Salt inducible kinase inhibition alters bone morphology in a site-specific manner in response to mechanical loading

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Introduction: Affecting one in three women, osteoporosis is the most common metabolic bone disorder, characterized by low bone mass and deterioration of the bone tissue, ultimately leading to bone fracture[1]. Parathyroid Hormone (PTH) is one of the few anabolic therapeutics approved by the FDA to treat osteoporosis, and the synergistic effect of PTH and mechanical loading stimulates bone formation further[2]. PTH affects bone formation and resorption by inhibiting Salt Inducible Kinases (SIK) and promotes the nuclear translocation of HDAC4/5 and CRTC2[3]. However, efficacy of PTH is limited to 2 years[4]. Therefore, understanding the anabolic window and the mechanism of PTH action through SIK inhibition are necessary to find alternative treatments for osteoporosis. SIK inhibition by genetic knock-out in the mouse and through an inhibitor compound (SK-124) demonstrated similar effects on bone as PTH treatment, increasing cancellous bone in young male mice[5]. The focus of this study was to investigate the effect of SIK inhibition on adult female mice and the potential anabolic synergism with mechanical loading.

Method: Following IACUC approval, 17-week-old female C57BL/6J mice were treated with SIK inhibitor SK-124 (40mg/kg via intraperitoneal injection) or Vehicle (15% HPBCD in sterile water, VEH) for two weeks (five days of injections and loading per week, followed by two days of rest) with concurrent in vivo cyclic tibial mechanical loading at 9N and 4Hz for 1200 cycles (n=10/group). The left tibia was loaded (L), and the right limb was the contralateral control (C)[6]. Animals were euthanatized on day 15. Blood serum was collected by cardiac puncture for ELISA analyses of P1NP and CTX. Tibiae were dissected and prepared for microCT imaging (n=10/group) and immunohistochemistry (IHC: n=5/group). MicroCT scans were analyzed at the cancellous core and cortical shell of the metaphysis and cortical mid-diaphysis. IHC was performed on 6 µm thick paraffin embedded sections. Anti-SOST and anti-RANKL antibodies were used to analyze sclerostin expression and RANKL expression in osteocytes. Significance was set at $p < 0.05$ for linear mixed model (loading and treatment as fixed effect and mouse as random effect) and Tukey HSD post hoc test was performed when the interaction term was significant.

Results: SK-124 treated mice had significantly higher systemic P1NP levels compared to vehicle mice (+46.2%, p=0.039, Fig.1a). Systemic CTX serum levels did not differ with SIK inhibition (Fig. 1b). In the metaphyseal shell, control limbs in the SK-124 group had a lower cortical area (-4.6%, Ct.Ar, p=0.015), cortical thickness (-5.1%, Ct.Th, p=0.01) and tissue mineral density (-1.7%, Ct.TMD, p=0.016) compared to the VEH group (Fig. 1c,d). When combined with mechanical loading, in the cancellous core, loaded limbs had 10.4% higher trabecular thickness (Tb.Th) in both groups (p<0.0001, Fig. 2a). SK-124 limbs had a 1.9% lower tissue mineral density (Cn.TMD) compared to vehicle group (p=0.012). Cn.TMD was 1.6% lower in loaded limbs compared to control limbs in both groups (p=0.020, Fig. 2b). Neither treatment altered bone volume fraction (BV/TV). In the metaphyseal shell, loaded limbs had higher maximum and minimum moment of inertia (+12.3% Imin & +17.9% Imax, both p<0.0001), Ct.Th (+7.2%, p<0.0001) and Ct.Ar (+11.8%, p<0.0001) in both groups (Fig. 3a,b). With loading the SK-124 group had a greater increase in Ct.Th compared to the VEH group (203.6%, p=0.0002, Fig. 3b). Loading decreased the percentage of sclerostin positive osteocytes by 24.8% (p=0.0072) in both groups and decreased the percentage of RANKL positive osteocytes by 80.0% in VEH group (load*tx, p=0.009) in the metaphyseal shell. SIK inhibition blunted the load-induced RANKL decrease (Fig. 3c,d).

Discussion: SIK inhibition reduced metaphyseal shell bone mass, as shown by the decreased Ct.Th and Ct.Ar. The higher change in Ct.Th with loading and SIK inhibition group indicates an anabolic synergistic effect between SIK inhibition and mechanical loading in the metaphyseal shell. Furthermore, loading attenuated SIK inhibition-induced metaphyseal cortical bone loss, indicating that mechanical loading prevented metaphyseal shell bone loss and potentially increased fracture risk due to SIK inhibition. Lower cancellous bone tissue mineral density suggested that newly formed bone was less mineralized. However, cancellous bone volume did not differ, which could reflect the short experimental duration, mouse age and sex, and the administration route. SIK inhibition did not alter mid-diaphyseal cortical bone morphology. Metaphyseal shell bone loss due to SIK inhibition potentially could increase the loads borne by the cancellous bone, leading to the increased cancellous bone in the metaphysis observed by Sato et al[4]. Mechanical loading decreased the sclerostin expression level in metaphyseal shell osteocytes, suggesting an anabolic effect of loading. SK-124 diminished the load-induced anabolic effect as demonstrated by the stable RANKL expression between control and loaded limbs, which could reflect the catabolic effect of SIK inhibition on metaphyseal shell. Future work should study the spatial and temporal effect of SIK inhibition on cortical and cancellous bone.

Significance: Understanding the synergism between SIK inhibition and mechanical loading and the advantage of SIK inhibition over PTH will provide insights for clinical therapeutics for osteoporosis.

References: [1]Imel et al. 2014. [2]Sugiyama et al. 2008. [3]Wein et al. 2016. [4]Aslan et al. 2012. [5]Sato et al. 2022. [6]Fritton et al. 2005

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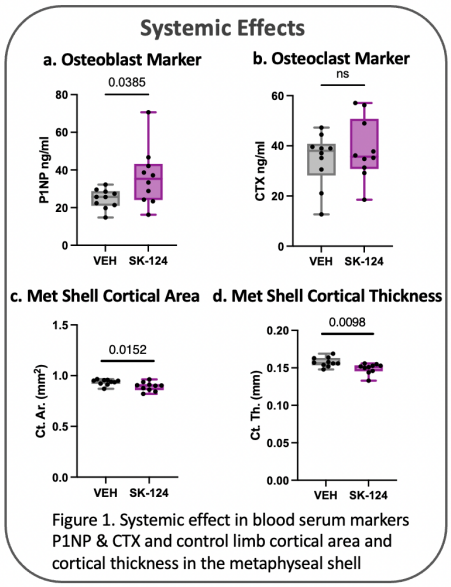

Figure 1. Systemic effect in blood serum markers P1NP & CTX and control limb cortical area and cortical thickness in the metaphyseal shell

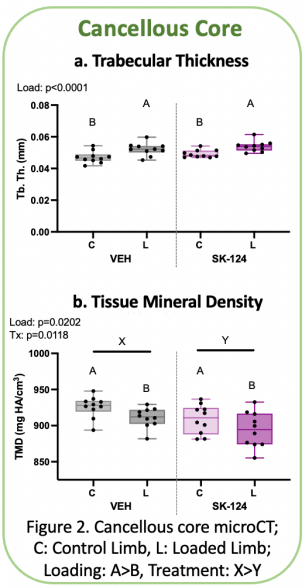

Figure 2. Cancellous core microCT; C: Control Limb, L: Loaded Limb; Loading: A>B, Treatment: X>Y

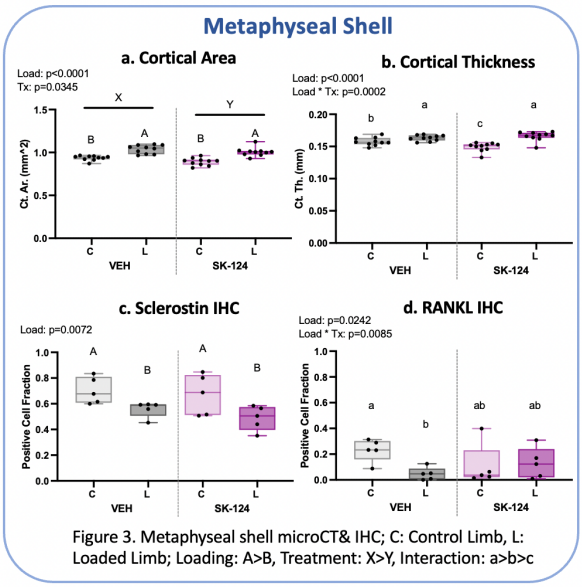

Figure 3. Metaphyseal shell microCT& IHC; C: Control Limb, L: Loaded Limb; Loading: A>B, Treatment: X>Y, Interaction: a>b>c