The role of translational research in development and clinical introduction of novel regenerative therapies

Piotr Buszman M.D., PhD, Ass.Prof.,

Co-Director
Center for Cardiovascular Research and Development
American Heart of Poland.
Silesian Center for Heart Diseases
Innovations in Cardiovascular Medicine
Key Elements to Success

Organization & Structure

Technology or systems

Therapy: Biomedical or bio-pharmacological
The Innovation Circle and the Role of Translational Research

Juan F. Granada, Skirball Center for Innovation
Pre-clinical evaluation of regenerative therapies

In-vitro: i.e. cell cultures

In-vivo: Animal models
Key pre-clinical models for evaluation of novel regenerative therapies and devices

• Porcine Myocardial infarction, reperfusion and heart failure:
  – Regenerative/Cardioprotective therapies and devices

• Rabbit model:
  – Regenerative/Cardioprotective
Myocardial Reperfusion: A Double-edged Sword?

Eugene Braunwald and Robert A. Kloner
Department of Medicine, Harvard Medical School, Brigham and Women's Hospital, and Beth Israel Hospital, Boston, Massachusetts 02115; and Wayne State University School of Medicine and Harper Hospital, Detroit, Michigan 48201

“While reperfusion, by definition, relieves or at least greatly reduces ischemia, it also results in a complex group of phenomena, some of which may initially appear to be deleterious”

Despite the recent progress in interventional and pharmacological treatment of MI, a Reperfusion Injury remains significant limitation of the pPCI
Contribution of Lethal Reperfusion Injury to Final Myocardial Infarct Size and the role of cardioprotection

The Porcine Model of Myocardial Ischaemia / Reperfusion

- An acute infarction is induced by inflation of a Over The Wire balloon catheter in the **medial LAD for 60 minutes**

- **Minutes Before or at reperfusion** through central lumen of the OTW catheter intracoronary downstream study drug or palcebo is injected

- Coronary angiography and ventriculography is performed **before, 30 minutes and 48h after ischemia**

- A series of blood samples for troponin I and biochemical inflammation markers are carried out

- Animals are sacrificed after **48h of reperfusion**

- The infarct size area (ISA) and the area at risk (AAR) are evaluated with TTC and Evans Blue staining
TTC / Evans Blue staining of myocardium

- **White**: infarcted area
- **Red**: viable myocardium
- **White + Red**: area at risk (AAT)
- **Blue**: non ischemic area
Cardiovascular magnetic resonance imaging and histopathology.
Intracoronary Adiponectin at Reperfusion Prevents Reperfusion Injury in Porcine Myocardial Infarction Model

Dębiński M, Buszman PP, Milewski K et al., Int J Mol Med. 2011
Secondary Endpoints: Biochemical Evaluation

Dębiński M, Buszman PP, Milewski K et al., Int J Mol Med. 2011
What can we learn from a negative study?

“Controlled Reperfusion with Intravenous Bivalirudin and Intracoronary Abciximab Combination Therapy in the Porcine Myocardial Infarction Model”


23 Pigs

Bivalirudin

60 min ischaemia and reperfusion
Randomization 1:1

N=12

Intacoronary Placebo
0,9% NaCl

N=11

Intracoronary Abciximab (ReoPro®)
0.25 mg/kg downstream i.c. bolus
Primary Endpoint: Infarct Size

- IA/ AAR%: Placebo 57.6%, Abciximab 57.1%
- IA/ LV%: Placebo 28.3%, Abciximab 27.2%
- AAR%: Placebo 41.7%, Abciximab 40.7%
# Histopathological evaluation and enhancement of Apoptosis

<table>
<thead>
<tr>
<th></th>
<th>Abciximab + Bivalirudin N=10</th>
<th>Bivalirudin N=10</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophile extravasation*</td>
<td>0,5</td>
<td>0,4</td>
<td>0,7</td>
</tr>
<tr>
<td>Erythorcyte extravasation*</td>
<td>0,9</td>
<td>0,4</td>
<td>0,05</td>
</tr>
<tr>
<td>No. of heamorrhagic infarcts</td>
<td>7</td>
<td>2</td>
<td>0,07</td>
</tr>
</tbody>
</table>

![Graph showing number of TUNNEL (+) cell per 100 nuclei in the AAR for Bivalirudin and Bivalirudin + Abciximab.](chart.png)

Piotr Buszman et al. Thrombosis Research 2011
Bone marrow cells with HO-1 in experimental MI

Viral transduction (AdV 50 MOI)
Evaluation of endothelial potential:
- Ac-LDL uptake
- Lectin binding
- Capillary formation

Wojtek Wojakowski et al.
Thrombosis Hemostasis 2012
Bone marrow cells with HO-1 in experimental MI - The recognition of underlying molecular mechanisms

A

SDF-1a

B

mRNA level (2^ΔCt)

0.000

0.025

0.050

0.075

0.100

LV HEALTHY EMPTY
LV HEALTHY GFP
LV HEALTHY HO-1
LV infarction EMPTY
LV infarction GFP
LV infarction HO-1
LV near inf. EMPTY
LV near inf. GFP
LV near inf. HO-1
Results: Primary Endpoints

Infarct size and Area at Risk

Dębiński M, Buszman PP, Milewski K et al., Int J Mol Med. 2011
The enhancement of apoptosis

Number of TUNNEL (+) cell per 100 nuclei in the AAR

- Placebo: 8.92
- Adiponectin: 3.13

Control

Study
Large animal preclinical research allows testing of feasibility of novel local delivery devices
Large animal preclinical research allows testing of feasibility of novel local delivery devices
The Peregrine Catheter (Ablative Solutions)
Validation of the rabbit critical limb ischemia model

Vaclav Prochazka et al et al. Cell Transplantation 2015
Adipose-Derived Therapeutic Factor Concentrate treatment enhances ischemic limb perfusion, capillary density and increases the level of circulating growth factors.

Vaclav Prochazka et al. Cell Transplantation 2015
# Cardiac protection and regeneration: A spectrum of negative clinical trials

<table>
<thead>
<tr>
<th>Type of Agent</th>
<th>Study</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibitors of WBC adhesion</td>
<td>HALT MI, LIMIT MI</td>
<td>No reduction in IS</td>
</tr>
<tr>
<td>Calcium blocker (Nifedipine)</td>
<td>SPRINT-2</td>
<td>Increased mortality</td>
</tr>
<tr>
<td>h-SOD</td>
<td>Flaherty JT, et al.</td>
<td>No increase in LV function</td>
</tr>
<tr>
<td>Rheoth RX (poloxamer 188)</td>
<td>CORE Study</td>
<td>No difference in death, shock or re-infarction</td>
</tr>
<tr>
<td>Trimetazidine (antioxidant)</td>
<td>EMIP-FR</td>
<td>No effect on mortality</td>
</tr>
<tr>
<td>Molsidomine (nitric oxide donor)</td>
<td>ESPRIM</td>
<td>No effect on mortality or other clinical outcome</td>
</tr>
<tr>
<td>Fluosol</td>
<td>TAMI-9</td>
<td>No reduction in infarct size or improvement in LV function</td>
</tr>
<tr>
<td>Hyaluronidase</td>
<td>Pre-thrombolytic era</td>
<td>No effect on infarct size</td>
</tr>
<tr>
<td>Complement Inhibition</td>
<td>COMPLY Trial APEX Trial</td>
<td>No reduction in infarct size No reduction in mortality in large APEX AMI trial</td>
</tr>
<tr>
<td>Na+-H+ Exchange Inhibitor</td>
<td>ESCAMI CASTEMI</td>
<td>No effect on infarct size, clinical outcomes, LVEF</td>
</tr>
<tr>
<td>Magnesium</td>
<td>MAGIC</td>
<td>Most recent study showed no effect on mortality, CHF, VT</td>
</tr>
<tr>
<td>Nicorandil</td>
<td>Kitakaze et al 2006</td>
<td>No effect on mortality, infarct size, LVEF</td>
</tr>
</tbody>
</table>
Cardiac protection and regeneration: positive studies

<table>
<thead>
<tr>
<th>Type of agent</th>
<th>Study</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine</td>
<td>AMISTAD 1 and 2</td>
<td>Reduced Anterior MI infarct size. With early (≤ 3 hours) reperfusion improved clinical outcome (AMISTAD 2).</td>
</tr>
<tr>
<td>Therapeutic Hypothermia</td>
<td>COOL MI</td>
<td>No overall difference in infarct size, clinical events. However patients with anterior AMI cooled &lt; 35°C before PCI had smaller infarcts. Hyeroxemic reperfusion AMIHOT No overall difference</td>
</tr>
<tr>
<td>Hyeroxemic reperfusion</td>
<td>AMIHOT</td>
<td>No overall difference. However, patients with anterior AMI reperfused &lt; 6 hours had greater improvements in function, smaller infarct size, improved ST resolution.</td>
</tr>
<tr>
<td>GIK</td>
<td>A large number of studies – both negative and positive. IMMEDIATE TRIAL – will give GIK in ambulance</td>
<td>Variable results but avoidance of hyperglycemia and early therapy may be key.</td>
</tr>
<tr>
<td>Ischemic postconditioning</td>
<td>Staat et al.</td>
<td>4 x 60 sec. inflations and deflations of angioplasty balloon after stenting reduced infarct size and improved reperfusion</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>Piot C et al.</td>
<td>Reduced Infarct Size, CK and TnI AUC</td>
</tr>
<tr>
<td>Protein Kinase C- delta inhibitor</td>
<td>DELTA MI. Roe et al. 2007</td>
<td>Reduced infarct size, improved ST resolution</td>
</tr>
</tbody>
</table>
Why haven’t animal always models predicted human clinical response

● Experimental occlusion/reperfusion does not reproduce clinical disease process (not dynamic, no embolization)
● Differences in animal coronary circulation
● Non consistent protocols and methodology between investigators
● Imprecise tools to measure impact on infarct size in clinical MI
● Publication bias – “The need for speed” of positive results
What can we learn from preclinical cardiac protection-regeneration experiments?

• Feasibility – delivery systems
• Safety – underlying tissue response
• Rapid development through series of pivotal studies
• Efficacy, however, only therapies that have been conclusively shown to be cardioprotective in experiments in animals by multiple investigators should be investigated in the clinical setting
• If interventions have not shown conclusive cardioprotection in experimental studies, they are also unlikely to be cardioprotective in the clinical setting – high specificity *
Preclinical research makes novel medical technologies fly

“It is time to plant the seed for a new code of “experimentation in humans” based on a much more rigorous and coded approach to “first in man”, using a host of evidence collected in situations that do not endanger the patient.”

Patrick W. Serruys. Eurointervention 2015
Contact details

Center for Cardiovascular Research and Development
American Heart of Poland, Inc.
41 Czajek Street, 40-534 Katowice, Poland
tel/fax: +48 32 251 02 11
e-mail: info@ahp-ccrd.org

Department of Preclinical Research
Kostkowice, 19A Osiedlowa St.
43-426 Dębowiec, Poland
tel. +48 33 472 29 00

Katowice International Airport in Pyrzowice:
http://www.katowice-airport.com/en/index/index/0
- about 125 km from the cath-lab
- about 1 hour 30 minutes drive

Krakow International Airport in Balice:
http://www.lotnisko-balice.pl/eng/
- about 140 km from the cath-lab
- about 1 hour 45 minutes

Ostrava Airport
- about 60 km
- about 55 minutes

www.ahp-ccrd.org
Thank you for your attention