Δ Effect of Cold Therapy on Bone Healing

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Purpose: The purpose of this study is to investigate the effect of local cold therapy on bone healing. The hypothesis is that the group exposed to cold therapy will show more signs of healing in both microCT and histologic analysis when compared to the control group.

Methods: Twelve C3H wild-type mice aged 2-3 months were used in the study. A 1-mm burr was used to create a 1 × 3 mm unicortical rectangular bone window in the lateral aspect of the femoral diaphysis. The right side was designated as the experimental side in all mice. A temperature sensor implanted on the quadriceps of the index mouse was able to confirm that using a 6°C cold bath, the legs were cooled down to 19°C within 3 minutes. The experimental side was immersed daily in a 6°C cold bath for a total of 15 minutes. A total of 12 mice underwent the abovementioned protocol without complications. MicroCT analysis was performed using a Skyscan 1172 microCT (Bruker Corp). A region of interest (ROI) was defined using a 1-mm fixed diameter circle centered on the medullary canal. A constant total volume (TV) was created by extrapolating the ROI over a distance of 2.3 mm, centered at the middle of the cortical defect. Using a threshold of 55 Hounsfield units, the total bone volume (BV) was extrapolated from the ROI. For histologic analysis, the femora were fixed and cut in 5-μm sections. Staining for alkaline phosphatase (ALP), CD34, and tartrate-resistant acid phosphatase (TRAP) was then performed.

Results: The average bone window length was 2.68 ± 0.16 mm. The percent bone volume (BV/TV) in the experimental group was 34.1 ± 5.0, which was significantly higher than that of the control group 26.9 ± 7.1 ($P < 0.001$). Histological analysis revealed a significant decrease ($P < 0.001$) in the percentage of ALP stained cells in the experimental group (0.44 ± 0.2) when compared to the control group (1.2 ± 0.4). There was also a significant decrease ($P = 0.03$) in the percentage of CD34 stained cells in the experimental group (0.22 ± 0.08) when compared to the control group (1.58 ± 0.6). Finally, there was no significant difference ($P = 0.4$) in the percentage of TRAP stained cells between both groups ($P = 0.4$).

Conclusion: The results of our experiments show that daily treatments with cold therapy stimulate the bone growth/healing process in our murine model. Furthermore, the histological analysis reveals that the mechanism by which cold therapy stimulates growth is not necessarily linked to osteoblast activity since osteoblast activity was not increased in the experimental group despite increased bone formation. Further studies aimed to characterize the mechanism of action of cold therapy on bone healing are warranted based on the results of this pilot study.

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