Myocyte Preservation After Mechanical Reperfusion

Status 2003

William W. O’Neill, M.D.
Future Reperfusion Algorithm

Chest pain onset

- Protective measures
- PCI
- Filters, thrombectomy

TIMI III flow

- Microcirculatory agents

Recovery assessment

- ST resolution
- MRI
- MCE

Good recovery

- Home

Poor recovery

- Myocyte regeneration
TOPCARE AMI
Wall Motion Scores

Assmus et al. Circ 2002;106:3009
TOPCARE AMI

A LV-Angiography (RAO initial)

B LV-Angiography (RAO follow-up)

Assmus et al. Circ 2002;106:3009
Study protocol

20 patients with acute myocardial infarction successfully reperfused by stent implantation
LV-angiography

intracoronary infusion of circulating progenitor cells
n = 11

105 ± 29 h
1 patient discontinued due to additional acute myocardial infarction

n = 7

intracoronary infusion of bone-marrow cells
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104 ± 48 h

stress echocardiography

coronary flow reserve

FDG-PET

repeat cardiac catheterization at 4 months
n = 10

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LV-angiography
coronary flow reserve

echocardiography

FDG-PET

Assmus et al. Circ 2002;106:3009
ILCOR Advisory Statement

Therapeutic Hypothermia After Cardiac Arrest
An Advisory Statement by the Advanced Life Support Task Force of the International Liaison Committee on Resuscitation

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Cellular Therapy for Cardiac Myocardial Repair

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ASAIO June 20, 2003
Case Presentation

• A 16 year old boy was shot in the heart with a 3” finishing nail from an air powered nail-gun while being taunted by a coworker.

• The nail embedded into the RV free wall approx. 1 cm from the LAD.

• The nail was emergently removed under direct surgical visualization.
Case Presentation

- Initially, the patient did well.

- 2 days post-op, the patient was noted to have acute AMI by ECG.

- Echocardiography confirmed an extensive area of myocardial infarction with wall thinning.
Coronary Arteriography
Case Presentation

- Following LAD stent implantation, both cardiac MRI and PET scanning confirmed a large area of nonviable myocardium.

- The same region appeared thin and necrotic by echocardiography.
Prognosis

Lousy!

• Progressive LV dilation.
• Deterioration of LV systolic function.
• Worsening CHF.
• Eventual aneurysmectomy, cardiac transplant…or worse.
Ischemic Cardiomyopathy
Progress in the treatment of advanced ischemic cardiac disease has dramatically affected both its morbidity and mortality.

1. Risk-factor modification and patient education
2. ASA, clopidogrel, β-blockers, ACE inhibitors, HMG Co-A inhibitors
3. Refined PCI techniques
4. More aggressive CABG strategies
Despite such advances, the prevalence of progressive ischemic heart disease continues to increase and remains the leading cause of death in the U.S.
The great paradox: As the treatment for ischemic heart disease improves, the level of patient complexity increases.

- Older
- More advanced and complex disease
- Restenotic lesions
- Graft failures

We are victims of our own success.
Alternative Therapies?

- Gene and Gene product therapy
  - VEGF, Ad-VEGF
  - FGF, Ad-FGF
- Cellular therapy
  - Skeletal myoblast therapy
  - Endothelial progenitor cells
  - Autologous stem cell therapy
    - Bone marrow
    - Circulating
  - Fetal Stem Cells
Gene and Gene Product Therapy

To date, clinically significant improvements with gene or gene product therapy using angiogenic cytokines (i.e. VEGF, FGF) has been limited or disappointing.

- VEGF – VIVA Trial
- FGF2 (bFGF) – FIRST Trial
- Ad5-FGF4 – AGENT Trial
Problems with GT or GPT

- Angiogenesis may be too complex for a single gene product
- Delivery strategies
  - Dosage of angiogenic substance
  - Duration of infusion with gene product therapy
  - Use of viral vectors
- Significant placebo effect in comparative trials in both clinical and objective measures.
Problems with GT or GPT

- Angiogenic reperfusion treats ischemia.
- If the affected myocardial area is dead, so what?!
- Angiogenesis does not produce myocardial regeneration.
Cellular Therapy
Objectives of Cellular Therapy

• Angiogenesis

• Myogenesisis
Science Fiction?

Rules of myocardial cellular biology

- Cardiac myocytes are terminally differentiated, post-mitotic cells.
- Myocyte regeneration is not feasible
- OR IS IT?
Cardiac myocytes in post-mortem patients with recent MI demonstrated increased expression of Ki-67, a nuclear antigen associated with cellular division.

Beltrami AP, Anversa P. NEJM 2001;344:1750.
• Mitosis of cardiac myocytes in the same post-MI patients was also observed.

Beltrami AP,…Anversa P. NEJM 2001;344:1750
Female hearts transplanted in male patients demonstrated Y chromosomes (arrowheads) in up to 10% of myocytes, arteriolar smooth muscle cells and endothelial cell.

Ki-67 expression was also increased.
• In combination, these studies suggest that myocardial proliferation in adult hearts occurs.

• The source of the cellular proliferation is undetermined.

• Perhaps endogenous and circulatingprimitive cells migrate to and proliferate within injured (from AMI or chronic rejection) myocardium.

Figure by Schwartz RS, Curfman GD
NEJM 2002;346:2
“Studies Show…”

- Myocardial injury mobilizes pluripotent stem cells and EPCs from bone marrow.

- Once mobilized, stem cells and EPCs home specifically to ischemic areas and trans-differentiate into cardiomyocytes and endothelial cells.

Homing of Endothelial Progenitor Cells

However!

• The process of migration and proliferation of primitive cells to repair clinically significant myocardial injury is inadequate in humans.

• There remains a need to artificially amplify or accelerate cellular migration and proliferation.
Transplantation of Endothelial Progenitor Cells

- Human EPCs were cultured for 7 days.
- Human EPCs were transplanted intravenously into athymic (immunodeficient) nude rats after coronary ligation.
- Human EPCs were labeled with Dil dye which appears red on fluorescent microscopy.
Transplantation of Endothelial Progenitor Cells

Human EPCs accumulated in ischemic areas and differentiated into mature endothelial cells
Transplantation of Endothelial Progenitor Cells

Transplanted EPCs

- Increased capillary density
- Reduced scar formation
- Improved myocardial function
Bone Marrow Cell Transplantation


- Canine study of LAD ligation
- At 30 days, equal groups of 7 dogs underwent injection of concentrated bone marrow cells or PBS into infarcted, border and normal areas of the left ventricle.
Bone Marrow Cell Transplantation


Normal | Border-zone | Infarct-zone
Bone Marrow Cell Transplantation

• Cultured bone marrow cells secrete the angiogenic factors VEGF and MCP-1

• Bone marrow cell culture medium can induce endothelial cell proliferation in vitro.
Bone Marrow Cell Transplantation
Orlic et al. Nature 2001;410:701

- Lin⁻ c-kit⁺ bone marrow cells transplanted into myocardium of transgenic mice shortly after coronary ligation.

- The border-zone was targeted.

- Newly formed myocardium occupied 68% of the infarcted region at 9 days.
14 patients with end-stage ischemic CMP underwent bone marrow harvest from the iliac crest.

After isolation, washing and resuspension, mononuclear bone marrow cells were transplanted into the LV endocardium using a NOGA catheter.

7 matched patients were followed for control.
Cellular Therapy in Humans
Perin EC et al. Circ 2003;107:2294

The NOGA catheter is a multifunctional devise that can measure myocardial viability, local LV contractility and provide for transendocardial injections.
EMM using a NOGA catheter
Fuchs S, et al. JACC;41:1721

LV EMM evaluating local shortening (contractility) and unipolar voltage (viability)
## Symptoms & Exercise Tolerance (2-Months)

<table>
<thead>
<tr>
<th></th>
<th>Treatment (n=14)</th>
<th>Control (n=7)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NYHA class</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>2.21±0.89</td>
<td>2.71±0.75</td>
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<tr>
<td>After treatment</td>
<td>1.14±0.36</td>
<td>2.71±0.76</td>
<td>0.0001</td>
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<tr>
<td>P</td>
<td>0.0003</td>
<td>1.0</td>
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<tr>
<td><strong>CCSAS class</strong></td>
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<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>2.64±0.84</td>
<td>2.57±0.97</td>
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</tr>
<tr>
<td>After treatment</td>
<td>1.28±0.61</td>
<td>2.14±0.89</td>
<td>0.0001</td>
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<tr>
<td>P</td>
<td>0.0001</td>
<td>0.06</td>
<td>0.001</td>
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<tr>
<td><strong>Ramp treadmill METs</strong></td>
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<tr>
<td>Before treatment</td>
<td>5.09±2.5</td>
<td>5.07±1.96</td>
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<tr>
<td>After treatment</td>
<td>6.68±2.35</td>
<td>5.16±2.45</td>
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<tr>
<td>P</td>
<td>0.0085</td>
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<tr>
<td><strong>V̇O₂max</strong></td>
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<tr>
<td>Before treatment</td>
<td>17.96±8.78</td>
<td>17.75±6.85</td>
<td></td>
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<tr>
<td>After treatment</td>
<td>23.38±8.31</td>
<td>18.08±8.58</td>
<td>0.08</td>
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<tr>
<td>P</td>
<td>0.01</td>
<td>0.84</td>
<td></td>
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</tbody>
</table>

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<tr>
<th></th>
<th>Before Treatment</th>
<th>After Treatment</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LV angiogram</strong></td>
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<tr>
<td>EDV, cc</td>
<td>213.5±81.6</td>
<td>181±51.3</td>
<td>0.1</td>
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<tr>
<td>ESV, cc</td>
<td>174.1±78.7</td>
<td>133.5±54</td>
<td>0.03</td>
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<tr>
<td>EF, %</td>
<td>20±9</td>
<td>29±13</td>
<td>0.0003</td>
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<tr>
<td><strong>EMM</strong></td>
<td></td>
<td></td>
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<tr>
<td>Unipolar voltage, mV</td>
<td>10.5±3.5</td>
<td>10.3±2.7</td>
<td>0.65</td>
</tr>
<tr>
<td>Local linear shortening, %</td>
<td>5.7±3.7</td>
<td>10.8±7.5</td>
<td>0.0005</td>
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</tbody>
</table>
Transmyocardial BMC Txp in Chronic Ischemia

• NOGA
  – 10 pts treated with autologous BMC — ↑ myocardial perfusion in injected areas and ↑ exercise duration. Fuchs S et al. JACC 2003;41;1721

• Direct Surgical Injection
Cellular Therapy in Humans

TOPCARE-AMI. Assmus et al. Circ 2002;106:3009

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echocardiography
Cellular Therapy in Humans

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Ejection Fraction (%)

Subjects | Controls
--- | ---
Baseline | 55 | 45 | \( p = 0.003 \) | P = NS
Follow-up | 60 | 55 | \( p = 0.01 \) | P = NS

End Systolic Volume (ml)

Subjects | Controls
--- | ---
Baseline | 60 | 50
Follow-up | 55 | 50 | \( p = 0.01 \) | P = NS

Baseline | Follow-up
Cellular Therapy in Humans

TOPCARE-AMI. Assmus et al. Circ 2002;106:3009

Regional Wall Motion (SD/chord)

-1.6

Infarct Zone Infarct Center Infarct Border

Baseline Follow-up

P<.001 P<.001 P<.001
Cellular Therapy in Humans

TOPCARE-AMI. Assmus et al. Circ 2002;106:3009

A LV-Angiography (RAO initial)

enddiastolic

B LV-Angiography (RAO follow-up)

diastolic

enddiastolic

endsystolic

endsystolic
Quantitative F-18-fluorodeoxyglucose PET demonstrated a significant increase in viability in the infarct zone ($p < 0.01$)
Cellular Therapy in Humans


- IC administration of BMC in post MI pts.
- Reduction in size of infarct region
- Enhanced myocardial perfusion
- Improved contractility
  - SVI
  - ESV & ESP: ESV
WBH Experience

- **Protocol**
  - 2 weeks after stent implantation
  - G-CSF 10mg/kg SQ x 4days
  - On day 5, a Quinton dialysis catheter was placed in the right femoral vein
Leukapheresis

- COBE/BCT apheresis machine was used to perform leukapheresis
- 150 cc removed
Stem Cell Product

- The leukapheresis product was centrifuged to remove excess plasma and platelets.
- A 55cc concentrate of mononuclear cells enriched with stem cells (CD34 and AC133) remained.
The concentrate was administered into the distal LAD using an infusion catheter.

During infusion, a second balloon cath was inflated proximally to occlude blood flow.

Each 5cc aliquant was delivered over 5 minutes.
Intracoronary Stem Cell Administration

Figure by Strauer BE, et al. Circ 2002;106:1913
3 Month Follow-Up

Echo EF (%)

MUGA EF (%)
3 Month Clinical Follow-Up

- NYHA Class I
- Returned to school to complete the semester
- Playing street basketball and ignoring most advise to take it easy
Stem Cell Therapy

- Early results are promising
- Larger comparative clinical trials are needed
- WBH is proposing a 100 patient randomized trial pending FDA clearance