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An evaluation of the effect of bone morphogenetic protein-2 in a hydroxyapatite carrier on the rate of cortical restoration of large bone defects using the dog ulna model

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Abstract

To evaluate the rate of establishment of cortical continuity and union of large bone defects with the use of recombinant bone morphogenetic protein (rh-BMP-2)/hydroxyapatite implants. Six adult male dogs were used to evaluate the effect of bone morphogenetic proteins (BMP) in filling large cortical defects. A 1.5cm cortical ulnar defect was created in two groups of dogs. First group had 1.5cm BMP implant in a carrier of hydroxyapatite in the cortical defect; the control (group 2) defect was left intact. Evaluation was through serial radiographic determination of mean fracture gaps. There was progressive filling of osseous defects in group 1, which was total at the 16th week post-surgical (PS); group 2 dogs had radiographic non-union at the 16th PS week. It was concluded that BMP implanted with a hydroxyapatite carrier significantly enhanced the rate of cortical restoration of massive bone defects in dogs.

Keywords: Bone healing, Bone morphogenetic protein-2, Cortical growth, Dogs, Segmental bone defects, Ulna
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Introduction

The release of growth factors and osteo-progenitor cells by biocompatible scaffolds have been shown to enhance the regenerative capacity of bone (Yang *et al.,* 2004; Huang *et al.,* 2005, Kaigler *et al* 2006) resulting in the replication of the natural bone environment by provision of appropriate synthetic extracellular matrix scaffolds, (Putnam & Mooney, 2006) presentation of growth factors (Richardson *et al* 2001) and osteo-progenitor cells. Two important signals that have been found to induce osteogenesis are bone morphogenetic proteins and the mammalian homologue of wingless in drosophila (Wnt) (Uwagie-Ero *et al.,* 2014; Canalis *et al.,* 2003, Krishnan *et al.,* 2006) Bone morphogenetic proteins and Wnt play fundamental roles in the differentiation of osteoblastic lineage cells into mature osteoblasts. (Canalis *et al.,* 2003; Gazzerro & Canalis, 2006; Krishnan *et al.,* 2006) Bone morphogenetic proteins, Wnt, and IGF-I have been shown to be regulated at the level of their synthesis and their receptors by specific extracellular and intracellular regulatory proteins. (Nohe *et al.,* 2004) The application of bio-factors for new bone regeneration has brought a new trend in the management of fracture with segmental bone loss. Various biological factors, such as bone morphogenetic proteins (BMP), fibroblast growth factors (FGF), platelet-derived growth factor (PDGF), insulin like growth factors (IGFs) and Lim mineralization protein-1 (LMP-1), have been investigated for application in bone regeneration and skeletal repair since autologous bone graft which has been the standard application for most orthopaedic procedures with severe loss of bone has major and significant disadvantages. Therefore new approaches are being tried to achieve osteo-induction and osteogenesis. The use of Bone Morphogenetic proteins as a substitute to autologous bone graft have been intensely researched in the last few years in order to provide a burden of proof for its clinical applications. Extensive research is ongoing to develop injectable formulations for minimally invasive application, and/or novel carriers for prolonged and targeted local delivery (Blokhuis, 2009). The specific aim of the study was to evaluate the rate of establishment of cortical

continuity and union of segmental bone defects using bone morphogenetic protein implanted in hydroxyapatite using the dog ulna bone as a model.

Materials and Methods

Six skeletally mature male local dogs were used for this study and they were randomized into two groups of three animals each. Hydroxyapatite (Sigma Aldlich, Germany) paste was used as the carrier device for the BMP-2 (Sigma Aldlich, Germany). For implantation, 2 ml (1µg/ml) of BMP-2 solution was made into a thick paste with hydroxyapatite powder, rolled into a cylindrical mold of about 0.5cm diameter and 1.5cm height and allowed to dry to form a cylindrical mold before grafting directly to fracture sites. Dogs in group 1 were treated with BMP/hydroxyapatite implant and dogs in group 2 were not treated with any implant (control).

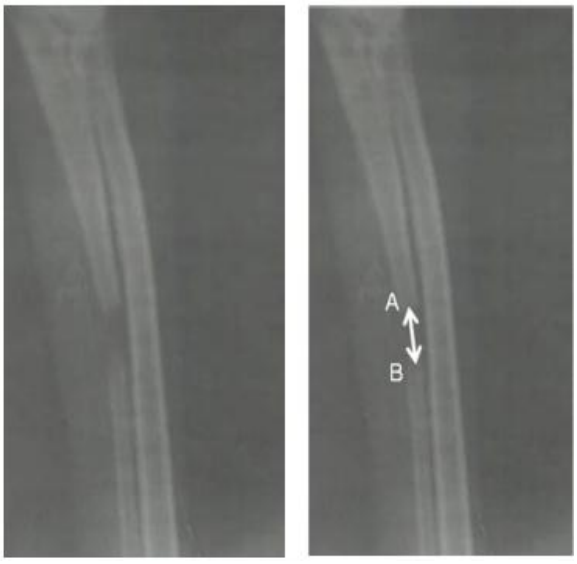
Prior to surgery clinical parameters of the experimental animals were determined and found to be within normal limits. Animals were pre-medicated with xylazine 0.05mg/kg intramuscularly and maintained with 1.5% halothane. Tylosin tartarate 10mg/kg intravenous (i/v) injection was administered prior to surgery for prophylaxis. A 4-5cm caudolateral incision was made over the mid to distal one-third of the left forelimb. The lateral digital extensor muscle was separated from the extensor carpi ulnaris to expose the ulna. A 1.5cm section of bone was excise using an osteotome with the aid of a bone ronguers. The gap created was replaced with the implant for dogs in group 1 while dogs in group 2 received no implants. There was no attempt to repair the fracture with any internal fixation device, there was no attempt made to secure the implant in place at the fracture site. The muscle fascia and skin incisions were closed with 2.0 catgut and 2.0 nylon respectively and a sterile compression bandage applied to the limb. Postoperatively, all animals were permitted free exercise within their pens. Four days after surgery 2 ml of the liquid BMP-2 was injected into the defect site of dogs in group 1 transcutaneously to enhance availability of BMP-2 at the osteotomy site. Radiographs were taken to assess cortical growth and restoration rate of the fracture, rate of callus formation, and cortical bridging and repeated every two weeks post operatively for 20 weeks in dogs in both groups. Fracture gaps (FG) in millimeters were measured and recorded on lateral radiographs for each animal in both groups from week 2 to the termination of the experiment at week 20 (Figures 1, 2 and 3). Mean Fracture Gap (MFG) was determined and evaluated against time.

Data generated were analyzed using student t-test with Statistical Package for Social Sciences (SPSS) version 16.0 for windows to determine significance difference between the means of the two groups (Table 1). Significance was accepted at 5% probability level..

Table 1: Mean Fracture gaps for the two groups of dogs

Weeks	Group 1	Group 2
Week 4	1.13±0.33[a]	1.50±0.00[b]
Week 6	0.87±0.33[a]	1.16±0.12[b]
Week 8	0.50±0.06[a]	0.90±0.05[b]
Week 10	0.40±0.03	0.70±0.10
Week 12	0.27±0.01[a]	0.50±0.06[b]
Week 14	0.15±0.29[a]	0.30±0.00[b]

Different superscripts in the rows indicate significant difference between the groups ($p<0.05$). Parameters reported as Mean ± SEM



AB (mm) = Fracture Gap (FG)

Figure 1: Determination of fracture gaps (FG) on the radiographs of dogs

Results

Surgeries were successful and well tolerated by the dogs. All dogs recovered from anaesthesia and were stable 2-3 days post-surgery. Mean weight of animals was 13.9 ± 0.50kg. There was no wound dehiscence at the surgical sites. Weight bearing began within two days post-surgery. There were no post-surgical complications and implant failure. The mean fracture gaps of the dogs in the treated group were significantly ($p<0.05$) lower than for dogs in

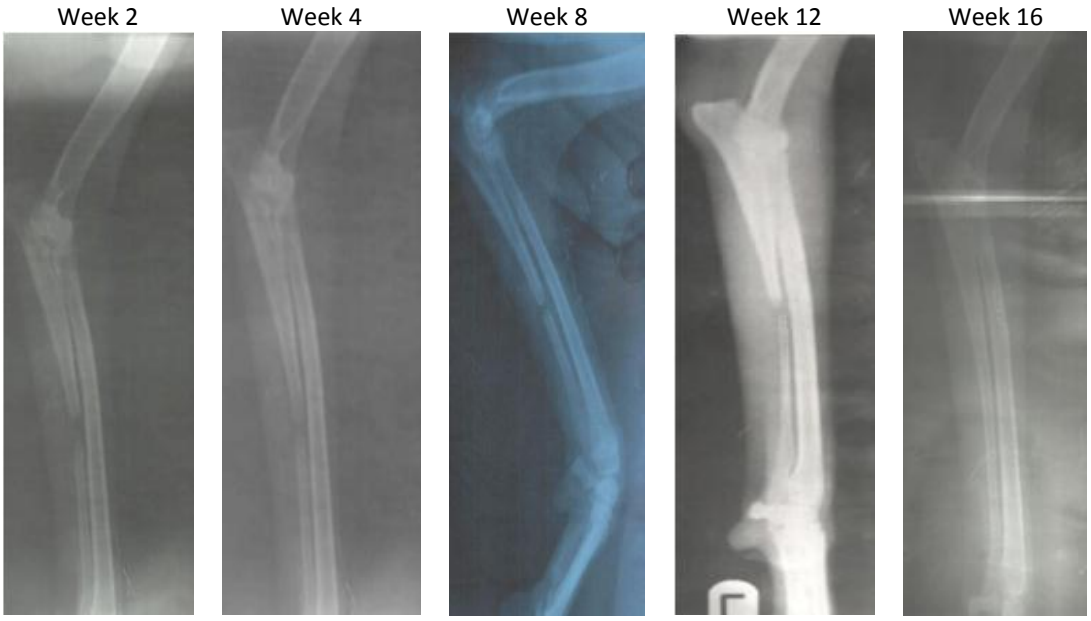


Figure 2: Serial radiograph of dogs treated with BMP-2/HA implants

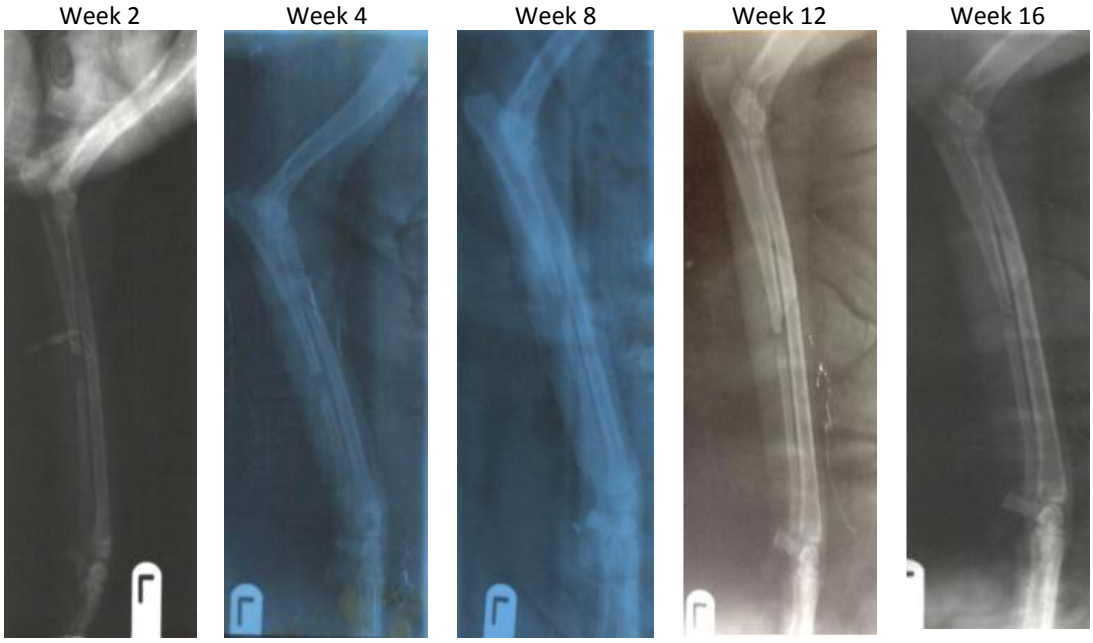


Figure 3: Serial radiographs of untreated dogs

the untreated group from week 2 to week 16 except on week 10 that showed no significant difference (Table 1).

Discussion

The study showed that BMP-2/hydroxyapatite enhances rates of union and repair of large defects. Certain common parameters were examined for each group. These were: i) the time of first appearance of callus (when the fracture gap reduced from 1.5 or when the ratio of group 1 compared to group 2 was below unity); ii) the minimum mean fracture gap achieved; and iii) the time taken to reach the minimum mean fracture gap as this can indicate approximately the point where callus growth ceases and fracture union is established. More so, the time it took for the mean fracture gap to return to zero was an appropriate estimation of the time of resorbtion and evidence of clinical union and remodeling. In evaluating the results, various methods of analysis were used, the principal method being radiography. Several radiographic features are easily observed during indirect or secondary fracture healing with the production of an external callus, these being the formation and growth of a calcified callus and the bridging of the fracture callus. As shown in Table 1; the mean fracture gaps in the treated group reduced progressively when compared to the untreated control. At week 16 the cortical integrity was totally restored in all animals in the treated group while the mean fracture gap of the control dogs was 0.3cm. The rate of restoration of cortical

integrity of the ulna bones was faster in the treated group when compared to the control. Callus resorbtion and remodeling to restore the integrity of the ulna bone occurred earlier in the treated group when compared to the control. BMP-2/hydroxyapatite significantly enhanced the rate of restoration of lost cortical bones in treated dogs.

Autogenous iliac bone graft has been always been considered the best standard in the management of fractures with severe bone loss because of its advantage of structural support and osteo-inductive capacity (Bloemer *et al* 2003). However procurement of this graft requires a second surgical procedure, creating injury and pain at an obviously uninjured and non-traumatized site. Apart from the risk of iatrogenic infection complications have been report to have resulted from these procedures (Goulet *et al* 1997; Hierholzer *et al.,* 2006). Injectable BMP implants, applicable with minimally invasive technique, would be suitable for the treatment of different bone healing problems (Uwagie-Ero *et al.,* 2014). The success of the use of bovine BMP in a canine ulna nonunion model has been demonstrated; when bovine BMP implanted with gelatin induced healing of bone defects in twelve weeks. (Nilsson *et al.,* 1986) Heckman *et al.,* (1991) using canine radius model treated established non-unions with BMP in a polylactic acid carrier. Yasko *et al.,* (1992) used guanidine hydrochloride-extracted demineralized rat bone matrix in a rat femoral model to test the efficacy of BMP. Segmental long bone defects have been used as models for bone reconstruction to evaluate different transplant materials as well as the efficiency of BMPs. The dog ulna model was used in this study, this model is valid in studying osteo-inductive agents because the defect does not heal spontaneously.

Since fracture healing involves a local cascade of molecular and cellular events, targeting the fracture environment systemically has not been reproduced successful (Harris *et al.,* 1975, Moskilde & Bak 1993). Local injection of various growth factors other than BMPs has produced mixed results (Uwagie-Ero *et al.,* 2014; Adeyanju *et al.,* 1982; Joyce *et al.,* 1990; Nash *et al.,* 1994; Bland *et al.,* 1995) and other studies have shown the successful use of rhBMP-2 with various carriers to heal large segmental bone defects, enhance periodontal regeneration, and promote spinal fusion (Uwagie-Ero *et al.,* 2016; Gerhart *et al.,* 1993, Cook *et al.,* 1994a). For effective delivery of growth factors into a fracture or segmental bone defect, biological carriers are required to prevent rapid dispersion of the proteins (Cook *et al.,* 1994b; Sandhu *et al.,* 1995; Wang *et al* 1998) and prolong the exposure time of the growth factor to osteoprogenitor cells hydroxyapatite was used as a carrier in this study and it served as a suitable bio-carrier for BMP. A major limitation to the study however, was the institutional ethical restraints so histopathology to assess the cellular involvements at various levels of healing was not done.

In conclusion, BMP-2 in a hydroxyapatite carrier resulted in significantly higher rates of cortical ulna bone growth and restoration; the increased cortical bone growth in the BMP-2/hydroxyapatite treated group proved effective in the primary treatment of fractures and closure of the large segmental bone defects. BMP-2 served as an alternative to bone graft in the management of segmental ulna bone defects. Results from the study showed that BMP-2 can be used to stimulate new bone growth – osteo induction, bone fracture healing can be significant enhanced by the use of BMP-2 and Hydroxyapatite is an effective bio-carrier for BMP-2.

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