Therapeutic Capacity of Apoptotic Mononuclear Cell Secretome in Experimental Spinal Cord Injury

Doctoral viva

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Vienna, March 29, 2016
Introduction

Stem Cell Therapy

Number of papers dealing with stem cells and stroke

Years


Introduction

![Image of brain with various labels: Neuroprotection, Immune suppression, Normalization of metabolic profiles, Promote autophagy, Angiogenesis, Neurogenesis, Synaptogenesis, Trans differentiation.

NSC Neuroblast, Astrocyte, Neuron, Microglia, Activated microglia, Newborn astrocyte, Newborn neuron, Adult stem cell, Extracellular vesicle, Protein mRNA, miRNA]
## Introduction

### 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><strong>Acute myocardial infarction</strong></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 †</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Infarct size ‡</td>
</tr>
<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>
</tr>
<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>
</tr>
<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><strong>Ischemic heart failure</strong></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>

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

Introduction

Myocardial Infarction

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

Introduction

Stem Cell Engraftment and Transdifferentiation?

• Low number of transplanted cells
• Permanent engraftment not observable
• Therapeutic effect in less than 72h
• Secretome (CM) alone delivers comparable results
Introduction
Introduction

Comparability of BMCs and PBMCs Secretome

- Secretome: Difference in 35 out of 174 analyzed factors
  - 25 secreted factors were higher in BMC-secretome
  - 10 secreted factors were higher in PBMC-secretome

Introduction

Introduction

- Attenuation of acute myocardial infarction
  (Lichtenauer et al; Basic Res Cardiol. 2011)

Macroscopic analysis after 24 hours

(a) Medium IV

(b) APOSEC IV
Introduction

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  (Lichtenauer et al; Basic Res Cardiol. 2011)

- Immunosuppression in an experimental myocarditis model
  (Hoetzencker et al; Eur Heart J. 2013)
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- **Experimental stroke model**
  (Altmann et al; F1000Res. 2014 Jun 19 [revised 2014 Oct 28])
Introduction
Introduction

Induction of Apoptosis

Non-irradiated

Irradiated

HLF

γ-IR

-      -      +      +

0.0

0.5

1.0

1.5

20h

Beer et al. BMC Genomics 2014
Introduction

Compounds of the Secretome

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8</td>
<td>2305,8 ±136,4</td>
</tr>
<tr>
<td>GRO-alpha</td>
<td>487,7 ±89,5</td>
</tr>
<tr>
<td>ENA-78</td>
<td>37857,5 ±12734,2</td>
</tr>
<tr>
<td>MCP-1</td>
<td>739,9 ±175,5</td>
</tr>
<tr>
<td>NAP-2</td>
<td>9,9 ±0,5</td>
</tr>
<tr>
<td>RANTES</td>
<td>22251,2 ±3641,9</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>2068,2 ±415,2</td>
</tr>
<tr>
<td>VEGF_{165}</td>
<td>640,1 ±35,2</td>
</tr>
<tr>
<td>IL-16</td>
<td>1254,2 ±77,6</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>410,7 ±167,0</td>
</tr>
<tr>
<td>IL-10</td>
<td>7,1 ±0,5</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>5,8 ±3,2</td>
</tr>
<tr>
<td>IGF-I</td>
<td>72,9 ±19,1</td>
</tr>
<tr>
<td>HGF</td>
<td>534,2 ±11,6</td>
</tr>
<tr>
<td>TGF-beta</td>
<td>87,3 ±20,4</td>
</tr>
<tr>
<td>MMP9</td>
<td>3612,3 ±597,7</td>
</tr>
<tr>
<td>MIF</td>
<td>20147,5 ±1140,2</td>
</tr>
<tr>
<td>PAI-1</td>
<td>5060,6 ±3247,5</td>
</tr>
<tr>
<td>SDF-1</td>
<td>148,5 ±7,1</td>
</tr>
</tbody>
</table>

Introduction

Compounds of the Secretome

• Secreted Proteins

• Oxidized Lipids

• Extracellular vesicles
  • Microparticles
  • Exosomes

Beer et al. BMC Genomics 2014
Introduction

Spinal Cord Injury
Introduction

Spinal Cord Injury

• 50 per 1 million annually worldwide (200/year in AUT)

• Average age of patients : 31 years

• 50% experience total loss of motor function

• 2 out of 3 – cervical spinal cord affected

Introduction

Spinal Cord Injury

Introduction

Spinal Cord Injury – Treatment

• Early surgical decompression

• Corticosteroids (?) – Methylprednisolone (MP) regime

• Rehabilitation
Introduction

Spinal Cord Injury – Treatment

• 30 years of SCI research -> 1 approved treatment option (MP) aside from surgical therapy

• Recommendation for the use of MP in SCI was revoked in 2013 by the AANS
Introduction
Introduction
Hypothesis

Compounds of secretome of apoptotic peripheral blood mononuclear cells exert therapeutic capacity
Methods

Contusion Injury Model

BBB-Score

# Methods

<table>
<thead>
<tr>
<th>Venous Blood Withdrawal</th>
<th>Cell Separation</th>
<th>Irradiation</th>
<th>Incubation for 24h</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Centrifugation</th>
<th>Lyophilization</th>
<th>Lyophilized MNC-secretomes</th>
<th>Virus Elimination (GMP)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Medical University of Vienna
Methods

Impact | Treatment | Histology | Histology

0 | 40 min | 24h | d3 | d7 | d14 | d21 | d28

Locomotor Evaluation – BBB-score

-RESULTS-
Results

![Graph showing BBB-Score over days after trauma compared to MNC-secretome and medium groups.](image)

- **MNC-secretome**
- **Medium**

Days after Trauma vs BBB-Score graph with statistical significance markers (*** and **) and sample size (n=12).

Results

Results

Results

Results

Results
Results


Medium

MNC-secretome

E

F

VWF-count/mm²

n=3-5

*
Results
Previous Results

Control  viable PBMC i.v.  IA-PBMC i.v.  IA-PBMC i.c.

H&E  CD68  c-kit

Results
Results

![Graph showing CXCL1 expression levels in different fractions: Supernatant, Microparticle, Exosome, Lipid, Protein. The graph indicates significant differences marked with asterisks (**, ***).]
Results

A

<table>
<thead>
<tr>
<th></th>
<th>Medium</th>
<th>MNC-secretome</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-ERK 1/2</td>
<td><img src="A" alt="Image" /></td>
<td><img src="A" alt="Image" /></td>
</tr>
<tr>
<td>p-CREB</td>
<td><img src="A" alt="Image" /></td>
<td><img src="A" alt="Image" /></td>
</tr>
<tr>
<td>p-p38</td>
<td><img src="A" alt="Image" /></td>
<td><img src="A" alt="Image" /></td>
</tr>
<tr>
<td>p-HSP27</td>
<td><img src="A" alt="Image" /></td>
<td><img src="A" alt="Image" /></td>
</tr>
</tbody>
</table>

B

*P-ERK 1/2 Fold Induction*

- Untreated
- Medium
- MNC-secretome

Summary

- Secretome of apoptotic PBMCs previously attributed with
  - Cytoprotection/Inhibition of apoptosis
  - Immunomodulation
  - Inhibition of microvascular obstruction/thrombocyte activation
- Translatable to Spinal Cord Injury

Summary

- Increment of systemic CXCL-1 and BDNF levels
- Recruitment of monocytes
- Up-regulation of anti-apoptotic/pro-survival pathways
- Neuroprotection
- Inhibition of thrombocyte activation
- Vasodilation
- Neonangiogenesis
- Modulation of the inflammatory response
- Accelerated resolution of inflammation

Reduced morphological detriment
Improved Neurologic Outcome

TTND. 2016; 3:e1198.
Summary

- Secretome of apoptotic PBMCs lead to
  - Improvement of neurologic outcome
  - Attenuation of morphological damage
  - Improvement of vascularity
  - Recruitment of peripheral monocytes

-> Multilayered therapy

Outlook

• Crossing of the blood-brain barrier?

• Alternative administration route/Combination with novel approaches

• Mechanisms involved in monocyte recruitment?

• Translation to bed-side
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  Jens Hartmann
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  Christian Gabriel
Addendum

MNC-secretome

MNC-secretome + aVEGF

Addendum

TNF and Increased Intracellular Iron Alter Macrophage Polarization to a Detrimental M1 Phenotype in the Injured Spinal Cord

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