Stem cell based therapy in experimental osteoarthritis

Thesis

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Abstract

Osteoarthritis is a degenerative joint disease generally characterized by progressive cartilage degeneration,. Stem cell therapy holds a great promise for the repair of injured tissues and organs, including OA. The present study aims to detect the effect of mesenchymal stem cells on osteoarthritis in rabbits and also to detect its possible mechaism of action.. The study was carried on 20 New Zealand White rabbits. They were divided into 6 groups as follow: control group, phosphate Buffer group injected intra articularly(IA) by phosphate buffer, stem cell group recieved stem cells, the arthritis group induced by LPS, LPS & Stem cell treatment group injected by LPS then treated after one week by stem cells (IA), LPS &stem cell prophylactic group**,** rabbits were injected prophylactically by stem cells then by LPS. The results of the present study showed significant increase CRP and TNF-α in OA group compared to control group however when this group is treated with MSCs there was significant decrease in these parameters. Also, there was a significant decrease in TGFβ and CTGF in OA group compared to control group and increased when treated with MSCs. Histopathological results shows improve of OA after stem cell therapy Conclusion, MSC can exert beneficial effect on OA possibly by a paracrine mechanism through regulation of inflammatory cytokines such as TNF-α and growth factors such as TGF-β and CTGF.

Key Words:

Mesenchymal stem cells

Osteoarthritis

Paracrine action of MSCs

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List of abbrevations

AMH	**Anti mullerian hormone**
BM	**Bone marrow**
BMPs	**Bone morphogenetic proteins**
BMI	**Body mass index**
BMCs	**Bone marrow cells**
BMSCs	**Bone marrow stem cells**
bp	**Base pair**
bFGF	**Basic fibroblast growth factor**
BIO	**6-bromoindirubin-3'-oxime**
BM-MSC	**Bone marrow mesenchymal stem cells**
COX	**Cyclooxygenases**
CFU	**Colony forming units**
cDNA	**Complementary DNA**
CXCR4	**Chemokine receptor type 4**
CTGF	**Connective tissue growth factor**
CD	**Cluster of differentiation**
CFU-F	**Fibroblastoid colony forming unit**
CB	**Cord blood**
CMV	**Cytomegalovirus**
DNA	**Deoxyribonucleic acid**
DC	**Dendritic cells**

DMEM	**Dulbecco's modified Eagle's medium**
dNTPs	**Deoxynucleotide triphosphate**
ECM	**Extracellular matrix**
EBV	**Epstein-Barr virus**
ES cells	**Embryonic stem cells**
EB	**Ethidium Bromide**
EULAR	**European League Against Rheumatism**
EGF	**Epithelial growth factor**
Fms	**Fetal membranes**
FBS	**Fetal bovine serum**
FOXP3	**Forkhead box P3**
GVHD	**Graft-versus-host disease**
GDFs	**Growth and differentiation factors**
G-CSF	**Granulocyte-colony stimulating factor**
GTC	**Guanidine thiocyanate**
HPRI	**Human Placental Ribonuclease Inhibitor**
HSCs	**Hematopoietic stem cells**
HPC	**Hematopoietic progenitor cell**
HSC cells	**Hematopoietic stem cells**
HLA	**Human leukocyte antigen**
hUC-MSCs	**Human umbilical cord-derived mesenchymal stem cells**
IL-1	**Interleukin-1**

IFNγ	**Interferon-gamma**
IFNβ	**Interferon-β**
IDO	**Indoleamine 2,3-dioxygenase**
iNOS	**Inducible nitric-oxide synthase**
ICM	**Inner cell mass**
ICAM-1	**Inter-Cellular Adhesion Molecule 1**
IDO	**Indoleamine 2,3-dioxygenase**
KS	**Keratan sulfate**
LPS	**lipopolysaccharide**
LFA-1	**lymphocyte function–associated antigen 1**
MSC	**Mesenchymal stem cells**
MTP joints	**Metatarsophalangeal joint**
MMPs	**Matrix metalloproteinases**
MHC	**Major histocompatibility complex**
M-MLV	**Moleny – Murine Leukemia virus**
MBCs	**Mature blood cells**
MMP	**Matrix metalloproteinase**
MT1	**Membrane type 1**
MI	**Myocardial Infarction**
MAPCs	**Multipotent adult progenitor cells**
NSAID	**Non Steroidal Anti inflammatory Drug**
NO	**Nitric oxide**
NK	**Natural killer**

OA	**Osteoarthritis**
PIP joints	**Proximal interphalangeal joint**
PG	**Prostaglandins**
PDGF	**Platelet derived growth factor**
PCR	**Polymerase chain reaction**
RT-PCR	**Reverse transcriptase PCR**
RT	**Reverse transcriptase**
RNA	**Ribonucleic acid**
ROS	**Reactive Oxygen Species**
SDF-1	**Stromal-derived factor 1**
SCNT	**Somatic cell nuclear transfer**
SCF	**Stem cell factor**
Treg	**Regulatory T cells**
TIMP	**Tissue inhibitors of metalloproteinases**
TNF α	**Tumor necrosis factor alpha**
TENS	**Transcutaneous Electrical Nerve Stimulation**
TGF-β	**Transforming growth factor beta**
TMB	**Tetramethyl benzidine**
Th	**T-helper**
Taq	**Thermus aquaticus**
TAE	**Tris-Acetate EDTA**
UCB	**Umbilical cord blood**
UCBT	**Umbilical cord blood transplantation**

VEGF	**Vascular endothelial growth factor**
VCAM	**Vascular cell adhesion molecule**
VLA-4/5	**Very late antigen 4/5**
WBC	**White Blood Cell**

Introduction and Aim of the Work

Mesenchymal stem cells (MSCs) have the capacity to differentiate into a variety of connective tissue cells ***(Owen et al., 1988)*** including bone, cartilage, tendon, muscle, and adipose tissue ***(Colter et al., 2001).*** These cells may be isolated from bone marrow with ease and expanded in culture through many generations, while retaining their capacity to differentiate when exposed to appropriate signals.

The isolation of these cells from adult tissues raises opportunities for the development of novel cellular therapies without the ethical considerations associated with the use of embryonic stem cells. Multipotent cells have been isolated from various mesenchymal tissues in adults, including skeletal muscle, fat, and synovial membrane as well as hematopoietic, neural and hepatic tissues***(Deasy et al., 2001 and Weissman 2000).***

Because of their multipotentiality and capacity for self-renewal, adult stem cells may represent units of active regeneration of tissues damaged as a result of trauma or disease ***(Gage , 2000).*** In certain degenerative diseases such as osteoarthritis (OA), stem cells are depleted and have reduced proliferative capacity and reduced ability to differentiate***(Murphy et al., 2002).*** The systemic or local delivery of stem cells to these individuals may therefore enhance repair or inhibit the progressive loss of joint tissue.

OA is characterized by degeneration of the articular cartilage, with loss of matrix, fibrillation, formation of fissures, and ultimately complete loss of the cartilage surface. Other articular tissues are also affected, including the subchondral bone, ligaments, joint capsule, synovial membrane, and periarticular muscles. Although OA affects a large proportion of the

population, there are few, if any, effective therapies available today that alter the pathobiologic course of the disease ***(Felson et al., 2000).***

Several tissue-engineering approaches have been used for the repair of joint lesions. For example, the fixation of implanted chondrocytes beneath a sutured flap of ectopic tissue, such as periosteum, has been widely used for the treatment of cartilage defects ***(Brittberg et al., 2001)***. Other approaches have centered on the use of cells loaded on a scaffold and delivered to a lesion site. These methods are applicable to the repair of focal defects of defined dimensions, but not to the treatment of complex lesions that cover a large surface area of the joint and that may be associated with severe and progressive inflammatory conditions such as OA . MSCs have the capacity to repair fibrillated cartilage by a direct resurfacing of the articular cartilage or act to preserve subchondral or trabecular bone structure-associated mechanical integrity of the joint ***(Kavalkovich et al., 2000 and Henry, 2004)***

Aim of the work

The present study aims to detect the effect of mesenchymal stem cells on osteoarthritis in rabbits and also to detect its possible mechaism of action.

Osteoarthritis

Definition of Osteoarthritis:

Osteoarthritis is a clinical syndrome of joint pain and dysfunction, which not only affects the the articular cartilage, but also involves the entire joint, including the subchondral bone, ligaments, capsule, synovial membrane and periarticular muscles ***(Brandt, 2001a).*** Ultimately the articular cartilage degenerates with fissures, ulceration and progressive loss of proteoglycans, leading to tissue destruction ***(Venkatesan et al., 2004).*** It occurs frequently in the hand, foot, knee, spine and hip joint and rarely in the ankle,wrist,elbow, and shoulder, the most common risk factors are age, excessive joint loading, and joint injury.

In spite of being common in the elderly, it also affects young people. The disease symptoms are debilitating and causes physical impairment ***(Buckwalter et al., 2004).***

The American College of Rheumatology categorizes OA as a heterogeneous group of conditions that lead to joint signs and symptoms associated with defective integrity of articular cartilage, in addition to related changes in underlying bone and joint margins ***(Hicks et al., 2001).***

Epidemiology of Osteoarthritis:

Osteoarthritis can be defined epidemiologically (using radio graphic criteria) or clinically (using radio graphs and clinical symptoms/ signs). Using radio graphic criteria, 30% of individuals between the ages of 45 and 65 are affected, and more than 80% are affected by their eighth decade of