

Effects of low dose BMP-2 and immunomodulation targeting IL-1 β on fracture healing in a femur defect model in rats

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INTRODUCTION: Stimulating bone healing using osteo-inductive growth factors, delivered via an osteoconductive scaffold, represents a common strategy when healing is impaired like in large segmental bone defects or complex comminuted fractures [1]. Clinically applied bone morphogenetic growth factors like BMP-2 show good healing capacity but have been associated with excessive and prolonged pro-inflammatory cytokine release and heterotopic ossification in muscle at high doses [2]. Immunomodulation approaches may represent a novel strategy to improve bone healing. Local administration of interleukin-1 receptor antagonist (IL-1Ra), has recently been shown to enhance bone healing in a femoral segmental defect in rats when used in combination with a low dose of BMP-2 [3]. However, due to the rapid degradation of IL-1Ra *in vivo* [4], more effective strategies to inhibit interleukin 1 β (IL-1 β) activity in the fracture microenvironment are needed. This study aims to investigate (1) the effect of a low dose of BMP-2 (1 μ g) alone, delivered locally via a collagen scaffold, on callus formation and pro-inflammatory cytokine production at the fracture site (fracture repair tissue (hematoma, callus) and adjacent muscle) at early time points in a femur segmental defect model in rats and (2) the effect and optimal timing of administration of a monoclonal anti-IL-1 β antibody in combination with low dose BMP-2 (1 μ g) on bone healing over 14 weeks in the model.

METHODS: 2 mm segmental femoral defects were created in skeletally mature (22-27 weeks old) female Fischer F344 rats, internally fixed with a plate (animal license: GR/19/2022; nat. number: 35156) using established protocols for analgesia and anesthesia. To study BMP-2 mediated effects on early fracture healing, animals (n=3 per group) received either no treatment (empty defect), a Lyostypt collagen sponge, or a Lyostypt collagen sponge + 1 μ g BMP-2 (InductOs, Medtronic) and were sacrificed at either 3, 7 or 14 days. Radiographs were taken at surgery and at regular intervals throughout the study. In addition, the fracture repair tissue, as well as the adjacent muscle, were collected, snap frozen in liquid nitrogen, and stored at -80°C. Afterwards, the tissues were collected in T-PER tissue protein extraction reagent (Thermo Fisher) and the levels of cytokines, e.g. IL-1 β , were determined by ELISA (R&D Systems DuoSet). In a follow up study, animals (n=4 per group) received either a collagen sponge, a collagen sponge + 1 μ g BMP-2 or collagen sponge + 1 μ g BMP-2 in combination with a monoclonal anti-IL-1 β antibody (InVivoMAB anti-mouse/rat IL-1 β , clone B122, BioXCell, 10 mg/ml), starting either from day 0 or 3. The antibody was administered intravenously under anesthesia every third day until day 15. New bone formation within the defect site was assessed using *in vivo* micro-CT immediately after surgery and at 2, 3, 4, 6, 8, 10 and 14 weeks post-OP. The mechanical properties of the operated femurs were assessed by non-destructive 4-point bending using an Instron 5866 material testing system and compared to the contralateral femurs. Data in Fig. 2 was statistically tested by two-way ANOVA followed by Bonferroni post-hoc analyses, data in Fig. 3 by one-way ANOVA (GraphPad Prism 8 software).

RESULTS SECTION: In the early healing phase following the fracture, low dose BMP-2 had a strong local effect on new bone formation while maintaining IL-1 β levels in the hematoma and adjacent muscle comparable to controls. Radiographs demonstrated that collagen scaffolds with 1 μ g BMP-2 induced pronounced new bone formation and cortical bridging 2 weeks post-operatively (Fig. 1). IL-1 β levels in the fracture repair tissue of all groups showed a decline from day 3 to 14 (Fig. 2A). Lower IL-1 β levels were detected in the muscle than in the fracture repair tissue (Fig. 2B). Moreover, evaluation of the micro-CT scans of the second part of the study demonstrated that administration of anti-IL-1 β (starting from day 0) + 1 μ g BMP-2 led to faster cortical bridging (3/4 femurs bridged by week 4) than 1 μ g BMP-2 alone (0/4 femurs bridged by week 4), this effect was even more pronounced in the group anti-IL-1 β (starting from day 3) + 1 μ g BMP-2 (4/4 femurs bridged by week 4). Non-destructive 4-point bending showed higher mechanical competence for the IL-1 β groups (average stiffness 106% of contralateral femurs for group anti-IL-1 β + (day 0) + 1 μ g BMP-2 and 117% for group anti-IL-1 β + (day 3) + 1 μ g BMP-2) compared to BMP-2 alone (average stiffness 72% of contralateral femurs) (Fig. 3).

DISCUSSION: In the early healing phase following the fracture, low dose BMP-2 alone had a strong local effect on new bone formation in the femur segmental defect model in rats without inducing excessive and pro-longed cytokine expression. Moreover, anti-IL-1 β administration in the first two weeks following surgery had a beneficial effect on late fracture healing with faster cortical bridging and new bone formation than low dose BMP-2 alone. Anti-IL-1 β administration starting from day 3 may be more effective in promoting cortical bridging compared to day 0.

SIGNIFICANCE/CLINICAL RELEVANCE: Our study shows a potential for combined immunomodulatory treatments e.g., monoclonal anti-IL-1 β and recombinant BMP-2, to promote bone healing with relevance for clinical applications.

REFERENCES: [1] Ball *et al.* Bioengineering 10(11) (2023): 1052 [2] James *et al.* Tissue Eng. Part B Rev 22(4) (2016):284-297 [3] Lackington *et al.* Acta Biomater. 1(149) (2022):149-189 [4] Furst DE. Clin. Ther. 26(12) (2004) 1960-1975

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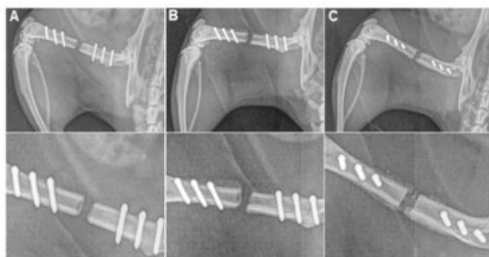


Figure 1: Representative radiographs of femoral defects 2 weeks post-surgery. (A) Empty, (B) collagen sponge, (C) collagen sponge + 1 μ g BMP-2.

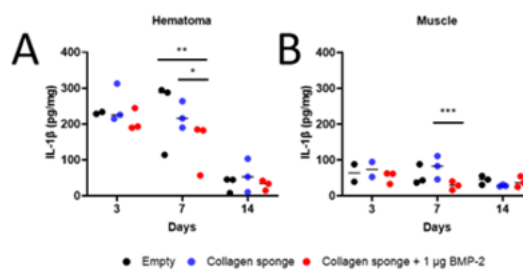


Figure 2: IL-1 β concentration in (A) hematoma and (B) adjacent muscle on days 3, 7 and 14 of fracture healing. Data represents the mean \pm standard deviation (n=3). *** indicates p<0.001, **p<0.01 and * p<0.05, two-way ANOVA.

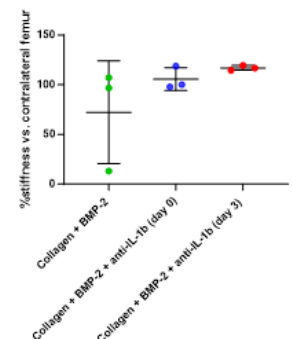


Figure 3: None destructive mechanical testing of operated femurs. Bone stiffness is given in % of contralateral femur stiffness (n=3).