Bone regeneration with mesenchymal stem cells

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Summary

Bone possesses the intrinsic regeneration capacity as part of the repair process in response to injury, during skeletal development or continuous remodeling throughout adult life. However, some complex clinical conditions require bone regeneration in too large quantity, and tissue engineering approach was developed to favor the regeneration of a new functional tissue. Mesenchymal stem cells (MSCs) have emerged as a promising alternative to the traditional surgical techniques. The purpose of this mini-review is to investigate the role of MSCs in clinical practice for bone regeneration, documenting the state of art and indentifying future research directions.
We performed a search of the literature on PUBMED database between 2001 and 2011 using the key words "MSC and bone regeneration". Inclusion criteria were clinical studies regarding the use of MSC in bone regeneration, for both bone repair and metabolic bone diseases, and in English language. References from selected papers were also screened.
Our search resulted in 516 articles. Among these a total of 18 articles were included: 12 case series, 5 case reports and 1 comparative studies.
MSCs represent an exciting and promising stem cell population for regeneration of bone in skeletal diseases, especially when tissue engineering or biomaterials are applied. However, literature results are limited, because of the small number and the low quality of trials, the lack of controls and the short follow-up. Researchers have to perform more high quality studies in order to document results and increase the potential of MSCs use in clinical practice, to develop a minimally invasive treatment to favor high quality bone tissue regeneration.

KEY WORDS: bone regeneration; mesenchymal stem cells; bone marrow transplantation.

Introduction

Bone is a highly vascularized and innervated connective tissue, subjected to continuous remodeling and renovation; it possesses the intrinsic regeneration capacity as part of the repair process in response to injury, as well as during skeletal development or continuous remodeling throughout adult life (1). Some complex clinical conditions require bone regeneration in large quantity, like in case of large bone defects (due to trauma, infection, tumor resection, skeletal abnormalities) or atrophic nonunions and osteoporosis (1). Other pathologic conditions in which regenerative process is compromised are osteonecrosis of the femoral head, characterized by insufficient blood perfusion and subsequent necrosis of bone in a defined area (2), osteogenesis imperfecta, a heterogeneous group of inherited disorders of the connective tissue characterized by bone fragility and other evidences of connective tissue malfunction (3), and hypophosphatasia, characterized by defective bone, teeth-mineralization and deficiency of serum and bone alkaline phosphatase (AP) activity (4).
Current therapies have several limitations and sometimes result in treatment failure, delay in normal activities recovery and therefore high costs for patients and health care system. Thus, in the last 10 years a tissue engineering approach was developed aiming to induce a new functional tissue, rather than just to implant new spare parts, and in this field stem cells have emerged as a promising alternative to the traditional surgical techniques. Among this cell population, mesenchymal stem cells (MSCs) have demonstrated their ability to function as a 'natural system of tissue repair', confirmed by clinical studies in different fields of medical application (5). Clinically, MSCs can be used as cell suspension expanded by culture or just as bone marrow concentrate (6), that differ markedly for composition. MSCs concentration in bone marrow transplants is lower respect to cultured cells. It also has to be considered that cell amplification by culture is not free from the dangers of bacterial contamination, xenogenic risk or cellular transformation (7), influencing MSC's differentiation capacities. It is necessary to perform more clinical trials to compare these techniques, evaluating clinical outcome, adverse events, and thus the best treatment modality for each bone tissue pathology.
The purpose of this mini-review is to document the available evidence on the role of MSCs in clinical practice for bone regeneration, reporting both the state of art and the future research directions.

Methods

We performed a search of the literature on PUBMED database between 2001 and 2011 using the key words "MSC and bone re-

generation". Inclusion criteria were clinical studies regarding the use of MSC in bone regeneration, for both bone repair and metabolic bone diseases, and in English language. References from selected papers were also screened.

Results

Our search resulted in 516 articles. The retrieved articles were initially screened for relevance by the title and abstract and the remaining by text study. A total of 18 articles were included: 13 are case series, 3 case report and 2 comparative studies. These trials were divided according to bone pathologies and MSCs technique employed.

Bone defect repair

Bone marrow transplantation

Wongchuensoontorn et al. (8) reported good clinical outcome in the healing of a mandible atrophic non-union with particulate bone implant and bone marrow transplantation (BMT). The fracture healed uneventfully without complications. Jager et al. (9) presented 10 patients treated with BMT. They found no complications and sufficient new bone formation within follow-up period (8.3 months). All patients returned to their profession after treatment. Previously, Hernigou et al. (10) had demonstrated the effectiveness and safety of percutaneous autologous BMT for treating 60 non-infected atrophic non-unions of the tibia. A positive correlation was noted between the volume of mineralized callus at 4 months and the number and concentration of fibroblast colony-forming units in the graft. In 7 patients in whom union was not achieved, both the concentration and the total number of stem cells injected were significantly lower than in patients with osseous union.

Cultured mesenchymal stem cells

Marcacci et al. (11) investigated the clinical use of culture-expanded osteoprogenitor cells in conjunction with porous hydroxyapatite (HA) scaffolds for the treatment of 4 patients with diaphyseal segmental defects in a tibia, a humerus, and 2 ulnar fractures. Good integration of the implants with the preexisting bone was maintained during follow-up period, and no major adverse reactions were observed. All patients showed recovery of limb function and at last follow-up (6-7 years after surgery) good integration of the implants was maintained.

Quarto et al. (12) used bone-marrow-derived stem cells cultured for three weeks and seeded on to macroporous HA scaffolds to treat nonunions. At seven months the three treated patients showed good integration of the implants. Angiographic evaluation after seven years showed vascularization of the grafted zone, which is vital for the survival and future stability of the graft. Morishita et al. (13) used an HA scaffold to differentiate MSCs ex vivo into osteoblasts to heal the defect in a patient after curettage of a tumour, which illustrated that tissue-engineered osteogenic ceramics may be an alternative to autologous bone grafting.

MSCs have also been used together with growth factors (GFs) or other treatments: the combined approach could increase the healing potential, but it makes difficult to determine which is the key factor in the healing process enhancement. For example, Warnke et al. (14) described a new bone-muscle-flap technique for the treatment of a mandibular defect. The scaffold was placed in an external titanium mesh loaded with HA blocks coated with BMP-7 and MSCs and then implanted into latissimus dorsi for growth of blood vessels and bone. After seven weeks, the constructed mandible was removed and fixed to the stumps of the original mandible. The patient regained full function of his jaw four weeks after operation.

Osteogenesis imperfecta

Bone Marrow Transplantation

Horwitz et al. (15) conducted a pilot study in 5 children with osteogenesis imperfecta (OI), who underwent engraftment of donor osteoblasts and were evaluated at 18 to 36 months of follow-up. The investigators were able to demonstrate improvement in linear growth, total body bone mineral content, and fracture rate in 3/5 children. However, with increasing time post-transplantation, growth rates slowed and eventually reached a plateau while bone mineral content continued to increase.

Cultured Mesenchymal Stem Cells

Horwitz et al. (16) used gene-marked, donor marrow-derived MSCs to treat 6 children who had undergone standard BMT for severe OI. Each child received 2 infusions of the allogeneic cells. All patients had increase in total body bone mineral content and growth velocity, and a reduction in bone fracture frequency.

Le Blanc et al. (17) transplanted allogeneic HLA-mismatched MSCs of fetal liver origin into the uterus at 32 weeks gestation to treat a fetus with severe OI. A considerably high level of donor cell engraftment (7.4%) was estimated and the transplanted cells participated in bone turnover, providing a continual source of osteoblastic progenitor cells. This report showed that the fetal liver MSCs were capable of engrafting and differentiating into bone in the human fetus, even when the recipient was immunocompetent and HLA incompatible, without provoking any graft-vs-host disease in the absence of immunosuppressive therapy.

Hypophosphatasia

Bone Marrow Transplantation

Whyte et al. (18) studied an 8-month-old child with infantile hypophosphatasia that underwent, after full myeloablation, BMT. During the first 6 months after BMT, the patient showed clinical and radiographic improvement without correction of the biochemical features of hypophosphatasia. However, clinical deterioration with skeletal demineralization occurred 13 months after BMT (21 months of age). Therefore, she further received, by intravenous infusion, bone marrow cells that had been expanded *ex vivo*. Six months later, a considerable lasting clinical and radiographic improvement was obtained, even if still without correction of her biochemical abnormalities.

Osteonecrosis

Bone Marrow Transplantation

Good clinical outcome were reported by Hernigou et al. (19) in 116 patients (189 hips) with osteonecrosis. Core decompression and autologous bone marrow transplantation were used in combination and patients were followed-up from 5 to 10 years. The outcome was determined by the changes in the Harris hip score, by progression in radiographic stages, and by the need for hip replacement. When patients were operated on before collapse (Stage I and Stage II), hip replacement was done in nine of the 145 hips. Total hip replacement was necessary in 25 hips among the 44 hips operated on after collapse (Stage III and Stage IV). This case series was subsequently extended in another study performed by the same group: 342 patients with a vascular hip osteonecrosis at early stages were described (20). Patients with low osteonecrosis stage and great number of progenitor cells transplanted were the best candidate for the treatment.

Previously, Gangji et al. (21) studied 13 patients (18 hips) with stage I or II osteonecrosis of the femoral head, comparing core decompression alone with core decompression plus BMT. BMT group

resulted in minor side effects with pain and joint symptoms reduction.

Cultured Mesenchymal Stem Cells

A series of clinical cases (22) showed that a combination of percutaneously injected autologous adipose-tissue-derived stem cells, hyaluronic acid, platelet-rich plasma (PRP) and calcium chloride may be able to regenerate bones in human femoral head osteonecrosis. The MRI data from all the patients showed significant positive changes and the measured physical therapy outcomes, subjective pain, and functional status improved.

MSCs cultured with beta-tricalcium phosphate (β-TCP) ceramics and with a free vascularized fibula were transplanted into three patients with steroid-induced osteonecrosis of the femoral head (23). Sclerotic change in the implant area were observed in two patients but at 6 months and 1 year revascularization were observed and patients were pain free. One patient presented preoperative collapse that severely progressed after surgical treatment. This tissue-engineered approach has potential for the treatment of osteonecrosis, but results suggested that the present procedure cannot be used in cases with severe preoperative collapse.

Promising results were obtained using MSCs in the treatment of other bone pathologies: Kitoh et al. (24) injected differentiated bone-marrow-derived stem cells (cultured with osteogenic supplements and differentiated into osteoblast-like cells) and PRP into three femora and two tibiae in two patients undergoing distraction osteogenesis. The target lengths were obtained without major complications. PRP could shortened the treatment period by accelerating bone regeneration. Finally, allograft bone chips containing bone-marrow-derived cells have been used for spinal fusion in 41 patients (23) by Gan et al. (25): 95.1% of patients had good spinal fusion results.

Discussion

In orthopaedic research, adult MSCs have emerged as a cell type candidate with great potential for cell-based therapies (26). In fact, it has been shown that these cells are able to differentiate into a variety of connective tissues such as bone, cartilage, fat, tendon, ligament, marrow stroma, and others (27). The regenerative effects of MSCs are due to their structural contribute to tissue repair and to their immunomodulatory and anti-inflammatory activity, through direct cell-cell interaction or secretion of various factors (28).

MSCs have been firstly identified in bone marrow, but nowadays they have been isolated also from other human sources as adipose tissue, umbilical cordon blood, synovial membrane, synovial fluid, periosteum, dermis, trabecular bone, infrapatellar fat pad, and articular cartilage (27). They show similar phenotypic characteristics but different propensities in proliferation and differentiation potentials (28).

Our findings reported for both BMT and MSCs transplantation good and promising results: however, regarding osteonecrosis, it results clear that BMT or cultured MSCs showed good clinical outcome only in early stages before collapse, while in hypophosphatasia MSCs provides symptoms relief but they don't represent a long term solution.

Three interesting aspects came from our literature review: the possible use of MSCs in combination with coadjuvant agents, the use of a scaffold to improve MSCs effect and the possibility to apply cultured MSCs through an injective approach.

MSCs can be used together with GFs or PRP that could improve their healing properties; promising results were reported, but sometimes it is difficult to understand which element is really effective, because of the lack of comparative and randomized studies. Combination of MSCs and biomaterials, in particular when bone tissue healing required a structural support, were used in clinical practice with good clinical outcome; however several researches have to be performed to select the best biomaterial and cell-biomaterial combination for bone tissue healing. Finally, the goal in orthopaedic research remains the stimulation of bone regeneration with injections of MSCs, because this approach could offer an autologous cell source with great regeneration potential applied through a minimally invasive procedure. Some preclinical researches focusing on this approach show promising results (29), but nowadays literature presents still a too limited number of clinical trials to confirm the real potential of this biological treatment approach.

MSCs represent an exciting and promising stem cell population for regeneration of bone in skeletal diseases, as shown by the various studies discussed above, especially when biomaterials are applied. However, literature results are limited so far because of the small number and the low quality of trials, the lack of controls and the short follow-up (30). Researchers have to perform more high quality studies in order to document results and increase the potential of MSCs use in clinical practice, aiming to develop a minimally invasive treatment to favor high quality bone regeneration.

Disclosure

The authors declare that they have no conflict of interest.

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