istological Evaluation of Bone Marrow Derived Stem Cell Therapy on Experimentally Induced Osteoarthritis in Albino Rats' Knee Joint

Thesis

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التقييم الهستولوجي لتـأثير العلاج بالخلايا الجذعية النخاعية في الالتهاب العظمي المفصلي المستحدث تجريبيا في مفصل الركبة للفئران البيضاء

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Abstract

Aim of work: This work aims to evaluate the efficacy of intra-articular injection of bone marrow derived-mesenchymal stem cells (MSCs) in treatment of mono-iodoacetate (MIA) induced osteoarthritis in rat knee joint monitored by histological and immunohistochemical methods.

Materials and methods: this study was carried out on 45 adult male albino rats. They were classified into 4 groups: group I (control group), group II (osteoarthritic group) in which rats received 1 mg of MIA and sacrificed after 2 weeks (in subgroup IIa) and after 4 weeks (in subgroup IIb), group III (stem cell treated group) in which rats received MSCs 2 weeks after MIA injection (in subgroup IIIa) or 4 weeks after MIA (in subgroup IIIb) and sacrificed 2 weeks later and group IV (untreated group) in which rats received PBS 2 weeks after MIA injection (in subgroup IIIa) or 4 weeks after MIA (in subgroup IIIb) and sacrificed 2 weeks later. Sections were taken from rats' knee joints and stained with Hematoxylin and Eosin, toluidine blue, immunohistochemichal stains for collagen type II. Sections were examined by light microscopy & immunofluroscent microscopy for PKH26-labeled MSCs. The mean articular cartilage (AC) thickness, optical density of cartilage matrix proteoglycan and area percent of collagen type II immunoreactivity were measured using image analyzer and statistically analyzed.

Results: Sections of group II showed features of osteoarthritis in the form of disorientation & degeneration of chondrocytes, Exfoliation of the superficial part of AC with many osteoblasts and osteoclasts were noticed with significant reduction in cartilage thickness, optical density of AC matrix and area % of collagen type II immunostaining compared to control. Sections of AC in the MSCs treated group showed apparent improvement of the osteoarthritic features in the form of increase in the AC thickness with normal zonation and proliferation of chondrocyte with significant increase in optical density of AC matrix and collagen type II immunoreactivity. PKH26-labeled cells were found in the AC of groups III. Sections of the AC in group IV (untreated group) showed

deterioration of the OA features with complete loss of zonation and many degenerated chondrocytes.

Conclusion: Treatment with bone marrow derived mesenchymal stem cells (MSCs) could significantly treat the osteoarthritic changes induced by MIA in rat knee joint.

Key words:

Knee joint- AC- osteoarthritis- MIA- MSCs- PKH26.

Contents

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List of Abbreviations

AC	Articular Cartilage.
ACL	Anterior Cruciate Ligament.
ANOVA	Analysis of variance.
ASCs	Adult Stem Cells.
BM	Bone Marrow.
BMP	Bone Morphogentic Protein.
BSS	Balanced saline solution.
CILP	Cartilage Intermediate Layer Protein.
CRP	C-reactive protein.
CT	Computed tomography.
DAB	Diaminobenzedine.
DJD	Degenerative Joint Disease.
DMEM	Dulbecco's Modified Eagle's medium.
DNA	Dioxyribonuclic Acid.
DPBs	Dulbecco's phosphate Buffered Saline.
ECM	Extracellular Matrix.
EDTA	Ethylene Diamine Tetra Acetate.
ESCs	Embryonic Stem Cells.
FBS	Fetal bovine serum.
FDA	Food & drug administration.
FRZB	Frizzled Related Protein.
GAGs	Glycosaminoglycans.
HA	Hyaluronic Acid.
HBSS	Hanks' Balanced Salt Solution.
HSCs	Hematopoietic stem cells.
IGF	Insulin- like growth factor.
IL	Interleukin.
iPSCs	induced pluripotent stem cells.
IVF	in-vitro fertilization.
LCL	Lateral Collateral Ligament.

LFA-1	Lymphocyte function- associated antigen 1.
MCL	Medial Collateral Ligament.
MIA	Mono-iodoacetic acid.
MRI	Magnetic resonance imaging.
MSCs	Mesenchymal Stem Cells.
MMP	Matrix Metalloproteinase
NSAIDS	Non steroidal anti-inflammatory drugs.
OA	Osteoarthritis.
OST	Osteophyte.
PBS	Phosphate buffer saline.
PCL	Posterior Cruciate Ligament.
PDGF	Platelet derived growth factor.
SC	Stem cell.
SOX9	Sry-related HMG box-9.
SPSS	Statistical package for the social sciences.
TRAP	Tartrate-resistant acid phosphatise
TGF β	Transforming Growth factor-beta.
TNF ALP6	Tumor Necrosis Factor alpha-induced Protein 6.
TNF-α	Tumor Necrosis Factor Alpha.
US	Ultrasonography.

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Introduction:

Osteoarthritis (OA) is a degenerative joint disease characterized by pain and dysfunction which represents a major cause of disability worldwide ***(Buckwalter and Martin, 2006).***

The disease is characterized by chronic joint pain with various degrees of joint deformity and destruction of the articular cartilage. OA commonly affects weight-bearing joints. There is a decrease in proteoglycan content, with subsequent reduction in the intercellular water content in the cartilage matrix. Chondrocytes also play an important role in the pathogenesis of osteoarthritis. By producing interleukin-1 (IL-1) and tumor necrosis factor α (TNF-α), the production of metalloproteinases is stimulated, whereas synthesis of type II collagen and proteoglycans by the chondrocytes is inhibited ***(Ross and Pawlina, 2011).***

Although articular cartilage is a metabolically active tissue, the chondrocytes in the matrix have a relatively slow rate of turnover. The tissue itself lacks a blood supply to support repair and remodeling ***(Chang et al., 2005).***

OA is characterized by a catabolic and inflammatory joint environment. To this date, no drugs are available to structurally modify OA processes or prevent progression of the disease ***(Harvey and Hunter, 2008).***

The available treatment strategies of OA are different surgical procedures including debridement, drilling, osteochondral transplantation, autologous perichondral and periosteal grafts and autologous chondrocyte implantation but these procedures were shown to be poor and restricted to relieving the symptoms ***(Hunziker et al., 2002)***.

Khan et al., (2010) stated that the management of cartilage defects is currently not ideal with evolving techniques and technologies. The use of stem cells has the potential to replace the damaged cartilage with hyaline cartilage.

Mesenchymal stem cells (MSCs) have emerged as a candidate cell type with great potential for cell-based articular cartilage repair technologies. MSCs can be isolated from a variety of adult tissues including bone marrow, adipose tissue and synovial membrane ***(Jorgensen et al., 2004).***

MSCs are multipotent cells present in adult bone marrow. They can replicate as undifferentiated cells and have the potential to differentiate to lineages of mesenchymal tissues, including cartilage, bone and fat. MSCs are therefore a promising cell source for the regeneration of cartilage, as they possess chondrogenic differentiation potential and are easy to obtain in high numbers ***(Singh et al., 2014)***.

Agung et al., (2006) demonstrated the possibility of intra-articular injection of MSCs for the treatment of intra-articular tissue injuries including anterior cruciate ligament, meniscus or cartilage. If this treatment option is established, it can be minimally invasive compared to conventional surgeries for these tissues. MSCs have chondrogenic potential and are experimentally being implanted in focal cartilage defects, showing promising results ***(Van Buul et al., 2012).***

The intra-articular injection technique involves administration of a suspension containing therapeutically active stem cells. The procedure of intra-articular injection itself is abundantly described and has been established for decades. It is technically easy to perform because it is less invasive, and is suitable for outpatients. Also, the risks associated with stem cell injections are less severe compared with an open surgical treatment ***(Orth et al., 2014)***.