2nd EU-CARDIOPROTECTION COST Action MC and WG Meeting
March 19th - 21st 2018, Vienna, Austria

Meeting Venue
Lecture Hall,
Josephinum Medical Museum,
Währinger Str. 25,
1090 Vienna, Austria.

Program committee
COST Action Core Group committee
Bruno Podesser
Mariann Gyongyosi

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 Owen (UK)

Day 1: Mon Mar 19th 2018

1300-1400 **COST Action Core group meeting** – library (Core Group Members only)

1400-1500 **COST Action MC meeting Part 1** – lecture hall (All invited to attend)

1500-1530 Coffee Break/Poster view - library

1530-1700 **COST Action MC meeting Part 2** (All invited to attend)

1700-1800 **Welcome and introductions to WG meeting**
Derek Hausenloy, Ioanna Andreadou, Peter Ferdinandy
Bruno Podesser, Mariann Gyongosi

Opening Keynote Lecture
Old and new mechanisms in cardioprotection: Role of nitric oxide and mitochondria
Invited specialist: Prof Charles Steenbergen (US) (45,15)

1830 Travel to traditional "Viennise Heurigen" from Josephinum by bus

1900-2200 **Meeting dinner**
Austrian Restaurant
"Heurigen Wieninger"
Stammersdorfer Str. 78
1210 Vienna

2200-2230 Bus transfer back to Hotels

Day 2: Tue Mar 20th 2018

0830-1000 **WG1 NEW TARGETS**
Chairs: Ioanna Andreadou, Hector Cabrera-Fuentes, Derek Hausenloy

Cardiovascular therapy with dipeptidyl peptidase-4 (DPP-4) inhibitors (gliptins) and glucagon-like peptide-1 (GLP-1) receptor agonists – effects beyond glucose lowering
Invited specialist: Andreas Daiber (Germany) (25,5)

SGLT2 inhibitors as a novel cardioprotective therapy (10,5)
Ioanna Andreadou (Greece) (10,5)

Red blood cells as novel target for cardioprotection
John Pernow (Sweden) (10,5)

-Omics in cardioprotection
Mariann Gyöngyösi (Austria) (10,5)

Mitochondria/cytoskeleton interaction and cardioprotection
Tuuli Käämbre (Estonia) (10,5)

1000-1030 **Chairs:** Ioanna Andreadou, Derek Hausenloy
Discussion
- CVR Spotlight issue

1030-1100 Coffee Break/Poster view

1100-1230 **WG2: COMBINATION THERAPY**
Chairs: Sean Davidson, Attila Kiss, David Garcia-Dorado

Identifying cardioprotective strategies with clinical potential in P2Y12 inhibitor-treated animal models
Invited specialist: James Downey (US) (25,5)

Combination therapy as a novel cardioprotective strategy: rationale
Sean Davidson (UK) (10,5)

RIC + Exenatide – 2 agents, 2 different pathways
David Garcia-Dorado (Spain) (10,5)

Mitochondrial acylcarnitine as a new target for cardioprotection
Edgars Liepins (Latvia) (10,5)

Inflammation - new targets for cardioprotection
Soren Pischke (Norway) (10,5)

1230-1300 **Chairs:** Sean Davidson, David Garcia-Dorado
Discussion
- Review on combination Therapy

Day 2: Tue Mar 20th 2018

1300-1400 Lunch/Poster review

1400-1530 **WG3 CONFOUNDERS**
Chairs: Rainer Schulz, Alina Serban, Peter Ferdinandy

Network biology to reveal cardiovascular comorbidities: the Diseaseome
Invited specialist: Joerg Menche (Austria) (25,5)

Risk factors and cardioprotection: focus on hyperlipidemia.
Peter Ferdinandy (Hungary) (10'5)

Myocardial infarction in the presence of chronic treatment with the COX2 inhibitor rofecoxib: cardioprotection and/or hidden cardiotoxicity?
Gabor B Brenner (Hungary) (10'5)

Effect of aging on cardioprotection
Marisol Ruiz-Meana (Spain) (10'5)

miR and exosomes in cardiovascular diseases
Henrique Girao (Portugal) (10'5)

1530-1600 **Chairs:** Rainer Schulz, Peter Ferdinandy
Discussion
- Horizon 2020 grant
- BJP Themed issue
- Hans Erik Botker- GTN
- Derek Hausenloy- GTN
- Sean Davidson- GTN

1600-1630 Coffee Break/Poster review

1630-1800 **Introductory talk to Josephinum Medical Museum**
"From Wax Models to Modern Imaging"
Bruno Podesser (Austria)
Visit to **Josephinum Medical Museum**

Evening Dinner on own or come to:
Stiegl Ambulanz (Old Ambulance)
Altes AKH, Alser Str. 4, 1090 Wien, Austria

Day 3: Wed Mar 21st 2018

0830-1000 **WG4 CONSORTIUM**
Chairs: Hans Erik Botker, Jacob Lonborg, Gerd Heusch

Setting up a Cardioprotection Consortium, the CAESAR Experience
Invited specialist: Charles Steenbergen (US) (25,5)

Long term myocardial remodelling after IR injury and its modification by conditioning strategies
Bruno Podesser (Austria) (15'5)

CMR-based salvaged index in preclinical and clinical cardioprotection trials
Derek Hausenloy (UK) (15'5)

Optimal design of a clinical trial for cardioprotection in STEMI patients
Hans Erik Botker (Denmark) (15'5)

1000-1030 **Chairs:** Hans Erik Botker, Gerd Heusch
Discussion
- BRC review

1030-1100 Coffee Break/Poster review

1100-1200 **Closing Keynote Lecture**
Chair: Derek Yellon

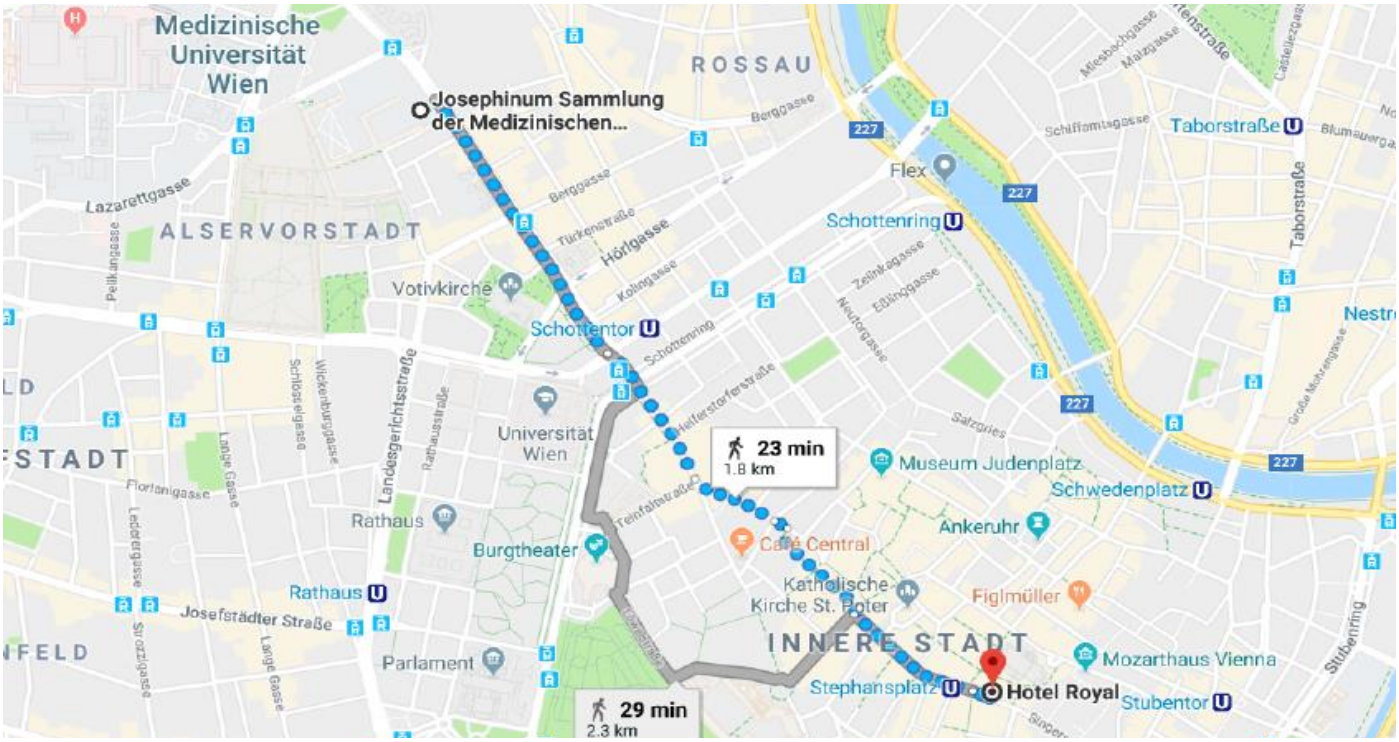
The inflammasome: the hidden killer of reperfused myocardium?
Invited specialist: James Downey (US) (45,15)

1200-1300 **Chairs:** Derek Hausenloy, Peter Ferdinandy
All WGs Discussion and Action Points

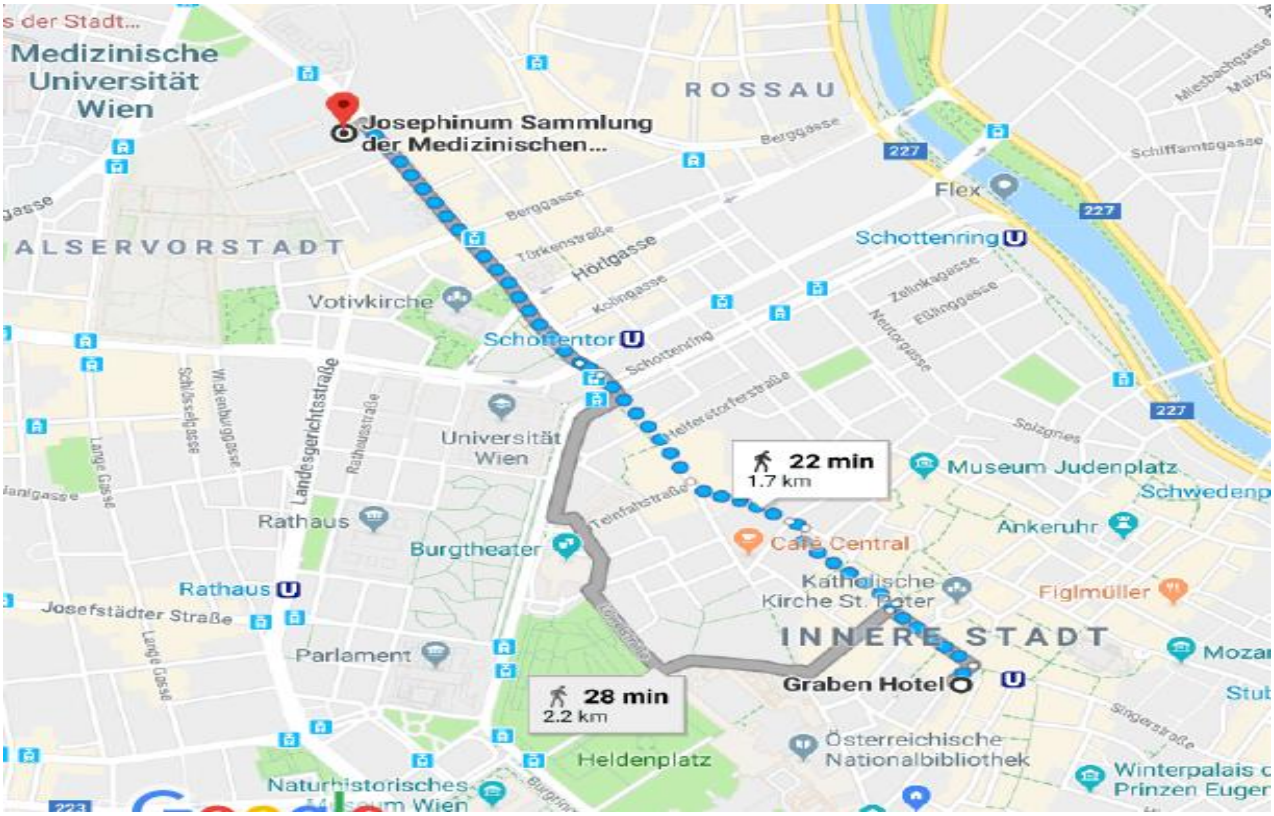
1300-1400 Lunch

1400-1500 Visit local research labs (optional)

Hotel Royal to Josephinum



Hotel Graben to Josephinum



Abstracts (in alphabetical order)

Superior Exosome-mediated Paracrine Effects of Cardiac Progenitor Cells Compared to Bone Marrow Mesenchymal Stem Cells Derived from the Same Patient for Cardioprotection

Lucio Barile, *Alessandra Ciullo, Vanessa Biemmi, Giuseppina Milano, Sara Bolis, Claudia Altomare, Tiziano Moccetti and Giuseppe Vassalli*
Cellular and Molecular Cardiology Laboratory, Cardiocentro Ticino Foundation, Lugano, Switzerland

OBJECTIVE(S)
Available evidence supports paracrine effects as the mechanism of benefit of both bone marrow-derived mesenchymal stem cells (BM-MSCs) and cardiac progenitor cells (CPCs) in patients after acute myocardial infarction (MI). Recently, we have shown that exosomes (Exo), secreted extracellular nanovesicles, are the critical component of the paracrine activity of CPCs. However, Exo-MSC and Exo-CPC have not been compared so far.

MATERIAL AND METHOD
Both a sternal aspirate and a right atrial appendage explant were obtained from each of 12 patients who underwent heart surgery for valve disease to derive BM-MSCs and CPCs, respectively. Exo was purified by ultracentrifugation from the respective conditioned media. Exo were injected intramyocardially in rats after permanent left coronary artery ligation. Exo-F was injected as a control. One and 4 weeks after MI and Exo injection, LVEF was evaluated by echocardiography.

RESULT(S)
Exo-CPC inhibited staurosporin-induced apoptosis in mouse cardiomyocytes more effectively than Exo-MSC ($p<0.05$). Both Exo-CPC and Exo-MSC promoted angiogenesis in vitro. Infarcted rat hearts injected with Exo-CPC showed significantly less cardiomyocyte apoptosis and scar, more angiogenesis, higher end diastolic thickness, and an improved LV ejection fraction (LVEF) at 4 weeks post-MI (75.37±3.15%) compared to those injected with Exo-MSC (58.71±6.49%) or Exo-F derived from human dermal fibroblasts (48±4.5%; $p<0.05$ for Exo-CPC vs Exo-MSC; Exo-CPC vs Exo-F; Exo-MSC vs Exo-F). Exo-MSC showed intermediate efficacy between Exo-CPC and Exo-F. Both Exo-CPC and Exo-MSC were enriched for cardioprotective and/or proangiogenic miRNAs such as miR-132, miR-146a-3p and miR-210 and compared to Exo-F. Proteomics analyses identified around 1000 proteins, of which about 40 and 30 were up- and down-regulated, respectively, in Exo-CPC over Exo-MSC

CONCLUSION
Exo fully accounts for paracrine cardioprotective effects by CPCs and BM-MSCs. On a same patient-basis, Exo-CPC was superior to Exo-MSC in this regard. Thus, Exo-CPC may represent an attractive cell-free therapeutic approach for MI.

Combination therapy as a novel cardioprotective strategy: rationale

Sean M Davidson
The Hatter Cardiovascular Institute, University College London, UK

Coronary artery disease and myocardial infarction are major health-care challenges worldwide, causing significant morbidity and mortality. Finding ways to reduce the damage to the heart muscle is a vital step in the overall long-term management of patients who experience a myocardial infarction. Early reperfusion is crucial but paradoxically causes reperfusion (IR) injury. Single-target agents have been largely unsuccessful in addressing the multifactorial nature of IR injury in the clinical setting. One explanation for this is that there are multiples possible pathways to cell death, including necrosis, apoptosis, necroptosis and pyroptosis, and protection from one type of cell death may simply result in cells dying by another pathway. Furthermore, the increasing prevalence of co-morbidities such as obesity, diabetes, and an aging patient population “raises the bar” for cardioprotective strategies to be effective.

In order to overcome this raised bar and maximize the opportunities for successful clinical translation of cardioprotection, new approaches are needed. One possibility is to combine treatments activating more than one of the known cardioprotective signalling pathways, which include the “RISK” pathway, “SAFE” pathway, and NO-cGMP-PKG pathways. Another approach is to combine drugs in order to maximize or prolong activation of a particular signalling pathway. Alternatively, it may be more effective to use a combination of drugs targeting alternative cell death pathways. Most approaches to date are intended to target cardiomyocytes, but it may be necessary to combine these with drugs targeting other cell types in the heart. Also relevant to the concept of “combination therapy” are the clinical co-treatments that are commonly present already in patients with acute coronary syndromes, such as propofol and nitrates. With this overview I will present evidence that supports the rationale for the use of combination therapy as a novel cardioprotective strategy.

The inflammasome: the hidden killer of reperfused myocardium?

James Downey *and Michael V. Cohen*
University of South Alabama, Mobile Alabama, USA

ABSTRACT TEXT

Postconditioning or $P2Y_{12}$ receptor blockers (P2RB) reduce but do not eliminate infarction in hearts experiencing ischemia/reperfusion (IR). We are trying to identify what is responsible for that remaining infarction and determine if it results from a treatable reperfusion injury. We noted that removing extracellular DNA with iv DNase given at reperfusion and that it further reduced infarct size in rat heart when combined with a P2RB (a drug that is routinely given to patients treated for acute myocardial infarction). A mitochondrially-directed DNA repair enzyme did the same suggesting that the toxic DNA came from mitochondria. Oxidized DNA fragments are known to trigger inflammasome formation. Cardiac fibroblasts are the likely downstream targets as they express all inflammasome components and have been implicated in myocardial infarction. The bactericidal payload for the inflammasome is activation of caspase-1. We found that the highly selective caspase-1 inhibitor VX-765 given at reperfusion was protective and that the protection could again be added to that from a P2RB. The protection against infarction from a single treatment with VX-765 plus a P2RB persisted when reperfusion was extended to 3 days indicating that the salvage was long-lasting. Wall motion was also dramatically preserved indicating that the salvaged myocardium was also contractile. Earlier studies suggested that caspase-1 attracts white cells to the ischemic zone which then kill the heart muscle. However, when we gave VX-765 to Krebs-perfused isolated hearts which are blood-free, it was just as effective at reducing infarct size suggesting that caspase-1 in the absence of white blood cells also contributes to infarction in hearts experiencing IR. We conclude that Inflammasomes kill an appreciable amount of reperfused heart muscle through the action of caspase-1 following an ischemic insult. Several pharmaceutical grade caspase-1 inhibitors are currently available, and adding one to a P2RB should produce an effective anti-infarct therapy.

Identifying cardioprotective strategies with clinical potential in P2Y12 inhibitor-treated animal models

James Downey *and Michael V. Cohen*
University of South Alabama, Mobile Alabama, USA

Ischemic postconditioning (IPOC) has a powerful anti-infarct effect in animal models of acute myocardial infarction. IPOC is easy to perform in patients treated with primary percutaneous intervention (PCI). But, surprisingly IPOC produced little or no clinical benefit in more than a dozen clinical trials. A number of explanations have been proposed (sick patients vs. healthy animals, co-medications, or even a fundamental species difference). We suggest that $P2Y_{12}$ receptor blockers (P2RB) probably explain much of IPOC's failure. P2RBs caused a dramatic improvement in clinical outcomes especially if given as a loading dose prior to PCI. P2RB came into use at the same time that IPOC was being tested clinically, but the effect of P2RB on infarct size was never measured. Cangrelor in rabbits has a strong anti-infarct effect but, surprisingly adding IPOC to cangrelor produced no further reduction in infarct size. We next tested whether signalling elements used by IPOC might also be used by cangrelor. We tested 7 inhibitors that block IPOC's protection and all blocked protection from cangrelor as well. None of the blockers interfered with cangrelor's inhibition of platelet aggregation. Similar behaviour was seen with ticagrelor and clopidogrel. We conclude that P2RBs are as a class postconditioning-mimetics and protect the heart through a similar mechanism as IPOC. We suspect P2RBs reduce infarct size in patients which would account for their marked clinical benefit although that can never be proven. At the same time they render IPOC redundant. We are finding that anti-inflammatory interventions like caspase-1 inhibition can add protection to that from a P2RB. We propose that any anti-infarct intervention being considered for clinical testing be first tested in an animal infarct size model to see if it can add to the protection from a P2RB. If it cannot, there is little chance it will benefit PCI patients.

RIC and exenatide, two treatments with different protective pathways

David Garcia-Dorado
Vall d'Hebron University Hospital and Research Institute,CIBERCV, Universitat Autonoma de Barcelona, Spain

Translation of therapies against reperfusion injury to patients with ST segment elevation myocardial infarction (STEMI) faces the limitations of small and inconsistent effects, and susceptibility to co-treatments and comorbidities. We investigated the potential of a strategy based on the combination of treatments with different cardioprotective pathways and different susceptibility to confounders to obtain a stronger and more robust protection. In pigs submitted to transient coronary occlusion, we investigated the pathways involved in the cardioprotection afforded by different interventions that have been found protective in more than one pilot clinical trial and were safe. We studied the metabolic fingerprints of interventions with NMR spectrometry of myocardial tissue, the activation of SAFE and RISK cascades, and the effects of interventions on ROS mediated injury of eNOS, that we had found to be a hallmark of ischemic postconditioning protection in previous studies. Glucose K+ Insulin (GKI) treatment and exenatide had similar metabolic effects consisting in increased glucose utilization, but we did not detect AKT stimulation with exenatide. Remote ischemic conditioning produced no detectable metabolic changes or SAFE/RISK activation, but had a marked protective effect on the NOS, cGMP, PKG axis, similar to that of local postconditioning. RIC, exenatide and GKI reduced infarct size and combination of RIC with any of the other two treatments had a significant additive effect. The combination of RIC and exenatide is now been tested in the multicentric placebo-controlled COMBAT-MI clinical trial, with a 2 x 2 factorial design, and IS assessed by CMR as primary end-point. Close to 50% of the 460 patients sample size calculated have been so far enrolled.

miR and exosomes in cardiovascular diseases

Henrique Girao
Center for Innovative Biomedicine and Biotechnology - Faculty of Medicine of University of Coimbra

A well balanced communication between the different cells that form the heart is vital to ensure a synchronized and coordinated cardiac pumping activity. In general, intercellular communication can occur between adjacent cells, through gap junctions, or at long distances, via extracellular vesicles (EVs). It has been widely described that deffects in intercellular communication are associated with several cardiac diseases, including myocardial infarction (MI), heart failure or hyprtrophy. Among the various molecules that can cross gap junctions or be conveyed in EVs are miRNAs. Indeed, the transfer of miRNAs through EVs has been shown to regulate different aspects of cardiac pathophysiology. Studies carried out in our group have shown that miRNAs carried in EVs can mediate the crosstalk between cardiomyocytes-endothelial cells, cardiomyocytes-macrophages and pulmonary arterial endothelial cells- cardiomyocytes. We have shown that cardiomyocytes subjected to ischemia release EVs that once taken up by endothelial cells elicit an angiogenic response. Moreover, we demonstrated that miR-222 and miR-143, the relatively most abundant miRs in ischaemic exosomes, partially recapitulate the angiogenic effect of EVs. Importantly, intramyocardial delivery of ischaemic exosomes improved neovascularization following MI. Furthermore, we showed that the levels of circulating miR-424(322) are higher in pulmonar hypertension (PH) patients when compared with healthy subjects, suggesting that miR-424(322) has diagnostic and prognostic value in PH. Hypoxia led to an increased secretion of miR-424(322) by PAECs, which after being taken up by cardiomyocytes resulted in down-regulation of SMURF1. In the monocrotaline rat model of PH, we found an association between circulating miR-424(322) levels and the stage of right ventricle hypertrophy, and an inverse correlation between miR-424(322) and SMURF1 levels in the hypertrophied right ventricle. Additionally, miR-424(322) can target proteins with a direct effect on heart function, suggesting that this miRNA can act as a messenger linking pulmonary vascular disease and right ventricle hypertrophy.

-Omics in Cardioprotection

Mariann Gyöngyösi
Medical University of Vienna

We have investigated the effects of the repetitive (3x10 min) ischemia-reperfusion (r-I/R) stimulus without subsequent myocardial infarction on the gene expression profile in translational porcine experiments, using next generation sequencing (NGS) (transcriptomics). We have analyzed the relevant genes in the early and second windows of cardioprotection, revealing temporospatial differently expression of genes of the Ca-signaling, adipocytokine and insulin signaling pathways with key regulator STAT3, which was also upregulated in the remote areas together with clusterin (CLU) and TNF-alpha. During the second window of cardioprotection, antigen immunomodulatory pathways were activated with overexpression of STAT1 and CASP3 and downregulation of neprilysin. Additionally, by using our straightforward method, we have displayed the gene expressions of the entire left ventricle in 2D and 3D images (image-omics). Five or 24h after r-I/R, the pig hearts were explanted and myocardial biopsy samples from 52 equally distributed left ventricular locations were collected. Based on NGS results, seven genes of interest (HIF-1α; caspase-3, transcription factor GATA4, MEF2c HK2 CLU and ERCC4) were selected, and their myocardial expression levels were measured by qPCR. 2D and 3D gene expression maps were constructed by exploiting the electroanatomical NOGA 2D and 3D mapping principles, and the gene expressions of the 52 locations were displayed in 2D and 3D. At 5 h post r-I/R injury caspase-3, GATA4, HK2, CLU, and ERCC4 were overexpressed region-specifically in the ischemic zone. Upregulation of GATA4, CLU and ERCC4 persisted after 24 h. HK2 showed strong up-regulation in the ischemic zone and down-regulation in remote areas at 5 h, and was severely reduced in all heart regions at 24 h. The temporospatially differently activated genes and pathways revealed a global myocardial response to r-I/R injury and revealed cardioprotection also in remote, non-ischemia-affected myocardial regions, termed “intrinsic remote conditioning” responsible for prevention of left ventricular adverse remodeling.

Effects of Ischemia and Proton Pump Inhibitors Preconditioning on Functional Recovery of Isolated Rat Heart

***Nevena Jeremic*[1]**, *Vladimir Zivkovic*[2], *Ivan Srejovic*[2], *Jovana Jeremic*[1], *Anica Petovic*[1], *Jovana Bradic*[1], *Vladimir Jakovljevic*[2,3]
[1]*Department of Pharmacy, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia*
[2] *Department of Physiology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia*
[3]*Department of Human Pathology, 1st Moscow State Medical, University IM Sechenov, Russian Federation*

OBJECTIVE
The aim of this study was to compare protective effects of ischemic and potential protective effects of pharmacological preconditioning with proton pump inhibitors on isolated rat heart subjected to ischemia/reperfusion.
Matherial and METHODS
The hearts of male, Wistar albino rats (12 in each group, BM 180–200 g) were retrogradely perfused at a constant perfusion pressure (70 cmH_2O), using the Langendorff technique, and cardiodynamic parameters were determined. Hearts of IPC group (ischemic pregonditioning group) were subjected to 2 minutes of ischemic preconditioning and 4 minutes of reperfusion before 20 minutes of ischemia and 30 minutes of reperfusion. In OMPC (omeprazole preconditioning), PAPC (pantoprazole preconditioning) and LAPC (lansoprazole preconditioning) groups hearts underwent preconditioning with 100 μM of PPIs, and then submitted to global ischemia and reperfusion.
RESULTS
Administration of omeprazole before ischemia induction had protective effect on myocardium function recovery especially regarding to values of systolic left ventricular pressure and dp/dt max. Also our findings are that values of coronary flow did not change between OPC and IPC groups in last point of reperfusion. Obtained results has demonstrated that preconditioning with lansoprazole, and especially with pantoprazole, showed better results in terms of both parameters of contractility (dp/dt max and dp/dt min) compared with ischemic preconditioning.
CONCLUSION
Based on our results it seems that ischemic preconditioning could be used as first window of protection after ischemic injury especially because all investigated parameters showed continuous trend of recovery of myocardial function. On the other hand, preconditioning with omeprazole induced sudden trend of recovery with positive myocardium protection, although less effective than results obtained with ischemic preconditioning not withstand, we must consider that PPIs may be used in many clinical circumstances where direct coronary clamping for ischemic preconditioning is not possible.

Antioxidant activity and cardioprotective effects of wild garlic (*Allium ursinum*) extract against ischemia/reperfusion injury on isolated rat heart

***Jovana Jeremic*[1]**, *Jovana Bradic[1], Anica Petkovic[1], Tamara Nikolic Turnic[1], Nevena Jeremic[1], Vladimir Zivkovic[2], Ivan Srejovic[2], Isidora Milosavljevic[1], Marina Tomovic[1], Vladimir Jakovljevic[2,3]*
[1]Department of Pharmacy, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia
[2]Department of Physiology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia
[3]Department of Human Pathology, 1st Moscow State Medical, University IM Sechenov, Russian Federation

OBJECTIVE(S)
The aim of this study was to ascertain whether chronical uses of the methanol leaves extract of wild garlic (*Allium ursinum*) can protect the heart against ischemia/reperfusion injury using the Langendorff heart preparation model.

MATERIAL AND METHOD
Two groups of adult male *Wistar albino* rats (n=20; 8 weeks old; 200±50g body weight) were used: one group was treated with methanol leaves extract of *Allium ursinum* at a dose of 500 mg/kg/day per os for 4-week; the other one of untreated rats served as control. At the end of the treatment, rats were sacrificed and isolated hearts were perfused on a Langendorff apparatus. After stabilisation period, hearts were subjected to global ischemia for 20 minutes and 30 minutes of reperfusion. During experiment parameters of heart function in the left ventricle was measured: the minimum and maximum rate of pressure, systolic and diastolic pressure, heart rate and coronary flow. Markers of oxidative stress: index of lipid peroxidation, nitric oxide, superoxide anion radical and hydrogen peroxide in the coronary venous effluent were assessed spectrophotometrically.

RESULT(S)
Chronic administration of methanol leaves extract of *Allium ursinum* led to improving functional recovery, cardiac contractility and systolic and diastolic function. Also, our findings are that oxidative stress induced by acute myocardial ischemia-reperfusion injury is reduced after chronic administration of extract.

CONCLUSION
Our results strongly suggest that methanol leaves extract of *Allium ursinum* exaggerates the cardioprotection offered by ischemic preconditioning. This study is very encouraging and indicates that wild garlic should be studied more extensively to confirm these results and reveal other potential therapeutic effects.

Mitochondria/cytoskeleton interaction and cardioprotection

Tuuli Käämbre[1], *Rafaela Bagur* [2,3], *Rita Guzun*[4]
[1] *National Institute of Chemical Physics and Biophysics*
[2] *University Grenoble Alpes, Laboratory of Fundamental and Applied Bioenergetics, INSERM U1055, Grenoble, France*
[3] *University Grenoble Alpes, TIMC-IMAG, CNRS, UMR5525, Grenoble, France*
[4] *Hospital of the University Grenoble Alpes, Department Thorax (EFCR), France*

OBJECTIVES

In adult cardiomyocytes, the MOM permeability to ADP is regulated by the interaction of voltage-dependent anion channel with cytoskeletal proteins, particularly with β tubulin II. The cardiac ischemia–reperfusion (IR) injury alters the expression and the intracellular arrangement of cytoskeletal proteins. The objective of this presentation was to clarify the impact of IR injury on the intracellular arrangement of β tubulin II and its effect on the regulation of mitochondrial respiration.

MATERIAL AND METHOD

Perfused rat hearts were subjected to total ischemia for 20 minutes and 45 minutes or to ischemia followed by 30 min of reperfusion in both groups. High resolution respirometry was used to study the kinetics of mitochondrial respiration and, fluorescent confocal microscopy for studies of β tubulin II and mitochondrial arrangements in cardiac fibers.

RESULTS

The results of these experiments show a heterogeneous response of mitochondria to IR-induced damage. The intracellular rearrangement of β tubulin II, which in the control group colocalized with mitochondria, was associated with increased apparent affinity of oxidative phosphorylation (OxPhos) for ADP, decreased regulation of respiration by creatine without altering mitochondrial CK activity and the ratio between octameric to dimeric isoenzymes. Cardiac IR injury compromises mitochondrial OxPhos and compartmentalized intracellular energy transfer via the phosphocreatine/creatine kinase (CK) network.

CONCLUSION

This study allowed us to evaluate the impact of cardiac IR injury on mitochondrial interactions with cytoskeletal protein β tubulin II and its effect on the regulation of mitochondrial respiration. IR-induced alterations of cellular cytoarchitecture, such as the displacement of β tubulin II and affect cytoskeleton-mitochondria interactions, causing the decrease in apparent Km for ADP due to the increase in MOM permeability to adenine nucleotides. The loss of adenine nucleotide compartmentalization affects the functional coupling of MtCK to ATP Synthase and thereby, decreases the control of respiration by creatine. The impaired feedback regulation of mitochondrial function may lead to ADP accumulation within the intracellular energetic units.

Mitochondrial acylcarnitine as a new target for cardioprotection

Edgars Liepins
Latvian Institute of Organic Synthesis

Acylcarnitines as long-chain fatty acid intermediates are known for decades, but many aspects of their molecular action are still unclear. A growing body of evidence suggests that acylcarnitines impact important pathological consequences of cardiometabolic diseases. The accumulation of long-chain fatty acids and their intermediates is observed in the ischemic myocardium after acute ischemia/reperfusion. Recently we showed that long-chain acylcarnitines, but not acyl-CoAs, accumulate at concentrations that are harmful to mitochondria. Acylcarnitine accumulation in the mitochondrial intermembrane space is a result of increased carnitine palmitoyltransferase 1 (CPT1) and CPT2 activity in ischemic myocardium and it leads to inhibition of oxidative phosphorylation, which in turn induces mitochondrial membrane hyperpolarization and stimulates the production of reactive oxygen species (ROS) in cardiac mitochondria. In the isolated rat heart set-up, the supplementation of perfusion buffer with palmitoylcarnitine before occlusion resulted in a 2-fold increase in the long-chain acylcarnitine content of the heart mitochondria and increased the infarct size (IS) by 33%. A pharmacologically induced decrease in the mitochondrial acylcarnitine content reduced the IS by 44%. Moreover, increase of long-chain acylcarnitine content in heart and muscle induced marked insulin insensitivity and decreased glucose uptake and oxidation both in vivo and ex vivo. Increase in the content of long-chain acylcarnitine induced insulin resistance by impairing Akt phosphorylation at Ser473. The pharmacological decrease of acylcarnitine content significantly improved insulin sensitivity and facilitated insulin-dependent glucose metabolism. In conclusion, long-chain acylcarnitine accumulation in ischemic heart is harmful to mitochondria and decreasing the acylcarnitine content via cardioprotective drugs may represent a novel treatment strategy. Moreover, the reduction of acylcarnitine content is an effective strategy to improve cardiac insulin sensitivity.

Red blood cells as novel target for cardioprotection

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Development of ischemia-reperfusion injury involves a complex series of events. Accordingly, therapeutic strategies to protect from ischemia-reperfusion injury may be directed against various cellular and molecular targets. Several studies have established that reduction in bioavailability of endothelium-derived nitric oxide (NO) and increased oxidative stress are key events in ischemia-reperfusion injury. Furthermore, increased arginase activity may via substrate competition be a key factor reducing NO production and increased formation of reactive oxygen species (ROS). It has been shown that red blood cells (RBCs) may be involved in the regulation of cardiovascular function via export of NO bioactivity and redox regulation. Since RBCs also contain high levels of arginase, we hypothesized that RBCs arginase is an important regulator of endothelial NO synthase activity (eNOS) and NO production that mediates protection against myocardial ischemia-reperfusion injury. Using isolated rat and mouse hearts we have explored the role of RBC as a target for protection against ischemia-reperfusion injury. Inhibition of arginase significantly improves myocardial functional recovery following ischemia-reperfusion in rat hearts if administered with isolated RBCs, but not when administered with buffer or plasma. The protective effect of arginase inhibition was lost in the presence of a NOS inhibitor and when given with blood from eNOS$^{-/-}$ mice. Arginase activity and ROS formation are increased in RBCs from mice with type 2 diabetes (db/db). The increased ROS production was reduced by arginase and NOS inhibition. Post-ischemic recovery of myocardial function was impaired in db/db mice compared to WT mice. RBCs from db/db mice depressed myocardial recovery of WT hearts via an arginase-dependent mechanism. These data demonstrate a novel role of arginase in control of eNOS function in RBCs. Inhibition of arginase unravels an important functional effect of RBC-derived NO that mediates protection against myocardial ischemia-reperfusion injury. RBC arginase is a potential therapeutic target in ischemia-reperfusion.

Inflammation – new targets for cardioprotection

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The pathophysiology of myocardial ischemia/reperfusion (I/R) injury is complex consisting of several cellular and molecular mechanisms. Activation of the highly conserved innate immune system, including the complement system and the Toll-like receptor (TLR) system, is crucial in the events leading to direct I/R induced tissue damage and to long-term detrimental effects through priming and activation of the adaptive immune system. Once the innate immune system has been activated, successful inhibition is difficult due to the characteristics of this system with rapid escalation. Hence, interventions targeting innate immunity have to be initiated as early as possible during the ischemic period and should target central components of its main branches. Inhibition of the complement system at the central level of C5 has been evaluated in clinical studies in myocardial infarction and coronary artery bypass grafting without translation to standard clinical practice and the reasons and pit-falls of the clinical studies will be presented. The TLR system is a promising target candidate for inhibition together with complement. However, difficulties arise as multiple distinct TLRs are known to be involved in the pathophysiology of I/R injury. The TLRs are dependent on interaction with extracellular accessory proteins in order to elucidate full-scale signalling. We present an approach with inhibition of the central co-factor CD14, which plays a key role in signalling through several TLRs, including the important TLR4 and TLR2. CD14 is present both as a membrane and a soluble form. CD14 can bind multiple damage associated molecular patterns from dying cells including RNA and DNA. Once bound, CD14 chaperones these pathogenic molecules to the correct TLR. Thus, we hypothesize that double inhibition of the complement system and the TLRs may lead to a lasting and beneficial anti-inflammatory effect in myocardial I/R injury.

Long term myocardial remodelling after IR injury and its modification by conditioning strategies

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Cardioprotective strategies aim to salvage myocardium from ischemia/reperfusion (IR) injury and to reduce infarct size as well its consequences such as the development of heart failure. There is large body of evidence that short episodes of IR insult at sites remote from the heart initiates cardioprotection on the heart in a setting of myocardial ischemia/reperfusion insult. This phenomenon called ischemic remote conditioning. The efficacy of remote conditioning of the heart has been established in preclinical studies, although recent large clinical trials provided conflicting results and failed to demonstrate the beneficial effect of remote ischemic preconditioning in patients undergoing elective cardiac surgery. More recently, it has been demonstrated that repeated remote ischemic conditioning over a number of days has the potential to augment the protective process to improve cardiac and vascular function in patients with heart failure. However, the underlying cardioprotective mechanisms are not fully understood. This presentation will focuses on current evidence based on two levels of remote and repeated remote conditioning and its resulting profound cardioprotection: 1) the effectiveness of repeated remote conditioning on infarcted myocardium and in heart failure in different animal models and patients and 2) potential novel mediators and signalling mechanism of cardioprotection by repeated remote conditioning.

Effect of aging on cardioprotection

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Advanced age increases the extent of myocardial infarcts and exacerbates mitochondrial damage. Recently, mitochondrial ATP synthase has been proposed to conform the molecular entity of mitochondrial permeability transition (mPTP). We investigated the contribution of aging on the accumulation of advanced glycation end-products in the senescent heart and their impact on mitochondrial damage and ATP synthase. Our results disclosed a significant increase in AGEs content both in human and murine myocardium of aged individuals secondary to deficient glyoxalase-dependent detoxification system. Proteomic analysis identified mitochondrial ATP synthase as one of the target proteins of glycative damage. Mitochondria from human and murine aged hearts exhibit a reduction in ADP-dependent O2 consumption. In mouse cardiomyocytes submitted to transient ischemia-reperfusion, glycative damage of mitochondrial ATP synthase is associated with more pronounced failure in mitochondrial energy recovery upon reperfusion and higher susceptibility to undergo mPTP. These effects were paralleled by an increased cell death. These results identify a new pathophysiological mechanism that may underlie the increased vulnerability of senescent heart to ischemia-reperfusion damage.

Setting up a Cardioprotection Consortium, the CAESAR Experience

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Because of variability in the effects of different cardioprotective interventions, the NIH sponsored a consortium to develop models of ischemia-reperfusion in different species, in different laboratories, at different institutions, to evaluate rigorously cardioprotective interventions, and hopefully to separate those interventions which work well in different models from those which are not reproducible. This was not the first time that the NIH had sponsored a group of laboratories to test cardioprotective interventions. The first initiative took place in the early 1980s. The CAESAR initiative had one major difference from the earlier study; there would be complete standardization of methods between the different laboratories using the same species and same protocol, and this would allow the reproducibility to be better quantified. Using ischemic preconditioning at the first test intervention, the methods were refined so that control and experimental infarct size was essentially the same between laboratories using the same model. After this was completed, a protocol for testing cardioprotective agents in a reperfusion treatment protocol was developed, and various drugs that had been shown to be protective in earlier studies from a single center, were tested in a defined protocol, with adequate sample size, with multiple parameters measured to assess the amount of damage, and with complete blinding of the experimental procedure and the data analysis, performed at different centers. The CAESAR initiative demonstrated the difficulties in achieving a standardized protocol that could be reproduced in different laboratories. Although the rigid adherence to a single protocol achieved a degree of reproducibility, it did not allow for adjustment when the adopted protocol did not achieve protection, and ultimately, despite considerable effort by the participants, the effort failed to identify specific cardioprotective drugs that could be administered at the time of reperfusion and would significantly reduce infarct size.

Old and New Mechanisms in Cardioprotection. Role of Nitric Oxide and Mitochondria.

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Cardioprotection has been studied for many years and many different mechanisms have been proposed. Initially, many studies of cardioprotection showed small effects which could not be easily reproduced in other laboratories. With the discovery of ischemic preconditioning, a robust cardioprotective intervention was uncovered, that was easily reproduced in different laboratories, in different animal models, and using different protocols of brief intermittent ischemia and reperfusion to induce the protected phenotype. Shortly thereafter, it became clear that this was primarily a receptor mediated process, which activated a variety of intracellular signalling cascades that ultimately lead to protection. To be maximally effective, these interventions needed to be instituted prior to ischemia, limiting the clinical applicability. More recently, it has been shown that ischemic postconditioning is also effective at reducing infarct size. This has the potential to be more clinically relevant, but the effect seems to be protocol dependent, may vary from species to species, and is less reproducible. This potentially limits its clinical use. Perhaps better understanding of the mechanisms of cardioprotection would allow a more targeted approach. We have become interested in the role of nitric oxide in cardioprotection, and specifically the role of cysteine S-nitrosylation as a post-translational modification that may be involved, and more specifically the targets of S-nitrosylation. In addition to a role for S-nitrosylation, we have also found differences in cardioprotection between males and females, and some of these differences may be related to a difference in eNOS activity and the ability to achieve higher levels of S-nitrosylation in female myocardium. Using the SNO-RAC method, we have found some potential targets that may mediate the protective effect of S-nitrosylation, as well as highlighting how this could be protective. Among the targets are mitochondrial proteins, suggesting that the mitochondria are important in ischemia-reperfusion injury and cardioprotection.

Discovery of Isoform Selective Voltage-Gated Sodium and Potassium Channel Modulators

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OBJECTIVES

Voltage-gated sodium (Nav) and potassium (Kv) channels are transmembrane proteins present in neurons and other electrically excitable cells, such as myocytes, and are crucial for neurotransmission. Currently there are nine identified Nav channel isoforms (Nav1.1–Nav1.9) and eight known isoforms of the Kv1 subfamily (Kv1.1- Kv1.8). Their abnormal activity can result in various neurological disorders, chronic pain, epilepsy, and arrhythmias. $Na_v1.5$ and $K_v1.5$ channels are mainly expressed in the myocytes and their abnormal activity has been linked to a range of cardiac disorders. Marketed drugs that target Nav channels, e.g. local anesthetics, antiarrhythmics and antiepileptics, are relatively non-selective, therefore there is a need for the development of isoform selective modulators.
Alkaloids from the Caribbean sponge of the genus *Agelas*, *e.g.* clathrodin, were reported to possess modulatory activity on Na_V channels. We have designed and synthesized a series of clathrodin analogues and biologically evaluate them on different Na_V and K_V channel subtypes using electrophysiological assays.

MATERIALS AND METHODS

Compounds were evaluated for their modulatory activities against different Nav1 (Nav1.1-Nav1.8) and Kv1 (Kv1.1, Kv1.2, Kv1.4, Kv1.5 and Kv1.6) channel isoforms using an automated patch clamp electrophysiology assay or a two-electrode voltage clamp method.

RESULTS

We have designed and synthesized a series of potential Nav and Kv channel modulators. Four compounds displayed IC_{50} values of less than 3 µM on the P3 state of the Nav1.3 channels and one compound displayed an IC_{50} value of 20 nM against the Nav1.3 channels, and a good state selectivity. Most active analogues had IC_{50} values lower than 1 mM against Kv1.3-Kv1.6 channels. Compounds were inactive against Nav1.5 and Kv1.5 channels.

CONCLUSION

Low and sub-micromolar IC_{50} values against selected Nav and Kv channels and relatively low molecular weights of the prepared compounds highlight their potential for further optimisation of their inhibitory activity.

Empagliflozin effects on ischemic contracture and I/R injury in isolated mouse hearts perfused with or without insulin

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OBJECTIVE(S)

We recently reported that EMPA has sodium hydrogen exchanger (NHE) inhibition properties in isolated cardiomyocytes. Classic NHE inhibitors such as Cariporide (CARI) delay ischemic contracture onset and reduce ischemia reperfusion (I/R) injury in the heart. Here we examined whether EMPA delays contracture onset and reduces I/R injury. Both normal- and low-glycogen hearts were used, because the rate of glycogen depletion during ischemia affects contracture onset and IR injury.

MATERIAL AND METHOD

Isolated mouse hearts were perfused with and without 50 mU/L insulin, to create normal- and low-glycogen hearts respectively, and subjected to 25 min I and 120 min R. 1µM EMPA, 10µM cariporide (CARI; positive control) or vehicle was administered before ischemia until 10 minutes post-reperfusion. NHE activity was measured after NH_4^+-pulse in isolated cardiomyocytes. Glycogen, lactate and mitochondrial-bound hexokinase II (mtHKII) levels were determined at baseline and end-ischemia.

RESULT(S)

In normal-glycogen hearts, EMPA and CARI did not change ischemic contracture (in seconds, vehicle 1056±56, EMPA 900±44, CARI 880±84) and IR injury (infarct size (%): vehicle 59±6, EMPA 62±10, CARI 68±4), however in low-glycogen hearts, both EMPA and CARI delayed ischemic contracture onset (vehicle 459±25, EMPA 559±22, CARI 588±31). Higher EMPA concentration further delayed contracture onset. Only CARI reduced I/R injury (from 51±6 to 34±5). 50mU/L insulin did not alter NHE activity, nor the ability of CARI or EMPA to inhibit NHE, in isolated cardiomyocytes. The increased IR injury in normal-glycogen hearts was associated with increased end-ischemic lactate and glucose-6-phosphate accumulation, and consequently decreased mtHKII.

CONCLUSION

EMPA does not protect against I/R injury in isolated hearts, yet EMPA delayed ischemic contracture onset in low-glycogen hearts, suggesting a direct cardiac, ATP preserving, effect of EMPA during ischemia. Cardiac glycogen content interferes with the effects of NHE inhibitors on I/R injury in isolated hearts. These data indicate that empagliflozin and cariporide may inhibit NHE in different manners.