Comparative Study on the Effect of Bone Marrow versus Adipose Tissue Derived Mesenchymal Stem Cells on Experimental Skeletal Muscle Injury in Rats

Thesis

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Marwa Aboelkasim Saleh

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List of Abbreviations

ADSCs .....Adipose-Derived Stem Cells

ANOVA.....Analysis of Variant

ASCs.....Adipose Derived Stromal/Stem cells

BFGF .....Basic Fibroblast Growth Factor

BM-MSCs.....Bone Marrow Derived Mesenchymal Stem Cells

BMP.....Bone Morphogenic Protein

CD .....Cluster of Differentiation

CFU .....Colony Forming Unit

CKM .....Muscle Certain KINASE

CTx.....Cardiotoxin

DAB .....Diaminobenzidine

DMEM.....Dulbecco's Modified Eagles Medium

DNA .....Deoxyribonucleic Acid

ECM.....Extracellular Matrix

EDTA.....Ethylene Diamminetetracetic Acid

EGF .....Epidermal Growth Factor

EPC .....Endothelial Progenitor Cells

ESCs.....Embryonic Stem Cells

FBS.....Fetal Bovine Serum

FCS.....Fetal Calf Serum

FGF-2 .....Fibroblast Growth Factor-2

HGF.....Hepatocyte Growth Factor

HSC .....Hematopoietic Stem Cell

IGF1 .....Insulin-Like Growth Factor 1

IL.....Interleukin

IM.....Intramuscular

List of Abbreviations

MDSCs.....Muscle-Derived Stem Cells

MHC .....Myosin Heavy Chains Complement

MHC-II.....Class II Major Histocompatibility Complex

MMPs.....Matrix Metalloproteinase

MRFs/MYF.....Myogenic Regulatory Factors

MSCs .....Mesenchymal Stem Cell

MSTN.....Myostatin

MVS.....Micro-Vesicles

MyoD.....Myogenic Differentiation Factor

MyoG.....Myogenin

NK .....Natural Killer Cell

NMJ.....Neuromuscular Junctions

No.....Nitric Oxide

NSAIDs .....Non-Steroidal Anti-Inflammatory Drugs

NTx.....Notexin

PBS.....Phosphate Buffered Saline

PCNA .....Proliferating Cell Nuclear Antigen

PCR .....Polymerase Chain Reaction

PDGF .....Platelets Derived Growth Factor

PGE .....Prostaglandins

PIC .....PW1+/pax7- Interstitial Cells

PLA.....Processed Lipoaspirate Cell

SCs .....Satellite Cells

SP .....Side Population Cells

SRY .....Sex Determining Region of Y Chromosome

SVF.....Stromal Vascular Fraction

List of Abbreviations

TGFβ .....Transforming Growth Factor β

TIMPs .....Tissue Inhibitors of Metalloproteinase

VCAM1 .....Vascular Cell Adhesion Molecule

VEGF .....Vascular Endothelial Growth Factor

α-SMA.....Alpha-Smooth Muscle Actin

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Abstract

Introduction: Skeletal muscle injury can result from a variety of mechanisms, including contusion, strain, laceration, or a combination of these mechanisms. Lesions originating by trauma are associated with loss of healthy muscular tissue and development of fibrous tissue scar with irreversible atrophy .Stem cells as a part regenerative therapy could overcome these limitations and improve skeletal muscle regeneration. **Aim of the work:** to compare between the effects of mesenchymal stem cells derived from the bone marrow versus those derived from the adipose tissue in treatment of skeletal muscle injury in female albino rats. **Materials and Methods:** Fifty adult female albino rats weighing 150-200gm were included in the study. They were divided into four groups: Group I (Control), Group II (untreated group) in which skeletal muscle laceration injury was induced and left for spontaneous healing, Group III in which the rats received a single dose of 10^6 BM-MSCs via IM at the site of injury, Group IV in which the rats received a single dose of 10^6ADSCs via IM at the site of injury. Skeletal muscle specimens were prepared for histological examination using H/E and Mallory trichrome stain. Also muscle specimens for detection of y chromosome in stem cells treated group by using PCR. Morphometric study and statistical analysis were performed. **Results:** Histological examination of the skeletal muscle laceration injury in the untreated group revealed a gap filled with granulation tissue. There was disruption of the muscle fibers with vacuolation in the cytoplasm and pyknotic nuclei. After 2 weeks, there was significant increase in the collagen fiber deposition in the interstitium. The BM-MSCs treated group showed better regeneration of the muscle fibers through the appearance of centronucleated regenerating myotubes but the interstitium also showed increased collagen deposition. Meanwhile, the ADSCs treated group showed significant increase in the regenerating centronucleated myotubes without an increase in the collagen fiber deposition. **Conclusion:** Inramuscular injection of ADSCs is preferable than BM-MSCs in the treatment of skeletal muscle laceration injury.

Key words: Skeletal muscle laceration injury, BM-MSCs Bone marrow mesenchymal stem cells, ADSCs Adipose derived stem cells, Rat.

Introduction

Skeletal muscle injury can result from a variety of mechanisms, including contusion, strain, laceration, or a combination of these mechanisms. Lesions originating by trauma are associated with loss of healthy muscular tissue and development of fibrous tissue scar with irreversible atrophy **(Pereira et al., 2013).**

The capacity of skeletal muscle tissue to regenerate resides in a reserve population of mono-nucleated precursors, which are termed *satellite cells* **(Pecanha et al., 2012)**. **Filareto et al., (2015)** reported that the low frequency of satellite cells in skeletal muscle fibers limits its regenerative capacity.

Complete functional recovery of skeletal muscle after severe injury remains a challenge. Accordingly, significant efforts are being made to improve the current treatment of skeletal muscle trauma. An approach is needed to limit fibrosis and atrophy, improve myocyte regeneration and local revascularization **(Quintero et al., 2009).**

Stem cells and regenerative medicine is a fast emerging field with rapid progress. Mesenchymal stem cells (MSCs) are rapidly proliferating and can differentiate into special types including skeletal muscle fibers. On the other hand, satellite cells are poorly expandable in vitro

with poor survival rate and rapidly undergo senescence **(Pecanha et al., 2012).**

By definition, stem cells are characterized by being primitive undifferentiated non-specialized, and by having the ability to generate not only new stem cells, but also a diverse range of specialized cell types under certain physiological and experimental conditions. In this process of self-renewal and differentiation, the stem cell can go through two basic division processes: a) the deterministic model, which corresponds to the division of a stem cell that generates a new stem cell and a cell that will differentiate (progenitor cell) and, b) the random or stochastic model, in which some stem cells generate only new stem cells while others generate differentiated cells **(Samira and Okamoto, 2010).**

Many tissues have been investigated as a source of adult mesenchymal stem cells including adipose tissue, bone marrow, periosteal tissue, peripheral blood, skeletal muscle and the synovium **(Murphy et al., 2013).**

Several researchers have reported that bone-marrow-derived mesenchymal stem cells (BM-MSCs) have the potential to differentiate and fuse with myoblasts in vitro and to contribute to muscle-healing and treatment of muscle disorders. Like bone marrow, adipose tissue is

derived from the embryonic mesoderm and contains a heterogenous stromal cell population such as endothelial cells, smooth muscle cells, pericytes and mesenchymal stem cells **(Pecanha et al., 2012)**

Horwitz et al., (2007) stated that adipose tissue and BM are the most readily available sources of MSCs. Although adipose tissue derived MSCs and BM-MSCs show the same immunoregulatory properties and supporting hematopoiesis, BM-MSCs have a higher degree of commitment to differentiate into chondrogenic and osteogenic lineages than adipose tissue-derived MSCs.

Of known MSC-containing tissues, adipose tissue is a particularly attractive source due to its availability and accessibility **(Herrera et al., 2007).**

Adipose-Derived Stromal/Stem Cells (ASCs) have the advantage of being safely harvested in abundant quantity. Per gram of adipose tissue 5×10^3 colony-forming stromal cells can be isolated, which is estimated to represent up to 500 times more cells than for bone marrow stromal cells **(Murphy et al., 2013 and Bourin et al., 2013)**.

In culture, ASCs have displayed good proliferative capacities as well as an impressive developmental

plasticity, including the ability to undergo multi lineage differentiation **(Baer and Geiger, 2012)**.

Cui et al., (2007) showed that ASCs are less immunogenic and immuno-suppressive. Adipose-derived stem cells have several advantages over bone marrow in clinical trials, such as easy accessibility, abundance, and higher stem-cell proliferation rates than BM-MSCs. Recently, ASCs were shown to secrete multiple angiogenic and anti-apoptotic cytokines, supporting tissue regeneration and minimizing tissue damage. These cytokines were also classically described to be crucial for satellite cell proliferation and fusion in vitro and in vivo **(Pecanha et al., 2012).**

Aim of the Work

To compare between the possible effects of mesenchymal stem cells derived from bone marrow versus those derived from adipose tissue in treatment of skeletal muscle injury in female albinos rats.

Development of muscle fibers

A muscle fiber is formed during development by fusion of small, individual muscle cell called myoblast. These cells derived from self-renewing population of multipotential myogenic stem cells that originated in the embryonic mesoderm. Early in embryonic development, these cells express MyoD transcription factor, which along with other myogenic regulatory factors (MRFs) played key role in activation of muscle-specific gene expression and differentiation of all skeletal muscle lineages **(Charg and Rudnick 2004).**

Myoblast also express negative regulatory myostatin gene which has a balancing effect on skeletal muscle development through the synthesis of myostatin (MSTN). Myostatin protein belongs to the bone morphogenic protein/ transforming growth factor B (BMP & TGF-B). Myostatin has an inhibitory effect on muscle growth and differentiation. It was observed that inactivation of myostatin gene results in hyper muscular phenotypes.

There are two types of myoblasts. First is the early myoblast that is responsible for formation of primary myotubes through synchronous fusion. By light microscope, primary myotubes show a chain of multiple central located nuclei that are surrounded by myofilament. Myotubes undergo further differentiation into skeletal