Secretome of apoptotic cells causes cardioprotection and inhibits ventricular remodeling after acute myocardial infarction

Doctoral viva
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### Background

**Table 1 | Randomized trials in patients with acute myocardial infarction or ischemic heart failure**

<table>
<thead>
<tr>
<th>Trial name</th>
<th>Number of patients</th>
<th>Cell type</th>
<th>Dose</th>
<th>Route of delivery</th>
<th>Timing of delivery</th>
<th>Primary end point</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute myocardial infarction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BOOST</td>
<td>60</td>
<td>nBMC</td>
<td>128 ml</td>
<td>i.c.</td>
<td>Day 6 ± 1</td>
<td>LVEF ↑</td>
<td>Effect diminished after 18 and 61 months</td>
</tr>
<tr>
<td>REPAIR-AMI</td>
<td>187</td>
<td>mnBMC</td>
<td>50 ml</td>
<td>i.c.</td>
<td>Day 3–6</td>
<td>LVEF ↑</td>
<td>NA</td>
</tr>
<tr>
<td>Leuven-AMI</td>
<td>66</td>
<td>mnBMC</td>
<td>130 ml</td>
<td>i.c.</td>
<td>Day 1</td>
<td>LVEF ↔</td>
<td>Regional contractility ↑ Infarct size ↓</td>
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<tr>
<td>ASTAMI</td>
<td>97</td>
<td>mnBMC</td>
<td>50 ml</td>
<td>i.c.</td>
<td>Day 6 ± 1</td>
<td>LVEF ↔</td>
<td>NA</td>
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<tr>
<td>FINCELL</td>
<td>77</td>
<td>mnBMC</td>
<td>80 ml</td>
<td>i.c.</td>
<td>Day 3</td>
<td>LVEF ↑</td>
<td>NA</td>
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<tr>
<td>REGENT</td>
<td>117</td>
<td>mnBMC (unselected vs CD34+/CXCR4+)</td>
<td>50–70 ml (unselected) 100–120 ml (selected)</td>
<td>i.c.</td>
<td>Day 3–12</td>
<td>LVEF ↑ with both cell types</td>
<td>NA</td>
</tr>
<tr>
<td>HEBE</td>
<td>189</td>
<td>mnBMC vs mnPBC</td>
<td>60 ml (mnBMC) 150 ml (mnPBC)</td>
<td>i.c.</td>
<td>Day 3–8</td>
<td>Regional contractility ↔</td>
<td>NA</td>
</tr>
<tr>
<td>Ischemic heart failure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAGIC</td>
<td>97</td>
<td>SkM</td>
<td>400 or 800 × 10⁶</td>
<td>i.m.</td>
<td>&gt;Week 4</td>
<td>LVEF ↔</td>
<td>LVEDV ↓ LVESV ↓</td>
</tr>
<tr>
<td>TOPCARE-CHD</td>
<td>58</td>
<td>mnBMC vs CPC</td>
<td>50 ml</td>
<td>i.c.</td>
<td>Month 81 ± 72</td>
<td>LVEF ↑ (mnBMC) LVEF ↔ (CPC)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Only patients with complete imaging studies are considered here. Dose refers to the average amount of bone marrow or peripheral blood that was harvested, or the number of transplanted skeletal myoblasts. Abbreviations: ↓, decreased; ↑, increased; ↔, no significant change; CPC, circulating blood-derived progenitor cells; i.e., intracoronary; i.m., intramuscular; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; mnBMC, mononucleated bone marrow cells; mnPBC, mononucleated peripheral blood cells; NA, not applicable; nBMC, nucleated bone marrow cells; SkM, skeletal myoblasts.

Background

Myocardial Infarction

Necrosis

Attraction of immune cells
Secretion of pro-inflammatory cytokines
 IL-1  IL-6  TNF-α
Amplification of inflammation

The Dying Stem Cell Hypothesis
by Anker et al.

up to 25% of all transplanted cells are in the state of apoptosis

apoptotic cells induce transient immunosuppression

Experimental Design

Peripheral Blood Mononuclear Cells (PBMC)

Irradiation & Induction of Apoptosis

Cell Culture for 18-24h

Flow Cytometry
Annexin-positivity >70%

Intramyocardial Injection

Intravenous Injection

Controls
Injection of
Non-irradiated viable PBMC

Cell Culture Medium

Sham Operation

Model of Experimental AMI

anesthetized and mechanically ventilated rat

intercostal thoracotomy

ligation of the coronary artery
Results

Histology and Immunohistology 3 days after induction of MI

<table>
<thead>
<tr>
<th>Medium Control</th>
<th>Viable PBMC IV</th>
<th>Apoptotic PBMC IV</th>
<th>Apoptotic PBMC IM</th>
</tr>
</thead>
<tbody>
<tr>
<td>H&amp;E</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-kit</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n=5-6 per group
Results

Scar Dimension 6 Weeks after Induction of MI

Control
Intravenous Administration of viable PBMC
Intravenous Administration of IA-PBMC
Intramyocardial Injection of IA-PBMC

Area of fibrosis after 6 weeks
% of left ventricle

IA-PBMC suspensions of irradiated apoptotic peripheral blood mononuclear cells
Results

Composition of Scar Tissue

- **Control**
- **Intravenous Administration of viable PBMC**
- **Intravenous Administration of IA-PBMC**
- **Intramyocardial Injection of IA-PBMC**

**Percentage of elastic fibres in fibrotic scar tissue**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Percentage (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>medium IV</td>
<td>0.0 ± 0.5</td>
</tr>
<tr>
<td>viable PBMC IV</td>
<td>5.0 ± 1.0</td>
</tr>
<tr>
<td>apoptotic PBMC IV</td>
<td>15.0 ± 2.0</td>
</tr>
<tr>
<td>apoptotic PBMC IM</td>
<td>20.0 ± 3.0</td>
</tr>
</tbody>
</table>

n=10-12

IA-PBMC suspensions of irradiated apoptotic peripheral blood mononuclear cells
## Results

### Evaluation of Cardiac Function

<table>
<thead>
<tr>
<th></th>
<th>sham</th>
<th>medium IV</th>
<th>viable PBMC IV</th>
<th>apoptotic PBMC IV</th>
<th>apoptotic PBMC IM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LVEDD (mm)</strong></td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td><strong>LVESD (mm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ejection Fraction %</strong></td>
<td>60</td>
<td>50</td>
<td>40</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td><strong>Shortening Fraction %</strong></td>
<td>40</td>
<td>30</td>
<td>20</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

(* indicates p < 0.05, ** indicates p < 0.01, *** indicates p < 0.001)
Conclusion

Administration of irradiated apoptotic PBMC after myocardial infarction induces ...

- Reduction of Pro-inflammatory Signals
  - IL-1β ↓
  - IL-6 ↓
- Up-regulation of Pro-angiogenic mediators
  - Interleukin-8 ↑
  - MMPs ↑
- Increased Homing of CD68⁺ and c-kit⁺ Cells
- Favorable Elastin/Collagen Ratio
- Better Recovery of Cardiac Function
  - Ejection Fraction ↑
  - Shortening Fraction ↑
  - Dilation ↓
Experimental Set-up

PBMC Suspension

Irradiation and Induction of Apoptosis

Cell Culture for 24h and Secretion of various proteins

Centrifugation

Cell-free supernatant

Used in in vivo experiments

Hypothesis:
Factors secreted during cell culture induce cardioprotection

Lyophilization

Cell pellet discarded

Control

Suspensions of apoptotic PBMC

Irradiated cultured apoptotic peripheral blood mononuclear cells regenerate infarcted myocardium.
Production of APOSEC
(Cell culture supernatants of apoptotic PBMC)

Venous Blood Withdrawal

Ficoll Cell Separation

Irradiation

Incubation for 24h

Centrifugation

Dialysis

Lyophilization

Lyophilized Cell Culture Supernatant - Aposec -

PBMC    peripheral blood mononuclear cells

Wien, 11.01.2012
APOSEC
AMI – Small Animal Model

Results after 72h

Area of necrosis after 3 days
% of left ventricle

Medium IV
APOSEC IV

Wien, 11.01.2012

n=5-6 per group
Results after 6 Weeks

Area of fibrosis after 6 weeks
% of left ventricle

Sham
Medium IV
APOSEC IV

Ejection Fraction %

Sham
Medium IV
APOSEC IV

n=10 per group

Wien, 11.01.2012
APOSEC
AMI – Large Animal Model

Start of large animal experiment
APOSEC
Reperfused AMI
Large Animal Model

Results after 24 Hours

Medium IV

APOSEC IV

n=5
APOSEC
Reperfused AMI
Large Animal Model

Results after 30 Days

Medium IV

APOSEC IV

n=7-9 per group

Wien, 11.01.2012
## APOSEC
Reperfused AMI
Large Animal Model

### Results MRI Analysis

**Cardiac MRI evaluation 3 and 30 days after Ischemia/Reperfusion Injury**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Medium control (n=8)</th>
<th>250.10^6 apoptotic PBMC (low dose APOSEC, n=7)</th>
<th>1.10^8 apoptotic PBMC (high dose APOSEC, n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>age (days)</td>
<td>90 ±0</td>
<td>90 ±0 ns</td>
<td>90 ±0 ns</td>
</tr>
<tr>
<td>LVEDV (ml)</td>
<td>67.59 ±2.7</td>
<td>64.19 ±5.4 ns</td>
<td>63.73 ±1.6 ns</td>
</tr>
<tr>
<td>LVESV (ml)</td>
<td>38.42 ±2.5</td>
<td>35.96 ±3.0 ns</td>
<td>33.93 ±2.1 ns</td>
</tr>
<tr>
<td>LVSV (ml)</td>
<td>29.17 ±1.3</td>
<td>28.23 ±3.2 ns</td>
<td>29.77 ±1.8 ns</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>43.38 ±1.9</td>
<td>43.63 ±2.8 ns</td>
<td>46.65 ±2.9 ns</td>
</tr>
<tr>
<td>HR/min.</td>
<td>111 ±6</td>
<td>109 ±5 ns</td>
<td>111 ±13 ns</td>
</tr>
<tr>
<td>CO (l/min.)</td>
<td>3.24 ±0.1</td>
<td>3.03 ±0.3 ns</td>
<td>3.28 ±0.3 ns</td>
</tr>
<tr>
<td>CI (l/min/m²)</td>
<td>3.64 ±0.13</td>
<td>3.59 ±0.4 ns</td>
<td>3.82 ±0.37 ns</td>
</tr>
<tr>
<td>Infarct %</td>
<td>18.17 ±1.7</td>
<td>14.01 ±1.9 ns</td>
<td>8.66 ±1.5 **</td>
</tr>
</tbody>
</table>

**after 3 days**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Medium control (n=8)</th>
<th>250.10^6 apoptotic PBMC (low dose APOSEC, n=7)</th>
<th>1.10^8 apoptotic PBMC (high dose APOSEC, n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>age (days)</td>
<td>120 ±0</td>
<td>120 ±0 ns</td>
<td>120 ±0 ns</td>
</tr>
<tr>
<td>LVEDV (ml)</td>
<td>54.74 ±4.1</td>
<td>53.43 ±3.5 ns</td>
<td>65.99 ±3.5 ns</td>
</tr>
<tr>
<td>LVESV (ml)</td>
<td>32.93 ±4.0</td>
<td>31.89 ±3.2 ns</td>
<td>28.71 ±3.5 ns</td>
</tr>
<tr>
<td>LVSV (ml)</td>
<td>21.84 ±1.8</td>
<td>21.54 ±2.0 ns</td>
<td>37.29 ±1.7 ***</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>40.54 ±3.6</td>
<td>40.64 ±3.5 ns</td>
<td>57.05 ±3.3 **</td>
</tr>
<tr>
<td>HR/min.</td>
<td>114 ±7</td>
<td>108 ±8 ns</td>
<td>107 ±5 ns</td>
</tr>
<tr>
<td>CO (l/min.)</td>
<td>2.44 ±0.1</td>
<td>2.28 ±0.1 ns</td>
<td>3.98 ±0.2 ***</td>
</tr>
<tr>
<td>CI (l/min/m²)</td>
<td>2.46 ±0.12</td>
<td>2.40 ±0.15 ns</td>
<td>3.51 ±0.15 ***</td>
</tr>
<tr>
<td>Infarct %</td>
<td>12.80 ±1.3</td>
<td>11.50 ±1.6 ns</td>
<td>6.92 ±1.4 **</td>
</tr>
</tbody>
</table>

**after 30 days**
Analysis of Protein Content of APOSEC (Cell culture supernatants of apoptotic PBMC)

Membran Array – Angiogenic Factors

pos

TF
GDNF
MIP-1a
Serpin B5
pos

neg

CXCL16
GM-CSF
MMP-8
PAI-1
Serpin F1

Activia A
ADAMTS-1
Angio
genin
Angiopoietin-1
Angiopoietin-2
Plasminogen
Amphiregulin
Artemin
FGF-4
FGF-7
FGF-8
Leptin
MCP-1
Prolactin
VEGF-C
APOSEC
Mechanism of Action

Cell Culture of human Cardiomyocytes

Cell Starvation Assay

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Medium</th>
<th>APOSEC&lt;sup&gt;H&lt;/sup&gt;</th>
<th>5 min</th>
<th>10 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
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<tbody>
<tr>
<td>Phospho-p38 MAPK</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Total p38 MAPK</td>
<td></td>
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<tr>
<td>Phospho-c-Jun</td>
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<td>Total c-Jun</td>
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<tr>
<td>Phospho-CREB</td>
<td></td>
<td></td>
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<tr>
<td>Total CREB</td>
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<tr>
<td>Phospho-AKT</td>
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<td>Total AKT</td>
<td></td>
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<tr>
<td>Phospho-Erk1/2</td>
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<tr>
<td>Total Erk1/2</td>
<td></td>
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<tr>
<td>Phospho-Hsp27 (Ser15)</td>
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<tr>
<td>Phospho-Hsp27 (Ser85)</td>
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<tr>
<td>Total Hsp27</td>
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n=3
APOSEC
Mechanism of Action

Cell Culture of human Cardiomyocytes – Factor Inhibition Assay

<table>
<thead>
<tr>
<th>APOSEC</th>
<th>Medium</th>
<th>mouse IgG</th>
<th>goat IgG</th>
<th>anti VEGF</th>
<th>anti IL-8</th>
<th>anti ENA-78</th>
<th>anti MMP9</th>
<th>isotypes comb.</th>
<th>Abs comb.</th>
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<tbody>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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</tbody>
</table>

Fold induction

<table>
<thead>
<tr>
<th></th>
<th>Phospho-CREB</th>
<th>Phospho-Hsp27 (Ser85)</th>
<th>Bcl-2</th>
<th>BAG1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fold induction</td>
<td>4.9</td>
<td>4.2</td>
<td>5.1</td>
<td>4.8</td>
</tr>
<tr>
<td>Fold induction</td>
<td>7.3</td>
<td>7.0</td>
<td>7.2</td>
<td>7.4</td>
</tr>
<tr>
<td>Fold induction</td>
<td>3.8</td>
<td>4.7</td>
<td>3.9</td>
<td>3.9</td>
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<tr>
<td>Fold induction</td>
<td>2.2</td>
<td>2.3</td>
<td>2.6</td>
<td>2.5</td>
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</table>
APOSEC
Mechanism of Action

Apoptotic PBMC

APOSEC
Secretion of soluble factors

Cardioprotective effects

Cardioprotection
Special thanks

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Moritz Rauch

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Department of Cardiology
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