Role of CaMKK2 in Mechanically Induced Bone Formation

Adam J. Warrick[1], Uma Sankar[1]

[1] Department of Anatomy and Cell Biology, Indiana University School of Medicine

Background and Hypothesis: Mechanical stimulation of bone results in the translation of external forces into a cascade of structural and biochemical changes which work to increase bone density and decrease fracture healing time. The specific mechanisms contributing to these processes are areas of active investigation. Ca^{2+}/calmodulin-dependent protein kinase kinase 2 (CaMKK2) is a serine-threonine protein kinase with key roles in both the anabolic and catabolic pathways of bone remodeling. We hypothesize that the absence of CaMKK2 potentiates an increase in bone density as a response to mechanical stimulation.

Experimental Design or Project Methods: The right ulna of anesthetized C57BL/6 mice were loaded for 220 cycles at 2 Hz and with peak forces specific to both sex and genotype. Loading was completed using an electro actuator (Bose ElectroForce 3200; EnduraTEC, Minnetonka, MN, USA) and was repeated on days 3, 5, 8 and 10 after the initial procedure. The non-loaded left ulna served as an internal control. Calcein and alizarin red were administered intraperitoneally on days 9 and 16 respectively. Mice were sacrificed on day 19 after the initial load; blood and long bones of the lower limbs were collected for analysis.

Results: Bone volumetric analyses will be measured using microcomputed tomography, bone formation rate will be assessed using dynamic histomorphometry measurements of double fluorochrome labeling, and cellular and molecular mechanisms will be assessed using histology, immunohistochemistry and real-time reverse transcription-polymerase chain reaction. These data are currently forthcoming.

Conclusion and Potential Impact: Clinical outcomes of conditions ranging from stress fractures to osteoporosis may be improved by an increased understanding of the mechanisms through which bone growth is augmented. Expanded knowledge of these pathways may provide opportunities for the development of novel therapies which decrease healing times in the event of injury and increase bone density to combat degenerative disease states.