ROLE OF BONE MARROW-DERIVED MESENCHYMAL STEM CELLS IN SKIN WOUND HEALING IN MALE ALBINO RAT

A Thesis

Submitted for Partial Fulfillment of
Master Degree in Histology

Presented By

Heba Fikry Abd El Galil Abd El Baky

M.B.B.Ch.
Candidate of histology
Faculty of Medicine, Ain Shams University

Supervised By

Prof. Dr. / Kawthar Abd El Raheem Farrag

Professor of Histology
Faculty of Medicine, Ain Shams University

Prof. Dr. / Manal Hassan Moussa

Professor of Histology
Faculty of Medicine, Ain Shams University

Dr. / Abeer Abd El Mohsen Abd El Samad

Lecturer of Histology
Faculty of Medicine, Ain Shams University

Faculty of Medicine
Ain Shams University
2009

Acknowledgement

First and foremost thanks Allah the most kind and most merciful, to whom I relate any success in achieving any work in my life.

I would like to express my deepest gratitude and profound respect to ***Prof. Dr. Kawther Abd Elraheem Farrag****, Professor of Histology, Faculty of Medicine-Ain Shams University, for her everlasting encouragement, meticulous help, wide experience, precious instructions and kind supervision. It was such a great honor to work under her guidance.*

I am also deeply grateful ***to Dr. Manal Hassan Moussa,*** *Professor of Dermatology, Faculty of Medicine-Ain Shams University, for her precious opinions, contributive comments and sincere effort that surved much in the construction of this work.*

I am deeply indebted and extremely grateful to ***Dr. Abeer Abd Elmohseen Abd Elsamad,*** *lecturer of Histology, Faculty of Medicine-Ain Shams University, for her valuable advices and supervision during various stages of the development of this work. Without her endless help, patience, continuous encouragement and skillful scientific guidance, this work could not have been completed.*

I would like to extend my thanks to ***Prof. Dr.Hoda Nada,*** *Professor and Head of Histology department, Faculty of Medicine-Ain Shams University, for her support and encouragement.*

Acknowledgement

*I would like to extent my thanks to **Dr.fatma Abd Elkarim** and **DR.Mohmed Kamal** in medical research center Ain Shams University for their remarkable effort and kind help*

*I wish to also to express my sincere appreciation to **my mother in-law** who devoted much of her time to help me during this work.*

Special thanks to all my professors and my colleagues in Histology department, Faculty of Medicine-Ain Shams University, for their continuous support and valuable advices.

Dedication

*Words can not express my thanks, gratefulness, respect and love to my **parents**, my **husband** and all members of my **family**. Without their help, support, patience and encouragement I would have never achieved any success.*

*To all of them and to **Abd ElRahman** & **Sara**, I dedicate my work.*

Heba fikry

Cairo, 2010

LIST OF CONTENTS

Page

LIST OF TABLES:

Page

List of figures

Page

List of Histograms

Page

LIST OF ABBREVIATIONS

BM	Bone marrow.
BM-HSCs	Bone marrow-derived HSCs.
BM-MSCs	Bone marrow-derived mesenchymal stem cells.
CD44	Cluster of differentiation 44.
DMEM	Dulbecco's Modified Eagles medium.
EGF	Epidermal growth factor.
ESCs	Embryonic stem cells.
DAB	Diaminobenzidine .
DCM	Dilated cardiomyopathy.
FBS	Fetal bovine serum.
HC	Hematopoietic cell.
Hpf	high power field.
HSCs	Hematopoietic stem cells.
IL-12	Interleukin 12.
MSCs	Mesenchymal stem cells.
MC	Mesenchymal cell.
PBS	Phosphate buffer saline.
TC	Tissue culture.
OI	Osteogenesis imperfect.

ABSTRACT

ROLE OF BONE MARROW-DERIVED MESENCHYMAL STEM CELLS IN SKIN WOUND HEALING IN MALE ALBINO RAT

Bone marrow contains two types of stem cells; HSCs and MSCs. BM-MSCs can differentiate into different mesenchymal lineages, when cultured in appropriate environments. Wound healing is a complex process of the replacement of dead tissue by a vital tissue. The aim of the work was to isolate, grow, cultivate (culture) BM-MSCs from adult male albino rat and evaluate its benefit in wound healing. Thirty five adult male albino rats of average weight 150 grams were used. They were divided into three groups. Group I: BM was isolated and cultured, skin specimens were obtained as a control. Group II: a standardized wound was done on the mid back and injected intradermal by 0.5ml of PBS at 6 different sites and were examined on day 3 and day 7. Group III: a wound was performed as in group II and then treated with BM-MSCs and examined as in group II. From the current study, BM-MSCs were isolated from the BM of adult male albino rat. They were identified by their morphology, their ability to adhere to tissue culture dish and being CD44 positive. Results showed that treated wound with BM-MSCs, there were evidence of reepithelization and increased collagen and elastic formation. It is concluded that local administration of BM-MSCs was effective in promoting wound healing. These findings indicate that BM-MSCs cell therapy might provide a powerful technique to augment healing of clinically problematic wounds.

Keywords: Mesenchymal stem cells, incision wound, rat.

ROLE OF BONE MARROW-DERIVED MESENCHYMAL STEM CELLS IN SKIN WOUND HEALING IN MALE ALBINO RAT

A Thesis

Submitted for Partial Fulfillment of
Master Degree in Histology

Presented By

Heba Fikry Abd El Galil Abd El Baky

M.B.B.Ch.
Candidate of histology
Faculty of Medicine, Ain Shams University

Supervised By

Prof. Dr. / Kewther Abd El Raheem Farrag

Professor of Histology
Faculty of Medicine, Ain Shams University

Dr. / Manal Hassan Moussa

Assistant Professor of Histology
Faculty of Medicine, Ain Shams University

Dr./ Abeer Abd El Mohsen Abd El Samad

Lecturer of Histology
Faculty of Medicine, Ain Shams University

Faculty of Medicine
Ain Shams University
2009

INTRODUCTION

Stem cell is a cell that has the ability to divide for indefinite periods. Scientists primarily work with two kinds of stem cells from animals and humans: embryonic stem cells and adult stem cells. Embryonic stem cells (ESCs) can be isolated from the embryo at a gestational age of 3-5 days (blastocyst). ESCs have a great capacity for self-renewal and plasticity. However, the use of human embryonic stem cells, in researches as well as in therapeutics, is limited by scientific, political, and ethical considerations. The use of adult stem cells, especially mesenchymal stem cells (MSCs), is not burdened by many of these consideration ***(Fu X B & Li HH .2009).***

Adult stem cells can be isolated from bone marrow and other tissues such as adipose tissue, nerve tissue, umbilical cord blood, and dermis. Adult bone marrow contains two types of stem cells, which are the hematopoietic stem cells (HSCs) and the bone marrow-derived mesenchymal stem cells (BMSCs) ***(Fu X B & Li HH.2009).***

BMSCs are multipotential stem cells capable of differentiation into numerous cell types, including fibroblasts, cartilage, bone, muscle, and brain cells***(* Pittenger M *et al.2002),***they are able to repair tissue damaged by trauma or disease, and partially restore normal function ***(Yoshikawa T et al. 2008; Sasaki M et al. 2008).***

Because of their multipotency, easy isolation and culture, highly expansive potential, and immunosuppressant properties, these cells may be an attractive therapeutic tool for regenerative medicine and tissue engineering. BMSCs not only participate in the regeneration of tissues of mesenchymal lineages, such as intervertebral disc cartilage, bone, cardiomyocytes, and articular cartilage at knee joints, but also appear to differentiate into cells derived from other embryonic layers, including skin ***(Fu X B & Li HH .2009).***

Several studies have found that MSCs can contribute to skin reconstitution in cutaneous wounds ***(Yoshikawa T et al. 2008; Sasaki M et al. 2008).***

Wound repair is a highly complex biological process involving many kinds of cells, the extracellular matrix, and regulatory factors. Two main sources of stem cells are probably involved in the repair of the injured tissues: stem cells from injured tissues, stem cells migrating to injured tissues from bone marrow, such as (BMSCs) and (HSCs)***(Li HH et al .2006).***

BMSCs have the capacity to migrate to remote tissues and play a critical role in wound repair and tissue regeneration ***(Fu X B & Li HH .2009)***. Direct injection of BMSCs into injured tissues shows improved repair through mechanisms of differentiation and/or release of paracrine factors ***(Wu YJ et al .2007).***

Wound healing proceeds normally in the vast majority of patients; however, healing of wounds is significantly compromised in a number of medical conditions, such as diabetes, chronic renal failure and arterial or venous insufficiency. Impaired wound healing is also a secondary effect of chronic chemotherapeutic regimens**.** The mechanism of compromised wound healing is multifactorial, including the impaired inflammatory cell migration to the wound, and reduced growth factor and collagen synthesis. So better and more effective treatment strategies are needed to treat difficult wounds ***(McFarlin K et al. 2006).***

AIM OF THE WORK

The aim of the current study is to isolate and culture bone marrow-derived mesenchymal stem cells (BMSCs) from adult male albino rats, and also to evaluate their benefit in wound healing.

MATERIALS AND METHODS

Thirty five adult male albino rats of average weight 120-150 grams will be included in this study. The animals will be purchased and raised in medical research center Ain Shams University. They will be housed in plastic cages with mesh wire covers and will be given food and water ad libitum.

The animals will be divided into three groups:

Group I (control group):will include 7 rats. In each rat bone marrow will be collected from both tibia and femur and BMSCs will be cultured. One of the culture plates will be stained with Giemsa stain for demonstration of BMSCs. A skin specimen will be obtained from the mid back.

Group II (experimental group): will include 14 rats. In each rat the dorsal hair will be removed and the skin will be prepared for generation of a standardized 1.5 cm^2 full-thickness wound on the mid back. The wound will be injected by 0.5ml of phosphate buffered saline solution (PBS) at different sites of the wound. This group will be further subdivided into the following two subgroups:

Subgroup IIa (7rats): In which the skin wound specimen will be examined on day 3.

Subgroup IIb (7rats): In which the skin wound specimen will be examined on day7.

Group III(experimental group): will include 14 rats. In each rat the dorsal hair will be removed and the skin will be prepared for generation of a standardized 1.5n cm^2 full-thickness wound on the mid back. Local treatment with BMSCs suspended in 0.5 ml PBS will be done .This cell suspension will be injected at different sites of the wound .This group will be further subdivided into two subgroups:

Subgroup IIIa(7rats): In which the skin wound specimen will be examined on day 3.

Subgroup IIIb(7rats): In which the skin wound specimen will be examined on day7.

After the determined post injection period, the experimental animals of each group will be sacrified using ether inhalation. Skin specimens will be fixed in buffered formalin for 48 hours. Then paraffin blocks will be prepared. Serial sections of 5 μm thickness will be cut and stained with histological

and immuno-histochemical techniques including:

- Hematoxylin & Eosin stain.
- Stainings for Collagen and Elastic fibers.
- Immuno-histochemical stain for CD 44(a sample of the attatched vital rat BMSCs will be fixed by (methanol :acetone 1:1) and characterized for CD 44 to make sure that injected cells are mesenchymal stem cells).

The bodies of the dead animals will be get rid of by using the incinerator. All animal procedures will be performed in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Animal ethical Committee of the Ain Shams University.

Method of obtaining bone marrow specimens for isolation and culture of BMSCs:

- The animals will be sacrificed by diethyl ether.
- Isolation and culture of BMSCs will be done according to ***(McFarlin K et al. 2006)*** with some modification by using the followings steps:
  1. Rats will be sterilized with betadine before cutting the skin.
  2. The femurs and tibia of the rats of group I will be carefully dissected from adherent soft tissues. Then will be placed into sterilized container containing 70 % alcohol.
  3. The epiphyses of both ends of the bones will be removed, using a sterilized bone scissors.
  4. The specimens will be delivered to the hood (lamina flow), to extract the bone marrow.
  5. Bones will be flushed with 2mL of cold (PBS) and cells washed two to three times with PBS.

The cells will be suspended in DMEM (Dulbecco's modified Eagles medium 85%) and L.Glutamine.and supplemented with fetal bovine serum (FBS) 10% and 100 µg/ml penicillin and streptomycin.

The culture plates will be followed every day. One of the plates will be stained with Giemsa stain for demonstration of BMSCs.

Incision Wound model:

The rats will be anesthetized through intraperitoneal injection of ketamine and xylazine mixture (15 and 1 mg/kg, respectively) ***(Fathke C et al. 2004, McFarlin K et al. 2006).***

The dorsal hair will be removed and the skin will be cleaned with Betadine solution. The skin will be prepared for generation of a standardized 1.5 cm^2

full-thickness wound on the mid back. The wound will be injected by 0.5ml of phosphate buffered saline solution (PBS) at different sites 1mm lateral to the wound along the entire length of the incision immediately after covering with a strei-strip (3mmX75mm) and surgical absorbent dressing to prevent desiccation. ***(McFarlin K et al. 2006).***

After recovery, the animals will be returned to their housing and allowed chow and water.

On days 3 and 7, rats will be sacrified and the entire wound including the adjacent 2mm skin margins will be excised and examined by histological and immuno-histochemical techniques ***(McFarlin K et al. 2006).***

Incision Wound model treated by BMSCs:

The rats will be anesthetized through intraperitoneal injection of ketamine and xylazine mixture (15 and 1 mg/kg, respectively) ***(Fathke C et al. 2004 McFarlin K et al. 2006).***

Skin will be prepared as in the incision wound model then local treatment with BMSCs suspended in PBS and cell suspension will be injected at different sites 1mm lateral to the wound along the entire length of the incision immediately after covering with a strei-strip (3mmX75mm) and surgical absorbent dressing to prevent desiccation. ***(McFarlin K et al. 2006).***

After recovery, the animals will be returned to their housing and allowed chow and water.

On days 3 and 7, rats will be sacrified and the entire wound including the adjacent 2mm skin margins will be excised and examined by histological and immuno-histochemical techniques ***(McFarlin K et al. 2006).***

Sample size:

Based on previous studies on the role of the BMSCs injection on the wound and their benefit in wound healing. The highest proportion was (P_1=0.63) while the lowest proportion was (p_2=0.03).

The sample size in this study is based on 95% level of confidence (α error = 5%) and power of study 90% (β error =10%).

The equation will be used for calculation of animal's numbers per group:

$$N \text{ (each group)} = \frac{2PQ\,(Z_{\alpha/2} + Z_{\beta/2})^2}{(P1-p2)^2}$$

N=minimum number of animals per each group.