Cellular and molecular mechanism of heart failure

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Translational medicine – strategic trend in development of clinical science and practice

Everyday clinical practice:

- Diagnostics
- • Treatment
- • Primary prevention
- • Secondary prevention and rehabilitation
- • Cardiovascular surgery and Interventions

Basic research
Thus we should find out:

- What specific biological events or molecular pathways play a role in certain diseases?
- What biomarker(s) can we monitor to assess target therapy in the clinics?
- How can we best use this information to discover and develop new therapeutics and associated diagnostics that will help with patient selection?
Translational research in heart failure: main projects

- Novel in etiology and pathogenesis of heart failure
  - Molecular imaging modalities
  - Cardiac protection against ischemia-reperfusion injury
  - Targeted therapy
  - Circulating stem cells and resident progenitor cells in heart failure
Etiology of heart failure

- Coronary artery disease
- Primary and secondary hypertension
- «Non-coronary heart diseases»
  - cardiomyopathies
  - myocarditis
  - infiltrative disorders (amyloidosis)
  - storage diseases
  - congenital and acquired heart diseases
- Endocrine disorders
Etiology of diastolic heart failure depending on the age of manifestation

20-40 y.o.
Genetic cardiomyopathies and multiorgan syndromes with cardiomyopathy phenotypes

40-90 y.o.
Infiltrative and storage diseases (including senile amyloidosis 46-70%)

AA-amyloidosis
Haemohromatosis
TTR-amyloidosis

Genetic cardiomyopathies with late manifestations

40-90 y.o.
Metabolic syndrome resulting in heart dilation
There are no rare diseases there are “rare diagnosis”
ECG in 45 y.o. patient with TTR amyloidosis. QS and ST elevation (pseudo MI) in V1-V4

23.06.2003

24.09.2004
TTR amyloidosis constitutes a rare (1%) but important cause of HF
• Prevalence of **AL- amyloidosis**
• were investigated in 212 patients with CMP (RCMP, HCMP, DCMP genesis) including patients with complicated biventricular predominantly right CHF by immunohistochemical methods
• Results: Cardiac form of AL-amyloidosis was diagnosed in 22 patients (10.4%)

**AL- amyloidosis is NOT a rare disease !!!**
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Molecular imaging: advantages and state-of-the-art

- Early detection of biochemical and metabolic processes underlying the disease;
- Combination of diagnostics and therapeutic effect (teranostics);
- Individual characteristics of the disease course at the molecular level (personalized therapy);
- Understanding molecular pathogenesis of the disease
Diagnostic fluorescent systems

**Single-dot spectral**
Evaluation of the metabolism of certain substances

**Monochromatic**
Evaluation of structure (anatomical imaging)

**Multispectral**
Evaluation of metabolism + structure
Ischemia-induced imbalance between NAD and NADH can affect the intensity of autofluorescence.

Reduced of the oxidized form: NADH

Oxidized form: NAD+

Absorption

Wavelength, nm
Intraoperative imaging of tissue viability.
Autofluorescence method

Before ischemia

30 sec after coronary occlusion
Intraoperative evaluation of myocardial metabolism in cardiac surgery with use of fluorescent spectroscopy

Baseline
End of anoxia
Start of cardioplegia
End of cardioplegia

NADH

![Graph showing changes in NADH levels during the surgical process.](image)
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Pre- and postconditioning in open heart surgery: main benefits

1. Reduced myocardial ATP breakdown

2. Lower troponin T in the postoperative period – lower myocardial injury

3. Hemodynamic benefit and improved cardiac performance: greater LV and RV ejection fraction, higher cardiac index

4. Reduced duration of mechanical ventilation

5. Less need for inotropic support

6. Fewer ventricular tachyarrhythmias
Current concept of preconditioning: *cardioprotective phenotype can be elicited by a wide spectrum of mildly noxious stimuli applied either locally or systemically*

**Ischemic preconditioning:** local and remote

**Non-ischemic preconditioning:**
- Pharmacological preconditioning
- Preconditioning with physical factors
- Metabolic preconditioning
Local ischemic preconditioning (LPC): significant infarct limitation occurs when preconditioning stimulus is strong enough.

**Personal communication, 2001**

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<thead>
<tr>
<th></th>
<th>Risk area size</th>
<th>Infarct size</th>
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<tbody>
<tr>
<td>Controls</td>
<td>64%</td>
<td>0%</td>
</tr>
<tr>
<td>LPC 1×(5'/5')</td>
<td>53%</td>
<td>27%</td>
</tr>
<tr>
<td>LPC 2×(5'/5')</td>
<td>27%</td>
<td>9%</td>
</tr>
<tr>
<td>LPC 3×(5'/5')</td>
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** - p<0.01 vs. controls
First description of antiarrhythmic effect of ischemic postconditioning

Ischemic postconditioning: brief ischemia during reperfusion converts persistent ventricular fibrillation into regular rhythm

Michael Galagudza\textsuperscript{a,c}, Dmitry Kurapeev\textsuperscript{b}, Sarkis Minasian\textsuperscript{a}, Guro Valen\textsuperscript{c}, Jarle Vaage\textsuperscript{d,*}

\begin{itemize}
\item Restoration of ischemic environment for 2 min resulted in 100% reversal of persistent VF in the isolated rat heart
\end{itemize}
New method of preconditioning induction in open heart surgery

Standard CPB and cardioplegia scheme

- CP
- Global ischemia with repeated CP
- Reperfusion

CPB and cardioplegia scheme with preconditioning

- 3-min episodes of reperfusion
- Induction of cardioplegia
- Global ischemia with repeated CP
- Reperfusion
Clinical trial on the effectiveness of ischemic preconditioning in cardiac surgery

• 200 patients with ischemic heart disease and valvular pathology;

• Randomization into the groups of preconditioning, controls, and parallel circulatory support;

• Main end points: troponin I, CK-MB prior to cardiopulmonary bypass, and 12, 24, and 48 h after surgery;

• Transmyocardial oxygen gradient (paired blood samples from the cardioplegic cannula);

• Myocardial biopsies for electron microscopy and molecular studies (Western blot analysis);

• Secondary end points: hemodynamic and clinical parameters
Myocardial protection and regeneration with product of apoptosis: hypothesis

Our hypothesis: products of apoptosis might be a “rescue signals” for cardiac resident stem cells and circulating bone marrow stem cells thereby promoting cardiac regeneration after injury.

Genesis of the hypothesis: endothelial apoptotic bodies dose-dependently stimulate proliferation of endothelial progenitor cells (Hristov et al., 2004)
Apoptotic body-enhanced proliferation and maturation of cardiac myocyte colonies

G. Belostotskaya et al., 2011

25th day, 46 beats/min

25th day, 99 beats/min
Aptoptotic products-mediated amelioration of LV function

Experimental design:

- Left coronary artery ligation
- Infusion of apoptotic bodies or culture medium
- 7 days
- 28 days
- Langendorff heart perfusion

Personal communication, 2011
• Additional proof-of-concept experiments in different models of cardiac injury;

• Identification of the molecular pattern of the “rescue signal” from the apoptotic bodies may contribute to the development of novel drugs for heart failure;

• The payload of the apoptotic body can vary depending on the cell source, type and severity of injury, etc. Molecular profiling of these natural “cocktails” may provide unique opportunity of tissue- and cell-targeted repair.
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Targeted drug delivery to the ischemic heart: *advantages*

- Decreased volume of drug distribution
- Reduced drug toxicity
- Increase in the solubility of hydrophobic drugs
- Improvement in the stability of the drugs (proteins, peptides, oligonucleotides)
- Increased biocompatibility
- Increased patient adherence to treatment
Active nanoparticle-based heart targeting: use of targeting ligands ("anchors")
The algorithm of heart targeting with nanoparticles

1. Silica of carbon nanoparticle
2. Engraftment of organic spacer
3. Binding of drug to the functional groups of spacer (e.g., NH2)
4. Intravenous administration
5. Binding of annexin 5 to the surface of nanocarrier

Accumulation of nanoparticles within the area of ischemia

Release of the drug during biodegradation of the coating

Uptake?

Translocation of phosphatidylserine

Specific recognition

Cardiac myocyte (area of ischemia)
Augmentation of infarct-limiting effect of adenosine after its adsorption on the surface of silica nanoparticles

** - p<0.01 versus control; * - p<0.05 in comparison to free adenosine
Targeted drug delivery into reversibly injured myocardium with silica nanoparticles: surface functionalization, natural biodistribution, and acute toxicity

Targeted drug delivery to ischemic heart with use of nanoparticulate carriers
Concepts, pitfalls and perspectives

Michael Galagudza
V.A. Almazov Federal Heart, Blood and Endocrinology Center, Institute of Experimental Medicine, St-Petersburg, Russia and
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SICA-HF

Studies Investigating Co-morbidities Aggravating Heart Failure

Consortium funded by European Commission under the 7th Framework Programme and the Russian Ministry of Science and Education within the Federal Programme “R&D in priority fields of the S&T complex of Russia 2007 - 2012”
We hypothesize that estimation of angiogenic potential of the patient's own stem cells and progenitor cells can serve as a novel valuable diagnostic and prognostic criteria/markers for heart failure (also in combination with diabetes and body mass disorders), and potentially constitute therapeutic targets.
Material and Approaches

Study participants
(HF / DM / Obesity & Healthy controls)

Biosamples
Bone marrow, blood, fat tissue

Clinical Data
(Complete evaluation)

Laboratory Data
(Blood biochemistry, SNP assay, etc.)

Cell Properties
(Proliferation, differentiation, interactions)

DNA
Serum, Plasma, etc.

Cells:
MSC BM
MSC FT
EPC

Merging a volume of clinical and laboratory data promises identifying candidate targets for diagnostics and prognostics
Comparative study of two patient-derived MSC populations

The study compares MSC derived from bone marrow (BM-MSC) and subcutaneous adipose tissue (F-MSC) of the same patient.

Frequency of Colony Forming Units (CFU) changes with successive passages in MSC derived from bone marrow (BM-MSC) and adipose tissue (F-MSC)
The population of CD146+ cells was more abundant in BM-MSC than in F-MSC at early passages and declined dramatically by P4.
Correlation of HF patient's stem cell functional properties with clinical laboratory parameters

Serum NT-proBNP and population doubling time of BM MSC

\[ r=0.35; p=0.01 \]

Blood glucose and population doubling time of BM MSC

\[ r=0.51; p=0.0005 \]
Correlation of HF patient’s stem cell functional properties with clinical laboratory parameters

Abdominal fat volume and IL-8 secretion by F MSC

Correlation of blood glucose and TGFβ secretion by F MSC

$r=-0.81; p=0.02$

$r=0.51; p=0.04$
BM MSC from HF obesity patients secrete greater amount of angiogenic factors but not VEGF
MSC derived from HF and HF&Cm are altered between the groups when cultured in vitro.

BM MSC have greater capacity to produce some proangiogenic and proinflammatory factors comparing to FMSC.

MSC from HF patients with obesity are more potent in producing angiogenic factors comparing both to patients with isolated HF and healthy subjects.
Clinical trial
“Intramyocardial Multiple Precision Injection of Bone Marrow Mononuclear Cells in Myocardial Ischemia” (acronim: IMPI)

**Goal:** investigation of the effect of mononuclear bone marrow cell transplantation after precise intramyocardial injection for treatment of coronary artery disease and heart failure

**Trial characteristics:** Double-blind randomized placebo-controlled trial

**Dates:** Total duration of the study: September 2010 – September 2014. Patient enrollment: 18 months after beginning of the study. Follow up period – 36 months.
Intramyocardial Multiple Precision Injection of Bone Marrow Mononuclear Cells in Myocardial Ischemia (IMPI)

This study is currently recruiting participants.
Verified on May 2011 by Almazov Federal Center of Heart, Blood and Endocrinology
First Received on May 13, 2011  Last Updated on May 16, 2011  History of Changes

Sponsor: Almazov Federal Center of Heart, Blood and Endocrinology
Information provided by: Almazov Federal Center of Heart, Blood and Endocrinology
ClinicalTrials.gov Identifier: NCT01354578

Purpose
Randomised placebo-controlled study of efficiency and safety of bone marrow mononuclear cells transplantation by intramyocardial multiple precision injection in ischemic heart failure patients.

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<th>Condition</th>
<th>Intervention</th>
<th>Phase</th>
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<td>Heart Failure</td>
<td>Procedure: NOGAXP Cardiac Navigation System</td>
<td>Phase I</td>
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Clinical data of the patient NM-01

Age: 62 years

Diagnosis

Main: CAD, effort angina
Postinfarction cardiosclerosis (STEMI in 1996)
CABG, LV aneurism surgery in 1997
Arterial hypertension

Complications: Atrial fibrillation with impaired AV conduction.
LBBB. Ventricular premature beats. Paroxysmal ventricular tachycardia. Implantation of CRT-device. CHF II (NYHA)
Patient NM-01 data

- Voltage
- Contractility

● = sites of injection of cells
13 injections 200 microL each