Evaluation of rhBMP-2/ACS/TCP-HA bone graft composite with addition of unprocessed Bone Marrow cells in a Canine Femoral Multi Defect Model

INTRODUCTION:
The combination of recombinant human bone morphogenetic protein-2 (rhBMP-2) on an absorbable collagen sponge (ACS), is a novel osteoinductive therapy recently made available to accelerate bone formation in certain applications. rhBMP-2 upregulates crucial processes in the activation, recruitment, and differentiation of stem cells and progenitor cells involved in skeletal homeostasis, fracture repair, and bone graft incorporation. Many clinical studies have also shown that improved graft performance could also be achieved with addition of bone marrow aspirate (BMA), as a source of osteogenic connective tissue progenitors (CTP-Os). This study was designed to test the hypothesis that the addition of bone marrow aspirate to the rhBMP-2/ACS graft in conjunction with TCP-HA granules (“fajita technique”) will increase the local bone formation response in the Canine Femoral Multi Defect (CFMD) Model.

METHODS:
Study design: This study was conducted after approval from the Cleveland Clinic Institutional Animal Care and Use Committee. Comparative competition between rhBMP-2/ACS/TCP-HA bone graft mixed with BMA clot or peripheral blood (PB) clot was performed in ten skeletally mature coonhounds (31.6 ± 2.2 kg). In each subject, four identical 10 mm diameter x 15 mm deep unicortical cylindrical defects were created. The two composite grafts were distributed randomly among the four defects, enabling paired scaffold comparison within the same subject. Due to a therapeutic focus on rapid bone formation, animals were euthanized at 4 weeks.

Graft Preparation: BMA was aspirated from the proximal humerus. PB was collected from the open wound during soft tissue exposure. Both graft composites were prepared intraoperatively. rhBMP-2/ACS/TCP-HA bone graft was prepared by uniformly distributing Tri-Calcium Phosphate (TCP)-Hydroxyapatite (HA) granules onto an absorbable type 1 collagen sponge (ACS) trimmed to dimension of 1.8 cm (height) x 3.2 cm (length) x 0.35 cm (thickness). The graft composite was then wetted with 0.5 cc rhBMP-2 solution at 0.2 mg/ml concentration. 0.5 cc bone marrow or 0.5 cc peripheral blood was added and allowed to clot. The graft was rolled into a 10 mm diameter x 15 mm height cylinder.

Quantitative µCT analysis was used to define the distribution of bone formation in each defect. These data were visualized in radially oriented two-dimensional contour plots based on Percent Bone Volume (%BV). Histological samples were fixed in 10% neutral buffered formalin for 48 hours, then changed to 70% EtOH and sent to the Bone Histomorphometry Laboratory at Mayo Clinic where they were dehydrated, and embedded in PMMA without decalcification. Sections were stained with Goldner’s trichrome or Hematoxylin & Eosin (H&E).

RESULTS:
Mineralization was found to be greater in the center region and lower at the periphery of the defect volume. After adjusting %BV measurement for unresorbed TCP-HA (bright intensity pixels), mean %BV for the overall defect region for rhBMP-2/ACS/TCP-HA with BM clot was 10.8% ±1.22% and mean %BV for rhBMP-2/ACS/TCP-HA with PB was 11.2% ±1.22%. There was no statistically significant difference between groups (ANOVA). Both rhBMP-2/ACS/TCP-HA treatments with BMA clot and with PB clot showed active bone formation and remodeling within the defect and resorption of the collagen sponge. There was no evidence of acute inflammation and rare cyst formation.

DISCUSSION:
This study found no early benefit (4 weeks) on bone formation of adding unprocessed clotted bone marrow aspirate as a supplemental source of local osteogenic CTP-Os to the rhBMP-2/ACS/TCP-HA composite graft when compared to clotted peripheral blood. rhBMP-2/ACS/TCP-HA with both with BMA or PB resulted in bone formation within the defect that was greater near the center than at the periphery, even after correction for residual TCP-HA granules. This pattern of central mineralization differs significantly from the pattern of bone formation that was previously reported using a similar model using the OP-1 Device (Stryker, Hopkinton, MA) as well as allograft bone and other polymeric scaffold materials in which bone formation was most robust at the periphery of the defect and decreased toward the center. This suggests that rhBMP-2 and TCP-HA granules may interact synergistically to induce a greater biological response where they are co-localized.

SIGNIFICANCE:
The CFMD model is providing new insight for the use of rhBMP-2/ACS bone graft and other biomaterials. It has not yet been shown to be sensitive to modifications of cell sourcing or progenitor cell transplantation within the defect. When using the “fajita” technique, it may be valuable to add some TCP-HA granules to the surface of the implant in order to more uniformly distribute the biological effect at the interface between graft and host.

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REFERENCES: