EFFECT OF BONE MARROW DERIVED MESENCHYMAL STEM CELLS ON MUSCLE REGENERATION IN RAT LIMB ISCHEMIA MODEL

Thesis

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ABSTRACT

MSCs are a heterogenous population of self renewable, pluripotent cells that can be isolated from bone marrow and other sources. They are capable of restoring the hematopoietic microenvironment after back-transplantation of even a single clone into the body.

The aim of this study was to examine the effect of local delivery of MSCs on muscle regeneration in a murine limb ischemia model. To achieve this aim, rat hindlimb ischemia model was established by surgical ligation of the left femoral artery. Animals were grouped; control rats, ischemic group and, ischemic with hMSCs group. In vitro, hMSCs were isolated from human bone marrow and characterized by flow cytometry. hMSCs were labeled by red fluorescent PKH dye. Peak isometric twitch force (Pt), was assessed 4 weeks after surgery. After rats were sacrificed, muscle tissues of the three studied groups were harvested for pathological assessment, tissue tracing of labeled MSCs and for vascular endothelial growth factor gene expression using quantitative real time PCR. Our results showed that hMSCs were positive for mesenchymal stem cell marker. Histopathologically, ischemic muscle transplanted with hMSCs showed definite angiogenesis& neovascularization. Red fluorescent PKH dye for best cell tracing showed that the red fluorescence was found in ischemic muscle injected with hMSCs. Ischemic with injected hMSCs group induced a significant improvement in blood reperfusion, detected by improved muscle performance, high significant level of VEGF gene expression compared to ischemic group.

Key words: rat hindlimb ischemia - hMSCs – VEGF- skeletal muscle - angiogenesis.

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List of Abbreviations

ABPI	**Ankle brachial pressure index**
AEC	**Amniotic epithelial cell**
AECs	**Amniotic epithelial cells**
AF	**Amniotic fluid**
AMSC	**Amniotic mesenchymal stem cell**
BFGF	**Basic fibroblast Growth factor**
BM	**Bone marrow**
BMP-2	**Bone morphogenetic protein-2**
BP	**Blood pressure**
BSC	**Biological safety cabinet**
CCM	**Complete culture medium**
CD	**Cluster of differentiation**
CFUs- F	**Colony forming units- fibroblastic**
CMSC	**Chorionic mesenchymal stem cell**
CNS	**Central nervous system**
CT	**Computerized tomography**
Ct	**Cycle threshold**
DMEM	**Dulbecco's modified eagle's medium**
dNTPs	**Deoxynucleotidetriphosphate**
DPBS	**Dulbecco's phosphate buffered saline**
ECM	**Extra cellular matrix**
eNOS	**Endothelial NO Synthase**
EPCS	**Endothelial progenitor cells**
ESC	**Embryonic stem cell**
FBS	**Fetal bovine serum**

FCS	**Fetal calf serum**
FGFs	**Fibroblast growth factors**
FITC	**Fluorescein isothiocyanate**
G-CSF	**Granulocyte colony stimulating factor**
GFs	**Growth factors**
GVHD	**Graft versus host disease**
HE	**Hematoxylin and Eosin**
HIF- 2α	**Hypoxia induced factor -2α**
HLA	**Human leucocyte antigen**
HPRI	**Human placental ribonuclease inhibitor**
HSCs	**Hematopoietic stem cells**
HSPGs	**Heparan sulphate proteoglycans**
hTERT	**Human telomerase reverse transcriptase**
hUCMSCS	**human umbilical cord blood mesenchymal stem cells**
IM	**Intramuscular**
iPS	**Induced pluripotent stem**
Klf 4	**Kruppel like family 4**
LIF	**Leukemia inhibitory Factor**
MAPC	**Multipotent adult progenitor cell**
MAPC	**Multipotent adult progenitor cell**
M-CSF	**Macrophage Colony stimulating factor**
MHC II	**Major histocompatibility complex type II**
MMLV	**Moloney murine leukemia virus**
MMPS	**Matrix metallo proteinases**
Ms	**Millisecond**
MSCs	**Mesenchymal stem cells**

NO	**Nitric Oxide**
Oct 4	**Octamer binding factor 4**
PAD	**Peripheral arterial disease**
PD	**Population doublings**
PE	**Polyerythrin**
PECAM1	**Platelet- endothelial- cell adhesion molecule-1**
PKB	**Protein Kinase B**
PLGF	**Placenta growth factor**
Pt	**Peak isometric twitch force**
RQ	**Relative Quantification**
SDF-1	**Stromal cell- derived factor-1**
SMA	**Smooth muscle actin**
Sox 2	**Sex determining region Y Factor 2**
TGF- β	**Transforming growth factor beta**
T-PA	**Tissue- type plasminogen activator**
UCB	**Umbilical cord blood**
VCAM-1	**Vascular cell adhesion molecule-1**
VE	**Vascular endothelial**
VEGF	**Vascular endothelial growth factor**
VPF	**Vascular permeability factor**
Wnts	**Drosophilia wingless**
αSMA	**α Smooth muscle actin**

INTRODUCTION

Stem cells are a unique source of self-renewing cells within the human body. Several sources of stem cells have been proposed as sources for cell therapy. Embryonic stem cells are the most potent in terms of their differentiation potential but may be tumorigenic when transplanted in vivo, and their use is limited by ethical issues. Adult stem cell therapy could solve the problem of degenerative disorders, including liver disease, in which organ transplantation is inappropriate or there is limitation as host age of organ donors. This view is predicated upon the evidence that stem cells, particularly those in hematopoietic tissue, have the ability to develop into endodermal, mesodermal, ectodermal cell types (**Preston et al., 2003).**

Several bone marrow subpopulations, such as endothelial progenitor cells and marrow stromal cell fraction (marrow-derived stromal cells) may be able to differentiate into 1 or more of the cellular components of the vascular bed. Thus, therapeutic delivery of bone marrow donates cells with potential to incorporate into new or remodeling blood vessels. However, the magnitude of incorporation of bone marrow-derived cells in to vascular structures varies between studies **(Jiang Y et al., 2002).**

MSCs play an important supportive role in the marrow microenvironment mediated partly through cell-to-cell contact but importantly also via paracrine mechanisms involving release of cytokines that exert effects on surrounding cells. MSCs are

multipotent progenitor cells that have often been reported to have the potential to differentiate into lineages of mesenchymal tissues, including muscle **(Pittenger et al., 1999)** and also into vascular endothelial cells **(Reyes and verfaillie**, **2002).**

Bone marrow was the first reported source of MSCs, but adipose tissue and umbilical cord blood (UCB) are also sources of MSCs. There is data suggesting that MSCs from UCB possess the greatest Capacity to proliferate **(Kern and Bieback, 2006).**

AIM OF WORK

The present study aims to examine the effect of local delivery of MSCs on muscle regeneration in a murine limb ischemia model.