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Mesenchymal Stem Cells for Treatment of Tendon and Ligament Injuries-clinical Evidence

Suad Trebinjac[1], Mourad Gharairi[2]

[1]Czech Rehabilitation Hospital, FIFA Medical Center of Excellence, St - Deira. Dubai, UAE
[2]Al Garhoud Private Hospital1. Dubai, UAE

Corresponding author: Associate Professor Suad Trebinjac, MD. PhD. FIFA Medical Centre of Excellence Dubai. UAE. E-mail: s4965@hotmail.com. ORCID ID:000-0001-6927-346X.

ABSTRACT

Introduction: Sport injuries, most of the time affect muscles, tendons, ligaments, cartilage and bones and range from very mild to severe, prompting different therapeutic approaches. Overuse is the most common cause of sports injuries and half of those injuries affect tendon, tendon sheet and tendon insertion to the bone. The number of ligament injuries, particularly anterior cruciate ligament (ACL) increasing. Aim: We were searching PubMed, Google Scholar and Medline focusing on human clinical studies related to stem cell therapy for tendinopathies and ligament injuries. Considering small number of published articles, we accepted papers with all level of evidence without following strict PRISMA guidelines. **Results:** The number of studies related to ligament injuries is very low compared to tendon injuries. In human clinical trials there have been only a few studies published so far. In double blind randomized control trial (RCT) Wang and coauthors combined allogenic precursor mesenchymal stem cells (MPC) with hyaluronic acid (HA) and compared with hyaluronic acid alone in 17 patients underwent ACL reconstruction. Intensity of pain and quality of life were assessed by Knee Injury and Osteoarthritis Outcome Score (KOOS) and SF-36v2 scores. A width of joint space, volume of cartilage and bone were recorded by magnetic resonance imaging (MRI). Moderate arthralgia and swelling were detected within 24 hours after the injection in 4 out of 11 patients in the group receiving MSC+HA. In the group receiving only HA, there were no adverse reactions. The signs of slowing down of regenerative process were presented on MRI by preserving joint space and reducing degradation of cartilage volume. **Conclusion:** Clinical application of MSCs for treatment of tendon and ligament injuries might be good alternative option for athletes. Published clinical studies confirmed clinical improvement and integrity of impaired tissues. However, RTCs are needed to confirm real potential of cell therapy and their advantages comparing to other treatment options.

Keywords: Tendon and Ligament injures, Mesenchymal Stem Cells.

1. INTRODUCTION

Professional and recreational sport practice is increasing but unfortunately so is the rate of sports injuries as mentioned in many epidemiological studies (1, 2, 3). Some injuries can cause serious impairment with prolonged pain and functional restrictions. One example is secondary knee osteoarthritis, common in retired football players reaching more than 27 percent (4). Aside from medical consequences, the economic burden related to costs of conservative treatment, surgery and rehabilitation is very high.

Sport injuries, most of the time affect muscles, tendons, ligaments, cartilage and bones and range from very mild to severe, prompting different therapeutic approaches. Overuse is the most common cause of sports injuries and half of those injuries affect tendon, tendon sheet and tendon insertion to the bone (5). The number of ligament injuries, particularly anterior cruciate ligament (ACL) increasing (6).

Standardized treatment including rest, ice, compression, elevation, short course of pain modulators and anti-inflammatory medications provides relief of symptoms without complete healing (7). It is known that poor vascular supply of tendons and ligaments slows down healing processes and extends time of full recovery. An injured player wants to return to sport as soon as possible because prolonged rest has a detrimental effect on the body.

The concept of regenerative medicine attracts the attention of researchers and clinicians offering a promising potential to regenerate the damaged tissue rather than to alleviate symptoms. In the last 30 years it spread through-

out the different medical specialties and recently it made a strong advancement in sports medicine as well.

What is orthobiologics ?

Orthobiologics is a new term ascribed to the use of different biological substances to promote better healing of musculoskeletal conditions (8). Their effect is based on mimicking of the body's natural healing development by stimulating the cascade of physiological reactions ensuing after the injury. The process of regeneration is extremely complex and still not well understood. Some injuries heal spontaneously without specific interventions. Others never heal even with intensive treatment because of limited regenerative potential. It is well known that the healing of muscles is much faster than healing of tendons or ligaments. The results of animal studies are not always applicable to humans. Finally, many factors like age, sex, general health, genetics, nutrition etc. have an impact on healing outcome.

The most common biological substances used for healing of skeletal system are mesenchymal stem cells (MSCs), blood derivates (Platelet rich plasma-PRP) and recently, extracts of placenta. In this article we will focus mainly on the effect of cell therapy, particularly mesenchymal stem cells on sports injuries.

Mesenchymal stem cells

Stem cells are undifferentiated cells with potential to differentiate under certain conditions into other type of cells. Theoretically, this allows them to regenerate damaged part of the body and eventually to restore impaired functions. Contrary to previously held opinions, the stem cells can be found in many tissues, raising the question of most efficient source of cells.

Based on origin, stem cells can be classified as an embryonic, fetal, adults and induced pluripotent (9). Considering their differentiation potential, they are defined as a totipotent, unipotent, oligopotent, multipotent and pluripotent. Embryonic cells can differentiate into all germ layers; endoderm, ectoderm and mesoderm with the ability to produce any type of cells. However, their use is restricted due to ethical issues. It is confirmed that they might have teratogenic effect. Collection of these cells would result in a fatal effect on embryos (10, 11).

The most commonly used in clinical practice are adult multipotent cells of mesenchymal origin. They are recognized among the other cells by identification of protein markers on their surface. Mesenchymal stem cells (MSCs) have expressed following markers: CD29, CD44, CD90, CD49a-f, CD51, CD73 (SH3), CD105 (SH2), CD106, CD166, and Stro-1 and not expressed CD45, CD34, CD14 or CD11b, CD79a orCD19 and HLA-DR. Other features defining stem cells are adherence to plastic and potential for self-renewal and differentiation. (12).

MSCs are able to differentiate into mesodermal lineage forming osteocytes, chondrocyte and adipocytes (13). They are most commonly harvested from the patient's own body (autologous cells), mainly from bone marrow (pelvic area) and adipose tissue.

The concentration of stem cells in bone marrow is very low, only about 0.001–0.01 % (14), as opposed to that in fat tissue where it ranged from 1-10% (15). With aging it decreases even more. However, the other type of cells existing in bone marrow or adipose tissue have also a certain role in the healing process.

The mechanism of action of MSCs is very complex and it is subject to intensive research. Different theories are proposed, none of which have been conclusively supported. The initial hypothesis that undifferentiated stem cells can migrate to the injured area, engraft themselves and start the process of differentiation is quite simple to explain the complexity of cell communication with their environment and the role of different bio molecules involved in this process (16). Considering the short life of stem cells, it is unlikely that direct differentiation is the main mechanism of action. Stem cells release cytokines, growth factors and extracellular vesicles providing communication between cells through signaling system. Aside from this, they activate the host stem cells triggering their self-renewal and differentiation. Stem cells have an immunomodulatory and anti-inflammatory effect. They suppress catabolic mediators (IL1, TNFα. nitric oxide) and convert pro inflammatory M1 macroglobulin to immunosuppressive M2 by the action of PGE2. Also, stem cells facilitate angiogenesis and prevent apoptosis and fibrosis (17).

Effect of MSCs on sports injuries

The number of preclinical and clinical studies related to the effect of cell therapy increasing. Different components were used for therapeutic purpose; skin fibroblast, tenocytes, chondrocytes and stem cells from different sources. The route of applications was either direct injections to the lesion or by using biomaterials (scaffold) or as a part of surgical procedures. Most investigations explore the effect on knee cartilage, but number increasing for tendons and ligaments (17-28).

2. MATERIAL AND METHODS

We were searching PubMed, Google Scholar and Medline focusing on human clinical studies related to stem cell therapy for tendinopathies and ligament injuries. Considering small number of published articles, we accepted papers with all level of evidence without following strict PRISMA guidelines.

3. RESULTS

There were 1 RCT, 2 non RCTs and 8 non controlled studies related to treatment of tendon injuries. The all studies reported clinical improvement, and in majority of them structural changes were verified by MRI and US (Table 1).

The number of studies related to ligament injuries is very low compared to tendon injuries. In human clinical trials there have been only a few studies published so far. In double blind randomized control trial (RCT) Wang and coauthors combined allogenic precursor mesenchymal stem cells (MPC) with hyaluronic acid (HA) and compared with hyaluronic acid alone in 17 patients underwent ACL reconstruction. Intensity of pain and quality of life were assessed by Knee Injury and Osteoarthritis Outcome Score (KOOS) and SF-36v2 scores.

Authors	Type of Injury	Study design	Source of MSCs	Clinical Outcome	Structural changes	Follow up	Level of evidence
Usuelli et al. (18)	Achilles tendinopathy	RCT SVF versus PRP	SVF	On short term (15-30 days) better for SVF, mid term-6 months, no difference	MRI: no significant difference	6 months	1
Stein et al. (19)	Achilles tendon rupture	Case series	BMAC + arthroscopy	At mean follow up of 29.7±6.1 months, no re rupture of tendon		24 months	4
Tate-Oliver K. and Alexander RW (20)	Achilles tendon- partial thickness tear	Case series	SVF + PRP	Return to full activities after 12 weeks	US: tendon structure become normal after 12 months	3-4 years	4
Kim YS. et al. (21)	Rotator cuff tear	Non RCT- arthroscopy versus ASCs	ASCs	Reduced re tear in ASCs group – 14.3 %, comparing with control group -28.5%	MRI: complete healing in ASCs group 85.7% and in control group 71.4%	21 months	3
Hernigou P. et al. (22)	Rotator cuff tear	Non RCT- arthroscopy versus BMC	BMC	Tendon intact in 87% in BMC group versus to 44% in control group after 10 years	MRI and US: 100% healing of tendon in BMC group versus 67% in control group after 6 months.	10 years	3
Gomez E.et al. (23)	Rotator cuff complete tear	Pilot study: BMMC added as augmentation to acromioplasty	BMMC	Pain reduction and functional improvement	MRI: tendon integrity restored	More than 12 months.	4
Centeno CJ. et al. (24)	Rotator cuff tear < 1.5 cm and OA	Case series	Hypertonic dextrose +PRP +BMAC	Pain reduction and functional improvement		Up to 2 years.	4
Lee SY. et al. (25)	Lateral epicondyle tendinopathy	Case series	ASCs mixed with fibrin glue	Pain reduction and functional improvement	US : structural defects significantly decrease	52 weeks	4
Moon YL.et al. (26)	Lateral and/or medial epicondylitis	Case series	Arthroscopy with BMC	Pain reduction and functional improvement	US: evidence of tendon healing.	6 months	4
Sing A.et al. (27)	Lateral epicondylitis	Case series	BMC + Lidocaine	Functional improvement		12 weeks	4
Pascual-Garrido C.et al. (28)	Patella tendinopathy	Case series	Non expanded BMC	Functional improvement		5 years	4

Table 1. The effect of MSCs on tendons healing. Abbrevations: SVF – stroma vascular fraction, PRP – platelet rich plasma, BMAC – bone marrow aspirate concentrate, BMC- bone marrow concentrate, ASCs- Adipose derived stem cells, BMMC-bone marrow mononuclear cells, UCLA-The University of California Los Angeles shoulder score, OA- osteoarthritis.

A width of joint space, volume of cartilage and bone were recorded by magnetic resonance imaging (MRI). Moderate arthralgia and swelling were detected within 24 hours after the injection in 4 out of 11 patients in the group receiving MSC+HA. In the group receiving only HA, there were no adverse reactions. The signs of slowing down of regenerative process were presented on MRI by preserving joint space and reducing degradation of cartilage volume (29).

In another case series study Centeno and coauthors investigated the effect of autologous BMC combined with PRP on 29 patients with grade 1,2 and 3 ACL tear with less than 1 cm of retraction. After 36 moths 77% of patients showed functional improvement followed by MRI confirmed integrity of ligament (30).

4. DISCUSSION

Two systematic reviews published in 2017 and 2020 documented the positive therapeutic effect of orthobiologics on tendon injuries in humans. Clinical improvement and in some studies structural changes detected by MRI and US were reported (31, 32). Positive effects were maintained even 3 to 4 years after the treatment.

It is interesting that other type of cells injected into the damaged tendon can also provide long term effect. Researchers from Australia used tenocyte instead of stem cells for treatment of lateral epicondylitis. Functional and radiological improvement was confirmed in the period up to 5 years (33). Another study confirmed that dermal fibroblast can be used for treatment of patella tendinopathy. Functional and morphological improvement were observed 6 months after the treatment (34). These results raised questions about the role and possible mechanism of action of other cells in the process of regeneration of impaired tendons. The lack of comparative studies in this matter makes, thus far, inconclusive any evidence of eventual advantages with regards to stem cells.

We found only 2 studies investigating the effect of MSCs on ACL injury. In both studies additional treatment was combined with MSCs. The only one study was RCT. In this study MSCs is combined with Hyaluronic

acid. In other study patients received MSCs, dextrose and PRP injections. Clinical and MRI improvement was documented in both studies, but it is unclear what was the particular and unique effect of MSCs.

Most published articles are without control group so it is difficult to conclude whether treatment by MSCs alone is more efficient than other therapeutic modalities traditionally applied.

Future research must focus on selection of most suitable sources of MSCs, type and quantity of cells needed for regeneration of impaired tissues and controlled randomized trials with larger sample size.

5. CONCLUSION

The number of people practicing professional and recreational sports increasing all over the world. Unfortunately, it is in proportion with number of injuries. Tendon and ligament injuries are very common and known with their chronicity. Standardized treatment is mainly symptom oriented and does not provide complete healing in majority of patients. It has detrimental effect on sports carrier. Orthobiologic is a new science incorporated in different medical specialties including sports medicine. It changes the paradigm by offering regeneration of damaged structure rather than relieve of symptoms. Mesenchymal stem cells are front runner in the field of regenerative medicine with thousands of scientific papers published in the last 10 years. This manuscript presents the latest clinical results related to application of mesenchymal stem cells in treatment of chronic ligament and tendon injuries. Clinical application of MSCs for treatment of tendon and ligament injuries might be good alternative option for athletes. Published clinical studies confirmed clinical improvement and integrity of impaired tissues. However, RTCs are needed to confirm real potential of cell therapy and their advantages comparing to other treatment options.

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