Effective Systemic Mesenchymal Stem Cell Therapy for Vertebral Compression Fractures

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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. We have already shown that BMP-modified MSCs induced vertebral defect regeneration in rat model of VCF. Alternatively, systemically administrated 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 this MSC-based therapy, we investigated the strategy to regenerate vertebral defects in both, a rodent (rat) and pig models.

Methods: Human or porcine MSCs were isolated from bone marrow and expanded in culture. 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 (BM)-MSCs were labeled with Luciferase reporter gene using lentiviral vectors. Multiple vertebral defects were created in the lumbar spine of osteopenic rats. Bone voids were created in lumbar vertebrae of nude rats (1.8mm in diameter and 2.5mm in depth) and lumbar vertebrae of minipigs (4mm in diameter and 15mm in depth). Treatment included multiple i.v. injections of labeled cells and daily SQ injections of PTH (in different concentrations) or saline for 4 weeks (for both animals). Cells were aliquoted (2x10^6 for rat and 50x10^6 for pigs) and injected i.v. (total of 5 injections, twice per week for rats and total of 4 injections, once per week for pigs). For pigs, porcine BM-MSCs were administrated i.v. once a week (total of 4). Cell survival and homing to the defect site were monitored using bioluminescent imaging (BLI) in rats. Bone regeneration was monitored using µCT in vivo for rats and clinical X-ray scanner and µCT ex vivo in pig studies after harvesting. Histological analysis and immunofluorescent staining were done in the treated vertebrae.

Results: Ovariectomy and LCD resulted in 15-20% loss of bone mineral density and over 30% reduction in trabecular thickness in nude rats. BLI detected MSC homing to the lumbar region of the animals few days after the intravenous delivery (Fig. 2). 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. 3). 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 5 weeks after the surgery (Fig. 3). Notably the combined stem cell-and-PTH therapy succeeded to regenerate the defect in a much more efficient way than each treatment alone. Importantly, no signs of neurological side effects, bone growth into the spinal canal or immunogenicity were detected.

**Discussion:** Our results show 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 imaging system, 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. This is a critical step towards the development of allogeneic, gene-modified, MSCs as a therapeutic candidate to treat VCFs.

**Significance:** 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.
Fig. 1. A. Experimental design. Stem cell tracking using bioluminescence. Cells were labeled with lentiviral vector to constitutively express Luc2 reporter gene. The homing of systemically administered cells was monitored throughout the experiment using BLI of the lumbar region (B). The total homing of the cells in the defect site proximity was significantly increased the groups treated with 4μg/Kg and 40μg/Kg PTH over cell only group (C). The accumulative effect of injected cells was more significant in rats injected with 4μg/kg PTH (D).
Fig. 2. Combined PTH therapy and systemically injected MSCs promote vertebral defect repair in osteopenic rats as early as 2 weeks after surgery, as shown above with µCT imaging (A) and quantitative analysis of the bone volume density (B) and bone mineral density (C). Histological analysis and standard H&E staining shows that untreated vertebrae did not heal after 8 weeks (D). Immunofluorescent staining and microscopy shows that Dil labeled hMSCs contributed to the bone healing and expressed osteogenic markers hBSP and Osteocalcin (E).
Fig. 3. Combined PTH therapy and systemically injected MSCs has synergistic effect on vertebral defect healing in porcine model as early as 5 weeks after surgery, as shown above with μCT imaging. Remarkable healing of the defect was observed in the group treated with MSCs and 5μg/Kg PTH (A). The injected pMSCs were identified in the repaired defect site by immunofluorescent staining for the Luc reporter gene and Dil lipophilic dye (B).