Developing novel diagnostic and therapeutic strategies for patients with critical limb ischaemia?

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LEAD, COMPLEX ENDOVASCULAR AORTIC INTERVENTION

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THE ARTERIAL TREE

- Superficial temporal artery
- Posterior auricular artery
- Common carotid artery
- Subclavian artery
- Brachiocephalic trunk
- Axillary artery
- Deep brachial artery
- Brachial artery
- Aorta
- Radial artery
- Interosseous artery
- Ulnar artery
- Deep palmar arch
- Superficial palmar arch
- Descending genicular artery
- External carotid artery
- Internal carotid artery
- Vertebral artery
- Aorta and arch
- Pulmonary artery
- Cardiac artery
- Thoracic aorta
- Celiac trunk
- Superior mesenteric artery
- Renal artery
- Gonadal artery
- Inferior mesenteric artery
- Common iliac artery
- External iliac artery
- Internal iliac artery
- Deep femoral artery
- Femoral artery
- Popliteal artery
- Anterior tibial artery
- Peroneal artery
- Posterior tibial artery
CO-MORBIDITIES CLI PATIENTS

• 30% DIABETIC
• 50% HYPERCHOLESTEROLAEMIC
• 80% HYPERTENSIVE
• 80% CURRENT/EX-SMOKERS

• 30% NOT ON ANTIPLATELET
• 50% NOT ON LIPID LOWERING AGENT
ODDS CARDIOVASCULAR EVENT WITH ABPI
LIMB ISCHAEMIA

- Poor blood supply to the limb
- Narrowing/blockage of artery
- Spectrum of disease
- Risk factors: Smoking, diabetes, high cholesterol

Claudication → Rest Pain → Gangrene
• 25,000 patients per year in the UK
• Severe pain
• Bed bound
• Ulceration and gangrene
• Over a third require an amputation
• Over a third are dead one year after amputation
• Quality of life similar to terminal cancer
INTERVENTIONS FOR LOWER LIMB ISCHAEMIA
POPLITEAL ANGIOPLASTY/STENTING
5 Year patency of femoro-pop bypass
Vein: 66%
Prosthetic above knee: 47%
Prosthetic below knee: 33%

*Hunink et al 1994*

5 year Patency femoro-distal bypass
Vein: 70%
Prosthetic: 25%

*Norgren et al 2007*
ANY FOOL CAN CUT OFF A LEG – IT TAKES A SURGEON TO SAVE IT
ANY FOOL CAN CUT OFF A LEG – IT TAKES A SURGEON/SCIENTIST TO SAVE IT

GEORGE G ROSS
1834-1892
CAN WE GROW NEW BLOOD VESSELS TO REVASCULARISE THE LIMB?
Endothelial Progenitor Cells Are Recruited Into Resolving Venous Thrombi

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Background—The purpose of this study was to determine whether endothelial cells of bone marrow origin are involved in thrombus recanalization.

Methods and Results—Irradiated mice were reconstituted with bone marrow from transgenic donors expressing green fluorescent protein (GFP) linked to the Tie2 promoter. Thrombi were formed in 2 groups of 6 mice. GFP-expressing cells were located and quantified in sections of the thrombi taken after 7 and 14 days. The cell markers Mac-3, F4/80, CD68 (macrophage), and vascular endothelial growth factor receptor 2 (VEGFR2; endothelial cells) were used to determine colocalization with GFP expression in tissue sections and peritoneal macrophages. The markers CD34 and VEGFR2 were used to quantify changes in circulating endothelial cells by flow cytometry of blood from 3 cohorts of wild-type animals that had either a thrombus induced (n = 18), a sham operation (n = 18), or no operation (n = 10). The number of GFP-expressing cells was found to increase by 3-fold in thrombi formed in transplanted animals between 7 and 14 days after induction (P = 0.0022). No GFP-expressing cells were found lining the new vascular channels that formed at either time interval, but many of the GFP-expressing cells also expressed Mac-3, CD68, and VEGFR2. Approximately twice as many circulating CD34⁺/VEGFR2⁺ cells were found by day 3 in animals with thrombi compared with sham controls (CD45⁺, P = 0.046 and CD45⁺, P = 0.016).

Conclusions—Bone marrow–derived, Tie2-expressing cells were recruited into the thrombus during resolution but did not line the new vessels. Many of these cells expressed a macrophage phenotype and may represent a population of plastic stem cells that orchestrate thrombus recanalization. (Circulation. 2005;111:2645-2653.)

Key Words: thrombus ▶ cells ▶ revascularization ▶ angiogenesis

Venous thrombi resolve by a process that is similar to the formation of granulation tissue in wound healing. Neutrophils, monocytes, and endothelial cells enter the thrombus as it organizes. In the initial stages of organization, the thrombus retracts away from the vein wall, which leads to the appearance of peripheral pockets and clefts that enlarge with time and eventually become lined by cells. These channels coalesce and enlarge, and blood flow is established through and around them. New vessels also appear within the body of the thrombus and contribute to restoration of a patent vein lumen (see Data Supplement Figure). This is associated with an increase in local expression of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor, which are thought to drive neovascularization within the thrombus.

Bone marrow–derived circulating endothelial progenitor cells appear to play an important part in physiological and pathological neovascularization in the adult. They may also have a role in thrombus resolution, because thrombus failed to resolve in urokinase-type plasminogen activator knockout mice but was restored to normal by transplantation of normal bone marrow.

Tie-2 is a tyrosine kinase receptor that is expressed on endothelial cells and is essential for normal vascular development in the embryo and for stabilization of blood vessels in the adult. In FVB/N-Tg(Tie2-GFP) 287 Sato transgenic mice, the reporter gene green fluorescent protein (GFP) is expressed under transcriptional regulation by the Tie2 receptor promoter, and therefore green fluorescence can be used to identify cells that express the Tie2 receptor. In wild-type mice transplanted with bone marrow from the transgenic strain, the presence of GFP signifies a Tie2-positive cell that has been derived from the bone marrow rather than local tissues.

The purpose of the present study was to determine whether the new vessels that appear during thrombus resolution develop by angiogenesis from local endothelial cells in the vessel wall beneath the thrombus or originate from bone marrow–derived endothelial progenitors. Fluorescence-activated cell sorter (FACS) analysis was used to quantify changes in the numbers of circulating endothelial cells in animals with organizing venous thrombi.

Methods

Bone Marrow Harvest and Transplantation

The study was performed under the Animals (Scientific Procedures) Act 1986. Bone marrow cells were obtained from donor Tie2-GFP
NEOVASCULARISATION AND COLLATERALISATION

Qureshi et al. Neurosurgery 2002
Clinical evidence of angiogenesis after arterial gene transfer of phVEGF in patient with ischaemic limb

Jeffrey M Isner, Ann Pieczek, Robert Schainfeld, Richard Blair, Laura Haley, Takayuki Asahara, Kenneth Rosenfield, Syed Razvi, Kenneth Walsh, James F Symes
<table>
<thead>
<tr>
<th><strong>Trial</strong></th>
<th><strong>Treatment</strong></th>
<th><strong>Patients</strong></th>
<th><strong>n</strong></th>
<th><strong>Design</strong></th>
<th><strong>Endpoints</strong></th>
<th><strong>Outcome</strong></th>
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<tr>
<td>TACT</td>
<td>BM MNC</td>
<td>CLI: rest pain</td>
<td>22</td>
<td>Not randomised</td>
<td>Safety Feasibility</td>
<td>ABPI: Imp tCpO2: Imp</td>
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<tr>
<td></td>
<td>PB MNC</td>
<td>Bilateral</td>
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<td>Not blinded</td>
<td>ABPI tCpO2 Rest pain</td>
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<td>Contralat leg: control</td>
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<td>PROVASA</td>
<td>Intra-arterial BM-MNC</td>
<td>Buergers CLI</td>
<td>40</td>
<td>Phase II Double blind</td>
<td>ABPI Ulcer healing</td>
<td>AFS: NS ABPI:NS Ulcer: Imp Up to 3mnths</td>
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<td>Placebo</td>
<td>AFS</td>
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<td>RESTORE-CLI</td>
<td>BM MNC</td>
<td>CLI: rest pain/gangrene</td>
<td>33</td>
<td>Phase II Double blind</td>
<td>AFS Time to rx Major amputation</td>
<td>AFS:sig Time to rx:sig Major amp: NS</td>
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<td>Placebo</td>
<td>AFS</td>
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<tr>
<td>ACT34-CLI (Baxter)</td>
<td>Leukopheresis CD34+ cells</td>
<td>CLI: rest pain/gangrene</td>
<td>28</td>
<td>RCT Double blind</td>
<td>Safety AFS</td>
<td>AFS: trend</td>
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<td>Placebo</td>
<td>AFS</td>
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<tr>
<td>REVIVE</td>
<td>Lxmyelocel-T (MSC+macrophages)</td>
<td>CLI:tissue loss</td>
<td>594</td>
<td>Phase III Randomised Double Blind</td>
<td>AFS Ulcer MACE</td>
<td>Terminated</td>
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<td>Placebo</td>
<td>AFS</td>
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<td>Harvest</td>
<td>Concentrated bone marrow aspirate</td>
<td>No-option CLI: tissue loss</td>
<td>210</td>
<td>Randomised Double Blind</td>
<td>AFS Rutherford Dec 2016</td>
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NEOVASCULARISATION

Furmston, Modarai et al., J Cardiovasc Surg, 2014
Effect of fibroblast growth factor NV1FGF on amputation and death: a randomised placebo-controlled trial of gene therapy in critical limb ischaemia.
Belch J¹, Hiatt WR, Baumgartner I, Driver IV, Nikol S, Norgren L, Van Belle E; TAMARIS Committees and Investigators.

Regional angiogenesis with vascular endothelial growth factor in peripheral arterial disease: a phase II randomized, double-blind, controlled study of adenoviral delivery of vascular endothelial growth factor 121 in patients with disabling intermittent claudication.
Rajagopalan S¹, Mohler ER 3rd, Lederman RJ, Mendelsohn FO, Saucedo JF, Goldman CK, Blebea J, Macko J, Kessler PD, Rasmussen HS, Annex BH.
Murine hindlimb ischaemia

- Complete excision of femoral artery

Soup of cells not specific angiogenic cell type(s)

Lack of objective endpoint measures

Poor cell retention
MONOCYTES IN COLLATERAL ARTERIOGENESIS

Fung et al. Frontiers in Physiol 2012
- Tissue culture
- Biochemistry
- Histology
- Microscopy
- Animal models
Angiogenic factors e.g. VEGF, bFGF

THE ANGIOGENIC MONOCYTE

Angiogenic factors e.g. VEGF, bFGF

FUNCTIONAL IMPAIRMENT IN CLI

Age-matched control TEMs

CLI TEMs
Gene expression analysis CLI monocytes vs Control
Functional Role of HTATIP2 in Angiogenic Monocytes

76.8% knockdown

siHTATIP2 TEMs

siCONTROL TEMs
Molecular Imaging of Bone Marrow
Mononuclear Cell Survival and Homing in Murine Peripheral Artery Disease

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ROAM-CLI study
Retention Of Angiogenic Monocytes in Critical Limb Ischaemia
• Ethical Approval

• Investigator Brochure
  - Efficacy/Biology in animals
  - Toxicology

• Investigator Medicinal Product Dossier
  - Batch Analysis Data:
    sterility, endotoxin, mycoplasma, viability, phenotype
REGULATORY SUBMISSION TO MHRA

• Ethical Approval

• Investigator Brochure
  - Efficacy/Biology in animals
  - Toxicology

• Investigator Medicinal Product Dossier
  - Batch Analysis Data:
    sterility, endotoxin, mycoplasma, viability, phenotype
ENHANCING CELL RETENTION: ENCAPSULATION

Patel, Modarai et al. Integrative Biology. 2013
CELL ENCAPSULATION

3.5mm
500 μm
400 μm
250 μm
MONOCYTE ENCAPSULATION

Day 1  Day 2  Day 3  Day 5  Day 8  Day 15  Day 18

Microsphere diameter

Alginate concentrations

2% 2.50% 3.00% 3.50% 4.00% 4.50%

0 50 100 150 200 250 300 350

Live  Apoptosis  Dead  Debris

24h  48h  72h
Need for Sensitive Endpoints

Laser Doppler Imaging

Positron Emission Tomography (PET)

Contrast Ultrasound

Healthy volunteer

Rest

Adenosine

PAD subject

Bajwa, Modarai et al. Circulation Imaging 2014
Blood Oxygenation Level Dependent MRI

- **Baseline**
- **Ischaemic Phase**
- **Reactive Hyperaemia Phase**

Cuff Inflated: 2 mins
Cuff Deflated: 5 mins
5 mins

Thigh Cuff
BOLD Imaging Plane
BOLD-MRI Based Assessment of Perfusion in the Lower Limb

Pre-Intervention

Post-Intervention

Normalised T2*

Time (sec)

Normalised T2*

Time (sec)

Gradient (ms/s)

Pre Post

Pre Post

SRi (%)
FATE OF PATIENT WITH CRITICAL LIMB ISCHAEMIA

Primary treatment

- Medical treatment only 25%
- Primary amputation 25%
- Revascularisation 50%

A year later

- CLI resolved 25%
- Continuing CLI 20%
- Alive amputated 30%
- Dead 25%
Promote Limb Salvage and Rehabilitate
Summary

- Global drive for regenerative medicine

- Translational studies: Clinicians, scientists, industry

- Potent cells

- Improve retention

- Objective outcome measures

- Well designed clinical trials

- Address general risk factors to promote patient survival
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