Inhibition NF-κB Signaling Pathway Leads to Improved Bone Quality without Interfering with Bone Healing

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Introduction: The transcription factor nuclear factor kappa B (NF-κB) plays a critical role in inflammatory and immune responses. Many studies indicate that NF-κB antagonists hold great promise for the treatment of metabolic bone diseases, such as osteoporosis, however, concerns about the inhibition of the NF-κB signaling pathway may also impair bone healing through the influence of inflammatory processes. In fact, other compounds used for the treatment of metabolic bone disorders including corticosteroids, NSAIDS, and bisphosphonates, which all impact inflammatory processes, have been shown to be detrimental to fracture healing. It has been shown that RANK inhibition (NF-κB inhibition) is sufficient to reduce bone resorption without being detrimental to bone healing [1]. However, exploring more specific targets in the NF-κB signaling pathway that could inhibit bone loss, without affecting the bone healing process, remain important for developing novel and improved strategies for treating osteoporosis and inflammatory bone diseases. Here we report that the inhibition of the NF-κB signaling pathway by the partial ablation of the P65 subunit, through the use of p65+/- mice, leads to improved bone quality without interfering with bone healing.

Methods: The mice lacking one allele of the NF-κB subunit p65 (p65+/-) and wild type (WT) mice at 1 year of age were used in this study. All animal protocols used for these experiments were approved by the University of Pittsburgh’s Animal Care and Use Committee. Bone quality of p65+/- and WT mice on L6 vertebrae and mid-shaft of femur were evaluated by Micro-CT analysis and histomorphometric analysis. In order to study the influence of P65 partial ablation on the bone healing process, a 1mm diameter unicortical bone defect was created on the medial side of proximal tibia. The bone healing process was monitored with Micro-CT scanning at weeks 1 and 2 after surgery, and compared between p65+/- and WT mice groups.

Results: Inhibition of NF-κB by partially ablation of the P65 subunit increases bone quality: Micro-CT revealed that at age one year, p65+/- mice displayed more dense bone tissues both in the lumber vertebra and the mid-shaft of femur compared to their WT littermates, as visualized by H&E, von Kossa stains and measured by Micro-CT(n=6). Quantitative analyses by Micro-CT demonstrated that p65+/- mice had significantly more bone tissue (BV/TV) (p=0.0378), a greater number of trabeculae (Tb. N) (p=0.0089), and less trabecular spacing (Tb. Sp) (p=0.0066) in their lumber vertebra (Fig.1, n=6). Moreover, they had thicker cortical bone (p=0.0352), and higher bone mineral density (BMD) (p=0.030) in the mid-shaft of their femur when compared with WT mice (Fig.2, n=12). These data demonstrated that the inhibition of NF-κB by partial ablation of P65 subunit increases bone quality when compared to their WT littermates. In addition, in vivo dynamic labeling revealed that bone turnover rates were significantly lower in p65+/- mice than those in WT mice (data not shown). Histological analysis showed that the partial ablation of P65 did not affect osteoblast differentiation (data not shown), while significantly less osteoclasts were found in the p65+/- mice than that in the WT mice (data not shown).

Inhibition of NF-κB by partial ablation of the P65 subunit does not influence bone healing: At all time points after surgery, X-ray radiographs showed evidence of callus formation at the defect areas in both the p65+/- mice and WT mice. No significant differences were found in either of the groups regarding the volume of callus and BMD (Fig. 3, n=8). HE von Kossa staining of cross sections of the defect area showed evidence of newly formed bone. All specimens in both groups showed woven bone-bridging across the defect areas, no significant differences were found between p65+/- mice and WT mice regarding collagen deposition and calcification (data not shown).

Discussion: Our results demonstrated that the specific inhibition of the NF-κB signaling pathway by partial ablation of the P65 subunit leads to improved bone quality without impairing bone healing. These findings suggest that inhibiting the NF-κB signaling pathway through partial P65 ablation may represent an important therapeutic target for developing novel and improved strategies for treating osteoporosis and inflammatory bone diseases without interfering with the healing process.

Significance: Understanding more specific regulation of the NF-κB signaling pathway during bone homeostasis and bone healing is important for developing novel and improved strategies for treating osteoporosis and inflammatory bone diseases.

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Figure 1. Micro-CT analysis of the L6 vertebrae.
Figure 2. Micro-CT analysis of the mid-shaft of femur
Figure 3. Micro-CT analysis of the bone defect area

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