Local Co-delivery of BMP2 and Clodronate Liposome for Treatment of Juvenile Femoral Head Osteonecrosis

[1,2]Chi Ma, [1,2] Ningning Lei, [1,2] Takuya Usami, [1,2] Yinshi Ren, [1]Sarah Madero, [1,2]Harry K.W. Kim

[1] Center of Excellence in Hip, Scottish Rite for Children, Dallas; [2] Department of Orthopedic Surgery, UT Southwestern Medical Center, Dallas

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INTRODUCTION: Legg–Calvé–Perthes disease (LCPD) is a juvenile idiopathic femoral head osteonecrosis characterized by extensive ischemic cell death and the release of damage-associated molecular patterns that activate macrophage pro-inflammatory response and abundant osteoclastic bone resorption. Bone morphogenetic protein-2 (BMP2) is a potent osteogenic factor but also known to activate osteoclasts. Clodronate liposomes (CL) selectively deplete macrophages and their lineage of cells, such as osteoclasts, through phagocytosis-induced apoptosis. We hypothesized that local BMP2 treatment following necrotic bone wash will stimulate early osteoclastic resorption followed by osteogenesis, while co-delivery of BMP2 and CL will attenuate early bone resorption, preserve the trabecular network, and stimulate osteogenesis, thus improving the healing process by preventing femoral head weakening associate with osteoclastic resorption.

METHODS: The experimental design to test this hypothesis and to determine the therapeutic effects of BMP2 and CL co-delivery following osteonecrosis in a piglet model of LCPD is illustrated in Fig. 1A. BMP2 and CL (BMP2+CL) were locally injected into the necrotic femoral heads (right side, n=8) using a heparin–gelatin–tyramine hydrogel carrier [1], following a bone wash procedure in the piglet LCPD model [2]. Bone wash alone (BW, n=12) and BMP2-only (n=7) groups served as treatment control groups. The contralateral left femoral heads were used as normal controls. Three weeks after treatment, the femoral heads were harvested and evaluated by gross and X-ray examination, hematoxylin and eosin (H&E) staining, tartrate-resistant acid phosphatase (TRAP) staining, and micro-computed tomography (μCT). Statistical analyses were performed using one-way ANOVA.

RESULTS: 1) Gross morphology: The normal femoral heads displayed a uniform reddish hematopoietic bone marrow, whereas the BW group showed white, fibrotic marrow tissue. In contrast, both BMP2 and BMP2+CL groups exhibited dark reddish marrow tissue, suggestive of revascularization and hematopoiesis (Fig. 1B). Quantitatively, the BMP2 and BMP2/CL groups showed comparable revascularization rates (84% and 81%, respectively), both significantly higher than the BW group (62%, $p < 0.05$; Fig. 1C). 2) Radiographic analysis: X-ray images revealed a uniform trabecular network in the normal control epiphysis, while the BW and BMP2 groups exhibited large areas of bone voids due to resorption (asterisks). However, no notable bone voids were observed in the BMP2+CL group (Fig. 1D–E). 3) Histological evaluation (H&E): Both BW and BMP2 groups exhibited large fibrotic areas devoid of trabeculae in the femoral epiphysis (red dashed line, Fig. 2A, upper panel). The BW group contained significantly higher amount of necrotic bone, as indicated by a high percentage of empty lacunae (82%, $p < 0.0001$, Fig. 2B, lower panel). This percentage was significantly reduced in the BMP2 group (7%, $p < 0.0001$). In the BMP2+CL group, new appositional bone was observed superficial to the remaining necrotic trabeculae with empty lacunae (25%, $p < 0.05$ for BMP2 versus BMP2+CL), indicating that some of the necrotic trabeculae were retained and served as a scaffold for new bone formation (Fig. 2A–B). 4) TRAP staining: TRAP-positive osteoclasts were found at the interface between necrotic bone and the revascularization front in the BW and BMP2 groups, with the BMP2 group showing higher number of osteoclasts compared to BW. In contrast, osteoclast numbers were substantially reduced in the BMP2+CL group (Fig. 3). 5) μCT analysis: 3D micro-CT reconstructions showed similar bone void volumes in the BW (32%) and BMP2 (31%) groups, both significantly higher than in the BMP2+CL group (12%, $p < 0.05$). The bone volume/tissue volume (BV/TV) was significantly higher in the BMP2+CL group compared with the normal ($p < 0.01$), BW ($p < 0.05$), and BMP2 ($p < 0.01$) groups. Trabecular separation in the BMP2+CL group was comparable to the normal group but significantly lower than that of the BW ($p < 0.01$) and BMP2 ($p < 0.01$) groups. Moreover, the BMP2+CL group exhibited a significantly higher trabecular number than the normal ($p < 0.0001$), BW ($p < 0.0001$), and BMP2 ($p < 0.0001$) groups.

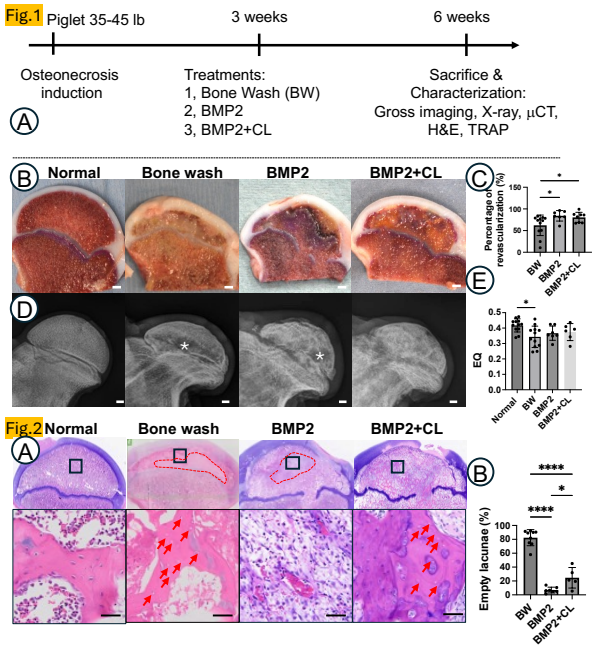
DISCUSSION: A previous study found that local BMP2 therapy can significantly increase bone regeneration in the piglet model of LCPD under non-weight-bearing condition [1]. Current study reveals that BMP2 treatment alone is associated with rapid early-phase bone resorption followed by new bone formation, raising concerns about potential bone collapse due to bone loss during resumption of weight bearing. The results of this study demonstrate that a combination of BMP2 and CL mitigates this risk by preventing early-phase bone loss through depletion of macrophage-lineage cells while promoting accelerated bone regeneration, thereby improving bone healing and trabecular morphometry after osteonecrosis.

CLINICAL RELEVANCE: LCPD affects 1 in 1200 children between the ages of 2 to 14. LCPD is a devastating hip disorder for children because of permanent femoral head deformity that results in early-onset osteoarthritis. Currently, no effective joint-preserving treatment is available, and 50% of LCPD patients will develop debilitating osteoarthritis requiring a joint replacement. The results of the study show that local co-delivery of BMP2 and CL is a promising joint-preserving treatment for LCPD patients.

REFERENCES:

[1] Ma C, et al., NPJ Regen. Med, 2023, PMID: 37709818

[2] Kim HK, et al., J Bone Joint Surg Am, 2021, PMID: 33877059



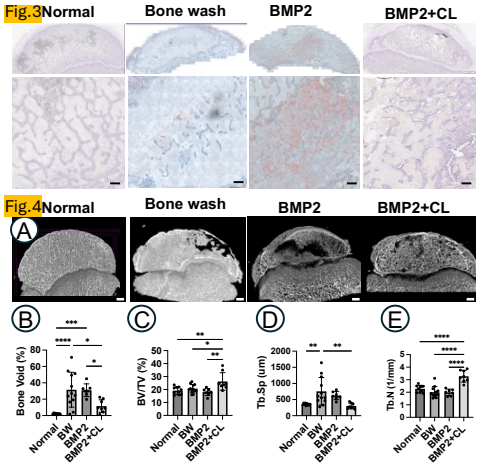


Fig.1. A) In vivo experimental design for testing the co-delivery of BMP2 and CL for LCPD treatment. (**B,C**) Representative gross images of cross-sectioned femoral heads (**B**) and quantification of revascularization in the femoral head (**C**) following different treatments. (**D,E**) Representative X-ray images of femoral heads (**D**) and bar graph showing the epiphyseal quotient (EQ, **E**) under various treatments. Scale bar: 500 μm.

Fig.2. A) Representative H&E images of femoral heads (upper panel: whole head; lower panel: enlarged view of the boxed region). (**B**) Quantification of empty lacunae from H&E images. Scale bar: 50 μm. Red arrows showing the empty lacunae.

Fig.3. Representative TRAP images of femoral heads following different treatments. Scale bar: 50 μm.

Fig.4. A) Representative cross-sections of 3D reconstructed μCT images showing femoral heads after different treatments. (**B–E**) Quantification of bone parameters, including bone void volume (**B**), bone volume/tissue volume (BV/TV, **C**), trabecular separation (Tb.Sp, **D**), and trabecular number (Tb.N, **E**). Scale bar: 500 μm.