

Δ Effects of Endothelial Progenitor Cell Therapy on Diabetic Rat Fracture Healing*Clifford Lin, MD; Aaron Nauth, MD, FRCSC; Emil H. Schemitsch, MD, FRCSC;**University of Toronto, St. Michael's Hospital, Toronto, Ontario, Canada*

Background/Purpose: There has been substantial interest in tissue engineering strategies that employ the use of stem/progenitor cells for the treatment of bone defects and bone loss in orthopaedic patients. Furthermore, several studies have shown increased complications with bone healing in patients with associated comorbidities such as diabetes. The effectiveness of tissue engineering strategies in these healing-compromised hosts is not well understood. This study sought to investigate the effects of an endothelial progenitor cell (EPC) type on a model of impaired fracture healing, using a diabetic rat model.

Methods: EPCs were isolated from rat bone marrow, cultured for 10 to 14 days in endothelial cell culture media, then harvested and reimplanted into either a control rat fracture model or a diabetic rat fracture model. This model consisted of creating a 3-mm segmental bone defect in the right femur then filling the defect with an empty gelfoam scaffold (control treatment) or EPC-seeded gelfoam. The femur was then stabilized with a plate and screw construct and the rat allowed to bear weight as tolerated. Rats were then sacrificed at 10 weeks and the femurs harvested then submitted for clinical and radiological analysis. In the diabetic group, diabetes was induced via intraperitoneal injection of 35 mg/kg of streptozotocin 2 weeks prior to creation of the bone defect. Hyperglycemia was confirmed with glucometer testing on a regular basis throughout the study period.

Results: In control (nondiabetic) rats, 0 of 8 rats (0%) that were implanted with gelfoam only went on to radiographic union. Of those that were implanted with EPC-seeded gelfoam, 5 of 8 (62.5%) were healed. In diabetic rats, 0 of 10 (0%) with implanted gelfoam only went on to radiographic union. Of those implanted with EPC-seeded gelfoam, 5 of 12 (41.7%) went on to heal.

Conclusion: Implantation of EPCs into bony defects can increase the incidence of union in segmental bony defects. This effect is seen in both healthy control rats as well as healing-compromised diabetic rats, although the incidence of union is lower in the diabetic group. Continued research in this area is required to identify effective therapies for the enhancement of fracture vascularity and bone regeneration.

The Effects of Aminobisphosphonate In Vitro and In Vivo Treatment on the Osteogenic Capacity of Bone Marrow Stromal Cells from Senile Osteoporotic Hip Fracture Patients

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Purpose: Aminobisphosphonates (BPs) prevent age-related bone loss and osteoporosis-associated fractures through the inhibition of osteoclast resorptive activity. However, the effects of these potent synthetic compounds on cells of the osteoblastic lineage of senile osteoporotic patients is unclear so far, although resident bone marrow stromal cell (BMSC) populations are known to play a critical role in determining bone quality. The purpose of this study therefore was to determine whether both zoledronate (ZA) in vitro and alendronate (ALN) in vivo treatment enhance the osteogenic differentiation capacity of BMSCs obtained from senile osteoporotic hip fracture patients.

Methods: BMSCs were intraoperatively harvested from 7 senile osteoporotic hip fracture patients not receiving BP therapy and from 3 patients receiving alendronate therapy. BMSCs were cultured in osteogenic medium ± ZA (10 and 100 nM) for up to 21 days. The effects of ZA in vitro treatment on BMSC viability and proliferation were evaluated using Annexin-V/PI FACS (flow cytometry) analysis and WST-1 assay, respectively. The effect of ZA on osteogenic differentiation was assessed using Alizarin Red staining, alkaline phosphatase (ALP) enzyme activity, and quantitative real-time polymerase chain reaction (qRT-PCR) of osteogenic marker genes. Furthermore, osteogenic potential of BMSCs obtained from 3 patients receiving ALN therapy in vivo and from 3 matched controls without BP therapy were compared.

Results: In vitro exposure to ZA (10 and 100 nM) up to 72 hours did not significantly affect BMSC viability and proliferation. BMSCs cultured in osteogenic medium supplemented with ZA (10 and 100 nM) for 21 days showed a significant increase in mineralized matrix formation as assessed by Alizarin Red staining when compared to BMSCs cultured in osteogenic medium alone ($P < 0.01$). However, no significant differences were found for ALP enzyme activity and gene expression levels of osteogenic markers ALP, bone sialoprotein (IBSP), and basic fibroblastic growth factor (FGF2). Similarly, BMSCs obtained from osteoporotic hip fracture patients receiving ALN treatment in vivo also showed a markedly enhanced mineral deposition as compared to BMSCs obtained from matched osteoporotic controls not receiving bisphosphonate therapy ($P < 0.01$).

Conclusion: Our results for the first time show that aminobisphosphonate in vitro and in vivo treatment enhances osteoblastogenesis and subsequent mineralized matrix formation of osteoporotic BMSCs and thus supports an osteoanabolic effect of bisphosphonates in senile osteoporosis.

- The FDA has not cleared this drug and/or medical device for the use described in this presentation (i.e., the drug or medical device is being discussed for an "off label" use). For full information, refer to page 496.

Healing Segmental Bone Defects With Endothelial Progenitor Cell Subtypes

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Purpose: Angiogenesis is critical for osteogenesis, and vascular cell-based therapy can be used to stimulate healing in segmental bone loss. The purpose of the study was to compare early endothelial progenitor cells (EPCs) and late outgrowth endothelial progenitor cells (OEC) for fracture healing potential in vitro and in vivo.

Methods: Primary EPC subtypes were isolated from rat marrow via Ficoll density gradient centrifugation. Endothelial assays, immunosorbent assays, and multicolor flow cytometry for population surface markers (CD11, CD31, CD34, CD45, CD133, and Flk-1) were used to characterize EPC and OEC monocultures. Cocultures of EPC subtypes with and without primary rat osteoblasts (pObs) were analyzed for tube length and connectivity using Image J to evaluate cell-cell effects on angiogenic potential. In vivo, EPCs or OECs (1×10^6) were seeded to gelfoam scaffold and implanted in a critical-size (4-mm) fixed diaphyseal defect in a rat femur; control animals received empty scaffold in the defect. Radiography was used to monitor bone formation over 10 weeks.

Results: OECs expressed significantly more bone morphogenetic protein (BMP)-2 and significantly less vascular endothelial growth factor (VEGF) than EPCs ($P < 0.05$). Surface marker analysis showed decreased CD34+ / CD133+ / Flk-1+ (48% EPCs vs 22% OECs), CD133+ (77% EPCs vs 13% OECs), and CD45+ (46% EPCs vs 2.6% OECs) populations in OECs while the CD34+ / CD31+ / Flk-1+ (33% EPCs vs 49% OECs) population increased. pObs significantly inhibited tubulogenesis of OECs while enhancing connectivity and sprout length of EPCs in coculture ($P < 0.05$). In vivo, 0 of 6 control and 1 of 5 OEC rats achieved partial union at 10 weeks while 4 of 5 EPC rats achieved full union at this time point.

Conclusion: Despite favorable tubulogenic and osteoinductive profiles of OEC monoculture, EPCs displayed enhanced tubulogenic behavior in coculture and superior bone healing in vivo. No previous studies have directly compared subtypes of this novel progenitor population for healing bone defects. The results suggest an early EPC subtype may be more biologically pertinent for this application.