Mechanistic Propensities of Cryotherapy in a Bone Injury Site

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Purpose: It has been previously established that when the vascular network in and around bone is compromised, hypoxia occurs near the fracture site. Furthermore, acute cold exposure has also been shown to trigger upregulation of RBM3 and PGC-1α, both of which have essential roles in osteogenesis. The objective of this study is to elucidate the mechanism by which cold therapy affects bone formation in vivo at the injury site. Here we hypothesize that localized application of cold temperature will lead to elevated induction of hypoxia in the bone injury site indirectly in conjunction with increased detection of RBM3 and PGC-1α, which is responsible for the upregulation of osteoblast differentiation.

Methods: A bilateral cortical bone defect model was applied to 13 male C3H strain mice aged 2-3 months. Following the formation of the defect, the region was flushed with phosphate-buffered saline (PBS) to remove any remaining bone fragments. Hypoxyprobe was then intraperitoneally injected 7 days post-operation into the mice. The experimental hindlimb of the mouse was then exposed to an ice-water bath for 15 minutes. An internal temperature of 19°C within the mouse hindleg was measured in previous studies done at our lab using the same methodology.

Results: A 5.6% increase ($P<0.01$) compared to untreated controls in the number of hypoxic cells within and around the cortical bone defect in the hindlimbs of mice following exposure to an acute cold stimulus demonstrates detectable localized hypoxia induction through the application of cold exposure. Increased detection of PGC-1α ($P<0.005$) and RBM3 ($P<0.008$) were prevalent in newly forming bone cells within cortical defects following cold exposure, signifying elevated presence of proteins involved within the osteoblastogenesis pathway.

Conclusion: The results illustrate the development of a detectable hypoxic environment that provides a conjunctive avenue to explore certain regenerative pathways dependent upon hypoxic conditions within the early stages of bone repair. PGC-1α and RBM3's increased presence within a bone injury site such as a cortical defect may reflect their importance in the bone repair process in conjunction with the mechanistic propensities of cold therapy. Osteoblastogenesis, a pathway both proteins stimulate, results in the formation of new osteoblasts that has an essential role in the formation of new bone tissue and a possible pathway for bone repair.