Role Of Autologus Bone Marrow Adult Stem Cell Transplantation In Neoangiogenesis And Lower Limb Salvage In Patients with Critical Limb Ischemia

A Thesis Submitted For Partial Fulfillment Of MD Degree In CardioVascular Medicine

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To My family

My father and mother

Who saw great potentials in me and kept pushing me too hard to achieve them

My sister and her family

Who gave me support when I was in extreme need of it

My husband and kids

Who gave my life a wonderful meaning

Ghada Sayed Mahmoud

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There is no area of the world that should not be investigated by scientists. There will always remain some questions that have not been answered. In general, these are the questions that have not yet been posed.

Linus Carl Pauling
(1901 – 1994)
An American chemist, biochemist, peace activist, author, and educator

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Abbreviations

ABI	Ankle Brachial Index
AP	Ankle Pressure
ASO	Atherosclerosis
bFGF	Basic Fibroblast Growth Factor
BM-MNC	Bone Marrow MonoNuclear Cells
CABG	Coronary Artery Bypass Grafting
CAD	Coronary Artery Disease
CLI	Critical Limb Ischemia
CXCR4	SDF-1 receptor
DNA	DeoxyriboNuclic Acid
ECG	ElectroCardioGram
ECM	Extra Cellular Matrix
ECs	Endothelial Cells
EPCs	Endothelial Progenitor Cells
FDA	Food and Drug Administration
G-CSF	Granulocyte - Colony stimulating Factor
GFs	Growth Factors
GVHD	Graft Versus Host Disease
hESCs	human Embryonic Stem cells
HGF	Hepatocyte Growth Factor
HSC	Hematopoietic Stem Cells
IPSCs; iPSCs	Induced pluripotent stem cells
MAPC	Multipotent Adult Progenitor Cells
MDCTA	Multi-Detector Computed Tomography Angiography
MMPs	Matrix MetalloProteinases
MRA	Magnetic Resonance Angiography
MSC	Mesenchymal Stem Cells
NIH	National Institute of Health
PAD	Peripheral Arterial Disease
PB	Peripheral Blood
PCI	Percutaneous Coronary Intervention
PTA	Percutaneous Transluminal Angioplasty
PTFE	PolyTetraFluoroEthylene- coated grafts
PVD	Peripheral Vascular Disease
PVR	Pulse Volume Recording

QoL	Quality of Life
REACH	Reduction of Atherothrombosis for Continued Health
SDF-1	Stromal-cell Derived Factor-1
siRNA	Small Interfering RiboNucleic Acid
SLP	Segmental Limb Pressure
TAO	ThromboAngiitis Obliterans
TcO2	Transcutaneous Oxygen tension
VEGF	Vascular Endothelial Growth Factor
VSMC	Vascular Smooth Muscle Cell

Abstract

Background: Patients with critical limb ischemia (CLI) who are not candidate for surgical or percutaneous revascularization carry a poor prognosis. Stem cell injection is emerging as a novel therapy in those patients. Stem cell injection aim at critical limb salvage through improving peripheral vascular collaterals.

Patients and Methods: Thirty patients were included in the study and divided into 2 groups. Group 1 patients (active treatment group, n=15) were subjected to autologous bone marrow injection into their critical limbs. Group 2 patients (control group, n=15) were observed for the natural history of their critical limbs. Patients were followed up for 6 months for subjective and/or objective evidence of vascular improvement.

Results: The mean amount of injected bone marrow-mononuclear cells (BM-MNC) was (67.1 $\pm$ 33.7 x10^7 /ml), of which (94.1 $\pm$ 2.7%) were viable cells. The mean CD34+ cells was (4.5 $\pm$ 1.0%), mean CD133+ cells was (2.1 $\pm$ 0.5%) and mean dual CD34+ CD133+ cells was (1.2 $\pm$ 0.2%). After 6 months follow up, there was no significant difference in the clinical outcome or ankle brachial index (ABI) between the two studied groups. Three patients in each group showed clinical vascular improvement. Seven patients in each group were amputated (median time to amputation was 30 days for group (1) patients and 21 days for group (2) patients, *p* value was 0.4). Three patients in group 1 and two patients in group 2 died before completing the follow up period.

Conclusion: This study shows that injection of autologus BM-MNC in patients with "no-option" CLI was a safe and feasible procedure. Yet, its therapeutic benefit could not be demonstrated. It's noticeable that all patients who improved (3 patients in each group) were at a lower (less than category 6) Rutherford grade. Larger placebo-controlled studies are needed to verify these findings.

Keywords: Critical limb ischemia. Stem cells. Limb salvage. Therapeutic neoangiogenesis.

INTRODUCTION

INTRODUCTION

Peripheral arterial disease (PAD) broadly encompasses the vascular diseases caused primarily by atherosclerosis and thromboembolic pathophysiological processes that alter the normal structure and function of the aorta, its visceral arterial branches, and the arteries of the lower extremities (***Hirsch et al, 2006***).

Lower extremity PAD is a common syndrome that affects a large proportion of most adult populations worldwide. PAD can be present in subclinical forms that can be detected by the use of sensitive vascular imaging techniques, or can be present in symptomatic forms (***Criqui et al, 1997; Murabito et al, 1997***).

Critical limb ischemia (CLI) is defined by most vascular clinicians in most patients who present with lower extremity ischemic rest pain, ulceration, or gangrene (***Hirsch et al, 2006***). The term CLI should only be used in relation to patients with chronic ischemic disease, defined as presence of symptoms for more than 2 weeks (***Rutherford et al, 2000***). CLI develops when atherosclerosis causes significant arterial narrowing that the blood flow to the distal limb does not meet the metabolic needs of tissues at rest (***Santilli et al, 1999***).

CLI confers a prognosis of high risk for limb loss and for fatal and nonfatal vascular events, myocardial infarction, and stroke. Observational studies of patients with CLI, who are not candidates for revascularization, suggest that one year after the onset, approximately 25% will have died and 25% will have required a major amputation. Their prognosis is, in many ways, similar to that of some malignancies. The diagnosis of CLI thus predicts poor prognosis for life and limb (***Rutherford et al, 2000)***.

The primary goals of treatment of CLI are to relieve ischemic pain, heal ischemic ulcers, prevent limb loss, improve patient function and quality of life and prolong survival. A primary outcome would be amputation-free survival. To achieve these outcomes, most patients will ultimately need a revascularization procedure, whether endovascular or surgical. A successful revascularization may reduce pain and improve quality of life for a limited period of time, but frequently, this goal is not achieved (***Rutherford et al, 2000***). Limb preservation by means of revascularization is cost-effective, leads to a better quality of life for most patients and is associated with lower perioperative morbidity and mortality

than amputation. Limb preservation should be the goal in most patients with chronic critical limb ischemia (***Albers et al, 2003***).

When open or endovascular intervention is not technically possible or has failed, the question arises as to whether pharmacological treatment is an option (***Rutherford et al, 2000***). Medical care strategies have included the use of antiplatelet agents, anticoagulant medications, intravenous prostanoids, modification of atherosclerotic risk factors and maintenance of the limb in a dependent position. However, none of these clinical interventions has been adequately evaluated or proven in prospective clinical trials to offer predictable improvements in limb outcomes. Amputation maybe, sometimes, the only solution to reduce pain, though amputees may have an even more reduced life expectancy (***Hirsch et al, 2006***).

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

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 (***Zhang et al, 2002***).

Adult stem cells typically generate the cell types of the tissue 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 (***www.stemcells.nih.gov***).

Recent studies have suggested that unpurified marrow mononuclear cells and/or subsets of adult hematopoietic stem cells have been reported to contribute to neoangiogenesis (***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 fractions 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 (***Kamihata et al, 2001; Shintani et al, 2001***). In one trial, patients were selected for chronic ischemic extremity pain or nonhealing ischemic ulcers or both. Significant improvement in the ankle brachial index (ABI), transcutaneous oxygen pressure, and a pain-free walking occurred following treatment. Implantation of bone marrow-mononuclear cells 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-MNC transplantation in CLI. The aim in most trials is to reduce the number of necessary leg amputations, reduce pain and induce wound healing.

Successful injection of BM-MNC in CLI may be the only hope in patients who seem to have no other alternative therapy to save their limbs.