Stem cells in bone diseases: current clinical practice

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Introduction: Bone is an obvious candidate tissue for stem cell therapy. This review provides an update of existing stem cell-based clinical treatments for bone pathologies.

Sources of data: A systematic computerized literature search was conducted. The following databases were accessed on 10 February 2011: NIH clinical trials database, PubMed, Ovid and Cochrane Reviews.

Areas of agreement: Stem cell therapy offers new options for bone conditions, both acquired and inherited.

Areas of controversy: There is still no agreement on the exact definition of ‘mesenchymal stem cells’. Consequently, it is difficult to appreciate the effect of culture expansion and the feasibility of allogeneic transplantation.

Growing points: Based on the sound foundations of pre-clinical research, stem cell-based treatments and protocols have recently emerged.

Areas timely for developing research: Well-designed prospective clinical trials are needed in order to establish and develop stem cell therapy for bone diseases.

Keywords: stem cells/mesenchymal stem cells/bone/fracture non-union/osteogenesis imperfecta/infantile hypophosphatasia

Accepted: July 8, 2011
Introduction

Bones are known for their regenerative capacity. While damage to most organs and tissues often leads to scarring, which is the substitution of functioning tissue with fibrous tissue, injured bones tend to heal. Although this response may be attenuated in the elderly, fractured or infected osseous tissue responds by creating new functioning filling in the gap. This woven unorganized bone later remodels and becomes fully incorporated into the pre-existing bone. Although new bone formation and repair of the damaged tissue are common they are not guaranteed. Through the history caregivers tried to support this process by providing optimal environment for bone regeneration by means of mechanical stability, establishment of a systemic anabolic state and manipulations that were aimed to trigger bone tissue formation.

While bone grafting was widely used and bone marrow aspirate was injected into fractures to facilitate union in the 1980s, the concept of progenitor or stem cells emerged only closer to the end of the 20th century. Isolation of cells that can proliferate but may also differentiate into several different lineages created a whole new field of regenerative medicine in which bone was, and still is, one of the most studied tissues. Bone is easy to identify both by histology and by various imaging modalities and therefore easy to investigate. More importantly and unlike most other tissues, bone form defines its function and the mere existence of bone tissue is often sufficient to exert mechanical role.

During the 20th century bone conceptually stopped being a tissue and instead was related to as an organ that holds several types of tissues and serves many functions in addition to its obvious mechanical role. Failure to function, mechanical or metabolic, may be addressed using a cellular approach that utilizes stem cells.

As our knowledge of stem cells rapidly expanded over the past two decades, new modalities of treatment were created. Some have matured beyond the stage of an in vivo animal model. Thus far, only few reached the stage of clinical trials and may potentially soon become part of our practice. This review summarizes the existing data regarding available stem cell treatments for bone pathological conditions. Appreciating the vast resources employed in bone regenerative research and mesenchymal stem cell (MSC) research, there is a surprising paucity of high-quality clinical data in this field.
Sources of data

A systematic computerized literature search was conducted using an iterative manipulation process of the following keywords used singularly or in combination: keyword ‘stem cell’ in combination with ‘bone disease’, ‘clinical’, ‘treatment’, ‘therapy’ and ‘human’, with no limit regarding the year of publication. The following databases were accessed on 10 February 2011: PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez/), Ovid (http://www.ovid.com) and Cochrane Reviews (http://www.cochrane.org/reviews/). Given the linguistic capabilities of the research team, we considered the publications in English only. We excluded case reports, letter to editors and articles not specifically reporting clinical procedures. The authors read the abstract of each publication identified (if an abstract was available). The National institute of Health (NIH) clinical trial database (www.clinicaltrials.gov) was also searched for studies involving ‘stem cell’, ‘mesenchymal stem cells’ or ‘bone’. In addition, the references section of all the publications identified were studied to ascertain whether other relevant material could be found. The personal collection of scientific material of the authors was consulted for the same purpose. If deemed relevant, all relevant publications were retrieved. The most relevant material was drawn between the years 2000 and 2011. A large number of publications focusing on basic research, in vitro assays and in vivo animal experiments were not included.

Stem cells

Stem cells are defined as having the potential for self-renewal (proliferation) as well as becoming one of the several specialized cell types (differentiation). Bone tissue has the potential to be healed and cured by stem cells and stem cell-based therapy. In addition, bones and specifically the medullary cavity host the biological niches of several types of stem cells. At least three different adult stem cell populations were identified within the bone, namely hemopoietic stem cells (HSCs), endothelial stem cells, and MSCs. Embryonic stem cells that may be recovered only from early stage embryos are totipotent and can theoretically be also used for bone tissue regeneration and treatment. Unlike adult stem cells, they possess an unlimited differentiation potential. However, complex biological and ethical issues have driven many investigators to prefer adult stem cell. While all three adult stem cell types were shown to have a positive effect on reconstitution of bone tissue, the most investigated and widely known cell type is
the MSC population. These cells, also named bone marrow stromal stem cells or multipotent adult progenitor cells, give rise to the many cell lineages of the mesenchyme. These include bone, cartilage, adipose, muscle, tendon, ligament and more. It is for this reason that these cells were investigated thoroughly in order to establish a therapeutic platform for cell-based therapy for bone disorders. Easily isolated using their affinity to tissue culture plastics, these cells are somewhat difficult to identify. Unlike HSCs that carry the specific antigen CD34, MSCs do not express any exclusive surface marker or antigen hence they are often identified functionally by their ability to proliferate and to differentiate into different cell types of the mesenchyme, most commonly bone, adipose and cartilage. That, in addition to a pattern of antigen molecules that should be presented on their cell membrane, while other should be absent. Formerly believed to reside within the bone marrow only, these cells were later isolated from nearly every mesenchymal tissue as well as from some non-mesenchymal ones. These populations of MSCs from different source tissues share the differentiation range as well as a set of cell markers (‘classical MSC markers’) but differ in the pattern of other surface markers that they display as well as in their frequency and their osteogenic potential. Recent data suggest that at least some of the MSCs are in fact pericytes—cells that line the outer side of the capillary basement membrane and therefore are distributed all across the body. MSCs were investigated for their potential to migrate, and compelling evidence show that these cells translocate into the circulation and migrate to the site of injury. MSCs are capable of rolling—decelerating by adhering to the capillary wall—a unique characteristic exerted only by cells that move by means of peripheral vasculature. The attenuated healing of skeletal tissues in the elderly may be attributed, in part, to the age-related decrease in MSC concentration.

It is assumed that activated MSCs exert all these functions and many more, yet to date there is no data regarding identification of activated MSCs. Since MSCs are considered the direct progenitors of bone-forming cells in the hierarchy of differentiation and as the natural reservoir for renewal of all mesenchymal tissues, this review will focus on the clinical application of these cells in the treatment of pathologic bone conditions.

Immune modulation by stem cells

Tissue injury typically triggers an immune response that may lead either to the formation of fibrotic non-functioning scar tissue or to a regenerative response that reconstitutes functional tissue that will
replace the injured one. With bone being no exception, a delicate balance of signals dictates the actual response upon injury. It is accepted that the type of the immune response elicited by the host will dictate the clinical outcome of a fracture for example that may result in a solid bony union or a fibrotic scar—termed ‘non-union’. MSCs are also known to be powerful immune modulators. This capacity, demonstrated both in vitro and in vivo, is exerted through complex cell–cell interactions and results in profound alterations in cell maturation, activation and function across the different arms of the immune system. Several mechanisms were suggested by which this effect may be induced, including contact dependent and non-contact dependent. MSCs were shown to inhibit lymphocyte response to a third party and to a toxin triggering in vitro, prevent maturation of antigen presenting cells, attenuate natural killer (NK) cell response and induce a shift of an otherwise classic Th1 immune response to a Th2 in a mixed lymphocyte reaction. When introduced in vivo these cells can delay rejection of third party skin graft, assist injected tumor cells to evade host immune system and produce tumors and even suppress graft versus host disease after bone marrow transplantation. By exerting this powerful capability, MSC modulate classic immune response that normally yields scar and fibrosis and shift it by inducing local (and perhaps systemic) environment favoring regeneration and tissue reconstitution. The exact mechanism by which MSC act to modulate the immune system is still not fully understood, yet studies are being conducted focusing on regulation of immune and inflammatory diseases using MSC. To date it is debated whether MSCs main regenerative effect is carried through their proliferation, differentiation and incorporation in the reconstituting tissue (bone in this case) or is it carried through their immune modulatory activity.

Fracture non-union

Fracture non-union, benign bone defects and spine fusion surgeries are three of the most commonly encountered conditions where filling of a given space with bone tissue is needed. Of these, fractures are the most prevalent bone pathology. While most often heal with no complications whether treated conservatively or surgically, some 5–20% of all fractures result in delayed union or non-union. When no improvement is detected in a fracture for consecutive 3 months or no union is achieved over 8–9 months the fracture is considered ‘non-union’. In this situation, based on animal studies, introduction of stem cells and specifically MSC to the fracture site may promote bone healing and regeneration.
The role of MSC in fracture healing was demonstrated by Hernigou et al.\textsuperscript{45} They treated 60 cases of atrophic non-union of the tibia with percutaneous injection of concentrated bone marrow. In addition to a high union rate (43/60), the researchers were able to detect a correlation between the number and concentration of colony forming units—fibroblast (CFU-F) in the graft and the volume of mineralized callus at 4 months. Quarto et al.\textsuperscript{46} reported a 15–27 months follow-up of three patients in whom a segmental defect in a long bone (4–7 cm) was filled using a size matching hydroxyapatite (HA) scaffold loaded with culture expanded autologous MSC. A defect at this magnitude in a long bone is often related to as a ‘critical size defect’, meaning that healing without grafting is not anticipated. Graft integration was detected as early as 2 months after surgery and all three patients regained function of their limbs with no adverse events. A similar study utilizing HA scaffold loaded with autologous culture expanded MSC with 7 years follow-up was reported by Marcacci et al.\textsuperscript{47} They reported incorporation of the graft within 7 months, but noted that the graft was not resorbed at long-term follow-up. Similar to these studied, Soleymani et al. and Meijer et al.\textsuperscript{48} reported small series of six patients utilizing autologous culture expanded cells loaded onto ceramic scaffolds to fill intraoral bone defects. Both groups report overall good results. Other reports concerning MSC application in fracture healing, treatment of non-union or bone fusion is in a scale of a case report or a mini-series. In addition to these, MSCs were reportedly injected or applied in a variety of benign cases where bone tissue was needed to fill a cyst, expand bone stock for dental implants, treat a comminuted fracture or augment intervertebral fusion; however, these reports add very little data and are not discussed here.

Exception to those is a study by our group (NCT 00250302) that will be concluded soon. We have established a method for safe and rapid isolation of MSC from bone marrow aspirate. The ability to reproducibly obtain high numbers of freshly isolated MSC obviates the need to wait for culture expansion with its risks for infection as well as loss of function while \textit{in vitro}. With the graft readily available within several hours and the entire grafting performed percutaneously the morbidity associated with this procedure is moderate and so is the cost. We used an injectable composite graft as a preventive treatment for fractures of the distal third of the tibia. All procedures have been completed and we are currently in the stage of follow-up.

Canine studies demonstrated that MSC loaded scaffolds may be an effective treatment in fractures of long bones and even in acute segmental bone defects. Allogeneic MSCS were as effective as autologous cells in dogs with no immune rejection of the graft. Recent human study that was similarly designed utilized ceramic scaffold loaded with MSCs
that were expanded in culture to treat successfully bone defects. Different scaffold materials have been introduced in animal studies and later on into the market. These include soft and injectable scaffolds, bio-absorbable scaffolds, 3D-printed made-to-fit scaffolds and sheets of scaffold material carrying layer(s) of MSC.\textsuperscript{12,16,18,23}

Not all approved for clinical use, several manipulations on stem cells were shown effective in enhancing the effect of MSC in fracture healing and bone formation. These include application of MSC in conjunction with different pro-osteogenic factors such as TGF-B, BMP2, FGF, EGF, VEGF and others, culturing MSC in pro-osteogenic differentiation medium prior to transplantation and even genetic manipulation of MSC. None of these was reported to induce tumor formation or to result in any detected adverse event.\textsuperscript{19}

**Osteogenesis imperfecta**

Abnormality of the collagen type I gene product is the basis for this heterogeneous group of diseases. Clinically, these conditions are manifested by connective tissue malfunction disorders such as increased susceptibility to fractures, fragile deformed bones, slow growth and low bone mass. Severity of osteogenesis imperfecta (OI) ranges between in-uterine death due to multiple fracture complications and rather normal life span with mild tendency for fractures and decreased bone mass. Treatment options for these conditions were limited up until recently since the genetic etiology could not be addressed. Instead, fractures were treated as they occurred and bisphosphonates were administered to increase bone mass.\textsuperscript{18} This treatment reduced significantly the occurrence of fractures in affected individuals however concern was expressed regarding long-term treatment for children with drugs from this class due to potential adverse effects. Human experiments followed animal studies that showed increased bone mass and improved bone morphology and architecture of OI mice following engraftment of transfused culture expanded MSC. In the first report of stem cell treatment for OI dated to 1999 Horwitz \textit{et al.}\textsuperscript{49} report allogeneic bone marrow transplantation (BMT) in three children suffering severe form of OI. In their study they describe significant increase in bone mass and histologic evidence for new bone formation followed by reduced incidence of fractures and marked increase in growth velocity. The investigators concluded that allogeneic BMT may result in engraftment of functioning MSC that migrate and incorporate into host bones thus alter overall bone biomechanics. In their following study\textsuperscript{50} the researchers recruited five children with severe OI for BMT. Two of the subjects failed to exhibit engraftment of donor bone-forming cells and
were thus excluded from the study. In the remaining three children, an improvement in vertical (linear) growth was demonstrated along with increase in total body bone mineral content and decrease in the incidence of fractures. This study had a longer follow-up than the first (18–36 versus 6 months) that was sufficient to reveal a plateau in the rate of growth rate after transplantation but a steady increase in bone mineral content. Similar results which further supported the use of MBT and MSC transfusion for OI were obtained in the third study where six children were treated with BMT and MSC transfusions for OI,51 this time labeled MSC were used so that their engraftment could be easily confirmed. Taken together these promising results led Le Blanc et al.52 to transfuse allogeneic male MSC of fetal liver origin into a 32 weeks female fetus in-utero that was diagnosed with multiple fractures and severe OI. Engraftment was confirmed and integration of cells from donor origin was confirmed by detection of cells exhibiting Y chromosome. This transplantation was carried out with no immune suppression, and the child was followed for at least 2 years developing fairly well.

Hypophosphatasia

Hypophosphatasia is a rare inherited metabolic bone disease. It is manifested by an electrolyte imbalance where a low level of blood phosphate results from a loss of activity of the tissue non-specific alkaline phosphatase (TNSALP) gene product. Bone mineralization is disturbed and clinical rickets and osteomalacia may present at several degrees of severity with infantile hypophosphatasia being the most severe. Currently there is no medical treatment for this disorder, although one case was reported where an adult with hypophosphatasia was treated with recombinant parathyroid hormone with subsequent improvement in biochemical and clinical parameters.53 Cahill et al.54 reported in 2001 a transfusion of 4/6 HLA match cultured osteoblasts obtained from a crushed iliac bone of the patient’s father. Donor cells were not detected in the peripheral blood yet the female patient demonstrated clinical and radiological improvement. A bone biopsy sample obtained several years later revealed male (donor) cells.54 Based on that experience Whyte et al.53 performed a 5/6 HLA match BMT from a sibling to an 8-month-old baby. Clinical and radiological improvement lasted 6 months, and when deterioration began the child was transfused with culture expanded marrow cells, resulting in clinical and radiological improvement but without normalization of blood biochemistry. Cahill et al.54 reported in 2007 of another case of infantile hypophosphatasia in another female baby. This time donor cells were introduced by three
routes: intravenous, intraperitoneal and subcutaneous, in order to allow for donor cells to reach not only the bones but the thymus thus inducing tolerance towards the foreign antigens. Again, clinical and radiological improvement took place, and at the age of 8, 7 years after the treatment, the girl was active and expressed only the mild form of hypophosphatasia. Taken together these reports describe a process by which transplantation of allogeneic MSC becomes a viable option with favorable long-term outcome to a potentially fatal metabolic disease.

Discussion

Stem cells in general and MSC specifically present a great hope and a challenge to modern physicians. Bone conditions that were formerly addressed mainly by orthopedic surgeons performing procedures that are mechanic in nature are becoming candidates for biological interventions. Basic research in this field presents exciting data learned from \textit{in vitro} as well as \textit{in vivo} assays. The latter addressed many conditions related to the bone both as a tissue and as an organ using animal models. It is thus disappointing somewhat to witness the discrepancy between the wealth of basic literature and the paucity of clinical data that currently exist.

Since their discovery towards the turn of the millennium stem cells has been thoroughly investigated and in particular MSC. Their potential to be used in regenerative medicine as well as in the treatment of inflammatory conditions and metabolic diseases was noted, and first few examples of transfer of knowledge from the bench to the clinical practice were presented here.

Delayed or non-union of a fracture, probably the most intuitive indication for MSC utilization, drew researchers who implemented knowledge earned in the laboratory, for example when MSCs were introduced loaded on a carrier in a specific concentration that often required culture expansion. Application of MSC in metabolic bone diseases is another emerging field, however in this field the mere presence of viable grafted cells or even their incorporation into host bone does not yet ensure function. In light of these some controversies still exist that need to be settled for us to speak in the same terms. There is still no agreement on the exact definition of ‘mesenchymal stem cells’. Both their phenotype and their function are still under debate and it is possible that what we name MSC is in fact a mixed population of several cell types that are selected by many \textit{in vitro} passages. Consequently, it is difficult to appreciate the effect of culture expansion on the cells and on their potential in clinical applications. A true consensus in this
matter of MSC identity and a clear definition may assist in the evaluation of the feasibility of allogeneic MSC transplantation.

Based on the sound foundations of pre-clinical research, stem cell-based treatments and protocols have recently emerged. In order for MSC to become a tool in the foreseeable future, it is mandatory that well-designed prospective clinical trials be carried out.

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