Adipose derived stem cells based regenerative therapy in Wrocław. Current state and future prospects

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Therapeutic angiogenesis

Stem cells:
- peripheral blood
- bone marrow
- adipose tissue
- Wharton Yelly
Therapeutic angiogenesis
stem cells application

intraarterial
intra tissue
MATERIAL

20 CLI – patients IV Fontaine, Rutheford 5

Age 41 – 61 y 50,3 ± 10,1

Duration of CLI: 6 m – 8 y mean 3,7 ± 2,7 lat
Methods

• Bone marrow aspiration under general/spinal anaesthesia
  550ml/ACD-A 10:1 (multiple aspiration biopsy)
• Separation of the mononuclear cells using the COBE Spectra separator
• Final volume of the preparation 40 - 50ml

Phenotype: **CD34+: 15.8%±0.25**, CD45-CD34-: 10.8%±0.96,
CD45-CD34- CD90+: 0.1%±0.02, CD45-CD34-CD105+: 2.8%±0.4,
CD45-CD34-CD73+: 0.07%±0.01, CD45-CD34-CD117+: 0.02%±0.007
Methods

- Intramuscular implantation by 40-50 injections
- Number of implanted cells: $30.2 \times 10^8 \pm 4.5 \times 10^8$
- Arteriography prior to implantation and after 3 months
- ABI
- Pain assessment by VAS scale
- Ulcers planimetry
- Follow up 24 months
Stem cells separation:

COBE Spectra separator. Cleanness 90%

Final volume 40-50ml

Phenotype:

CD34+: 15.8%±0.25, CD45-CD34-: 10.8%±0.96, CD45-CD34-CD90+: 0.1%±0.02, CD45-CD34-CD105+: 2.8%±0.4, CD45-CD34-CD73+: 0.07%±0.01, CD45-CD34-CD117+: 0.02%±0.007
Intramuscular implantation

40-50 injections 15 - 20mm depth

Number of implanted cells: $30.2 \times 10^8 \pm 4.5 \times 10^8$
Reduction of pain

<table>
<thead>
<tr>
<th></th>
<th>przed</th>
<th>1 mies</th>
<th>3 mies</th>
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<tr>
<td>VAS</td>
<td>6.17</td>
<td>4.625</td>
<td>3.66</td>
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<tr>
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<td>5.23</td>
<td>4.63</td>
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Healing of ulcers

<table>
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<tr>
<th>Time</th>
<th>Area (cm²)</th>
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<tr>
<td>Przed</td>
<td>502.27</td>
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<tr>
<td>10 dni</td>
<td>156.53</td>
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<tr>
<td>1 mies</td>
<td>77.32</td>
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<td>2 mies</td>
<td>32.34</td>
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<tr>
<td>3 mies</td>
<td>146.53</td>
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<td>168.6</td>
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Results

Healing of ulcers

Pat No 1

22.12.2003r

10.04.2004r
Results

Pat No 8

04.03.2004
10.04.2004
16.12.2004

10.04.2004

16.12.2004
Angiography

Pat No 2

15.12.2003r

19.03.2004r
Results

- Reduction of pain 18/20
- Complete healing 9/20
- Partial healing (>50%) 6/20
- Progression of necrosis/ulcers 5/20
- Amputation 2/20
- Neovascularisation 9/20

Follow-up 24 months
Mesenchymal stem cells have now been isolated from various tissues including: - bone marrow, - peripheral blood, - cord blood, - muscle, - skin, - synovium, - adipose tissue

Adipose tissue could be one of the most suitable cell sources for cell therapy, because of its - easy accessibility, - minimal morbidity and - abundance of mesenchymal stem cells.
Aim of the study

To assess the effectiveness and safety of autologous transplantation of adipose tissue-derived stem cells (adipose derived stem cells) in the treatment of chronic wounds (diabetic foot ulcers and venous leg ulcers)
Patients

21 patients with t. 2 DM
7 woman
14 men
Mean age: 60.2 ± 5.64 y

Standard follow-up 6 months
Patients

- 14 patients
  - 11 women
  - 3 men

Mean age: 66.6 ± 9.5 y

Standard follow-up 6 months

venous leg ulcers
Methods

Adipose tissue was harvested by aspiration after infiltration by local anesthetic and tumescent isotonic solution.

Stem and regenerative cells were separated using a closed and fully automated system CELLUTION 800 from Cytori Therapeutics USA.

5 ml of a concentrated cell suspension were implanted into subcutaneous tissue around the wound and the wound bed.

The phenotype of the cells obtained was determined immediately after separation and after 7 days of culture on RPMI with 10% fetal calf serum.
Methods

Harvesting of the adipose tissue by tumescent – aspirations method
5 ml of a concentrated cell suspension were implanted into subcutaneous tissue around the wound and the wound bed.
Methods
Results

Preparation - 5ml

5.6 x 10^6 + 4 x 10^6 cells
# Results

## Phenotype of the cells after separation

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<tr>
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<th>CD45</th>
<th>CD31</th>
<th>CD34</th>
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<tr>
<td>X ± SD</td>
<td>25,52</td>
<td>22,83</td>
<td>59,33</td>
<td>0,5 ± 0,84</td>
<td>18,83</td>
<td>28,5</td>
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<td>± 7,49</td>
<td>± 13,47</td>
<td>± 28,45</td>
<td>± 16,66</td>
<td>± 25,99</td>
<td>± 27,84</td>
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## Phenotype of the cells after culture

<table>
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<tr>
<td>X ± SD</td>
<td>7,4</td>
<td>8,4</td>
<td>34,8</td>
<td>0,0</td>
<td>16,8</td>
<td>81,25</td>
<td>93,8</td>
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<td></td>
<td>± 10,59</td>
<td>± 11,84</td>
<td>± 27,69</td>
<td>± 2,17</td>
<td>± 34,18</td>
<td>± 9,55</td>
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Clinical results
Clinical results

No significant side effects of the lipoaspirations or applications of the stem cells were observed.

Clinical improvement was observed in 17 patients suffering from diabetic foot ulcers (77.3%) including 8 completely healed and 9 with >50% reduction of ulcer area.

Clinical improvement was observed in 10 patients with venous leg ulcers (71.4%) including 3 completely healed and 7 with >50% reduction of ulcer area.
Clinical results

No improvement was observed in 4 patients (with diabetic foot ulcers), including 2 with progression of necrotic lesions resulting in amputation.

No improvement was observed in 4 patients (with venous leg ulcers)
Conclusion

Autotransplantation of adipose tissue stem cells is a safe and effective treatment method for diabetic foot ulcers, however with transient effect after the single application.

The use of stem cells propagated in vitro will provide a good continuation of the current study.
Projects for the future

ADMSC autotransplantation for patients with scleroderma and advanced Raynaud phenomenon with acral ulcers

Allogenic ADMSC in autologic plasma gel as a biological dressing for chronic wounds

Allogenic ADMSC in autologic plasma gel as a biological dressing for corneal ulcers

Stem cells conditioned medium for chronic wounds

Therapeutic angiogenesis with stem cells conditioned medium
Thank for your attention