• Modulation of Wnt Signaling During Fracture Repair

+1Komatsu, D E; 1Mary, M N; 1Schoeder, R J; 2Robling, A G; 2Turner, C H; 2Warden, S J
+1InMotion Musculoskeletal Institute, Memphis TN
+3Indiana University, Indianapolis IN
Senior author dkomatsu@inmotionmemphis.org

INTRODUCTION:
Fracture healing involves the precisely coordinated activation of multiple signaling pathways. Recently, evidence has emerged that the canonical Wnt signaling pathway is one of these. Though much is known about the role of Wnt signaling in skeletal development and regulation, its role in fracture repair has yet to be clearly elucidated.

At the cellular level, Wnt signaling is initiated when Wnt ligands bind to Frizzled and the Wnt co-receptor Low-Density Lipoprotein Receptor-Related Protein 5/6 (LRP5/6). Loss of function mutations in LRP5 (and the mouse ortholog Lp5) are associated with low bone mass and skeletal fragility, while activating mutations result in high bone mass phenotypes. The availability of LRP5 to bind Wnt ligands is regulated by several secreted proteins including Dickkopfs (Dkkks), SOST (the sclerostin gene product), and Wnt-1-induced secreted protein (WISE); all of which bind to LRP5 and prevent signal transduction.

In order to elucidate the importance of Wnt signaling in fracture repair, a study was conducted in Lp5 knockout mice and wild type littermates, as well as C57BL/6 mice treated with anti-Dkk1 antibodies (Dkk-1 Ab), to test the hypothesis that inhibition and activation of Wnt signaling impairs and enhances bone regeneration, respectively.

METHODS:

Animal Model - Male and female mice with global Lp5 deficiency (Lp5-/-), wild-type littermates (Lp5+/+), and male C57BL/6 mice were subjected to unilateral closed femoral fractures at 17 weeks of age. Lp5-/- and Lp5+/+ mice (5 mice/group; N=13, 8+/+ N=16, 5-/- N=12, 8+/+ N=9) received no treatment while C57BL/6 mice were subjected to one of 3 treatments: 1) Vehicle (PBS, 2x/wk initiated post-op, N=9); 2) Dkk-1 Ab (25 mg/kg s.c., 2x/wk initiated post-op, N=9); 3) Dkk-1 Ab (25 mg/kg s.c., 2x/wk initiated 4 days post-op, N=8). Animals were radiographed post-operatively and weekly thereafter until sacrifice 4 weeks later. All procedures were approved by the Indiana University IACUC.

Dual-Energy X-Ray Absorptiometry (DXA) - Fractured and intact femurs were scanned using a PIXImus II (GE-Lunar Corp.) and bone mineral density (BMD), bone mineral content (BMC), and area were quantified at a threshold of 1341 mg/cm^2.

Peripheral Quantitative Computed Tomography (pQCT) - Fractured and intact femurs were scanned in a Stratec XCT (Stratec Electronics). Three 0.07 mm slices were acquired for each sample (callus center ± 0.5 mm). Total and mature callus area and density were calculated for fractured femurs using thresholds of 250 and 600 mg/cm^3, respectively. Total area and density for intact femurs was measured at the 600 mg/cm^3 threshold.

Mechanical Testing - All femurs were subjected to 4-point monotonic bending to failure (0.1 mm/min) using an ELF3200 (Bose Corp.). Force versus displacement curves were plotted in Excel and energy to failure, stiffness, and ultimate force were calculated using a set of custom written macros.

Statistical Analyses - Responses from fractured Lp5-/- and Lp5+/+ femurs were normalized to their contralateral intact femurs, in order to account for baseline genotypic differences. Effects of sex and genotype on the responses were assessed using ANOVA. Responses from Dkk-1 Ab treated mice were compared using ANOVA, with pairwise comparisons made using Dunnett’s procedure. Intact and fractured samples were compared separately.

RESULTS:
Fractured femurs from Lp5-/- mice displayed significant reductions in area (25%), BMC (15%), and BMD (10%), with no effects observed for sex. Measurement of cross-sectional callus area revealed significant reductions of 20% for both total and mature callus area in female and Lp5-/- mice with no significant sex or genotype effects seen for density. Concomitant with these radiographic impairments, Lp5 deficiency significantly diminished biomechanical integrity of the fractured femurs for all parameters measured with losses of 48, 26, and 38% seen for energy to failure, stiffness, and ultimate force, respectively. In addition, a sex effect was observed with female mice showing a 35% reduction in energy to failure. No interactions between genotype and sex were seen.

Dkk-1 Ab treatment resulted in differing effects on intact and fractured femurs depending on the timing of treatment initiation. Following post-op initiation, intact femoral BMD significantly increased by 6%, compared to vehicle. In contrast, when treatment began 4 days post-op, intact femurs had significant reductions in cross-sectional area (14%) and density (6%), as well as whole bone BMD (9%), which contributed to a significant 13% reduction in ultimate force. Comparison of fractured femurs revealed that post-op Dkk-1 treatment enhanced fracture repair (compared to vehicle) with significant increases seen for total (58%) and mature callus area (29%), whole bone BMD (15%) and BMC (23%), contributing to biomechanical gains of 40% in stiffness and 26% in ultimate force. Delaying Dkk-1 treatment to day 4 resulted in no observable effects, with fractured femurs from these mice indistinguishable from vehicle treated.

DISCUSSION:
These experiments sought to test the complementary hypotheses that inhibition of Wnt signaling adversely affects fracture repair and activation of Wnt signaling enhances this process. The data supports these hypotheses, as fractured femurs from Lp5-/- mice were smaller, less mineralized, and biomechanically inferior to those from wild-type littermates. Conversely, elevation of endogenous Wnt signaling by neutralization of Dkk-1 mediated Wnt suppression increased the size, mineralization, and biomechanical properties of fractured femurs.

The degree of impairment seen in Lp5-/- mice by radiographic analyses was mild compared to their loss of biomechanical integrity. This may indicate that during fracture repair, Lp5mediated Wnt signaling predominantly acts to establish architecturally sound and well organized bone and contributes little to simple mineral accrual.

This study identified timing of Dkk-1Ab treatment initiation as a key determinant of this compound’s efficacy in enhancing fracture repair. The success of post-operative initiation suggests clinical utility as treatment can be administered immediately and would possibly only require a single dose.

This study demonstrates that activation of Wnt signaling predominantly acts to establish architecturally sound and well organized bone and contributes little to simple mineral accrual.

REFERENCES:

ACKNOWLEDGEMENTS:
The anti-Dkk-1 antibody was kindly supplied by Amgen, Inc. This work was generously supported from a grant by NIH R01-AR53237.