Effective Systemic and Local Mesenchymal Stem Cell Therapies for Vertebral Compression Fractures

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Disclosures:


Introduction: Osteoporosis-related vertebral compression fractures (VCFs) occur at a rate of 750,000 per year twice the rate of hip fractures. Importantly, the 3-year mortality rate is nearly 50% in patients with acute VCFs, which is twice the mortality rate of age-matched control groups. Once VCFs occur, there are limited treatment options. Since surgery entails significant risks of morbidity and implant failure, non-operative management options such as medications and/or bracing are usually recommended. New nonbiological procedures of vertebroplasty and kyphoplasty have been developed to restore the vertebral body. However, the AAOS recommended against vertebroplasty for patients with symptomatic VCF following controversial evidence of efficiency. We have already shown that BMP-modified MSCs induced vertebral defect regeneration in rat model of VCF. Alternatively, systemically administered MSCs may be a potent, minimally invasive and more effective solution for a multiple level vertebral fractures. We proposed to develop a new approach to accelerate bone repair based on systemic administration of MSCs and PTH. It has been already established that PTH alone can accelerate fracture repair in healthy animals by activating bone marrow MSCs. However, osteoporotic patients have decreased numbers and/or dysfunctional MSCs. Therefore, we hypothesized that an intravenous injection of MSCs combined with PTH administration would induce stem cell homing to vertebral defects followed by osteogenesis and defect repair. In order to test these two MSC-based therapies, we investigated both strategies to regenerate vertebral defects in a rodent (rat) and pig models.

Methods: The Cedars Sinai Medical Center IACUC approved all animal procedures. Human or porcine MSCs were isolated from bone marrow and expanded in culture. For the local approach, the cells were transfected with a BMP6-encoding plasmid using electroporation (Lonza), as previously shown. Bone voids were created in lumbar vertebrae of nude rats (1.8mm in diameter and 2.5 mm in depth) and lumbar vertebrae of minipigs (4mm in diameter and 15 mm in depth). Cells were aliquoted (1x106 for rat and 4x106 for pigs), suspended in fibrin gel (Tisseel, Baxter) and implanted into the voids. Bone regeneration was monitored using µCT for rats and clinical CT scanner for pig studies through 2 and 6 months, respectively. Porcine vertebrae were quantitatively analyzed for bone regeneration with high-resolution µCT after harvesting. In addition, the treated vertebrae were subjected to histological analysis. For the systemic therapy approach, we induced osteopenia in Nude rats by ovariectomy and four months of low calcium diet (LCD). Osteopenia was confirmed using µCT scans and analysis of the vertebral trabecular bone. Human bone marrow-derived MSCs were labeled with Luciferase or human sodium iodide symporter (NIS) reporter genes using lentiviral vectors. Multiple vertebral defects were created in the lumbar spine of osteopenic rats. Treatment included multiple i.v. injections of labeled cells and daily SQ injections of PTH (40μg/Kg body weight) or saline for 4 weeks. Cell survival and homing to the defect site were monitored using bioluminescent imaging (BLI) or µSPECT/µCT following iv injection of the 99mTecnecium. In the porcine model, porcine BM-MSCs were administrated i.v. once a week for three weeks and PTH injections for 4 week. Vertebral defect repair was monitored and analyzed using X-ray imaging and histology.

Results: Local approach: In vivo µCT monitoring of vertebral defects in rats showed that BMP6-overexpressing MSCs were able to regenerate bone tissue in the vertebral defects as opposed to controls (Fig. 1). In the porcine model, clinical CT imaging, 20 weeks post-operation, revealed considerable repair in the MSC-BMP6 group compared to the control defects implanted only with fibrin gel (Fig. 2). No bone overgrowth was found in any of the treated vertebrae in both animal models. Moreover, we did not detect any signs of an inflammatory response in the histological sections analyzed. Systemic approach: ovariectomy and low calcium diet resulted in 15-20% loss of bone mineral density and over 30% reduction in trabecular thickness in Nude rats. BLI and nuclear imaging detected MSC homing to the lumbar region of the animals few days after the intravenous delivery (Fig. 3, 4). Vertebral defects in osteopenic rats treated with the combined stem cell-and-PTH therapy resulted in 2-fold increase in bone volume density two months after treatment when compared to defects treated with PTH only (Fig. 5). The vertebrae in the untreated rats did not heal after 8 weeks. In the porcine model of multiple vertebral defects, remarkable healing of the defect was observed as early as 4 weeks after the surgery (Fig. 6). Notably the combined stem cell-and-PTH therapy succeeded to
regenerate the defect in much more efficient way than both treatments alone.

**Discussion:** We were able to determine that allogeneic, BMP6 gene-modified, bone marrow-derived MSCs induced bone regeneration in a local rat and pig vertebral fracture models. Importantly, no signs of neurological side effects, bone growth into the spinal canal or immunogenicity were detected. Our results also showed that vertebral defects in osteopenic rats and pigs were efficiently repaired when treated with human MSCs and PTH, compared to the controls. Moreover, when we tracked labeled MSCs using optical and nuclear imaging systems, we could detect cell homing to the lumbar region of the animals that were treated with PTH. This study provided evidence for future therapies that could revolutionize the treatment of vertebral and other complex fractures especially in osteoporotic patients.

**Significance:** This is a critical step towards the development of allogeneic, gene-modified, MSCs as a therapeutic candidate to treat VCFs. The advantage of allogeneic cells is that they do not require the patient to undergo an additional medical procedure such as bone-marrow aspiration. The systemic approach holds additional revolutionary advantage, since it opens an opportunity to treat VCFs non-invasively on multiple levels.

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**References:**
Fig. 1. High-resolution µCT imaging of locally transplanted human MSC-BMP6 in to rat vertebral defects. Sagittal cross-sections of vertebrae are shown in 3D and 2D images. Arrows indicate the defect site.
Fig. 2. CT imaging of MSC-treated pig vertebrae. Clinical CT was used to image the vertebral defect repair 4 months after surgery. Blue arrow: fibrin gel control, Orange arrow: MSC-BMP6

Fig. 3. PTH enhances MSC homing to the site of injury: μSPECT/μCT imaging. MSCs were labeled with hNIS reporter gene and imaged homing to the proximity of the defect.
Fig. 4. Stem cell tracking using bioluminescence. When we compared local versus systemic administration of cells to the defect site, we found that starting from 7 days time point, the amount of cells labeled with Luc reporter gene in proximity of the defect was similar in both groups. Arrows indicate multiple cell injections.
Fig. 5. Combined PTH therapy and systemically injected MSCs promote vertebral defect repair in osteopenic rats. Synergistic effect was observed when PTH therapy and systemic injection of MSCs were applied as shown in μCT images. Untreated
vertebrae did not heal after 8 weeks.

Fig. 6. Combined systemic MSC+PTH treatment has synergistic effect on vertebral defect healing in porcine model as early as 4 weeks after surgery, as shown above with μCT imaging.

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