ROLE OF BONE MARROW-DERIVED MESENCHYMAL STEM CELLS IN HEALING OF CORNEAL INJURY IN ADULT MALE RABBIT

Thesis

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Introduction

Bone marrow-derived mesenchymal stem cells (MSCs) were originally described as plastic-adherent fibroblast-like cells and can differentiate into osteoblasts, adipocytes, and chondrocytes **(friedenstein et al., 1976).**

Clinical development of mesenchymal stem cells (MSCs) formulations for therapeutic use has involved the isolation of MSCs from bone marrow and expansion in culture(***Pittenger et al 1999***)

Their plasticity has been expanded to include contribution to cell lineages in brain (***Brazelton et al., 2000***), liver (***Alison et al., 2000***), and kidney tissue (***Poulsom et al., 2001***).

Bone marrow stromal cells are progenitors of skeletal tissue components such as bone, cartilage, the hematopoiesis-supporting stroma, and adipocytes.

In addition, they may be experimentally induced to undergo unlimited differentiation, possibly forming neural and myogenic cells. As such, they represent an important paradigm of post-natal non hematopoietic stem cells, and an easy source for potential therapeutic use(***Paolo Bianco et al ,2001***).

INTRODUCTION

The term "stem cell" can be applied to a remarkably diverse group of cells. These cells, regardless of their source, share two characteristic properties. Firstly, they have the capacity for prolonged or unlimited self-renewal under controlled conditions, and secondly they retain the potential to differentiate into a variety of more specialized cell types (***Barry et al ,2004***).

(Fathke et al., 2004) found that bone marrow stroma contains precursor cells that are capable of differentiating along hematopoietic cell (HC) and mesenchymal cell (MC) lineages

Moreover,***Ryan et al***.,(***2005***) reported that MSCs are easy to isolate ,expand in culture and have minimal immunogenic response when allogeneic or syngeneic cells are used Given these qualities and the current barriers limiting embryonic stem cell research, MSCs have become a recent focus of interest for cellular therapy in tissue regeneration.

MSCs became an attractive therapeutic tool for regenerative medicine and tissue engineering due to threir multipotency ,easy isolation,culture,highly expansive potential and immunosuppressant properties ***(FU and li.,2006***).

Later on***,Wu et al.(,2007).*** Reported that adult bone marrow-derived hematopoietic stem cells (BM-HSCs) have long been recognized to give rise to all blood cell lineages including: red blood corpuscle, B lymphocytes, T lymphocytes, natural killer cells, neutrophils, basophils, eosinophils, monocytes, macrophages, and platelets. In contrast to Hematopoietic stem cells (HSCs) that are unable to Trans differentiate into other cell types, bone marrow-derived mesenchymal stem cells (BM-MSCs) are self-renewing for clonal precursors of non hematopoietic tissues **.**

Altough ESCS have a great capacity for self –renewel and plasticity, their use is limited due to scientific, political and ethical considerations (***Fu and LI ,2009***).

Corneal alkali burn and its sequale

Corneal alkali burn often causes extensive damage and results in permenant visual impairment.Recurrent epithelial erosions, corneal ulceration, severe stromal inflammation, and neovascaularization are common clinical consequences of alkali burn (***Kao* et *al.,1996).***

Treatment of the severe disorder of the ocular surface remains a challenge. Recent studies have illustrated that BM-MSCs participate in severe ocular surface disease

regeneration.(**Schwatz et *al*.,*1996***).

Although reports of the transplantation of corneal epithelial stem cells are promising.The feasibility and the long term efficacy have been questioned **(Hollan et al .,1996)**

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AIM OF THE WORK

The aim of the current study was to isolate and culture bone marrow-derived mesenchymal stem cells (BM-MSCs) from adult male rabbits and to evaluate their benefit in healing of corneal alkali burn .


بسم الله الرحمن الرحيم
وَأَنْزَلَ اللهُ
عَلَيْكَ الْكِتَابَ
والْحِكْمَةَ
وَعَلَّمَكَ مَا لَمْ
تَكُنْ تَعْلَمُ
وَكَانَ فَضْلُ
اللهِ عَلَيْكَ
عَظِيمًا
صدق الله العظيم
سورة النساء آية
(113)

Abstract

Introduction: Corneal alkali burn often causes extensive damage and results in permenant visul impairment Treatment of the severe disorder of the ocular surface remains a challenge.Mesenchymal stem cells (MSCs) are non haematopoietic progenitor cells with multi lineage differentiation potential that could be isolated from bone marrow and other tissues.

Aim;The aim of this study was to assess the role of stem cells in the healing of corneal alkali burn in adult male NewZealand rabbits.

Materials and methods: Forty five male New Zealand rabbits were used in this study. The animals were divided into four groups. Group I; the control group. Group II; bone marrow was collected from both tibia and femur of each rabbit. Group III; corneal alkali burn was created . Group IV; rabbits with corneal alkali burn treated with BM-MSCs. Both wounds of groups III and IV were examined after fourteen and twenty eight days.

Results: The corneal epithelium of animals subjected to alkali burn(group III).showed marked lesions. Vascularization ,cellular infiltration and irregularty of the collagen fibers were seen in the substantia propria .Moer obvious changes were recorded after 28 days following alkali burn.Increase in the thickness of the Descemet's membrane was also noticed. Pronouced improvement was noticed after MSCs injection.Minimal epithelial lesions could be detected after 14 days.More obvious improvement was detected after 28 days .

Conclusion: It was concluded that BM-MSCs could be effectively used in the treatment of corneal alkali burn.

Keywords: BM-MSCs, corneal alkali burn

List of Abbreviations:

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BMSCs	Bone marrow-derived mesenchymal stem cells.
CD44	Cluster of differentiation 44.
DMEM	Dulbecco's Modified Eagles medium.
ESCs	Embryonic stem cells.
FBS	Fetal bovine serum.
HSCs	Hematopoietic stem cells.
MSCs	Mesenchymal stem cells.
PBS	Phosphate buffer saline.

List of Abbreviations

Activin B ……..bBbB
Adenosine tri-phosphate……...ATP
Analysis of variance…….ANOVA
Angiopoietin…….Ang-1
Basic fibroblast growth factor……...bFGF
Bone marrow stromal cells……..BMSCs
Bone marrow–derived cells ……..BMDCs
Bone marrow-derived endothelial progenitor cells……BMD-EPC
Bone marrow-epidermal cells ……..…….BM-EC
Bone marrow-Mesenchymal stem cells…….……BM-MSCs
Carbon dioxide……CO_2
Chemokine receptor…….....CXCR
Cluster of differentiation…….CD
Corneo-desmosomes……...CD
Deoxyribonucleic acid……DNA
Dermal fibroblasts…….……....DF
Desmocollin-1……...….DSC1
Desmoglein 1…….DSG1
Diabetes mellitus ……....…….DM
Dicilitre…….dl
Dominant-negative activin receptor IB mutant …...….dnActRIB
Dulbecco's modified Eagles medium……..DMEM
Embryonic stem cells……..……ESC
Endothelial Nitric Oxide Synthase…….eNOS
Enhanced green flourscent protein……..EGFP
Epidermal growth factor ……..…….……EGF
Ethylenediaminetetraacetic acid…….EDTA
Extracellular matrix.…….……ECM
Fasting blood sugar……FBS
Fibroblast growth factor……..……FGF
Glial fibrillary acidic protein……..……GFAP

Gluccocorticoids……GC
Glycoprotein……..gp
Green fluorescent protein……GFP
Haemopoeitic stem cells……..……HSC
Hair follicle…….HF
Hematopoietic stem cell transplantation ……HSCT
High power field……HPF
Human breast milk stem cells…….hBSCs
Human mesenchymal stem cells……....hMSCs
Hypoxia induced factor 1alpha……...…HIF1-α
I.V…….intravenous
Inducible nitric oxide Synthase……iNOS
Insulin growth factor ……..IGF
Interfollicular epidermis……..……IFE
Interleukin…….……IL
Isoform of Nitric Oxide Synthase……iNOS
Keratinocyte growth factor…….……..……KGF
Lamellar bodies……..…….……..LB
Least significant difference…….……..…..…....LSD
Lymphocyte function-associated antigen 1…...……LFA-1
Mesenchymal stem cells……MSCs
Metalloproteinases…….MMP
Microlitre……....µl
Micrometer…….....µm
Microtubule associated protein …….……MAP
Milligram……mg
Millilitre……..ml
Mitogen activated protein kinase 5…….……MAPK5
Monocyte chemotactic protein-1……..……..MCP-1
Natural moisturizing factor …….…..NMF
Neuronal nuclear antigen……..…….NeuN
Nitric oxide……NO
Papanicolaou stain…….……Pap
Phosphate buffered saline…….…...…….PBS
Placental growth factor …….PLGF
Plasminogen activator inhibitor-1…….……PAI-1

Plasminogen-deficient ……..PG_/_
Platelet derived growth factor ……..PDGF
Probability of error or chance…….……..p
Profilaggrin endopeptida……..…PEP-I
Progenitor Cell ……PC
Rate per minute……rpm
Reactive oxygen species……..ROS
Stem cells……SC
Stratum compactum……..……...SC
Stratum corneum……..SC
Streptozotocin diabetic…….STZ-d
Stromal-derived factor-alpha……SDF1-α
The International Diabetic Federation ……..IDF
Tight junctions……...TJ
Transforming growth factor b…….……...TGF-b
Transmission electron microscope……...TEM
Tumour necrosis factor x……TNFx
Tumour necrosis factor α……TNFα
Umbilical cord derived mesenchymal cells…….UCMSCs
Umbilical cord derived MSCs…….……..……..…UCDMSCs
Uracil tri-phosphate…….UTP
Vascular endothelial growth factor…….VEGF
World Health Organization……WHO

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