4rd EU-CARDIOPROTECTION COST Action MC and WG Meeting

Feb 12th – 15th 2019, Kragujevac, Serbia

Meeting Venue

Hotel Šumarice
Address: Desankin venac, Kragujevac, Serbia
Phone: +381 34 336180
http://www.hotelsumarice.com/
Kragujevac, Serbia

Program committee

COST Action Core Group committee
Prof Vladimir Jakovljevic (local organisor)

General Information

Local organiser:
Prof. Vladimir Jakovljevic
E-mail: drvladakgbg@yahoo.com
Administrative person:
Djordje Djukic
E-mail: djordje.djukic@medf.kg.ac.rs
Faculty of Medical Sciences, University of Kragujevac, Serbia

International Partner Country - Mexico

Tecnológico de Monterrey | Centro de Biotecnología FEMSA

Cost Action CA16225

Chair:
Derek Hausenloy
(UK)

Vice Chair:
Peter Ferdinandy
(Hungary)

WG1: New Targets
Ioanna Andreadou
(Leader, Greece)
Derek Hausenloy
(Co-leader, UK)
Hector Cabrera-Fuentes
(Co-leader, Germany)

WG2: Combination Therapy
Sean Davidson
(Leader, UK)
David Garcia-Dorado
(Co-leader, Spain)
Attila Kiss
(Co-leader, Austria)

WG3: Confounders
Rainer Schulz
(Leader, Germany)
Peter Ferdinandy
(Co-leader, Hungary)
Alina Scridon
(Co-leader, Romania)

WG4: Consortium
Hans Erik Botker
(Leader, Denmark)
Gerd Heusch
(Co-leader, Germany)
Jacob Lonborg
(Co-leader, Denmark)

STSM Chair:
Coert Zuurbier
(Netherlands)

Science Communications Manager:
Jelena Jovanic
(Bosnia)

Webmasters:
Søren Erik Pischke
(Norway)
Hector Cabrera-Fuentes
(Germany)

Grant Manager:
Elizabeth Hendricks (UK)

Tuesday February 12th 2018

1730-1930 Tour of Belgrade (leave from Hotel Park)
1930-2130 Pre-meeting dinner in Skadarlija (Bohemian Part of Belgrade)

Hotel Park
Address: Njegoševa 2/a, Beograd 11000, Serbia
Phone: +381 11 4146800

Wednesday February 13th 2018

0900-1100 Transport from Hotel Park to Kragujevac by bus for those staying overnight in Belgrade
Various times Transport from Belgrade airport to Kragujevac by car/minibus (please add your arrival info to meeting spreadsheet here)

1200-1300 **COST Action Core group meeting** – (COST Core Group Members only)

1300-1330 Lunch and registration - put posters up (Approx 25 posters, size 120cm x 80cm)

1330-1500 **Welcome and introductions to WG/MC meeting**
Derek Hausenloy, Ioanna Andreadou, Peter Ferdinandy, Vladimir Jakovljevic

COST Action MC meeting – (All invited to attend)
Discuss JCMM special issue on Mito (Lucio Barile)

1500-1530 Coffee Break - Poster viewing

1530-1615 **Opening Keynote Lecture**
Chair: Peter Ferdinandy

Invited specialist: (30 min talk, 15 min QA)
Clinical Cardioprotection following STEMI: Elusive but Attainable
Michael Cohen (US)

1615-1715 **Short-Term Scientific Missions - REPORTS** (10 min talk, 5 min QA)
Chair: Coert Zuurbier

1. Neuroprotection by Remote Ischaemic Conditioning and Liraglutide
   Maryna Basalay (UK)
2. Cardiomyocytes sensitivity to TNF- induced necroptosis
   Veronica Kormanova (Slovakia)
3. Cardioprotection by P2Y12 receptor antagonist (Ticagrelor) and NLRP3 Inflammasome Inhibitor (INF4E) in an in-vivo mouse model
   Saveria Femmino (Italy)
4. New therapies to reduce ischemia-reperfusion injury
   Laura Valls-Lacalle (Spain)

1715-1815 **Oral abstract ECI presentations** (7 min talk, 3 min QA)
Chairs: Ioanna Andreadou

1. Mechanical Postconditioning Stimulates Glucose Metabolism in parallel with Improved Post-Ischemic Recovery in an Isolated Rat Heart Model of Donation after Circulatory Death
   Maria Arnold (Switzerland/Slovakia)
2. Endothelial Cell-Derived Extracellular Vesicles Display Cardioprotective Effects
   Stefano Comità (Italy)

3. Short-term E-cigarette vapor exposure causes vascular oxidative stress and dysfunction - evidence for a close connection to brain damage and a key role of the phagocytic NADPH oxidase (NOX-2)
   Marin Kuntic (Germany)
4. Application of Mitochondria-Targeted Antioxidant Restores Mitochondrial Function in Senescent Cardiomyocytes
   Yusuf Olgar (Turkey)
5. Effect of ischemic pre-conditioning on expression of cardio- and thrombomiRs in a porcine model of acute myocardial infarction
   Denise Traxler (Austria)
6. *Cardiac Mitochondrial Integrity During Early Reperfusion – Consequences of Ischemic Duration and Association with Post-Ischemic Cardiac Recovery*
   Rahel Wyss (Switzerland)

1930 Meeting dinner in Hotel Sumarice
Traditional entertainment

Thursday Feb 14th 2018

International Partner Country - Mexico

Tecnológico de Monterrey | Centro de Biotecnología FEMSA

WG1 NEW TARGETS
Chairs: Ioanna Andreadou, Hector Cabrera-Fuentes, Derek Hausenloy

WG2 COMBINATION THERAPY
Chairs: Sean Davidson, Attila Kiss

0830-0915 **Invited specialist:** (15,5)
Chair: Hector Cabrera-Fuentes

Mitochondrial dynamics and autophagy
David Sebastian (Spain)

The Platelet-coagulation interaction in vascular inflammation and hypertension
Philip Wenzel (Germany)

0915-1015 **Workshops to select new targets and combinations**
Chairs: Ioanna Andreadou, Sean Davidson, Derek Hausenloy

Mitochondria/Metabolism
Inflammation

1015-1045 Coffee Break

1045-1130 **Workshops to select new targets and combinations**
Chairs: Ioanna Andreadou, Sean Davidson, Derek Hausenloy

Coronary vascular/Endothelial
Cell death pathways
Others

1130-1145 WG1/2 Summary and Action Plan

1145-1230 **Invited specialists:** (each 15,5)
Chairs: Peter Ferdinandy

Network Medicine - From protein-protein to drug-drug and human-machine interactions
Jörg Menche (Austria)

A personalized, multi-omics approach identifies genes involved in cardiac hypertrophy and heart failure
Marc Santolini (France)

1230-1330 Lunch

1300-1400 Moderated poster session
Chairs: Sean Davidson, Ioanna Andreadou, Peter Ferdinandy, Rainer Schulz

WG3 CONFOUNDERS
Chairs: Peter Ferdinandy and Rainer Schulz

1400-1445 **Workshops to select co-morbidities**
Chairs: Peter Ferdinandy and Rainer Schulz

Diabetes
Metabolic syndrome
Age
Gender

1445-1530 **Workshop to select co-medications**
Chairs: Peter Ferdinandy and Rainer Schulz

Anti-platelets
GTN
Statins
Beta-blockers
Anaesthetics
Anti-diabetics
Others

1530-1545 WG Summary and Action Plan

1600 Excursion to visit Royal Family Mausoleum
1930 Meeting dinner in local winery
2130 Transfer back to Hotel Sumarice

Friday February 15th 2019

WG3 CONFOUNDERS

0830-0900 **Invited specialist:** (25,5)
Chairs: Peter Ferdinandy and Rainer Schulz

Sex differences in cardiovascular disease
Georgios Kararigas (Germany)

WG4: CONSORTIUM
Chairs: Hans Erik Botker

0900-0945 **Invited specialist:** (15,5)
Microvascular obstruction: A target for cardioprotection
Ingo Eitel (Germany)

Targeting insulin resistance in heart failure
Qutuba Karwi (Canada)

0945-1015 Coffee Break

1015-1115 **Chair:** Hans Erik Botker
Setting up our European Cardioprotection Consortium
Choosing the animal models, protocols, and sites
SYNERGY grant application
Discuss 3 SYNERGY proposals
Other SYNERGY ideas

1115-1130 WG Summary and Action Plan

1130-1215 **Closing Keynote Lecture**
Chair: Derek Hausenloy

Invited specialist: (30 min talk, 15 min QA)
CMR in clinical cardioprotection studies
Borja Ibanez (Spain)

1215-1245 **Chair:** Derek Hausenloy
Summary and action plan

1300 Lunch

1500 Transport from Hotel Sumarice to Hotel Park in Belgrade by bus

Various times Transport from Hotel Sumarice to Belgrade Airport Kragujevac by car/minibus (please add your departure info to meeting spreadsheet here)

1900 Dinner in Hotel Park for those staying overnight

Oral and poster abstracts

Poster 1

Mechanical Postconditioning Stimulates Glucose Metabolism in parallel with Improved Post-Ischemic Recovery in an Isolated Rat Heart Model of Donation after Circulatory Death

Maria Arnold[1], Natalia Méndez Carmona[1], Patrik Gulac[1,2], Rahel K. Wyss[1], Nina Rutishauser[1], Adrian Segiser[1], Hendrik Tevaearai Stahel[1], Thierry Carrel[1], Sarah Longnus[1]

[1]Department of Cardiovascular Surgery, Inselspital, Bern University Hospital, Department for BioMedical Research (DBMR), University of Bern, Switzerland
[2]Department of Pharmacology and Toxicology, Comenius University, Bratislava, Slovakia

OBJECTIVES
Donation after circulatory death (DCD) could substantially improve donor heart availability. However, the inevitable warm ischemia raises concerns about graft function. Mechanical postconditioning (MPC) may limit injury, but the underlying mechanisms remain controversial. Therefore, we investigated the roles of glucose metabolism and key signaling molecules in MPC using a rat heart model of DCD.

METHODS
Isolated, working rat hearts underwent 20' aerobic perfusion, 30' global ischemia, and reperfusion without (control) or with MPC (2 cycles of 30'' ischemia, 30'' reperfusion). Contractile function (left ventricular (LV) work; developed pressure * heart rate), glycolysis (GLY), and glucose oxidation (GO) were monitored during reperfusion, and phosphorylation of key signaling proteins, oxygen efficiency (O2E) and cytochrome c (Cyt c) release were assessed.

RESULTS
Percentage recovery of LV work was either significantly increased (high recovery=HiR; 59±7%; $p<0.05$), or decreased (low recovery=LoR; 32±5%; $p<0.05$) by MPC compared with control (47±9%; n=7-11/group). Correspondingly, O2E was significantly higher and Cyt c release lower in HiR vs LoR hearts. In MPC hearts, LV work recovery correlated positively with GLY ($p<0.05$), but not with GO. Higher phosphorylation of the AMPK pathway, namely AS160, appeared to be present in the HiR vs LoR group. Furthermore, glucose uptake correlated positively with LV work ($p<0.05$) in MPC hearts.

CONCLUSION
MPC affects positively, but also negatively, post-ischemic contractile function. The AS160 phosphorylation pattern corresponds with increased glucose uptake and GLY, which seems to be relevant for a good functional recovery in MPC. These findings should help to establish effective reperfusion strategies.

Poster 2

Boosting Endogenous Cardioprotection via Human Fetal Stem Cell Paracrine Effects

C. Balbi[1,2], K. Lodder[3], A. Costa[1], S. Moimas[4], F. Moccia[5], V. Rosti[6], T. van Herwaarden[3], F. Santini[7], M. Giacca[4], A. Palmeri[8], P. De Biasio[8], M.J. Goumans[3], L. Barile[2], A.M. Smits[3], S. Bollini[1]

[1]Regenerative Medicine Lab., Dept. of Experimental Medicine, University of Genova, Genova, Italy, [2]Molecular and Cell Cardiology Lab., CardioCentro Ticino, Lugano, Switzerland, [3]Dept. of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands, [4]International Centre for Genetic Engineering and Biotechnology, Trieste, Italy, [5]Dept. of Biology and Biotechnology "Lazzaro Spallanzani", University of Pavia, Pavia, Italy, [6]Myelofibrosis Study Centre, IRCCS Ospedale Policlinico San Matteo, Pavia, Italy, [7]Cardiac Surgery Unit, IRCCS Ospedale Policlinico San Martino, Genova, Italy, [8]Dept. of Obstetrics and Gynecology, IRCCS Ospedale Policlinico San Martino, Genova, Italy.

OBJECTIVES

Following injury, cardiovascular disease may arise from inefficient cardioprotection, defective repair and lack of myocardial renewal. We reported that human amniotic fluid stem cells (hAFS) are cardioprotective on rodent ischemic myocardium and on cardiac cells exposed to cardiotoxicity. Here we analyze the hAFS secretome potential to: i) enhance cardiac repair and ii) trigger endogenous regenerative mechanisms.

METHODS

c-KIT+ hAFS were isolated from leftover samples of II trimester amniotic fluid for prenatal screening and stimulated under 1% O_2 to enrich their conditioned medium (hAFS-CM) with paracrine factors. Paracrine anti-apoptotic, angiogenic, and proliferative effects were evaluated on rodent neonatal cardiomyocytes (NVCM), human endothelial colony forming cells (hECFC) and human epicardial progenitors (hEPDC). A preclinical mouse model of myocardial infarction (MI) was treated with a single intra-myocardial injection of: total hAFS-CM; conditioned medium depleted of extracellular vesicles (hAFS-DM), and hAFS-extracellular vesicles (hAFS-EV).

RESULTS

hAFS-CM improved survival of NVCM undergoing oxidative and hypoxic damage; it induced Ca^{2+}-dependent angiogenesis in hECFC and triggered hEPDC and NVCM proliferation. In contrast to hAFS-DM, hAFS-CM enriched with EV counteracted scarring, supported cardiac function and triggered cardiomyocyte cell cycle progression ($p<0.05$). hAFS-CM also induced reactivation of endogenous EPDC, similarly to hAFS-EV ($p>0.05$). Although no EPDC cardiovascular differentiation was observed, our data suggest paracrine contribution to local angiogenesis. Transfer of hAFS-EV microRNA into myocardial tissue following MI might explain the increase in resident cardiomyocyte cell-cycle progression

CONCLUSION

From our data the secretome soluble component (hAFS-CM and hAFS-DM) is more likely to drive therapeutic angiogenesis, while hAFS-EV improved cardiac function and triggered EPDC reactivation and cardiomyocyte cell cycle re-entry. Thus, the hAFS secretome fractions may be relevant for cardiac regenerative medicine.

Poster 3

Ubiquitination mediates the selective sorting of Cx43 into extracellular vesicles

Henrique Girao[1], Teresa Ribeiro-Rodrigues, Lino Gonçalves, Monica Zuzarte e Tania Martins-Marques

[1]Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, University of Coimbra, Coimbra, Portugal

OBJECTIVES

A well-balanced intercellular communication (IC) between the different cell types that compose the heart is vital to ensure cardiac homeostasis and function. IC can occur either directly through connexin43 (Cx43)-containing gap junctions (GJ), or via extracellular vesicles (EVs) that sustain flow of information at longer distances. Recently, we demonstrated that Cx43 forms functional channels at the EVs surface, facilitating cargo release into recipient cells. Several disorders, including myocardial ischemia, have been associated with altered trafficking of Cx43-GJ. Although the mechanisms regulating GJ-mediated IC are well established, the signals underlying protein sorting into EVs and the consequences of ischemia upon the levels and/or function of EV-Cx43 are currently undefined. Hence, this study aimed to characterize the signals that govern Cx43 release in EVs, emphasizing the role of ubiquitin.

METHODS

Chemical and genetic tools to manipulate ubiquitination levels of Cx43 were used in different models, including cardiac cell lines HL-1 and H9c2. The amount of Cx43 in ischemic EVs was assessed *in vitro*, in the *ex vivo* Langendorff heart and in human subjects.

RESULTS

Inhibition of Cx43 ubiquitination decreased the levels of EV-Cx43, whereas PKC activation, known to promote Cx43 phosphorylation and ubiquitination, led to an accumulation of EV-Cx43. Moreover, simulated ischemia downregulated secretion of Cx43 in EVs from cardiac cell lines. The levels of Cx43 in serum circulating vesicles from patients with ST-segment elevation myocardial infarction were also decreased, comparing with controls without epicardial coronary artery disease.

CONCLUSION

Our results demonstrate that ubiquitination promotes the targeting of Cx43 into EVs, which is affected during ischemia.

Poster 4

TITLE

Is there convincing evidence for H_2S donor compounds for cardioprotection against myocardial ischemia-reperfusion injury?

AUTHOR(S)

Salah Michael Hayek

AFFILIATIONS

School of Life, Health and Chemical Sciences, the Open University, Milton Keynes, UK.

OBJECTIVES

I. Review the literature on the biochemical role of hydrogen sulfide (H_2S) in myocardial ischemia-reperfusion injuries (mIRI) discussing the current understanding of the endogenous H_2S in hypoxia.
II. Review the evidence from in-vivo studies on H_2S donor agents evaluating the molecular mechanisms for the proclaimed therapeutic role in cardioprotection against mIRI.

METHODS

Academic and public bibliographical databases (Medline, PubMed, BIOSIS) identify primary and secondary literature on: cardioprotection and/or pharmacological conditioning against mIRI; in-vivo studies for H_2S compounds in cardioprotection; Gasotransmitters role in myocardial ischaemia.

RESULTS

- Equilibrium ratio between unionised and ionised form $[H_2S]:[HS^-]$ is pH and oxidants sensitive making it highly reactive physiologically.
- H_2S plays a role in mitochondrial electron transfer chain to support anaerobic ATP production cycle. How H_2S travels the cellular compartments during hypoxia or mIRI is inconclusive.
- Unclear if reduction in plasma H_2S concentration is causation to increased risk of MI.
- Basal H_2S plasma concentration is inconclusive.
- Narrow therapeutic window. No consensus on specific biomarker(s) and chemistry analytical toolkits have limited selectivity to H_2S reactive species.

CONCLUSION

H_2S donor agent is likely to have limited / no potential clinical translation because:

- In-vivo studies lack of in-situ chemical analysis to determine if observed reduction in MI size corresponds to activity of H_2S.
- H_2S reactive species are biochemically volatile and transient in different cellular compartments, hence endogenous H_2S release mechanism is complex and elusive.

However, H_2S role in anaerobic metabolism may involve macromolecule modifications e.g. K_{ATP} -channels persulfidation, altering the endogenous H_2S biosynthesis. Their role in mitochondrial degeneration and the undesirable formation of the mPTP during mIRI requires further investigation.

Poster 5

TITLE

Deep Neural Networks as supporting tools to cardiology

AUTHOR(S)

Ljubinka Sandjakoska[1], Atanas Hristov[1]

AFFILIATIONS

Bioengineering Laboratory, University of Information Science and Technology St Paul the Apostle, Ohrid, Macedonia

OBJECTIVES

The main goal of this research is to show that deep neural networks, as advanced concepts of machine learning, are promising tools in developing software solutions for improving cardiology services. Also, we aim to give the state-of-the-art review of using artificial neural networks in cardiology and related fields. Depicting the efficiency and effectiveness of its application in cardiology is in the focus of this research.

METHODS

In order to depict its applicability followed by efficiency and effectiveness we present two different case studies. First we have developed deep neural network for classifications of foetal heart rate signals. It is used cardiotocography dataset that include 2126 instances of 23 attributes. In the second case study, we try to distinguish between the presence and absence of cardiac arrhythmia and classify it in one of the 16 groups. The dataset contains 279 attributes, 206 of which are linear valued and the rest are nominal, including multivariate data of 452 instances.

RESULTS

The findings show that deep learning classification achieves excellent sensitivity and specificity.

CONCLUSION

Developing models based on deep neural networks can improve the computational approaches used in cardiology. Also, deep learning techniques are able to solve specific task that include work with big and complex data, such as frequency and time domain analysis, foetal hypoxia detection or drug response in patients undergoing percutaneous coronary intervention.

Poster 6

Time response for development of vascular oxidative stress, endothelial dysfunction and high blood pressure by aircraft noise exposure

Sanela Kalinovic[1], Katie Frenis[1], Ahmad Al Zuabi[1], Matthias Oelze[1], Sebastian Steven[1,2], Miroslava Kvandova[1], Benjamin P. Ernst[3], Sebastian Strieth[3], Andreas Daiber[1,4], Swenja Kröller-Schön[1], Thomas Münzel[1,4]

[1] Center for Cardiology, Cardiology I – Laboratory of Molecular Cardiology, University Medical Center Mainz, Mainz, Germany
[2] Center for Thrombosis and Hemostasis, University Medical Center Mainz, Mainz, Germany
3 Department of Otolaryngology, University Medical Center Mainz, Mainz, Germany
4 German Center for Cardiovascular Research (DZHK), Partner Site Rhine-Main, Mainz, Germany

Objectives: Transportation noise is recognized as an important cardiovascular risk factor. It was shown that it is associated with cardiovascular diseases. Aircraft noise causes endothelial dysfunction, inflammation and increases oxidative stress in mice. We recently demonstrated that sleep phase noise exposure has bigger impact on the circadian clock and causes oxidative stress, endothelial dysfunction and high blood pressure comparing to awake phase noise exposure.
Methods and Results: C57Bl/6JRj mice were exposed to aircraft noise for 4, 7, 14 and 28 days, we ensured that used exposure protocol did not cause hearing loss by using audiometry**.** The induction of endothelial dysfunction and elevation of blood pressure was comparable in all exposure groups. Oxidative burst in the whole blood was increased in all exposed groups with a maximum at 4 or 7 days. Vascular ROS formation as investigated by dihydroethidium (DHE) staining was increased in noise-exposed mice without a significant time response. Noise-induced aortic, cardiac and cerebral superoxide formation as measured by DHE HPLC analysis increased in dependence of noise exposure duration. Similarly, levels of interleukin-6 (IL-6) were time dependently increased by trend in plasma and brain tissue in all noise exposed mice. We also established increased NOX2 expression in aortic tissue of noise exposed mice, while levels of P-VASP were decreased.
Conclusion: We here present novel data on the effects of different aircraft noise exposure protocols indicating that most adverse vascular effects are established already after 4 days. Thus our data provide important technical insights in noise exposure protocols for future translational studies.

Poster 7

HUMAN IPS-DERIVED CARDIOMYOCYTES SHOW ENHANCED MATURATION AND RESPONSE TO LOW-OXYGEN CONDITIONS IN MICROFLOW CONDITIONS - NOVEL SYSTEM FOR IN-DEPTH CELL BIOLOGY AND PHARMACOLOGICAL STUDIES

T. Kolanowski[1,2#], M. Busek[3#], A. Dmitrieva[2], S. Grünzner[4], N.Rozwadowska[1], F. Sonntag[4+], K. Guan[2+]*
1) PAS, Institute of Human Genetics, Dept. of Molecular Pathology, Strzeszyńska 32, 60479 Poznań, Poland;
2) TU Dresden, Institute of Pharmacology and Toxicology, Fiedlerstraße 42, 01307 Dresden, Germany;
3) TU Dresden, Faculty of Manufacturing Technology, Nöthnitzer Straße 64, 01187 Dresden, Germany;
4) Fraunhofer Institute of Material and Beam Technology IWS, Winterbergstraße 28, 01277 Dresden, Germany;
 # Authors contributes equally to this work; + Authors share senior authorship;
** tomasz.kolanowski@igcz.poznan.pl*

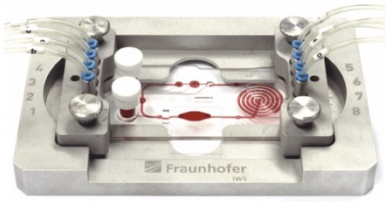
OBJECTIVES

Animal models deliver the majority of our knowledge concerning development of human heart, yet this causes severe discrepancies in translational research. Therefore, an advanced *ex-vivo* system for human cardiac cell interaction studies is needed to change that situation.
The aim of this study was to develop a microfluidic platform for perfused cell cultivation that would allow detailed characteristic of the biological interaction between differentiating cells or cell populations.

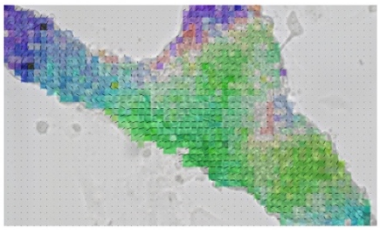
METHODS

The presented microfluidic platform features a micro pump, micro channels and an oxygenator for gas exchange (a). The human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) were employed for 1 week-long culture and subsequently characterized by Mitotracker, α-actinin and MLC2a/v stainings, together with molecular markers evaluation. We have modified and developed a software for image analysis as well (b and c).

a)



b)



c)

RESULTS

The iPSC-CMs cultured within the system show more efficient contraction, which is probably a synergic effect of increased cell alignment, when compared to the static culture (b and c, respectively). Moreover, cells within the microfluidic culture show increased sarcomere length and more developed mitochondrial network. They do respond on decreased oxygen levels with lowered functional output as well.

CONCLUSION

Taken together, microdevice-cultured cells show more mature characteristics when compared to standard culture and response to hypoxia conditions. Therefore, the system allows to study advanced CMs-features, giving a new insight into development of the human heart tissue diseases. Additionally, the platform is easily expandable and maintain stable hypoxia conditions without any additional equipment needed, which holds great potential for high-throughput applications.

Poster 8

Short-term E-cigarette vapor exposure causes vascular oxidative stress and dysfunction - evidence for

a close connection to brain damage and a key role of the phagocytic NADPH oxidase (NOX-2)

Marin Kuntic[1], Matthias Oelze[1], Sebastian Steven[1,2], Swenja Kröller-Schön[1], Sanela Kalinovic[1], Katie Frenis[1], Ksenija Vujacic-Mirski[1], Konstantina Filippou[1], Omar Hahad[1], Frank P. Schmidt[1], Andreas Daiber[1,3], Thomas Münzel[1,2,3]

1 Center for Cardiology, Cardiology I – Laboratory of Molecular Cardiology, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany;
2 Center for Thrombosis and Hemostasis, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany;
3 German Center for Cardiovascular Research (DZHK), Partner Site Rhine-Main.

OBJECTIVES
The clinical impact of E-cigarette smoking is not characterized in detail but this life style drug is known to cause vascular damage via oxidative stress and inflammation. The underlying mechanisms, especially the enzymatic sources of oxidative stress are not well characterized. Here, we investigated effects of phagocyte-type NADPH oxidase (Nox2) knockout on vascular and cerebral complications in response to short-term e-cigarette vapor exposure in mice.

METHODS
C57BL/6j and Nox2-/- (gp91phox-/-) mice were exposed to e-cigarette vapor from liquids without flavor and plus/minus nicotine for 1, 3 and 5d. Adverse effects of e-cigarette vapor on the vasculature and brain were mostly prevented by Nox2 deficiency.

RESULTS
The exposure to e-cigarette vapor without nicotine for 3d had the most detrimental effects characterized by endothelial dysfunction, increased markers of vascular/systemic oxidative stress and inflammation. E-cigarette vapor also caused increased blood pressure as well as cerebral oxidative stress and inflammation, nNOS mRNA and protein downregulation. Treatment of cultured human endothelial cells with e-cigarette vapor condensate and e-cigarette liquid revealed a more toxic effect of the condensate on cell viability and phenotype (IL-6, NOX-2). In human healthy subjects, single e-cigarette smoking caused impaired flow-mediated dilation as a surrogate of endothelial function.

CONCLUSION
E-cigarette vapor exposure increases vascular and cerebral oxidative stress via NOX-2 leading to vascular inflammation and endothelial dysfunction in mice (and men). Our data point to similar pathophysiological pathways for e-cigarette smoking as reported for tobacco smoking and other metabolic or environmental health risk factors.

Poster 9

Human iPSC-derived cardiomyocytes as cell-based platform to study cardiac senescence

Edoardo Lazzarini1, Alessandra Maria Lodrini2, Sara Bolis1, Giuseppe Vassalli1, Marcella Rocchetti2, Claudia Altomare1, Lucio Barile1

1Laboratory of Cellular and Molecular Cardiology, Fondazione Cardiocentro Ticino, Via Tesserete 49, CH-6900 Lugano.
2Department of Biotechnology and Bioscience, University of Milano-Bicocca, Milan, Italy.

OBJECTIVES

Ageing of Cardiomyocytes (CM) involves a series of structural and functional adverse remodelling, including QTc prolongation and metabolic imbalance resulting in the insurgence of heart failure. This study exploits the potential of human CMs derived from induced pluripotent stem cells (hiPSCs) as an in vitro model for cardiac senescence. Such platform will be exploited to characterize mechanisms involved in cardiac ageing and to screen anti-aging drugs. Cardiac progenitor cells (CPC)-derived exosomes (EXO), which has been shown as a source of cardioprotective factors, will be tested as anti-senescence agent.

METHODS

Patient-derived CPCs were reprogrammed into hiPSCs and subsequently differentiated into spontaneously beating cardiomyocytes (hiPSC-CMs). Finally, senescence phenotype was induced by exposure to doxorubicin (DOX) at sub-lethal concentration (0.2μM). Senescence induction was highlighted by protein and gene expression analysis and senescence-associated β-gal assay. Electrical activity of hiPSC-CMs was evaluated recording extracellular field potentials through a multielectrode arrays.

RESULTS

DOX treatment in hiPSC-CMs induced senescence, as confirmed by activation of p21 and p16 pathway and increase of β-gal staining as compared to untreated cells (CTRL). DOX-CMs showed prolonged QTc in comparison to CTRL and this effect was prevented by EXO treatment. Biochemical and gene expression analysis revealed a metabolic switch of DOX-CMs towards glycolysis, with a reduction in the □-oxidative metabolism which provokes a decrease in the ATP/AMP ratios.

CONCLUSION

Preliminary results highlight how the hiPSC-CMs based cellular model recapitulates the phenotype of aged CMs. Moreover, preliminary findings suggest the potential cardioprotective role of EXO in reducing age-related modifications.

Poster 10

Chronic voluntary exercise-induced molecular signaling in myocardium of rats: beneficial versus detrimental effects

Lubomir Lonek[1], Agnes Puhova[2], Lucia Kindernay[1], Veronika Farkasova[1], Sagar P. Patel[1,3], Daniela Jezova[2], Tanya Ravingerova[1]

[1]Institute for Heart Research, Centre of Experimental Medicine SAS, Bratislava, Slovakia;

[2]Institute of Experimental Endocrinology, Biomedical Research Center SAS, Bratislava, Slovakia;

[3]The M.S. University of Baroda, Vadodara, Gujarat, India

OBJECTIVES. Exercise-induced preconditioning (PC) exerts anti-ischemic cardioprotection proposed to involve "survival" RISK pathway, similar to other forms of conditioning including remote PC. However, molecular mechanisms of this form of cardioprotection are less elucidated. Moreover, negative effects of long-term exercise are also known. This study aimed to investigate effects of voluntary running on components of RISK pathway, expression of heat shock proteins and antioxidant system. Cell death mechanisms (pro-/anti-apoptotic), as well as cell proliferation were also explored.

METHODS. Male Sprague-Dawley rats (eight-weeks old) were randomly divided into groups of Run, where animals freely exercised in the cages equipped with running wheels and group of sedentary Controls placed in standard cages. After 23 days, left ventricular (LV) myocardial tissue samples were collected from rats of both groups for detection of expression and activation of RISK pathway proteins (WB analysis), while injection of 5-bromo-2'-deoxyuridine (BrdU), a marker of cell proliferation, was given to detect BrdU incorporation into DNA of the cells in LV. .

RESULTS. Voluntary running increased activation of Akt and ERK1/2, as well as levels of eNOS and PKCε, while PKCδ levels and caspase-3 activation were decreased. Moreover, expression of SOD and HSP70 was increased in the hearts of exercising rats, whereas incorporation of BrdU into DNA in the LV cells was similar in both experimental groups. However, exercise induced pro-apoptotic signaling - enhanced Bax/Bcl-2 ratio and reduced phosphorylation of GSK-3β kinase.

CONCLUSION. Results suggest that in the rat myocardium adapted to physical load, cell proliferation is not modified, but the natural cardioprotective processes associated with physiological hypertrophy are stimulated. However, long-term uninterrupted exercise might lead to maladaptation since chronic activation of kinases of RISK cascade may be detrimental due to their pro-hypertrophic effect on the myocardium. Up-regulation of pro-apoptotic markers supports potential induction of cell death mechanisms.

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Poster 11

EHD proteins mediate gap junction (GJ) remodelling during myocardial ischemia

AUTHOR(S)

Tânia Martins-Marques[1], Steve Catarino[1], Cláudia G Almeida[2], Henrique Girão[1]

AFFILIATIONS

[1]Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, University of Coimbra, Coimbra, Portugal

[2]Chronic Diseases Research Center (CEDOC), NOVA Medical School, Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, Lisboa, Portugal

OBJECTIVES

Electrical conduction in the heart is ensured by an efficient gap junction intercellular communication (GJIC) between cardiomyocytes. Mounting evidence has shown that GJ channel closure, increased degradation and lateralization of connexin43 (Cx43), the most abundant ventricular GJ protein, contribute to a loss of intercellular communication, with consequences on both infarct size and arrhythmia in the setting of myocardial infarction (MI). Previous studies demonstrated that Cx43-channels are degraded by autophagy in the ischemic heart. However, the molecular mechanisms underlying GJ remodelling are not fully understood. Recently, in a proteomic analysis we showed that ischemia enhances interaction of Cx43 with Eps15 homology domain-containing protein 1 (EHD1) in rat hearts. Therefore, the objective of this study was to investigate the role of EHD1 on ischemia-induced remodelling of GJ.

METHODS

We used *ex vivo* Langendorff-perfused rat hearts to evaluate the association of EHD proteins with Cx43. To gain further insight on the role of EHDs, we performed siRNA-mediated knockdown of EHD1 in HL-1 cardiomyocytes.

RESULTS

Our results show that ischemia increased the co-localization of EHD proteins with mislocalized Cx43-channels, namely at the lateral sarcolemma. During reperfusion, co-localization between Cx43 and EHDs return to basal levels, suggesting that the transient association between the two proteins is involved in the regulation of Cx43 trafficking during ischemia. EHD1-knockdown led to a stabilization of Cx43 at the plasma membrane of HL-1 cardiomyocytes, by decreasing Cx43 internalization. Moreover, silencing of EHD1 was sufficient to enhance GJIC.

CONCLUSION

Altogether, we identified EHDs as novel players involved in the remodelling of Cx43-channels during MI.

Poster 12

ENDOTHELIAL FUNCTION MORE SUSCEPTIBLE TO ISCHEMIA-REPERFUSION THAN CONTRACTILE FUNCTION IN AN ISOLATED RAT HEART MODEL OF DONATION AFTER CIRCULATORY DEATH (DCD)

Natalia Méndez-Carmona[1], Rahel K. Wyss[1], Maria Arnold[1], Anna Joachimbauer[1], Georg M. Fiedler[2], Thierry P. Carrel[1], Hendrik T. Tevaearai Stahel[1], Sarah L. Longnus[1]

[1]Department of Cardiovascular Surgery, Inselspital, Bern University Hospital and Department for BioMedical Research, University of Bern, Bern, Switzerland
[2]Center of Laboratory Medicine, University Institute of Clinical Chemistry, University Hospital, Inselspital, Bern, Switzerland

OBJECTIVES: Donation after circulatory death (DCD) could significantly improve cardiac graft availability. However, DCD hearts undergo potentially deleterious warm ischemia and reperfusion (I/R). As endothelial damage is a key factor in cardiac I/R injury, we aimed to investigate the tolerance of cardiac and endothelial function following various durations of warm ischemia, in order to improve the timing and choice of cardioprotective therapies.

METHODS: Isolated, working rat hearts were perfused for 20 min aerobically, then underwent various periods of warm global ischemia and either 30 or 60 min reperfusion.

RESULTS: Compared with non-ischemic hearts, recovery of left ventricular work (heart rate – developed pressure product) was significantly reduced after 60 min reperfusion following ≥27 min ischemia ($p < 0.05$ for all), but was unchanged after 21 or 24 min ischemia. Markers of cell death and edema significantly increased ≥27 min ischemia compared with non-ischemic hearts ($p < 0.05$ for all). Endothelial-dependent vasodilation was significantly impaired compared to non-ischemic hearts with ≥24 min ischemia, while endothelial-independent vasodilation was impaired ≥27 min ischemia ($p < 0.05$ for all). Furthermore, with ≥24 min ischemia, superoxide production by nitric oxide synthase and peroxynitrite levels were significantly increased compared to non-ischemic hearts, suggesting endothelial nitric oxide synthase (eNOS) uncoupling ($p < 0.05$ for both).

CONCLUSION: The first signs of endothelial dysfunction following cardiac ischemia occur with less ischemia than cardiac functional alterations, and may result from increased eNOS uncoupling. Strategies aiming at improving eNOS coupling may thus help to optimize both endothelial and myocardial recovery, ultimately facilitating DCD heart transplantation.

Poster 13

The role of GSK-3beta kinase as a molecular target for cardioprotection revisited using pharmacological inhibitors with indirubin scaffold

Panagiota-Efstathia Nikolaou[1], Kerstin Boengler[2], Panagiotis Efentakis[1,3], Konstantina Vougogiannopoulou[1], Anastasia Zoga[4], Nicholas Gaboriaud-Kolar[1,5], Vassilios Myrianthopoulos[1], Nikolaos Kostomitsopoulos[6], Alexios Leandros Skaltsounis[1], Andreas Papapetropoulos[1,6], Efstathios K. Iliodromitis[4], Rainer Schulz[2], Ioanna Andreadou[1]

[1]National and Kapodistrian University of Athens, Faculty of Pharmacy, Athens, Greece; [2]Institute for Physiology, Justus-Liebig University Giessen, Giessen, Germany; [3]Center for Thrombosis and Hemostasis, University Medical Center of the Johannes Gutenberg-University, Mainz Germany; [4]National and Kapodistrian University of Athens, Medical School, Attikon University Hospital, Athens ,Greece [5] Bioval Océan Indien, 73 rue Jean-François Breton, B95/16, 34398 Montpellier Cedex 5, France; [6]Academy of Athens Biomedical Research Foundation, Centre of Clinical Experimental Surgery and Translational Research, Athens, Greece

OBJECTIVES The role of glycogen synthase kinase 3 beta (GSK3β) in cardioprotection and its link with the mitochondrial Permeability Transition Pore (mPTP) is debated. We investigated the role of GSK3β in Ischemia (I)/ Reperfusion (R) injury by using novel pharmacological tools.

METHODS: Inhibition of GSK3β using the inhibitor BIO and several novel selective analogues (MLS2776-MLS2779) was investigated in anesthetized rabbits. Infarct sizes (IS) were determined and the two most protective inhibitors were tested in anesthetized mice. IS was determined and myocardial tissue was obtained to investigate GSK3β inhibition. In order to address the GSK3β-mitochondria interaction, murine hearts were Langendorff-perfused (30'I/10'R or normoxic conditions), subsarcolemmal (SSM) and interfibrillar mitochondria (IFM) were isolated, and GSK3β localization was determined. Calcium Retention Capacity (CRC) was determined 1) on mitochondria after administration of the inhibitors 2) in vitro on SSM derived from wild type and cyclophyllin D knock out mice. Cyclosporine A (CsA) was co-administered with the inhibitors to address putative additive cardioprotective effects.

RESULTS: Rabbits treated with MLS compounds had smaller IS compared to control group. MLS2776 and MLS2778 possessed the greatest infarct-sparing effects and reduced IS in mice compared to control. GSK3β inhibition was confirmed as a decrease in p(Y216)-GSK3β and p(S33/37/T41)-β-catenin, but not by a p(S9)-GSK3β increase at the 10th min of R. The mitochondrial amount of GSK3β was similar in normoxic SSM and IFM and it was not altered by I/R. The inhibitors did not affect CRC under normoxia or I/R. The co-administration of CsA ensured that cardioprotection was CypD independent.

CONCLUSIONS: Pharmacological inhibition of GSK3β attenuates IS beyond mPTP inhibition.

Poster 14

Application of Mitochondria-Targeted Antioxidant Restores Mitochondrial Function in Senescent Cardiomyocytes

Yusuf OLGAR, Erkan TUNCAY, Belma TURAN

Department of Biophysics, Ankara University, Faculty of Medicine, Ankara, Turkey.

OBJECTIVES Aging in humans are descending processes in cardio-protective systems while mitochondria plays pivotal role due to the higher energy demand of heart senescence. Although it has been mentioned the important contribution of the mitochondrial dysfunction during aging we aimed to analyse how mitochondrial targeted antioxidants affects mitochondria-related proteins that are responsible for mitochondrial function in aging subjects.

METHODS We used cardiomyocytes isolated from adult (6-month old) and aged (24-month old) rats. We measured reactive oxygen species (ROS), mitochondrial membrane potential and mitochondrial superoxide formation from cardiomyocytes. Mitochondrial function also evaluated via expression levels of mitochondrial fission/fusion related proteins isolated from cardiomyocytes.

RESULTS Freshly isolated cardiomyocytes incubated with 1 μM MitoTEMPO, mitochondria-targeting antioxidant, for 3 hours. While intracellular reactive oxygen species $[ROS]_{in}$ and mitochondrial superoxide formation $[SOX]_{Mit}$ increased, MitoTEMPO exhibited remarkable recovery of $[ROS]_{in}$ and $[SOX]_{Mit}$ in aged cardiomyocytes. We showed significant improvement in mitochondrial membran potential following antioxidant treatment in aging cardiomyocytes. Western blotting analysis exerted that mitochondrial fission protein levels of Fis-1 decreased in aging and restored after antioxidant treatment. Mitochondrial fusion protein levels of Mfn-2 and OPA-1 decreased while Mfn-1 unchanged in aging. Antioxidant treatment did not affect protein levels of Mfn-1 but increased protein levels of Mfn-2 and OPA-1 in aged cardiomyocytes.

CONCLUSION In summary present data demonstrated that improvement of mitochondrial antioxidant capacity with MitoTEMPO, directly targeting mitochondrial reactive oxygen species, could preserve mitochondrial dysfunction in aged-rats.

Poster 15

Cardioprotective effect of ellagic acid on ISO-induced pathological cardiac hypertrophy

Semir Ozdemir, Bilge Eren Yamasan, Tanju Mercan, Orhan Erkan

Akdeniz University Faculty of Medicine Department of Biophysics, Antalya, Turkey

OBJECTIVES

Sustained β-adrenergic stimulation leads to hypertrophy and heart failure due likely to defective Ca^{2+} handling and impaired excitation-contraction coupling of cardiomyocytes. On the other hand, ellagic acid (EA) has been suggested to exert protective effects in adverse cardiovascular events through modulation of Ca^{2+} signalling. The aim of this study was to determine whether EA can restore Ca^{2+} handling abnormalities and improve contractile activity of myocardium.

METHODS

Cardiac hypertrophy was induced by injection of 5 mg/kg of isoproterenol (ISO) subcutaneously for 4 weeks. The rats in the ISO+EA group were administered 20 mg/kg of EA inragastrically in addition to 5 mg/kg ISO injection. Following treatment period, fractional shortening and intracellular Ca^{2+} transients were recorded under the electrical field stimulation in isolated ventricular myocytes and the relationship between ionic and contraction parameters were evaluated. Ca^{2+} currents were also examined using patch-clamp technique.

RESULTS

The fractional shortening amplitude decreased and time to 75, 90% re-lengthening of ISO-group myocytes increased, compared to that of control group ($p<0.05$). EA administration in the ISO+EA group achieved a significant improvement in the altered contractile responses compared to the ISO-group ($p<0.05$). Similarly, the Ca^{2+} transient amplitude and decay rate of ISO-group myocytes were significantly decreased compared to that of control group myocytes ($p<0.05$). Moreover, L-type Ca^{2+} current was decreased significantly in the ISO-group myocytes and it was not restored with EA administration.

CONCLUSION

Our results showed that EA supplementation is capable of improving significantly the impaired contraction parameters and intracellular Ca^{2+} transients of myocardium in ISO-induced pathological hypertrophy.

Poster 16

Monoamine Oxidase Is A Source Of Oxidative Stress In Obese Patients With Chronic Inflammation

Adrian Sturza[1,], Mihaela Ionică[1], Oana Duicu[1,*], Norbert Pop[2], Sonia Rațiu[2], Sorin Olariu[2], Danina M. Muntean[1,*]*

[1]Department of Pathophysiology - Functional Sciences, [*]Centre for Translational Research and Systems Medicine, [2]First Department of Surgery, "Victor Babeş" University of Medicine and Pharmacy Timișoara, Romania

OBJECTIVES: Obesity has been systematically associated with an increased risk of cardiometabolic diseases and oxidative stress plus a chronic state of low grade inflammation are the major underlying mechanisms. Monoamine oxidases (MAOs), with 2 isoforms, A and B, have emerged in the past decade as an important source of oxidative stress in the cardiovascular system. The present study was purported to evaluate the role of MAO as source of reactive oxygen species (ROS) in visceral adipose tissue and vessels in obese patients with chronic inflammation. METHODS: To this aim, visceral abdominal adipose tissue and mesenteric artery branches were harvested from patients undergoing elective abdominal surgery, transferred to the laboratory and used for organ bath evaluation of vascular reactivity, ROS assessment, qRT-PCR and immune-histology (IH) studies. RESULTS: qRT-PCR and IH revealed that human visceral adipose tissue and mesenteric artery branches contain MAO, predominantly the A isoform. A significant upregulation of MAO expression together with a marked impairment of the vascular reactivity and increased ROS production were found in samples from obese patients with chronic inflammation. Acute incubation of the adipose tissue samples and vascular rings with the MAO-A inhibitor (clorgyline, 10 μM, 30 min) was able to significantly improve vascular reactivity and decrease ROS production, respectively. CONCLUSION: In this pilot study, we showed that MAO-A is the predominat isoform in human adipose tissue and mesenteric artery branches, are overexpressed in patients with inflammation, and contribute via H_2O_2 generation to the endothelial dysfunction.
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Poster 17

Pathophysiologically relevant TNF concentrations fail to elicit notable TNFR-mediated signalling in cardiomyocytes

Szobi Adrian[1], Kormanova Veronika[1], Hlavackova Marketa[2], Kolar Frantisek[2], Adameova Adriana[1]

1 - Faculty of Pharmacy, Comenius University in Bratislava, Slovak Republic
2 - Institute of Physiology, Czech Academy of Sciences, Prague, Czechia

OBJECTIVES
Elevated myocardial tissue levels of TNF acting on TNFR1 have been suggested to induce cardiomyocyte loss and dysfunction in pathologies such as heart failure. On the other hand, TNF can also be cardioprotective by activating prosurvival pathways through TNFR2. Therefore, our aim was to investigate the response of rat primary adult left ventricular cardiomyocytes (ARLV-CMs) to pathophysiologically relevant TNF concentrations during 48h.

METHODS
Isolated ARLV-CMs were treated with increasing TNF concentrations (0pg/ml, 10pg/ml, 25pg/ml, 50pg/ml, 100pg/ml, 1ng/ml) with or without AZD5582 (Smac mimetic, promoter of TNFR1-mediated cell death) and zVAD-fmk (pancaspase inhibitor) for 48h. Viability of ARLV-CMs was monitored with SYTOX Green. Additionally, Western blot analysis of apoptotic, proinflammatory and prosurvival signalling linked to TNFR stimulation was performed in groups treated with 0pg/ml or 1ng/ml of TNF after 24h and 48h.

RESULTS
TNF at any concentration demonstrated no effect on the survival of ARLV-CMs. In addition, TNF failed to activate apoptosis (csp8, csp3, PARP1 cleavage) or induce proinflammatory signalling (pSer536-NF-κB, pSer32/36-IκBα, IL-1β). Mirroring these results, changes in prosurvival pathways (pThr308-Akt, pTyr202/204-Erk1/2) were also absent. Concentration-dependant effects did not emerge when co-treated with AZD5582 or zVAD-fmk.

CONCLUSION
Primary cardiomyocytes display low sensitivity to pathophysiological levels of TNF at least in the short-term; therefore, TNF by itself is unlikely to significantly influence their survival. Other factors might be necessary to change the fate of cardiomyocytes in pathologies with elevated levels of TNF.

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Poster 18

Effect of ischemic pre-conditioning on expression of cardio- and thrombomiRs in a porcine model of acute myocardial infarction

Denise Traxler[1], Alfred Gugerell[1], Martin Riesenhuber[1], Jakob Prömer[1], Katrin Zlabinger[1], Andreas Spannbauer[1], Johannes Winkler[1], Mariann Gyöngyösi[1]

[1] Department of Internal Medicine II, Division of Cardiology, Medical University of Vienna, Austria

OBJECTIVES

Several microRNAs play role in cardioprotection. The aim of this work was to assess the effect of ischemic pre-conditioning on circulating cardio- and thrombo-microRNAs (miR) in a pig model of acute myocardial infarction (AMI).

METHODS

Closed chest reperfused AMI was induced in farm pigs by 90 minutes percutaneous balloon occlusion of the LCX, followed by balloon deflation. In four pigs IPC in the LAD for 60 minutes (3 circles of 10 min occlusion + 10 min reperfusion) was performed 24h prior to AMI to induce cardioprotection in the late window. Blood sampling was performed before and after IPC, before AMI induction, at the 25th and 90th min during balloon occlusion, and 60min after reperfusion. We measured expression levels of six cardiomiRs and four thrombomiRs using qPCR.

RESULTS

Hypoxia led to downregulation of miR-130a, miR-146a, miR-92a, miR-221, mir-150, miR-191 and mir-21, while reperfusion increased the level of miR-1-3p, miR-27b-3p, and miR-126-3p, not influenced by IPC. However, IPC induced decrease in circulating miR-143 and miR145-5p, and a trend to lower level of miR-223-3p, miR-92a-3p during coronary occlusion, with inhibitory effect on apoptosis, inflammation and necrosis.

CONCLUSION

Hypoxic myocardial stress followed by reperfusion resulted in deregulation of several cardiac- and platelet-associated miRs playing role in angiogenesis, cell proliferation and migration. IPC further decreased the level of circulating miR-s responsible for vascular injury, thereby exerting protective effect in the ischemic heart in the late window of cardioprotection.

Poster 19

β_3-Adrenergic Receptor Activation Contributes to Cardiac Dysfunction through alterations in cytosolic labile Zn^{2+} and Ca^{2+}

Erkan Tuncay, Yusuf Olgar, Aysegul Durak, Sinan Degirmenci, Ceylan Verda Bitirim, Belma Turan

Department of Biophysics, Faculty of Medicine, Ankara University, Ankara, Turkey

OBJECTIVES β-adrenergic receptors (β-ARs) are cell surface receptors, known as G protein-coupled receptors, regulates multiple cellular processes. In this study, we aimed to determine the molecular mechanism of β_3-AR activation and contribution in the depressed myocardial contractility.

METHODS We used cardiomyocytes either isolated from control and metabolic syndrome (MetS) rats or H9c2 cell-line. We measured intracellular labile Zn^{2+} level($[Zn^{2+}]_i$) and Ca^{2+} level ($[Ca^{2+}]_i$) as well as mitochondrial membrane potential(MMP), reactive oxygen/nitrogen species (ROS/RNS) from cardiomyocytes or β_3-AR overexpressed H9c2 cells. We also measured single cell action potential (AP) and the ionic-channel activities contributing to the AP parameters.

RESULTS We determined the significantly high protein expression level of β_3-AR, whereas no significant changes in both β_1-AR and β_2-AR in MetS rat cardiomyocytes. A β_3-AR stimulation induced significant increases in the $[Zn^{2+}]_i$, and $[Ca^{2+}]_i$ as well as in ROS and RNS levels, marked depolarization in the MMP and prolongation in the AP, and significant decreases in the K^+-and Ca^{2+}-currents. Furthermore, we observed a significant restoration in the prolonged APs in MetS cardiomyocytes with β_3-AR stimulation through the significant preservation in the depressed ion-channel activities. However, that stimulation induced significant depression in the contractility of the heart preparations.

CONCLUSION This study presents the important evidence of the role of β_3-AR activation in the depressed myocardial contractility *via* both elevated $[Zn^{2+}]_i$ and RNS under pathological conditions besides the increased ROS and $[Ca^{2+}]_i$. Taken into consideration our data in MetS-rats emphasize the important role of β_3-AR activation in cardiac pathological remodeling, through NO-signalling-$[Zn^{2+}]_i$ pathways. (This project supported by TUBITAK-SBAG217S254).

Poster 20

Cardiac Mitochondrial Integrity During Early Reperfusion – Consequences of Ischemic Duration and Association with Post-Ischemic Cardiac Recovery

Rahel K. Wyss[1,3], Natalia Méndez-Carmona[1,3], Maria-Nieves Sanz[1,3], Maria Arnold[1,3], Adrian Segiser[1,3], Georg M. Fiedler[4], Thierry P. Carrel[1,3], Siamak Djafarzadeh[2,3], Hendrik T. Tevaearai[1,3], Sarah L. Longnus[1,3]

[1]Department of Cardiovascular Surgery and [2]Department of Intensive Care Medicine, Inselspital, University Hospital Bern; [3]Department for BioMedical Research, University of Bern, Switzerland

OBJECTIVES: Cardioprotection and reliable means for graft evaluation following ischemia-reperfusion are essential in facilitating heart transplantation with donation after circulatory death (DCD). Given the key role of mitochondria in ischemia-reperfusion, we aimed to:
i) investigate mitochondrial tolerance to warm, global ischemia, and early reperfusion, and
ii) determine the predictive value of mitochondrial integrity for post-ischemic cardiac recovery.

METHODS: Isolated, working rat hearts underwent 0/21/24/27/30/33 min warm, global ischemia followed by 60 min reperfusion. Functional recovery was determined at 60 min reperfusion, whereas mitochondrial integrity was measured at 10 min reperfusion.

RESULTS: Contractile recovery at 60 min reperfusion decreased with ≥27 min ischemia ($p<0.01$ vs. no ischemia, n=7-8/group). ≥21 min ischemia decreased ventricular ATP content, mitochondrial coupling, and Ca^{2+} retention capacity, and also increased oxidative damage vs. no ischemia ($p<0.05$ for all). Reverse electron transfer (RET)-derived ROS emission increased with 21/27 min ischemia vs. no ischemia and 33 min ischemia ($p<0.05$), while forward electron transfer-derived ROS emission increased only with 33 min ischemia vs. no ischemia ($p<0.05$). Contractile recovery correlated positively with mitochondrial coupling and ATP content, while it correlated negatively with cytochrome c and succinate release, oxidative damage, and mitochondrial Ca^{2+} content ($p<0.05$).

CONCLUSION: Mitochondrial dysfunction occurs with shorter periods of ischemia than cardiac dysfunction. Mitochondrial coupling, ROS emission (RET) and Ca^{2+} retention capacity are particularly sensitive to early reperfusion injury, reflecting potential targets for cardioprotection and supporting the potential of immediate, mitochondria-directed cardioprotection after ischemia. Indicators of mitochondrial integrity may be of aid in evaluating suitability of DCD grafts for transplantation.

Poster 21

Kv1.3 channel blockade improves insulin resistance and reduces risk of arrhythmia in a type 2 diabetic animal model

AUTHOR(S)

Julián Zayas-Arrabal, Amaia Alquiza, Juan Manuel Rodríguez-Robledo, Oscar Casis, Mónica Gallego.

AFFILIATIONS

Facultad de Farmacia, Universidad del País Vasco UPV/EHU, Vitoria-Gasteiz, Spain.

OBJECTIVES

Kv1.3 channel is present in macrophages and lymphocytes where modulates the cell activation. Its inhibition improves insulin resistance in obesity models. Thus, we investigated if immunomodulation, induced by Kv1.3 channel blockade, could normalize hyperglycemia by reducing insulin resistance, as well as improve the ECG alterations and reduce the risk of ventricular tachyarrhythmia (VT) present in T2D.

METHODS

For the T2D model Sprague-Dawley rats were fed for 6 weeks with high fat diet and received an IP injection of STZ (35 mg/Kg) at week 2. Then, the animals were treated with either metformin, first therapeutic choice for T2D, or the Kv1.3 channel blocker PAP-1. Concentrations of metformin (50-100 mg/kg daily) or PAP-1 (5-10 mg/kg daily) were adjusted individually to correct hyperglycemia.

RESULTS

Our T2D model showed insulin resistance, hyperglycemia and the classical alterations in the electrocardiogram. Weekly glucose measurements and the intraperitoneal insulin and glucose tolerance test (IPIGTT) showed that PAP-1 normalized insulin resistance better than metformin. Electrocardiograms to conscious animals showed that, whereas metformin did not normalize ECG alterations, PAP-1 treatment normalized RR interval duration and the prolonged QTc interval. An *in vivo* arrhythmia susceptibility protocol with caffeine/dobutamine showed that only treatment with PAP-1 reduced the probability of developing ventricular tachycardia.

CONCLUSION

Kv1.3 channel blockade is a promising new target for T2D treatment with special relevance to cardiac arrhythmia prevention.

Notes

Notes