Osteoblast and Osteocyte Specific Connexin43 Knockout Mice have Impaired Bone Remodeling During Fracture Healing

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Introduction: Connexin 43 (Cx43) is the most abundant gap junction protein in bone. Cx43 has been shown to play many important roles in both bone formation and homeostasis including regulation of osteoblastic proliferation, differentiation and survival. In vitro work from our laboratory has shown that gap junction intercellular communication (GJIC) is required between osteocytes and osteoblasts in order to transduce biophysical signals such as fluid flow induced shear stress. Inhibition of GJIC can also impair osteoclastogenesis. Mice with osteoblast/osteocyte specific (Osteocalcin-Cre) loss of Cx43 display decreased gap junctional intercellular communication, bone density, cortical thickness and load at failure. The purpose of this study was to examine the role of Cx43 in mature osteoblasts during fracture healing, and test the hypothesis that loss of Cx43 results in delayed fracture healing due to decreased bone remodeling.

Methods: A closed fracture was produced by three-point bending in Osteocalcin-Cre+; Cx43<sup>fl/fl</sup> (KO) and Cre- Cx43<sup>2/2</sup> (WT) mice. Ten week old female mice were used in this study. Healing was assessed by X-ray, µCT, histology and in situ hybridization to detect osteoclasts. A novel method of µCT analysis was used to identify actively remodeling bone in the fracture callus. Low density bone volume and high density bone volume were calculated by thresholding. Actively remodeling bones had a greater proportion of low density woven bone. Osteoclasts were quantified from three sections/ specimen, and the number of osteoclasts in a defined area of the callus counted.

Results: A bridging callus was present in WT and KO mice 14 days after fracture, with Alcian blue staining of the cartilaginous callus in both groups. By 21 days, CoI2 and CoI2<sup>+</sup> positive cells were still present in the fracture callus of KO mice, but less so in WT, indicating a persistence of cartilage in KO. By 28 days, there was an increase in the area of mineralized tissue in WT callus compared to KO (Figure 1A&B). In addition, there was a greater area of CoI1 expression in WT callus at 28 days compared to KO, indicating new bone formation.

The total volume of callus was increased in KO, relative to WT, at 21 days (WT: 4.15±0.17; KO: 9.47±0.37, p=0.017; Figure 2A), consistent with a larger callus volume. Using a novel µCT analysis method to identify remodeling bone, we found that low density bone volume (BV), within the callus was increased in WT mice compared to KO at 28 and 35 days post-fracture (28- WT: 2.69±0.75; KO: 1.29±0.49, p=0.036; 35- WT: 1.19±0.29; KO: 0.73±0.12, p=0.41 Figure 2B), indicating more remodeling in WT bones.

Based on the impaired ability of cells with inhibited gap junctional communication to form osteoclasts in vitro, we explored changes in osteoclast number as a potential mechanism for decreased callus remodeling in Cx43<sup>−/−</sup> femur fractures. There was a significantly greater number of TRAP<sup>+</sup> osteoclasts in the callus of WT mice at 14 and 28 days compared to KO at the same time-points (Day 14- WT: 62.5±2.58, KO: 31.64±1.89 p=0.021; Day 28- WT: 33.9±2.76, KO: 13.41±3.60, p=0.037; Figure 3). There was abundant expression of RANKL at 28 days in the callus of WT mice, while there was minimal expression in KO mice at this time by in situ hybridization. Interestingly, OPG, the RANKL decoy receptor was strongly expressed in the callus of KO, relative to WT mice at 21 days post-fracture.

Discussion: Expression of OPG in the callus of KO mice suggests that the decreased presence of osteoclasts in callus of Cx43 deficient mice may be due to OPG, rather than decreased expression of RANKL, although minimal RANKL expression was detected at all times in the callus of KO mice. Interestingly, osteoclast number was increased and RANKL expression decreased in non fractured bone from Cx43 deficient mice relative to wild type (not shown). This suggests a differential Cx43 regulation of osteoclasts in normal bone and fracture callus. In any case these data indicate that loss of Cx43 in mature osteoblasts results in delayed completion of endochondral bone formation and decreased bone remodeling during fracture healing. These studies identify a novel role for the gap junction protein Connexin 43 during fracture healing, suggesting that loss of Cx43 can result in decreased remodeling and osteoclast formation. Therefore, enhancing Cx43 expression or GJIC may provide a novel means to enhance fracture healing. Indeed, parathyroid hormone, which increases osteoclastic cell GJIC also enhances fracture healing, supporting the concept that targeting connexins and GJIC will increase fracture healing.

References:
1. Donahue HJ: Bone 2000, 26:417-422