Stem cell implantation in treatment of peripheral vascular ischemia

By

Noha AbdelHafeez Abdelkader

M.B., B.Ch, Cairo University, 2003

Thesis

SUBMITTED FOR PARTIAL FULFILMENT OF THE MASTER DEGREE **(M.Sc.)**

IN CLINICAL AND CHEMICAL PATHOLOGY

Under supervision of

Prof. Dr. Mervat Mohamad El-Ansary
Prof. of clinical pathology, Faculty of Medicine, Cairo University

Prof. Dr. Sanaa Sayed AbdelShafy
rof. of clinical pathology, Faculty of Medicine, Beni Suef University

Prof. Dr. Alaa AbdelHalim Mrzouk
Prof. of general and vascular surgery, Faculty of Medicine, Cairo University

Clinical pathology Department
Faculty of Medicine
Cairo University

2009

ACKNOWLEDGEMENT

First of all, thanks to God for his grace and mercy, and for giving me the effort to complete this work.

I was fortunate enough to carry out this work under the supervision of Prof. DR.Mervat Elansary Professor of Clinical and Chemical Pathology, Cairo University. Thanks for her generous help and advice. It was a great honor to work with a great professor like her.

I would like to express my sincere thanks to Prof .Dr .Sanaa Abdel Shafy, professor of clinical and chemical pathology, Beni Suef University, for her kindness, great patience and continuous support throughout the work.

Words will not be able to express my deepest gratitude and appreciation to Prof .Dr.Alaa abedelHalim, Professor of general and vascular surgery Beni Suef University, for his unlimited help, support and guidance through this work.

Special thanks to all my colleagues in the department of Surgery and the department of Radiology for their co-operation through out this work.

My deep gratitude is to my friends who helped me a lot and supported me in hard times.

At last but not least I would like to thank the members who were behind me in every successful step in my life, and who afford me the best circumstances to realize my goal... to my family specially my great mother.

ABSTRACT

There is recent evidence from clinical trials that implantation of stem/progenitor cells improve limb ischemia. In the present study implantation of autologous peripheral blood mononuclear cells (PBMNCs) mobilized by granulocyte-colony stimulating factor (G-CSF) - investigated in patients with chronic limb ischemia.

Twenty-four patients with chronic lower limb ischemia were enrolled and randomized (1:1) to either the implanted group or the control group. In the implanted group, the patients received subcutaneous injections of recombinant human G-CSF (300µg/day) for 5 days to mobilize stem/progenitor cells, and their PBMNCs were collected and implanted by multiple intramuscular injections into ischemic limbs while control group injected by sterile saline and receive medical treatment. All of the patients were followed up after at 12 week.

At the end of the follow-up period, the main manifestations were significantly improved in the patients of the implanted group compared to the control group. Mean of rest pain decreased from 6.42±2.15 to 1.67±3.89 ($P<0.001$). Mean of pain free walking distance increased from 25±29 to 409±204 ($P<0.001$). Mean ankle-brachial pressure index increased from 0.45± 0.32 to 0.79± 0.38 ($P = 0.005$). A total of 7 of 9 limb ulcers and wounds (77.8%) of implanted patients healed after cell implantation. Two lower limb amputations occurred in the implanted patients. In contrast, eight control patients had to receive a lower limb amputation.

Key Words: G-CSF -Chronic limb ischemia-PBMNC-stem/progenitor cells.

LIST OF CONTENTS

List of tables

List of FIGURES

List of Abbreviations

Abbreviation	The full term
ABI	Ankle-brachial index
AECs	Amniotic epithelial cells
BM	Bone marrow
BMMNCs	Bone marrow mononuclear cells
BMP	Bone morphogenic protein
BMSC	Bone marrow stem cells
C	Complement
CD	Cluster of differentiation
CE	Corneal epithelial
CLI	Critical limb ischemia
CRP	C-reactive protein
CSFs	Colony stimulating factors
CTA	Tomographic angiography
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
DNMT3B	DNA methyltransferase 3 beta
EBAF	Endometrial Bleeding-Associated Factor
ECM	Extracellular matrix
ECs	Endothelial cells
EGCs	Embryonic germ cells
eNOS	Endothelial nitric oxide
EPCs	Endothelial progenitor cells
ESC	Embryonic stem cells
FGF	Fibroblast growth factor
FITC	Fluorescein iso thiocyanate
Flk-1	Fetal liver kinase
FSCs	Fetal stem cells
G-CSF	Granulocyte-colony stimulating factor
GFR	Glomerular filtration rate
Hct	Hematocrit
HDAC	Histone deacetylase
HESC	Human embryonic stem cell
HGF	Hepatocyte growth factor
HIF-1	Hypoxia-inducible factor-1
HMGB1	High-mobility group box 1
HSCs	Hematopoietic stem cells
HSPC	Hematopoietic stem/progenitor cells
hTERT	Human active subunit of the telomerase reverse Transcriptase

ICAM-1	Intercellular adhesion molecule 1
ICMs	Inner cell masses
IFN	Interferon
Ig	Immunoglbulin
IGF	Insulin-like growth factor
IL	Interleukin
iPSCs	Induced pluripotent stem cells
mAbs	Monoclonal antibodies
MAPC	Multipotent adult progenitor cells
MIF	Macrophage migration inhibitory factor
MMP-9	Matrix metallopeptidase 9
MPCs	Mesodermal progenitor cells
MRA	Magnetic resonance angiography
mRNA	Messenger RNA
MSCs	Mesenchymal Stem Cells
NO	Nitric oxid
Oct	Octamer-binding protein
OI	Osteogenesis imperfecta
OR	Odds ratio
PAD	Peripheral arterial disease
PAOD	Peripheral arterial occlusive disease
PB	Peripheral blood
PBMNCs	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PMT	Photomultiplier tube
RBCs	Red blood cells
RNA	Ribonucleic acid
RPE	R-phycoerythrin
SC	Stem cells
SCID	Severe combined immunodeficiency
SDF-1	Stromal derived factor-1
SNS	Sympathetic nervous system
SSEA	Stage-specific embryonic antigen
TA	Transit-amplifying
TACT	Therapeutic Angiogenesis by Cell Transplantation
TASC	TransAtlantic Inter-Society Consensus
TNF	Tumor necrosis factor
TRA	Tumor-rejection antigen
UCB	Umbilical Cord blood
UCE	Umbilical cord epithelium
UKPDS	United Kingdom Prospective Diabetes Study

V	Variables
VEGF	Vascular endothelial growth factor
VEGFR2	Vascular endothelial growth factor receptor
WBCs	White blood cells
wt	Wild-type

Introduction

Peripheral arterial disease (PAD) is a serious condition that increases individual and population-based risk of heart attack, stroke, death, and amputation and decreases quality of life and functional independence ***(Steffen et al., 2008).***

Despite considerable advances in the therapy of patients with peripheral arterial occlusive disease (PAOD) and critical limb ischemia (CLI), a substantial number remain, in whom amputation has to be considered the only and final option ***(Lenk et al., 2005).***

Endothelial progenitor cells (EPCs) circulate in adult human peripheral blood and are mobilized from bone marrow by cytokines, growth factors, and ischemic conditions. Vascular injury is repaired by both angiogenesis and vasculogenesis mechanisms. Circulating EPCs contribute to repair of injured blood vessels mainly via a vasculogenesis mechanism ***(Murasawa and Asahara, 2005).***

Therapeutic angiogenesis in patients with lower limb ischemic disease was studied by many researchers. Researchers have tried to overcome limitations of the natural angiogenic response by substantially increasing the local concentrations of angiogenic growth factors either by administering recombinant protein for the gene that codes for an angiogenic growth factor or by administering endothelial progenitor cells (EPCs) that will synthesize a cocktail of growth factors in the vicinity of new vessel formation . The EPCs were harvested from peripheral blood, autologous bone marrow, and human umbilical cord blood ***(Kim et al., 2006).***

Aim of the work

To assess the application of implantation of autologous granulocyte colony–stimulating factor (G-CSF)–mobilized peripheral blood mononuclear cells (PBMNCs) in the treatment of chronic limb ischemia of patient not eligible for arterial reconstruction or endovascular procedures and to evaluate the safety, efficacy, and feasibility of this novel therapeutic approach.

Stem cells

Human life begins as single fertilized cell. The journey from single cell to complex being is attributable to the role of the stem cells (SC) (i.e. cells that produce all different types of cells and tissues that make up the Human body) ***(Semsarian, 2002).***

In general, embryonic, fetal, and adult stem cells show several common functional properties. Common properties include their high self-renewal capacity and potential to generate differentiated cell progenitors of different lineages under simplified culture conditions in vitro and after transplantation in the host in vivo ***(Mimeault and Batra, 2006).***

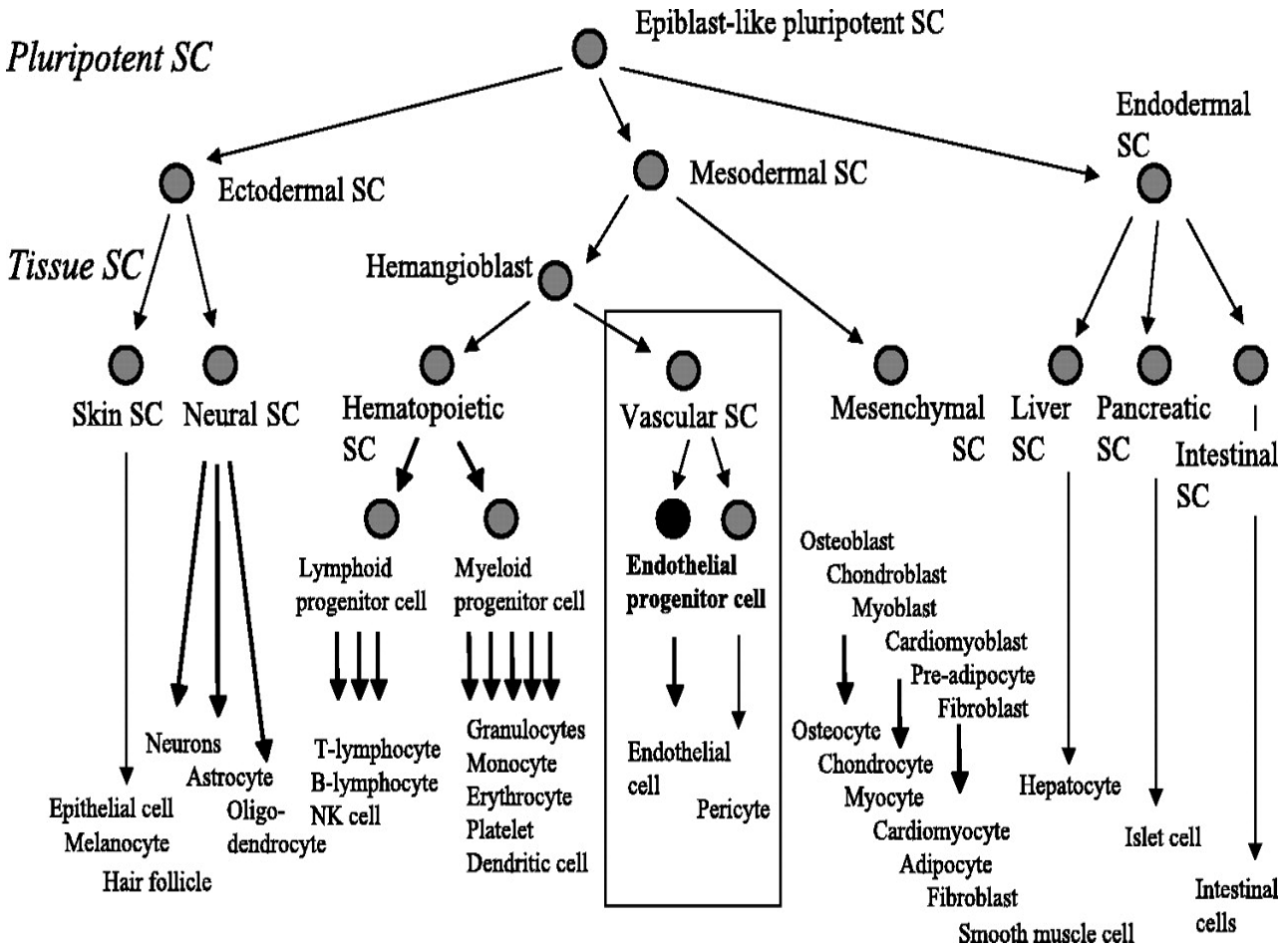
More particularly, the establishment of the functional properties of stem cells and their progenitors in vitro and in vivo has indicated that they may contribute to the regeneration of damaged tissues. Therefore, the use of stem cells and their progenitors is a promising strategy in cellular and genetic therapies for multiple degenerative disorders, as well as adjuvant immunotherapy for diverse aggressive cancer types ***(Leri et al., 2005).***

Parkinson and Alzheimer diseases, muscular degenerative disorders, chronic liver and heart failures, and type 1 and 2 diabetes, as well as skin, eye, kidney, and hematopoietic disorders, could be treated by the stem cell-based therapies(Table 1) ***(Mimeault and Batra, 2006).***

Potency definitions: Potency specifies the differentiation potential (the potential to differentiate into different cell types) of the stem cell (Fig.1). A totipotent stem cell (e.g. fertilized egg) can develop into all cell types including the embryonic membranes. A pleuripotent stem cell can

develop into cells from all three germinal layers (e.g. cells from the inner cell mass). Other cells can be Multipotent, oligopotent, or unipotent depending on their ability to develop into many, few or one other cell type(s) ***(Sell, 2004).***

Unipotent cells can produce only one cell type, their own, but have the property of self-renewal which distinguishes them from non-stem cells (e.g. muscle stem cells).(***Lantz and Huff, 1995).***



(Fig.1): stem and progenitor cells. SC, stem cell. ***(Asahara and Kawamoto 2004).***