Emerging Ideas

Treatment of Precollapse Osteonecrosis Using Stem Cells and Growth Factors

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Abstract

Background Osteonecrosis (ON) of the femoral head is a devastating disease affecting young patients at their most productive age, causing major socioeconomic burdens. ON is associated with various etiologic factors, and the pathogenesis of the disease is unknown. Most investigators believe the disease is the result of secondary microvascular compromise with subsequent bone and marrow cell death and defective bone repair.

Questions/Hypotheses We hypothesize that local delivery of vascular endothelial growth factor (VEGF) and bone morphogenetic protein-6 (BMP-6), which induces angiogenesis and osteogenesis respectively, will reverse the disease process and provide a treatment for precollapse ON.

Method of Study We will use genetically engineered bone marrow stem cells, carrying VEGF and BMP-6 genes, to enhance angiogenesis and osteogenesis in necrotic bone of an animal model, by local delivery of growth factor in addition to the bone-forming property of the stem cells. The participation, localization, and fate of the stem cells in

the repair process will be evaluated by tracing marker-gene product. Osteogenesis and angiogenesis will be assessed using high-resolution xray CT and immunohistomorphometry quantitatively. Mechanical properties of the repair tissue will be determined using an indentation test of the femoral head.

Significance We envision that a deliverable or injectable bone graft substitute containing engineered stem cells and therapeutic growth factors will be developed through this proposed study and will provide a much needed treatment for ON.

Questions/Hypotheses

We therefore hypothesize that local delivery of VEGF and BMP-6, which induces angiogenesis and osteogenesis respectively, will reverse the disease process and provide a treatment for precollapse ON.

Background

ON (sometimes called avascular necrosis or aseptic necrosis) of the femoral head, is thought to be a disease of impaired osseous blood flow [2, 28, 31]. Many investigators believe pathogenesis of ON is the result of secondary microvascular compromise with subsequent bone and marrow cell death and defective bone repair. Mechanical blood vessel interruption, thrombotic intravascular occlusion, and extravascular compression are the three most commonly accepted general mechanisms leading to ON [1, 22]. Attention has been given to the interplay between individual genetic predisposition and environmental factors related to ON [6, 41]. Heritable and

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acquired risk factors for hypercoagulability have been identified in many patients [18, 41]. Advances of technologies in molecular biology have enabled studies of molecular mechanism of steroid- and alcohol-induced ON [11, 12, 27]. Still, the exact pathogenesis of ON is unknown. This limited knowledge has impeded the development of prophylaxis or treatment for this debilitating disease. To some extent, this is the result of the lack of an ideal animal model [3–5, 12, 31]. However, ON of the femoral head in the emu [7] and chicken [12, 39] mimics human pathologic features of the disease (Fig. 1), indicating the bipedal animal model may fill a long-standing need in the field of ON research [10, 12].

Current treatment options for ON depend on the progression of the disease [29, 31]. THA is the most effective treatment once collapse occurs [21, 28, 31, 32]. Femoral osteotomies and free vascularized fibular graft have a relative low success rate, high complication rate, and may compromise subsequent THA [21, 32]. The preferred surgical treatment for early-stage ON is core decompression

with or without adjunct bone grafting [14, 29, 31]. In a study by Mont et al., BMP-enriched allograft was used in 19 patients (21 hips) with a mean followup of 48 months (range, 36–55 months); 18 of 21 hips (86%) were clinically successful at latest followup [30]. Gangji et al. studied 13 patients (18 hips) with ARCO Stage I or II ON [17] of the femoral head [15]. The hips were randomized with either core decompression only or core decompression and implantation of autologous bone marrow mononuclear cells. After 24 months, five of the eight hips in the control group had deteriorated to Stage III, whereas only one of the 10 hips in the bone marrow graft group had progressed. However, the limitations of their studies are the small number of patients and short-term followup [14, 15]. Therefore, well-designed basic science studies and prospective, randomized, controlled clinical trials are needed [14, 15, 32].

A novel approach for treatment of ON is to combine angiogenic and osteogenic factors in the form of VEGF and BMPs respectively. Combined use of VEGF and BMP-2 [19, 24, 34, 35, 37, 40] and BMP-4 [20, 25, 36] showed synergistic effects in osteogenesis. It has been shown that combined use of VEGF with one of the most potent BMPs, BMP-6 [23, 26], enhances osteogenesis and angiogenesis of mouse bone marrow stem cells in vitro and in vivo [8], however, the effectiveness of this therapy for ON has not been determined.

Proposed Program

Bone marrow stem cells offer promise for bone regeneration by not only providing cellular machinery directly to the sites of the ON, but also serving as a vehicle to deliver therapeutic genes and factors (Fig. 2) [13–16]. We will use genetically engineered chicken bone marrow stem cells, transfected with adenovirus carrying VEGF and BMP-6, to enhance angiogenesis and osteogenesis in avascular necrotic bone in a chicken ON model, by local delivery of angiogenic and osteogenic factors in addition to the bone-forming property of the stem cells, on a sintered polylactic-co-glycolic acid (PLGA) scaffold. The participation, localization, and fate of the stem cells in the repair process will be evaluated by tracing marker-gene product: green fluorescent protein (GFP). Osteogenesis and angiogenesis will be assessed using high-resolution xray CT (micro-CT) and immunohistomorphometry quantitatively. The location, function, and fate of the host and transplanted stem cells in the development and treatment of avascular necrosis of the femoral head will be determined.

To establish the best angiogenic and osteogenic effects of chicken bone marrow stem cells transduced with VEGF

Fig. 1A–B (A) A photomicrograph of subchondral bone death of the femoral head in a chicken treated with steroids for 8 weeks shows marrow necrosis and new bone formation covering dead trabecular bone, similar to the human form of osteonecrosis. (B) The photomicrograph for a normal control is shown (Stain, hematoxylin and eosin; original magnification, $\times 63$)

Fig. 2A–C The photomicrographs show (A) new bone formation in the renal capsule, (B) hindquarter muscle, and (C) a subcutaneous site at 2 weeks after a cloned bone marrow stem cell (D1-BAG) was

transplanted by injection (Stain, X-gal and hematoxylin and eosin; original magnification, $\times 25$)

and BMP-6, the material will be tested in vitro for osteogenesis and angiogenesis by measuring mRNA, protein production, and biologic activity. The optimal ratio of VEGF and BMP-6 will be determined. To evaluate the effectiveness of local delivery of the material in vivo, ON of the femoral head will be developed by surgical hip dislocation and disruption of blood supply to the chicken femoral head. Four weeks later, while ON is developed but no collapse has occurred, core decompression of the femoral head will be performed and the graft will be implanted in the core tract in the femoral head with different treatment combinations. Angiogenesis and osteogenesis will be determined using micro-CT, image-based 3-D stereologic techniques, and conventional immunohistomorphometry at three different times after implantation of the graft material. The quality of newly formed bone in the necrotic femoral head will be tested mechanically.

To assess the location, function, and fate of the host and transplanted stem cells, and their interaction with other cellular and molecular elements in the development and treatment of avascular necrosis of the femoral head, the specimens (the femoral heads) will be decalcified and embedded in paraffin. Sections will be obtained and will be examined by fluorescence microscopy to detect GFP and to localize transplanted cells. The location and total areas of positive fluorescent cells/matrices will be calculated quantitatively at each time using a computerized immunohistomorphometric analysis, the method recommended by the American Society of Bone and Mineral Research [12, 33]. Apoptosis of the marrow stem cells and osteoblasts/osteocytes, and distribution of osteoclasts will be determined using specific stains: TUNEL (terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate nick-end labeling) and TRAP (tartrate-resistant acid phosphatase).

Successful completion of the project will advance our understanding and knowledge of angiogenic factors, their

role in tissue engineering, and the association between angiogenesis and bone regeneration in necrotic bone. The results may establish scientific bases for clinical application of angiogenic growth factors in the treatment of human ON.

Limitations

Using GFP as a trace marker will provide a convenient approach to localize the transplanted cells and to determine the degree of expression of target gene products, in this case, the VEGF and BMP-6 proteins. However, procedures necessary for processing the repair tissue may decrease or even eliminate the fluorescence of GFP. The alternative is to detect GFP immunochemically using antibody to GFP.

Direct VEGF administration can improve blood flow and neovascularization in necrotic bone [38]. In addition to its effects on angiogenesis, VEGF plays an important role in bone growth and repair by interacting with other growth factors [35, 36]. However, overexpression of VEGF actually can adversely affect osteogenesis and decrease bone formation [25, 34–37]. In our in vitro study, the optimal ratio of VEGF and BMP-6 will be determined so that the undesired effects of VEGF can be avoided in the in vivo study.

Another potential problem is rejection of transplanted engineered bone marrow stem cells. The cells may not survive long enough to provide therapeutic levels of growth factors. A surviving study of the cell will be performed in vivo in chicken by transplanting GFP-labeled engineered chicken bone marrow stem cells to subcutaneous space, and survival of the cells will be examined by monitoring the marker-gene product: GFP and bone formation by the cells. The alternative is to deliver growth factors without using stem cells, in the form of protein.

Next Steps

BMPs have the capability to enhance fracture and bone defect repair in human and animal models. BMP-7 and BMP-2 are used clinically in patients, but limited information is available regarding treatment of ON [29, 30]. Studies with Level I or II evidence are lacking. Prospective, randomized, double-blinded, controlled clinical trials and well-designed animal studies are needed in this area.

Bone marrow stem cells could serve as graft material and a vehicle to deliver therapeutic factors that enhance bone repair [9, 13]. The efficacy of bone marrow implantation in the osteonecrotic zone was studied in prospective trials in humans and showed progression of the disease was avoided in nine of the 10 hips that received bone marrow implantation, whereas five of the eight hips in the control group had deteriorated to a late stage of the disease [14–16]. The mechanisms involved were not studied. The interpretation of the results is limited by a small number of patients and short duration of followup. Therefore, additional laboratory and clinical studies are needed.

Stem cell-mediated bone regeneration augmented with combined use of VEGF and BMPs has been reported but only for BMP-2 [19, 24, 34, 35, 37, 40] and BMP-4 [20, 25, 36]. It has been shown that VEGF enhances BMP-6-induced osteogenesis of mouse stem cells in vitro and in vivo, however, the effectiveness of this therapy for ON has not been determined [8]. If it is effective in treating ON induced in an animal model, then the composite graft material can be used to treat the human form of the disease. Bone marrow stem cells can be harvested and engineered before or during surgery, and then delivered to the site of ON. A prospective, randomized, controlled study will be needed to determine the effectiveness of such an approach.

Vision of the Future

Stem cell-based gene therapy is clinically relevant. The synergistic effect of VEGF and BMPs together with stem cells for treatment of ON is innovative and has abundant clinical applications. Successful treatment of precollapse ON of the femoral head could preserve the hip and avoid THA, which will have a major socioeconomic impact. Stem cell-based gene therapy also can be used in the treatment of many other musculoskeletal diseases, including genetic disorders, bone tumors, bone defects, fractures, and osteolysis. We envision that a deliverable or injectable bone graft substitute containing engineered stem cells will provide a much needed treatment for ON.

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