Safety and Effect of Adipose Tissue derived Mesenchymal Stem Cell Implantation in Patients with Critical Limb Ischemia

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Critical Limb Ischemia

- Revascularisation: 60%-70% in CLI
- 30-40% cannot be successfully revascularised
- PTA and bypass op. are difficult in Buerger’ disease

- Severe pain at rest, tissue loss, infection, amputation
- 1 yr major amputation risk for CLI patients with failed revascularisation: 80-90%

*TASC Working Group JVS 2000:31;51*
## Cell Therapy: BM-MNC therapy in PAD/TAO

<table>
<thead>
<tr>
<th>Author/yr</th>
<th>Study Level</th>
<th>Subjects,</th>
<th>ABI</th>
<th>TcP02</th>
<th>Pain</th>
<th>Amp.</th>
<th>+/-</th>
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</thead>
<tbody>
<tr>
<td>Tateishi (Lancet 2002)</td>
<td>1b</td>
<td>45, PAD, Diabetes</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
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<tr>
<td>Esato (CellTransplant2002)</td>
<td>4</td>
<td>8, PAD, TAO</td>
<td>↑</td>
<td>—</td>
<td>↓</td>
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<td>Saigawa (2004)</td>
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<td>8 PAD, Diabetes</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
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<tr>
<td>Higashi (Circulation 2004)</td>
<td>4</td>
<td>8, PAD</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>+</td>
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<tr>
<td>Durdu (J Vasc Surg2006)</td>
<td>1b</td>
<td>28, TAO</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>+</td>
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<tr>
<td>Huang (Diabetes Care 2005)</td>
<td>3</td>
<td>25, PAD, Diabetes</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>+</td>
</tr>
</tbody>
</table>
BM EPC : Critical Limb Ischemia

BONMOT 1: Harvesting + Separation

Extraction of bone marrow

BM-Purification/separation of the monocytic cell fraction

Pts #1-12 with Ficoll®

Pts #13-71 with Harvesttech SmartPrep® centrifugation (bed-side)
Adipose Tissue Derived Mesenchymal Stem Cell in CLI

- Convenient and easy to get the adipose tissue
- Larger abundance of MSC and stromal cells
- Basically same potency compared to BMC

- Effects: paracrine effect, homming effect and differentiation into endothelial cell, smooth m. cell

Zuk PA et al. Mol Biol Cell 2002:13(12);4279-95
Adipose Tissue Derived Mesenchymal Stem Cell in CLI

Zuk PA et al. Mol Biol Cell 2002:13(12);4279-95
Background: Animal Model

- Mice hindlimb ischemia model:
  ADSC $1 \times 10^5$, $5 \times 10^5$, $1 \times 10^6$ intramuscular injection

- 60% autoamputation in the control group
  0% autoamputation in the ADSC group

PNUH Jung JS et al, Cell Physio Biology 2006;17:279-90
Safety and Effect of Adipose Tissue derived Stem Cell Implantation in Patients with Critical Limb Ischemia : Animal Model

PNUH Jung JS et al, Cell Physio Biology 2006;17:279-90
Human Study: Inclusion Criteria

- PNUH IRB and KFDA Approved this human study (생물의약품 정책과-1273호, 2008. 12. 23.)

- Candidates: 15 patients

- At least 6 months since the onset CLI (Chronic ASO, DM foot or Buerger disease)

- 20 yrs < Age < 80 yrs

- Rutherford’s class is II-4, III-5 or III-6, Rest pain or ischemic ulcer/necrosis

- Patients with CLI were not suitable for percutaneous vascular intervention and bypass operation

- Patients who wrote informed consent
Method

- Liposuction 10 cc
- Culture during 2 weeks, 3 passage
- Increase dose: ADSC $1 \times 10^8 \rightarrow 3 \times 10^8$

- Intramuscular injection, lower leg
  0.5 cc * 20-60 points

- Mouse model dose: $1 \times 10^5 - 1 \times 10^6$
- Human dose: $1 \times 10^8 - 3 \times 10^8$
  reduction of dose
Method

Before 2 wks  Adipose tissue derived stem cells obtained

In Hospital

Intramuscular injection of ADSCs in lower leg

Dose : 1-3x10^8 ADSC 0.5cc * 20-60 points

Check ABI,  DSA, Thermography, Pain scale Rate

Treadmill test

(Initial pain walking distance, Maximum walking distance)

Hospital Adverse Events Analysis (MAE)
Method

At 1, 3 months:
3-month adverse events Analysis (MAE)

At 6 months:
Check ABI, DSA, Thermography, Pain scale rate, Treadmill test
6-month adverse events Analysis (MAE)
Safety and Effect of Adipose Tissue derived Stem Cell Implantation in Patients with Critical Limb Ischemia

- **Surface Antigen:**
  - CD73(+) (mesenchymal stem cell marker)
  - CD90(+) (mesenchymal stem cell marker)
  - CD31(-) (endothelial cell marker)
  - CD34(-) (hematopoietic or hemangioblast marker)
  - CD45(-) (blood-derived cell marker)

![Graphs showing CD90, CD31, CD34, CD45 distributions]
Method: ADSC proliferation and differentiation

- **Proliferation assay**: CFU (colony forming unit) assay
  - SVF (a seeding density of $10^5$ cells/10cm²)
  - ADSC (a seeding density of 100 cells/10cm²)

- **Differentiation**
  - AM (adipogenic differentiation)
    - Adipogenic medium (incubation period 7-10 days)
    - Oil Red O stain (an indicator of intracellular lipid accumulation)
  - OM (osteogenic differentiation)
    - Osteogenic medium (incubation period 14-16 days)
    - Alizarin red S stain (extracellular matrix calcification)
Results

- Enrolled 15 patients: 12 Buerger’s disease
  3 DM foot
- Mean follow up: 6 months
- We followed up 15 patients during 6 months
## Results: Patient characteristics

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age /Gender</th>
<th>Diagnosis</th>
<th>Ischemic Site/ Status</th>
<th>Past History</th>
<th>Previous Tx. For CLI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33/M</td>
<td>Buerger’s ds</td>
<td>Left toe/ Resting pain(II-4)</td>
<td>Smoking</td>
<td>Minor amputation, sympathectomy</td>
</tr>
<tr>
<td>2</td>
<td>52/M</td>
<td>Buerger’s ds</td>
<td>Left toe/ non-healing ulcer(II-5)</td>
<td>Smoking, quit hyperlipidemia</td>
<td>F-F bypass op Major amputation</td>
</tr>
<tr>
<td>3</td>
<td>24/M</td>
<td>Buerger’s ds</td>
<td>Left toe/ non-healing ulcer(II-5)</td>
<td>Smoking, quit</td>
<td>Minor amputation</td>
</tr>
<tr>
<td>4</td>
<td>46/M</td>
<td>Buerger’s ds</td>
<td>Right toe/ Necrosis(III-6)</td>
<td>Smoking, quit</td>
<td>Minor amputation</td>
</tr>
<tr>
<td>5</td>
<td>36/M</td>
<td>Buerger’s ds</td>
<td>Right toe / Resting pain(II-4)</td>
<td>Smoking, quit</td>
<td>Rt F-P bypass op</td>
</tr>
<tr>
<td>6</td>
<td>42/M</td>
<td>Buerger’s ds</td>
<td>Left foot/ Necrosis(III-6)</td>
<td>Smoking, quit</td>
<td>SFA-PTA bypass op</td>
</tr>
<tr>
<td>7</td>
<td>64/M</td>
<td>DM foot</td>
<td>Left foot/ non-healing ulcer(II-5)</td>
<td>DM, HT</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>55/M</td>
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<td>Buerger’s ds</td>
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<td>Smoking</td>
<td>Rt F-P bypass op</td>
</tr>
<tr>
<td>10</td>
<td>69/M</td>
<td>DM foot</td>
<td>Right foot/ Necrosis(III-6)</td>
<td>DM, HT</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>60/M</td>
<td>Buerger’s ds</td>
<td>Left foot/ non-healing ulcer(II-5)</td>
<td>Smoking, HT</td>
<td>Rt Axillo-F-F bypass</td>
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<tr>
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<td>46/M</td>
<td>Buerger’s ds</td>
<td>Left toe/ non-healing ulcer(II-5)</td>
<td>Smoking</td>
<td>Minor amputation</td>
</tr>
<tr>
<td>13</td>
<td>73/M</td>
<td>Buerger’s ds</td>
<td>Left foot/ Resting pain(II-4)</td>
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<tr>
<td>14</td>
<td>39/M</td>
<td>Buerger’s ds</td>
<td>Left toe/ non-healing ulcer(II-5)</td>
<td>Smoking, DM, HT, Hyperlipidemia</td>
<td>Minor amputation</td>
</tr>
<tr>
<td>15</td>
<td>73/M</td>
<td>DM foot</td>
<td>Left toe/ non-healing ulcer(II-5)</td>
<td>HT, DM</td>
<td>PTA</td>
</tr>
</tbody>
</table>
Results

: CFU assay of Stromal vascular fraction (SVF)

A

SVF
Buerger’s SVF
Diebatic SVF

B

Colony Numbers/10 cm²

SVF  Buerger’s SVF  Diebatic SVF

*
Results

CFU assay of culture-expanded cells

A

Colony Numbers/10 cm²

ADSC  Buerger’s ADSC  Diebatic ADSC

B

Cell Numbers/Single Colony

ADSC  Buerger’s ADSC  Diebatic ADSC

C

Cell Numbers (10⁶)

Days

- ADSC
- Buerger’s ADSC
- Diabetic ADSC

* Significant difference
Results

:CFU assay of culture-expanded cells

Figure A

AM

ADSC  Buerger’s ADSC  Diebatic ADSC

OM

ADSC  Buerger’s ADSC  Diebatic ADSC
Results

CFU assay of culture-expanded cells
Results

: Wong-Baker FACES Pain Rating Scale

P=0.032 by paired Wilcoxon test
Results
: Claudication Distance

Distance (m)

P=0.046 by paired Wilcoxon test
Results

: Maximal Walking Distance

\[ \text{Distance (m)} \]

\[ P = 0.075 \] by paired Wilcoxon test
Results
: Ankle Brachial Index

P = 0.21
Results

: Digital Subtraction Angiography

P = 0.005
Results
: Thermography

P=0.007
Results
: Thermography

Patient 8

Baseline

6 month
## Results: Clinical Outcomes

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age/Gender</th>
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<th>Ischemic Site/ Status</th>
<th>Past History</th>
<th>Clinical symptom</th>
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<td>Smoking</td>
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<td>Smoking, quit hyperlipidemia</td>
<td>Ulcer healing Well improved</td>
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</tr>
<tr>
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<td>46/M</td>
<td>Buerger’s ds</td>
<td>Right toe/Necrosis(III-6)</td>
<td>Smoking, quit</td>
<td>Minor amputation No change</td>
</tr>
<tr>
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<td>36/M</td>
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<td>Right toe /Resting pain(II-4)</td>
<td>Smoking, quit</td>
<td>Well improved</td>
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<td>42/M</td>
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<td>Left foot/Necrosis(III-6)</td>
<td>Smoking, quit</td>
<td>Minor amputation Mild improved</td>
</tr>
<tr>
<td>7</td>
<td>64/M</td>
<td>DM foot</td>
<td>Left foot/non-healing ulcer(II-5)</td>
<td>DM, HT</td>
<td>Ulcer healing Well improved</td>
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<td>55/M</td>
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<td>HT, DM</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Ulcer healing</td>
</tr>
</tbody>
</table>

# well ≥2step, mild ≥1step in Wong-baker Score
Results: Clinical Outcomes
# Results: Adverse Events

<table>
<thead>
<tr>
<th>NCI CTCAE(Version 4) Grade</th>
<th>Adverse Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 5 (death)</td>
<td>None</td>
</tr>
<tr>
<td>Grade 4 (life-threatening)</td>
<td>None</td>
</tr>
<tr>
<td>Grade 3 (Severe)</td>
<td>None</td>
</tr>
<tr>
<td>Grade 2 (Moderate)</td>
<td>None</td>
</tr>
<tr>
<td>Grade 1 (Mild)</td>
<td>Clinical symptoms</td>
</tr>
<tr>
<td></td>
<td>Fever (n=1)</td>
</tr>
<tr>
<td></td>
<td>Flu-like symptom (n=1)</td>
</tr>
<tr>
<td></td>
<td>Injection site pain (n=2)</td>
</tr>
<tr>
<td></td>
<td>Headache (n=1)</td>
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</table>
## Results: Clinical Outcomes

<table>
<thead>
<tr>
<th></th>
<th>Responders (n=10)</th>
<th>Non-Responders (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>suprapopliteal</td>
<td>4 (40%)</td>
<td>2 (50%)</td>
</tr>
<tr>
<td>infrapopliteal</td>
<td>6 (60%)</td>
<td>2 (50%)</td>
</tr>
<tr>
<td>DM</td>
<td>4 (40%)</td>
<td>0</td>
</tr>
<tr>
<td>Age</td>
<td>48.7 yr</td>
<td>51.8 yrs</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current Smoker</td>
<td>8 (80%)</td>
<td>3 (75%)</td>
</tr>
<tr>
<td>Initial Claudication Distance (m)</td>
<td>137</td>
<td>244</td>
</tr>
<tr>
<td>Maximum Walking Distance (m)</td>
<td>263</td>
<td>413</td>
</tr>
<tr>
<td>ABI</td>
<td>0.75</td>
<td>0.60</td>
</tr>
<tr>
<td>DSA</td>
<td>1.96</td>
<td>1.08</td>
</tr>
</tbody>
</table>

*# P value: insignificant*
Conclusion

- Intramuscular ADSC therapy for CLI is effective and safe.
- Response rate is about 71% in our study.
- Factors that influence on therapeutic response were not definite.
- Proliferation and differentiation ADSC in Buerger’s disease are normal compared to normal control group.

But, SVFs are fewer than normal control group.
Next Step

- Large scaled study as Phase II, III

- Strategy to increase activity of ADSC
  1. Selective collection and culture of high activity ADSC
  2. Heterogenous ADSC implantation
Our Team

- Han Cheol Lee, MD PhD, Cardiology
- Yong Chan Bae, MD PhD, Plastic Surgery
- Sung Wun Chung, MD PhD, Vascular Surgery
- Jin Sup Jung, PhD, Physiology
Thank you for your attention
DSA : Patient 1
DSA : Patient 6
DSA: Patient 10
DSA : Patient 14
Results: Thermography

Patient 5

Baseline

6 month
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