LIMB SALVAGE USING ANGIOGENESIS INDUCED BY STEM CELL INJECTION

Thesis
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بسم الله الرحمن الرحيم

(وما أوتيتـــــــم من العلـــــم إلا قليـــــلا)

صدق اللـــــــــــه العظيم

Abstract

This is a prospective clinical trial conducted in Kasr El-Aini hospitals over a period of 9 months starting from September 2008 till the end of June 2009 on 20 patients who presented with CLI not eligible for open vascular or endovascular interventions or failed one or both of them.

Among the 20 patients included in this study 18 patients had atherosclerosis and 2 patients proved to had Burgers' disease. Among 20 patients included in this study 14 patients (70%) had history of previous intervention in the form of failed angioplasty in 6 cases (30%) and failed surgical procedures in 8 of them (40%).

All patients in this study were complaining from CLI (rest pain in all patients, ulceration in 4 patients and gangrene in 8 patients).

Finally, it can be concluded that stem cell therapy is a rising hope for patients with PAD. It worth trial in all patients with CLI with good results in early CLI and less favorable result in delayed stages of CLI.

Keywords:

Limb salvage
Stem cell injections
CLI
Burgers' disease
Atherosclerosis

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List of Abbreviations

- ABI: Ankle brachial index.
- ACC/AHA: American college of cardiology/American heart association ADP**:** adenosine diphosphate.
- AKA**:** Above knee amputation.
- ASO: Arteriosclerosis obliterans.
- BKA: Below knee amputation.
- BM: Bone marrow.
- BM-EPCs: Bone marrow endothelial progenitor cells.
- BM-MNCs: Bone marrow-mononuclear cells.
- BP: Blood pressure.
- CABG: Coronary artery bypass grafting.
- CR: Case report.
- CBC: Complete blood count.
- CLI: Critical limb ischemia.
- CRI: Chronic renal insufficiency.
- CRP: C-reactive protein.
- CTA: Computed tomogram angiography.
- DM: Diabetes mellitus.
- DSA: Digital subtraction angiography.
- ECs: Endothelial cells.
- ECG: Electrocardiogram.
- ECM: Extracellular matrix.
- EPCs: Endothelial progenitor cells.
- ePTFE grafts: Expanded polytetrafluoroethylene.
- FGF: Fibroblast growth factor.
- G-CSF: Granulocyte-macrophage colony-stimulating factor.

- GFs: Growth factors.
- HB $A1_c$: Hemoglobin $A1_c$.
- HDLs: High density lipoproteins.
- HGF: Hepatocyte growth factor.
- HTN: Hypertension.
- IHD: Ischemic heart disease.
- LDLs: Low density lipoproteins.
- MDCTA: Multidetector computed tomography angiography.
- MRA**:** Magnetic resonance angiography.
- NIH: National Institute of Health.
- PAD: Peripheral arterial disease.
- PB: peripheral blood.
- PB-MNCs: peripheral blood mononuclear cells.
- PCI: percutaneous catheter intervention.
- PDGF: platelet-derived growth factor.
- PGI2: Prostacyclin.
- PS: Patient series.
- PTA: Percutaneous transluminal angioplasty.
- PVR: Pulse volume recordings.
- RBCs: Red blood cells.
- RCT: Randomized controlled trial.
- SMCs: Smooth muscle cells.
- SWMA: Segmental wall motion abnormalities.
- TACT Study: Therapeutic Angiogenesis using Cell Transplantation Study.
- TAO: Thromboangitis obliterans.
- TBI: Toe-brachial index.
- TcPO2: Transcutaneous oxygen tension.

- TOPCARE-AMI study: Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction study.
- US: United States.
- FDA: Food and Drug Administration.
- VAS: Visual analogue scale.
- VEGF: Vascular endothelial growth factor.
- VWF: Velocity wave form.

INTRODUCTION & AIM OF THE WORK

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Stem cells are a population of immature tissue precursor cells capable of self renewal and provision of de-novo and/or replacement cells for many tissues. Stem cells may be embryonic, derived from the inner cell mass of the embryonal blastocyst, or adult human stem cells which are found in mature tissues, e.g., bone marrow, fat, blood and other organs like the heart. Because of immunogenicity and rejection, as well as ethical consideration, embryonic cells research is restricted to in vitro studies ***(Zhang et al., 2002).***

Adult stem cells typically generate the cell types of the tissues in which they reside. However, a number of experiments over the last several years have raised the possibility that stem cells from one tissue may be able to give rise to cell types of a completely different tissue, a phenomenon known as trans-differentiation or plasticity ***(Bodo et al., 2003).***

Preliminary trials of intramuscular injection of autologus bone marrow mononuclear cells (BM-MNCs) to stimulate vascular growth have shown promising results. Yet, the appropriate use of gene therapy in vascular practice remains to be proven ***(Tateishi et al., 2002).***

Recent studies have suggested that unpurified marrow mononuclear cells and/or subsets of adult hematopoietic stem cells have been reported to contribute to neoangiogenesis ***(R Burt et al., 2003).*** A variety of different cell types from the mononuclear bone marrow cell fraction contribute to the regeneration of damaged vessels. In this regard, therapeutic use of mononuclear cell population of bone marrow may be more useful and promising than single isolated cell fraction alone ***(Bodo et al., 2003).*** The unfractionated mixture of hematopoietic mononuclear cells includes more

differentiated cells that are thought to provide angiogenic cytokines as well as stem cells that become incorporated into collateral vessels by a process of neoangiogenesis. ***(Tateishi et al., 2002).***

In animal models, marrow mononuclear cells injected into ischemic extremities improve regional blood flow ***(Shintani et al., 2001) & (Kamihata et al., 2001).*** In one trial, patients were selected for chronic ischemic extremity pain or non-healing ulcers or both. Significant improvement in the ankle brachial index (ABI), transcutaneous oxygen pressure, and a pain- free walking distance occurred following treatment. Implantation of BM-MNCs strikingly improved rest pain in most patients (complete regression in half), and ischemic ulcers or gangrene were improved in just under the half of all limbs, showing successful limb salvage in these legs ***(Tateishi et al., 2002).***

A number of trials are now ongoing to evaluate the safety and efficacy of autologus BM-MNCs transplantation in CLI. The aim in most trials is to reduce the number of necessary leg amputations, reduce pain and induce wound healing ***(Tateishi et al., 2002).***

Successful injection of BM-MNCs in CLI may be the only hope in patients who seem to have no other alternative therapy to save their limbs ***(Tateishi et al., 2002).***

Aim of the work

This thesis aims at evaluating the role of bone marrow-mononuclear cells (BM-MNCs) injection to improve blood supply in CLI as well as its effect on clinical symptoms and signs of ischemia, wound healing and delaying the decision of amputation.