabolic dysregulation observed in cardiac diseases.
Conclusion. In conclusion, the evolving understanding of GRK2's role in cardiac pathologies, specifically its impact on cardiac metabolism is crucial. Studies on the connections between GRK2 and metabolism, paves the way for the development of novel therapeutic interventions that target GRK2 to ameliorate metabolic derangements and improve cardiac function in various pathological states.

Key words: GRK2, mitochondria, heart, cardiac metabolism

Funding: The author is supported by TUBITAK-SBAG 121S487.

THE INCREASED EXPRESSION OF EXTRACELLULAR VESICLES IN PATIENTS WITH RHEUMATOID ARTHRITIS: NOVEL POTENTIAL BIOMARKER FOR DISEASE MONITORING?

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Background & Aim: The pathogenesis of rheumatoid arthritis (RA) is complex and comprises interactions between genetics, epigenetic modifications, and environmental factors, contributing to the systemic inflammatory response. Recently, the role of microparticles was implicated in the pathogenesis of RA. Microparticles, also called extracellular vesicles (EVs), are small membrane-coated vesicles 0.1–1.0 μm in diameter that are released from various cells during cell activation and apoptosis. EVs also have important procoagulant properties based on the availability of phosphatidylserine (PS) exposed on the surface after stimulation. The aim was to identify different subpopulations of EVs in the plasma of RA patients in relation to the activation of coagulation and fibrin formation.
Materials & Methods: Twenty women with established RA were included in the study (mean age 51.85 ± 9.43 years), mean disease duration 13.0 ± 6.6 years with medium to high disease activity (DAS28 was 4.1 ± 1.2). The PS^+ EVs; platelet ($CD42a^+$), leucocyte ($CD45^+$), monocyte ($CD14^+$) and endothelial ($CD144^+$)-derived EVs as well as and EVs-expressing tissue factor ($CD142^+$), P-selectin ($CD62P^+$) and E-selectin ($CD62E^+$) were determined by flow cytometry analysis. Overall hemostasis potential (OHP) was assessed to follow the hemostatic disturbances, including the parameters for overall coagulation potential (OCP) and overall fibrinolytic potential (OFP).
Results: Increased concentrations of PS+, CD42a+, CD142+, CD45+, CD14+, and CD62P+ EVs were found in plasma from patients with RA compared to healthy controls. EVs were positively correlated with the inflammatory parameters in RA patients. Positive correlations were also found between the levels of EVs and OCP and OHP. The levels of EVs were negatively correlated with OFP.
Conclusion: Elevated levels of circulating EVs of different cell origins were found in patients with established RA, in relation to the inflammatory burden and coagulation activation in the disease.

Key words: rheumatoid arthritis; extracellular vesicles; inflammation; coagulation

RAS INHIBITION THERAPY IN DIALIZED PATIENTS AND THEIR EFFECTS IN POSITIVE REMODELING

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Background: A haemodialysed patient has a particular cardiac and vascular condition with high cardiovascular risk with the disease growth rapidly because his metabolic condition - acidotic milieu, the entire enzimatic mechanism favoring the atherogenesis and oxidative stress. The angiogenesis develops slower than hypertrophy so oxigen diffusion is prolonged at least 25%. High blood pressure precipitates left ventricular hypertrophy which also represent a high risk for sudden cardiac death due to arrhythmic and ischaemic events. The aim of this study is to investigate the impact of blood pressure control with ACE inhibitors or AT1 receptor blockers both strategies known to be efficient in reducing left ventricular hypertrophy.
Materials & Methods: We studied 1200 dialysed patients with mean follow-up of 3 years. Mean age 57.8±2.3 years old, 52% men, being on dialysis treatment of the average duration of 6.5±2.3 years. We assessed the impact of different medications on survival. Those which had the most important impact were also those which proved to induce positive remodeling. Efficient hemodialysis procedure is an important step in order to control high blood pressure but LV hypertrophy of hypertensive dialysed patient (DP) has some peculiarities.
Results: ACE inhibitors and betablockers at the highest tolerated dose decreased cardiovascular morbidity and mortality and improved quality of life. Small doses of these drugs proved to be effective even in patients where hemodialysis alone was enough to control blood pressure. ACE inhibitors reduced hypertrophy and improved diastolic filling.
Conclusion: Antiischemic treatment, active surveillance of arrhythmias, control of blood pressure and electrolytes are of paramount importance. Changes in treatment according to guidelines improved blood pressure control, echo parameters and dialysis flow.

Key words: RAS inhibition therapy, dialysis, ventricular remodeling

UNUSUAL FORM OF LEFT VENTRICULAR TUMOR

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Intratrial and, in particular, intraventricular heart tumors often rise diagnostic and therapeutic challenges. We present the case of a 42-year-old patient with multiple cardiovascular risk factors (hypertension, dyslipidemia, smoking) in whom the routine echocardiographic examination revealed a pediculate, floating formation in the left ventricle. The patient is known to have a history of coronary artery disease for which he was thrombolyzed on the LAD and stented on the RCA 10 and 5 years ago, respectively. Also, the patient is known to have thrombangeitis obliterans at the lower limbs. A transmitral excision was performed, revealing a tumoral formation of approx. 5/2 cm, at which the histological examination described an amorphous cardiac tumor with an hyalin/fibrinoid appearance, with areas of dystrophic calcification. Genetic examination of thrombophilia risk revealed a genetic profile favoring thrombembolic events and decreased fibrinolytic activity (MTHFR A 1298C and PAI-1 4G/5G – heterozygotic forms). The particularity of the case consists in the association of a large, pediculate formation at the level of the LV with genetic changes that advocate for thrombembolic complications.

CONCURRENT CAUSES FOR ACUTE SEVERE RIGHT VENTRICULAR FAILURE IN AN APPARENTLY HEALTHY PATIENT

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A 52 year-old man, heavy smoker, with previous medical history of diabetes and obesity was presented to the emergency department with acute dyspnea, chest pain and cardiogenic shock. The echocardiography revealed the presence of pericarditis with cardiac tamponade and severe pulmonary hypertension. The lung CT angiography showed multiple bilateral filling defects (central, segmental, and subsegmental) with features compatible with acute PE and also signs of pre-existing CTEPH, mixed emphysematous dystrophy and mediastinal polymacroadenopathy. A pleuro-pericardial window was urgently performed with the evacuation of 850 mL of sero-hemorrhagic pericardial fluid, with characteristics of exudate and positive cytology for adenocarcinoma. Laboratory tests revealed very high values for D-dimers and inflammatory syndrome. The genetic profile of the thrombophilia risk revealed the heterozygous MTHFR A1298C variant, PAI-1 4G/4G homozygous genotype and EPCR with the presence of A2/A2 alleles (they represent a cardiovascular risk factor, the decrease in fibrinolytic activity). Further investigations ruled out lung, pancreas, prostate, gastric and colon cancer. Collagenosis, sarcoidosis, vasculitis, infectious pathology (including HIV, tuberculosis) were also excluded. A mild form of COPD and a moderate form of obstructive sleep apnea syndrome (IAH=25.1/hour) were confirmed. Urgently was initiated chronic anticoagulant treatment, diuretics, calcium blocker, bronchodilators and AutoCPAP, The right heart catheterisation confirmed precapillary pulmonary hypertension (PAPs/d=29/14mmHg, PAPm=20mmHg, PCW=11mmHg, RVP=3.28mmHg/l/min, RV dilated with mild wall hypertrophy). After three months the patient evolution was good, with stable general condition (no clinical signs of cancer or autoimmune disease) and with mild pulmonary hypertension. Regular follow-up with a CTEPH expert was indicated to assess the need for surgical intervention or/and PH treatment. In conclusion, acute right ventricular failure can be fatal by the association of different physiopathogenic mechanisms induced by undiagnosed chronic pathologies (in this case an acute PE superimposed on a CTEPH, OSA, COPD and thrombophilia mutations).

Key words: right ventricular failure, chronic thromboembolic disease, pulmonary hypertension

VITAMIN D MITIGATES OXIDATIVE STRESS IN VARICOSE VEINS EXPLANTS FROM OBESE AND NON-OBESE PATIENTS

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Background & Aim: Chronic venous disease and varicose veins of the lower extremities are common pathologies with high socio-economic impact with the ageing of the population. Elevated oxidative stress has been associated with vein wall injury, mainly in the setting of obesity. A high prevalence of varicose vein disease in obese patients has been reported to occur in association with a low plasma level of vitamin D. The aim of the current study was to evaluate the acute effects of $1,25(OH)_2$-D_3, the active form of vitamin D, on the oxidative stress in varicose veins explants from patients subjected to superficial veins surgery by cryostripping.
Materials & Methods: Patients were randomized into 2 groups, obese (n=12) and non-obese (n=17). Venous samples were incubated or not with $1,25(OH)_2$-D_3 (100 nM, 12 hours) and used for the assessment of: i) reactive oxygen species production by 2 methods (spectrophotometry - FOX assay for hydrogen peroxide and immune fluorescence - dihydroethidium probe for superoxide anion) and ii) nitric oxide synthases (endothelial-eNOS and inflammatory-iNOS) expression by qPCR.
Results: Acute incubation with $1,25(OH)2$-D_3 was responsible for the: i) reduction of reactive oxygen species generation, ii) up-regulation of eNOS and down-regulation of iNOS, in both obese and non-obese patients. Moreover, a significant reduction of $25(OH)$-D_3 serum levels was found in obese patients vs. non-obese patients that negatively correlated with the amount of H_2O_2 generated by the venous samples.
Conclusion: *Ex vivo* acute treatment with the active form of vitamin D mitigated the oxidative stress in the venous walls. Vitamin D might be useful in patients with varicose veins in short term administration prior to surgery but a careful dose titration is required.

Key words: chronic venous insufficiency, varicose veins, obesity, vitamin D, oxidative stress

EVOLUTION AND NEW ETIOLOGICAL TRENDS IN PATIENTS WITH PULMONARY ARTERIAL HYPERTENSION

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Background: Currently, approximately 50 diseases causing pulmonary arterial hypertension (PAH), heart failure and premature death are recognized, with varied treatments and prognosis.
Aim: To determine the current etiological trend and survival in PAH specific treated patients.
Method: A retrospective data analysis regarding patients treated in PAH Timisoara center of the national program in the 15 years of activity, between January 1st 2008 – December 31st 2022.
Results: A total of 112 patients were treated, with a mean inclusion age of 49.80±16.60 years, 62.83% women, 58% in NYHA class III. The PAH etiology distribution was 44.64% idiopathic, 31.25% congenital heart diseases (CHD), 11.60% CTEPH, 8.92% collagenosis and 3.57% with other causes (vasculitis, HIV associated, veno-oclusive disease). Patients had progressive access to specific medication (sildenafil, bosentan, ambrisentan, macitentan, riociguat, treprostinil, ralinepag/from clinical study). The majority were treated with monotherapy (70.53%, especially until 2020). Throughout the program, 62 deaths were recorded (representing 55.35% from total, with an average of 54.87±13.80 years at moment of death) and 10 patients were lost or transferred (9.82%). The average lifespan under PAH specific treatment was 4.13±3.33 years (14.16 years longest versus one month shortest). The best survival was in PAH secondary CHD (5.12±3.53) and the worst in collagenosis (2.87±2.07). At the end of 2022 the monotherapy percentage dropped to 40.90% and patients mean age increased to 53.13±17.09 years. The annual rate of newly diagnosed patients with PAH was 7.46, which severely decreased to 4.66 due to the pandemic between 2020-2022, but without impact on mortality rate. The incidence of CHD decreased (most were surgically solved), but the cases of CTEPH and rare disease increased thanks to diagnostic advances.
Conclusions: The etiology of PAH became more varied, the therapeutic options are more numerous, diverse and associated, and the survival rate undoubtedly increased.

Key words: pulmonary hypertension, etiology, trend, survival

ABSTRACTS OF THE POSTERS

1. MOLECULAR AUTOPSY IN LONG QT SYNDROME IN SUDDEN UNEXPLAINED DEATH CASES

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Background & Aim. In all cases of sudden unexplained death (SUD), forensic autopsy aims to identify the cause of death that is important for the risk prediction for the rest of the family members. But in almost 40% of SUD no definite cause of death is identified. It is known that an important percentage of SUD in young people aged 1-40 years old is caused by inherited arrhythmia syndromes or inherited cardiomyopathies. The aim of our study was to determine the susceptibility of long QT syndrome (LQTS) associated mutations in a cohort of 35 negative autopsy in sudden unexplained death (SUD).
Materials & Methods. Post-mortem DNA samples were isolated from the biological blood or tissue samples using the Maxwell RSC Whole Blood DNA kit (Promega, USA). The isolation was performed on the Automate DNA/RNA Maxwell RSC 48 System (Promega, USA). All biological samples were obtained during the autopsy procedure. Using polymerase chain reaction (PCR), and DNA sequencing on 3500 Genetic Analyzer (Thermo Scientific, USA), the genetic analysis was conducted for the genes (KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2) implicated in the pathogenesis of long QT syndrome.
Results. Mutations in genes KCNQ1, KCNH2, SCN5A, were more common among men 22 from 35 cases (62,85%) with than women 13 from 35 cases (37,15%). 8 cases were identified as having a family history of sudden death in first degree relative, thus suggesting a predisposition to cardiac arrhythmia.
Conclusion. Our data demonstrated that analyzing the mutational susceptibility to cardiac arrhythmia from postmortem samples can aid in establishing the cause of death and in the health management of the left relatives.

Key words: sudden unexplained death, long QT syndrome, molecular autopsy, genes, post-mortem

2. INVESTIGATION OF ACTION POTENTIAL ALTERNANS IN HUMAN HEART FAILURE

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Background & Aim: Heart failure is a progressive, multifactorial clinical syndrome leading to development of several life threating arrhythmias such as ventricular fibrillation. A possible mechanism contribute to arrhythmia development in heart failure is the so-called alternans, indicating periodic short-long oscillation of the action potential that leads to repolarization inhomogeneity. Alternans are considered suitable predictors of sudden cardiac death, however, there is no specific pharmacological intervention to avoid or reduce alternans. Here we test a novel, selective Na^+/Ca^{2+} exchanger inhibitor ORM-10962 on alternans development in human heart failure and in undiseased human hearts.
Materials & Methods: Left and right ventricular papillary muscles and trabecules were obtained from explanted hearts of heart failure patients undergoing cardiac transplantation. Undiseased human hearts were obtained from an organ donor after removal of pulmonary and aortic valves for transplant surgery. Action potentials were measured by conventional microelectrode technique. Alternans were evoked by rapid pacing from 700 ms to 250 ms. All experiments were made at 37 °C.
Results: Results show moderate action potential duration (APD) alternans in human heart failure which was unaltered after application of 1 μM ORM-10962. The magnitude of APD alternans was identical with APD alternans recorded from undiseased human heart. However, failing hearts often exerted action potential amplitude alternans, typically in higher pacing rates, leading to 2:1 conduction block then electrical disturbance. It seems that 1 μM ORM-10962 delays the development of action potential amplitude alternans.
Conclusion: These data indicate that APD alternans in human heart failure are not increased compared to undiseased human hearts. In contrast, human failing hearts often exert large action potential amplitude alternans leading to dynamic conduction block and irregular electric activity that could be important link between sudden cardiac death and alternans. Selective NCX inhibition may decrease amplitude alternans presumably due to a Ca^{2+}-dependent mechanism however, it requires further experiments.

Key words: heart failure, arrhythmia, alternans, Na^{2+}/Ca^{2+} exchanger, ORM-10962

Funding: TKP2021-EGA-23, RRF-2.3.1-21-2022-00003, K134939

3. PERIOSTIN KNOCK OUT IS CAUSAL TO DIFFERENTIAL RESPONSES AMONG MALE AND FEMALE MICE: CARDIAC EXTRACELLULAR MATRIX PROTEINS AND SURVIVAL

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Background & Aim: Ischemic heart disease presaged by myocardial infarction (MI) remains a significant cause of heart failure (HF). HF is often preceded fibroblast activation to myofibroblasts and by enhanced deposition of the extracellular matrix (ECM). Myofibroblasts secrete periostin (POSTN), a matricellular protein. Periostin is reexpressed in the adult heart subsequent to pathological injury ~ day 4 post-MI to support wound healing by the promotion of collagen fibrogenesis. We examined sex-related distinctions in mice with a periostin knockout (POSTN KO) phenotype, particularly emphasizing variations in the reconfiguration of the ECM subsequent MI.
Materials & Methods: An analysis was conducted on a cohort of 42 wild-type (WT) and 78 POSTN KO mice. The POSTN KO phenotype was confirmed using quantitative polymerase chain reaction (qPCR), Western blotting (WB), and immunohistochemistry (IHC). A subset of genes associated with collagen fibrogenesis and collagen crosslinking were explored via the use of qPCR.
Results: After MI, male POSTN KO mice exhibited a survival rate of 15.4% (±7.95%) at 1-week post-MI, contrasting with significantly higher survival of 66.7% (±9.01%) in females POSTN KO (*$P < 0.001$), as determined by log-rank test. Cardiac rupture was linked to mortality in all cases. Additionally, analysis of the infarct scar area (5 – 7 days post-MI) revealed significant overexpression of the Fmod gene, coding for fibromodulin protein, in female POSTN KO *vs* Male POSTN KO.
Conclusion: Reduced periostin secretion is associated with a heightened susceptibility to cardiac rupture in male mice compared to female counterparts. Notably, periostin contributes to acute wound healing after MI in both male and female hearts; however, males display a greater sensitivity to its absence. The data from this study supports the hypothesis that sex-related variations in post-MI cardiac wound healing within POSTN KO mice are dependent upon differential fibromodulin expression in females *vs* males.

Key words: sex differences, survival rate, periostin, cardiac extracellular matrix, myocardial infarction

4. METFORMIN ATTENUATES HUMAN ATRIAL FIBROBLAST ACTIVATION

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Background: Structural remodelling, particularly atrial fibrosis, drives the persistence of atrial fibrillation and is a major contributor to its high therapeutic resistance This study explores the antifibrotic potential of metformin as a possible upstream approach to AF management.
Objective: To investigate the antifibrotic potential of metformin on human atrial fibroblasts (HAFs).
Methods: Human atrial fibroblasts, cultured and subjected to control media or media containing 100µM metformin, were assayed on days 1, 2, and 4. Gene expressions related to fibroblast state and extracellular matrix remodelling were investigated, as were procollagen-I production and mammalian target of rapamycin (mTOR) activity.
Results: Metformin modulated HAF gene expression, upregulating Transcription Factor 21, a marker of resting fibroblasts (mean ΔΔCT ± SD on day 4 = 2.4 ± 0.6, p=<0.001) and supressing TGF-ß1 (ΔΔCT on day 1 = 0.77 ± 0.04, p=0.046) and COL3A1 (ΔΔCT on day 4 = 0.45 ± 0.05, p=0.005). Additionally, procollagen-1α1 levels were markedly reduced in metformin treated cells (20.54 pg/mL vs 222.26 pg/mL on day 4, p<0.001). The activity of mTOR, indicated by Thr389 phosphorylation, declined in metformin-treated cells across days 1 to 4, reaching 66% (95% Cl = [0.54;0.77] of control by day 4 (p = 0.003).
Conclusion: Results from this preliminary study suggest that metformin may reduce HAF activation *in vitro*, possibly through an mTOR-inhibitory mechanism. This highlights metformin's potential role in preventing atrial fibrillation-associated structural remodeling and underscores the need for further *in vitro* investigations.

Key words: atrial fibrillation, metformin, atrial fibroblasts, structural remodelling, fibrosis

5. THE HEART-COVID CONNECTION: AUTONOMIC DYSFUNCTION - HEART RATE VARIABILITY AND ATRIAL FIBRILLATION

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Background & Aim: The COVID-19 pandemic has shown not only the respiratory distress caused by the SARS-CoV-2 virus but also its multifaceted impact on various physiological systems, including the cardiovascular system. Data has shown a strong connection between the presence of the SARS-CoV2 infection and cardiovascular pathology generated especially from autonomic disturbance. Concomitant with autonomic dysfunction, there has been an increasing recognition of the link between COVID-19 and supraventricular arrhythmias, atrial fibrillation (AFib) being the most common rhythm disturbance in clinical practice. Emerging evidence suggests that the systemic inflammation caused by viral infection, and direct cellular damage may contribute to the initiation and perpetuation of these arrhythmias. A non-invasive method of evaluating autonomic function is the use of heart rate variability (HRV). The aim of the present study was to evaluate the relationship between COVID-19 and cardiovascular pathology, with a specific focus on autonomic dysfunction and supraventricular arrhythmias.
Materials & Methods: This retrospective study includes patients of the Cardiology clinic starting from November 2020, which were diagnosed with SARS-CoV2 infection or were in the first month post-COVID19. The patients were evaluated at admission and further after 1 to 6 months post-COVID19. ECG monitoring was obtained with HRV being evaluated in time-domain. The presence or absence of AFib, response to cardiological treatment, persistence or conversion to sinus rhythm, inflammatory markers and severity of SARS-CoV2 were assessed.
Results & Conclusion: HRV was significantly lower at the initial evaluation of symptomatic patients with SARS-CoV2 infection and new diagnosis of atrial fibrillation. The difficult rate control occurring at the onset or acute period of SARS-CoV2 highlights a close temporal relationship between the viral infection, inflammatory status and both autonomic dysfunction and supraventricular arrhythmias.

Key words: heart rate variability, atrial fibrillation, COVID19

6. THE EFFECTS OF HYPERBARIC OXYGENATION AND INSULIN TREATMENT ON THE MYOCARDIAL FUNCTION AND OXIDATIVE STRESS IN DIABETIC RATS

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Background & Aim: Diabetes mellitus (DM) is a chronic metabolic disorder characterized by impaired glucose homeostasis, oxidative stress, and increased risk of cardiovascular complications. The management of diabetes includes lifestyle modifications, pharmacological interventions, and emerging therapies such as Hyperbaric Oxygen Therapy (HBOT). HBOT involves exposing patients to high levels of oxygen in a pressurized chamber, leading to various physiological effects.
Materials & Methods: This study investigated the therapeutical effect of hyperbaric oxygenation (HBOT) on the cardiovascular system and oxidative stress status in rats with streptozotocin-induced diabetes. We divided Wistar albino rats into four groups: DM group (diabetic rats), DM+HBOT group (diabetic rats exposed to 100% oxygen 1 hour per day, five days a week, at 2.8 ATA for two weeks), DM+ INS group (diabetic rats treated with NPH insulin at a dose of 5 U/day), and DM+ HBOT +INS group (diabetic rats treated with both NPH insulin and oxygen under elevated atmospheric pressure for two weeks). During the study, we evaluated the glycemia levels, oxidative stress parameters and cardiac function parameters.
Results: Treatment with NPH insulin decreased blood glucose levels but not to values that could be considered normoglycemic. In the DM+ HBOT +INS, we measured the lowest values of pro-oxidation markers. Treatment with NPH insulin improved cardiac function, and the combined therapy contributed to an almost complete recovery of cardiac function in experimental animals.
Conclusions: Treatment with NPH insulin led to a reduction of hyperglycemia and improvement of cardiac function in diabetic rats. The combined therapeutical effect of NPH insulin with HBOT led to a decrease in pro-oxidation markers. These findings provide valuable insight into cardiovascular complications and oxidative stress in diabetes and could provide new opportunities for their potential remediation and treatment.

Key words: diabetes mellitus type 1, streptozotocin, hyperbaric oxygenation, neutral protamine hagedorn (NPH) insulin

7. MOLECULAR MECHANISMS BEHIND THE LATE CARDIOPROTECTIVE EFFECTS OF INORGANIC NITRITES

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The administration of inorganic nitrites 24 hours before occlusion and reperfusion (I/R) of the LAD ('late effect') significantly reduced the infarct size and the incidence of the life-threatening ventricular arrhythmias and increased survival (0% vs 50%) in dogs. The sudden increase of free radicals and the impaired calcium homeostasis are responsible for creating arrhythmias upon reperfusion. Our focus has centered on mitochondria due to their pivotal role in regulating both. Our previous results have revealed that sodium nitrite attenuated ROS production by partially suppressing the mitochondrial respiration, decreased the diastolic calcium level during reperfusion in simulated ischaemia, and prolonged the ERP/APD90, thereby contributing to the observed antiarrhythmic effects. We have now focused on the molecular mechanisms underlying the cardioprotective effects in this delayed protection, particularly the involvement of protein S-nitrosylation. In this study, we have determined the total protein S-nitrosylation (SNOs) in left ventricular tissue samples by the biotin switch method (ABC technique). Dog and rat left ventricular tissue samples were collected in the sham-operated and nitrite-treated groups (at 3 h, 6 h, 12 h, and 24 h). Our results have shown that sodium nitrite induces a significant increase in total protein SNOs levels after 24 hours in both dogs and rats compared to the sham-operated control group. Our results suggest that protein S-nitrosylation might play a role in the late antiarrhythmic effect of sodium nitrite. In the future, we aim to identify the individual proteins (targeting MCU and mNCX) altered by nitrite, providing further insights.

Key words: arrhythmia, S-nitrosylation, mitochondrial bioenergetics, biotin switch

8. ASSESSMENT OF FACTORS DECEPTIVELY LOWERING ACE ACTIVITY

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Elevated angiotensin-converting enzyme (ACE) activity supports the diagnosis of sarcoidosis and may be an indicator of diseases with macrophage activation, such as inflammatory bowel disease (IBD). Taking ACE inhibitor drugs (ACEI) and loss-of-function mutations can cause deceptively low ACE activity. With monoclonal antibodies (mAbs) we can detect subtle conformational changes in the enzyme due to inhibitors or mutations. The aim was to assess what factors (exo- or endogenous inhibitors, mutations, differences in glycosylation) may lower ACE activity making diseases get overlooked or the success of treatment interpreted incorrectly. Serum ACE activity of patients with sarcoidosis or IBD was measured by a fluorescent kinetic assay. The binding pattern of a set of mAbs and the ACE inhibition were determined. ACEIs significantly reduced serum ACE activity in the sarcoidosis population (median [interquartile range], treated with ACEI: 4.42 [2.93-6.75] U/L, n=302; untreated with ACEI: 11.32 [8.79-13.92] U/L, n=1521, $p<0.01$. Serum ACE was significantly higher in patients with sarcoidosis (11.84 [10.1-13.5] U/L, n=80) than in controls (9.19±2.1 U/L, n=133). A similar result was observed in IBD. Few patients with IBD had saliently low ACE activity (2.26 U/L, n=2) compared to the IBD population (9.445±3.3 U/L, n=190), where the effect of ACEI can be excluded. The binding of mAb 2D1 and 5F1 in the N domain, both sensitive to sialylation to sites Asp45 and Asp117, were significantly lower compared to the control. This raises the possibility of a genetic mutation in the exons of the ACE gene encoding these amino acids. Determination of serum ACE activity is important in establishing the diagnosis of sarcoidosis, although ACEIs and genetic mutations make correct assessment difficult. The ACE activity measurement we use with mAbs is a new tool in our hands that may shed light on some interindividual differences and is a promising way towards personalised medicine.

Key words: ACE, angiotensin-converting enzyme, diagnostics

Supported by the ÚNKP-22-3-I-DE-256 New National Excellence Program of the Ministry of Culture and Innovation from the source of the National Research, Development and Innovation Fund.

9. SGLT2 INHIBITORS REDUCE MONOAMINE OXIDASE EXPRESSION AND OXIDATIVE STRESS IN HUMAN ATRIAL TISSUE: A NOVEL OFF-TARGET CLASS EFFECT

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Background & Aim: Monoamine oxidases (MAOs) are mitochondrial enzymes with 2 isoforms, MAO-A and MAO-B, responsible for biogenic amines (norepinephrine, serotonin) degradation with the constant generation of hydrogen peroxide. An increasing body of evidence has documented their contribution to the oxidative stress in cardiometabolic pathologies. Sodium-glucose-cotransporter 2 inhibitors (SGLT2i) elicit cardiovascular protection via several pleiotropic, partially elucidated effects. The aim of the current study was to investigate the contribution of MAO to the cardiac oxidative stress in human atrial tissue and investigate whether SGLT2i, dapagliflozin and empagliflozin can alleviate it.
Materials & Methods: Right atrial appendages were isolated from patients subjected to elective open-heart surgery harvested from non-diabetic patients with HF with mildly reduced ejection fraction (HFmrEF, EF = 41-49%) and used for the evaluation of MAO gene expression (qPCR) and hydrogen peroxide production (Ferrous OXidation xylenol orange, FOX assay). Experiments were performed after incubation with dapagliflozin or empagliflozin (1μM, 10 μM; 12 h).
Results: Both MAO isoforms are expressed in human atrial tissue. Incubation with either dapagliflozin or empagliflozin mitigated oxidative stress and downregulated MAO expression in a dose dependent manner, suggesting a novel off-target class effect of these drugs.
Conclusion: In conclusion, MAO contributes to cardiac oxidative stress that can be acutely targeted by SGLT2i in non-diabetic patients with HFmrEF.

Key words: human atrial tissue, monoamine oxidase, oxidative stress, dapagliflozin, empagliflozin

10. FIBROSIS IN THE HEART OF THE MOST COMMONLY USED ANIMAL MODELS IN ATRIAL FIBRILLATION RESEARCH – A COMPARATIVE STUDY

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Background & Aim: Atrial fibrillation (AF) is the most common arrhythmia in humans, affecting more than 43.6 million people worldwide, and is associated with a higher risk of stroke and heart failure. The rising prevalence proves the need for a better understanding of the mechanisms underlying AF and especially structural changes, which are believed to drive the progression of the disease. Such studies can be achieved by using translational animal models. However, already published studies are characterized by significant variation in the amount of fibrosis, both between studies and species, making it challenging to identify the most appropriate animal model. The aim of this study was to investigate and compare the fibrotic content in mice, rats, goats, pigs, and horses in the healthy heart.
Materials & Methods: Quantification of fibrosis was performed using a robust reproducible histological analysis with samples from the left and right atrial appendage and free wall from all animals. The samples were stained with picrosirius red and fully automated software was used to quantify the level of fibrosis.
Results: The average amount of fibrosis found in the left atrium on average (mice: 12.8±2.0%, rats: 13.3±0.6%, goats: 16.3±1.6%, pigs: 18.8±0.9% and horses: 9.1±0.6%) was significantly lower in horses compared to pigs, goats and rats ($p<0.05$). In the right atrium the average amount of fibrosis (mice: 17.9±1.7%, rats: 14.1±1.5%, goats: 20.0±2.3%, pigs: 21.0±0.6% and horses: 6.9±0.3%) was significantly lower in horses when comparing to all four remaining species in the study ($p<0.05$).
Conclusion: The mice, rats, goats and pigs present with significantly higher fibrotic content than the horses. Horses, the only animal in the study known to develop AF spontaneously, have more fibrosis in left atrium than right atrium.

Key words: atrial fibrillation, atrial fibrosis, comparative study, histology, translational model

11. A POSSIBLE EXPLANATION FOR THE LOW PENETRANCE OF PATHOGENIC KCNE1 VARIANTS IN LONG QT SYNDROME TYPE 5

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Long QT syndrome type 5 (LQT5) is caused by dominant mutant variants of KCNE1, an important regulatory subunit of the I_{Ks} channel. While mutant LQT5 KCNE1 variants are known to inhibit I_{Ks} amplitudes in heterologous expression systems, cardiomyocytes from a transgenic rabbit LQT5 model showed unchanged I_{Ks} amplitudes, suggesting that additional factors play a critical role in the development of the LQT5 phenotype in vivo. In this study, we explore the effect of KCNE3 on the development of the LQT5 phenotype. KCNQ1, the pore-forming subunit of the I_{Ks} channel, WT-KCNE1, LQT5-KCNE1 variant (G52R-KCNE1) and KCNE3 were co-expressed in different combinations. The currents were characterized by whole-cell patch clamp technique. The NanoBiT structural complementation assay was applied to explore whether KCNE3 and KCNE1 co-assemble in the same ion channel complex or whether they are represented in a distinct ion channel population. Average current density was significantly lower in group 3 (KCNQ1+WT-KCNE1+G52R-KCNE1) compared to the group 1 (KCNQ1+WT-KCNE1) and group 2 (KCNQ1+WT-KCNE1+KCNE3). However, the mean current density in the presence of KCNE3 was significantly increased in group 4 (KCNQ1+WT-KCNE1+G52R-KCNE1+KCNE3) compared to the group 3. The KCNQ1 and KCNE1 were co-expressed with varying amount of KCNE3 for the NanoBiT experiments. The KCNQ1:KCNE1:KCNE3 ratio was 1:2:0 (group 1), 1:2:1 (group 2) and 1:2:2 (group 3). Average relative luminescence (RLU) was 194 in group 1 which was not significantly different from group 2 (129.3 RLU). However, group 3 showed significantly lower RLU (96.7) compared to group 1. We conclude that KCNE3 rescues the inhibitory effect of the LQT5-KCNE1 variant on I_{Ks} in vitro. Furthermore, KCNE3 is probably able to replace KCNE1 within the macromolecular complex of the I_{Ks} ion channel, therefore, KCNE3 possibly modulate the development of the disease phenotype in LQT5 patients.

Key words: LQT5, I_{Ks} channel, KCNE3, NanoLuc® Binary Technology

12. PLATELET MITOCHONDRIAL RESPIRATORY DYSFUNCTION IN SEPSIS AND COVID-19 IS ALLEVIATED BY CELL-PERMEABLE SUCCINATE

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Background & Aim: Sepsis is a severe pathological condition caused by different pathogens, including COVID-19. A major hallmark of severe COVID-19 patients is the acute inflammation with cytokine storm associated with multi-organ dysfunction. Mitochondrial dysfunction has been linked to multi-organ failure in patients with sepsis. Cell-permeable succinates are novel synthetic compounds able to rescue electron transport system impairment by improving complex II-supported respiration. The present study of mitochondrial respiratory function in platelets was double-aimed: i) to assess mitochondrial respiratory dysfunction in permeabilized platelets isolated from patients with sepsis and COVID-19 and ii) to investigate the effect of NV118, a cell-permeable succinate, on respiratory parameters in intact platelets.
Materials & Methods: Participants were included in two main groups: I) the sepsis study group (n=10) and II) the COVID-19 study group further subdivided into moderate (n=5) and severe (n=14) disease. Mitochondrial respiration was assessed by means of high-resolution respirometry according to a protocol adapted to measure complex I and complex II-dependent respiration. The main respiratory parameters were: routine respiration, active respiration and the maximal uncoupled respiration. Intact platelets of each main group were acutely exposed to NV118 (vs DMSO as solvent) with the assessment of the following respiratory rates: routine, LEAK and maximal uncoupled respiration.
Results: Permeabilized platelets harvested from patients with sepsis showed a significant decrease of all respiratory parameters as compared to the age-matched controls. The severe forms of COVID-19 infection expressed a significant decrease in platelet active respiration for both respiratory complexes and an increase of routine, leak and uncoupled respiration. Moderate forms of disease also presented a significant decrease in active respiration, particularly for CI. Cell-permeable succinate elicited a significant increase of the respiratory parameters for both mitochondrial complexes.
Conclusion: Platelet mitochondrial respiratory function is globally depressed in patients with sepsis, whereas in COVID-19 infection, mitochondrial respiration was influenced by the severity of the disease. Cell permeable pro-drug succinate alleviated mitochondrial respiratory dysfunction in both conditions.

Key words: mitochondrial respiration, platelets, sepsis, COVID-19, cell-permeable succinate

13. HESPERETIN DECREASES THE REPOLARIZATION RESERVE AND INHIBITS THE SLOW DELAYED RECTIFIER POTASSIUM CURRENT (I_{KS}) IN DOG AND RABBIT CARDIAC VENTRICULAR MUSCLE PREPARATIONS AND ISOLATED MYOCYTES: A PROARRHYTHMIC CANDIDATE

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Background & Aim: Hesperetin is the main flavonoid in oranges. Flavonoids are known to reduce cardiovascular mortality, however, their effects on cardiac electrophysiology may have both antiarrhythmic and proarrhythmic consequences as they can attenuate the repolarization reserve. The present work aimed to study the inhibitory effect of Hesperetin on the repolarization of the action potential duration (APD) in dog and rabbit ventricular preparations with normal and attenuated repolarization reserve. The hesperetin effect on different cardiac transmembrane currents was also investigated.
Materials & Methods: Action potentials were recorded in dog right ventricular preparations using conventional microelectrode techniques. To attenuate the repolarization reserve, Dofetilide 0.1 μM (I_{Kr} blocker) and Veratrine 50 μg (late Na^+ channel activator) were added both together to the tissue bath. Transmembrane currents were measured using the whole-cell configuration of the patch-clamp technique at 37°C.
Results & Discussion: Hesperetin 10 μM alone has no notable effect on action potential duration (APD), however, during the impaired repolarization reserve, 10 μM Hesperetin caused a significant prolongation of the steady APD (from 466±18 ms to 512±23 ms (n=14), $p<0.05$). In agreement with APD data, a moderate but statistically significant inhibitory effect of 10 μM Hesperetin was observed on the transmembrane I_{Ks} ((n=6), $p<0.05$). 10 μM Hesperetin did not but 30 and 60 μ m hesperetin significantly reduced the amplitude of I_{Kr} ((n=6), $p<0.05$), ((n=5), $p<0.05$). I_{K1} current was not significantly affected by 10 μM Hesperetin but 30 and 60 μM Hesperetin significantly reduced its amplitude ((n=9), $p<0.05$), ((n=3), $p<0.05$). 10 μM Hesperetin did not change but 30 and 60 μm hesperetin significantly decreased the amplitude of I_{to} ((n=9), $p<0.05$), ((n=3), $p<0.05$). 30 μM hesperetin not but 60 μM Hesperetin moderately but significantly decreased I_{NaL} in rabbit ventricular myocytes ((n=7), $p<0.05$).
Conclusion: Normally, in preparations where the repolarization reserve is intact, Hesperetin did not prolong the action potential duration, therefore the proarrhythmic risk of hesperetin is low among healthy people. However; if the repolarization reserve has been attenuated due to pharmacological block or some pathological conditions such as heart failure, genetic mutations, etc., a high amount of orange juice consumption might lead to an increased risk of ventricular arrhythmia due to the inhibition of the cardiac potassium currents and prolongation in the action potential duration resulting in enhanced dispersion of repolarization.

Key words: hesperetin, the slow delayed rectifier potassium current (I_{ks}), repolarization reserve

Funding: This work was funded by the National Research Development and Innovation Office (NKFIH K-119992, K-128851, and GINOP-2.3.2-15-2016-00006), the Ministry of Human Capacities Hungary (20391- 3/2018/FEKUSTRAT, EFOP-3.6.2-16-2017-00006-LIVE LONGER and EFOP 3.6.3-VEKOP-16-2017- 00009) and by the Hungarian Academy of Sciences. The GINOP and EFOP projects are co-financed by the European Union and the European Regional Development Fund.

14. ATRIAL FAT INFILTRATION POST MYOCARDIAL INFARCTION IN A GÖTTINGEN MINIPIG MODEL OF OBESITY

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Background & Aim: Myocardial infarction (MI) can contribute to alterations in atrial electrophysiology creating a substrate for atrial fibrillation (AF) post MI, but the mechanisms for these alterations are unexplored. Obesity enhances the accumulation of epicardial adipose tissue (EAT) and is an independent risk factor involved in the pathogenesis of AF. Enhanced EAT accumulation also leads to infiltration of adipocytes deep into the myocardium promoting fibrosis and leading to functional disorganization and the formation of a structural arrhythmogenic substrate. Whether obesity related atrial fat infiltration is further promoted upon MI has not been investigated yet. The aim of this study was to investigate the effects of obesity and MI on the development of the atrial arrhythmogenic substrate post MI.
Materials & Methods: 12 lean and 12 obese Göttingen Minipigs were enrolled in the study. MI was induced by 120 min occlusion of the left anterior descending coronary artery in 12 lean pigs and 9 obese pigs, while 3 obese pigs served as a sham operated controls. Tissue was harvested 8 weeks post MI from the right atrium free wall (RA), right atrial appendage (RAA), left atrium free wall (LA) and left atrial appendage (LAA) and fixed in 10% formalin and cryosectioned at 30µm before they were stained either with Sirius Red (fibrosis) or Oil Red O (adipocytes/fat). Total amount of fibrosis and fat including EAT was quantified using automated image segmentation. Furthermore, atrial myocardial fat infiltration was quantified excluding the EAT.
Results: Obesity tendentiously increases total atrial fat in RA, RAA, LA and LAA. MI further increases the total atrial fat in RA, RAA and LAA ($p<0.05$) in obese pigs with MI compared to lean pigs with MI. Furthermore, atrial myocardial fat infiltration in RA and LA was found to be significantly increased in obese pigs with MI compared to lean pigs with MI ($p<0.05$). The amount of total fibrosis remained unchanged at all sites investigated.
Conclusion: These data indicate that MI enhances atrial fat infiltration in obesity suggesting an additive effect of obesity and MI in the formation of an atrial arrhythmogenic substrate post MI. Furthermore, these data indicate that atrial fat infiltration predisposes atrial fibrosis formation.

15. ASSESSMENT OF DRUG-INDUCED MITOCHONDRIAL TOXICITY IN HUMAN PLATELETS ISOLATED FROM BUFFY-COAT

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Background & Aim: Ibuprofen and acetaminophen are two of the most commonly used over-the-counter analgesic and antipyretic drugs, while amiodarone is one of the most extensively prescribed antiarrhythmic agents. Mitochondrial impairment has been recognized as a central pathomechanism underlying the organ toxicity of these drugs. Assessment of mitochondrial respiratory dysfunction in peripheral platelets represents a novel non-invasive approach to characterise drug toxicity. The present study was aimed at investigating the acute dose-dependent effects of each drug on mitochondrial respiration in intact and permeabilized human platelets isolated from the buffy-coat.
Materials & Methods: Peripheral blood platelets were isolated from healthy blood donor-derived buffy-coat by differential centrifugations. Respiratory capacities of intact and digitonin-permeabilized cells were measured by high-resolution respirometry using the Oxygraph-2k (Oroboros Ltd) in the presence of increasing concentrations of acetaminophen (0.1-10mM), ibuprofen (0.05-3mM) or amiodarone (60-930µM).
Results: In both intact and permeabilized cells, each drug elicited a concentration-dependent reduction of oxygen consumption. While acetaminophen and ibuprofen inhibited mostly complex I-supported active respiration, amiodarone was particularly toxic for complex II-supported respiration.
Conclusion: Acute exposure to acetaminophen, ibuprofen or amiodarone induced a dose-dependent inhibition of respiration in human platelets. Whether these results can be recapitulated in patients treated with these medications is worth further investigation as potential peripheral biomarker of mitochondrial damage of drug overdose.

Key words: mitochondrial respiration, acetaminophen, ibuprofen, amiodarone, platelets, buffy coat

16. INVESTIGATION OF THE EFFECTS OF THE NEXT-GENERATION MYOSIN INHIBITOR AFICAMTEN ON CANINE LEFT VENTRICULAR ISOLATED CARDIOMYOCYTES

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Background & Aim: Hypertrophic cardiomyopathy (HCM) is one of the most common inherited cardiovascular diseases and the leading cause of sudden cardiac death in young adults. Myosin inhibitors are used to reduce disease-specific hypercontractility by directly inhibiting the myosin motor. The present study is focused on aficamten, a next-generation selective myosin inhibitor. Our aim was to investigate the concentration-dependent effects of aficamten on contractility of isolated cardiomyocytes.
Materials & Methods: Enzymatically isolated canine left ventricular myocytes were loaded with FURA-2-AM, a Ca^{2+}-sensitive fluorescent dye. Contractions and relaxations were recorded at baseline and following exposure to a range of aficamten concentrations (0.1 μM - 1 μM) at room temperature. Cardiomyocyte contractions were induced by field excitation at 0.5 Hz, while changes in sarcomere length and in intracellular Ca^{2+} concentrations were monitored in parallel.
Results: Both contraction duration and systolic ejection time were shortened in the presence of 1 μM aficamten (0.90±0.03 s vs. 0.48±0.14 s and 0.67±0.02 s vs. 0.40±0.04 s, respectively, $P<0.05$ for both, n=41), while the kinetics of contractions and relaxations were both decelerated (1.08±0.10 μm/s vs. 0.31±0.11μm/s and 1.38±0.09 μm/s vs. 0.24±0.09 μm/s, respectively $P<0.05$ for both, n=41). The negative inotropic effect of 1 μM aficamten was characterized by a reduction in fractional shortening (14.56±0.62% vs. 2.95±0.83%, $P<0.05$, n=41). Treatment with aficamten did not alter intracellular Ca^{2+}-levels, regardless of the applied drug concentration.
Conclusion: Our results suggest that aficamten exerts its negative inotropic effect through a combination of shortened contraction duration and systolic ejection time and slowed kinetic parameters. These data predispose aficamten, a selective myosin inhibitor, as a potential therapeutic option to mitigate HCM-induced hypercontractility.

Key words: negative inotropy, HCM, aficamten, myosin inhibitor, isolated cardiomyocytes

17. THROMBOPHILIA AND VENOUS THROMBOEMBOLISM IN YOUNG PATIENTS

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Background and Aim: Venous thromboembolism with two clinical presentations, deep vein thrombosis and pulmonary embolism, can occur in the presence of thrombophilia associated with other risk factors even in young patients.
Materials & Methods: Painful unilateral oedema of lower limb, dyspnoea, chest pain and syncope are part of the clinial presentation of venous thromboembolism and need further investigation through electrocardiogram, echocardiography, lower limb compression ultrasonography and CT pulmonary angiogram for confirmation of diagnosis and further management.
Results: Young patients with an episode of venous thromboembolism in the presence of risk factors for VTE such as major trauma and post-partum period, could be carrier of some form of hereditary thrombophilia. ECG changes of right ventricular strain such as inversion of T waves in leads V1-V4, incomplete or complete right bundle branch block or the S1Q3T3 pattern are found in more severe cases of pulmonary embolsim. Sinus tachycardia and atrial arrhythmias can also be found on ECG. Echocardiography can detect right ventricular pressure overload and dysfunction, decreased TAPSE, flattened intraventricle septum, the 60/60 and McConell sign even in young patients with intermediate or high risk pulmonary embolism. In patients with low risk PE echocardiographic results can be normal.
Conclusion: Even though thrombophilia is a moderate risk factor for venous thromboembolism, young patients with confirmed diagnosis and family history of VTE associated with transient moderate or strong risk factors for VTE should be tested for thrombophilia in order to decide the best strategy for long-term anticoagulation treatment.

Key words: young age venous thromboembolism, thrombophilia, echocardiography

18. INVESTIGATION OF THE BIOMARKER ROLE OF ANGIOTENSIN-CONVERTING AND CHITOTRIOSIDASE ENZYMES IN VARIOUS INFLAMATORY DISEASES

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Background & Aim: Inflammatory bowel disease (IBD) is a term that describes disorders involving chronic inflammation of tissues in the digestive tract. Types of IBD include Crohn's disease (CD) and ulcerative colitis. In macrophage-mediated inflammatory diseases such as IBD, activated macrophages can produce high amounts of angiotensin-converting enzyme (ACE) and chitotriosidase enzyme (CTO), whose activity can be measured in serum. The aim of our study was to investigate the role of serum ACE and CTO activities as biomarkers in IBD and to examine their correlation with drug therapy.
Materials & Methods: Patients with IBD were included in the study between 2019 and 2022 and their serum ACE and CTO activities were determined by fluorescent kinetic assays. Patients' clinical data and medication were recorded and evaluated.
Results: A total of 70 patients were selected during the study period, of which 6 patients' results were not evaluated due to missing data. In 5 (8%) cases ACE inhibitor (ACEI) effect could be observed, resulting a significant decrease in serum ACE activity compared to patients not taking ACEI (median [interquartile range]: 3.53 [2.09-8.95] U/L vs. 8.63 [5.86-10.09] U/L; $p<0.05$, respectively). Oral steroid therapy significantly decreased serum ACE activity among patients not taking ACEI (5.26 [4.70-7.73] U/L, n=12 vs. 8.96 [7.38-10.37] U/L, n=47; $p<0.0005$, respectively). ACE activity showed significant correlation with BMI (Pearson $r=0.3126$; $p<0.05$) and CTO activity with age (Spearman $r=0.3371$; $p<0.05$). Serum ACE and CTO activities were significantly higher in smokers with CD (11.29 [10.00-13.30] U/L; 667.10 [550.90-902.90] mU/L, n=6 vs. 8.50 [6.63-10.14] U/L; 320.60 [192.00-693.80] mU/L, n=20; $p<0.05$, respectively).
Conclusion: Serum ACE and CTO activities can be good biomarkers in IBD. ACE activity is suitable for monitoring the effect of steroid and ACEI therapy in clinical practice. Moreover, the elevation of these biomarkers highlights us the enhancing effect of smoking on macrophage-mediated inflammation in CD.

Key words: ACE, chitotriosidase, macrophage

Funding: The research and results publicised here were reached with the sponsorship of Gedeon Richter Talentum Foundation in framework of Gedeon Richter Excellence PhD Scholarship of Gedeon Richter (Attila Ádám Szabó). The research was funded by OTKA-FK128582.

19. EFFECTS OF CHRONIC SUPPLEMENTATION WITH *Urtica dioica* L. LEAF EXTRACT AND CHLOROGENIC ACID ON HEMODYNAMIC AND BIOCHEMICAL PARAMETERS IN SPONTANEOUSLY HYPERTENSIVE RATS

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Background & Aim: *Urtica dioica* L. has been used in traditional medicine for centuries. The aim of this study was to evaluate the effects of a 4-week supplementation with *Urtica dioica* L. leaf extract (UE) and with one of its main phenolic compounds - chlorogenic acid (ChA) - on hemodynamic and biochemical parameters in spontaneously hypertensive rats (SHR). **Materials & Methods:** Adult male SHR (300 g B.W.) were divided into 3 experimental groups: The control group (SHRC) received tap water, the SHR+UE group received 200 mg/kg/day UE, and the SHR+ChA group received 2.5 mg/kg/day ChA (a dose equivalent to the ChA content in 200 mg/kg/day UE). At the end of the four-week supplementation, diuresis, mean arterial pressure (MAP), heart rate (HR), cardiac output (CO), stroke volume (SV), total vascular resistance (TVR), and nitrite excretion (exc NO_2^-) were determined.

Results:

	Diuresis [mL/24h]	pTBARS [nmol/mL]	MAP [mmHg]	HR [min^{-1}]	CO [mL/min/kg]	SV [mL/kg]	TVR [mmHg x min/mL]	exc NO_2^- [nmol/24h]
SHRC	8.7±0.8	9.95±1.19	162±3	409±4	221.7±12.7	0.96±0.05	0.53±0.03	208±18
SHR+UE	8.6±0.6	6.57±0.48	140±4***	404±6	179.9±10.8	0.95±0.05	0.45±0.02	360±45**
SHR+ChA	10.7±1.7	6.05±1.46*	146±7*	379±29	211.8±21.1	0.93±0.11	0.55±0.05	195±36##

*p<0.05, **p<0.01, ***p<0.001 vs SHRC; ##p<0.01 vs SHR+UE

Conclusions: Chronic supplementation of SHR with 200 mg/kg/day UE and 2.5 mg/kg/day ChA decreased MAP. In addition, UE supplementation increased NO_2^- excretion, whereas supplementation with ChA decreased oxidative stress in SHR.

Key words: spontaneously hypertensive rats, *Urtica dioica* L., chlorogenic acid, oxidative stress

Funding: This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (No. 451-03-47/2023-01/200015).

20. ANGIOTENSIN CONVERTING ENZYME GENE D-ALLELE AND STENT RESTENOSIS , A TANGLED STORY- CASE PRESENTATION

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In-stent restenosis is a possible complication which can occur after successful coronary revascularisation in 1 out of 4 patients. The incidence became significantly smaller after the introduction of drug eluting stents (DES), as compared to bare metal stents (BMS) and balloon angioplasty. Still, 10% of the patients develop this complication even after percutaneous coronary revascularisation (PCR) with a DES.
We present the case of a 62 year old man, admitted for chest pain, with a history of PCR with DES of the left circumflex artery 8 months before. We performed coronary angiography, which revealed stent restenosis of the LCX DES.
This case is part of a larger study on 154 patients with coronary syndromes which aimed to investigate the relationship between the genetic polymorphism (I/D mutations) of the angiotensin converting enzyme gene and intrastent restenosis following successful revascularisation in patients with coronary artery disease. We initially determined the ACE genotype for each patient and after percutaneous revascularisation, performed a one-year follow up. A strong correlation was observed between restenosis the D-allele, with 37.2% of the restenotic patients having the DD genotype and 62.8% having the ID genotype. II genotype patients did not experience intra-stent restenosis (p=0.001). The patient presented in this case study was a DD genotype, therefore a D allele carrier.
It is worth noting that the existing body of research in this area remains contradictory and further data is required. Nonetheless, our study contributes valuable insights to our specifical geographical population.

Key words: D-allele, angiotensin converting enzyme gene, ACE mutations, stent restenosis

21. PULMONARY THROMBOEMBOLISM IN ADULTS UNDER 50 YEARS OLD

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Although it is well recognised that incidence of pulmonary embolism (PE) increases with age, PE has been shown to be an important cause of mortality in younger age groups. Venous thromboembolism is favoured by the existence of predisposing factors, pregnancy, oral contraceptives and trauma have been shown to be more important within a younger group age. Another important predisposing factor that has become easier to identify in recent years is thrombophilia. In this paper we will focus on the role played by thrombophilia in the occurrence of pulmonary thromboembolism in patients under 50 years of age. Starting from 2019, the extended profile for thrombophilia was determined for patients diagnosed with pulmonary thromboembolism with high intermediate risk or high risk, diagnosis established by pulmonary angio CT, under the age of 50, without identifiable risk factors, admitted to the Coronary Intensive Care Clinic and Clinic of Cardiology of the Emergency County Hospital "Pius Brânzeu" Timișoara. The extended profile for thrombophilia included determination of antithrombin III deficiency, protein C and protein S, determination of Factor V Leiden, mutation of prothrombin G20210 A, of Plasminogen Activator Inhibitor 1 (PAI-1), evidence of hyperhomocysteinemia associated with methylene tetrahydrofolate reductase (MTHFR) mutation. Patients with antithrombin III deficiency, protein C and S deficiency, homozygous factor V Leiden mutation, and homozygous 20210A prothrombin gene mutation require permanent oral anticoagulation in therapeutic dose. Regarding patients with heterozygous factor V Leiden mutation the recommendations are less clear, requiring periodic evaluations to determine the duration of anticoagulant treatment. The obtained data help to individualize the therapeutic recommendations and at the same time to reduce the recurrence of thromboembolic events in a category of young patients, with an important impact at a socio-economic level as well.

Key words: venous thromboembolism, thrombophilia, anticoagulant treatment

22. NATURAL VERSUS SYNTHETIC ANTIOXIDANT SUPPLEMENTATION OF LOSARTAN TREATMENT IN EXPERIMENTAL MODEL OF HYPERTENSION AND CHRONIC KIDNEY DISEASE: IS TOGETHER BETTER?

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Background & Aim: Oxidative stress is responsible for the development of hypertension and focal-segmented glomerulosclerosis (FSGS, type of chronic kidney disease). Natural antioxidant-olive leaf extract (O) is rich in phenolic compounds. Synthetic antioxidant-tempol (T) is a membrane-permeable radical scavenger. We showed that losartan (L, angiotensin II type-1 receptor blocker) decreased renal structural and functional changes in spontaneously hypertensive rats (SHR) with adriamycin (ADR)-induced FSGS, but these changes were still higher than in control. Thus, we investigated if natural or synthetic antioxidants together with losartan could be more effective in slowing down the progression of FSGS.
Materials & Methods: SHRs were divided in five groups. Control received vehicle. Four groups received ADR (2 mg/kg, *i.v.*) twice in a 3-week-interval, and three groups received L, L+O, and L+T (10, 10+80, and 10+100 mg/kg/day, respectively) by gavage for 6-week-period. Blood pressure, kidney function and histology, along with renal klotho, 6-nitrotryptophan (nitration of proteins marker), and nitric oxide (NO_x) levels were analyzed.
Results: Losartan significantly reduced blood pressure, urine protein loss, glomerulosclerosis, tubulointerstitial inflammation, and fibrosis compared to model, but renal changes still were higher than in control. Losartan reversed the NO_x level to control, significantly increased 6-nitrotryptophan but unchanged klotho protein expression compared to model. L+O treatment normalized NO_x content, significantly decreased blood pressure, protein loss, and renal injury score compared to model. Interestingly, renal injury was lowered to the level not significantly different from control, while klotho and 6-nitrotryptophan levels were significantly increased compared to model. L+T treatment significantly decreased blood pressure and increased NO_x content, while klotho, 6-nitrotryptophan, renal functional and structural changes were not significantly different compared to model group.
Conclusion: Our results showed that losartan treatment supplemented with natural antioxidant was better in slowing down the progression of FSGS in SHR.

Key words: olive leaf extract, tempol, losartan, hypertensive rats, chronic kidney disease.

Funding: This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (No. 451-03-47/2023-01/200015).

23. CHEMOTHERAPY - A FOCUS ON MECHANISMS OF CARDIOTOXICITY

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Oncologic pathology and the management of patients with cancer are real challenges in cardiology practice. Unpredictable situations triggered by this pathology, as well as adverse reactions of the cardiovascular system to oncological therapy, are increasing. The cardiovascular disease as a consequence of previous cancer treatment requires a multidisciplinary teams involving specialists in cardiology, oncology and other related fields. The optimal management of cardiovascular risk factors and pre-existing cardiovascular disease is essential for facilitating cancer therapy and improving prognosis. ECG is recommended in all patients before and during treatment. Echocardiography is the method of choice for the detection of myocardial dysfunction before, during and after cancer therapy; global systolic longitudinal myocardial strain has been reported to accurately predict a decrease in LVEF.
Myocardial dysfunction and heart failure, are the most significant cardiovascular complications of cancer therapies. Some oncology drugs are associated with a specific cardiotoxicity profile. Type 1 - irreversible cardiac injury caused by anthracyclines; Type 2 - reversible cardiotoxicity caused by monoclonal antibodies. One of the accepted pathophysiological mechanism of anthracyclines cardiotoxicity is the oxidative stress hypothesis, which suggests that the generation of reactive oxygen species and lipid peroxidation of the cell membrane damage cardiomyocytes. On the other hand, the mechanism of anti-HER2 drug-induced cardiotoxicity includes structural and functional changes in contractile proteins and mitochondria, but it rarely leads to cell death, explaining the potential for reversibility. Other conventional chemotherapies that can induce myocardial dysfunctionare cyclophosphamide, cisplatin, ifosfamide and taxanes. If patients are carefully followed up during cancer therapy, it is possible to detect cardiovascular toxicity as a result of specific oncological therapies. It is necessary to establish a plan to prevent the development of cardiotoxicity, as well as an accurate surveillance plan for potential cardiovascular complications.

Key words: cardiotoxicity, oncology, echocardiography

24. ELECTROPHYSIOLOGICAL CHARACTERISATION OF INDOMETHACIN IN A PORCINE MODEL OF OBSTRUCTIVE SLEEP APNEA

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Non-steroidal anti-inflammatory drugs are used daily by more than 30 million people worldwide to treat inflammatory pain. Cardiovascular diseases (CVD) are the leading cause of death worldwide and are highly associated with obstructive sleep apnea (OSA), which moreover shows a strong association with other CVDs. The aim was to investigate the effect of indomethacin on cardiac electrophysiology and arrhythmia susceptibility and whether it intensifies the arrhythmogenic substrate during obstructive events simulated by intermittent negative upper airway pressure (INAP). The study population consisted of 12 sedated (4.2% alpha-chloralose), spontaneously breathing LYD-pigs (8 indomethacin, four vehicles). INAP was applied four times for 75 seconds. Utilising a pacing protocol, indomethacin's effect on the sinus node recovery time and the refractoriness of the atria (AERP) and the atrioventricular node were investigated. Furthermore, the electromechanical window (EMW), the QTc-interval and the occurrence of brady-/tachyarrhythmia and extra-systoles, were measured. The pigs showed a tendency in AERP shortening (10 min: -17%±9%, 20 min: -17%±9%) and a significant shortening of the QTc-interval (10 min: -20.50±4.30 ms, 20 min: -15.88±3.70 ms) within the first 20 minutes post indomethacin. Furthermore, the occurrence of 2. Degree AV-blocks correlated with a high indomethacin plasma concentration. INAP-induced electrophysiological changes, such as AERP and EMW, did not worsen in the presence of indomethacin. The effect of indomethacin was most pronounced in the ventricles. However, indomethacin did not worsen the arrhythmogenic substrate during INAP. Further research is needed to elucidate potential ion channel involvement in indomethacin-induced electrophysiological changes.

25. ROLE OF MONOAMINE OXIDASE IN THE PATHOGENESIS OF SEVERE MITRAL REGURGITATION AND THE POSSIBLE LINK WITH THE PATIENTS' COMORBIDITIES

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Background & Aim. Oxidative stress plays an important role in tissue structural and functional damage. Inflammation and shear stress increase the production of reactive oxygen species (ROS) which in turn maintain a proinflammatory status. Monoamine oxidase (MAO) with its isoforms (MAO-A, MAO-B) is an important source of ROS, inducing pathological remodelling, tissue degeneration and function alteration. In the cardiovascular system this was mostly demonstrated in the vascular endothelium. There is scarce data in the literature regarding MAO expression in valvular tissue. Our aim was to investigate MAO implication in mitral valve damage as well as the influencing factors.
Materials & Methods. 30 patients with severe myxomatous and/or degenerative mitral regurgitation and surgical indication were prospectively included in the study. Valvular fragments were obtained from each of them during the intervention and analysed for the presence of ROS (confocal microscopy - DHE staining, spectrophotometry- FOX assay) and MAO isoforms (immune fluorescence, qRT-PCR). Several comorbidities that may favour ROS production (chronic kidney disease, diabetes mellitus, arterial hypertension, dyslipidaemia, atrial fibrillation, coronary artery disease, clinically advanced heart failure, dilated left ventricle, reduced ejection fraction) and the treatment received previously by the patient, were thoroughly analysed.
Results. Both MAO A and MAO B expression (gene and protein) was demonstrated in the damaged mitral valve tissue. Angiotensin II increased the amount of ROS and both MAO isoforms expression while angiotensin II receptor type 1 antagonist blocked this effect. Tissue incubation with MAO A and B inhibitors decreased ROS production demonstrating MAO as a source of ROS in mitral tissue. This effect was significantly more intensively expressed in patients with multiple favouring factors (at least 3 from the above mentioned comorbidities, without pretreatment with RAAS inhibitors) especially regarding the MAO B isoform. There was a significant correlation between decreasing LVEF and increasing ROS.
Conclusion. MAO contributes to the oxidative stress in mitral valvular tissue and its expression increases in the presence of comorbidities.
Key words: severe mitral regurgitation, comorbidities, reactive oxygen species, monoamine oxidase; angiotensin II, RAAS inhibitors

26. CARDIOPROTECTIVE EFFECTS OF POMEGRANATE PEEL EXTRACT IN TAKOTSUBO-LIKE MYOCARDIAL INJURY IN RATS

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Background and Aim: Takotsubo (TT) syndrome is a stress-induced cardiomyopathy triggered by extreme physical or emotional stress and characterized by elevated levels of catecholamines. Isoprenaline is a synthetic catecholamine that causes intense stress in the myocardium similar to Takotsubo cardiomyopathy. Pomegranate peel extract (PoPEx) has a high concentration of polyphenols that have several beneficial effects such as antioxidant, lipid-lowering, anti-inflammatory, and antihypertensive. The study aimed to investigate the cardioprotective effects of PoPEx on isoprenaline-induced TT-like myocardial injury (MI) in rats. **Materials and Methods:** Male Wistar Albino rats were divided into four groups:
C - 0.5% carboxy methyl cellulose (CMC) was administered via oral gavage for 7 days and saline s.c. was given on days 6 and 7; I – rats were treated with CMC via oral gavage for 7 days, and on days 6 and 7 they were treated with isoprenaline 85 mg/kg s.c.; P group – treated with PoPEx 100 mg/kg dissolved in CMC and given via oral gavage for 7 days and saline s.c. on days 6 and 7; P+I group – treated with PoPEx 100 mg/kg via oral gavage for 7 days and isoprenaline 85 mg/kg s.c. on days 6 and 7. Blood samples are taken for biochemical analysis, and cardiac tissue for histological and immunohistochemical analysis. **Results:** PoPEx was found to be effective in preventing isoprenaline-induced TT-like MI. PoPEx prevented oxidative stress by reducing pro-oxidative markers, such as thiobarbituric acid reactive substances (TBARS), nitrites, hydrogen peroxide and superoxide anion radical, and also by increasing antioxidative markers, such as catalase and reduced glutathione. Histological analysis revealed the anti-inflammatory effect of PoPEX. Immunohistochemical analysis demonstrated decreased isoprenaline-induced TT-like MI apoptosis in rats, including decreased CC3 and BAX and increased BCL-2. **Conclusion:** PoPEx prevented myocardial injury, oxidative stress, inflammation, and apoptosis in TT-like MI in rats.

Key words: Takotsubo syndrome, isoprenaline, pomegranate peel extract, oxidative stress

27. UNDERSTANDING DEADLY INTRAVENOUS PARACETAMOL INDUCED HYPOTENSION

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Background. Intravenous acetaminophen (APAP; paracetamol) is used commonly in the intensive care unit or post-surgery for its analgesic effect. Intravenous administration of APAP is well documented to cause severe transient hypotension. The mechanism underlying these hemodynamic changes is still not clear, but the APAP metabolite N-acetyl-p-benzoquinone-imine (NAPQI) can directly and indirectly active voltage-gated Kv7 channels in vascular smooth muscle cells promoting a vasodilation. APAP metabolism to NAPQI requires cytochrome P450 enzymes. Furthermore, another pathway of NAPQI formation could be activated myeloperoxidase (MPO) and the circulating level of MPO is elevated in critically ill patients. MPO could be the missing link of why critically ill patients experience the most severe hypotension caused by intravenous paracetamol.
Materials & Methods: Human Coronary Artery Endothelial Cells (HCAEC) and HEK293 cells were treated with APAP (0 – 50 mM) or APAP metabolites (0 – 50 μM) to see the effect on viability and thiol concentration levels. The formation of APAP-adducts by cytochrome P450 and MPO was investigated by immunocytochemistry, thiol assays, Western Blot and Mass Spectrometry. **Results:** APAP decreased viability and thiol levels in a concentration dependent manner similar to NAPQI, but not AM404 in HCAEC cells. The cytochrome P450 inhibitor Ketoconazole partially rescued the drop in viability and thiol levels. We showed that APAP-adducts are formed in HCAEC. CYP20A1 is expressed in both HEK293 and HCAEC and are upregulated in response to treatment of APAP. Additionally, by blocking of normal pathways of MPO metabolites, we show that MPO can form NAPQI.
Conclusion: Our findings show that NAPQI formation is a combination of cytochrome P450 and MPO. By improving our understanding of the metabolism of IV APAP, we might be able to prevent the iatrogenic hypotension.

28. THE ROLE OF TRPA1 CHANNELS IN REGULATION OF VASCULAR TONE - 3D QSAR MODELING WITH DESIGN OF NOVEL TRPA1 AGONISTS

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Background & Aim: Transient Receptor Potential Ankyrin 1 (TRPA1) channels are present in various tissues such as sensory neurons, fibroblasts, T cells, cardiomyocytes and endothelial cells of blood vessels. Carvacrol is a monoterpene compound found in essential oil of *Labiatae* family members and it causes a very strong vasorelaxant effect on isolated blood vessels. It is also a potent activator of TRPA1 channels that are involved in regulation of vascular tone. Aim of the study: In the first part of this study the aim was to investigate the bond between carvacrol-induced vasodilation and TRPA1 channels. Second step was to create a proper QSAR model and to design novel TRPA1 activators based on carvacrol structure with enhanced activity.
Materials & Methods: Isolated human mesenteric arteries were cut into rings, which were then placed in an isolated tissue bath system. The carvacrol concentration-response curves were obtained on PE-precontraction in presence of A967079, a TRPA1 receptor antagonist, and compared with the control curve. Quantitative structure-activity relationship (QSAR) modeling was performed on 30 compounds with activity against TRPA1. Information obtained from the 3D-QSAR model was then used to design new molecules with improved activity.
Results: In the presence of A967079 the maximal effect of carvacrol-induced vasodilation was reduced compared with the control curve. Reliable PLS model with good statistical parameters (R_{pred} = 0,84 and Q^2 = 0,59) was obtained and new highly selective and potent TRPA1 inhibitors were designed.
Conclusion: Carvacrol-induced vasodilation was diminished by blocking TRPA1 channels showing involvement of these channels in regulation of vascular tone. *In silico* 3D-QSAR technique can be a useful method to evaluate and design new TRPA1 inhibitors.

Key words: 3D-QSAR, carvacrol, TRPA1, vasodilation

29. BUERGER'S DISEASE WITH UPPER LIMBS ISCHEMIA IN A FEMALE PATIENT

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Trombangeiitis obliterans (Buerger's disease) is a rare disease that affect young smokers. At first it was diagnosed almost exclusively in men. Nowadays, the disease is occuring more and more frequently in women, probably due to the increasing number of women smokers. We present a case of 41-year-old smoking female with a personal history of ischemic events (right toe gangrene). She was admitted in Surgical Clinic presenting the classical symptoms and signs for left hand ischemia (fingers gangrene). She also had signs of Raynaud's phenomenon on her right hand. Standard lab examination and imaging tests (ultrasonography and CT-Angiography) together with signs and symptoms confirmed the diagnosis. The patient was treated with injectable vasodilators, antiplatelet medication and mild anticoagulants. After discharge the evolution was less favorable and after one month the patient underwent long-term injectable treatment with a prostaglandin analogue (PGE1 for 4 weeks). The evolution was slowly favorable with delimitation of gangrene areas after completion of PGE1 treatment. Patients' lesion responded favorably to long-term injectable treatment with a prostaglandin analogue associated with smoke cessation.

Key words: trombangeiitis obliterans, woman, smoking

30. mTORC1 ACTIVATION IS ESSENTIAL FOR SACUBITRIL/VALSARTAN STIMULATION OF SUBCUTANEOUS WHITE ADIPOSE TISSUE BROWNING

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Background & Aim: This study aimed to investigate the potential of combined treatment with a neprilysin inhibitor (sacubitril) along with antagonist of AT_1 receptor (valsartan) to activate mTORC1 expression in subcutaneous white adipose tissue (sWAT) of rats with metabolic syndrome and thereby promote browning of white adipocytes.
Materials & Methods: Forty *Wistar albino* male rats (8 weeks old; body weight, bw: 200 ± 20g) were included in this study and randomly divided into four equal groups according to diet regime and applied treatment as follows: CTRL – healthy untreated rats; CTRL+SAC/VAL – healthy rats treated with combination of sacubitril and valsartan; MetS – rats with metabolic syndrome and MetS+SAC/VAL – rats with metabolic syndrome treated with combination of sacubitril and valsartan. Rats from experimental groups received combination of drugs in a dose of 68 mg/kg every day by oral gastric gavage for 4 weeks. After finishing the experimental protocol, sWAT sections were isolated for the hematoxylin/eosin (HE) staining and the assessment of the intensity of mTORC1 staining.
Results: The results of histological analysis with HE staining showed significantly larger lipid droplets in sWAT of rats from MetS compared to CTRL group ($p<0.05$). Moreover, 4-week treatment protocol with sacubitril and valsartan succeeded to decrease lipid droplet size in both healthy and rats with MetS inducing multilocular morphology in sWAT depots of these rats. By analyzing of mTORC1 staining, our results revealed that MetS induction resulted in negative mTORC1 expression compared to moderate staining found in sWAT of rats from MetS+SAC/VAL group. Additionally, applied treatment induced strong intensity of mTORC1 expression in sWAT of healthy rats compared to its weak staining in adipose tissue of untreated healthy rats.
Conclusion: The findings obtained from this study proved strong potential of the applied drugs to improve adipose tissue morphology and promote browning of sWAT through mTORC1 activation.

Key words: sacubitril, valsartan, mTORC1, adipose tissue, browning

31. CROSSTALK BETWEEN EARLY VASCULAR AGING, NONALCOHOLIC FATTY LIVER DISEASE AND INSULIN RESISTANCE IN PATIENTS WITH CARDIOVASCULAR RISK FACTORS

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Background & Aim. Early vascular aging refers to premature alterations in arterial structure and function and predicts cardiovascular events. The present paper aimed to study the possible association between blood lipid levels and vascular age in patients with cardiovascular risk factors.
Materials & Methods. A total of 76 patients, aged 63±10 years, 53% males, with cardiovascular risk factors, underwent pulse wave analysis using a Mobil-O-Graph. Standard blood lipids were assessed and non-conventional lipid markers, lipid ratios and triglyceride-glucose index (TyG), a marker of insulin resistance, were calculated. Information about other laboratory investigations, diagnosis and therapy were available from medical records.
Results. Early vascular aging (EVA) was detected in 27 patients (35.52%) and 53 patients (70%) were diagnosed with nonalcoholic fatty liver disease (NAFLD). Atherogenic index (AI), Castelli Risk Index I (CRI), remnant cholesterol (RC), triglyceride (TG) levels and TyG were, as follows: 3.32±1.67, 4.32±1.68, 24.56±28.44 mg/dL, 188±205 mg/dL and 4.84±0.38, respectively. No significant correlations were found between blood lipid levels and pulse wave analysis variables. ROC curve analysis revealed NAFLD and TyG as predictors of EVA (AUC=0.62, p=0.016 and AUC=0.645, p=0.026, respectively), and AI, CRI, RC and TG levels as a predictors of NAFLD (AUC=0.761, AUC=0.774, AUC=0.801, and AUC=0.979, respectively, with $p<0.01$).
Conclusion. Serum lipid levels predict NAFLD but not EVA in patients with cardiovascular risk factors. On the other hand, NAFLD and insulin resistance are predictors of EVA.

Key words: early vascular aging, nonalcoholic fatty liver disease, lipid profile, triglyceride-glucose index

32. THE SIGNIFICANCE OF THE BODY FAT INDEX AND OTHER INDICATORS IN IDENTIFYING METABOLIC SUBTYPES

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Background & Aim: The primary risk factors for CVD are central obesity and increased visceral adipose tissue. The visceral adiposity index (VAI) is an indirect indication of visceral adipose tissue using both anthropometric and lipemic characteristics. The role of the VAI score was analyzed to identify metabolic phenotypes in the current study.
Materials and Methods: In this retrospective study, age, and gender-matched 200 patients were grouped into 50 patients each group according to their body mass index. They are classified as metabolically healthy obesity, metabolically unhealthy obesity, Metabolically unhealthy normal, and metabolically healthy normal (MHO = 50, MUHO = 50, MUHNO = 50, and MHN = 50). The patients' demographic, clinical and biochemical parameters and anthropometric measurements were reviewed retrospectively. BMI and VAI scores were calculated.
Results: The VAI scores of the MUHNO group were greater than those of the MUHO and MHNO groups in males (P=0.041, 95% CI=0.234; 13.780; P=0.046, 95% CI=0.099; 13.730, respectively). The VAI scores of the MHO group were lower than the MUH groups in females (P=0.019, 95% CI=-6.788; -0.472; P=0.010, 95% CI=-11.732; -1.344, respectively). The lower 4.75 VAI score to identify the MHO patients among the study population in females; and the higher 4.49 VAI score to identify the MUHNO patients among the study population in males can be used as the cutoff point.
Conclusion: Metabolic health extends beyond BMI, and it is critical to identify people who are metabolically unhealthy. The VAI score is a simple technique to define high CVD individuals before metabolic syndrome appears, although it may not describe all unhealthy patients. Other variables such as WC, TG, HDL-C, and CRP levels, as well as the VAI score, can be used to distinguish different metabolic subtypes.

Key words: the visceral adiposity index, metabolically unhealthy normal weight, metabolically healthy obese, metabolically unhealthy obese

33. URSODEOXYCHOLIC ACID ATTENUATES SYSTEMIC AND LIVER INFLAMMATION INDUCED BY LPS IN RATS

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Background & Aim: Bacterial lipopolysaccharide (LPS) has shown to induce general inflammation. The systemic proinflammatory response involves the activation of multiple pathways, including cytokines, the blood coagulation system, the complement system, and the release of acute phase proteins; while the most important cellular components are leukocytes and endothelial cells. The consequences of inflammatory responses include tissue injury and organ failure. The aim of the study was to examine the effects of ursodeoxycholic acid (UDCA) pretreatment on oxidative stress and inflammatory parameters in rats with LPS induced endotoxemia.
Material & Methods: Male Wistar albino rats were used in this experiment. The endotoxemia was induced by administration of LPS (5,5 mg/kg bw) intraperitoneally. In order to alleviate the effects of LPS, the UDCA (25mg/kg bw) was administrated by gavage as a pretreatment for 10 days. The animals were divided into 4 groups. Control group (propilene-glycol, as vehiculum p.o. for 10 days and saline on day 10 ip; LPS group (vehiculum, p.o. for 10 days and LPS i.p on day 10); UDCA group (UDCA p.o. for 10 days and saline i.p. on day 10); UDCA+LPS group (UDCA p.o. for 10 days and LPS i.p. on day 10). The blood markers of oxidative stress (GSH, CAT, SOD, H2O2, O2-) and inflammation (homocysteine, troponine I, ICAM-1, CK, LDH, AST, ALT) were determined, and histological analysis of liver damage was evaluated.
Results: Antioxidative stress markers in plasma, GSH, CAT and SOD, were significantly decreased in rats treated with LPS, while the concentrations of H_2O_2 and O_2•, as prooxidative stress markers, were significantly increased in comparison to the control group. The UDCA significantly attenuated oxidative stress markers in LPS-treated rats; it increased GSH and CAT, and decreased H2O2 and O2-. In UDCA pretreated group the blood levels of homocysteine, TnI, ICAM-1 and CK were significantly attenuated compared to LPS-treated rats. Histologic analysis revealed that UDCA significantly reduced liver injury induced by LPS.
Conclusions: UDCA reduce oxidative stress by raising antioxidative enzymatic activity and decreasing prooxidative molecules. Pretreatment with UDCA significantly reduced LPS-induced inflammation. Histological evaluation also confirmed that UDCA attenuated LPS-induced liver injury.

Key words: LPS, ursodeoxicolic acid, inflammation, oxidative stress

34. IS A PACIENT WITH HEMOPHILIA PROTECTED AGAINST ISCHEMIA?

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Hemophilia A (AHA) is a rare autoimmune bleeding disorder in which antibodies attack clotting factor VIII. Although patients with hemophilia have been thought to be protected against acute ischemic vascular events because of the hypocoagulant status they posses such events cannot be fully excluded. We report the case of a patient with a severe form of AHA and hypercholesterolemia (under statin treatment) who underwent treatment with ELOCTA (FVIII) which could determine the development of an acute coronary syndrome. The patient is a 34 y.o. male, who presented in the E.R. department experiencing constricting chest pain which radiated towards the shoulders and cervical region. Primary evaluation showed a HR-74 bpm, BP-150/100 mmHg, SpO2-98%. ECG showed 1mm ST segment elevation in leads V4-V6 and Q wave in leads DII, DIII and aVF. High sensitive Troponin levels where high: 464.8 ng/L at presentation and 999.6 ng/L after 3 hours. The pacient was admitted and standard ACS medication was given as accordance with the ACS protocol. After admission the patient's complaints were relieved under medication but with a rising Hs Troponin level to 1602.3 ng/L. Transthoracic ecography showed lateral wall hypokinesys and a global strain (GLS) of -10.7. Coronary angio-CT was performed and a soft atheromatous plaque which determined complete stenosis of the anterior descending coronary artery on the 7th segment 23 mm in length was revealed. The patient underwent a coronary angiography but due to the extensive lessions (complete blockage of ADC and RCA, 80% stenosis of DI and 70% stenosis MO 1) stent placement was not performed and the patient underwent double by-pass surgery for myocardial revascularization AMIS/ADA, GVS/OMI with good outcome. At discharge antiplatelet therapy with aspirin was recommended at a dose of 100 mg od. Although thrombotic events are a rare complication of factor VIII therapy (FVIII) for patients with hemophilia, in some cases in the presence of associated risk factors, as presented above, patients with hemophilia could develop ACS.

Key words: haemophilia, myocardial infarction, by-pass revascularization, VIII factor

35. IMMUNOTHERAPY-MEDIATED CARDIOTOXICITY IN A PATIENT WITH METASTATIC MELANOMA

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Immunotherapies have been associated with a significant risk for cardiotoxicity particularly in patients with preexisting cardiovascular pathologies. The multidisciplinary approach is mandatory in order to both address the complex pathomechanisms and provide the rational therapeutic approach. We report here the case of patient with metastatic melanoma with BRAF V600 mutation, who underwent therapy with BRAF kinase inhibitors and MEK inhibitors responsible for the development of severe cardiotoxicity. A 72 year-old male with a history of arterial hypertension, myocardial infarction, dilated cardiomyopathy and chronic kidney disease, was diagnosed with metastatic melanoma and presents for the periodic cardiologic evaluation required for immunotherapy of the oncologic condition. Immunotherapy for a couple of months elicited oscillating values of the ejection fraction (EF) ranging from HFrEF to HFimpEF. Upon the last visit, transthoracic echocardiography revealed a significant decrease in EF and a left ventricle apical thrombus. Moreover, the electrocardiogram showed supraventricular and ventricular arrhythmia and right bundle branch block in the absence of acute ischemic changes. The underlying cardiologic condition was managed with beta-blocker, mineralocorticoid receptor antagonist, loop diuretic and sodium-glucose co-transporter 2 (SGLT-2) inhibitor. In this fortunate case, the combined sustained therapeutic approach allowed the EF improvement and the continuation of anticancer treatment. However, despite the fact that several classes of drugs when associated may prevent the progression of HFrEF, it is not uncommon for cancer patients to be ineligible for maximal treatment due to the risk of hypotension.

Key words: immunotherapy, melanoma, cardiotoxicity, cardio-oncology, heart failure

36. THE INFLUENCE OF THE RELATIVE PARIETAL THICKNESS ON THE POST-INTERVENTIONAL HEMOGLOBIN VALUE IN AORTIC STENOSIS AFTER TRANSCATHETER AORTIC VALVE REPLACEMENT

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Background and Aim: Severe aortic stenosis, a prevalent valvulopathy, requires valvular correction, and transcatheter aortic valve replacement (TAVR) has emerged as an alternative to surgical intervention especially in elderly individuals. The optimal therapeutic approach involves a multidisciplinary decision-making process based on various factors. Over the past decade, the transcatheter implantation of the aortic valve has emerged as an alternative to traditional valve prosthetic surgery, leading to an increased number of patients undergoing valvular correction procedures.Anemia is a prevalent condition among patients undergoing TAVR and has been associated with poor outcomes following the procedure. The study aimed to investigate the relation between RPT and the drop in hemoglobin levels after TAVR.
Materials and Methods: A retrospective analysis was conducted on a cohort of 50 patients who had undergone TAVR between 2021-2023 at the Institute of Cardiovascular Diseases, Timișoara. Relative parietal thickness (RPT) was assessed using pre-interventional imaging, and post-interventional hemoglobin values were recorded at specific time points following the procedure. Statistical analysis was performed using MedCalc Statistical Software version 20.015 (MedCalc Software Ltd, Ostend, Belgium) with a significant p-value<0.05.
Results: A statistical significant correlation was found between relative parietal thickness and post-TAVR hemoglobin levels. Patients with a higher RPT tended to experience a more pronounced drop in hemoglobin levels post-TAVR (r=-0.2, p-value=0.0234, CI: 2.1426 ; 0.1631).
Conclusion: This investigation sheds light on a novel aspect of TAVR management, emphasizing the significance of relative parietal thickness as a potential predictor of post-interventional hemoglobin levels in patients with aortic stenosis. The findings of this research could have significant implications for risk stratification and patient selection in TAVR procedures.

Key words: TAVR, aortic stenosis, parietal thickness, hemoglobin, age

37. LEVOSIMENDAN ATTENUATES LUNG INJURY IN INTESTINAL ISCHEMIA-REPERFUSION MODEL IN RATS

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Background & Aim: Intestinal ischemia-reperfusion (I/R) injury is a widely used experimental model to study mechanisms of tissue injury induced by mesenteric artery occlusion. The lungs are considered to be the most vulnerable organs in intestinal I/R injury and the reactive oxygen species and oxidative stress are thought to play a key role in the pathogenesis of lung damage. Experimental and clinical data have shown that levosimendan, known as an inotropic, vasodilatory drug, can elevate hemodynamic properties in conditions like heart failure or acute renal failure, and improve survival. However, it is not known whether levosimendan can alleviate oxidative stress and lung injury following mesenteric occlusion. The aim of the study was to investigate the effects of levosimendan on lungs following intestinal I/R injury in rats.
Materials & Methods: Intestinal ischemia was induced by clamping the superior mesenteric artery for 30 minutes followed by reperfusion for 90 minutes. Levosimendan was given intraperitonally 30 minutes prior to the ischemia. The rats were divided into 4 groups: S group: sham + dimethylsulfoxide; IR group: ischemia-reperfusion; LS group: levosimendan 1mg/kg + sham; LIR group: levosimendan 1mg/kg + ischemia-reperfusion. Bronchoalveolar lavage (BAL) was used as a dynamic tool for the analysis of oxidative stress markers: TBARS, NO_2^-, glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD). The lungs were used for pathohistological analysis.
Results: Levosimendan prevented I/R-induced oxidative stress, by reducing TBARS and $NO2^-$ in BAL. At the same time it increased GSH, SOD and CAT. The histological evaluation also confirmed that levosimendan had positive effects on lung injury following intestinal I/R.
Conclusion: Levosimendan has significant antioxidative potential and anti-inflammatory impact in the lungs during intestinal I/R injury in rats.

Key words: intestinal ischemia-reperfusion injury, oxidative stress, levosimendan, lungs, bronchoalveolar lavage

38. DYNAMIC RIGHT VENTRICULAR OBSTRUCTION COMPLICATING LAPAROSCOPIC CHOLECYSTECTOMY IN A PATIENT WITH KNOWN ATRIAL MYXOMA

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We present a unique case of a 51-year-old female patient with a known history of pulmonary embolism following a premature birth complicated by HELLP syndrome. At the age of 48, she underwent a pulmonary endarterectomy for secondary pulmonary hypertension. In the last year, she was diagnosed with atrial myxoma with a planned surgical tumor resection. Recently, at the age of 54, the patient sought laparoscopic cholecystectomy for symptomatic cholelithiasis. During the induction of pneumoperitoneum for laparoscopic cholecystectomy, the patient had a sudden drop in end-tidal carbon dioxide (ETCO2) levels and went into cardiogenic shock. These hemodynamic changes were attributed to dynamic obstruction caused by the atrial myxoma, suggesting it as a potential cause for pneumoperitoneum-induced shock, a rare complication scarcely described in the literature. Atrial myxomas are rare cardiac tumors that can obstruct blood flow within the heart chambers, posing a risk for hemodynamic instability during surgical procedures. In this case, the atrial myxoma presence became evident during laparoscopic cholecystectomy induction, raising suspicion of its role in pneumoperitoneum-induced shock, an infrequent complication with limited documentation in the literature. Prompt recognition and management of this dynamic obstruction were crucial to ensuring the patient's safety. This case underscores the importance of a thorough preoperative evaluation, including cardiac assessment, in patients with a history of atrial myxoma, especially when considering laparoscopic procedures. Awareness of potential dynamic obstructions, such as atrial myxomas, during surgical procedures is essential for timely intervention and optimal patient outcomes. The rarity of this complication emphasizes the need for further research and documentation in the medical literature.

Key words: atrial myxoma, dynamic obstruction, laparoscopic cholecystectomy, pneumoperitoneum-induced shock

39. THE INFLUENCE OF ULCERATIVE COLITIS ON MYOCARDIAL INFARCT SIZE IN A CO-MORBID MODEL: THE ROLE OF IMMUNO-CARDIOVASCULAR DISEASOME RESEARCH

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Background & Aim: Several immunological diseases have been shown to be associated with an increased risk of cardiovascular diseases. However, studies evaluating the connection between inflammatory bowel disease such as Ulcerative Colitis (UC) and risk of Acute Myocardial Infarction (AMI) reported inconsistent results. The purpose of our study was to preclinically assess the association between UC and risk of AMI.
Materials & Methods: The experiment was carried out in male C57BL6 mice. Dextran Sulfate Sodium (DSS, 2.5%) was given via drinking water for 7 days to induce ulcerative colitis prior to AMI. Transthoracic echocardiography was performed to assess cardiac function and morphology 7 days after DDS treatment, then animals were subjected to ischemia/reperfusion (I/R) injury by left anterior descending coronary artery occlusion for 45 minutes followed by 120 minutes reperfusion. Ischemic preconditioning (IPC) as endogenous cardioprotection was applied in either groups by 3 cycles of 5 min ischemia /5 min reperfusion. Myocardial infarct size was measured at the end of protocol by Evans blue and triphenyltetrazolium chloride double staining. Histological and biochemical analyses of colon were assessed for further evaluations.
Results: All-cause mortality rate was significantly higher in the colitis groups. Colon length was significantly decreased in colitis group, and signs of a mild inflammation were observed in their histological analysis. Although there was no difference in cardiac functional parameters, and the ischemic risk area did not differ in colitis and control groups, mice with an IBD showed a higher infarct size as compared to the control animals. IPC evoked a decreasing tendency in IS in colitis groups, however, in control there was no significant protection by IPC.
Conclusion: In summary, our results show that ulcerative colitis possess an increased risk for more severe outcomes of AMI.

Key words: acute myocardial infarction, ulcerative colitis, myocardial infarct size, diseaseome

Funding: This study was supported by Project no. RRF-2.3.1-21-2022-00003 "National Heart Laboratory, Hungary", as well as by Project ID: OTKA-138223, ÚNKP-23-5 -SZTE-704, and BO_481_21 – by the Hungarian Academy of Sciences

40. THE ROLE OF m_2R-$I_{K,ACh}$ PATHWAY IN THE GENESIS OF SECOND-DEGREE ATRIOVENTRICULAR BLOCK IN HORSES

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Background: Second-degree atrioventricular (AV) block is the most common arrhythmia in horses and is attributed to high vagal tone, although, the underlying molecular mechanisms are unexplored. **Aim:** To assess the expression levels of the muscarinic acetylcholine receptor (M_2) and the G protein-gated inwardly rectifying K^+ (GIRK4) channel that mediates the cardiac $I_{K,ACh}$ current, both of which are essential in the vagal signalling in the AV node. **Materials & Method:** Seventeen horses with a high burden of second-degree AV block (median: 408 block per 20 hours, IQR: 109-1545 per 20 hours) were included and compared to 18 horses with a low burden of second-degree AV block (median 8 block per 20 hours, IQR: 0-32 per 20 hours). The PR interval and incidence of second-degree AV block were assessed at baseline and after pharmacological blocking of the autonomic nervous system (ANS). Wenckebach cycle length was measured by intracardiac pacing (n=16). Finally, biopsies from the AV node were collected and processed for immunohistochemical quantification of the expression levels of the M_2 receptor and the GIRK4 subunit of the I_{KACh} channel using machine-learning based automated segmentation (n=9+9).
Results: The horses with a high burden of of second-degree AV block had significantly longer PR interval (mean±SD: 0.40±0.05 sec; $p<0.001$) and longer Wenckebach cycle length (mean±SD: 995±86 ms; $p=0.007$) at baseline. After blocking the ANS, all second-degree AV blocks were abolished. We identified a higher expression of the M_2 receptor ($p=0.02$), but not the GIRK4 ($p=0.25$) in the AV node in the horses with a high burden of AV block compared to the control group. Both M_2 and GIRK4 were highly expressed in the AV node and less expressed in the atria and the ventricles.
Conclusion: A higher expression level of the M_2 receptor facilitating the m_2R-$I_{K,ACh}$ pathway-induced slowing of the AV nodal conduction may be responsible for the high burden of second-degree AV blocks seen in some horses.

41. WOUND HEALING EFFECTS OF AN OINTMENT CONTAINING HELICHRYSUM ITALICUM ESSENTIAL OIL

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Background & Aim: *Helichrysum italicum (H. italicum)* is a typical Mediterranean plant belonging to the *Asteraceae* family and its anti-inflammatory, antioxidant, and antibacterial properties has been verified. *H. italicum* essential oil has been used traditionally for wound and burns treatment, but there is no scientific evidence that supports the traditional claim. The present study aimed to develop an ointment containing the *H. italicum* essential oil and investigate its wound healing effects on excision wounds in streptozotocin-induced diabetic rats.
Materials & Methods: Thirty-two *Wistar albino* rats with the confirmed diabetes were used to evaluate *in vivo* wound healing effects of ointment. Wounds were created one week after confirmed diabetes. Firstly, animals were anesthetized, and the back of the rats was shaved and the open excision wounds of size 2 × 2 cm were created with scalpel and scissors. The animals were randomly divided into four groups: Group I was untreated. Group II was vehicle control (ointment base). Group III was 0.5% *H. Italicum* ointment. Group IV was standard (1% silver sulfadiazine ointment). The response to the treatment was assessed by macroscopic and biochemical analysis.
Results: Topical application of the *H. italicum* ointment showed the highest wound contraction with the highest content of hydroxyproline in comparison to the all examined groups. The *H. italicum* ointment showed significant wound contraction from day 7 to day 21 as compared to other groups. On the day 21, there was an average of 99.32% wound contraction in the *H. italicum* group, whereas the mean wound contraction in the untreated group and ointment base group was 71.36% and 81.26% respectively.
Conclusions: Our findings revealed that the *H. italicum* ointment approach might serve as a promising and innovative tool for wound healing.

Key words: Helichrysum italicum; essential oil; wound healing; ointment

42. CHARACTERIZATION OF CHANGES IN PLATELET MITOCHONDRIAL RESPIRATION PRE- AND POST-CARDIOPULMONARY BYPASS

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Background & Aim: Open-heart surgery with cardiopulmonary bypass (CPB) is currently the standard therapy for complex cardiac pathologies, in particular the severe valvular defects and advanced coronary artery disease (CAD). Platelet dysfunction has been widely reported as elicited by the CPB circuit, yet literature data regarding changes in mitochondrial respiration are scarce. The aim of the present work was to assess mitochondrial respiratory function of platelets isolated prior and after CPB from a patient undergoing elective cardiac surgery for severe aortic stenosis and triple-vessel CAD under mild hypotermia.
Materials & Methods: A 67-year-old man was referred for progressive breathlessness, constrictive thoracic pain and fatigue. Mitochondrial respiratory function of permeabilized platelets was measured by high-resolution respirometry (HRR) using the O2-k oxygraph (Oroboros Instr.) in a patient who underwent aortic valve replacement and coronary artery by-pass surgery. Platelets were harvested pre-CPB (prior to heparin administration) and post-CPB (within 10 min after the administration of protamine sulphate), isolated via a 2 step-centrifugation protocol and used to measure complex I (CI) and complex II (CII) - supported respiration.The following respiratory parameters were measured: *ROUTINE* respiration (respiration based on endogenous substrates), *OXPHOS capacity* or the maximal coupled respiration for CI and CII (in the presence of ADP), *LEAK* or the non-phosphorylating respiration (in the presence of oligomycin), and *ET capacity* or the maximal uncoupled respiration (in the presence of FCCP).
Results: A significant increase in CI-supported OXPHOS (71.36 %) was observed in platelets isolated post-CPB *vs* pre-CPB at variance from the minor increase in maximal active respiration when succinate, the CII substrate, was further added to chamber (22.13 %). Net OXPHOS capacity, an index of ADP stimulation, showed a significant increase (76.67%) in platelets post-CPB as compared to the pre-CPB values. However, the ET reserve capacity that indicates the potential of substrate uptake was only mildly increased post-CPB (18.29%).
Conclusion: Changes suggestive for an early impairment of the CII-supported mitochondrial respiration occurred in platelets harvested post- *vs* pre-CPB in patient with complex cardiac pathology. Whether these changes can be recapitulated in a proof-of-concept study in patients with complex cardiac pathologies and whether therapeutically targeting CII postCPB will influence patients outcome remains to be determined.

Key words: cardiopulmonary bypass, platelet, mitocondrial respiration, aortic stenosis, coronary artery bypass

43. ALTERED CARDIAC REPOLARIZATION ASSOCIATED WITH CELLULAR ELECTROPHYSIOLOGICAL REMODELING FOLLOWING CHRONIC TESTOSTERONE ADMINISTRATION IN A LARGE ANIMAL MODEL

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Background & Aim: Despite the popularity of testosterone-based products, the direct effects of supraphysiological testosterone levels on cardiac structure and ion channels are not fully understood. We aimed to explore the potential structural and electrophysiological cardiac effects of chronic testosterone-undecanoate administration in a canine model through a variety of *in vivo* and *in vitro* studies.
Materials & Methods: Eight male beagle dogs were randomized into control ('Cont') and treated ('Tr') groups. The latter group received testosterone-undecanoate intramuscular injections (15 mg/kg) for 3 months. Testosterone levels were monitored via blood samples. Electrocardiography was used to study repolarization changes. Transmembrane ionic currents were recorded using patch-clamp technique, action potential duration (APD) was measured by perforated patch-clamp technique.
Results: Testosterone level was significantly higher in the 'Tr' group (47.02 nm/L vs. 15.23 nm/L). The treatment led to a shortening of QT (226±49 vs. 244.9±26.6 ms), QTc (26.06±2.5 vs. 29.6±3.01 ms), and Tp-Te (32.95 ± 7.45 vs. 53.46 ± 16.6 ms) intervals in the 'Tr' group, while prolonging PQ (112.1±15.5 vs. 61.65±12.7ms) and QRS (72.37±15.4 vs. 61.65±12.7 ms) intervals. APD of isolated left ventricular myocytes was significantly shorter in the 'Tr' group (235.2±26.7 vs. 283.6±28.5 ms). Patch-clamp experiments revealed increased magnitude of transient outward potassium current, the inward rectifier potassium current, and the slowed delayed rectifier potassium current in the 'Tr' group.
Conclusion: Constantly high levels of testosterone significantly altered the repolarization of the canine ventricular myocardium, suggesting that supraphysiological testosterone levels could lead to potentially harmful changes in cardiac repolarization, thereby potentially increasing the risk of arrhythmogenesis under certain conditions.

44. MITOCHONDRIAL DYSFUNCTION IN iPSC-DERIVED VENTRICULAR CARDIOMYOCYTES IN SPINAL MUSCULAR ATROPHY

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Background & Aim: Spinal Muscular Atrophy (SMA) is an autosomal recessive disease induced by the degeneration of spinal motor neurons. Although SMA is primarily considered a motor neuron disease, a growing number of SMA-related congenital heart defects have been recognized in recent studies. Some experimental data imply that the determination of the mitochondria dysfunctions at the developmental and progression stages in SMA pathogenesis may contribute to the cardiomyopathy. Therefore, here, we aimed to determine the impacts of SMN gene loss on mitochondrial functions during the congenital period in human induced pluripotent stem cells (hiPSCs) and hiPSC-derived ventricular cardiomyocytes (iPSC-vCM) in SMA patients.
Materials & Methods: The hiPSCs were isolated from blood samples of SMA patients (0-2 years-of-age) and healthy-control donors. The function of mitochondria was examined by monitoring the reactive oxygen species (ROS) production ($[ROS]_i$) and mitochondrial membrane potential (MMP) in both control and SMA-iPSCs groups by using DCFDA or JC-1 dyes, respectively. The iPSCs were cultured to induce ventricular CM differentiation. Following characterization steps by Immunefluoresence assays, a patch-clamp analysis was performed to measure action potential parameters and voltage-dependent Na^+ and Ca^{2+} channel-currents.
Results: Our data showed that cellular $[ROS]_i$ production was increased and MMP was significantly depolarized in SMA-iPSCs compared to control-iPSCs. Our electrophysiological data demonstrated an important contribution of SMA-related changes in electrical activities of iPSC-vCM besides the reprogramming and differentiation procedures in these groups.
Conclusion: Our data suggest that mitochondrial dysfunction may arise in the development of the early stages of SMA pathogenesis and may have a cross-talk with the deficiency of SMN protein, which in turn can impact the maturation of cardiomyocytes at the developmental stage. Also, the present data may also highlight the importance of restoring mitochondrial function during the reprogramming stages of patient-specific hiPS-based cell therapies.

Key words: spinal muscular atrophy, human induced pluripotent stem cells, mitochondria, ventricular cardiomyocyte, differentiation

45. ASSESSING THE CARDIOPROTECTIVE POTENTIAL OF USNIC ACID IN MITIGATING DOXORUBICIN-INDUCED CARDIOTOXICITY IN RATS

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Background & Aim: Usnic acid (UA) is a natural, dibenzofuranic secondary metabolite found in various lichens and has been widely studied for its biological activities. The significance of this substance arises from its prospective therapeutic applications, encompassing antimicrobial, antitumor, and antioxidant properties. While lichens that contain usnic acid (UA) have been utilized in traditional medicine and several beneficial properties of usnic acid have been established, there remains an absence of data regarding its cardioprotective effects. The aim of this study was to evaluate the effect of UA on doxorubicin-induced cardiotoxicity in rats.
Materials & Methods: UA was extracted from the acetonic extract of lichen *Xanthoparmelia* stenophylla (XSA) and identified by comparison with the standard. The study was conducted on 40 male Wistar albino rats. The UA was administered orally at a dose of 25 mg/kg for 28 days. After 28 days, doxorubicin was administered intraperitoneally at a cumulative dose of 15 mg/kg. Three days after doxorubicin administration, hearts were isolated and subjected to ex vivo examination on a Langendorff apparatus. Blood and coronary venous effluent samples were also collected in order to determine the markers of oxidative stress by spectrophotometric method.
Results: Administration of UA at a dose of 25 mg/kg for 28 days leads to the preservation of cardiac function in a model of doxorubicin-induced cardiotoxicity. Also, a reduction in cardiac oxidative stress can be observed in treated animals compared to the animals not treated with UA.
Conclusions: Our results showed that UA exhibits cardioprotective and antioxidant activity, which indicates that UA can potentially be used as a cardioprotective agent. Additional research is necessary to reveal possible mechanisms of action of usnic acid.

Key words: lichen, usnic acid, oxidative stress, doxorubicin, cardiotoxicity

46. EFFECTS OF STATIN THERAPY ON EPICARDIAL ADIPOSE TISSUE: FOCUS ON OXIDATIVE STRESS

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Background & Aim: Epicardial adipose tissue (EAT) is a particular fat depot that has been in the past decade at the forefront of research due to the complex metabolic changes and inflammatory remodelling. Statins, the most prescribed lipid lowering drugs with pleiotropic effects, have been reported to ameliorate EAT dysfunction/inflammation, while their effect on the local oxidative stress has been less investigated. The aim of this study was to measure reactive oxygen species (ROS) production in EAT samples isolated from patients, treated or not with statin therapy, who underwent elective cardiac surgery.
Materials & Methods: A total number of 25 patients were included, and divided in 2 groups (treated or not with statins). Adipose tissue samples were isolated from the anterior wall of the right ventricle during elective cardiac surgery, were placed in ice-cold buffer, transferred to the laboratory and used for ROS measurement via 2 techniques: spectrophotometry (using the FOX assay for hydrogen peroxide) and confocal microscopy (dihydroethidium probe for superoxide anion).
Results: Patients chronically treated with statins (atorvastatin or rosuvastatin) had a significant lower level of ROS in the EAT than those without treatment. Interestingly, the level of ROS from EAT positively correlated with the diameter of the right ventricle.
Conclusion: Statins treatment is associated with a lower level of oxidative stress in the EAT. ROS generation from EAT may be an important contributor to cardiac enlargement. Whether the lower oxidative stress is a consequence for reducing upstream chronic inflammation or is a direct effect of long term statin therapy remains to be demonstrated.

Key words: epicardial adipose tissue, oxidative stress, statins

47. INVESTIGATION OF ACTION POTENTIAL ALTERNANS IN HUMAN HEART FAILURE

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Background & Aim: Heart failure is a progressive, multifactorial clinical syndrome leading to development of several life threating arrhythmias such as ventricular fibrillation. A possible mechanism contribute to arrhythmia development in heart failure is the so-called alternans, indicating periodic short-long oscillation of the action potential that leads to repolarization inhomogeneity. Alternans are considered suitable predictors of sudden cardiac death, however, there is no specific pharmacological intervention to avoid or reduce alternans. Here we test a novel, selective Na^+/Ca^{2+} exchanger inhibitor ORM-10962 on alternans development in human heart failure and in undiseased human hearts.
Materials & Methods: Left and right ventricular papillary muscles and trabecules were obtained from explanted hearts of heart failure patients undergoing cardiac transplantation. Undiseased human hearts were obtained from an organ donor after removal of pulmonary and aortic valves for transplant surgery. Action potentials were measured by conventional microelectrode technique. Alternans were evoked by rapid pacing from 700 ms to 250 ms. All experiments were made at 37 °C.
Results: Results show moderate action potential duration (APD) alternans in human heart failure which was unaltered after application of 1 μM ORM-10962. The magnitude of APD alternans was identical with APD alternans recorded from undiseased human heart. However, failing hearts often exerted action potential amplitude alternans, typically in higher pacing rates, leading to 2:1 conduction block then electrical disturbance. It seems that 1 μM ORM-10962 delays the development of action potential amplitude alternans.
Conclusion: These data indicate that APD alternans in human heart failure are not increased compared to undiseased human hearts. In contrast, human failing hearts often exert large action potential amplitude alternans leading to dynamic conduction block and irregular electric activity that could be important link between sudden cardiac death and alternans. Selective NCX inhibition may decrease amplitude alternans presumably due to a Ca^{2+}-dependent mechanism however, it requires further experiments.

Keywords: heart failure, arrhythmia, alternans, Na^{2+}/Ca^{2+} exchanger, ORM-10962

Funding: TKP2021-EGA-23, RRF-2.3.1-21-2022-00003, K134939

48. EFFECT OF SELECTIVE IKUR INHIBITOR XEN-D0103 ON THE DOG CARDIAC VENTRICULAR AND PURKINJE FIBER ACTION POTENTIAL

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Background & Aims: XEN D0103 was developed as selective inhibitor of IKur/kv1.5 current by Xention/Servier pharmaceutical companies to treat atrial fibrillation because the currently used antiarrhythmic drugs like flecainide or dofetilide possess some proarrhythmic potential. XEN-D0103 was reported to inhibit Kv1.5 channels in CHO mammalian cell line with IC50 about 25 nM and lengthened action potential duration (APD) significantly in atrial muscle. Since it is generally believed that IKur is expressed and operates in the atria but not in the ventricle. Selective IKur inhibition seemed a promising mechanism for future development avoiding proarrhythmic complication in the ventricle. Our aim was to answer these questions: 1. Are Kv1.5 channels expressed in the cardiac ventricular muscle and if they are, how its inhibition impact cardiacventricular and Purkinje fiber APD? 2. What are their functional roles?

Methods: To answer these questions, we used different testing methods. Firstly, we used immunohistochemistry examinations to detect the channels. Action potentials were recorded with standard conventional microelectrode technique from canine right ventricle myocytes. We carried out patch-clamp measurements to investigate the effect of the drug in isolated single myocytes.

Results: From immunohistochemical experiments clearly revealed that Ik1.5 channels are expressed in canine ventricles. In the action potential measurements, we noticed that XEN-D0103 prolonged the APD at one micromolar concentration. The patch clamp experiments revealed that it does not block the IKr, IKs, Ito and IK1 currents in 1 μmol concentration. However, the Ito current can be significantly inhibited even at 3 μmol.

Conclusion: Because it can cause APD prolongation in 1 μmol concentration, but can not inhibit Ito in the same concentration, we suggest this effect can be a putative IKur inhibition.

Special acknowledgments to the organizations/companies below for their contribution and sponsorship of the meeting

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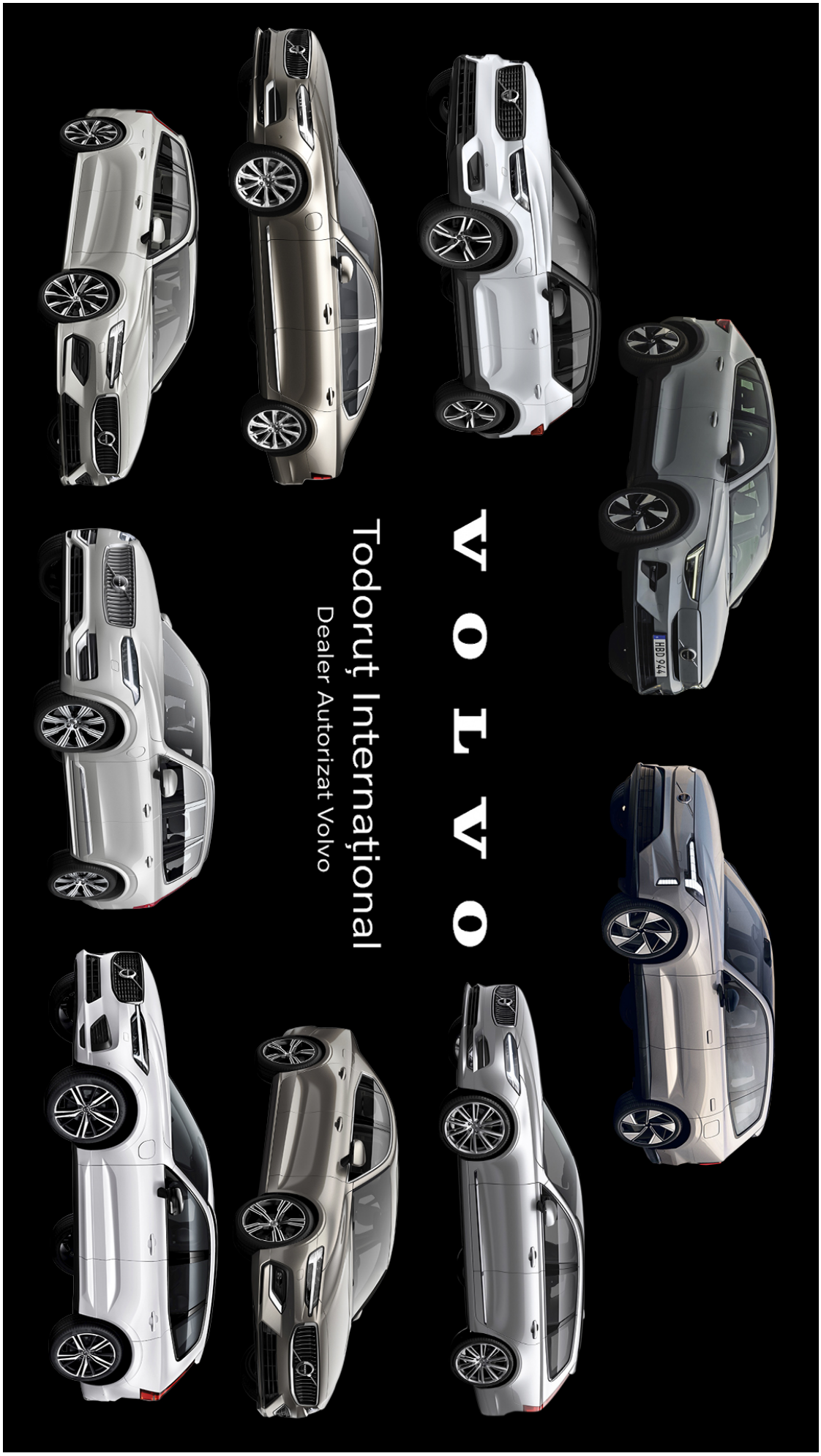
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