Cardioprotection: reduction of irreversible ischemia/reperfusion injury – prevention of HF

Cardiac regeneration by cell therapy: importance of protecting the therapeutic cells:


Ischemic conditioning: 30 years of hope for cardioprotection

Ischemia/reperfusion

Preconditioning (1986)
- < 120 min: “classic”
- 24-72 hours: “delayed”


Remote conditioning strategies (1993 - )

Cardioprotection
- Infarct size ↓
- Cardiac function ↑
- Arrhythmias ↓


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Why do we still not have cardioprotective drugs? „death valley for biotech companies”

CYCLE trial targeting MPTP FAILED
Circus trial targeting MPTP FAILED
Bendavia trial targeting mitochondria ROS FAILED

NOMI study to increase NO level FAILED

Intracoronary nitrite – Phase II FAILED

MITOCARE study targeting MPTP FAILED
EMBRACE STEMI trial targeting MPTP FAILED

Heusch et al., Circulation, 2008; Andreadou et al, Br J Pharmacol 2014; Ferdinandy et al, Pharmacol Rev, 2014
Why do we still not have cardioprotective drugs on the market?

Hypothesis-driven simplified approach in target identification and validation so far?

Still neglecting comorbidities and comedications in preclinical development
1. Functional genomics of cardioprotection: unbiased novel targets?

2. Effect of co-morbidities on cardioprotection and the functional genomics of the heart
One dominant pathway or multiple ones are responsible for cardioprotection? Importance of „omics” approach

- cardioprotection by preconditioning and postconditioning induces dramatic changes in cardiac gene expression profile:
  - Varga et al, Curr Drug Targets, 2015

- cardioprotective genomic and proteomic program?

- need for „omics” approach and bioinformatics for finding targets

Heusch et al., Circulation, 2008; Andreadou et al, Br J Pharmacol 2014; Ferdinandy et al, Pharmacol Rev, 2014
### Gene expression changes by pre- and postconditioning

<table>
<thead>
<tr>
<th>DNA chip studies</th>
<th>Genes over-expressed</th>
<th>Genes repressed</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3200 genes, rat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IR vs. precond</td>
<td>14</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>31000 genes, rat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>350 miRNAs, rat: altered miRNAs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IR vs precond</td>
<td>4</td>
<td></td>
<td>Varga et al, Am J Physiol, 2014</td>
</tr>
<tr>
<td>IR vs postcond</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IR vs pre &amp; postcon</td>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*ProtectomiRs*: miRNA-139-5p, 125b*, let-7b, antagomiR-487b
microRNA alteration patterns after I/R and cardioprotection

Pattern 1.
- Significantly affected by I/R
- Not affected by Pre/Post

miRNAs with cardioprotective potential:

Pattern 2.
- Not affected by I/R
- Significantly affected by Pre/Post

Pattern 3.
- Significantly affected by I/R
- Counter-regulated by Pre/Post

Cardioprotective microRNA expression pattern:
Significantly affected by I/R and counter-regulated by Pre/Post

- rno-miR-487b
  - preconditioning-induced down-regulation
  - postconditioning-induced down-regulation
  - protectomiR: miR-487b antagonomiR

- rno-miR-208
  - preconditioning-induced down-regulation
  - postconditioning-induced up-regulation
  - protectomiR: miR-208 antagonomiR

- rno-miR-125b*
  - pre- and postconditioning-induced up-regulation
  - protectomiR: miR-125b* mimic

Validation of protectomiRs: simulated ischemia/reperfusion injury in protectomiR transfected cardiomyocytes.

Dy-547 conjugated positive control miRNA

Viability test

ProtectomiR Transfection

Percent survival (%)

NORMOXIA

SIMULATED ISCHEMIA/REPERFUSION

Non-targeting Control

Non-targeting Control

miR-139-5p mimic

miR-125b* mimic

let-7b mimic

miR-487b inhibitor

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Extracellular vesicles are required to transfer cardioprotection in remote conditioning: carriers of ProtectomirRs?

Active substance carried by the vesicles are to be identified

Giricz et al, J Mol Cell Cardiol. 2014
Detection of extracellular vesicles from the blood: only technical difficulties?

Isolation of Exosomes from Blood Plasma: Qualitative and Quantitative Comparison of Ultracentrifugation and Size Exclusion Chromatography

Low-density lipoprotein mimics blood plasma-derived exosomes and microvesicles during isolation and detection

Barbara W Sódar¹, Ágnes Kittel², Krisztina Pálóczí³, Krisztina V Vukman¹, Xabier Osteikoetxea¹, Katalin Szabó-Taylor¹, Andrea Németh¹, Beáta Sperlágh², Tamás Baranyai³, Zoltán Gircz³, Zoltán Wiener³, Lilla Turiák⁴, László Drahos⁴, Éva Pállinger¹, Károly Vékey⁴, Péter Ferdinandy³, András Falus³ & Edit Irén Buzás¹
1. Functional genomics of cardioprotection: unbiased novel targets?

2. Effect of co-morbidities on cardioprotection and the functional genomics of the heart
# Influence of co-morbidities/risk factors on I/R injury and cardioprotection by conditioning

<table>
<thead>
<tr>
<th>Co-morbidities</th>
<th>I/R Injury</th>
<th>Protection by preconditioning</th>
<th>Protection by postconditioning</th>
<th>Protection by remote cond.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aging</td>
<td>↑ increased</td>
<td>↓ attenuated</td>
<td>↓ attenuated</td>
<td>no data</td>
</tr>
<tr>
<td>Hypertension, hypertrophy, remodelling</td>
<td>~</td>
<td>~</td>
<td>~</td>
<td>no data</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>↑ increased</td>
<td>↓ attenuated</td>
<td>↓ attenuated</td>
<td>no data</td>
</tr>
<tr>
<td>Diabetes</td>
<td>↑ increased</td>
<td>↓ attenuated</td>
<td>↓ attenuated</td>
<td>no data</td>
</tr>
<tr>
<td>Kidney failure</td>
<td>↑ increased</td>
<td>~</td>
<td>~</td>
<td>no data</td>
</tr>
</tbody>
</table>

Influence of acute hyperglycemia on I/R injury and cardioprotection by remote conditioning in rats

Biochimica et Biophysica Acta

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

High glucose-induced hyperosmolarity impacts proliferation, cytoskeleton remodeling and migration of human induced pluripotent stem cells via aquaporin-1

Rosalinda Madonna a,c, Yong-Jian Geng a,b, Harnath Shelat a, Peter Ferdinandy d,e, Raffaele De Caterina c,*

www.pharmahungary.com

Baranyai et al, Cardiovasc Diabetol, 2015
Effects of hyperlipidemia on cardiac gene expression profile (mRNA, miRNA, and protein microarray studies in rodent hearts)

<table>
<thead>
<tr>
<th>Microarray and RT-PCR</th>
<th>Overexp.</th>
<th>repressed</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3200 genes, rat - hyperchol vs. normal</td>
<td>26</td>
<td>25</td>
<td>Puskas et al, FEBS Lett, 2004</td>
</tr>
<tr>
<td>330 antibodies, rat - hyperchol vs. normal</td>
<td>10</td>
<td>3</td>
<td>unpublished</td>
</tr>
<tr>
<td>16 oxidative stress genes - hyperchol vs. normal</td>
<td>eNOS</td>
<td>Phox4, MMP9</td>
<td>Kocsis et al, Med Sci Monit, 2010</td>
</tr>
<tr>
<td>360 miRNA, rat - hyperchol vs. normal</td>
<td>6</td>
<td>2</td>
<td>Varga et al, JMCC, 2013</td>
</tr>
<tr>
<td>420 miRNA, rat - Statin vs normal</td>
<td>7</td>
<td>4</td>
<td>Szücs et al, in preparation</td>
</tr>
<tr>
<td>15000 genes, ZDF rat - Metabolic dis vs. normal</td>
<td>36</td>
<td>42</td>
<td>Sárközy et al, Cardiov Diab 2013</td>
</tr>
</tbody>
</table>
Cardiac microRNA expression pattern is altered hyperlipidemia: biased selection of microRNA-25

Cardiac miRNA expression change, cholesterol-fed vs. control

Target analysis

NADPH oxidase 4 upregulation
oxidative/nitrosative stress

Varga et al, J Mol Cell Cardiol, 2013
Unbiased network biology approach: novel targets affected by hyperlipidemia?
### Influence of co-medications on I/R injury and cardioprotection by conditioning

<table>
<thead>
<tr>
<th>Drug class</th>
<th>I/R Injury</th>
<th>Protection by preconditioning</th>
<th>Protection by postconditioning</th>
<th>Protection by remote cond.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate</td>
<td>decreased</td>
<td>no data</td>
<td>no data</td>
<td>no data</td>
</tr>
<tr>
<td>Nitrate tolerance</td>
<td>increased</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statins acute</td>
<td>decreased</td>
<td>(hyperlipidemia)</td>
<td>(hyperglycemia)</td>
<td>no data</td>
</tr>
<tr>
<td>Statins chronic</td>
<td>decreased</td>
<td>(chronic stenosis)</td>
<td>(chronic stenosis)</td>
<td>no data</td>
</tr>
<tr>
<td>β-Blocker</td>
<td>Drug-dependent</td>
<td></td>
<td></td>
<td>no data</td>
</tr>
<tr>
<td>ACE-inhibitor</td>
<td>Reduced threshold</td>
<td></td>
<td>no data</td>
<td>no data</td>
</tr>
</tbody>
</table>

Influence of co-medications on I/R injury and cardioprotection by conditioning

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<th>Protection by postconditioning</th>
<th>Protection by remote cond.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$AT_1$-antagonist</td>
<td>↓ increased</td>
<td>Reduced threshold ↑ (LVH)</td>
<td>no data</td>
<td>no data</td>
</tr>
<tr>
<td>Metformin</td>
<td>↓ decreased</td>
<td>no data</td>
<td>no data</td>
<td>no data</td>
</tr>
<tr>
<td>$K_{ATP}$-blocker</td>
<td>~</td>
<td>Drug-dependent</td>
<td>Drug-dependent</td>
<td>sulfonyleurea</td>
</tr>
<tr>
<td>Glitazone</td>
<td>↓</td>
<td>no data</td>
<td>no data</td>
<td>no data</td>
</tr>
<tr>
<td>DPP4-inhibitor</td>
<td>↓</td>
<td>no data</td>
<td>no data</td>
<td>no data</td>
</tr>
<tr>
<td>GLP-1 analogs</td>
<td>↓</td>
<td>no data</td>
<td>no data</td>
<td>no data</td>
</tr>
<tr>
<td>Insulin-K$^+$</td>
<td>↓</td>
<td>no data</td>
<td>no data</td>
<td>no data</td>
</tr>
<tr>
<td>Cox-inhibitor</td>
<td>~</td>
<td>no data</td>
<td>no data</td>
<td>no data</td>
</tr>
</tbody>
</table>


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www.semmelweispharma.com
The infarct size limiting effect of postconditioning is lost in hearts of nitrate tolerant rats.

Fekete et al, J Cardiovasc Pharmacol, 2013
Altered gene expression pattern of the heart and the aorta in nitrate tolerant rats

Out of the 7742 genes analyzed by DNA microarray:

25 genes changed significantly in the heart:
• **increased**: Tas2r119, Map6, Cd59, Kcnh2, Kcnh3, Senp6, Mcpt1, Tshb, Haus1, Vipr1, Lrn3, Lifr
• **decreased**: Ihh, Fgfr1, Cryge, Krt9, Agrn, C4bp, Fcer1a, Csf3, Hsd17b11, Hsd11b2, Ctnnb1, Prpg1, Hsf1

14 genes changed significantly in the abdominal aorta:
• **increased**: Tas2r119, Ihh, Rrad, Npm1, Snai1
• **decreased**: Tubb2b, Usp15, Sema6c, Wfdc2, Rps21, Ramp2, Galr1, Atxn1, Lhx1
Take home messages

• Cardioprotection involves a complex functional genomic and proteomic program:
  - mRNA, miRNA expression changes,
  - exosomal transport of …?

• Cardiovascular comorbidities and comedications modify cardiac functional genomics

• Relevance to protecting therapeutic cells (see Madonna et al, EHJ, 2016)

See for reviews: Ferdinandy et al, Pharmacol Rev, 2014
Hausenloy et al, Cardiovasc Res, 2013
Varga et al, Curr Drug Targets, 2015

www.pharmahungary.com
Target finding and validation for cardioprotection: comorbidities, comedications, and multi-omics

Modified from: Varga et al, Curr Drug Targets, 2015

Financing all these studies?
Semmelweis University, Budapest; University of Szeged, Szeged, and Pharmahungary Group, Szeged, Hungary

Bence Ágg
Tamás Baranyai
Péter Bencsik
Tamás Csont
Anikó Görbe
Gabriella Fodor
Zoltán Giricz
Krisztina Kupai
Krisztina Kiss
Márton Pipicz
Gergő Szücs
Zoltán Varga
Márta Sárközy
János Pálóczy
Veronika Fekete
Renáta Gáspár
Judit Pipis
Szilvia Török
Gábor Koncsos
Csilla Nagy

Univ. Chieti, Italy:
Rosalinda Madonna
Raffaele De Caterina

Cardiff University, Cardiff, UK:
Gary F. Baxter
Dwain S. Burley

University of Wien, Austria:
Mariann Gyöngyösi

JL University, Giessen, Germany:
Rainer Schulz
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Cape Peninsula Univ. Cape Town, South Africa:
Jacques van Rooyen
Dirk Bester

Comenius Univ, and Slovak Academy of Sci, Bratislava, SK:
Adriana Adameova
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