Combination Therapy with PTH and DBM Can Not Heal a Critical Sized Murine Femoral Defect

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Introduction: Orthopaedic surgeons continue to search for bone graft substitutes to enhance bone repair. The high cost and complications associated with bone morphogenetic proteins (BMPs) clearly demonstrates the need for alternative regimens. Teriparatide (PTH 1-34) and demineralized bone matrix (DBM) have been used to promote bone healing. Our goal was to evaluate the efficacy of combination therapy with systemic PTH and DBM in healing a mouse critical sized femoral defect. Novel lineage-specific reporter mice were used to evaluate the osteoprogenitor response to this treatment.

Methods: Critical sized femoral defects (2mm) were created in 4-5 month old male lineage-specific transgenic mice expressing Col3.6GFTopaz (a pre-osteoblastic marker), Col2.3GFPeamerald (an osteoblastic marker) and α-SMACherry (a pericyte/myofibroblast marker). Three groups were evaluated: Group I (PTH), Teriparatide (Bachem, Torrance, Ca) 30 µg subcutaneous injection daily and empty defect, Group II (PTH+DBM), Teriparatide 30 µg subcutaneous injection daily + local DBM (Musculoskeletal Transplant Foundation, Edison, NJ) and Group III (DBM), local DBM + 30µL saline injection (control group). There were 5 mice per transgene per treatment per time point. The Col3.6 and Col2.3 mice were sacrificed at 14, 28 and 56 days after surgery. The α-SMACherry mice were sacrificed at 7 and 14 days. Injections began on the first postoperative day for 28 days or up until the point of sacrifice if the latter came first. X-rays were taken to evaluate bone healing in biweekly intervals for Col3.6 and Col2.3 mice and weekly intervals for the α-SMA mice. We used a 5-point scoring system to evaluate bone healing by three independent reviewers. At the time of sacrifice, frozen bone sections were made to evaluate the osteoprogenitor response using fluorescent microscopy.

The site of the critical defect was chosen as the region of interest (ROI) for analysis (Figure 1). Within the ROI 3 volume ratios and 3 surface ratios were quantified using computer-automated bone histomorphometric techniques. Measured surface ratios were: 1) LS/BS (labeled surface / bone surface): newly mineralized bone surface to total bone surface, 2) Cell/BS (cell surface / bone surface): total GFP cell surface to total bone surface and 3) Label & Cell/BS (labeled and cell surface / bone surface): co-localization of newly mineralized bone surfaces and GFP cell surfaces to total bone surface. Volume ratios are: 1) BV/TV (bone volume / total volume): bone volume to total volume of ROI, 2) Cell/TV (cell volume / total volume): total GFP cell volume to total volume of ROI and 3) Cell/TV (cell volume / bone volume): total GFP cell volume to total bone volume.

Comparisons of radiographic data between groups were conducted with the Kruskal-Wallis test and Wilcoxon rank sum testing post hoc. Comparisons of cellular measurements by treatment and by time point were analyzed with ANOVA and Sidak post hoc testing. An alpha level of 0.05 was considered significant for all analyses. All statistical analysis was performed using Stata 12 (StataCorp. 2011. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP).

Results: Radiographic analysis consistently showed a lack of healing across all treatment groups at all time points (see Figure 2). The average healing score for Group I (PTH) was 1.65+/-.93, reaching a maximum score of 2.57 +/-1.04 at 28 days. None of the 20 mice that were sacrificed at 28 or 56 days had completely healed. Group II (PTH+DBM) had an average healing score of 1.70 +/-1.15, reaching a maximum score of 3.0 +/-1.28 at 56 days. One of twenty (5%) specimens at or beyond the 28 day time point completely healed. The average healing score for Group III (DBM) was 1.84+/-.13, reaching a maximum score of 2.9+/-.102 at 56 days. None of the 20 specimens at or beyond 28 days completely healed.

ANOVA revealed significant differences among the radiographic scores at 56 days. Post hoc testing demonstrated that Group II outperformed Group I (p<0.01) as did group III (P<0.01). A weighted Kappa value of 0.87 was achieved among the three radiographic reviewers suggesting high interobserver reliability.

Across all three treatment groups the histomorphometric findings mirrored the lack of radiographic healing seen on plain radiographs. The defects were largely absent of Col3.6, Co2.3 and α-SMACherry positive cells. The DBM in the defect site for specimens in Groups II and III was virtually always void of osteoprogenitor cells and mineral label suggesting minimal to no osteoinductive activity.

PTH treatment (Group I) did not lead to any significant differences in any of the surface or volume ratios in the defect region in Co3.6 and Co2.3 mice. α-SMACherry mice had significantly larger LS/BS surface ratios at 14 days (18.68 +/-11.62) compared to 7 days (0.76 +/- 1.47). Combination therapy (Group II) led to significant increases in BV/TV in the defect across all time points in
Col.3.6 mice. Col.2.3 mice had significantly smaller BV/TV values at 14 and 28 days compared to 56 days. DBM alone (Group III) had no significant effect on the defect for any of the three mouse lineages.

**Discussion:** We hypothesized that the combination of a DBM with its osteoconductive and osteoinductive activity and PTH’s anabolic effect on osteoblasts, could heal a critical sized defect. However, our data is clear that the biological activity of these two agents is not sufficient to promote healing in this stringent environment. There are two critical findings in this study. First, we observed no difference in the healing rate of a critical sized mouse femoral defect treated with PTH, DBM or combination therapy. Complete healing occurred in only 1/40 (2.5%) mice at the 28 and 56 day time points among PTH treated specimens (Groups I and II). Similar findings were noted in the DBM group alone. Second, quantitation of frozen histologic sections revealed a limited osteoprogenitor response with combination therapy in all 3 transgenic mice consistent with a lack of healing of the defects. In a variety of pre-clinical models PTH has been shown to enhance bone repair but this type of anabolic response is not sufficient to heal a critical sized defect. Critical sized bone defects require either a potent osteoinductive agent like rhBMP-2 or autogenous bone graft for healing to take place.

**Significance:** Combination therapy with PTH and DBM does not possess the biologic potential to promote healing in a stringent bone defect. Orthopaedic surgeons should not use this regimen to treat fractures with bone defects or nonunions.

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**References:** None