A Study in Vivo of the Effects of a Static Compressive Load on the Proximal Tibial Physis in Rabbits

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INTRODUCTION:

The effect of compression on the vertebrate growth plate is generally defined by the Heuter-Volkmann principle, which states that there is decreased linear growth at the physis in the presence of a compressive force and increased growth in the presence of distraction. Previous studies have shown that mechanical compression alters the microscopic architecture of the physis. However, little is known of the actual cellular processes or extracellular events that are influenced by these physical changes.

Many pediatric disorders are directly related to abnormalities of the growth plate and its response to environmental influences. Slipped capital femoral epiphysis (SCFE), Blount’s disease, Scheuermann’s kyphosis, scoliosis, and club foot are all examples of conditions in which mechanical factors are thought to contribute to the pathophysiology of the growth plate. Childhood obesity is also associated with SCFE and Blount’s, observations suggesting that increased weight across the physis contributes to the pathology. However, investigations of mechanical compression across the growth plate are limited.

This study attempts to define specific molecular, biochemical, cellular and extracellular responses of the growth plate to mechanical compression using a rabbit model. The authors hypothesize that a compressed physis will exhibit structural changes in cells and extracellular matrices that reflect down regulation of aggrecan and type II collagen genes (related to cartilage structural molecules) and up regulation of type X collagen and matrix metalloprotease 13 (MMP-13) genes (related to molecules affecting chondrocyte hypertrophy and cartilage degradation, respectively).

METHODS:

This study was approved by the Institutional Animal Care and Use Committee (IACUC). Static compressive loads (10 and 30N) were applied for 2 or 6 weeks using an external fixator placed across proximal tibial physes of 13-week-old female New Zealand white rabbits (n = 24). Some unperturbed limbs served as normal controls. The contralateral hind leg in all rabbits underwent a sham surgery with no load to serve as an internal control. Harvested tissues were divided into portions for histology, immunohistochemistry (IHC), and quantitative reverse transcription-polymerase chain reaction (QRT-PCR) analysis.

The samples for histology and IHC were placed in 10% neutral buffered formalin, decalcified, embedded in paraffin, and sectioned 6 µm thick. Samples were stained with 1% thionin for metachromasia and general morphology, Periodic Acid Schiff (PAS) for the presence of proteoglycans, and picrosirius red for qualitative measurement of collagen. Chondrocyte hypertrophy was assessed using immunostaining for type X collagen. QRT-PCR samples were ground to powders under liquid nitrogen in a freezer/grinder mill (Model 6750, Spex, Inc., Metuchen, NJ). Total RNA was isolated, DNase-treated, and reverse-transcribed as previously described. Rabbit-specific primers for PCR of aggrecan, MMP-13, type II and X collagen, SOX-9 and β-actin (the latter two primers as normalizing genes) were designed with the Primer Express program (Applied Biosystems, Foster City, CA) using (when possible) primers spanning at least one intron of the respective known gene sequences. An ABI Prism 7500 Fast Sequence Detector (Applied Biosystems) was used for quantitative PCR. Analyses followed the relative standard curve methodology outlined in User Bulletin #2 (Applied Biosystems).

Statistical analyses of gene expression were performed using ANOVA, Tukey’s multiple comparisons test, and a test of sham versus load contrasts.

RESULTS:

Compared to unloaded shams, all physes loaded for 2 weeks showed no significant histological changes. Compared to unloaded shams, 6-week 30N-loaded physes were decreased in height (Figure 1). The 30N specimens also appeared to have structurally altered chondrocyte columns and decreased extracellular matrix between them in proliferative and hypertrophic zones of the tissues (Figure 2). Picrosirius red and other staining revealed disorganized arrangements of collagen fibrils in the extracellular matrices. IHC staining intensity for type X collagen was greatest in unloaded shams and it decreased directly in physes with increasing load.

With β-actin as a normalizing gene, QRT-PCR analysis of frozen ground loaded samples compared to unloaded shams demonstrated statistically significant (p < 0.05) decreased gene expression of aggrecan and type II and X collagen with increasing load. No statistically significant changes in MMP-13 expression were measured between unloaded shams and 10N and 30N loaded specimens.

DISCUSSION:

This model of compressed rabbit physes generated changes in the biochemical character of proteoglycans and collagens in addition to cellular and extracellular matrix architecture of the tissues. Down regulation of gene expression determined by QRT-PCR and decreased protein staining found by IHC are consistent in indicating that type X collagen is inhibited under load. Decreases in aggrecan and type II and X collagen gene expression correlated with structural alterations observed histologically support the concept that sustained compression leads to abnormal changes in the extracellular matrix of the growth plate. Such changes potentially weaken overall physis strength. Consequences of the absence of measured differences in MMP-13 expression are not fully understood. The structural alterations identified in this study further define changes consistent with the Heuter-Volkmann principle and may lead to insight into the etiology of orthopedic pathologies such as SCFE and Blount’s disease, in which mechanical force appears to be a significant contributor to the evolution of the disease process.

REFERENCES: