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ARTICLE · MARCH 2014

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DYPJHS Volume 2, Issue 1 : Jan-Mar. 2014
D Y Patil Journal of Health Sciences

ISSN (Print) 2347-3665
ISSN (Online) 2347-8020

RESEARCH PAPER

A study of Cell Therapy Assisted Regeneration of Cartilage in Avascular Bone Necrosis.

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Abstract

Application of 'regenerative medicine' has given a new hope to surgeons for the treatment of several chronic diseases and disorders including severe orthopedic conditions. There are a myriad of orthopedic conditions and injuries that presently have limited therapeutic treatments and could benefit from new developing therapies in regenerative medicine with the help of stem cells[1]. Regenerative medicine therapies are mainly based on the applications of stem cells. Stem cells play a vital role in orthopedic treatments and the studies have shown promising results in repair of bone, tendon, cartilage including AVN, Spondylitis etc. Bone and Cartilage regeneration ability of stem cells has been demonstrated clinically; however success rate may not be same in every case and it depends on patient to patient. Several factors can be responsible for the same including patient's immune response, the type and the grade of the disease which altogether decide the fate of the treatment. In this paper we have presented some of the orthopedic case studies performed through autologous transplantation of the stem cells.

Keywords: AVN (Avascular Bone Necrosis), ECM (Extracellular Matrix), cytokines and chemokines, bone marrow mononuclear cells, adipose tissue, PRP (Platelet rich plasma)

Introduction

Stem cells- known as the building blocks of the body represent unspecialized cells, which have the ability to differentiate into different types of adult stem cells. The differentiation depends on the type of the stem cell and the niche. Niche and several signaling pathways are responsible for the differentiation of the stem cells into particular lineage of the cell[2, 3]. Broadly, stem cells are classified as 'embryonic' and 'adult stem cells'. Being truly pluripotent, embryonic stem cells (ESC) can renew indefinitely and differentiate into cells of all three germ layers thereby can regenerate a part or even a complete organ[2, 3]. Embryonic stem cells are not easy to harvest and have many ethical and legal issues associated so attempts were started to induce pluripotency in somatic cells to make them function like ESC's. Takahashi and Yamanaka in 2006 first reported the concept of induced pluripotent stem cells (iPS cells)[4]. However because of the teratogenic potential of induced pluripotent stem cells, the technique is yet in the frame of question mark for its commercial use in the mass[5,6]. However, in contrast, a variety of multipotent adult stem cells exist in almost all tissues of the organisms which reside in a specific niche in vivo where various microenvironmental cues form an intertwined signaling regulatory network that maintains stem cells fate and functions. In this niche there are different regulators such as ECM molecules (Extra Cellular Matrix) molecules, biochemical cues such as soluble growth factors and cytokines and mechanical cues such as intrinsic matrix stiffness and extrinsic forces which play a major role in deciding the fate of the stem cell.

Multipotent adult stem cells derived from bone marrow also known as mesenchymal stem cells are considered as most competent stem cells. These MSCs can be induced in vitro and in vivo to differentiate into a variety of cell lineages including bone, cartilage, tendon, muscles and other similar tissues[7]. However their differentiation into other types of tissue-specific cells, such as cardiac myoblasts, endothelial cells, hepatocytes and neural cells has also been demonstrated in experimental studies[8,9].

Stem Cells in the treatment of Orthopedic Conditions.

Mesenchymal stem cells derived from bone marrow are able to differentiate into different lineages as they come in contact with specific niches. MSCs are known to be capable of osteogenic, chondrogenic differentiation. Bone marrow is aspirated from the region of posterior superior iliac spine and the stem cells are isolated and processed to prepare the dose for the transplantation[10]. Modern day orthopedics with the use of cellular medicine for rejuvenation therapies looks promising and have overcome the traditional surgical therapies. Traditional replacement therapies involve the use of artificial joints with invasive operative procedures which take longer time to heal properly. There are numerous problems associated with the use of biological grafts including donor site morbidity, scarcity, and tissue rejection These types of problems can be solved by using stem cell transplant as they are based on less

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invasive applications of cellular medicine. Most of the orthopedic problems are because of the degeneration of the cartilage. When we transplant mesenchymal stem cells locally, these cells try to move in the micro-environment of the bones and tend to convert into osteogenic, chondrogenic cells[11]. These cells help in regenerating the damaged area by forming new hyaline cartilage and bone. In Non-unions, avascular necrosis (AVN), bone fractures and bone defects, tendinitis and cartilage defects stem cells and regenerative medicine have a definite role.

Bone fractures

Bone has a natural tendency to reform when fractured or damaged and while doing so it may show a development of the fibrous cartilage. MSCs having osteogenic potential tend to differentiate along the osteogenic pathway in response to the niche factors stimulation. Niche is usually composed of growth regulators, cell adhesion molecules, niche cells and extra cellular matrix which govern the differentiation of the stem cells[11, 12]. The process of the entire regeneration of the bone at the damaged site after the application of the stem cells can dependent on the factors like area of the dislocation or grade of the fracture. The cellular medicine with the use of grafts was studied by Fernandez et al. to study the effect of autologous bone marrow mononuclear cells (BM-MNCs) on pseudoarthrosis. They concluded that by coupling autologous BM-MNCs and allogenic bone graft could constitute and easy, safe, inexpensive and efficacious attempt to treat long-bone pseudoarthrosis[13]. These studies showed how stem cells are helpful in promoting union in cases of non-unions when they are used alone or in combination.

Stem cells in the treatment of avascular necrosis of femoral head and bony gaps

Avascular necrosis (AVN) of the femoral head is a pathologic process that results from interruption of blood supply to the bone. Loss of vascularity means the blood supply for these bones enters through very restricted spaces & there is limited collateral circulation which ultimately leads to the death of the osteocytes and collapse of the femoral head with change in shape of the femoral head associated with pain, limp and restriction of movements[14]. The rate of avascular necrosis of the femoral head is found higher in young patients following trauma, steroid intake, alcohol consumption etc. Treatment options available till date primarily focused on reducing the intra osseous pressure by drilling channels into the head through the neck. In advanced disease, replacement arthroplasty is commonly opted but now surgeons are looking forward to cellular medicine as an effective treatment over traditional surgical procedures. Recently, Wang et al. concluded bone-marrow mononuclear cells implantation as an effective procedure in patients with early-stage AVN of the femoral head[15]. The additive application of concentrated bone marrow aspirates, *ex vivo* expanded mesenchymal stem cells, holds great potential to improve bone regeneration[16]. Similarly using autologous mesenchymal stem cells from bone-marrow, Park et al and Zamzam et al have succefully treated voids (gaps) in simple bone cysts[17, 18].

Stem Cells to treat cartilage defects with scaffolds

Marcacci et al, used autologous MSCs used in combination with hydroxyl-appatite scaffolds for filling of cartilage defects and reported good integration of the grafts[19]. Cartilage once damaged has a very poor ability to repair itself so application of new chondrocytes at the damaged site may give rise to formation of new hyaline cartilage. Autologous chondrocyte transplantation have been used by Jager et al, to treat cartilage and bone defects[20]. Abrasion chondroplasty in which drill holes are made into the bone showed good results with processed cells in the combination with biodegradable gels. Wakitani et al used MSCs harvested from iliac crest in vitro and then cultured in the lab for 1 month and then transplanted them to the site of cartilage defect using collagen gel and covered the defect with a periosteal flap[21]. Similarly Buda et al, reported the use of MSC's for the treatment of osteochondral lesions of the femur and talus[22].

All above findings make it very clear that stem cells have the potential to treat several orthopedic conditions due to their ability to get differentiated into osteocytes, chondrocytes and muscle cells. Our study further shows that how patients got recovered from AVN by using autologous chondrocyte transplantation.

Materials and Methods

Here we present some of the case studies performed at the laboratory where application of stem cells in the patients of AVN of femur head were performed. A prospective randomized trial on 15 patients of different age group undergoing treatment for AVN of femur head was conducted (Table 1). All of them underwent stem cells therapy by which stem cells from their bone marrow, adipose tissue were isolated in the laboratory and later after processing were injected back in the body. After taking the follow-ups we found these patients were recovering that the success ratio of recovery depends on various factors.

AVN in basically graded at 4 stages as per the Ficat Staging

- **Stag e 0**
  - X-ray : normal
  - MRI: normal
  - clinical symptoms : nil
- **Stage I**
  - X-ray : normal or minor osteopaenia
  - MRI : oedema
  - bone scan: increased uptake
  - clinical symptoms: pain typically in the groin
- **Stage II**
  - X-ray : mixed osteopenia &/or sclerosis &/or subchondral cysts, without any subchondral lucency (crescent sign - see below)
  - MRI : geographic defect
  - bone scan : increased uptake
  - clinical symptoms: pain and stiffness
- **Stage III**
  - X-ray : crescent sign & eventual cortical collapse
  - MRI : same as Xray
  - clinical symptoms : pain and stiffness+/- radiation to knee and limp
- **Stage IV**
  - X-ray : end stage with evidence of secondary degenerative change
  - MRI : same as Xray
  - clinical symptoms : pain and limp

Table 1: No of patients treated for AVN of Femur head with Autologous Cell Therapy.

Subjects	Sex	Age	BMI	Stage	Treatment
1	M	27	24.3	1	
2	M	26	26.9	1	
3	M	45	31	3	Autologous
4	M	26	23	2	Cell
5	M	46	28.3	4	Therapy
6	M	27	25	1	
7	F	32	26	1	
8	F	47	28.3	3	
9	F	34	22	2	
10	F	26	25	2	Autologous
11	F	38	27.1	2	Cell
12	F	27	22	1	Therapy
13	F	33	26	3	
14	F	55	30.2	4	
15	F	54	29.3	4	

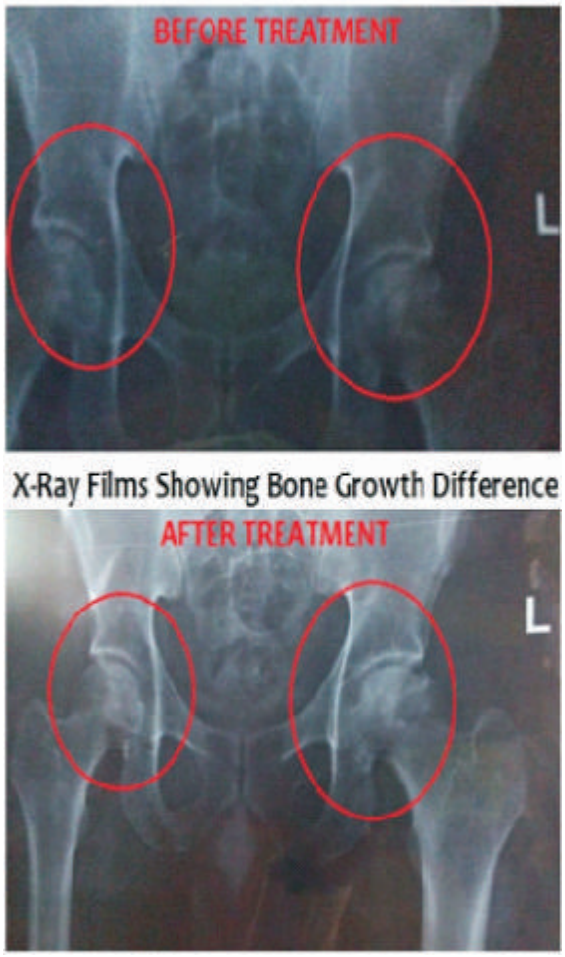
Methodology

For all 15 patients case history was taken and all were eligible for undergoing this treatment as per the standard eligibility criterion confirmed after clinical diagnosis. Consents were taken from all the 15 patients at the time of their autologous stem cells therapy. Following procedure was performed for every patient.

100 cc of bone marrow was taken from the iliac bone of the patient with the help of a bone marrow harvester in the medical facility and sample was sent to the laboratory in an aseptic sterile condition.100 cc of adipose tissue was taken from the patient's body and sample was sent to the laboratory in an aseptic sterile condition. Similarly 100 cc peripheral blood was collected from same patient's body and was sent to the laboratory in an aseptic sterile condition. All samples were processed by density centrifugation method to isolate mesenchymal stem cells from bone marrow and adipose tissue in separate batches[23]. The isolated cells from bone marrow were tested for cell count and cell viability after which cells were characterized for MSC markers CD73, CD 90, CD 105[24]. Molecular characterization of the processed cells confirmed their identity as MSC's. Similarly isolated MSC's from adipose tissue were tested for cell count and cell viability and molecular characterization with CD13, CD29 confirmed their identity[24]. Collected peripheral blood was processed and platelet rich plasma [PRP] was prepared. Bone marrow & adipose tissue derived stem cells with stromal factor were transplanted autologously along with PRP. After 3 months, reports showed the impressive results in all these cases. Follow up was taken for a year after every 3 months for individual patient. Patients showed good improvement symptomatically in following indications.

1. Clinical findings showed that Knee Pain, hip pain reduced and mobility and flexibility of joint increased than before.
2. Radiological findings: Restoration Joint space to near normalcy, articular surface was well defined, sub-articulor geodes were disappeared with signs of new cartilage formation**. (Figure 1)**

Figure 1. X-ray of a patient showing improvement in terms of bone growth difference after treatment.



Role of Niche in stem cells differentiation and proliferation

X-ray taken after 3 months follow up of the patient showed the signs of new cartilage formation which indicates the process of chodrogenesis. Studies have shown that the microenvironment - also known as 'niche' comprised of ECM (extracellular matrix), regulators, chemokines and cytokines play an important role in chondrogenic differentiation. TGFβs, IGF and FGF's have been implicated in chondrogenesis[26]. Beside the cell-cell signaling, cell-matrix interactions can also alter cell behavior and thus influence the commitment of MSC's into chondrocyte lineage[25,26]. Along with the above-discussed applications of the stem cells at our laboratory, some other conditions are also being investigated for their suitability for stem cell application including ankolysing spondylitis, bone fractures, non healing fractures, osteoarthritis, sports injuries. Though application of stem cells is well proven in orthopedic conditions, the therapy also looks promising for other neurological and other metabolic disorders and clinical for which clinical trials are being performed worldwide [27].

Summary

Stem cell therapy based on the principle of the regenerative medicine is as an attractive option for the treatment of intractable diseases. However, the use of stem cell therapy in all the conditions discussed above is subject of individual treatment. Many of these studies have shown quick good results but at the same time few cases have shown slow improvement in the conditions. As discussed earlier, this might also be linked to the selection of the patient, the type of cells used, concentration and dose of the cells used, application of cells , duration of follow up and evaluation tools among others. Many more long-term prospective randomized human trials need to have good results before one may actually recommend the use of these cells. It is certain that the future is going to be exciting with the use of stem cells. A paradigm shift from conventional mode of treatments to the novel stem cells therapy is the need of the modern healthcare. High-quality research coupled with practical applications in the cellular medicine has set a stage for successful tissue engineering in vitro. Stem cell therapy has brought in a lot of optimistic hope amongst researchers, doctors, and but obviously for patients. At the same time establishing the safety profile of these therapies is equally important to avoid complications as there is a strong demarcation between hype and hope regarding the potential use of these therapies.

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